Seventh Annual Meeting Program

IFATS09

INTERNATIONAL FEDERATION FOR ADIPOSE THERAPEUTICS AND SCIENCE

October 15-17, 2009

Hotel Inter-Burgo, Daegu, Republic of Korea

Colorful DAEGU

Institute of Medical Science, Yeungnam University

Department of Pathology, Yeungnam University Medical College

대한성형외과학회

The Korean Society of Plastic and Reconstructive Surgeons

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Mission

To improve world health through a better understanding of adipose biology.

Description

The Society began as the International Fat Applied Technology Society (IFATS), a 501 (c) (3) non-profit organization incorporated in August of 2002. The organization is dedicated to facilitating the development of new technology derived from and directed toward adipose tissue. IFATS has evolved to become the International Federation of Adipose Therapeutics and Science (IFATS) and is a leading source of information regarding adipose biology and related technology. The Society identifies new technology and brings together scientists and industry for productive collaborations. Such collaborations will relate to adipose tissue, with far-reaching applications in obesity reduction products and services, fat-derived stromal and cell-based therapies, and fat-applied instrumentation devices which may be directly translated to therapeutic application. IFATS' current scientific areas of interest relate to facilitating the development of treatments for excess body fat, the generation of new fat tissue for reconstruction after cancer or birth-related defects, and the use of adipose tissue as a source of mesenchymal stem cells that have the potential to regenerate and repair many different tissues of the body. IFATS is currently the world's only interdisciplinary fat tissue society. IFATS hope is to serve as the premier global incubator in the field of fat applied and derived technology.

Leadership of Society

Co-Presidents:

Jae-Ho Jeong, M.D., Ph.D.
Director, Noblesse Plastic Surgery Clinic and StemTec Korea
Daegu, Korea

Sang Hong Baek, M.D., Ph.D.
Professor, The Catholic University of Korea School of Medicine
KangNam St Mary's Hospital
Seoul, Korea

Immediate Past Presidents:

Anne Bouloumie, Ph.D.
AVENIR Team leader, INSERM U858
Toulouse, France

Louis Casteilla, Ph.D.
Professor, UMR 5241 CNRS UPS
Toulouse, France

Scientific Program Chair

Spencer A. Brown, Ph.D
Director of Research, University of Texas Southwestern Medical Center
Dallas, Texas, USA

Welcome Message

It is a great honor to welcome you to the 7th Annual Meeting of the International Federation of Adipose Therapeutics and Science (IFATS) which hold on 15-17 October 2009 in Daegu, Republic of Korea. IFATS 2009 is the first meeting in Asia and this is a wonderful chance to propagate this new field of science into Asian countries.

During the last decade of 20th century at Pittsburgh University, there had been pioneering experiments by several plastic surgeons in a small laboratory testing the characteristics of stromal cells harvested from adipose tissue. It was the beginning of discovery of adipose tissue-derived stem cells (ASCs). After ten years since the discovery of this attractive cell, they appear as one of the most powerful source of regenerative medicine.

Since the establishment of IFATS in 2003, it has been a wonderful academic center of international collaboration as well as sharing knowledge and ideas. Day by day, many researchers with great talents are joining in this society and their contribution to this cutting edge science of regenerative medicine is magnificent.

This meeting is not only for basic research disclosing the characteristics of the adipose stromal/stem cells, but also for active clinical application of ASCs. Clinically, the ASCs are already being used in limited situations in plastic surgery, such as soft tissue augmentation, wound healing, etc. and many preclinical experiments for other clinical fields are also being performed throughout the world.

Korea is referred to as one of the successful countries established after World War II. However, Korea is not a new country and its five thousand years of long history and cultural atmosphere have not been very well known in Western countries. Modern Korea is full of so many passionate rapid adaptors with dynamic energy. Daegu is located at southeast part of Korean peninsula and is a new center of medical science and education. Three of UNESCO's seven world cultural heritage sites are located around Daegu: Bulguksa Temple, Seokguram Grotto and Tripikata Koreana located in Haeinsa Temple.

We do sincerely thank you for joining IFATS 2009 and also enjoy the Korean hospitality.

Jae-Ho Jeong, MD., PhD.
Co-Presidents, IFATS 2009

Sang Hong Baek, MD., PhD.
Co-Presidents, IFATS 2009

Wednesday, Oct 14, 2009

6:00 - 7:30 PM **Social Gathering (Amante Hall, 2F, Inter-Burgo)**

7:00 - 9:00 PM **Exhibitor Set-up (Lobby, 1F, Inter-Burgo)**

Thursday, Oct 15, 2009

Room A (Clavel Hall)

8:00 - 8:30 AM **Registration**

8:30 - 9:30 AM **Poster Set-up (Room C)**

9:30 - 10:00 AM **Keynote Address Ⅰ** Moderator: Sang Hong Baek (The Catholic Univ. of Korea)
HDACs modulate differentiation of mesenchymal stem cells into adipocytes and osteoblasts
Jae Bum Kim (Seoul Nat'l Univ., KOREA)

10:00 - 11:00 AM **Symposium Ⅰ : ASCs Extraction -**"How do you extract ASCs?"
Moderators: Kotaro Yoshimura (Univ. of Tokyo.) & Adam J. Katz (Univ. of Virginia)

10:00 - 10:15 AM **Ⅰ-1 Isolation and culture methods of adipose-derived stem cells**
Il-Hwa Hong (Kyungpook Nat'l Univ. KOREA)

10:15 - 10:30 AM **Ⅰ-2 Stem cell isolation method for clinical uses; Aseptic manual system & simple cell concentration method**
Hee Young Lee (KangNam Plastic Surgery & Medikan Inc., KOREA)

10:30 - 10:45 AM **Ⅰ-3 Automated, point-of-care system for rapid ADSC isolation**
Stuart Williams (Tissue Genesis, Inc., USA)

10:45 - 11:00 AM **Ⅰ-4 Extraction of adipose derived stem cell in clinical urology: First patient treated for stress urinary incontinence**
Tokunori Yamamoto (Univ. of Nagoya/ Cytori Therapeutics, Inc., JAPAN)

11:00 - 11:15 AM **Coffee Break**

11:15 - Noon **Symposium Ⅱ : Basic Science of ASC 1**
Moderators: Jae Bum Kim (Seoul Nat'l Univ.) & Dae Hyun Lew (Yonsei Univ.)

11:15 - 11:30 AM **Ⅱ-1 Cell division regulator has a strong relationship with immaturity maintenance and differentiation capability of hASCs**
Ho Park (Adult Stem Cell Research Institute, Kangbuk Samsung Hospital, KOREA)

11:30 - 11:45 AM **Ⅱ-2 Differentiation of adipose derived stem cell into endocrine cell**
Jong Lim Park (Seoul Nat'l Univ., Boramae Hospital, KOREA)

11:45 - Noon **Ⅱ-3 Dedifferentiated fat cells as a new cell source for regenerative medicine**
Taro Matsumoto (Nihon Univ., JAPAN)

Noon - 1:15 PM **Luncheon Poster (Room C)**

1:15 - 1:30 PM **Opening Remarks** Chair: Yong Jin Kim (Yeungnam Univ.)

1:30 - 2:30 PM **Special Lecture 1** Moderator: Jae-Ho Jeong (Noblesse Plastic Surgery Clinic)
Cell-based therapies: All mesenchymal stem cells are not equal
Arnold I. Caplan (Case Western Reserve Univ., USA)

2:30 - 3:30 PM **Symposium Ⅲ: Basic Science of ASC 2**
Moderators: Louis Casteilla (UMR 5241 CNRS UPS) & Woo Seob Kim (Chung-Ang Univ.)

2:30 - 2:45 PM **Ⅲ-1 Comparative transcriptional profiling of tissues engineered using ASCs and BM-MSCs**
Julie Fradette (Universite Laval, CANADA)

2:45 - 3:00 PM **Ⅲ-2 Differentiated adipocytes do not elicit alloreactive immune response**
Mi-Hyung Kim (Anterogen Co., Ltd, KOREA)

3:00 - 3:15 PM **Ⅲ-3 Possible specific markers for differentiating human adipose-derived stem cells**
Hitomi Eto (Univ. of Tokyo, JAPAN)

3:15 - 3:30 PM **Ⅲ-4 The in vitro cell fate of photoaged fibroblast after ASCs application: Comparison between transwell and conditioned medium culture**
Yeo Reum Jeon (Institute for Human Tissue Restoration, Yonsei Univ., KOREA)

3:30 - 3:50 PM **Coffee Break**

3:50 - 4:50 PM **Special Lecture 2** Moderator: Kyu-Shik Jeong (Kyungpook Nat'l Univ.)
Molecular regulation of muscle stem cell fate
Thomas A. Rando (Stanford Univ., USA)

4:50 - 5:50 PM **Symposium Ⅳ : Clinical Application of ASCs**
Moderators: Hajime Ohgushi (AIST) & Ramon Llull (Stem Center)

4:50 - 5:10 PM **Ⅳ-1 Therapeutic potential of adipose stem cells**
Jae- Ho Jeong (Noblesse Plastic Surgery Clinic & StemTec, KOREA)

5:10 - 5:30 PM **Ⅳ-2 Adipose-tissue progenitor cells: Functions in adipose transplantation and clinical use for soft tissue enhancement**
Kotaro Yoshimura (Univ. of Tokyo., JAPAN)

5:30 - 5:50 PM **Ⅳ-3 Recent personal clinical trials ; ASCs - scaffolds injection**
Hee Young Lee (KangNam Plastic Surgery, KOREA)

6:00 PM - **Welcome Reception** (Poolside Garden, Hotel Inter-Burgo)

Friday, Oct 16, 2009

Room A (Clavel Hall)

8:30 - 9:00 AM **Keynote Address Ⅱ** Moderators: Ramon Llull (Stem Center)
The FAT revolution: Past, present, and future
Adam J. Katz (Univ. of Virginia, USA)

Symposium Ⅴ : Cardiovascular Science I Moderators: Satoshi Shintani (Nagoya Univ.)

9:00 - 10:00 AM **Ⅴ-1 Adipose stromal cells in angiogenesis, vasculogenesis, and tissue repair**
Keith L. March (Indiana Univ., USA)

9:00 - 9:20 AM **Ⅴ-2 Implantation of adipose-derived regenerative cells enhances ischemia-induced neovascularization. Role of SDF-1-mediated endothelial progenitor cell mobilization.**
Satoshi Shintani (Nagoya Univ., JAPAN)

Scientific Program

9:40 - 10:00 AM

Ⅴ-3 Clinical application of adipose tissue-derived mesenchymal stem cell implantation as a Novel therapeutic option for Buerger's disease: Preliminary report
Sang Hong Baek (The Catholic Univ., KOREA)

10:00 - 10:20 AM **Coffee Break**

10:20 - 11:20 AM **Symposium Ⅵ : Cardiovascular Science II**
Moderators: Keith L. March (Indiana Univ.) & Sang Hong Baek (The Catholic Univ. of Korea)

10:20 - 10:35 AM

Ⅵ-1 Adipose-derived stem cells posses a functional hepatocyte growth factor / c-Met receptor autocrine loop that is essential for in vivo potency
Brian Johnstone (Indiana Univ., USA)

10:35 - 10:50 AM

Ⅵ-2 Vascular cell differentiation of human adipose-derived stem cells based on FGF2- immobilized substrate
Sang-Heon Kim (Korea Institute of Science and Technology, KOREA)

10:50 - 11:05 AM

Ⅵ-3 Injury-associated growth factors improve ischemia through the activation of adiposederived stem/progenitor cells in obese and non-obese mice
Hitomi Eto (Univ. of Tokyo, JAPAN)

11:05 - 11:20 AM

Ⅵ-4 Tracking injected adipose-derived stem cells in vivo using a novel murine model of muscle ischemia
Blair Stocks (UVA, USA)

11:20 - 11:35 AM

Ⅵ-5 Adipose derived stromal vascular fraction cells potentiate neovascular sprout invasion of surrounding matrix.
James B. Hoying (Cardiovascular Innovation Institute, USA)

11:35 - 11:50 AM

Ⅵ-6 Adipose stromal cells induce stable vascular network formation by endothelial cells in in vitro co-culture settings
Dmitry Traktuev (Indiana Univ., USA)

11:50 - 12:20 AM **IFATS Business Meeting**

12:20 - 1:30 PM **Luncheon Poster (Room C)**

1:30 - 2:00 PM **Keynote Address Ⅲ** Moderator: Gou Young Koh (KAIST)
The dog - The ideal preclinical model for human orthopedic disease therapy with adipose-derived stem cells
Robert J. Harman (Vet-Stem, USA)

2:00 - 2:30 PM **Keynote Address Ⅳ** Moderator: Arnlod I. Caplan (Case Western Reserve Univ.)
Proliferation/differentiation capability of mesenchymal stem cells (MSCs) - Importance of transcription factor(s) expression in human MSCs -
Hajime Ogushi (RICE, AIST, JAPAN)

2:30 - 3:30 PM **Symposium Ⅶ : ASC and Adipogenesis**
Moderators: Spencer A. Brown (UT Southwestern Medical Center) & Sa Ik Bang (Sungkyunkwan Univ.)

2:30 - 2:45 PM

Ⅶ-1 Human adipose-derived stem cell enriched fat grafts: Effect on cutaneous radiation injury in the athymic rat
Spencer A. Brown (UT Southwestern Medical Center at Dallas, USA)

2:45 - 3:00 PM **Ⅶ-2 Co-culture of adipocytes and adipose stem cells with either resident or peritoneal macrophages results in preadipocyte producing stem cell spheres in Human and Mouse.**
Gregorio Chazenbalk (Cedars-Sinai Medical Center, USA)

3:00 - 3:15 PM **Ⅶ-3 Reversible adipose enlargement induced by external tissue suspension: Possible contribution of growth factors for its preservation**
Hirotaka Suga (Univ. of Tokyo, JAPAN)

3:15 - 3:30 PM **Ⅶ-4 PDK regulated Warburg-like phenotype protects against ROS production in differentiating human adipocytes**
William Roell (Indiana Center for Vascular Biology and Medicine, USA)

3:30 -3:45 PM **Coffee Break**

3:45 - 4:45 PM **Symposium Ⅸ: Tissue Engineering**
Moderators: Jin Sup Jung (Pusan Nat'l Univ.) & Gilson Khang (Chonbuk Nat'l Univ.)

3:45 - 4:00 PM **Ⅸ-1 Preparation and evaluation of PLGA-silica scaffold for bone tissue engineering**
Jong-Won Rhie (The Catholic Univ. of Korea, KOREA)

4:00 - 4:15 PM **Ⅸ-2 Hydrostatic pressure (HP)-driven 3-D cartilage regeneration using collagen gel and human adipose-derived stem cells**
Rei Ogawa (Nippon Medical School, JAPAN)

4:15 - 4:30 PM **Ⅸ-3 Novel electrospun polymer scaffolds with microencapsulated human ASCs for tissue regeneration**
Yihwa Yang (Univ. of Virginia, USA)

4:30 - 4:45 PM **Ⅸ-4 Osteogenesis of SFF-based scaffolds using osteoblasts derived from adipose-derived stem cells and HUVECs**
Jong Young Kim (POSTECH, KOREA)

4:45 - 5:45 PM **Special Lecture 3** Moderator: Yong Jin Kim (Yeungnam Univ.)
Robot technology and Future: The age of robot
Jun-Ho Oh (KAIST, KOREA)

5:45 -6:30 PM **Cocktail Reception (Lobby)**

6:30 PM - **Gala Award Dinner (Room A)**

Room B (Camellia Hall)

2:30- 4:00 PM **Symposium Ⅷ**
Moderators: Hee Young Lee (KangNam Plastic Surgery) & Daegu Son (Keimyung Univ.)

2:30 - 2:45 PM **Ⅷ-1 Safety and feasibility of expanded adipose mesenchymal stem cells**
Jeong-Chan Ra (RNLBIO Co., Ltd, KOREA)

2:45 - 3:00 PM **Ⅷ-2 Preliminary investigation of the effect of human adipose tissue-derived mesenchymal stem cell(ASC) in progressive hemifacial atrophy (Parry-Romberg's disease)**
Jong-Woo Choi (Ulsan Univ., KOREA)

3:00 - 3:15 PM **Ⅷ-3 Study for development of cell therapy for Alzheimer's disease using adipose-derived stem cells**
Yoo-Hun Suh (Seoul Nat'l Univ. KOREA)

3:15 - 3:30 PM **Ⅷ-4 Autologous adipose tissue derived mesenchymal stem cells: Clinical trials for osteoarthritis**
Kangsup Yoon (Seoul Nat'l Univ. KOREA)

3:30 - 3:45 PM **Ⅷ-5 Autologous adipose tissue derived mesenchymal stem cell intravenous infusions ameliorate osteoarthritis (OS), ulcerative colitis (UC), hashimoto thyroiditis (HT), atopic dermatitis (AD) with allergic rhinitis, and chronic obstructive pulmonary disease with bronchial asthma**
Tai June Yoo (Tennessee Univ. USA)

3:45 - 4:00 PM **Ⅷ-6 Safety and effect of adipose tissue derived stem cell implantation in patients with critical limb ischemia : Early clinical experiences**
Han-Cheol Lee (Pusan Nat'l Univ. KOREA)

Saturday, Oct 17, 2009

Room A (Clavel Hall)

8:30 - 9:00 AM **Keynote Address Ⅴ** Moderators: Jae-Ho Jeong (Noblesse Plastic Surgery Clinic)

Adipose derived stem cells in bone regeneration
Riitta Suuronen (Regea Institute for Regenerative Medicine, Univ. of Tampere, FINLAND)

9:00 - 10:30 AM **Symposium Ⅹ: ASC and Other Therapeutic Application**
Moderators: Riitta Suuronen (Regea Institute for Regenerative Medicine)
William Futrell (Univ. of Pittsburgh Medical Center)

9:00 - 9:15 AM **Ⅹ-1 In-vitro differentiation of adipose derived stem cells into Schwann cells and *in-vivo* implications for peripheral nerve defects**
Hakan Orbay (Nippon Medical School, JAPAN)

9:15 - 9:30 AM **Ⅹ-2 The role of human adipose-derived stem cells in 2-acetylaminofluorene-induced liver injury of rats**
Il-Hwa Hong (Kyungpook Nat'l Univ., KOREA)

9:30 - 9:45 AM **Ⅹ-3 Stem cells from adipose tissue: Applications in spinal disorders**
Marco N. Helder (VU Univ. Medical Center, NETHERLANDS)

9:45 - 10:00 AM **Ⅹ-4 Omentum as a potential source of cell therapy for acute liver damage**
Makoto Tokuhara (International Medical Center of Japan, JAPAN)

10:00- 10:15 AM **Ⅹ-5 Protective effects of adipose stem cells against cigarette-smoke induced lung injury**
Irina Petrache (Indiana Univ., USA)

10:15 - 10:30 AM **Ⅹ-6 Optimization of administration route for adipose derived stem cell therapy on random pattern skin flaps**
Dong Won Lee (Institute for Human Tissue Restoration, Yonsei Univ., KOREA)

10:30 - 10:45 AM **Coffee Break**

10:45 - 11:15 AM **Invited Lecture** Moderator: Ramon Llull (Stem Center)

New perspectives in plastic surgery: Adipose derived stem cells and enriched fat grafting in breast surgery

Michele L. Zocchi (CSM Institute of Aesthetic Plastic and Reconstructive Surgery, ITALY)

11:15 - 12:15 PM **Symposium Ⅺ: ASC and Diseases**

Moderators: Jong-Won Rhie (TheCatholic Univ. of Korea) & Ho Yun Chung(Kyungpook Nat'l Univ.)

11:15 - 11:30 AM **Ⅺ-1 Adipocyte differentiation influences proliferation and migration of normal and tumoral epithelial breast cells**

Luciano Vidal (Universidad del Salvador, ARGENTINA)

11:30 - 11:45 AM **Ⅺ-2 Aging, fat depot origin, fat cell progenitor senescence, and inflammation**

James Kirkland (Robert and Arlene Kogod Center on Aging, USA)

11:45 - Noon **Ⅺ-3 Cellular events in pathological adipose tissue hyperplasia: Lipoma, lipedema and obesity**

Hirotaka Suga (Univ. of Tokyo, JAPAN)

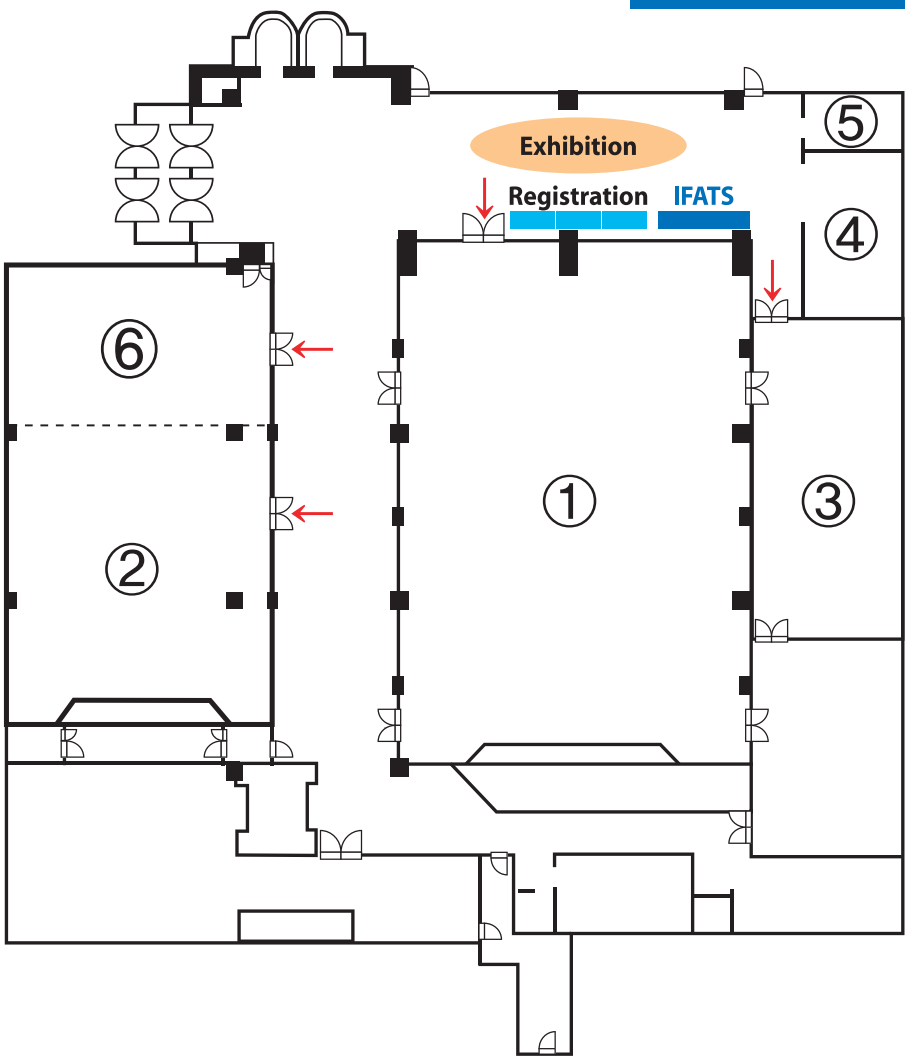
Noon - 12:15 PM **Ⅺ-4 Tumor-derived exosomes can induce differentiation of mesenchymal stem cells into tumor-associated myofibroblastic cells**

Jung Ah Cho (Adult Stem Cell Research Institute, Kangbuk Samsung Hospital, KOREA)

12:15 - 12:30 PM **Preview IFATS 2010 & Adjournment**

Floor Guide Map

1F, Hotel Inter-Burgo



① **Room A** (Clavel Hall)_ Main Symposium (October 15-17, 2009)
Gala Award Dinner (October 16, 2009)

② **Room B** (Camelia Hall)_ Video Presentation (October 15, 2009)
Parallel Symposium (October 16, 2009)

③ **Room C** (Dailia Hall)_ Poster Session

④ **Preview Room & VIP Room**

⑤ **Secretariat**

⑥ **Internet Lounge**

Conference Information

General Information

DATE October 15-17, 2009

VENUE Hotel Inter-Burgo, Daegu, Republic of Korea

LANGUAGE
The official language for the conference is English. Simultaneous translation will not be provided.

REGISTRATION
The on-site registration desk will be opened at the conference site as follows:

Date	Time & Place
October 15-16, 2009	8:00 am - 6:00 pm, 1F, Inter-Burgo 1
October 17, 2009	8:00 am - Noon, 1F, Inter-Burgo 1

SECRETARIAT OFFICE
The conference secretariat office will be located near conference halls and will be opened from 7:30 am to 6:00 pm on October 15-16, 2009 and from 7:30 to 12:00 on October 17, 2009

COMPUTER ACCESS
The wireless system is available at the conference site. Also you will find an Internet Lounge at Camelia Hall, 1F, Hotel Inter-Burgo. Computers will be equipped with the usual software: MS Office, an SSH client, Acrobat reader.

MESSAGE BOARD
Message boards will be located in front of the conference hall for communication among delegates.

INFORMATION DESK
IFATS 2009 information desk will be located in the registration areas to assist you with your inquiries and will be open from 8:00am to 5:00pm throughout conference. Inquires can include Daegu city tour, transportation information, restaurants, etc.

Scientific Information

INSTRUCTIONS FOR ORAL PRESENTERS
Presentations will start at their scheduled times in order for conference attendees to attend those talks they wish to hear. The conference will be providing LCD projectors and machines for presenters. You are also welcome to bring your own laptops, particularly if video and animation is included in the presentations or you are a Mackintosh user. The computers in the session rooms will be Windows XP-based PC with Microsoft PowerPoint 2007. Please plan to arrive at the podium 15 minutes before your presentation is scheduled to begin.

PREVIEW ROOM
We strongly recommend that all authors come to the preview room before their presentation and submit their presentation file to IFATS 2009 staffs at least 2 hours prior to the start of your session or email it to IFATS 2009 local office in advance. When reviewing your presentation make sure all fonts, images, and animations appear as expected and that all audio or video clips are working properly. If the presentation does not play properly in the preview room, it will not play properly in the conference room.

Conference Information

POSTER SESSION

Schedule for Display, Mounting, and Removal

Posters will be on display from Thursday, October 15 to Saturday, October 17. Poster mounting will be possible on Thursday, October 15, from 08:30. However, the posters will be up until 10:30 to allow IFATS 2009 participants to continue looking at the posters, also during the lunch breaks on October15-16, 2009. During the poster session, the authors are requested to be available at their display board to discuss their work with interested attendees.

Removal will be possible on Friday, October 16, from 19:00.

***Please note that posters remaining on display after the symposium closes will be taken down by the staff of the hotel and will not be stored or sent to the authors.*

AWARDS

The IFATS wishes to recognize the outstanding contributions made to the scientific program. The award will be made for the best oral and poster presentations given by pre-doctoral students or post-doctoral fellows. The selection of the winners of the awards will be made by a Scientific Committee of IFATS 2009. Awards will be presented at the Gala Awards Dinner on Friday, October 16, 2009.

Social Events

The following programs are arranged for all participants and accompanying persons. Participation in these programs is included in the registration fee.

SOCIAL GATHERING

Date & Time: 6:00-7:30 pm, Wednesday, October 14, 2009
Place: Amante Hall, 2F, Hotel Inter-Burgo 1

WELCOME RECEPTION

Date & Time: 6:00pm, Thursday, October 15, 2009
Place: Poolside Garden, Hotel Inter-Burgo 2 (Annex Building)

***Please be sure to carry a ticker jacket with you.*

GALA AWARD DINNER

Date & Time: 6:30pm, Friday, October 16, 2009
Place: Clavel Hall, 1F, Hotel Inter-Burgo 1

POST CONFERENCE TOUR

Date & Time: October 17-18, 2009 (1night 2days)
Place: Andong *(Hanok* Stay*)*

***Shuttle bus for Andong Tour will depart at 1:30 pm. in front of Hotel Inter-Burgo. To sign-up for this tour, please ask information desk.*

Oral Abstracts

Oral Abstracts

Oral Abstracts

KEYNOTE ADDRESS Ⅰ

HDACs modulate differentiation of mesenchymal stem cells into adipocytes and osteoblasts

Jae Bum Kim, Hyun Woo Lee, A Young Kim, Sang Yoon Kim

School of Biological Sciences, Seoul National University, Seoul, South Korea 151-742

Mesenchymal stem cells are able to differentiate into adipocyte, osteoblast, chondrocyte and myoblast depending upon environmental factors and tissue specific transcription factors. Adipogenesis is regulated by the complex transcriptional cascades that are essential for the execution of adipocyte differentiation. CCAAT/enhancer binding proteins (C/EBPs), peroxisome proliferator-activated receptor gamma (PPAR gamma), and adipocyte determination- and differentiation-dependent factor 1/sterol response element binding protein 1c (ADD1/SREBP1c) have been identified as the key transcription factors involved in adipogenesis. Although it is not clear how these genes are maintained quiescent in preadipocytes, it is possible to speculate that HDACs are involved.

Depending on various cellular and environmental signals, mesenchymal progenitor cells are also able to differentiate into osteoblasts. For instance, bone morphogenetic protein (BMP) -2 and -7 stimualte osteoblast differentiation through activation of osteogenic transcription factors such as Runx2 (also called as Cbfa1, Pebp2alphaA, and AML3) and osterix. Runx2 is a master transcription factor for osteoblast differentiation accompanied with osteoblast-specific gene expression.

Chromatin modifications, notably histone acetylation and deacetylation, are crucial for the regulation of gene expression and development in eukaryotes. During tissue differentiation, early inductive processes that determine cell fate leave traces at particular genes in the form of histone modifications. HATs and HDACs have been shown to respond to signals that regulate cell differentiation and directly modulate tissue-specific gene expression.

Past research on tissue differentiation has been mainly focused on understanding the regulatory roles of specific transcription factors. On the contrary, little is known about the functional roles of coregulators such as HATs and HDACs during differentiation processes. In this study, I will discuss novel roles of HDACs during adipogenesis and osteoblast differentiation. We demonstrate that the down-regulation of HDACs at the early stages adipogenesis and osteogenesis appears to be a prerequisite step to induce mature differentiation from mesenchymal cells. These findings add to list of the functional roles of coregulators in regulating cell differentiation and may provide a new target for discovering novel therapies for the treatment of fat-related disorders.

This study was supported in part by grants from the Stem Cell Research Center of the 21st Century Frontier Research Program.

Symposium Ⅰ: ASCS EXTRACTION -"How do you extract ASCs?"

Ⅰ- 1] Isolation and culture methods of adipose-derived stem cells

Il-Hwa Hong[1], Jin-Kyu Park[1], Ae-Ri Ji[1], Seon-Young Han[1], Mi-Ran Ki[1], Young-Mi Moon[2], Se-Il Park[1], Hae-Ryeong Jo[1], Jae-Ho Jeong[2], Kyu-Shik Jeong[1]

[1]Colleges of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Republic of Korea.

[2] Noblesse Plastic Surgery, Daegu 700-411, Republic of Korea.

Introduction

Adipose-derived stem cells (ASCs) have been concerned an exciting alternative stem cell source because adipose tissue allows extraction of a large volume of tissue with readily accessible. Cell isolation methods from adipose tissue were pioneered in the 1960s and then, adipose tissue has demonstrated to serve as multipotent stem cells which has the ability to differentiate into several mesodermal lineages including bone, muscle, cartilage, fat and neural progenitors by many researchers, since a putative stem cell population was identified from human lipoaspirates in 2001. Herein, methods for isolation and expansion of ASCs were reviewed and described in detail.

Methods

1) Collection of adipose tissue

① Adipose tissue was collected by needle biopsy or liposuction from humans and laparatomy from animals.

② Adipose tissue was washed with sterile phosphate-buffered saline (PBS) to remove contaminating debris and red blood cells (RBCs).

2) Isolation of SVF

① Adipose tissue was minced finely using surgical scissors.

② Preparation of digestion buffer: 2mg/ml of collagenase type Ⅰ in PBS filtered through 0.2㎛ pore and should be prepared freshly before use.

③ Minced adipose tissue were placed into digestion buffer for 10~30min at 37℃ with vigorous agitation (300rpm).

④ The collagenase was inactivated with an equal volume of complete low glucose (LG)-DMEM with 10% fetal bovine serum (FBS).

⑤ The cell suspension was filtered through 70㎛ nylon cell strainer and centrifuged at 3000 rpm for 3min

⑥ The cell suspension was separated into three layers: an upper layer of oily lipocytes, an intercellular layer of adipose tissue, a bottom layer of mononuclear cells.

⑦ Cells of the bottom layer were collected carefully and transferred to new centrifuge tubes and then washed with medium. The cells were centrifuged at 3000rpm for 3 min and re-suspended to wash with new medium. This washing step was repeated two or three times. The pellet cells of this step were stromal vascular fraction (SVF) and these cells can be applied to the patients for cell therapy.

3) Expansion of ASCs

① Cells were plated onto culture dishes and incubated at 37℃, 5% CO_2. These cells were defined as passage 0 (P0). One or two hours after plating, the entire old medium was aspirated and replaced the new medium.

② The media replaced every 2-3 days until they reached confluence completely. In human-ASCs, 1-2 $\times 10^6$ cells reached confluence completely in a 150mm dish.

③ Cells were split with 0.05% trypsin-EDTA solution for 37℃ at least 3min or until approximately 90% of the cells have detached from the dishes to subculture. When 3-5 $\times 10^5$ cells of human-ASCs plated onto a 150mm dish and they reached confluence completely in LG-DMEM within 7~10 days.

4) Storage of ASCs

ASCs could be harvested at 80% confluence for freezing with 90% fetal bovine serum and 10% demethylsulfoxide (DMSO) in the LN_2 tank.

Results

The isolated cells from the adipose tissue were SVF and they consisted of a heterogenous cell population, including red blood cells, fibroblasts, pericytes and endothelial cells as well as predipocytes. However, finally adherent cell population onto plastic culture dishes within the SVF was named by adipose-derived stem cells (ASCs) by the International Fat Applied Technology Society (IFATs). RBC, which is abundantly contaminated cells in SVF, removed by exchanging the medium to exclude ASCs injury caused by RBC lysis solution. P3~5 stem cells have been usually used for animal experiments or *in vitro* study, however, there is a report that stem cell-related transcription factors including Nanog, Oct-4, Sox-2 and Rex-1 still expressed in ASCs of P30. α-MEM, low glucose (LG) and high glucose (HG) DMEM have been used for culture medium of ASCs.

Conclusion

This isolation and expansion methods of ASCs have been generalized in human adipose tissue. In other species, this procedure can be applied with minimal modifications.

Symposium Ⅰ: ASCS EXTRACTION -"How do you extract ASCs?"

Ⅰ- 2] Stem cell isolation method for clinical uses; Aseptic manual system & simple cell concentration method.

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The clinical use of ASCs is still limited in several countries because stem cells are considered as a drug. However, in almost all the countries, when whole processes are made in clinic as a part of surgery, it is traditionally considered as a therapy. These rules still have many contradictions and argues. Off-label uses and new trials of therapy for doctors also need clear definition in several nations.

Korean FDA changed the law to permit use of collagenase as a legal minimal manipulation in 8th, June, 2009. It is the first time in the world as an official announcement related with clinical application of ASCs. Before this, there was no way to distinguish HCT from HCPs.

We used enzyme isolation for 5 years in our clinic. We invented aseptic, simplified methods using specialized system(Lipokit TM, Medi-khan, U.S.A.). It removes unsterilized pipets, tubes which can be a source of contamination and a technical mistake.

It also minimizes room air contacts and condensate through squeezing and centrifugation so it standardizes real fat concentration and enzyme concentration differences. It also can save times in ASCs isolation so can keep maximal viability. Isolated ASCs is always counted in number and checked viability. This method is similar with conventional manual method, but more aseptic and more standardized, more legal because it just uses licensed medical instruments. The contamination is one of the most frequent problems in isolation and subsequent culture, failure, so not use.

We also used fully automatic system (Lipokit IV TM Medikan, Korea). Almost robotic actions were designed to mimic conventional manual techniques. Cell yields were better than that of well trained technicians. As basic principles, viscosity, flow resistance, specific gravity of pellet are adapted. We regulate centrifugal forces and finally separate cell pellet in a sealed chamber.

Recently we use more condensed fat graft in which stem cell density is over 2-3times than normal fat. It is made through 'cell-saving micronization' using medical cutting mill (Filler Geller TM, Medikan, Korea). The rotary blades are very sharp and have 50 - 100um gaps from opposite blades, so they can save 10 um cells relatively. It is not real isolation of stem cells, but it can mimic cell enriched fat grafts just in the aspect of cell number. We can call this an incomplete ASCs isolation method if it can save real isolation. We also can obtain small number of cells in the pellet after centrifugation.

We can use various different methods in ASCs isolation. However, if it is for a real clinical use, we should choose the most optimal method considering feasibility and safety, cost, processing time including fat harvesting steps.

Symposium Ⅰ: ASCS EXTRACTION -"How do you extract ASCs?"

Ⅰ - 3] Automated, point-of-care system for rapid ADSC isolation

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Introduction

Adult stem cells occupy the forefront of regenerative medicine with clinical evaluations of bone marrow-derived stem cells (BMSCs) and adipose-derived stromal cells (ADSCs). Therapeutic targets for these cells have expanded from initial indications for bone[1 2 3]and blood illnesses[4] for BMSCs, and reconstructive applications[5 6 7 8]for ADSCs, to a myriad of applications such as treating peripheral vascular diseases[9 10] , arthritis[11], nervous system ailments[12 13], coating devices[14], wound care[15 16], and chronic heart disease[17 18]. A major obstacle to wide-spread clinical use of ADSCs has been the lack of operating room-compatible point-of-care systems for consistent isolation of therapeutic doses of cells. Investigators have used established "laboratory methods" employing tissue culture equipment to isolate adipose-derived cells. However, these methods are labor intensive and operator dependent, resulting in significant variability in cell yield and cell quality. They therefore do not scale well to clinical applications. An automated adipose cell isolation system (CIS) has been developed that permits point-of-care, operator-independent ADSC isolation in therapeutic doses in about an hour for a myriad of indications. During development of the CIS, patient demography was considered for possible influence on the outcomes of tissue digestion and cell isolation. The effect of patient demographics (age, gender, ethnicity, body mass index (BMI), tobacco use, and Type II diabetic status) was evaluated with respect to cell yield and viability with lipoaspirate samples from 65 patients undergoing cosmetic liposuction.

Methods

Lipoaspirate samples were obtained from the abdomen, flank, and hip region of patients undergoing tumescent liposuction for cosmetic purposes. Tissue samples were shipped chilled and processed the following day using the CIS instrument, which automates tissue digestion, wash, centrifugation, and cell suspension. Cell yield and viability were assessed using a trypan blue exclusion assay. Age, gender, ethnicity, body mass index (BMI), tobacco use, and Type II diabetic status were statistically analyzed to see if patient demographics affected cell yield or viability of the isolated SVF. BMI data were divided into three categories: BMI ranges from 18.5 to 24.9 were considered "normal" , from 25.0 to 29.9 "overweight", and 30.0 and above "obese". Statistical analysis was completed using the Mann Whitney Rank Sum Test to compare cell yield and viability for gender, tobacco use, and diabetic status. The effect of BMI, ethnicity and age on cell yield and viability were completed using the Kruskal-Wallis One Way Analysis on Ranks.

Results

The three ethnic populations compared in this study were African American (n=9), Caucasian (n=18), and Hispanic (n=37). Median cell yields of 0.824×10^6 cells/g, 0.916×10^6 cells/g, and 0.870×10^6 cells/g for African American, Caucasian, and Hispanic populations, respectively, were obtained. Median cell viabilities of 83.07%, 82.05%, and 85.60% for African American, Caucasian, and Hispanic populations, respectively, were obtained. According to statistical analysis, ethnicity did not significantly affect cell yield (H statistic of 0.412, p= 0.814) or viability (H statistic of 0.610, p=0.737).

Patients were categorized into three subgroups for BMI analysis: normal (n=13), overweight (n=29), and obese

(n=23). Patients with a normal BMI had a median cell yield of 0.874×10^6 cells/g. Patients with an overweight BMI had a median cell yield of 0.870×10^6 cells/g. And, patients with an obese BMI had a median cell yield of 0.871×10^6 cells/g. The median cell viabilities were 86.3%, 83.7%, and 83.07% for normal, overweight, and obese BMI's, respectively. Statistical analysis suggested no significant difference occurred for cell yield (H statistic of 0.457, p=0.796) and viability (H statistic of 4.394, p=0.111) between BMI's.

The majority of patients used for this comparison were female (n=57) compared to male patients (n=7). The median cell yields were 0.878×10^6 cells/g and 0.826×10^6 cells/g for females and males, respectively. The median cell viabilities were 84.90% and 80.10% for females and males, respectively. There was no significant difference for either cell yield (U statistic of 239.5, p=0.395) or viability (U statistic of 223.5, p=0.613) between female and male patients.

Patients were subdivided into six age groups 20-29 years (n=7), 30-39 years (n=18), 40-49 years (n=16), 50-59 years (n=12), 60-69 years (n=6), and 70-79 years (n=5). The median cell yields were 0.878×10^6 cells/g, 0.954×10^6 cells/g, 0.800×10^6 cells/g, 0.974×10^6 cells/g, 0.851×10^6 cells/g, and 0.935×10^6 cells/g for 20-29, 30-39, 40-49, 50-59, 60-69, and 70-79 age groups, respectively. The median cell viabilities were 85.40%, 85.00%, 81.38%, 83.75%, 85.80%, and 86.70% for 20-29, 30-39, 40-49, 50-59, 60-69, and 70-79 age groups, respectively. Statistical analysis suggested that there was no significant difference between age groups and cell yield (H statistic of 3.977, p=0.553) or viability (H statistic of 3.565, p=0.614).

The majority of patients were non-tobacco users (n=59) compared to tobacco users (n=6). The median cell yields were 0.936×10^6 cells/g and 0.860×10^6 cells/g for tobacco and non-tobacco users, respectively. Median cell viabilities were 81.55% and 84.85% for tobacco and non-tobacco users, respectively. Statistical analysis suggested that there was no significant difference between the use of tobacco and cell yield (U statistic of 159.5, p=0.700) or viability (U statistic of 186.0, p=0.847).

A total of 61 non-diabetic patients and 4 Type II diabetic patients were analyzed in this study. Median cell yields of 0.897×10^6 cells/g and 0.871×10^6 cells/g for diabetic and non-diabetic patients, respectively. Median cell viabilities were 87.70% and 84.80% for diabetic and non-diabetic patients, respectively. No significant difference was found between diabetic and non-diabetic patients for cell yield (U statistic of 112.5, p=0.806) and viability (U statistic of 64.0, p=0.116).

Conclusion

The CIS yields 0.945×10^6 cells/g $\pm 0.278 \times 10^6$ cells/g (mean $\pm$ SD; n=65) with a viability of 83.13% $\pm$ 4.93% (mean $\pm$ SD; n=65) regardless of patient demographics. Normalized methods for collecting lipoaspirate and use of the CIS provide a convenient, reliable, and repeatable cell product. This is accomplished by automation and use of a proprietary enzyme which takes out the lot to lot variation that is often associated with crude enzyme lots. After loading of a one-time-use disposable, the cell isolation process is completely automated with no user interaction; in about one hour 60 cc of lipoaspirate typically yields 50 million ADSC for clinical use.

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Symposium Ⅰ: ASCS EXTRACTION -"How do you extract ASCs?"

Ⅰ-4] Extraction of adipose derived stem cell in clinical urology: First patient treated for stress urinary incontinence

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Stress urinary incontinence can have a significant impact on a patient's quality of life, resulting in involuntary release of urine due a weakened urethral sphincter. The condition is more common in women and often comes about following child birth or menopause . It is estimated that greater than 50% of all women and approximately 9 million people in Japan, and more than 13 million women in the U.S., are affected by stress urinary incontinence. In addition, incontinence after radical prostatectomy in men as more severe case may be the result of damage to sphincteric structures, bladder dysfunction, an obstructive stricture, or some combination of these. We developed a novel cell therapy by autologous adipose tissue-derived stem cells using the Cytori's Celution® 800 for stress urinary incontinence after radical prostatectomy due to the result of damage as the severe cases and reported initial cases undergoing peri-urethral injection of stem cells for the treatment of urinary incontinence. . The stem cells isolated from own fat by the System were injected intra-muscularly into the sphincter as well as in combination with a measured volume of the patient's own fat tissue to create a bulking agent to support the urethra. The study evaluated safety, functional endpoints including intraurethral pressure and leak point pressure, as well as subjective assessments of patient and physician satisfaction. Current treatments include use of collagen as a bulking agent to provide pressure against and support the urethra.

The present preliminary study demonstrated that peri-urethral injection of the autologous adipose-derived stem cells are safe and feasible treatment modality for stress urinary incontinence.

Symposium Ⅱ : BASIC SCIENCE OF ASCS 1

Ⅱ - 1] Cell division regulator has a strong relationship with immaturity maintenance and differentiation capability of hASCs

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Objective

Mesenchymal stem cells (MSCs) are potentially very useful for regenerative and reparative medicine due to the capability of self-renewal and differentiation into multi-lineages. The aim of this study is to examine the biological characteristics and the bio-safety of human adipose tissue-derived stem cells (hASCs) for the clinical application by investigating proliferation and differentiation potentials according to the cell passage (passage 3, 20, 40 and 60).

Methods

hASCs at passage 3, 20, 40 and 60 were differentiated into adipocytes as confirmed by special staining for lipid vacuole (Oil red O staining) and RT-PCR for adipocyte-specific genes. hASCs at each passage were analyzed and compared for pluripotency marker expressions by RT-PCR, senescence by b-gal staining, proliferation by CCK-8 assay, cell cycle-related protein expressions (CDK2, CDC2) by immunobloting, and chromosome stability by karyotyping.

Results

The hASCs at each passage showed the decreased differentiation capability according to the passage number as confirmed in Oil Red O staining and adipocyte-specific marker expressions. The result for mRNA expressions of important dedifferentiation genes (Oct4, Sox2, Nanog, c-myc, lin28, klf-4) showed that the expressions of Oct4, Sox2, Nanog, lin28 and klf-4, excepting c-Myc, were decreased according to the cell passage number. Senescence was not prominently different by the passage number, while proliferation rate was significantly different at each passage. Chromosome at 60 passages was revealed as normal, indicating the genetic stability of hASCs for tumorigenesis potential at high passage. The protein level analysis of cell cycle regulators such as cyclin-dependent kinase CDC2 and CDK2 demonstrated that the protein expressions were remarkably different at each passage as decreased from the low passage to high passage.

Conclusion

In this study, we demonstrated for the first time that the expressions of the cell cycle regulator CDK2 and CDC2 decreased as the hASCs passed. Therefore, our study suggests that cell division, not senescence, had a strong relationship with immaturity maintenance and differentiation capability of hASCs, and may affect on clinical approaches and practical applications of hASCs.

Symposium Ⅱ : BASIC SCIENCE OF ASCS 1

Ⅱ - 2] Differentiation of adipose derived stem cell into endocrine cell

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Introduction

Eversince it was revealed that mesenchymal stem cells can be differentiated into not only mesodermal cells but into ectodermal or endodermal cells as well, further research into induction of pluripotent stem cells from adult somatic cells was propelled.

Our research aims to show induction and differentiation of adipose derived mesenchymal stem cells into pancreatic islet cells, an endodermal endocrine cell.

Methods

Adipose tissues were obtainted from patients undergoing TRAM breast reconstruction in our institute. The stem cells from the adipose tissue were obtained and cultured. The cells of passage 3rd to 4th were induced to differentiate into endodermal endocrine cells (pancreatic islet cells). The cells were acquired before differentiation (Day 0), and on days 3, 7,11, 14, 17, 21, 24, 28 of differentiation. RT-PCR was performed to observe the genetic expression of Oct4, Brachyury, SOX17, FOXA2, HNF4A, PDX1, NGN3, Isl1, Pax6, glucagon, and insulin on these respective days. 1 month after differentiation, the differentiated cells were then implanted into diabetes induced nude mice using streptozocin; the mice were observed for changes in blood glucose levels as well as body weight.

Result

After the differentiation process, the genes pertinent to the mesendoderm, definitive endoderm, posterior foregut, and pancreatic endoderm were expressed and we were able to postulate that differentiation into an endocrine cell had occurred. However, genes for the primitive gut tube, glucagon and insulin, hormones secreted by the endocrine pancreas as final by-products, were not expressed. Regulation of blood glucose levels in diabetes induced nude mice did not occur after implantation of the cells, however, the rate of increase in blood glucose was comparatively delayed in the experimental group than in the control group. Furthermore, the survival of the experimental group was longer than in the control group.

Conclusion

Final differentiation into pancreatic Islets of Langherans, sites of production of glucagon and insulin, did not occur, however, we were able to differentiate adipose derived mesenchymal stem cells into endodermal progenitor cells of pancreatic islet cells. More studies on the factors required in the maintenance, proliferation, and differentiation of pluripotent stem cells, and research into heightening the efficacy of stem cell differentiation should be done.

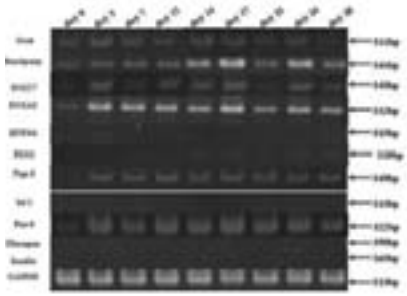

Fig 1. Chronological gene expression during differentiation.
Oct4, Brachyury, SOX17, FOXA2, NGN-3, Pax-6 genes are markedly expressed although PDX-1 gene is weakly expressed. HNF4A, ISL-1, glucagon and insulin genes are not expressed.

Symposium Ⅱ: BASIC SCIENCE OF ASCS 1

Ⅱ - 3] Dedifferentiated fat cells as a new cell source for regenerative medicine

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Background and Aim

Mature adipocytes are the most abundant cell type in adipose tissue. Using an *in vitro* dedifferentiation strategy, referred to as ceiling culture method, we established dedifferentiated fat (DFAT) cells from mature adipocytes. In the ceiling culture, mature adipocytes divide asymmetrically and generate fibroblast-like DFAT cells that show high proliferative activity. In the present study, we examined phenotypic characteristics and multilineage differentiation potential of DFAT cells.

Methods

Samples of human subcutaneous fat obtained from total of 18 donors (1-81 years) who underwent surgery. Patients provided written informed consent and the Nihon University School of Medicine ethics committee approved the study. DFAT cells were obtained by ceiling culture of floating adipocyte fraction of collagenase-digested adipose tissue. ASCs were also obtained by expansion of adherent cells derived from pellets of collagenase-digested adipose tissue. Expression of cell surface antigens and differentiation marker genes were analyzed by FACS and real-time RT-PCR, respectively. The differentiation potential of DFAT cells was analyzed by culturing the cells under conditions favorable for adipogenic, osteogenic, chondrogenic, and myofibroblast differentiation.

Results

DFAT cells were obtained from all tissue samples tested, although reduced proliferative activity was observed in the cells from donors over 70 years of age. Flow cytometric analysis revealed that DFAT cells comprised a highly homogenous cell population compared with that of ASCs, although the cell surface antigen profile of DFAT cells was very similar to that of ASCs. DFAT cells lost expression of mature adipocytes marker genes but retained or gained expression of mesenchymal lineage-committed marker genes such as PPARγ RUNX2, SOX9, and α-smooth muscle actin. *In vitro* differentiation analysis revealed that DFAT cells could differentiate into adipocytes, chondrocytes, osteoblasts, and myofibroblasts under appropriate culture conditions, although the cells were clonally expanded. DFAT cells also formed osteoid matrix when implanted subcutaneously into nude mice. In addition, transplantation of DFAT cells enhanced angiogenesis and improved blood flow in a mouse ischemic hindlimb model.

Conclusions

Our data indicate that mature adipocyte-derived DFAT cells exhibit multilineage differentiation potential and angiogenic activity. Because DFAT cells are easily isolated from small amount (approximately 1 g) of subcutaneous adipose tissue and readily expanded with high purity regardless of donor age, DFAT cells may be an attractive cell source of mesenchymal lineages for tissue engineering and other cell-based therapies.

SPECIAL LECTURE Ⅰ

Cell-based therapies: All mesenchymal stem cells are not equal

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Marrow derived adult Mesenchymal Stem Cells (MSCs) can be isolated and culture expanded. Although these cells are capable of differentiating into lineages that result in the fabrication of bone, cartilage, muscle, marrow stroma, tendon/ligament, fat and other connective tissues, MSCs have recently been shown to be intrinsically therapeutic. Such culture expanded adult/MSCs are immuno-modulatory especially in muting T-cells and, thus, allogeneic MSCs have been used to mute or cure graft-versus-host-disease and Crohn's disease and are now being tested in certain autoimmune diseases. Furthermore, these allo-MSCs set-up a regenerative micro-environment which is anti-apoptotic, anti-scarring, mitotic for tissue intrinsic progenitors and angiogenic.

These immuno and trophic activities result from the secretion of powerful bioactive molecules that, in combination, support localized regenerative event. The MSCs reside in every tissue of the body and function as perivascular cells (pericytes) until a focal injury occurs. At sites of injury the pericyte is released and functions as a MSC that provides molecular assistance in activities leading to tissue regeneration. Such assistance involves many tasks involving the immuno-protection and trophic activities provided by the MSCs. Although it is proposed that all MSCs are pericytes and have common capacities, it is expected that MSCs from different tissues location or anatomical sites of injury will not be equivalent. Thus, adipose-derived and marrow-derived MSCs naturally reside as pericytes and have differently functional capacities.

Symposium Ⅲ : BASIC SCIENCE OF ASCS 2

Ⅲ - 1] Comparative transcriptional profiling of tissues engineered using ASCs and BM-MSCs

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Introduction

Among the different sources of mesenchymal cells available for tissue engineering purposes, adipose-derived stem/stromal cells (ASCs) have now demonstrated their usefulness in a wide variety of settings. Since bone marrow-derived mesenchymal cells (BM-MSCs) are still considered the gold standard in the field, it is important for investigators to compare the behavior of these two cell populations using their engineering model systems. The main objective of this study was to compare the potential of cells from these two sources to generate entirely natural human three-dimensional adipose tissue substitutes produced using the selfassembly approach of tissue engineering.

Methods

ASCs were isolated from lipoaspirated adipose tissue of healthy patients and commercially available human BM-MSCs were used. A comparative characterization was performed for transcript and cell-surface protein expression by RT-PCR and flow cytometry, respectively, on cultures of thawed primary cells. Adipose tissue substitutes were generated using the self-assembly approach. This consisted of stimulating the mesenchymal cells with ascorbic acid and serum to make them produce and organize an endogenous extracellular matrix (ECM) while concomitantly inducing adipogenic differentiation. The resulting adipocyte-containing cell sheets were then superposed to produce thicker tissues. Transcription profiling of adipose tissues (AT) reconstructed from ASCs (n= 4) and BM-MSCs (n= 3), as well as their non-induced control counterparts (reconstructed connective tissues), was performed using the Illumina Whole-Genome Sentrix® Human-6 Expression BeadChips in comparison with samples of human fat tissues (n= 4). Finally, adipogenic differentiation, tissue thickness as well as expression levels (qRT-PCR) of selected adipogenic and ECM related genes were also evaluated.

Results

RT-PCR performed on primary cell cultures showed that genes normally associated with stem cells such as Kit, Nanog, and Gnl3 were expressed in both cell populations. Flow cytometry analyses revealed that they express CD73 and CD105 but are negative for CD34 and CD45 markers. Both cell sources allowed the production of adipose tissue substitutes using the self-assembly approach. However, lipid accumulation was greater when BM-MSCs were used whereas tissue thickness and extracellular matrix formation were enhanced when ASCs were used. Microarray data analysis revealed that reconstructed adipose tissues from both cell sources have a global transcript profile closer to human fat than to their respective non-induced controls. Analysis also revealed that 122 transcripts were commonly upregulated in reconstructed AT from both cell sources and were associated with PPAR, adipocytokine and insulin signaling pathways. Interestingly, 301 transcripts were differently expressed between tissues engineered from ASCs or BMMSCs, independently of the differentiation status. These transcripts were associated with cell cycle and ECM-receptor interaction pathways.

Conclusion

In conclusion, while both cell sources can be used to generate entirely natural human adipose tissue substitutes

using the self-assembly approach, differences were observed between the reconstructed tissues, mainly regarding the more limited capacity of the BM-MSC populations tested to generate human ECM components. The results suggest that ASCs, which are abundant and represent an easily accessible cell source, offer a greater potential for the reconstruction of manipulatable autologous adipose tissues using the self-assembly approach of tissue engineering. Moreover, microarray analyses informed us that a specific set of transcripts differentially expressed between reconstructed tissues from both cell sources, are more likely related to the tissue from which the MSCs originate than a difference in adipogenic potential within the tissue engineering context. Supported by CIHR.

Symposium Ⅲ : BASIC SCIENCE OF ASCS 2

Ⅲ - 2] Differentiated adipocytes do not elicit alloreactive immune response

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Background

Recent studies demonstrated that adipose derived stem cells (ASCs) can modulate the immune response as well as are non-immunogenic. Here, we investigated the immunologic properties of differentiated adipocytes that were induced from ASCs.

Methods and Results

Differentiated adipocytes were produced from ASCs with adipogenic inducers. Flow cytometric analysis showed that MHC class II molecules and co-stimulatory molecules including CD40, CD80 and CD86 were not expressed in differentiated adipocytes like ASCs, however, MHC class I expression was markedly decreased in differentiated adipocytes compared to ASCs. In mixed lymphocyte reactions (MLRs), differentiated adipocytes did neither stimulate proliferation of allogenic peripheral blood mononuclear cells (PBMCs) nor enhance secretion of interferon-γ. Splenocytes co-cultured with allogenic differentiated adipocytes did not elicit immune response on re-exposure to the same antigen. To investigate the memory response *in vivo*, splenocytes isolated from mice immunized with allogenic differentiated adipocytes were subjected to MLRs. Alloreactive immune response was not observed. When differentiated adipocytes were transplanted into immune competent, allogenic mice, no evidences of an immune rejection or lymphocytes infiltration were found. Furthermore, differentiated adipocytes formed adipose tissue at the transplantation site of allogenic host similar to that of syngraft control.

Conclusion

Differentiated adipocytes do not elicit alloreactive immune responses *in vivo* as well as *in vitro*. These results suggest that differentiated adipocytes are very low immunogenicity, even if no immunogenicity can be concluded without further study. It would be required further investigation to demonstrate differentiated adipocytes could be used as a “universal donor” in MHC- mismatched recipient for soft tissue regeneration.

Symposium Ⅲ : BASIC SCIENCE OF ASCS 2

Ⅲ - 3] Possible specific markers for differentiating human adipose-derived stem cells

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Introduction

Stromal vascular fraction (SVF) cells from mouse or human adipose tissue can differentiate into several lineages *in vitro*, and the multipotent component is called adipose-derived stem/stromal cells (ASCs). However, any specific marker reflecting the multipotency has not been identified yet. Recently, CD24 was identified as a marker for early adipocyte progenitor cells in mice that were capable of adipogenesis efficiently *in vivo* [Rodeheffer M.S, *Cell*, 2008]. In this study, we investigated expressions of selected stem-cell-associated markers (CD24, CD200, CD34, and CD271) in human adipose tissue and its cellular components.

Methods

Immunohistochemistry was performed to assess expressions of CD24, CD200, CD34, and CD271 in human adipose tissue. Single- and multi-color flowcytometry and colony-forming assay were also conducted to examine SVF cells freshly isolated from human aspirated adipose tissue or cultured in various mediums (DMEM, M199, EBM, and EGM-2).

Results

Flowcytometric analyses of fresh SVF revealed that there were few blood-derived cells ($CD45^+$) in $CD200^+$, $CD34^+$, or $CD271^+$ cells, while a part of $CD24^+$ cells were $CD45^+$. In adipose-derived cells ($CD45^-$), CD24 was expressed by 50.6% of $CD31^+$ cells (endothelial cells; ECs) and 24.2% of $CD31^-CD34^+$ cells (ASCs), CD200 was positive in 77.3% of ECs and 1.7% of ASCs, and CD271 was expressed by 11.4% of ECs and 76.9% of ASCs. After plating, CD34 and CD271 expressions decreased in ASCs gradually with culture passages. On the other hand, CD24 expression was preserved at a constant level of about 0.5%, and surprisingly, CD200 expression level increased gradually over culture period except when cultured in EGM-2.

Immunohistochemistry demonstrated that CD34 localized in the endothelium and adventitia of vessels, periendothelially in capillaries, and interstitial space between adipocytes. CD271 was expressed periendothelially in capillaries and vasa vasorum of vessels. $CD24^+$ cells were located periendothelially in capillaries and in a part of the adventitia of vessels. Most of $CD200^+$ cells were capillary endothelial cells.

Discussions

Our results suggested that ASCs are predominantly $CD45^-CD31^-CD34^+CD271^+$ and reside in the periendothelial region of capillaries. CD24 was also identified in human ASCs and may be a marker of adipocytes progenitor, though CD24 and CD200 were expressed predominantly by capillary endothelial cells in human adipose tissue. As shown in *in vitro* study, CD200 may be a marker of cells with highest expansion capacity in both ASCs and ECs. Although further studies are needed, it was suggested CD24 and CD200 are possible markers of stem cells in human adipose tissue.

Symposium Ⅲ : BASIC SCIENCE OF ASCS 2

Ⅲ - 4] The *in vitro* cell fate of photoaged fibroblast after ASCs application: comparison between transwell and conditioned medium culture

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Background

Extrinsic aging is mainly caused by ultraviolet B radiation, thus aptly named 'photo-aging'. UV radiation can damage dermal fibroblast, which plays a crucial role in maintaining youthful skin. Photo-damaged fibroblast can change the skin's character of regulating extracullar matrix, cellular senescence, cell death, and so on. The senescent cells are known to be resistant to apoptosis but irradiated by higher dose, accumulated cell damage finally cause cell death, so called necrosis. Adipose-derived stem cells (ASCs) are known to have direct and indirect beneficial effects, relaying restoration and regeneration of injured tissues. These effects are thought to have due influence on photo-damaged fibroblasts.

In this study, we investigate the effect of ASCs for improving photo damaged human dermal fibroblast. In particular, we focus on the influence of ASCs on UVB-induced death process and senescence of human dermal fibroblast.

Purpose

The purpose of this study was to investigate how the ASCs could reduce UVB induced cytotoxicity and cellular senescence.

Materials and Methods

Fibroblast and ASC were harvested from three healthy donors. In addition, UVB was irradiated to fibroblast with a subcytotoxic dose (100mJ/cm^2). Photo-aging was confirmed via microscope and β-galactosidase staining. Then this fibroblast was cultured with ASC from the same person. The experimental model consisted of 3 groups: only UVB irradiated fibroblast (Group 1), UVB irradiated fibroblast cultured with an ASC conditioned medium (Group 2), and UVB irradiated fibroblast co-cultured with ASC (Group 3).

The proliferation of fibroblast was measured with a CCK-8 kit at 24, 48, and 72 hours. Type I collagen, matrix metalloproteinase-1, and p16 production were measured by RT- PCR, and apoptosis was also measured using annexin V-PI double stain and FACS analysis in each study group.

Results

After irradiated with UVB, SA-β-galactosidase level of fibroblast was increased and we could confirm photoaging of fibroblast. Proliferation assay revealed an increased viable cell count of the conditioned medium (Group2), transwell co-culture (Group3), and control (Group1) at 48 hours (Fig 1). RT-PCR was also conducted at 48 hours. Experimental groups (Group2, Group3) showed an increase in type I collagen production and a decrease in MMP-1 and p16 compared to the control (Group1; Fig2). Annexin V-PI double staining and FACS analysis were done for the apoptosis assay. There are no significant differences between control and experimental groups in viable cell proportion (Fig3-1). However, experimental groups (Group2, Group3) showed a decrease in the necrotic portion but an increase in the apoptotic portion. (Fig3-2)

Conclusion

Adipose derived stem cells induced proliferation of photo damaged human dermal fibroblast and decreased MMP-1 production. These results suggest that ASCs can improve photo damaged skin. In addition, P16, which strongly correlates to aging, decreased in ASC-treated groups. Also, ASCs induced photo damaged dermal fibroblast in a more physiologic cell cycle, apoptosis. It decrease necrotic cells and increase early apoptotic cells fraction. It seems that ASCs can convert necrotic or late apoptotic cells cells to early apoptotic cells. In comparing transwell cultures and conditioned medium cultures, paracrine effects may play a more important role rather than cell-to-cell direct contact. In conclusion, ASCs may have an inhibitory effect on the UVB-induced cytotoxicity and it can be useful for the treatment of skin aging.

Fig 1) Cell viability test by CCK-8 (48hrs)

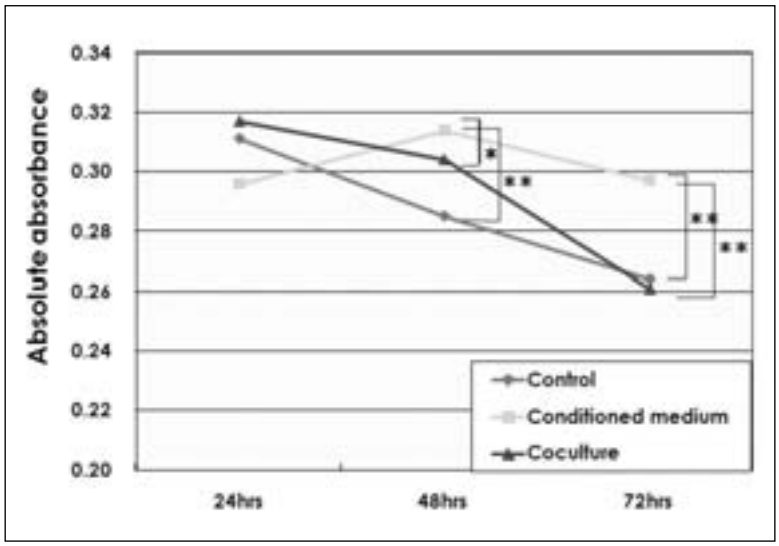


Fig 2) Measurements of type I collagen, MMP-1, and p16 by real time RT PCR (48hrs)

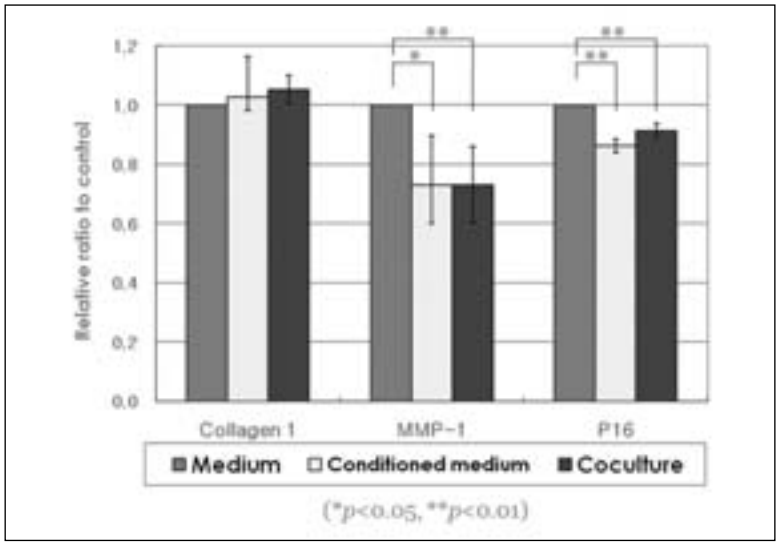


Fig 3-1) Apoptosis Assay by FACS analysis

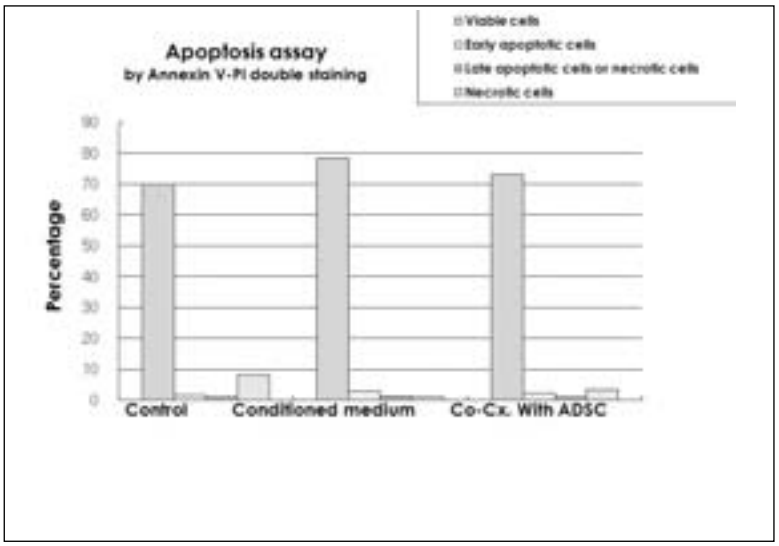
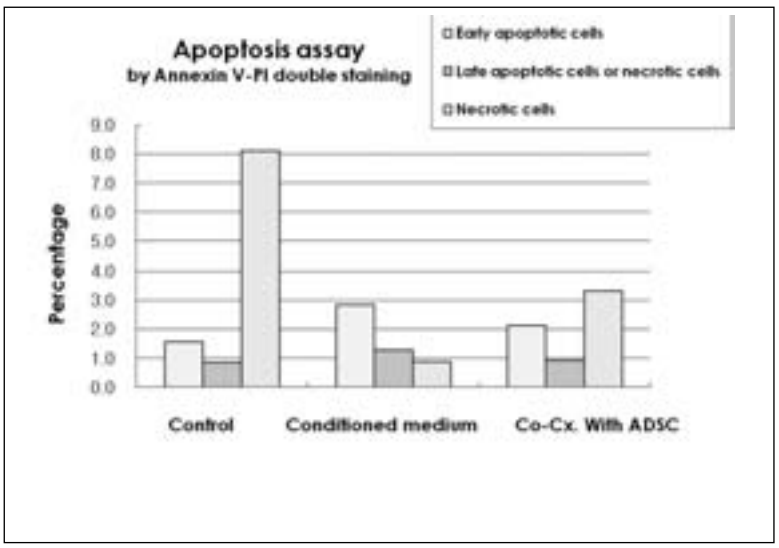


Fig 3-2) Apoptosis Assay by FACS analysis



SPECIAL LECTURE Ⅱ

Molecular regulation of muscle stem cell fate

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We study the molecular pathways that regulate the transitions between quiescence and activation of adult muscle stem cells (satellite cells), and the pathways the guide cell fate decisions of the progeny of those cells once they have begun to proliferate. We have focused primarily on the Notch and Wnt signaling pathways, and interactions between the two. Our results suggest that the Notch signaling pathway plays a critical role in the activation and proliferative expansion of satellite cells. Interestingly, we have found that Wnt signaling plays an important role in the commitment to differentiation of transit amplifying population of cells, with an increase in Wnt signaling in those cells leading to a more differentiated myogenic progenitor, or myoblast. Interaction between the Notch and Wnt pathways in mediating this transition from a more undifferentiated progenitor to a more differentiated progenitor occurs via the opposing effects on the state of activation of GSK3β. As well described, activation of the Wnt pathways leads to the inhibition of the enzyme. We found, interestingly, that activation of the Notch pathway maintains GSK3β in an active state. In further studies of the role of Wnt in promoting differentiation of myogenic progenitors, we have found that the differentiation-inducing effects of Wnt are dependent on the expression of the Wnt co-activator, BCL9, in myogenic progenitors. Using a muscle-specific driver of Cre recombinase and mice in which both BCL9 and BCL9-2 are flanked by loxP sites, we found muscle development to be normal but muscle regeneration postnatally to be impaired, suggesting that BCL9 is not essential for the effects of Wnt on muscle development but plays an important role in the Wnt signaling pathway in postnatal myogenesis.

The findings have led us to examine epigenetic modifications and transcriptional regulation of Notch and Wnt target genes involved in quiescence, activation, and self-renewal. In studies of both the Notch and Wnt pathway, we have found that regulation of the methylation status of lysine 4 of histone 3 (H3K4) plays a key role in regulating gene expression and cell fate. BCL9 binds specifically to di- and tri-methylated H3K4, and inhibition of myogenic differentiation is associated with a preferential enrichment of H3K4me2 at a key regulatory region of the promoter of the myogenic regulator, MyoD. Ongoing studies focus on the Jumonji family of histone demethylases, in particular JumonjiD1C and Hairless, which are specific for H3K4me2/3 and have a clear regulatory role in muscle stem cell function. Our goal is to obtain epigenetic profiles that are signatures of the different states of stem cell activation and correlate those profiles with the specific patterns of gene expression that define those states.

Symposium Ⅳ: CLINICAL APPLICATION OF ASCS

Ⅳ- 1] Therapeutic Potential of Adipose Stem Cells

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Although the function of adipose stem cells (ASCs) is not completely understood, some of basic function of ASCs has been identified during last 10 years by many pioneering scientists. Therapeutic potential of ASCs is also under active investigation. ASCs have strong angiogenic capability and tissue regeneration potential. There is no doubt that it could be a powerful armament for doctors confronting with intractable disease. I've been working on ASCs for clinical application for more than 10 years and most of the animal experiments showed positive results and some of them are already clinically available. I do believe it would be worthy to summarize and present at IFATS 2009, especially for beginners interested in adipose stem cells

Discovery of ASCs: During the last decade of 20th century at Pittsburgh university, there had been pioneering experiments by several plastic surgeons in a small laboratory testing the characteristics of stromal cells harvested from adipose tissue. It was the beginning of discovery of ASCs, and after ten years since the discovery, they are ready to be used in limited clinical situations, such as fat grafting, wound healing, scar remodeling, etc. We could even find many types of devices extracting adipose stem cells in the market. The major role of 'regenerative medicine' in 21st century is based on cell therapy and ASCs is going to take the core position.

Potential of ASCs: ASC is a kind of mesenchymal stem cells and shares a common characteristic of mesenchymal stem cells (MSCs). The number of stem cells within adipose tissue reaches more than hundreds of times compared with BMSCs contained in the same amount of bone marrow. Cellular plasticity is one of the most important features of ASCs as in other adult stem cells and strong angiogenic potential is another important nature of ASCs. In many reports, ASCs are known not only to be differentiated into osteoblast, chondrocytes, vascular endothelial cells, but also to be cardiomyocytes and neuronal cells. The cells also have a special function of immune modulation and immunosuppression which open a new possibility of utilizing these cells for chronic inflammatory disease and immunologic disorders such as graft-versus-host disease.

Clinical application at present: We are at the beginning of the new era of stem cell therapy and ASCs are probably the most powerful source of stem cells. At present, ASCs are clinically applied in limited conditions such as fat grafts, difficult wound healing, radiation necrosis, scar remodeling and skin rejuvenation, etc. Clinical trials of ASCs for the treatment of ischemic heart and CNS regeneration have started recently in Korea. Immunomodulatory therapy using ASCs for graft-versus-host disease is also readily available. Therapeutic indication is different according to countries and doctors preferences. I believe it is important time to collect multi-centric scientific data of clinical application to prove definitive therapeutic effect.

Positive therapeutic effect of ASCs in animal study: There are so many interesting experimental papers regarding therapeutic effect of ASCs in animal study. I think we need to recheck every possibilities and scientific advancement should be achieved. Possibility of hepatic regeneration, insulin-producing islet cell regeneration, leukemia treatment and recovery of renal function are very important topics. Possibility of peripheral nerve and skeletal muscle regeneration is also important.

In conclusion, we are at the beginning of active clinical application of adipose stem cell. Apart from all proven scientific evidence of ASCs in basic science which have been accumulated during the last decade, we have to make every effort to collect scientific clinical data from many different institutes. It may be necessary to organize a team within IFATS.

Symposium Ⅳ: CLINICAL APPLICATION OF ASCS

Ⅳ- 2] Adipose-tissue progenitor cells: Functions in adipose transplantation and clinical use for soft tissue enhancement

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Many features of adipose tissue-specific stem/progenitor cells, such as physiological function and localization, have recently been clarified. Adipose tissue turns over very slowly and its perivascular progenitor cells differentiate into new generation adipocytes to replace. The progenitor cells, called "adipose-derived stem/stromal cells (ASCs), play important roles in physiological turnover, hyperplasia, and atrophy of adipose tissue; as well as in incidental remodeling, such as post-injury repair and adipose tissue transplantation. Even in ischemic conditions such as those where adipocytes and endothelial cells die in several hours, ASCs can survive, divide, migrate, release important factors, and differentiate to repair the adipose tissue.

Adipose tissue has been used as an autologous filler for soft tissue defects, despite unpredictable clinical results and a low rate of graft survival, which may be due to the relative deficiency of progenitor cells in graft materials as we reported before. We have developed a novel strategy called cell-assisted lipotransfer (CAL), involving the concurrent transplantation of aspirated fat tissue and adipose-derived stem/stromal cells (ASCs). In the CAL strategy, the progenitor deficit was compensated for by supplementation with ASCs isolated from a separate volume of aspirated fat tissue. Speculated roles of ASCs in CAL are 1) divide and differentiate into adipocytes; 2) divide and differentiate into vascular endothelial cells or mural cells; 3) release angiogenic growth factors such as hepatocyte growth factor (HGF); and, 4) survive as tissue-resident progenitors (as original ASCs) for future tissue turnover and remodeling.

Our clinical study of CAL began in 2003 and a total of approximately 400 patients received CAL: more than 70% of the patients underwent breast augmentation including immediate breast augmentations after implant removal and breast reconstructions after mastectomy, and about 20% of patients underwent facial remodeling including treatments for aging changes and facial lipoatrophy. The results suggest that postoperative atrophy of transplanted fat was prevented by the improved ASC/adipocyte ratio achieved in the CAL technique. Although further studies are necessary for confirming the efficacy of this technique, clinical results are quite encouraging.

Symposium Ⅳ: CLINICAL APPLICATION OF ASCS

Ⅳ- 3] Recent personal clinical trials; ASCs - Scaffolds injection

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Medikan, Seoul, Korea

ASCs clinical uses are increasing in the field of plastic surgery. Cell enhanced(enriched/assisted) fat graft is actually the first clinical use for soft tissue replacement. We can sure that procedures improve the clinical outcomes not only in volume survival but also in skin textures and vascularity around grafted areas. However, there are little studies which can explain whole mechanism. We hypothesize that stem cell and scaffold effects of fat tissues would have major roles for tissue replacement. Cell membrane and own interstitial structures can induce cell differentiation and act as rail way for cell conduction.

We have been tried to increase cell density and interstitial structures through rupture of mature adipocytes and removal of lipid droplets. We used licensed medical cutting mill (Filler Geller ^{TM}. Medikan, Korea.) for removal of lipid of fat tissues at operation room. This fat tissue scaffold still have dense living stem cells, so cell density is increased 3 times than normal fat tissues. When we add isolated stem cells, cell density will increase maximally. We use these maximally condensed fat tissues for nasal tip augmentation in which minimal absorption is necessary.

We recently use ASCs with several other scaffolds including licensed absorbable polymers like PDS. We can change them to injectable powder easily with cutting mill. It would be an off-label use, but it is very safe materials and absolutely legal in Korea. These absorbable particles would help sustain initial graft structures and would guide tissue ingrowth.

In ASCs local injection, a composite use of scaffolds would increase local effects because it serves attachment surfaces. The scaffolds can be used as a natural injection filler which have lesser absorption rate because a part of them would be replaced with new tissues.

Isolated ASCs can be used with normal fat tissues and also with condensed fat tissues, micronized tissues, or other absorbable polymers as scaffolds. We experienced this 3 years without any complication. In the nasal tip, the most sensitive area, we have successful results than usual fat graft. Otherwise, it would help the surgeon's injection techniques for fine sculpturings with small volume, because consistency of graft is easily regulated.

We reported 'fat gel' as more condensed fat tissues in the aspect of ASCs density per graft volume, and 'ASCs + scaffolds' injection as a useful method for small volume augmentation even though statistics calculating exact volume maintenance are not enough.

KEYNOTE ADDRESS Ⅱ

The FAT revolution: Past, present, and future

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Adipose Tissue is a unique biological resource capable of providing vast amounts of matrix, bioactive factors, and a variety of cell types - including MSCs. Due to its unmatched abundance, expendability, ease of harvest, and mass donor appeal, adipose lends itself to a variety of therapeutic strategies. This presentation will highlight progress in the field over the past decade including seminal work in the field, adipose-related clinical studies/applications that are imminent and/or currently in progress, a discussion of the regulatory, clinical and commercial considerations associated with a given strategy, and the future promise of adipo-derived regenerative therapies.

Symposium Ⅴ: CARDIOVASCULAR SCIENCE Ⅰ

Ⅴ - 1] Adipose stromal cells in angiogenesis, vasculogenesis, and tissue repair

Keith March, MD, PhD

Vascular and Cardiac Center for Adult Stem Cell Therapy, Indiana University

The description of pluripotent cells in adipose tissue has led to the concept that adipose tissue may provide a novel autologous source of cells with significant potential for tissue modification. The ready accessibility of adipose stem / stromal cells (ASC) strongly supports the notion that they might provide for a particularly feasible and attractive form of autologous cell therapy requiring either no *ex vivo* expansion or relatively limited expansion. Work from our laboratory and Center, as well as from multiple other groups, has clearly demonstrated that ASCs can increase tissue perfusion and limit ischemic tissue damage in several circumstances, including models of acute skeletal muscle[1], myocardial[12], and cerebral ischemia[11], as well as in cutaneous wound healing[10], particularly when delayed post-irradiation. Evidence derived from each of these models has consistently indicated that these salutary effects are predominantly mediated by angiogenic and anti-apoptotic factors secreted by ASCs rather than differentiation to replace tissue parenchyma[1, 4, 6, 9, 10, 11, 12].

Recently, we have also found that ASCs are primarily located in the walls of adipose microvasculature, possess many characteristics of pericytes[3], and are capable of stabilizing endothelial networks *in vitro* as well as robustly synergizing with endothelial cells (EC) to participate in the *in vivo* formation of new vessels which connect with host vasculature, conduct blood flow, and exhibit network stability for several weeks[8]. This synergistic assembly of functional vessels from ASC and EC, two non-transformed[13] and readily available human cell components, coupled with the formation and remodeling of a vascular network in the context of physiologic flow, provides a novel model of postnatal vasculogenesis and permits study of the mechanisms underlying this process. Additionally, this observation led us to hypothesize that the synergy between ASC and EC would provide a practical approach to tissue vascularization for implants or regional ischemia[8]. Our long term goal is to fully develop the translational and therapeutic potential of adipose stromal cells in a variety of disease states through the characterization and exploitation of their complementary paracrine and pericytic pro-angiogenic properties.

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Symposium Ⅴ: CARDIOVASCULAR SCIENCE Ⅰ

Ⅴ - 2] Implantation of adipose-derived regenerative cells enhances ischemia-induced neovascularization. Role of SDF-1-mediated endothelial progenitor cell mobilization.

Satoshi Shintani[1], Kazuhisa Kondo[1], Yasuo Kitagawa[2], Toyoaki Murohara[1]

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Background

Therapeutic angiogenesis using endotherial progenitor cells may represent a novel approach for ischemic diseases. However, recent studies indicate that patients with severe vascular disease may have poor response to angiogenic therapy. We examined whether implantation of adipose-derived regenerative cells (ADRCs) might augment angiogenesis in ischemic tissues.

Method and Results

ADRCs were obtained by culture of C57BL/6J mouse inguinal fat pad-derived stromal cells. Fluorescence-activated cell sorter (FACS) analysis of ADRCs indicated that Sca-1 antigen, but not CD31, CD34, c-kit, Lin, and flk-1 was expressed. These cells expressed stromal cell-derived factor 1 (SDF-1) mRNA. Hindlimb ischemia was surgically induced in mice and ADRCs or PBS (control) was injected into the ischemic tissues on postoperative day 1. Three weeks after surgery, the ADRCs implanted group had a greater laser Doppler blood perfusion, and a higher capillary density compared to the control group. ADRCs implantation increased circulating EPCs on postoperative day 7 assessed by culture and FACS analysis. Both SDF-1 expression at the ischemic thigh muscles and serum SDF-1 levels were increased in the ADRCs group compared to the control group. Finally, intraperitoneal injections of anti-SDF-1 neutralizing antibody reduced therapeutic effect by ADRCs.

Conclusions

Adipose tissue is a valuable cell source for therapeutic angiogenesis. Moreover, SDF-1 may play an important role in mediating therapeutic angiogenesis using ADRCs for ischemic disease by mobilization of EPCs.

Symposium Ⅴ: CARDIOVASCULAR SCIENCE Ⅰ

Ⅴ - 3] Clinical application of adipose tissue-derived mesenchymal stem cell implantation as a Novel therapeutic option for Buerger's disease: Preliminary report

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Background

There is no curative therapeutic strategy for Burger's disease. The delivery of autologous progenitor cells into ischemic tissue is emerging as a novel therapeutic option.

Objective

We investigated safety and efficacy of autologous implantation of adipose tissue-derived mesenchymal stem cell (Ad-MSC) in patients with ischemic limbs because of Buerger's disease.

Methods

A total 6 patients was enrolled. Two patients had a nonhealing ulcer at affected foot. Ad-MSC was isolated from patient's abdominal adipose tissue. We injected the Ad-MSC ($5x10^6$cells/kg) into the calf muscles of the affected limbs. We evaluated a safety of treatment based on NCI-CTCAE grade and efficacy of it based on clinical (treadmill walking distance[TWD], pain free walking distance[PFWD],rest pain, non-healing ulcers) and laboratory(toe-brachial pressure index[TBPI],ankle-brachial pressure index[ABPI], laser Doppler, transcutaneous oxygen pressure[TcO2], conventional angiography, CT angiography)parameters after 24 weeks.

Results

No procedurally related complications or demonstrable side effects occurred at 24weeks. Nonhealing ulcer was disappeared completely in one patients and became smaller and thinner in another patient. Significant improvement of rest pain on VAS score and walking distance was observed in all patients (Baseline vs. after 24weeks: VAS [points] 5.8 vs. 2.2, p=0.027, TWD[m] 289 vs. 492, p=0.028, PFWD[m] 271 vs. 452, p=0.028) with resultant limb salvage at 24weeks. Laboratory parameters also showed improvement of ischemic status (ABPI 0.62 vs. 0.69, p=0.027, TBPI 0.58 vs. 0.75, p=0.028, TcO2 [mmHg] 51.6 vs. 60.6, p=0.028, laser Doppler [perfusion unit] 0.72 vs. 0.94, p=0.028). However, the results of angiography didn't show an increase in number of visible collateral vessels.

Conclusion

Autologous implantation of Ad-MSC is safe and effective to reduce rest pain and to improve clinical performance. Thus, it will be a promising and novel therapeutic modality for patients with Buerger's disease.

Symposium Ⅵ: CARDIOVASCULAR SCIENCE Ⅱ

Ⅵ - 1] Adipose-derived stem cells posses a functional hepatocyte growth factor / c-Met receptor autocrine loop that is essential for *in vivo* potency

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Background

Adipose-derived stem cells (ASC) secrete many beneficial factors and promote reperfusion and tissue repair in ischemia models. We have previously demonstrated that knock-down of hepatocyte growth factor (HGF) by RNA interference (RNAi) attenuates ASC potency *in vivo* in a murine hindlimb ischemia model. Interestingly, compared to ASC expressing normal levels of HGF, modified ASC demonstrated reduced persistence in repaired tissues. This implied that knock-down also disrupted an autocrine loop in HGF receptor- (c-Met) expressing ASC; thereby, reducing ASC homing to or survival in ischemic tissues. In the present study we conducted a series of *in vitro* and *in vivo* experiments to examine the function of c-Met in promoting ASC-mediated repair of ischemic tissues and present data describing a previously uncharacterized HGF / c-Met autocrine loop. These findings suggest possible strategies to increase the therapeutic efficacy and safety of ASC for treating diseases.

Methods

Dual-cassette lentiviral vectors, expressing GFP and either a small hairpin RNA (shRNA) specific for HGF mRNA (shHGF) or a control sequence (shCtrl), were used to stably transduce ASCs (ASC-shHGF or ASC-shCtrl). Activation of c-Met was blocked by incubating ASC for 1 hr in the presence of 10㎛ of the selective inhibitor PHA-665752 (Pfizer). Hyper-stimulation of c-Met phosphorylation was accomplished by treating for 1 hr with 100ng/ml purified recombinant HGF (R&D Systems). After both treatments, cells were washed with PBS before infusion. Cells were infused by tail vein infusion 24 hr after surgically inducing unilateral hindlimb ischemia in immunocompromised mice (N > 6 /group). Reperfusion was monitored by laser Doppler perfusion imaging (Moor Systems). GFP cells were quantitated by fluorescent microscopic imaging of thin sections from the gastrocnemius muscles of ischemic and non-ischemic limbs. Apoptosis of GFP cells was measured in situ with an ApopTag kit (Chemicon). The relative levels of phosphorylated-Akt (p-Akt) and total Akt were determined by Western blotting with antibodies specific to each (Upstate).

Results and Discussion

We first examined the effects of reduced HGF expression on ASC homing to the injury site and persistence in injured tissues. There was no difference in total GFP-positive cells in ischemic limbs at 5 d after infusion of 10^6 ASC, indicating similar homing potentials; however, significantly more ASC-shHGF cells were apoptotic than ASC-shCtrl cells (61 ± 0.1% vs. 41% ± 3.2%, respectively, $P<0.01$). There was no difference in apoptosis percentages in normal tissues (13.1 ± 6.8% and 14.9 ± 6.4% for ASC-shCtrl and ASC-shHGF, respectively). By 20 d following infusion, 3-fold fewer ASC-shHGF were present in ischemic tissues compared to ASC-shCtrl ($p<0.01$). The reduced survival of ASC-shHGF could be explained by 2-fold lower ratio of phophorylated-Akt (p-Akt), a potent survival factor regulated by c-Met, compared to ASC-shCtrl.

These data suggested that ASC possess a functional HGF/c-Met autocrine loop that is critical for survival in adverse environments and disruption of this cycle reduced the ability of ASC to withstand stresses associated with the ischemic environment and, thus, their ability to affect tissue rescue. This was examined directly either by specifically inhibiting c-Met phosphorylation with the selective inhibitor PHA-665752 or, conversely, by stimulating greater phosphorylation with excess HGF. Pretreated and untreated ASC ($3x10^5$) were infused into mice 1 d after creating

ischemia. At 21 d the relative (ischemic to non-ischemic) hindlimb perfusion of PBS-treated control mice was 35.9 $\pm$ 2.9%. The low dose of untreated ASC induced only 46.8 $\pm$ 4.3% relative perfusion, which was greater than control, but not sigficant ($P > 0.05$). Pretreating ASC with HGF enhanced the potency of the non-efficacious dose, leading to significant reperfusion (63.2 $\pm$ 5.3%; $P<0.001$ vs PBS and $P<0.05$ vs untreated ASC). Conversely, inhibiting c-Met abolished the effect of ASC on hindlimb reperfusion (30.3 $\pm$ 2.1%). To confirm the importance of c-Met in ASC-mediated repair of ischemia, experiments using knock-down by RNAi are currently in progress.

Conclusions

In addition to the important paracrine effects of HGF expression on recovery of host tissues from ischemic insult described earlier, these results establish that HGF is necessary for autocrine promotion of ASC survival and consequent efficacy. Importantly, it was shown that increased survival and potency can be attained with only a brief and reversible treatment immediately before administration. Enhanced donor cell survival is an important goal for increasing the efficacy and safety of cell-based therapies.

Symposium Ⅵ: CARDIOVASCULAR SCIENCE Ⅱ

Ⅵ - 2] Vascular cell differentiation of human adipose-derived stem cells based on FGF2- immobilized substrate

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Introduction

Cell adhesion to the extracellular matrix (ECM) is critical in determining cellular fates, such as proliferation, migration, and differentiation, in the living body or culture environment. Of interest in stem cell and tissue engineering research, studies involving cellular adhesion to an artificial ECM (art-ECM) have recently heightened. Human adipose stem cells (hASCs) can be isolated from human adipose tissue and differentiated into ECs like mesenchymal stem cells. Members of fibroblast growth factor (FGF) family are potent angiogenic factors. FGFs interact with cells through FGF receptor and heparin sulfate proteoglycan. In this study, we sought to develop FGF2-immobimized substrate (FGF2 substrate) as an art-ECM for hASCs differentiation and to demonstrate that hASCs cultured on FGF2 substrate were readily differentiated into vascular cells.

Methods

A MBP-FGF2 fusion protein was obtained from E coli carrying pMAL-FGF2 plasmids which generated by the insertion of human FGF2165 cDNA into pMAL vector. MBP-FGF2 was spontaneously adsorbed to culture dishes for the preparation of FGF2 substrates. For a cell adhesion assay, expanded hASCs were plated in triplicate into 96-well plates adsorbed with various proteins. The percent of cells adhered was determined from the amount of protein measured by the BCA assay. The phenotype of hASCs and endothelial lineage cells was assessed by immunofluorescense staining and FACS analysis. Angiogenic factors were detected by a human angiogenesis antibody array. A gel-based *in vivo* assay was carried out to examine the vaculogenic activity of hASCs-derived endothelial lineage cells.

Results

hASCs were adhered to FGF2 substrates in a dose-dependent manner. 3D floating cell clusters (FCC) were formed in hASCs cultured on FGF2 substrate. From immune-fluorescence analysis, it was proved that EC markers, CD34, KDR, vWF, and CD31, were highly expressed in FCC in 3 days after seeding. The induction of VEGF and IL-8 proteins, angiogenic factors, by FCC was most remarkable, leading to 5-fold expressional increase. Tubular network was formed on Matrigel by cells disaggregated from FCC. In FCCs-embedded gel implants, we observed a lot of CD31, CD34, KDR, and vWF positive cells as well as a-SMA positive cells.

Conclusion

In this study, we developed a novel method to make hASCs macroscopic 3-D floating cell clusters (FCC) based on FGF2 substrate. The great majority of cells in the FCC were proved to differentiate into cells similar to EC, expressing high Flk1 and CD31 and low CD34 and released various angiogenic factors. A rapid 3D cell cluster formation of hASCs on FGF substrate will be useful in the acquirement of angiogenic supplements and transplanted ECs for cell therapy and tissue engineering.

Symposium Ⅵ: CARDIOVASCULAR SCIENCE Ⅱ

Ⅵ - 3] Injury-associated growth factors improve ischemia through the activation of adipose-derived stem/progenitor cells in obese and non-obese mice.

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Introduction

An injury triggers wound healing process accompanied by angiogenesis in adipose tissue. By characterizing wound exudates after liposuction, bFGF, PDGF, EGF, and TGF-β, were determined as injury-associated soluble factors in the earliest stage. Adipose injury was simulated using the injury-associated growth factor cocktail (IAGFC), whose biological effects on human adipose-derived stem/progenitor cells (ASCs) were evaluated *in vitro*. Furthermore, we tested whether the IAGFC could treat ischemia in obese and non-obese mice by activating ASCs and promoting angiogenesis.

Methods

To evaluate ASC activation by IAGFC, proliferation, migration and capillary formation on Matrigel of hASCs were investigated. Surface marker expression using flow cytometry, gene expression using real-time PCR and differentiation capacity were also examined. In addition, IAGFC was injected into intact or ischemic inguinal fat pad of non-obese mice (n = 70) to see its effects on local oxygen partial pressure (pO_2) andcapillary density. It was also examined whether IAGFC could improve ischemia in adipose tissue of diabetic obese (db/db) mice (n = 20).

Results

By incubation with IAGFC, proliferation and migration of hASCs were significantly promoted, Flk-1 and CD31 mRNA expression were up-regulated, and cells binding lectin increased. ASCs stimulated by IAGFC showed a higher capacity for adipogenic differentiation and a lower capacity for chondrogenic or osteogenic differentiation. In capillary forming assay, IAGFC stimulated hASCs to form complex mesh-like networks, which were positive for vWF and lectin. In ischemic subcutaneous adipose tissue of mice (pO_2 was 17 mmHg, in contrast to 64 mmHg in intact adipose tissue), injection of IAGFC led to significant improvements of pO_2 and capillary density, and significantly decreased the size of fibrous area. In diabetic mice, whose adipose tissue exhibited moderate ischemia (pO_2 was 45 mmHg), adipogenesis/angiogenesis phenomena were seen and pO_2 and capillary density were significantly improved in the mice treated with IAGFC.

Discussions/Conclusions

It was revealed that IAGFC promoted proliferation and migration of hASCs, and tended to differentiate hASCs into not only adipocytes but endothelial cells, suggesting that hASCs were involved in adipogenesis and angiogenesis in the process of adipose injury repair. The *in vivo* results indicated the potential of IAGFC in therapeutic use for various kinds of tissue ischemia including ischemic limb in non-obese and diabetic patients. In some unusual circumstances such as a radiation ulcer, where ASCs were deficient, ASC transplantation in conjunction with IAGFC administration might be needed to induce therapeutic effects.

Symposium Ⅵ: CARDIOVASCULAR SCIENCE Ⅱ

Ⅵ - 4] Tracking injected adipose-derived stem cells *in vivo* using a novel murine model of muscle ischemia

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Injection of stem cells has been proposed to counteract the damaging effects of tissue ischemia, yet the mechanisms underlying their therapeutic benefit are largely unknown. The hind-limb ischemia model is the current gold-standard pre-clinical model for the quantification of gross-tissue level changes, however it prohibits detailed spatial visualization and analysis of cell-level behaviors (e.g. incorporation throughout the vasculature) due to reliance on histological tissue sectioning. Toward this end, we developed a surgical model of ischemia in a thin tissue, the murine spinotrapezius muscle, which we are able to visualize *en face*, preserving the spatial pattern of the microvasculature. We used this model to examine the homing and incorporation capabilities of intravenously injected human adipose derived stem cells (hASCs). Beneficial of this progenitor cell population, hASCs are obtainable in large quantities using minimally invasive procedures such as liposuction, posses robust homing capabilities to areas of injury, and extensively aid in tissue rescue and repair. Briefly, the feeder arteriole to the caudal-half of the left spinotrapezius was ligated in Balb/C mice. Twenty four hours after insult, fluorescently (DiI) labeled hASCs were injected in the mouse tail-vein and microvascular remodeling was quantified using intravital and confocal microscopy. Immunohistochemical analysis revealed that ligated tissues, when compared to non-ligated contralateral control tissues, were more heavily populated with injected hASCs: these cells were either found inside microvessels as whole cells (co-labeled with DiI and a nuclear stain) or in the extravascular space as intact cells or DiI positive fragments. Such observation suggests that hASCs specifically homed to areas of ischemia and furthermore, preferentially extravasated into damaged tissue to aid in repair. This surgical model enables the detailed analysis of injected therapeutic cells and their spatial incorporation into the microvasculature of ischemic vs. non ischemic tissues.

Symposium Ⅵ: CARDIOVASCULAR SCIENCE Ⅱ

Ⅵ - 5] Adipose derived stromal vascular fraction cells potentiate neovascular sprout invasion of surrounding matrix.

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Adequate vascularization of tissue-engineered constructs is a critical step in the integration of implants with the host tissue. The regulation of neovessel sprouting is closely related to extracellular matrix signals like stiffness and matrix fiber orientation. These conditions are typically encountered when neovessel sprouts traverse the boundary between different tissue structures, or at the interface of the host and implanted tissue, and can determine the fate of neovessel sprouts at such boundaries. Several studies have addressed strategies to improve vascularization of implanted tissue, but the boundary between the host and implant is not well investigated as a possible barrier to neovascularization. This study models such an interface *in vitro*, using a vascularized collagen construct based on adipose derived microvessels at the core, surrounded by cell free collagen on the sides. Though angiogenesis in the core of such models progresses to form well interconnected networks, instances of neovessels traversing the interface by themselves are infrequent. However, addition of stromal vascular fraction cells derived from adipose tissue in addition to the microvessels significantly increases the number of neovessels growing across the boundary. It is hypothesized that both regulatory modulation of existing neovessels and incorporation of cells from the SVF into neovessel sprouts could be the underlying mechanism for such invasiveness. The exact nature of this phenomenon is yet unclear, but addition of SVFs may have a pro -angiogenic role. Further, collagen at the interface between the cell free periphery and the vascularized-cellular core remains relatively well-defined suggesting that any matrix remodeling is a highly localized phenomenon. The absence of any conspicuous pooling of cells at the interfacial surface also suggests a pro-angiogenic and regulatory role for the SVFs rather than only matrix remodeling at the interface leading to a higher invasion of angiogenic sprouts into the surrounding matrix.

Symposium Ⅵ: CARDIOVASCULAR SCIENCE Ⅱ

Ⅵ - 6] Adipose stromal cells induce stable vascular network formation by endothelial cells in *in vitro* co-culture settings.

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As it was shown previously, adipose tissue stromal cells (ASCs) express markers and functional properties of pericytes *in vitro*, and in combination with endothelial cells (ECs), are able to establish stable multilayer functional vessels *in vivo*. However, the factors that coordinate EC-ASC communications to promote migration of these cells towards one another and their heterotypic assembly into vascular structures are not well-defined. To understand the mechanisms and dynamics of EC-ASC interaction we developed a new *in vitro* model of co-culturing ECs with ASCs in a system containing serum but no additional exogenous growth factors and extracellular matrix proteins. We demonstrated that ASCs have a profound potential to stimulate morphogenesis of ECs into branching networks of cord structures. The vascular networks developed in six days and were stable for at least three weeks. This process was associated with increase in fibronectin and perlecan-1 production by ASCs and collagen IV by ECs; α-smooth muscle actin expression by ASCs, and increased CD31/PECAM-1 surface presentation by ECs. The vascular network assembly was dependent on matrix metalloproteinases activities and cell communications through VEGF, HGF and PDGF-BB pathways. We have shown that based on the total tube length ASCs exhibited significantly higher (up to nine times) potential to stimulate vascular network formation (VNF) compared to smooth muscle cells (SMCs) and fibroblasts. Even though the ASC-conditioned media significantly promoted VNF by ECs cultures on SMCs and fibroblasts (up to four times), it was not able to replace the presence of ASCs in co-culture. The physical presence of ASCs in EC-fibroblast co-cultures even in a low fraction (5-20%) efficiently stimulated VNF. These findings demonstrate that the vasculogenesis-promoting potential of ASCs depend on interaction with ECs involving contact and likely bi-directional interaction, leading to modulated secretion of growth factors and ECM proteins.

KEYNOTE ADDRESS III

The dog - The ideal preclinical model for human orthopedic disease therapy with adipose-derived stem cells

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Introduction

The dog has long been used in human preclinical research as a relevant model of human disease. However, this use has traditionally been restricted to the creation of artificial models of human disease, such as ALC transection or menesectomy models of knee instability. More recently, data suggests that the naturally occurring orthopedic diseases in the dog may be useful as models for the corresponding diseases in people. Specifically, osteoarthritis, tendonitis, and ligament rupture and injury are very similar in pathogenesis and healing in the dog and human. In veterinary medicine, autologous stem cell therapy is allowed under regulatory discretion and it is possible to treat and evaluate large numbers of dogs with these types of orthopedic injuries using various strategies of dose, timing and delivery with intense diagnostics and follow-up imaging and clinical evaluations. This lecture will discuss two specific diseases and the results of treatment with adipose-derived stem and regenerative cells: osteoarthritis and rotator cuff injury.

Methods and Results - Osteoarthritis

Osteoarthritis: This study was a prospective, blinded, placebo-controlled study of clinical hip joint osteoarthritis. The veterinarians and owners were blinded as to treatment group. The treatment group received a single intraarticular dose of autologous adipose vascular stromal fraction cells and then followed for 90 days. Clinical measures of lameness and pain all showed statistically significant improvement in the treated versus the saline control group.

Methods and Results - Rotator Cuff Injury

Rotator Cuff Injury: The dog has a condition very similar to "rotator cuff tear" in the human, called Medial Shoulder Instability. In this disease, the soft tissue stabilizers of the shoulder are damaged due to repetitive sports/ trauma. Dr. Sherman Canapp has documented a series of over 20 consecutive cases using arthroscopy, MRI, clinical examination, and gait analysis to compare the shoulder condition before and after stem cell therapy. Treatment was a single intraarticular dose of autologous adipose vascular stromal fraction cells and then dogs were followed for 90 days. The common findings after cell therapy included: reduction in pain and inflammation, healing of frayed and torn tendon/ligament tissues, new cartilage formation, and neovascularization. These data will be used to design and conduct a blinded, prospective study.

Conclusions

Adipose-derived stem and regenerative cell therapy leads to a clinically-relevant, improved outcome in both osteoarthritis and rotator cuff injury in the dog and with no safety concerns. This data may be used to design and support human clinical studies in seeking regulatory approval for these conditions.

KEYNOTE ADDRESS Ⅳ

Proliferation/differentiation capability of mesenchymal stem cells (MSCs) - Importance of transcription factor(s) expression in human MSCs -

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Introduction

Mesenchymal stem cells (MSCs) derived from human bone marrow have capability to differentiate into cells of mesenchymal lineage. Especially, the differentiation capability towards bone/cartilage is very well known. We have already used the patient's MSCs for the treatments of osteoarthritis, bone necrosis and bone tumor cases. Interestingly, the MSCs also can differentiate into endotherial cells and produce cytokines such as vascular endothelial growth factor (VEGF) to promote new blood vessel formation. We thus utilized the MSCs for the treatment of heart failured patients. These our clinical experiences indicate the usefulness of the patient's MSCs for regeneration of various damaged tissues. However, the proliferation and differentiation capability of the MSCs are variable and many lose their capabilities after several passages. With the aim of conferring higher capability on human bone marrow MSCs, some genes could be transduced into the MSCs. This paper focuses on the importance of transcription factors to promote the osteogenesis as well as proliferation capabilities of human MSCs.

Methods

We used retrovirus vector in which IRES sequence was placed between the gene of interest and the Venus gene, a variant of GFP, so that expression of the construct was easily detectable during the cell culture. The gene of interests were either Nanog or Sox2 which has important role for maintaining the pluripotent state of human and mouse embryonic stem cells. The cells used were human bone marrow MSCs after several passages.

Results

Sox2-expressing cells showed distinct growth pattern in the presence of bFGF in culture media. In the presence of the bFGF protein in culture media, bone marrow MSCs show characteristic morphology changes, in which the cells become elongated in shape. In contrast, the Sox2-expressing MSCs responded to bFGF very differently, where the cells grew well as relatively round and small cells. The Sox2-expressing MSCs in the presence of bFGF had higher proliferation and osteogenic differentiation potential than control cells, in which only Venus was expressed.

We observed that Nanog-expressing MSCs were also relatively small and found that Nanog-expressing MSCs showed significantly higher proliferation potential than control cells. We failed to observe significant effects of addition of bFGF in culture media in the case of Nanog-expressing cells in terms of both cell growth ability and cell morphology change. We also found that Nanog-expressing cells showed higher differentiation abilities for osteoblasts than control cells both in terms of both ALP activity and calcium deposition assayed by Alizarin Red staining.

Conclusions

Recently Yamanaka et. al. reported that pluripotent stem cells can be directly generated from mouse and human fibroblasts by the introduction of several defined genes, one of which was Sox2. Thomson et. al also reported the generation of the induced pluripotent stem cells (iPS cells) by introduction of genes in which Nanog was included. These reports confirmed the importance of Sox2 and Nanog gene for the proliferation/differentiation capabilities of the stem cells. Though the single gene transduction as mentioned in this paper is not sufficient to generate the iPS cells, the functional importance of Sox2 and Nanog for altering the cell status was clearly demonstrated and might have clinical significance in regenerative medicine.

Symposium Ⅶ: ASC AND ADIPOGENESIS

Ⅶ - 1] Human adipose-derived stem cell enriched fat grafts: Effect on cutaneous radiation injury in the athymic rat

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Introduction

Freshly-isolated adipose-derived stem cells added to human fat grafts have been shown to enhance vascularization and improve volume retention.[1] Plastic surgeons now apply ASC-enriched lipoaspirate injections as fillers in cosmetic and reconstructive procedures.[2] Furthermore, enriched grafts carry unexplored potential as a novel therapy to revitalize damaged tissues. The quality of skin affected by radiation has been reported to improve markedly following subcutaneous engraftment of fat.[3] Supplementing lipoaspirate with ASCs may magnify therapeutic benefit due to superior viability, improved microvascular development, and delivery of ASCs to wound sites.

Methods

An athymic rat model was developed to test the effectiveness of human xenograft and stem cell-based therapies in treating acute cutaneous radiation injury. Loose skin was held in a non-occlusive acrylic clamp as X-ray radiation (27.5 Gy, 250 kV) was applied, sparing the body from significant exposure. The beam was centered at the edge of folded skin, creating a circular area of injury. Animals were assigned to an ungrafted control group and two treatment groups (N=10 each) employing human ASC-enriched vs. traditional fat injections. Two days post-irradiation, grafts were placed beneath affected skin. Primary endpoints include a photography-based temporal assessment of wound area and determination of microvascular density. Photographs were taken every two days until sacrifice (day 65), then final measurements were made and histological sections were prepared. Immunohistochemical determination of vascular tissue origin (donor vs. host), identification of ASC-derived cells through antihuman vimentin staining, and histological analysis of tissue characteristics are being conducted.

Results

Preliminary analysis indicates both traditional and ASC-enriched fat grafts placed beneath affected skin promote acceleration in the healing of acute irradiation injury. Significant differences ($p < 0.05$) in the fraction of ulcerated skin within irradiated areas were observable by day 19 (17 days postgrafting) and persist until day 33. Grafts in both groups were vascularized and accepted into the host, yet early observations support a higher rate of fat necrosis in the non-enriched group.

Discussion

Injectable human lipoaspirate may serve as an ideal vehicle for delivery of ASC therapy to cutaneous sites and into other areas of pathology into which fat can be grafted. Evidence supports the superior vitality of freshly-isolated vs. cultured and processed ASCs. Fresh cells may be added to lipoaspirates, which serve as a ready-made natural scaffold. Near-immediate application of ASCs following harvest obviates the need for prolonged exposure to unnatural conditions which may fundamentally alter the nature of stem cells.

1 - Matsumoto D, et al. Cell-Assisted Lipotransfer: Supportive Use of Human Adipose-Derived Cells for Soft Tissue Augmentation with Lipoinjection. *Tissue Eng.* 12(12):3375-82, 2006.

2 - Yoshimura K, et al. Cell-Assisted Lipotransfer for Cosmetic Breast Augmentation: Supportive Use of Adipose-Derived Stem/Stromal Cells. *Aesthetic Plast Surg.* 32(1):48-55, 2008.

3 - Rigotti G, et al. Clinical Treatment of Radiotherapy Tissue Damage by Lipoaspirate Transplant: A Healing Process Mediated by Adipose-Derived Adult Stem Cells. *Plast. Reconstr. Surg.* 119:1409, 2007.

Symposium Ⅶ: ASC AND ADIPOGENESIS

Ⅶ - 2] Co-culture of adipocytes and adipose stem cells with either resident or peritoneal macrophages results in preadipocyte producing stem cell spheres in human and mouse.

[1,2,4]Gregorio Chazenbalk, [3,4]Cristina Bertolotto, [3]Saleh Heneidi, [3,4]Charles F. Simmons and [1,2,4]Ricardo Azziz.

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Introduction

Direct cell-to-cell contact between adipose stem cells (ASCs) and mature cells appears to play a critical role for tissue regeneration by maintaining a high rate of ASCs ('seed') and also providing the necessary niche microenvironment ('soil') for the generation of endothelial progenitors. We have recently demonstrated that cell-to-cell co-culture between human adipocytes and a human 'macrophage fraction' containing adipose tissue macrophages (ATMs) and ASCs results in the robust proliferation of preadipocytes, at least in part due to the differentiation of ATMs to preadipocytes, which then proliferate. In addition to the ability to differentiate to preadipocytes, ATMs could also be part of the necessary microenviroment to facilitate the interaction of ASCs and adipocytes. Performing similar co-culture studies using murine cells could help to understand the range and nature of these cell-cell interactions *in vitro* and *in vivo*.

Hypothesis

Direct cell-to-cell contact of mouse adipocytes and ATMs/ASCs generate new preadipocytes through two primarily mechanisms: (i) ATMs differentiation to preadipocytes (ii) formation of stem cell spheres which produce ASCs/preadipocytes.

Methods

Adipose tissue was isolated from wild-type BALB/cByJ mice. After treatment with collagenase, adipocytes were isolated by centrifugation. A macrophage fraction was obtained from either adipose tissue or peritoneum by a Ficoll gradient. Adipocytes and ATMs/ASCs or peritoneal macrophage fractions were cultured separately for 24 hours. Adipocytes were then co-cultured with ATMs/ASCs or peritoneal macrophage fractions for an additional 24 hours, and then separated and cultured alone for another 24 hours or 48 hours. Co-culture in humans was only performed with ATMs/ASCs.

Results

As we previously observed in our human co-culture system, there was indeed generation and proliferation of mouse preadipocytes in the mouse ATM/ASC fraction, as well as the peritoneal macrophage fraction after 24 hr of co-culture and then removal of adipocytes. In mice, few preadipocytes were detected in the ATM/ASCs fraction, and none were detected in the peritoneal macrophage fraction cultured alone. The presence of new preadipocytes after co-culture was confirmed by immunofluorescence. Cells exhibiting preadipocyte morphology were positive for Preadipocyte Factor-1 (DLK). It was possible to observe inclusions with the appearance of lipid droplets inside of these preadipocytes. ATM/ASCs and the peritoneal macrophage fraction without co-culture were positive for CD68 and CD14, but not to DLK. We also detected the presence of cells grouped in CD68 (+)/DLK (+) and CD34 (+)/DLK (+) spheres from which many in preadipocytes were released. These cells were also CD105 (+)/DLK (+)

Conclusion

There are two observed sources of preadipocyte generation and proliferation after co-culturing adipocytes with the ATM fraction or peritoneal macrophage fraction through (i) ATM differentiation to preadipocytes (ii) formation of spheres of ATMs/preadipocyte and ASCs/preadipocytes. Two separate macrophage subsets differentiate using these two different mechanisms. This novel cellular differentiation pathway could have far reaching implications with wound healing and adipose stem cell plasticity. In addition, propagation of the sphere-forming stem cells could be an important tool for the ASC transplantation studies.

Symposium Ⅶ: ASC AND ADIPOGENESIS

Ⅶ - 3] Reversible adipose enlargement induced by external tissue suspension: possible contribution of growth factors for its preservation

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Introduction

There has reported no animal model for adipose tissue enlargement. We prepared a mouse model for continuous external suspension (having negative pressurelike effects) of the skin and subcutaneous tissue, and found that subcutaneous adipose tissue can be expanded by the external force but the effect was lost after removing the force 4 weeks later.

Methods

An original device for external suspension was applied to the back skin of nude mice for 4 weeks. On 0, 3, 7, 14, and 28 days, suspended skin samples were harvested and subjected to histological and biochemical analyses. On day 28, the suspension device was removed and one of the following gelatin microspheres was subcutaneously administered and the tissue was harvested 14 days later (day 42); bFGF, growth factor cocktail (GFC: bFGF, EGF, PDGF and TGFβ), or PBS was incorporated into gelatin microspheres for controlled release of the reagents.

Results

The suspended tissue especially the subcutaneous adipose tissue increased in size with time and reached the maximum enlargement on day 28, but after removing the device, it was substantially reduced on day 42. On the other hand, treatment with bFGF or GFC significantly contributed to preservation of the enlarged adipose tissue. By suspension stress, subcutaneous adipose tissue was expanded. GPDH activity of samples increased from day 3, and small adipocytes suggesting ongoing adipogenesis and capillary density also increased with a peak on day 7. Ki67+ proliferating cells increased from day 7, and most of them were also CD34+/lectin-, suggesting that they are ASCs. CD34+ ASCs increased in number, especially immediately under the dermis (superficial layer of adipose tissue), and some of them were also positive for lectin on day 14, which may suggest that they are transdifferentiating cells from ASCs into endothelial cells. Treatment with bFGF or GFC not only preserved the enlarged adipose tissue volume, but also significantly improved capillary density.

Discussions/Conclusions

Adipose tissue expansion can be achieved by continuous external suspension, which likely applies negative pressure to the tissue. The tissue enlargement, however, is reversible, as far as the force application period is within 4 weeks. Results suggested that ASCs plays crucial roles in the process of adipose tissue expansion by contributing to adipogenesis and angiogensis. The expanded adipose tissue volume can be preserved by administration of growth factors, even after removing the suspension device, suggesting potential use of external suspension in conjunction with growth factors to therapeutically induce soft tissue enhancement.

Symposium Ⅶ: ASC AND ADIPOGENESIS

Ⅶ - 4] PDK regulated Warburg-like phenotype protects against ROS production in differentiating human adipocytes.

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Generation of reactive oxygen species through elevated oxidative phosphorylation has been well documented as a mechanism of cellular damage, dysfunction, and even death. Many cancer cell types have adapted by enhancing glycolysis at normal oxygen tensions to decrease ox-phos generated ROS, a state known as the Warburg Effect or aerobic glycolysis. Using a human ASCs model of adipogenesis, we have identified a similar shift to an enhanced glycolytic phenotype at normal oxygen tensions with adipocyte maturation. This Warburg like state is characterized by a nearly fifty-fold increase in lactic acid production that coincides with a three-fold decrease in their oxygen consumption rate with respect to their overall oxygen consumption capability. Using small molecule inhibitors, we have demonstrated that this effect is achieved through PDK action whose expression we identified as markedly increased with adipocyte differentiation. This control of ROS generation is critical for generating and maintaining a healthy adipose tissue considering its elevated free fatty acid content and high rates of glucose flux. We believe that similar changes in glycolytic rate are potentially critical for other differentiation events as well and plan to expand our research to other mesenchymal and non-mesenchymal lineages.

Symposium Ⅷ

Ⅷ - 1] Safety and feasibility of expanded adipose mesenchymal stem cells

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Stem cells are unspecialized cells that have the ability to give rise to specialized cells. For example, a stem cell could differentiate into, or transform into a nerve cell or bone cell or blood cell. The ability that stem cells have to differentiate makes them ideal for possible cell degenerating disorders. Diseases in which cells are being damaged could then be treated by adding new stem cells. The use of stem cells brings new hope to treat diseases that have been incurable with traditional medicine.

Human adipose tissue-derived mesenchymal stem cells (hATMSCs) were isolated post abdominal liposuction. The hATMSCs were expanded *ex-vivo* culture and were found to have the following characteristics: spindle-shaped, tight attachment to the culture dish and self-renewal capacity during a serial passage. Immunophenotypically, these cells were positive for CD73 and CD90, while negative for CD31, CD34 and CD45. The hAdMSCs demonstrated a diverse plasticity including the capacity to differentiate into adiopocytes, osteocytes, neurocytes and myocytes. They also were able to generate sphere formations in the colony-forming assay. Further characterization using karyotyping revealed a normal, euploid set of chromosomes in hATMSCs up to 12 passages.

Toxicity tests for hATMSCs in SCID mice were conducted. Test groups were divided into saline control group, hATMSCs low dose group ($2x10^5$ cells/kg BW), medium dose group ($2x10^6$ cells/kg BW), high dose group ($2x10^7$ cells/kg BW), and maximum dose group ($2x10^8$ cells/kg BW). The cells were administered once intramuscularly or once intravenously and all groups were observed for 13 weeks. The toxicity tests showed no abnormalities. Therefore, a safe quantity for hATMSCs was determined as $2x10^8$ MSCs/kg BW or higher.

To compare tumor occurrence in BABL/c-nu nude mice, MRC-5 (human fetal lung cell line) cells were used as a negative control and A375 (human malignant melanoma cell line) cells were used as a positive control. Test groups were divided into hATMSCs low dose group ($2x10^6$ cells/kg BW), medium dose group ($2x10^7$ cells/kg BW), and high dose group ($2x10^8$ cells/kg BW). The cells were injected subcutaneously and all groups were observed for 26 weeks for tumorigenicity. Every subject in the positive control group that was injected with A375 developed tumors and no tumor was found in the negative control group and hAdMSC injected groups.

With all our technology we are performing commercials clinical trials for Burger's disease, degenerative arthritis and spinal cord injury approved by KFDA. We have done already the transplantation for a thousands of people in china that makes us confirmed the feasibility and safety of expanded-adipose derived mesenchymal stem cells.

Symposium Ⅷ

Ⅷ - 2] Preliminary Investigation of the effect of human adipose tissue-derived mesenchymal stem cell(ASC) in progressive hemifacial atrophy (Parry-Romberg's disease)

Jong Woo Choi[1], Eun Jung Park[1], Tae Suk Oh[1], Kang Woo Lee[1], Hoon Kim[1], Hyo BO Lee[1], Il Seob Shin[2], Jae Sub Chung[2], Chung Chan Rah[2], Kyung S Koh[1]

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Introduction

Parry-Romberg's disease is rare but crucial disease which result in progressive hemifacial atrophy including of skin, dermis, subcutaneous fat , muscle and finally cartilaginous and bones. The etiology of this diease are not known yet. Generally, this disease have been treated with microvascular free flap transfer because of limits in simple fat graft. Recently, microfat graft technique has been introduced for treatment of facial rejuvenation, however, it seems to have a potential limit owing to the unpredictable absorption ratio of grafted fat. Adipose-derived stem cells (ASCs) is a promising option for improving the angiogenesis. According to the recent studies, even though the exact mechanism were not known, ASC turned out to be a great contributor on vasculogenesis around the tissue. This investigation were done to evaluate the potency of ASC in microfat grafting and know whether the microfat graft with ASC therapy could treat the Parry-Romberg's disease or not. We submit that a clinical trials of therapeutic ASCs on 5 progressive hemifacial atrophic patients. Towards this end, we investigated fat absorption and facial volumes after the injection of ASC and microfat graft on atrophied hemiface objectively.

Methods

This investigation were done under the permission of Korean Food and Drug Administration(KFDA) and IRB of Seoul Asan Medical Center (Investigator – sponsored trials) at April, 2008. 10 volunteer with Parry-Robmberg disease were recruited and treated with ASC & microfat graft or microfat graft only from May, 2008 to January 2009. 5 patients were experimental group and the other 5 patients were control group. The follow up period was 10 months in average. The average age of the patients was 28 years. The 5 patients were male and the others were female patients. At operation day, the fat was harvested from the abdomen of the patients and injected on hemi-atrophic face. Microfat grafting technique of Coleman were used. The hASC were obtained from abdominal fat with liposuction. hASCs (P=0-3) were cultured and $1 * 10^7$ cells were obtained through usual methods. FACS confirmed these cells were the mesenchymal stem cells different from hematopoietic stem cells. At day 14, the secondary fat grating with simultaneous ASC injection were done. For postoperative evaluations, 3 dimensional camera and 3 dimensional CT scan were used and grafted fat volumes and absorption ratio were calculated objectively. And preoperatively and postoperatively, blood testings including CBC, SMA, U/A and opd follow up were repeated monthly until 6 months for detection of side effects of cell therapy.

Results

The results showed the successful outcomes in all 5 patients. Grafted fat much more survived in experimental group. According to the measurement with 3D Camera and 3D CT scan of hemifacial volumes, in the experimental group, preoperatively, the difference of volume between both hemiface were 27.2 cc in average. Postoperatively, those were 4.39cc in average. In the control group, preoperatively, the difference of volume between both hemiface were 16.8cc in average. Postoperatively, those were 8.4cc in average. The satisfaction VAS score was 4.5 (1-5) in the experimental group and 3.1 in the control group.

Conclusions

Our study suggests that hASCs enhances the survival of grafted fat on the face. And the microfat graft with simultaneous ASC injection technique showed the possibility of treatment of Parry-Romberg's disease without microvascular free flap transfer and clinical applications of ASC in various fields need of fat grafting.

Symposium Ⅷ

Ⅷ - 3] Study for development of cell therapy for Alzheimer's disease using adipose-derived stem cells

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Introduction

Delivery of human adipose-derived stem cells (hASCs) is a promising option for the treatment of Alzheimer's disease. Although hASCs have been capable of differentiating into different cell types, hASC transplantation represents an unexplored approach for treating neurodegenerative disorders associated with cognitive decline such as Alzheimer disease (AD). To elucidate the therapeutic effects of hASCs for Alzheimer's disease (AD), we transplanted hASCs into AD animal model, Tg2576.

Methods

We used aged transgenic mice (Tg2576) that express swedish form of amyloid precursor protein (APP) to investigate the effect of hASC transplantation on AD-related neuropathology and cognitive dysfunction. Early Passage hASCs (P=3) were transplanted by the intravenous deliveries or the brain direct delivery. For the intravenous delivery tail vein injections was performed every two weeks from 3 months to 9 months in Tg2576 mice. The brain direct delivery was performed in 9 month-old Tg2576 mice. For tracking the delivered hASCs, hASCs (1 $x10^6$ cells for intravenous deliveries; $1X10^5$ cells per each hemisphere for brain direct delivery) were prepared by CFDA-SE-labeling or BrdU-incorporation.

Results

In the preliminary experiments, following the intravenous deliveries as well as the brain direct delivery, hASCs resided in the injured extra-vascular space of the brain and incorporated into the injured tissues in hippocampus area. Immunohistochemical analysis revealed that the majority of the transplanted hASCs retained neuronal phenotype and some of them had a glial phenotype with no tumor formation, indicating that these may be safe for transplantation.

Conclusions

New cell therapeutic approaches for the replacement of degenerated cells are being evaluated. The aim of this study was to investigate the production of neurons from human adipose-derived stem cells (hASCs) and the potential for utilizing ASC-derived neuronal precursor cells (NPCs) and primed NPCs (PNPCs) for cell restorative therapy in a rodent model of AD. In further study, we will investigate whether the transplantation of human adipose-derived stem cells can promote behavioral recovery and reduce the pathological phenomena in a rodent model of AD.

Symposium Ⅷ

Ⅷ - 4] Autologous adipose tissue derived mesenchymal stem cells: clinical trials for osteoarthritis

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Introduction

Osteoarthritis is a common degenerative disease characterized by articular cartilage destruction. As limited inherent potential of articular cartilage regeneration, it has been a major challenge to clinicians. Although many treatment options have been reported and performed, there is no disease-modifying treatment for osteoarthritis. In early stage of disease, treatment strategy is based on symptomatic relief including, rest, rehabilitation exercise and medication of anti-inflammatory drugs, while surgical intervention including arthroscopy and arthroplasty is considered in late stage. To overcome limited capacity of articular cartilage regeneration, cell-based therapy has been increasingly studied and performed. Since autologous chondrocyte implantation is reported in 1994, it has been widely used for the repair of damaged articular cartilage. However, it was mainly for focal cartilage defect rather than generalized cartilage degeneration and its results are still controversial. Mesenchymal stem cells are multipotent cells and have been identified in most of human tissues including bone marrow, adipose tissue, and synovium. Due to their multipotentiality and self-renewal, mesenchymal stem cells present themselves as a promising tool for treatment of various musculoskeletal diseases. The purpose of the present study was to evaluate the clinical and radiological effects of intra-articular injection of autologous adipose tissue derived mesenchymal stem cells.

Methods

Three patients suffering from osteoarthritis of the knee was involved in the study. There were one man and two women. Average age was 62.7 years old (range, 55 – 72). Clinical and radiological variables were collected, measured and evaluated preoperatively and 6 months postoperatively. Clinical variables were range of motion, quadriceps circumference and power, visual analogue pain scale, and Korean Western Ontario and MacMaster University (K-WOMAC) score. Radiological variables were cartilage defect size, thickness and quality of the medial femoral condyle in magnetic resonance imaging. Autologous adipose tissue derived mesenchymal stem cells were isolated from the subcutaneous fat tissue of patients by liposuction technique. After 3 weeks of culture, 1x107cells/3ml were injected in the knee joint under arthroscopic monitoring. Patients were allowed immediate range of motion exercise, quadriceps setting exercise and non-weight bearing crutch walking till 2 months postoperatively.

Results

There were no postoperative complications such as infection, allergic reaction, injection site reaction and etc. Range of motion, quadriceps circumference, quadriceps isokinetic power, visual analogue pain scale, and K-WOMAC was slightly improved, but there was no statistical significance.

Conclusions

This is a preliminary report of the study results. While there were no serious immediate postoperative complications of injecting autologous adipose tissue derived mesenchymal stem cells, further identification of clinical and radiological results of more patients would be necessary.

Symposium Ⅷ

Ⅷ- 5] Autologous adipose tissue derived mesenchymal stem cell intravenous infusions ameliorate osteoarthritis (OS), ulcerative colitis (UC), hashimoto thyroiditis (HT), atopic dermatitis (AD) with allergic rhinitis, and chronic obstructive pulmonary disease with bronchial asthma.

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Intravenous delivery of adipose tissue derived mesenchymal stem cells is a promising option for many difficult diseases to manage, since it is regenerative, immunomodulatory, immunosuppressive and special homing activities to the diseased regions. It was found to be effective in many animal models of incurable diseases like stroke, spinal cord injury, diabetes mellitus, osteoarthritis, collagen induced arthritis, sensori-neural hearing loss, renal failures, cardiopulmonary diseases and allergic rhinitis. There are emerging evidence, which shows it is effective for Burger's disease, Romberg's disease, peri-anal fistula from Cohn disease, and epidermolysis bullosa.

We have successfully treated 60 years old white male who suffers from intractable osteoarthritis, unable to function as an artist and who had also an accompanying Ulcerative Colitis and mild form of Hemochromatosis. Upon receiving 600 millions and stem cells IV and local delivery of 40 million cells to interphalangeal joints ameliorate his hand joint pain and all of his other arthritis pain. 12 hours after the local delivery of stem cells made his finger joints flexible and within a week he improved his arthritis and he does not use any analgesics, function as a painter, and improved his vision also. His bowel movement became normal and not needing any oral intake of medication to control his UC. His anemia even improved. His CRP and anti CCP levels decreased.

38 y/Asian Female patient with Hashimoto Thyroiditis with angioedema and urticaria patient with high antithyroglobulin antibody level of 343U/ml did improve her symptoms after 100 millions of stem cell. Soon after IV infusion, she never had another attack of angioedema and the antibody level declined.

19 y/o Asian female of long standing intractable atopic dermatitis patient with peanut and soybean food allergy and house dust mite allergy improved immensely her AD conditions after 200 million of IV infusion of stem cells and TNF-α and INF-γ serum level decreased so as Il-6 and Il-β, level. Her skin became smooth and became silky. Her allergic rhinitis symptoms improved also.

69 y/o Asian male with COPD and asthma component for many years with heavy smoking history, allergic to house dust mite, cockroach and aspergillus with coughing, shortness of breath, rhinorhea and increased sputum production received immunotherapy for past two years. Received 200 million of autologous stem cells and one month later his symptom improved markedly; improved his wheezing, no sputum production and 40% increment of Fev 1. The 6 minutes walk distance test improved of 42 meters. Diffusing capacity remained the same so far. He breathes far better than before and sleep well now. Thus the stem cell IV infusion therapy is effective in several difficult human illnesses.

Symposium Ⅷ

Ⅷ - 6] Safety and effect of adipose tissue derived stem cell implantation in patients with critical limb ischemia : early clinical experiences

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Introduction

Adipose tissue derived stem cells(ADSCs) were very potential and effectives on damaged tissue regeneration. Adipose stem cell influenced on cell regeneration via paracrine effects and direct myogenesis. It is very difficult that treat critical limb ischemia by bypass operation or percutaneous vascular intervention. Intramuscular multiple injection of ADSCs may improve critical limb ischemia and limb salvage by angiogenesis and myogenesis. We performed intramuscular multiple injection of ADSCs to patients with critical limb ischemia which was composed of DM foot, Buerger's disease and ASO.

Methods

The study comprised 10 patients (10 men) with a median age of 44 years (range, 34-72 years) who had critical limb ischemia, defined as ischemic rest pain in a limb with or without nonhealing ulcers. The patients received intramuscular multiple injections of ADSCs into the gastrocnemius muscle, the intermetatarsal region, and the feet dorsum (n= 9) or forearm (n= 1). The patients were nonresponders to smoking cessation>6 months and were not candidates for nonsurgical or surgical revascularization. Primary end points were the total healing of the most important lesion while avoiding major or minor amputation, the relief of rest pain without the need for analgesics from baseline to 6 months' follow-up, and the safety and feasibility of the treatment. Secondary end points were the changes in ankle-brachial pressure index and peak walking time, the angiographic evidence of collateral vessel formation or remodeling, and the quality-of-life assessment. Two investigators blinded for treatment assignment performed image analyses.

Results

Intramuscular multiple injection of ADSCs was not associated with any complications. The mean follow-up time was 3 months (range, 1 to 8 months). Only one patient required toe amputation during follow-up. A change in the ankle-brachial pressure index >0.2 was achieved in 6 patients at 3 months. At 6 months, patients demonstrated a significant improvement in rest pain scores, peak walking time. Total healing of the most important lesion was achieved in 4 patients with ischemic ulcers, and relief of rest pain without the need of narcotic analgesics improved in 9 patients. Digital subtraction angiography studies before and 6 months after the ADSCs implantation showed vascular collateral networks had formed across the affected arteries.

Conclusions

Intramuscular multiple injection of ADSCs could be a safe alternative to achieve therapeutic angiogenesis in patients with critical limb ischemia which was composed of DM foot, Buerger's disease and ASO and critical limb ischemia refractory to other treatment modalities.

Symposium Ⅸ: TISSUE ENGINEERING

Ⅸ - 1] Preparation and evaluation of PLGA-silica scaffold for bone tissue engineering

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Introduction

Three dimensional scaffold used in bone tissue engineering is desired to posses not only good osteogenic potential, but also high mechanical strength. Poly(lactide-co-glycolide) (PLGA) scaffolds have been successfully used in bone tissue engineering, with ceramic materials, such as hydroxyapatite (HA), tricalcium phosphate (TCP), and bioglass, etc.. Silica, one of components of drug delivering scaffolds, which has high physical strength and chemical stability, still had been not reported on fabricating scaffolds of bone tissue engineering.

Methods

In the present study, porous PLGA-silica scaffolds were fabricated successfully through by indirect-microstereolithography technology. The scaffolds were evaluated *in vitro* by analysis of microscopic structure, porosity, and stiffness. Following culture *in vitro* the human adipose-derived stromal cells (hADSCs) seeded scaffolds, the expressions of osteocalcin mRNA and collagen type I protein were detected by RT-PCR and western bloting, respectively. And then the cells-seeded scaffolds were implanted subcutaneously into athymic mice.

Results

Results indicated that silica particles can improve the mechanical strength of PLGA scaffolds. PLGA-silica scaffolds exhibited better osteogenic potential than PLGA scaffolds. After implantation *in vivo*, the osteogenic events were consistent with that of culture *in vitro*.

Conclusions

This study demonstrated the potential of PLGA-silica scaffold seeded with hADSCs as a good tool candidate in bone tissue engineering.

Symposium Ⅸ: TISSUE ENGINEERING

Ⅸ- 2] Hydrostatic pressure (HP)-driven 3-D cartilage regeneration using collagen gel and human adipose-derived stem cells

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Backgrounds

We previously showed that a cyclic hydrostatic pressure (HP) combined with a collagen sponge induced differentiation of adipose derived stem cells (ASCs). We focused this study on HP-driven 3-D chondroinduction using human ASCs and collagen gels to develop chondron for augmentation / injection of cartilage.

Methods

Under an approved IRB protocol, discarded human adipose tissues (5.0 g) were used. After cell culture and expansion to three passages, 1 x 10*6 were suspended in neutralized type I collagen solution, and injected into semipermeable membrane tubing and incubated to allow gel formation. We then prepared three groups: an atmospheric pressure (AP) group, a 1st week loading of HP (HP1) group, and a 1st and 3rd week loading of HP (HP2) group. In the HP groups, cell constructs were incubated with treatment of cyclic HP at 0-0.5 MPa, 0.5 Hz, with a medium flow rate of 0.1 ml/min, at 37℃, 3% O_2, and 5% CO_2 using an automated pressure/perfusion bioprocessor. The cell constructs of AP control were incubated at the same bioprocessor. In every group, the medium was switched to chondrogenic differentiation medium including TGF-β1. One, two, three and four weeks after incubation, the cell constructs were harvested for histological, immunohistochemical, and genomic evaluation. Chondrogenic differentiation and cell proliferation were assessed by Toluidine blue staining, immunohistochemistry using antibodies of type II collagen, and real time RT-PCR for type I, II and X collagen, aggrecan, sox9, integrinβ1, MMP3 and13, and PCNA. Moreover, the size change of regenerated cartilage was evaluated.

Results

Production of a construct was produced in all groups. Accumulation of the matrix, expression of type II and X collagen, aggrecan, and Sox9 in the HP1 and HP2 groups were much greater than that of AP groups especially after 2 weeks. However, expression of type I collagen, MMP3 and 13, integrinβ1, and PCNA in the HP1 and HP2 groups were down-regulated much greater than that of AP groups. There were no significant differences between HP1 and HP2 groups. In observations of size changing with respect to time, degree of shrinkage was significant on HP2 groups.

Discussion

These results show that ASCs combined with a collagen gel could differentiate into a chondrocyte suspension capable of injection. Cyclic HP was effective in enhancing chondrogenic differentiation of ASCs and diminishing cell proliferation and collagen degradation. In consideration of shrinkage, it was suggested that a week of HP loading is efficient.

Symposium Ⅸ: TISSUE ENGINEERING

Ⅸ- 3] Novel electrospun polymer scaffolds with microencapsulated human ASCs for tissue regeneration

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Introduction

This study is designed around the central hypothesis that both chitosan and ASCs are natural accelerators of cutaneous wound healing and that their effects can be further amplified through the 1) use of nanofibrous scaffold resembling the structure of the native extracellular matrix and 2) encapsulation of 3-dimensional multicellular aggregates (MAs) of ASCs within the scaffold for improved bioactivity and extracellular interactions. The objectives of this study were to employ co-electrospinning method to create a nanofibrous cellular scaffold, and evaluate the effects of cell seeding, cell formulation, and scaffold composition *in vitro*.

Methods

15 w/v% polycaprolactone (PCL) or 3:1 (w:w) 15w/v% PCL: 8w/v% chitosan were dissolved in 3:1 (v:v) trifluoroacetic acid: methylene chloride solution. Human ASCs were isolated and culture expanded in monolayer adherent culture using 1% human serum culture media (LM1%). MAs (2k ASCs/MA) were synthesized by incubating a known cell density solution in agarose molds over 24hrs. To synthesize the cellular construct, simultaneous electrospinning method was employed. PCL (PN) or PCL-chitosan (PCN) solutions were electrospun at 25 kV, 20 cm, 1 mL/hr onto a rotating mandrel (800 rpm, ø : 4 cm). After 30 min, single cells or MAs suspended in LM1% were electrosprayed at 25kV, 7cm, 20mL/hr while continuing to electrospin the polymer. After 20 min, polymer solutions were electrospun for 10 min to secure the electrosprayed cells. Samples were sectioned (2.5cm2) and maintained in LM1% for 16 days. Single cells manually seeded onto acellular scaffolds (electrospun 1 hr) served as controls to evaluate the effects of scaffold composition (PN vs. PCN), cell formulation (single cell vs. aggregates), cell seeding method (seeded vs. electrosprayed), and cell density (67k vs. 167k/cm2). Scaffold properties and cell-scaffold interactions were evaluated by scanning electron/ confocal microscopy, hydrolytic degradation assay, antibacterial assay, MTT proliferation assay, and histological analysis.

Results

PN (ø : 117 $\pm$ 39nm) and PCN (ø : 131 $\pm$ 68nm) did not undergo significant reduction in mass after submersion in PBS for 16 days. PCN retained the antibacterial property of chitosan with reduction rates of 56 and 68% for E. coli and S. aureus, respectively. Although electrospraying resulted in lower cell loading efficiency compared to seeding, both methods supported the delivery and maintenance of viable cells in general. Largest fold-increase in ASC proliferation were observed in PN with electrosprayed cells within the low density seeding samples (13.9 ± 2.3) and in PN with electrosprayed MAs within the high density seeding samples (15.1 ± 2.7), respectively. This may be reflective of the environment within the scaffold in which the ASCs can attach and migrate in 3D. The effect of scaffold composition was significantly different and may be due to variations in polymer solution preparation methods ($p \leq 0.002$). The effect of cell seeding method was significant when electrosprayed vs. seeded cells were compared ($p \leq 0.001$), but not when MAs were compared ($p = 0.054$). This may be due to reduced cell loading efficiency of the MAs when electrosprayed. Statistical analysis: 3-way ANOVA followed by Holm Sidak Multiple Comparisons Test.

Conclusions

The composition and biomimetic morphology of the proposed cellular constructs make them promising alternatives to currently available skin substitutes. These studies demonstrate that both PN and PCN can support ASC proliferation, and the use of simultaneous electrospinning technique and MAs may enhance their potential as wound healing promoters. Further testing is planned to optimize PCN preparation methods and to confirm these initial findings through *in vivo* wound healings studies.

Symposium Ⅸ: TISSUE ENGINEERING

Ⅸ- 4] Osteogenesis of SFF-based scaffolds using osteoblasts derived from adipose-derived stem cells and HUVECs

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Introduction

We investigated the feasibility of using SFF(solid free-form fabrication)-based scaffolds seeded with osteoblasts, derived from human adipose-derived stem cells (hADSCs), and human umbilical vein endothelial cells (HUVECs) to enhance osteogenesis. To accomplish this goal, SFF-based PCL/PLGA/TCP scaffolds were fabricated using a multi-head deposition system (MHDS), which is one of SFF apparatus. The blended scaffolds were seeded with human osteoblasts and HUVECs and implanted into calvaria defects in rats. At 8 and 12 weeks after implantation, micro-computed tomography (μ-CT), real-time polymerase chain reaction (RT-PCR), and histological assays (hematoxylin and eosin (H&E) staining and Alizarin red staining) were conducted to determine the effects of SFF-based scaffolds on osteogenic potential.

Methods

The isolated hADSCs were cultured in DMEM supplemented with 10 % fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin in humidified air with 5 % CO_2 at 37°C. The cells were allowed to adhere and grow for 3 days, and then osteogenic differentiation was induced by adding osteogenic media. HUVECs were supplied by MCTT Inc., and were cultured in EGM-2 medium. We successfully fabricated SFF-based PCL/PLGA/TCP scaffolds with an external geometry of 8.0 x 8.0 x 2.0 mm^3. The pore size in each layer was about 400° $\pm$ 20 ㎛ and each pattern was staggered to increase the rate of adhesion of cells on the scaffold. The calculated porosity of the scaffold was approximately 66.7%. Scaffolds for *in vivo* experiments were divided into three experimental groups: scaffolds to be seeded with HUVECs only, scaffolds to be seeded with osteoblasts only, scaffolds to be seeded with both HUVECs and osteoblasts, and unseeded scaffolds (control group). A 50 $\mu\ell$ aliquot of suspension containing 10^5 HUVECs was seeded onto each scaffold in the HUVEConly group and the osteoblast-HUVEC group. Each scaffold in the osteoblast group and osteoblast-HUVEC group was seeded with 2 $\times$ 10^5 osteogenic hADSCs in 50 $\mu\ell$ osteogenic media. Cell-free scaffolds were immersed in osteogenic media but not seeded. Thirty adult male between 240-260 g were housed in groups of two and maintained in a room at 23°C with a 12 h light/dark cycle. The periosteum was elevated and an 8 mm (diameter) calvarial bone defect was created with a dental bur (ø 1.5 mm) without dura perforation. The defect area was then filled with each scaffold and the wound was closed. The scaffolds were collected 8 and 12 weeks after surgery. For immune-suppression, animals were treated with intraperitoneal injections of cyclosporin A.

Results

In vivo experiments were performed to investigate whether our SFF-based scaffolds seeded with osteoblasts and/or HUVECs had the potential to enhance bone formation. The μ-CT studies indicated that the osteoblastonly and osteoblast-HUVEC groups produced bone formation, whereas bone tissue did not appear to have developed in the control group and the HUVEC-only group. Additionally, scaffolds in the osteoblast-HUVEC group had the largest

area of new bone tissue. After 8 and 12 weeks *in vivo*, H&E and Alizarin red staining showed that the control group and HUVEC-only group had the lowest amount of bone formation. However, in the osteoblast-only group and osteoblast-HUVEC groups, bone-like tissue containing typical lacunae could be seen. Moreover, these data demonstrate that in the osteoblast-HUVEC group, calcium was distributed in a manner similar to that of normal bone tissue.

Conclusions

SFF-based PCL/PLGA/TCP scaffolds were fabricated using a MHDS. The blended scaffolds were seeded with human osteoblasts and HUVECs, and implanted into calvaria defects in rats. After implantation for 8 and 12 weeks, we demonstrated through μ-CT and histological assays that scaffolds seeded with both human osteoblasts and HUVECs were superior to other groups for effective bone formation.

SCIENTIFIC SESSION

SPECIAL LECTURE Ⅲ

Robot technology and Future: The age of robot

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World widely, various types of intelligent service robots are being on the research, and already became well acquainted with human beings. Also, many people expect these robots will be living together with mankind at work or home in the foreseeable future. Nevertheless, many people yet have a pessimistic point of view about the ultimate form of robot would be impossible to be realized in the real at the technology view. The intelligent service robot is distinguished from industrial robot in many aspects. The intelligent service robot is supposed to be working at human living environment with human. This simple-looking difference introduces tremendous technological challenges. Such challenge includes artificial intelligence, vision and voice recognition, communication, mechanical and hardware architecture as well. And also the technology level required to accomplish a task given by the user is very different according to the application requirement. Some simple task may be achieved by simple robotic technology but some others are not. That means that commercial realization of service robot strongly depends on the gap between market requirement and technology level available. Such kind of issues will be discussed in the lecture. The lecture will also mention the history of robot development and application of robot in the various fields.

KEYNOTE ADDRESS V

Adipose derived stem cells in bone regeneration

Riitta Suuronen

Institute for Regenerative Medicine, University of Tampere, Finland

Microvascular reconstruction is the state-of-the-art in many fields of defect surgery today. Currently, reconstruction of large bony defects involves harvesting of autologous bone causing donor site morbidity and risk of infection. Specifically, utilizing autologous adipose stem cells (autoASCs), large quantities of cells can be retrieved for cell therapy applications and the risk of tissue rejection is diminished.

Adipose derived stem cells have been used in clinical treatments in 20 patients in Finland. The longest follow up is three years. In this presentation the results will be shown and future perpectives elucidated.

Symposium Ⅹ: ASC AND OTHER THERAPEUTIC APPLICATION

Ⅹ - 1] *In-vitro* differentiation of adipose derived stem cells into Schwann cells and *in-vivo* implications for peripheral nerve defects

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Introduction

The study is performed to evaluate the results of repair of the peripheral nerve defects with adipose derived stem cells (ASCs) that are differentiated into schwann cells *in vitro*.

Materials and Methods

ASCs isolated from inguinal fat pads of Fischer rats were differentiated into schwann cells (dASCs) in a conditioning medium (DMEM supplemented with 10 % FBS, antibiotic / antimycotic, PDGF, bFGF, forskolin, GGF-2). At the end of the differentiation period, immunohistochemical staining and RT-PCR were performed for detecting schwann cell proteins namely; S-100, p-75, integrin-β4 and their genes. Sciatic nerve defects in Fischer rats was created in *in vivo* study and repaired either with Group I: silicone tube, Group II: silicone tube+collagen gel, Group III: nerve graft, Group IV: silicone tube filled with collagen gel embedded with ASCs and Group V: silicone tube filled with collagen gel embedded with dASCs. The cells were labeled with DiI for *in vivo* tracing. At the end of the *in-vivo* step, results were evaluated both functionally (walking track analysis, nerve conduction velocity study) and histologically (HE, toluidine blue staining; SEM; Immunohistochemical staining)

Results

In vitro: Immunohistochemically, 31 % of dASCs were positive for S-100, 27 % for p-75 and 12 % for integrin-β4. The genes of all these proteins were found to be positive in RT-PCR.

In vivo: Walking track analysis was performed at 3rd and 6th months postoperatively. At 3rd month the best results were obtained in dASCs group (mean SFI:-57 $\pm$ 50) but the results were insignificant (p=0.69). At 6th month ASCs group had slightly better results than the dASCs group: dASCs group (mean SFI:-52.6 $\pm$ 5.7), nerve graft (mean SFI:-74.8 $\pm$ 12.8), ASCs (mean SFI:-49.1 $\pm$ 13.1), silicone tube (mean SFI:-73.3 $\pm$ 5.07), silicone tube +collagen gel (mean SFI:-79.6 $\pm$ 12.01). The difference in-between the dASCs and ASCs groups was found to be statistically insignificant whilst the difference in-between these groups and the other groups were significant ($p<0.05$). The results of nerve conduction velocity study were as follows: dASCs group (4.26 $\pm$ 0.4 mm/msc), ASCs (4.44 $\pm$ 0.3 mm/msc), nerve graft (4.41 $\pm$ 0.6 mm/msc), silicone tube (3.67 $\pm$ 2.2 mm/msc), and silicone tube + collagen gel (3.4 $\pm$ 0.3 mm/msc)(p=0.084). The histological evaluation (mid and distal nerve myelinated/unmyelinated nerve fiber ratio and myelinated fiber density, SEM and *in vivo* immunohistochemical staining) further supported the results of functional tests.

Conclusions

Treatment of nerve defects with ASCs that were differentiated into Schwann cells *in vitro* yielded similar results with treatment with undifferentiated ASCs. This can be attributed to *in vivo* differentiation capacity of ASCs.

Symposium Ⅹ: ASC AND OTHER THERAPEUTIC APPLICATION

Ⅹ - 2] The role of human adipose-derived stem cells in 2-acetylaminofluorene-induced liver injury of rats

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Introduction

Adipose-derived stem cells (ASCs) have been demonstrated to be capable of differentiation towards hepatic lineage *in vitro* and *in vivo*. However, their transplantation efficacy into the liver has been reported that very low and there are still lack of theses demonstration *in vivo*. Moreover, there are a few reports of differentiation of bone marrow stem cells into the liver cells after liver injury models, but theses demonstration is still unclear with ASCs.

Methods

Eight-week-old Sprague-Dawley rats were received 2-acetylaminofluorene (AAF) for inhibition of hepatocyte proliferation, and the1n divided nine groups with a control group. Rats were performed with or without 70% partial hepatectomy (PHx), ASCs injection and orally administration of 5% glycine (kupffer cell inhibitor) in water. ASCs were isolated from the abdominal fat of human and labeled with 4×6-diamidino-2-phenylindole (DAPI). 1×10^6 of ASCs injected into the spleen and then all rats were sacrificed at 2 weeks. ASCs were detected by fluorescence in the liver and were examined by RT-PCR analysis and the immunolabelling with lineage markers such as albumin, ED1, senescence marker protein-30 (SMP30), PCNA, TGF-β, and TNF-α.

Results

DAPI-labeled ASCs were detected by nuclear blue fluorescence in the liver, and the number of transplanted ASCs was increased by 5% glycine treatment with decreased of kupffer cell appearance. CD3 (T lymphocyte marker) and CD79a (B lymphocyte marker) were not detected in human-ASCs transplanted liver. Transplanted ASCs were usually found in the hepatic sinusoids or presented in a cluster with kupffer cells in connective tissue. Specific human albumin sequence of mRNA was detected in the rat liver transplanted ASCs. Especially, co-treatment group of ASCs and glycine showed significantly increase of ASCs engraftment and decrease of liver injury factors.

Conclusion

Numerous approaches to cell transplantation of hepatic or extrahepatic origin into the liver are being developed. However, low transplant efficacy of these cells is still remains, moreover, most of them are destroyed by the phagocytic responses of kupffer cells. Therefore, inhibition of kupffer cell activity increases the number of transplant of ASCs. Consequently, ASCs transplantation protects AAF-induced liver injury, and glycine treatment give synergic effects to engraftment of ASCs and liver protection.

Symposium X : ASC AND OTHER THERAPEUTIC APPLICATION

X - 3] Stem cells from adipose tissue: Applications in sinal disorders

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Background

Mesenchymal stem cells from adipose tissue (ASCs) can be harvested with minimal patient discomfort with clinically relevant yields, thus eliminating the need for culture expansion prior to implantation. This allows one-step surgical procedures (harvesting, differentiation induction, scaffold seeding, and implantation within several hours within the operational theatre) for orthopaedic applications, either aimed at bone tissue engineering (bTE) (e.g. spinal fusion, fracture repair) or at regeneration of cartilaginous structures (cTE) (e.g. disc regeneration, articular cartilage repair. The concept may avoid loss of function (regenerating discs) and/or donor site morbidity (iliac crest bone harvesting) by effectively using the body's own regenerative potential.

Methods

Adipose tissue (AT) was obtained by resection or liposuction. The stromal vascular fraction (SVF) was isolated from the AT, followed by either seeding on calcium phosphate or polymeric scaffolds and implantation in a goat spinal fusion model (bTE), or directly injected in degenerating goat discs (cTE). Subsequent SVF characterizations were performed *in vitro*: ASC content within SVF by colony forming unit and FACS analyses, and verification of multi-differentiation potential using lineage-specific (adipogenic, chondrogenic, osteogenic) induction media. Evaluation was a.o. performed by X-ray, MRI, histology, and biochemical/molecular biological analyses

Results

The one-step surgical procedure appears feasible, and can be performed within 2-3 hours. Spinal fusion data are mixed, but show promising results in currently ongoing studies. For disc regeneration, initial results were discouraging, but optimization has been achieved since.

Conclusion

One-step surgical concepts using cell-based therapies are within reach, but need to be optimized for specific applications.

Symposium Ⅹ: ASC AND OTHER THERAPEUTIC APPLICATION

Ⅹ - 4] Omentum as a potential source of cell therapy for acute liver damage

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Introduction

We have reported that adipose tissue-derived stem cells (ASCs) were able to transdifferentiate into hepatocytes and would be a good source for hepatocytes cell therapy. The omentum, a sheet-like fat tissue attached to the greater curvature of the stomach, is easily obtainable in large quantities during abdominal surgery. However, relatively little attention has been paid to the omentum as a source of ASCs. We examined the capability of ASCs from omentum for the therapy of liver damage, using CCl_4 induced acute liver failure mouse model.

Methods

ASCs were isolated from omental adipose tissues of gastric cancer patients. ASCs were used for cell transplantation right after the isolation procedure. Six-weeks olds female Balb/c nude mice were treated with 0.25 ml/kg carbon tetrachloride (CCl_4) dissolved in olive oil. Twenty-four hours after the injection of CCl_4, $1.5x10^6$ human ASCs without culture or same volume of Hanks' balanced salt solutions (HBSS) as a control were injected into the tail vein. Then the mice were anesthetized and serum and liver tissue were harvested twenty-four hours after cell transplantation. Serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin (T-bil), were measured to assess the extent of liver damage. The liver tissue was also examined by histology and immunohistochemistry.

Results

The serum level of T-bil in transplantation group was significantly lower than in control group. Immunohistochemical analysis revealed that HLA-1 positive cells were found in transplant mice liver tissue. However, histology did not show amelioration of liver damage.

Conclusion

This present results indicated that transplanted human omental ASCs could migrate into damaged liver. Although the improvement of liver function was detected at present, omental ASCs might have protective effects to damaged liver.

Symposium Ⅹ : ASC AND OTHER THERAPEUTIC APPLICATION

Ⅹ - 5] Protective effects of adipose stem cells against cigarette-smoke induced lung injury

Irina Petrache, Kelly S. Schweitzer, Brian H. Johnstone, Dmitry Traktuev, Jana Garrison, Natalia I. Rush, Jeremy J. Adamowicz, Mary Van Demark, Amanda Fisher, Todd G. Cook, Dongni Feng, Stephanie Merfeld-Clauss, Krzysztof Kamocki, Robert G. Presson, Hal E. Broxmeyer, and Keith L. March

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Adipose-derived stem cells (ASCs) express produce critical factors for lung cell growth and survival which may be utilized to enhance the repair or recovery from lung injury such as that induced by cigarette smoking (CS). We evaluated the therapeutic effects of ASCs on the CSinduced emphysema model in mice. Animals treated with ASCs during the second half of their CS exposure had less alveolar space enlargement in response to CS, measured by mean linear intercept (p=0.032 compared to CS-only-exposed mice). This effect was associated with decreased lung cell apoptosis and reduced lung inflammation. Interestingly, mice treated with ASC were markedly protected against the suppressive effects of CS on hematopoietic progenitor cell numbers and had less weight loss during CS exposure. These results suggest a therapeutic effect of ASCs against both the lung and the systemic injurious effects of CS.

Funding: IU Signature Center Grant.

Symposium Ⅹ: ASC AND OTHER THERAPEUTIC APPLICATION

Ⅹ- 6] Optimization of administration route for adipose derived stem cell therapy on random pattern skin flaps

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Background

Adipose derived stem cells (ASCs) not only have the capacity for multilineage differentiation, but also for secretion of various growth factors, including hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF). It has been suggested that ASCs have angiogenic potential by promoting the production of angiogenic growth factors and by differentiating into a vascular phenotype, which can lengthen the survival of random pattern flaps. To maximize the therapeutic effects of ASCs, the choice of the optimal cell delivery route is very important. In current study, we compared the effectiveness of different administration routes of ASCs to improve random-pattern ischemic skin flaps.

Materials and Methods

Adipose tissues were harvested during abdominal liposuction, and ASCs were isolated and cultured. After identifying the surface marker, the ASC cell line was established and reproduced. The 3 x 8 cm sized random-pattern ischemic flap was elevated from the dorsal side of the rat and was placed back to the original site with silicone sheet of the same size applied between the bed and the flap to prevent blood supply. Twenty five rats were randomized into 5 groups. In the control group (Group I), phosphate buffered saline (PBS) was injected into the flap. The experimental groups were divided into 4 groups by ASC administration route. In each group, ASC suspension ($1x10^7$ cells/ml) was applied to the flaps with their carriers as follows: generalized intravenous injection (Group II), localized subcutaneous injection (Group III), application with fibrin glue (Goup IV; Tissucol®, Baxter, Austria) and application with collagen sponge seeding (Group V; Gelfoam®, Pfizer, Belgium). Cutaneous blood flow was measured in the 3 compartments of each flap (proximal, middle and distal), using laser Doppler flowmetry (Periflux 5000; Perimed AB, Sweden) preoperatively and at postoperative days 6 and 14. Flap viability was measured using computer planimetry, and histopathological examination was performed 2 weeks after flap elevation. The number and size of vessels were evaluated in the high-power (x100) fields. We established a new scoring system, which take all the factors influencing the flap survival rate into consideration.

Results

Viability measurements showed an increasing rate in localized injection group [56 ± 4.0%] and collagen sponge seeding group [50.0 ± 10.0%] compared to the control group [35 ± 3.0%]. However, generalized intravenous injection group and fibrin glue group made no difference with the control group (Fig. 1). Cutaneous blood flow was high in the proximal compartment of the flaps of the experimental groups 2 weeks after flap elevation in the following order: the collagen sponge seeding group, the generalized intravenous injection group, the localized injection group, and fibrin glue group (Fig. 2). On histopathologic examination, the number and size of vessels were greater in the experimental groups in the following order: the collagen sponge seeding group, the generalized intravenous injection group, the fibrin glue group, and localized injection group.

Four variables were incorporated into our new scoring system: (1) wound area, (2) cutaneous blood flow, (3) number of vessels and (4) total vascular area. All experimental groups scored higher than the control group. Among them, the collagen sponge group achieved the highest score (Fig. 3).

Conclusions

The findings of the present study suggest that ASCs can increase the survival of random-pattern cutaneous flaps in rats. Moreover, according to our new scoring system, the collagen sponge is the most effective scaffold for ASCs therapy in increasing flap survival.

Fig. 1. Surviving flap areas

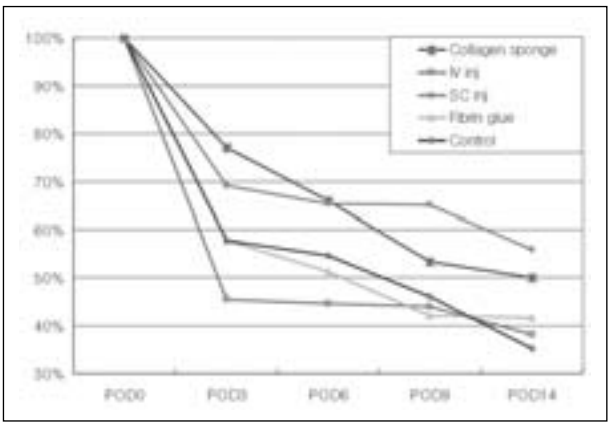


Fig. 2. Doppler report (proximal compartment)

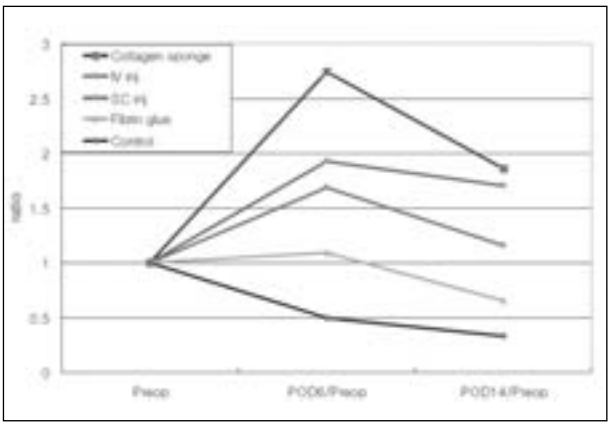
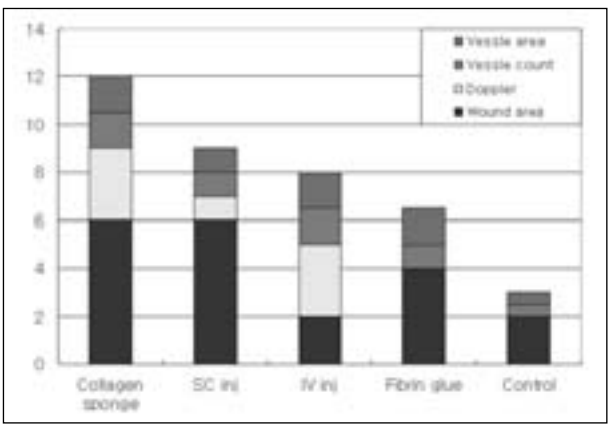


Fig. 3. Scoring system and its result



INVITED LECTURE

New perspectives in plastic surgery: Adipose derived stem cells and enriched fat grafting in breast surgery

Michele L. Zocchi

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NEW CONCEPTS IN ADIPOSE TISSUE PHYSIOPATHOLOGY

The adipose tissue is a real organ and is necessary for life.
Trace of fat organ can be detected between the 14 th and 16th weeks of prenatal life in some specific body areas like cheeks and buttocks. Then the fat lobules development follows. At the beginning of 3rd trimester adipocytes are present in all the main fat depot areas.
Along the evolution processes, mammals and humans have developed mechanisms to store energy during abundance periods. This nutriente excess is mainly stored as TGs (triacylglycerols) primarily in the adipose tissue.
Fat deposits in the different areas of human body serve as the main caloric store and help survivial during periods of food deprivation and famine. This efficient and advantageous mechanism of accumulation of fat stores, when humans were hunters and gatherers, has become deleterious with the modern style of life, characterized by food abundance and limitation of physical activity.
Excess of white adipose tissue (WAT) reflects the accumulation of TGs in fat deposits leading first to an increased filling of adipocytes and consequentially an increasing of their size (hypertrophy) and then in a second time to an increasing of the number of the adipocytes (hyperplasia). All these processes are controlled by a complex mechanism called lipogenesis. During the post-natal period the proliferative activity in adipose tissue is limited to adipocyte precursors only and remain totally undetected in adult adipocytes.
This observation is totally in contrast with the old believe that the adipocytes stop to proliferate after the sexual maturity and that the number of adipocytes stay constant through the time.
For this reason until very recently all the studies in humans were based only on determination of fat cells number (hyperplasia or hypoplasia) and fat cells volume (hypertrophy or hypothropy).
Therefore the estimation of cellularity was limited only to a posteriori definition of volume and number of adulte mature adipocytes, which do not proliferate, without taking into consideration cells precursors and progenitors which do proliferate and differentiate.
Therefore, based on the above mentioned observations, the most recent studies on adipose tissue have mainly focused on cell precursors and progenitors.

ADIPOSE DERIVED STEM CELLS

Since the moment of its conception lipoplasty has always been considered a specialized branch of plastic surgery which takes care of the remodelling of body adipose tissue.

Classic liposuction or ultrasonic lipoplasty, constituting the most advanced technical evolution has in fact as its main objective, the reduction of volumes through the taking away of excess adipose tissue.

Lipofilling and lipo-trasplant on the other hand allow for the addition of adipose cells to those anatomic areas where support volumes are naturally lacking and secondly as in the ageing process.

For many years then, the plastic surgeon found himself confronted with large quantities of adipose tissue,

underestimating its enormous intrinsic potential.

The most advanced research has been able to demonstrate that adipose tissue presents the same potentiality of growth as that of stem mesenchymal multi powerful bone marrow cells. But when taking away bone marrow the results are more traumatic and limitative, while adipose tissue can represent an inexhaustible source of easy and immediately available mesenchymal cells for clinical applications in all those areas of medicine that care for the regeneration of autologous tissues.

In fact from a modest quantity of adipose cells one can obtain, through rigorous isolation and culture techniques, large quantities of multi powerful stem cells which can eventually be differentiated according to needs (adipose, cartilaginous, bone, endothelial, muscular, hepatic tissue ...).

BI-COMPARTMENTAL BREAST LIPO-STRUCTURING

The techniques of additive mastoplastic which have been described over the years, require the use of artificial materials (silicone) which are often badly tolerated by the body and have access paths which could leave visible, unaesthetic, residual scars.

Furthermore there are universally known controversies on the use of pre-filled silicone gel breast implants, which at the beginning of the 90s, brought about a decree that caused the suspension and use of such products, which lasted for some years.

All of the above mentioned pushed Prof. Zocchi to look for alternative solutions to additive mastoplastic with silicone implants taking into consideration the breast lipotransplant technique.

As a matter of fact since almost a century the autologous adipose tissue has been used safely and with success in many other surgical techniques for the correction of volumetric defects of soft tissues.

Its natural, soft consistency, the absence of rejection and the versatility of use in many surgical techniques have always made autologous adipose tissue an ideal filler tissue.

All of these evaluations have allowed our Group to put in place a new surgical methodology, importantly taking into consideration the most modern interpretations of breast functional anatomy and of lipostructuring and lipotransplant methodologies.

Such methodology (B.B.L.S.) "Bi-compartmental Breast Lipostructuring" is based on the way adipose tissue is harvested, rigorously in closed cycle, with minimum manipulation by a so-called bi-compartmental technique of re-implantation, that is to say, exclusively in the pre-facial retro-glandular position, and in the under skin area and mainly at the upper pole breast level, so by avoiding the insertion of adipose tissue into the glandular structure context. Since 1998 we operated with this procedure on 263 patients. Total of 484 breast: 221 bilateral, 42 monolateral. Since October 2008 we consistently modified the way to harvest and to treat the adipose tissue for reimplantation, using a new revolutionary technique called "lipocondensation" in order to eliminate before the reimplantation all the fluid components of the fat by preliminary destroying the adulte mature adipocyte. The final aim of this new technical improvement is to obtain the more reliable and permanent material in order to dramatically improve the volume persistence and the tissue regeneration by reimplanting and higher

concentration of stromal fraction very rich in self precursor and adipose derived stem cells (A.D.S.C.). 17 patients have been already treated with this new procedure with a residual volume maintenance rate at six months up to 90%. With the traditional B.B.L.S., before lipocondensation, the grafted fat volume ranged from 160 cc. to 920 cc. per breast (average of 390 cc.), after lipocondensation the grafted fat volume ranged from 95 cc. to 360 cc. per breast (average of 245 cc.).

Complications encountered have been minimal and transitory (two cases of pseudo cysts which regressed spontaneously and a case of micro calcification at the upper pole level) but above all, thanks to the evolution of the way it is gathered and its re-insertion, it has been possible to sensibly increase the percentage of transferred adipose tissue survival and persistence.

Such operations must always be preceded and followed by a correct and rigorous radiography test (mammography and/or echography) which allows the safe evaluation of the evolution of transplanted tissue.

In the light of this above presented methodology, when carried out in the new described mode, and always respecting precise technical and anatomical parameters, it can constitute the most reliable therapeutic alternative to those cases where additive mastoplastic with silicone implants can prove either unsuitable or unacceptable by the patient herself.

Furthermore it is important to underline that this technique does not always need to be used in place of the additive mastoplastic with prosthetic implant. In fact the volumetric increase and the projection of the breast cone obtained, are more modest, even if noticeable, above all, at the upper breast pole level, a region that more frequently requires an earlier support intervention.

Symposium XI : ASC AND DESEASES

XI - 1] Adipocyte differentiation influences proliferation and migration of normal and tumoral epithelial breast cells

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Breast epithelial cells are in constant development and differentiation throughout a woman's life. Stromal tissue plays a key role in the regulation of those changes. Adipocytes are the main stromal cell type in breast tissue and can be found as preadipocytes, mature adipocytes and in intermediate differentiation states. Heparanase degrades heparan sulphate and the importance of this enzyme has been described in relation to tumoral metastasis. In addition, metalloproteinases (MMPs) cleave proteoglycans and therefore degrade the extracellular matrix. The aim of this work was to evaluate the effect of soluble and non-soluble factors obtained from distinct adipocyte differentiation states on proliferation and migration as well as the activity of heparanase and MMP-9 of normal (NMuMG) and tumoral (LM3) murine epithelial cells.

NMuMG and LM3 were grown on plastic or on 3T3-L1 irradiated cells at various degrees of differentiation (stromal support -SS-, preadipocytes -preA-, poorly differentiated adipocytes -pDA-, mature adipocytes -MA-), and in the presence or absence of conditioned medium (CM) from preA, pDA or MA 3T3-L1 cells. Proliferation was quantified by MTS and cell migration by wound healing. Heparanase activity was determined by heparin cleavage in native polyacrylamide gels dyed with Rubipy (a cationic dya), while MMP-9 expression was quantified by Western blot.

NMuMG and LM3 proliferation increased on all three SSs after 24 and 48 h of culture compared to controls ($p<0.05$). When NMuMG were cultured on the SS in the presence of CM from preA, pDA or MA 3T3-L1 cells for 24 h, proliferation was enhanced with respect to control ($p<0.05$). In the same growth conditions LM3 proliferation was not significantly modified. Initial NMuMG and LM3 adhesion to SSs did not show significant differences compared to plastic.

All three CM from 3T3-L1 increased LM3 migration since wound healing occurred in $75 \pm 3\%$ (preA); $53 \pm 4\%$ (pDA) and $39 \pm 4\%$ (MA) after 6h incubation ($p<0.05$). When LM3 were cultured on the three SSs migration was observed only on the preA SS ($33 \pm 3\%$, $p<0.05$). NMuMG migration was not observed when cultured on the SSs or with CM.
In order to evaluate the role of heparanase enzyme on NMuMG and LM3 cell proliferation and migration, we measured this enzyme's activity by means of heparin cleavage, in both cell lines previously incubated with the CM of preA, pDA and MA 3T3-L1 cells. We observed a significant decrease of heparin band intensity, corresponding to an increase of heparanase activity, in NMuMG cells previously incubated with the three CM. On the other hand, LM3 cells presented enzyme activity when previously incubated with the CM from pDA and MA 3T3-L1 cells. None of the cell lines presented a detectable basal heparanase activity.

Finally, we evaluated the expression of MMP-9 in NMuMG and LM3 cells previously incubated with CM from 3T3-L1 cells at various degrees of differentiation. Both cell lines presented basal expression of MMP-9. We observed a significant increase of MMP-9 expression in LM3 cells previously incubated with CM from preA; pDA and MA 3T3-L1 cells, with a significantly higher expression in presence of preA 3T3-L1 CM.
We conclude that adipocyte differentiation influences normal and tumoral epithelial breast cell proliferation and migration. Heparanase and MMP-9 seem to be involved in this regulation.
We present an experimental model that allows maintaining the characteristics of the physiological environment of epithelial breast cells, regarding both factors and stromal structure per se.

Symposium XI : ASC AND DESEASES

XI - 2] Aging, fat depot origin, fat cell progenitor senescence, and inflammation

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Robert and Arlene Kogod Center on Aging[1], Mayo Clinic, Rochester, MN, USA; Departments of Medicine[2] and Genetics and Genomics[4], Boston University, Boston, MA, USA; Biochemistry and Molecular Biology[3], University of Nevada, Reno, Reno, NV, USA; Newcastle University[5], Newcastle upon Tyne, UK.

Aging is associated with fat redistribution and increased metabolic syndrome prevalence. Age-related changes in fat cell progenitors may contribute, as fat cells turn over throughout life and aging and fat depot origin affect preadipocyte replication, differentiation into fat cells, susceptibility to apoptosis, and cytokine production. In genome-wide expression profiles of epididymal and perirenal rat fat cell progenitors, we found extent of age-related changes was fat depot-dependent, potentially contributing to fat redistribution with aging. Genes involved in stress responses increased with aging, while developmental regulators were prominent among depot-dependent genes. We tested if altered cytokine production contributes to age-related stress response activation.
Fat cell progenitor TNFα and IL-6 production was increased with aging. Cellular senescence is induced by repeated replication or cellular stress with loss of capacity to divide. Cellular senescence is associated with an altered, pro-inflammatory secretory phenotype similar to that we found in fat cell progenitors from old animals. Extensive progenitor replication, increased cytokine generation, and cellular stress due to inflammation occur during aging. We tested the hypothesis that senescent cells accumulate in fat tissue with aging. Abundance of senescent fat cell progenitors increased with aging in fat tissue from old rats and mice, more so in perirenal than epididymal fat. TNFα, IL-6, MCP-1, and MMP expression increased dramatically in senescent fat cell progenitors, as occurs in serially subcultured senescent human fibroblasts and fat tissue with aging. Treatment of primary fat cell progenitors with TNFα or IL-6 induced senescence in non-senescent fat cell progenitors, as well as inflammatory cytokine and chemokine expression. This even occurred in strains derived from single human fat cell progenitors by stably expressing human telomere reverse transcriptase. Thus, senescent fat cell progenitors with a pro-inflammatory secretory phenotype accumulate with aging to different extents in different fat depots. Inflammatory cytokines released by these cells may spread senescence and inflammatory cytokine generation to nearby cells and attract macrophages, initiating an inflammatory cycle, likely interfering with fatty acid handling and insulin responses. Differences in developmental gene expression may impact the timing of age- and obesity- related changes in fat cell progenitor function, contributing to altered fat tissue distribution and function.

Symposium XI : ASC AND DESEASES

XI -3] Cellular events in pathological adipose tissue hyperplasia: Lipoma, lipedema and obesity

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Introduction

Involvement of adipose-derived stem/progenitor/stromal cells (ASCs) in adipose tissue hyperplasia such as lipoma and lipedema has been suggested, but the pathogenesis and pathophysiology of these diseases remain unclear.

Methods

In this study, we analyzed cellular and transcriptional characteristics of pathological adipose tissue samples (5 lipomas and 1 lipedema), together with normal adipose tissue in the same patients, using immunohistochemistry, real-time PCR, and whole-mount staining.

Results

A increased numbers of small adipocytes surrounded by CD34+/lectin-ASCs (Fig 1) and Ki67+/CD34+ ASCs (Fig 2) indicated enhanced adipogenesis in lipoma tissue compared to normal adipose tissue. In contrast, cellular apoptosis was not enhanced in lipoma,suggesting that the enlargement of lipoma tissue may be due to a positive balance of adipocyte turnover (accelerated adipogenesis combined with non-enhanced apoptosis). Leptin mRNA was upregulated in lipoma, while adiponectin, TNFα, and GLUT1 mRNA were downregulated and there were no apparent changes in HIF1α, PPARγ, and PAI-1. These results suggested dysfunction of lipoma adipocytes similar to that in obesity, but indicated that lipoma tissue lacked several obesity-related phenomena such as hypoxia, macrophage infiltration, inflammatory reactions, and enhanced glycolysis. ASCs isolated from lipoma and normal adipose tissue showed similar proliferative and adipogenic capacity, suggesting that the increased adipogenesis may be dependent on microenvironmental factors of lipoma tissue. Lipedema tissue showed proliferation of ASCs, but also showed crown-like structures (necrotizing adipocytes surrounded by infiltrating CD68+ macrophages), that is a feature commonly seen in obese adipose tissue (Fig 3).

Discussions/Conclusions

Our findings indicated that ASCs were playing important roles in turnover process of pathological adipose tissue hyperplasia. Lipoma showed enhanced adipogenesis without any signs of ischemia, while lipedema and obesity adipose tissues exhibited degenerative changes presumably induced by ischemia, which may be due to enhanced adipogenesis and non-enhanced angiogenesis in these tissues.

Fig 1

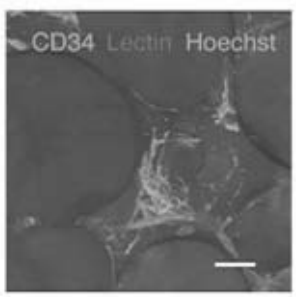


Fig 2

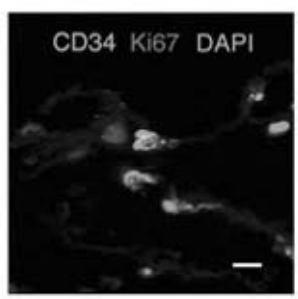
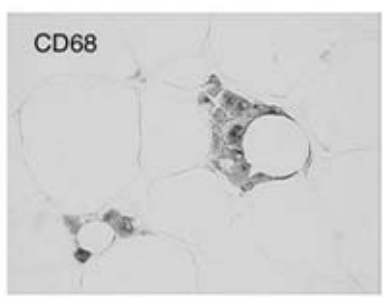


Fig 3



ASC AND DESEASES

XI -4] Tumor-derived exosomes can induce differentiation of mesenchymal stem cells into tumor-associated myofibroblastic cells

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Exosomes are small membrane vesicles secreted from various types of cells including tumor cells and enriched with a discrete set of cellular proteins, thereby expected to exert diverse biological functions according to cell origin. Mesenchymal stem cells possess the potential for differentiation into multilineages and play a role as precursors for tumor stroma including myofibroblast that provides a favorable environment for tumor progression.

In this study, we investigated whether and how tumor cell-derived exosomes can influence on MSCs by treating adipose tissue-derived MSCs (ADSCs) with ovarian cancer-derived exosomes and then analyzing the results of the treatment that occurred in the cells.

The exosome-treated ADSCs showed the phenotype of tumor-associated myofibroblasts including the increased expressions of α-SMA. Furthermore, exosome treatment on ADSCs also induced increased expression of an angiogenic factor SDF-1 in a dose-dependent way. This phenomenon was correlated with increased expressions of TGFβ receptor I and II. Analysis of SMAD2, an important player of TGFβ, receptor-mediated pathway, showed that its phosphorylation was increased in a time and dose-dependent way. Taken together, tumor-derived exosomes induced the myofibroblastic phenotype and functionality in ADSCs by activating TGFβ, receptor-dependent pathway.

In conclusion, this study suggests for the first time that tumor-derived exosomes contribute to progression and maglinancy of tumor cells possibly by converting MSCs into tumor-associated myofibroblastic cells.

Poster Abstracts

Poster Abstracts

P-01

Mature adipocyte-derived dedifferentiated fat cells can transdifferentiate into skeletal myocytes *in vitro*

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We have previously reported the establishment of preadipocyte cell lines, termed dedifferentiated fat (DFAT) cells, from mature adipocytes of various animals. DFAT cells possess long-term viability and can redifferentiate into adipocytes both *in vivo* and *in vitro*. Furthermore, DFAT cells can transdifferentiate into osteoblasts and chondrocytes under appropriate culture conditions. However, it is unclear whether DFAT cells are capable of transdifferentiating into skeletal myocytes, which is common in the mesodermal lineage. Here, we show that DFAT cells can be induced to transdifferentiate into skeletal myocytes *in vitro*. Myogenic induction of DFAT cells resulted in the expression of MyoD and myogenin, followed by cell fusion and formation of multinucleated cells expressing sarcomeric myosin heavy chain. These results indicate that DFAT cells derived from mature adipocytes can transdifferentiate into skeletal myocytes *in vitro*.

P-02

The role of calcium channel in diffentiation of osteogenic and neurogenic in adipose derived stem cells

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Introduction

Recently, there are many studies about stem cells to reconstruct bone defect because of congenital anomaly and trauma. Many reports are published that adipose derived stem cell differentiates to osteoblast. But the cell signaling system in this process is unknown. It is reported that calcium signaling is very important phase in this process. In this study, we study the process which adipose derived stem cell differentiates to osteoblast using calcium signaling which influences metabolism, differentiation.

Methods

After obtaining approval from the Human Subject Protection Committee, human liposuction aspirates were obtained from 3 elective surgery patients. Human ASCs were then obtained from these human aspirates and cultured by using the modified method as previously described. To briefly explain this procedure, these lipoaspirates were processed through a series of 3 sterile phosphate buffered saline washes, then centrifuged at 2000 RPM for 10 minutes, thus separating microlipid from the oil and PBS layers. The separated micolipid was washed once more with PBS, then digested with 0.05% collagenase type I in a 37 ℃ water bath shaker for 1 hour to release the cellular fraction. The collagenase type I was then neutralized with an equal volume of DMEM supplemented with 10% fetal bovine serum and 1% antibiotics. The solution was aliquoted into 50 ml tubes and centrifuged at 2000 RPM for 10 minutes. The supernatant was then discarded, and the resultant cellular pellet fraction containing processed lipoaspirate cells was resuspended in DMEM supplemented with 10% fetal bovine and penicillin-streptomycinglutamine mixture, filtered through an 100㎛ mesh filter and plated in a 175 cm2 cell culture flask. We regulated the condition of the culture medium to induce the osteogenic differentiation of ASCs. ASCs were washed with PBS, and cultured with the DMEM containing high glucose supplemented with 10% fetal bovine serum mixed with 0.01uM dehydroxy vitamin D3 , 1uM dexamethasone, 50uM ascorbic acidphosphate, 10mM beta-glycerol phosphate, 1% antibiotics/antimycotic for 3 weeks. After osteogenic differentiation, from 0 week to 3 weeks later, week after week, we measured TRPVs channel using by calcium imaging test and immunocytochemistry.

Result & conclusion

As undifferentiated adipose derived stem cell differentiates to osteoblast, TRPV2 channel which is manifested on erythroblast decreases in 1 weeks. In 2,3 weeks after differentiation, TRPA1, TRPV3, TRPV4 channel increases based on immunocytochemistry, calcium imaging test. The study will be meaningful whether the agonist of calcium channel increases the bone reproduction in bone defect animal model or not. In this study, we will find some kinds of agonists which increases channel could promote the differentiation of osteoblast. It will be important about reproduction of bone

P-03

Comparison of osteogenesis ability *in vitro* between adipose-derived stem cells and progenitor cells isolated from bone-related tissues

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Introduction

Recent studies have tried mesenchymal stem cells (MSCs) to use cell therapy in bone engineering. Progenitor cells from bone tissues are easy to use in clinical application but hard to obtain the cell. In this study, osteogenic ability was compared MSC in abdomen fat tissue (AD-MSC) with ability of cells from human periosteum PSMSC) and mandibular bone (MB-MSC).

Method

MSCs were isolated tissues using collagenase following well-known method. All MSCs cultured in DMEM containing fetal bovine serum (FBS) without discrimination. Gene expression was checked by RT-PCR and immunocytochemistry. Forth passage of cells had got down to osteogenesis induction, the results were analyzed by Von Kossa and Alizarin Red stain. To determine the effect in the potency after cryopreservation, same experiments for *ex vivo* expansion and osteogenesis of AD-, PS-, and MB-MSC were carried out.

Result

The AD-MSC, PS-MSC, and MB-MSC showed stable growth pattern like other stem cells. The proliferation rate of AD-MSC was similar to value of PS-MSC and MB-MSC *in vitro*. However the cumulative culture period of PS-MSC and AD-MSC was longer than MB-MSC. The MSC markers like as Desmin and Thy-1 were consistently expressed in all experimental groups, while an expression of Oct4, SCF, SSEA-4, and TRA-1-60 was not different from the results of AD- and PS-MSC. Expression of HLA class I was determined in AD- and PS-MSC, HLA class II was expressed in PS- and MB-MSC. Osteogenic differentiation didn't differ between the groups. To elucidate the change after cryopreservation, the potency of proliferation and differentiation were checked. As a results, the survival or recovery rate of AD-MSC was the better than the others after thawing in same cryo- or store condition. A change in the proliferation rate after cryopreservation has only observed in the experiment group of MB-MSC.

Conclusion

The osteogenic potential of AD-MSC was similar to those of PS- or MB-MSC regardless of tissue origin. However, the relative viability and proliferation rate of AD-MSC after cryopreservation during 1 year was higher than those of PS- or MB-MSC after thawing. These data showed that AD-MSC could be a good candidate for cell therapy in bone regeneration.

P-04

The brown adipose tissue has cardiogenic, myogenic and adipogenic progenitor cells

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Introduction

Adipose tissues are divided by two types of fat cells; brown adipose tissue (BAT) and white adipose tissue (WAT). WAT are specialized for the storage of chemical energy such as triglycerides, whereas BAT burns triglycerides for thermogenesis. Their similarities between WAT and BAT in cell morphology, lipid metabolism and patterns of gene expression were suggested by most investigators to assume that they share a common developmental origin. Culture of stromal vascular fraction (SVF) from WAT was demonstrated as multipotent stem cells which has the ability to differentiate into several mesodermal lineages including bone, muscle, cartilage, fat and neural progenitors and that cells were named by adipose-derived stem cells (ASCs) by the International Fat Applied Technology Society (IFATs). However, studies about SVF culture from BAT associated with characterization or multipotent potentials were very few. Therefore, we tried to exam of multipotent potentials for SVF from BAT.

Methods

BAT was isolated from interscapular region of postnatal day 1 (P1) to P2 neonates of C57BL/6 mice. Isolated BAT was washed with PBS and was digested with collagenase type Ⅰ at 37℃ for 30min. Isolated SVF cells were subcultured until passage 2 in low-glucose DMEM and then placed in the adipogenic, myogenic, cardiogenic, osteogenic and chondrogenic medium for 1~3weeks. Differentiation into mesenchymal cells were analysed by RT-PCR with each differentiation marker and some specific stains.

Results

BAT-ASCs were rapidly differentiated into adipocytes with numerous lipid vacuoles red stained by Oilred O, and PPAR-γ mRNA was also detected. Troponin Ⅰ mRNA was detected in the BAT-ASCs in cardiogenic medium and Myo D mRNA was detected in myogenic medium. However, BAT-ASCs in osteogenic and chodrogenic media were not showed into osteogenic and chodrogenic differentiation. Rex1, one of the stem cell-related transcription factors, was detected in BAT-ASCs.

Conclusion

There is hypothesis that multipotent cells are present in many connective tissue of the adult human for a promising pool of candidate cells for the engineered repair, regeneration of tissue and organ systems. Skeletal and cardiac progenitor cells have been identified in BAT and it is demonstrated that these cells were found in higher abundance than in WAT. According to our findings, BATs are one of the useful sources for a new strategy in cardiogenic and myogenic regeneration therapies. There are still controversies of treatment efficacy with WAT-ASCs in tissue regeneration. Therefore, identification of proper stem cell sources to each organ systems is important for increase of treatment efficacy.

P-05

BrdU labeling efficiency: toxicity, stability and effect on Adipose Stem Cells differentiation

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Introduction

5-bromo-2-deoxyurudine (BrdU) is a methodological tool for *in-vivo* investigation [1,2,3]. It has also been used for *in-vitro* prelabeling of transplanted cells in order to follow them in the recipient organism [1,2,3]. A long term stability of the incorporated BrdU is important for the recovery of BrdU labelled cells after the graft. The objective of this study is to test BrdU toxicity and stability over time and its effect on adipose stem cells (ASC) differentiation. For this purpose, we labelled ASC in passage 1 and then observed these cells by immunohistochemistry.

Materials and methods

Three pigs' cells on 1st passage were labelled with BrdU according the method [1]. Two concentrations (5 and 10 µm) and four contact times (2, 6, 24 and 48 hours) were tested. After the labelling, we evaluated i) BrdU toxicity by a MTT test, ii) stability by immunochemistry over 8 passages in monolayer culture and iii) effect on ASC differentiation into mature adipocytes by Oil Red O staining.

Results

Whatever the concentration and the contact time, BrdU is not cytotoxic. Viability is always superior to 85% in comparison with the negative control (medium only). Intensity and labelled cells number increase with the strongest concentration and the longest contact time. After 24 or 48 hours contact, 100% of cells are labelled, unlike 2 or 6 hours contact time. The labelling intensity decreases over time and totally disappears after 8 passages for each condition.

According the ASCs differentiation, BrdU doesn't affect the preadipocytes differentiation. There is no difference between the negative control and the labelled cells whatever the concentration and contact time used.

Conclusion

BrdU is a very good labelling method for investigate phenomena like adipose tissue engineering in order to differentiate donor cells versus recipient cells. Moreover, BrdU is not cytotoxic and can be detected after a long time.

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P-06

Effects of dexamethasone on *in vitro* expansion and *in vivo* bone formation of mesenchymal stem cells

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With extensive studies in the last decade, MSCs have been evaluated as one of the most promising tools for bone repair but culture-expanded MSCs may lose their stem cell characteristics and differentiated diversely or enter senescence.

The aim of this study was to determine optimal culture-expansion conditions for MSCs to allow bone defect repair. MSCs were supplemented with four different concentrations of dexamethasone: 0 M (Con), 0.2×10^{-8} M (D0.2), 1.0×10^{-8} M (D1.0), 5.0×10^{-8} M (D5.0), and analyzed every week up to 5 weeks (P5). Cells were analyzed using an Alkaline phosphatase assay, DNA quantification, Oil Red stain and flow cytometry for CD105 and CD90. Additionally, P3 and P5 cells were transplanted subcutaneously into nude mice after seeding in ceramic cubes. Proliferation of the cells was significantly higher in the D0.2 group. Alkaline phosphatase activities remained at low levels in the Con and D0.2 groups but increased to high levels in the D1.0 and D5.0 groups. CD105 expression at P5 was lower than at P1, P2, and P3. Adipocyte induced cells were highest in P3. *In vivo* bone formation of Con, D0.2, D1.0, and D5.0 with P3 and P5 cells as quantified by Cube Score were 0.00, 0.13, 0.79, 1.27 and 0.00, 0.29, 1.42, 1.75 respectively.

From these results, we concluded that supplementation of MSCs with low rather than physiological concentrations (2×10^{-9} M) of dexamethasone aids in the culture-expansion of these cells for osteogenic purpose by promoting cell proliferation without diverse differentiation and also enhances bone formation after *in vivo* transplantation.

P-07

Evaluation of reference genes for quantitative RT-PCR studies on adipose tissue-derived mesenchymal stem cells

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For the accurate determination of gene expression changes during growth and differentiation studies on adipose-derived stem cells (ASCs), quantitative real-time RT-PCR has become a method of choice. The technology is ery sensitive, however, without a proper selection of reference genes, to which the genes of interest are normalized, erroneous results may be obtained. In this study, we have compared the gene expression levels of a panel of twelve widely used reference genes during hypoxic culture, osteogenic and chondrogenic differentiation, and passaging of primary human ASCs. We found that the commonly used reference genes 18S rRNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin were unsuitablefor normalization in the conditions we tested, whereas tyrosine 3/tryptophan 5- monooxygenase activation protein (YWHAZ), cyclophilin A (PPIA), TATAA-box binding protein (TBP), beta-glucuronidase (GUSP), and were the most stable across all conditions. We recommend normalizing gene expression levels to the geometric mean af at least two of the above mentioned stable genes.

P-08

Human serum is suitable alternatives to fetal bovine serum for the expansion of adipose derived stem cells

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Introduction

Adipose derived stem cells (ASCs) are a promising cell source and are currently investigated for a variety of therapeutic applications. However, standard expansion protocols use fetal bovine serum (FBS) as growth factor supplement which is a potential source of undesired xenogeneic pathogens. For clinical safety, autologous human serum would be more appropriate. This study was to compare FBS supplemented and human serum (HS) supplemented condition for their enhancement of the proliferation potentials of human ASCs.

Materials and Methods

HS was obtained from 8 healthy volunteers with specially designed bags which could derive growth factors from platelets. Growth factors in HS or FBS were measured. ASCs were isolated with an established protocol from discarded human fat tissues obtained in an operation and cultured in DMEM supplemented with either 10% HS or 10% FBS. ASCs were collected at several time points for proliferation assay. The differences of the cell surface markers expression between HS and FBS group were determined with FACS analysis.

Results

Proliferation assay showed doubling time of ASCs in medium with HS was shorter than with FBS. The levels of platelet derived growth factor (PDGF) relatively correlated with the speed of cell proliferation. Cells expanded by this protocol met all the criteria for ASCs.

Discussion and Conclusion

It is demonstrated that ASCs expanded rapidly with human serum supplemented medium preserving their characters. This result might support establishing a safe and rapid expansion protocol with autologous serum for cell-based therapy using ASCs such as tissue engineering and regenerative medicine.

P-09

Differential proliferation and osteoblastic differentiation of mesenchymal stem cells isolated from bone marrow and adipose tissue

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Both human bone marrow derived mesenchymal stem cells (BMSCs) and human adipose tissue derived mesenchymal stem cells (ATSCs) are useful cell sources for cell therapy and tissue engineering. In this study we tried to determine and compare proliferation and osteoblastic differentiation of BMSCs and ATSCs isolated from the same patient. This may enable us to estimate which stem cells are more useful for higher bone generation. Both ATSCs and BMSCs expressed a similar level of MSC marker proteins at the cell surface such as CD29, CD44 and CD90. Upon osteogenic induction, both ATSCs and BMSCs accumulated a similar type of calcium phosphate when accessed by FTIR spectra. However, ATSC showed higher proliferation and enhanced osteoblastic differentiation compared to BMSCs in 6 cases. The enhanced osteoblastic differentiation of ATSCs was further confirmed by high expression of alkaline phosphatase (ALP), collagen type I (Col I), and Msx2 compared to BMSCs. We also investigated signal transduction pathways, leading to the enhanced osteoblast differentiation of ATSCs. Collectively, our data suggest that ATSCs is a useful cell source, compared to BMSCs, for regeneration of bone defects.

P-10

miR21 regulates adipose differentiation through the modulation of TGF-b signaling in mesenchymal stem cells derived from human adipose tissue

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A better understanding of the molecular mechanisms that govern human adipose tissue-derived mesenchymal stem cells (hASCs) differentiation could improve hASCs-based cell therapy and provide new insights into a number of diseases, including obesity. In this study, we examined the roles of microRNA-21 (miR-21) in adipogenic differentiation of hASCs. We found that miR-21 expression was transiently increased after induction of adipogenic differentiation, peaked at 3 days and returned to the baseline level 8 days. Lentiviral overexpression of miR-21 enhanced adipogenic differentiation. Overexpression of miR-21 decreased both protein and mRNA levels of *TGFBR2*. The expression of *TGFBR2* was decreased during adipogenic differentiation of hASCs in concordance with an increase in the level of miR-21. In contrast, inhibiting miR-21 with 2'-O-methyl-antisense RNA increased *TGFBR2* protein levels in hASCs, accompanied by decreased adipogenic differentiation. The activity of a luciferase construct containing the miR-21 target site from the *TGFBR2* 3'UTR was lower in LV-miR21-infected hASCs than in LV-miLacZ infected cells. TGF-β-induced inhibition of adipogenic differentiation was significantly decreased in miR-21 overexpressing cells compared with control lentivirus-transduced cells. RNA interference-mediated downregulation of SMAD3, but not of SMAD2, increased adipogenic differentiation. Overexpression and inhibition of miR-21 altered SMAD3 phosphorylation without affecting total levels of SMAD3 protein. Our data are the first to demonstrate that the role of miR-21 in the adipogenic differentiation of hASCs is mediated through the modulation of TGF-β signaling. This study improves our knowledge of the molecular mechanisms governing hASCs differentiation, which may underlie the development of obesity or other metabolic diseases.

P-11

Angiotensin II type 1 receptor blockade induces proliferation and differentiation of murine adipose stem cells by suppressing hox and activating peroxisome proliferator-activated receptor-gamma

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Introduction

Angiotensin II type 1 (AT1) receptor blockade induces adipocyte differentiation by increasing peroxisome proliferator-activated receptor-gamma (PPAR-γ) expression. Inflammatory cytokines and reactive oxygen species (ROS) inhibit the differentiation of pre-adipocytes into adipose cells, which, in turn, give rise to an insufficient absorption of triglycerides by adipose tissue from blood, causing several cardiovascular diseases. Hox genes encode transcription factors that are involved in stem cell maintenance and differentiation. The aim of this study was to investigate whether AT1 receptor blockade influences parameters which constitute self-renewal and/or differentiation of adipose stem cells (ASCs).

Methods

Abdominal fat from male C57BL/6 mouse was used for the isolation of ASCs. After expansion to three passages of ASCs, the cells were treated with losartan (0 to 100μM), an AT1 receptor blockade, for 1 hr. The expressions of inflammatory cytokines, antioxidant enzymes, PPAR-γ and some parameters related with cells proliferation and differentiation were investigated.

Results

Losartan decreased the expressions of Hox accompanied by an increase in PPAR-γ expression compared to those of negative control. IL-6 mRNA expression remained unchanged by addition of 1 μM losartant, strongly declining thereafter. MCP-1 expression was upregulated between 1 and 10 μM losartant but downregulated at 100 μM. Losartan steadily reduced the expression of peroxiredoxin (Prx) which is an H2O2 scavenger and existed in ASCs at a relatively high level, in a dose-dependent manner. In addition, proliferating cell nuclear antigen (PCNA) protein levels were also increased along with an increase in p53 and Fas mRNA expressions by losartan treatment.

Conclusion

Taken together, AT1 blockade may suppress Hox gene expression which might deprive ASC of its stemness and thereby drive ASC differentiation into adipocyte by PPAR-γ activation. In addition, AT1 blockade may modulate ASC self-renewal by activating PCNA as well as p53 and Fas expressions in response to IL-6, MCP-1 and/or H_2O_2. Therefore, angiotensin II might be involved in maintaining a balance between ASCs self-renewal and differentiation through regulating the expressions of transcription factors such as Hox and PPAR-γ and inflammatory cytokines and ROS production.

P-12

Features of L-NGFR$^+$ and CD34$^+$ subpopulations of human ASCs

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Human adipose-derived stem cells (hASCs) have been extensively characterized for their functional potential *in vitro*. However, the need for specific and selective mesenchymal stem cell markers is still a debated and unsolved issue. As previously reported, the low-affinity nerve growth factor receptor (L-NGFR - CD271) in adult bone marrow defines a subset of cells with high proliferative, clonogenic and multipotential differentiative ability. In this study we observed at early passages a high percentage of hASCs expressing on their cellular membrane either CD34 or L-NGFR; we investigated and compared positive-selected L-NGFR and CD34 subpopulations with the whole plastic-adherent (PA) hASCs. The phenotypic profile of freshly purified subpopulations shows an enrichment in stem cell markers expression compared to plastic-adherent (PA) hASCs: almost 50% of LNGFR$^+$ cells coexpresses CD34 and CD117, whereas the endothelial-committed progenitor markers KDR and P1H12 are mainly expressed on CD34$^+$ cells. The immuneselected populations proliferate and produce CFU-F as the well characterized PA hASCs; differences have been just observed in the long term period. Both L-NGFR$^+$ and CD34$^+$ cells are able to better differentiate into adipogenic, osteogenic and chondrogenic lineages compared with the unselected population as assessed by lipid drops formation, calcium deposition and glycosamminoglycans production. We conclude that immune-magnetic sorted L-NGFR$^+$ and CD34$^+$ hASCs are two homogeneous populations which may possess more suitable requirements for specific

applications in the field of the regenerative medicine. Furthermore we consider L-NGFR$^+$ hASCs progenitor cells at an earlier stage of cellular development, and it may represent a peculiar population of mesenchymal stem cells useful in tissue engineering.

P-13

The correlation between human adipose-derived stem cells differentiation and cell adhesion mechanism

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Introduction

Cell adhesion to the extracellular matrix (ECM) plays a fundamental role in regulating biological processes such as development, wound healing, organogenesis, and metastasis. Fibronectin is a multifunctional cell adhesive glycoprotein present in the ECM and plasma that is composed of a variety of binding domains for different bioactive molecules such as heparin, collagen, fibrin, DNA, and various integrin receptors. Heparan sulfate proteoglycans are involved in cell adhesion via the heparin-binding region of Fn. Our study focused on the adhesive characteristics of primary cultured hASCs on various substrates to determine uses for hASC cell sources in stem cell therapies or tissue engineering applications. Here, we sought to demonstrate the mechanism of hASC adhesion to Fn and to regulate cell function through the control of the adhesion mechanism by an art-ECM.

Methods

Human subcutaneous adipose tissue samples were obtained from the abdomen of 7 different female donors. A heparin binding domain (HBD) sequence (5081~5893bp) was determined from human fibronectin cDNA. The DNA fragment was cloned into plasmid pMAL to generate MBP-HBD. The recombinant MBP-HBD was dissolved in TES buffer, before coating the polystyrene (PS) plates. After 4 h at 37°C, non-adsorbed proteins solutions were removed from the plates that were then washed with PBS three times.

Results

We sought to determine the adhesive properties of human adipose-derived stem cells (hASCs) for extracellular matrix proteins. Cell adhesion on fibronectin (Fn) was inhibited by the heparin-binding peptide (HBP) in the presence of Mab 2253, but not by either Mab 2253 or HBP alone. These results indicate that both the b1 subunit and the heparin sulfate proteoglycan participated in the cell adhesion to Fn. Microscopic views showed extensive spreading of hASCs cultured on Fn, whereas the cells maintained a round shape when cultured on a heparin-binding domain (HBD) substrate. hASCs differentiated into adipocytes, which stained positive for lipid vacuoles by Oil Red-O analysis, more readily on HBD substrate than on FN substrate.

Conclusions

Our study suggests that hASCs have an adhesion mechanism for the HBD of Fn and hASC morphology is controlled by the adhesion mechanism and strongly correlated with adipogenic differentiation.

P-14

Derivation of mouse embryonic stem cells from C57BL/6J mice

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Although most mouse embryonic stem (ES) cells were derived from blastocysts of 129/sv strain, it has been known that this strain has many problems including spontaneous teratoma formation and behavioral and immunological responses. Therefore, it is necessary to backcross gene-manipulated mice derived from this ES cell with inbred C57BL/6 strain to overcome these problems. In this study, we successfully derived mouse ES cells from delayed blastocysts after ovariectomy and injection of progesterone. Total 15 different mouse ES cells were derived from 70 female C57BL/6 mice. Characterization of ES cells was performed by analysis of the expression of ES cells markers and teratoma formation after injection of mES cells into nude mice. The CJ42-1 ES cells, one of derived ES cells, showed high expression of Oct-4, Nanog, Rex1, alkaline phosphatase activity and SSEA-1 without expression of differentiated markers such as alpha fetoprotein, BMP4 and nestin. We also identified various tissues derived from all three germline layers in excised teratoma by injection of ES cells. We succeeded in making chimera mice by injection of mES cells into blastocystes. Collectively, derivation of ES cells from C57BL/6 mice was successfully performed by production of delayed blastocysts.

P-15

Clinical trials of adipose derived Mesenchymal Stem Cells for Melamine associated Canine Chronic Renal Failure

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Introduction

The kidney plays critical role in our body: secreting hormones such as erythropoietin and renin, eliminating many toxic metabolites from the body, controlling electrolyte balance, serum pH, and osmotic pressure. However, we can only offer supportive treatments, since there is no successful treatment for renal failure. We aimed to study the therapeutic ability of allogenic adipose derived mesenchymal stem cells (ASCs) for chronic renal failure in canines.

Methods

ASCs were isolated from abdominal adipose tissue of a healthy canine and cultured in 37℃ CO_2 incubator by passage 3 before intravenous injection. An eight-year-old female Golden Retriever and an eight-year-old neutered male Maltese, both with chronic renal failure, which was induced by a melamine contaminated diet (diagnosed in 2004), were injected with allogenic ASCs 2008 and 2009. We performed blood biochemical analysis, radiologic evaluation, and urine microalbumine test to evaluate renal functions.

Results

The eight-year-old female Golden Retriever and an eight-year-old neutered male Maltese showed 32.1mg/dl and 30.3 mg/dl of BUN, and 1.3mg/dl and 1.4mg/dl of Creatinine in the blood from biochemical analysis, but improved to 13.81mg/dl and 15.2 mg/dl of BUN, 0.9mg/dl and 1.2 mg/dl of Creatinine. Both of them represented mild reduced opacity in utrasonography and the Golden Retriever showed a change in urine microalbumine test from positive (+) to negative (-).

Conclusion

ASCs can improve the clinical condition of dogs with chronic renal failure without any adverse affects associated with the injection of ASCs.

P-16

Production in serum-free medium of human tissue-engineered multilayered conjunctive and adipose tissues

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As tissue-engineered constructs will increasingly be implemented into the clinical realm, the use of serum-free and animal-derived free products will be imperative. Here we describe the production of tissue-engineered stroma (conjunctive tissues) as well as reconstructed adipose tissues using a defined animal-free protein medium, in comparison to standard culture conditions involving 10% fetal calf serum and DMEM-F12-based media. The cell sheet self-assembly approach of tissue engineering was successfully used in conjunction with human adipose-derived stromal/stem cells (ASCs). This method is traditionally based on ascorbic acid and serum stimulation of cells to enhance their secretion and organization of endogenous extracellular matrix components. The use of serum-free media for the production of multilayered conjunctive tissues generated stroma that were easily manipulated and similar in thickness to tissues cultured under standard conditions. However, contraction of the tissues was reduced almost three fold when using serum-free medium. When the self-assembly approach with concomitant adipogenic conditions was used for the production of reconstructed adipose tissues, the use of serum-free medium impacted noticibly on the tissues. At the histological level, these reconstructed adipose tissues were 1.8 fold thicker than those cultured under standard conditions, and featured smaller adipocytes embedded into the matrix. These preliminary results are very promising for the development of « clinical grade » tissue-engineered soft tissues from human ASCs. Importantly, these substitutes can not only be produced in absence of animal-derived products, but they are also devoid of exogenous biomaterials since the scaffolding is generated by the matrix-producing human cells themselves. Profiling of the secreted products as well as matrix component expression will be investigated in order to characterize further these new tissues. *(Supported by NSERC and CIHR)*

P-17

Clinical trials with adipose-derived stem cells in two canine osteoarthritis cases

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Introduction

Osteoarthritis is a common disease caused by various factors in dogs. It is painful and can be a serious threat to the quality of life for the dog. Most pharmacological agents and surgical techniques have been used for the treatment of osteoarthritis like in humans, and alternative therapies, nutraceutical agents and physical therapies also have been tried as an assistant treatment of osteoarthritis. However, there are complications by pharmacological agents or sometimes low efficacy of treatment. Adipose-derived stem cells (ASCs) have the ability to self-renew and differentiate into many tissue types. There are enthusiastic efforts to utilize stem cells orthopedic tissue regeneration in recent stem cell research. We tried to clinical trial with autologuous or allogeneic ASCs in the treatment for osteoarthritis of two dogs.

Methods

A-2-year-old female Chowchow presented to veterinary clinic for lameness of a hind right limb with cranial cruciate ligament (CrCL) rupture by accident. Infra-patellar fat pad was collected from joint capsule during surgery of CrCL reconstruction, and then ASCs were isolated via enzyme digestion and cultured in 37℃, 5% CO_2 incubator. 2-5 × 10^7 of ASCs were injected two times into damaged joint capsule for 3 months without any other medical treatment. A-6-year-old female Maltese presented to veterinary clinic for rheumatoid like polyarthritis. Clinical signs was shifting lameness progressed from hind to fore limbs and then that dog became not to walk or move by severe angular deformation. The dog was received 5-10 × 10^6 of allogeneic ASCs, derived from healthy donor's abdominal fat, once a month for 5 months without any other medical treatment. Those dogs were evaluated according to check list by veterinarian and owner, and examined changes of cytokines associated with osteoarthritis including TNF-α and IL-6.

Results

No adverse events associated with injection of the autologuous or allogeneic ASCs in PBS were identified. Two dogs showed moderate improvement in all parameters measured by both owner and veterinarian. Chowchow dog showed that decrease of TNF-α and IL-6 in both synovial fluid and plasma without medical treatment. Surprisingly, the Maltese dog showed increase of mobility of all joint of the
body, decrease of the pain and then can crawl to owner in short distance. Owners were pleased with the outcome and would have the procedure done again.

Conclusion

This clinical trial showed the safe and effective use of autologuous and allogeneic ASCs for relieve of osteoarthritis. Therapy using the stem cell has been researched in human plastic and reconstructive surgery, however, clinical application of stem cells in human still has potential problems unsolved yet in various aspects. These clinical trials of stem cells in veterinary patients can be used useful informative data of cell therapy for the human beings as well as a new treatment method for the pets.

P-18

Soft tissue augmentation using *in vitro* differentiated adipocytes - A clinical pilot study

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Introduction

The demand for effective and durable soft tissue fillers to correct facial wrinkles or to augment soft tissues has grown dramatically. Although various commercially available filler materials are now commonly used, their varing degrees of resorption require repeated percutaneous injections. To overcome these drawbacks, a new injectable *in vitro* differentiated adipocyte bioimplant has been developed and previous animal studies have demonstrated that implantation of the bioimplant successfully enhanced *in vivo* adipose tissue formation. This study was undertaken to evaluate the clinical efficacy of this method, particularly for soft tissue augmentation cases.

Methods

Patients' own adipose derived stromal cells were obtained by liposuction, isolated, cultured, and differentiated to adipocytes *in vitro*. Immediately differentiated immature adipocytes were suspended in Dulbecco's Modified Eagle Medium. Eight patients were treated with this injectable bioimplant for soft tissue augmentation. A long-term follow-up for more than 1 year was possible. The contour of treated area, the degree and time of volume decrement or increment, the occurrence of complications, and patients' satisfaction were investigated.

Results

Two or three weeks after injection, volume increment could be observed around the injection area to the following 1-5 weeks. Thereafter, the additional volume augmentation was not shown and the augmentation effect was well maintained without volume resorption. All patients agreed on clinical effectiveness of the procedure and no complication occurred.

Conclusions

The results obtained indicate that this method is well tolerated and may have a potential to be an effective means of performing soft tissue augmentation.

P-19

The effect of adipose tissue-derived stem cells and platelet-rich plasma on fat injection : an animal experiment using nude mice

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Introduction

This study aimed to evaluate the effects of human adipose-derived stem cells (hASCs) and human platelet-rich plasma (hPRP) on routine fat grafting as Coleman technique.

Methods

Human ASCs were isolated from liposuction tissues of healthy patients. Human PRP was obtained by traditional two-step centrifugation. Fat injection was done into subcutaneous tissue on the back of nude mice as Coleman technique (group I, n=6), cell-enriched fat injection with hASCs (group II, n=6), fat injection supplied with hPRP (group III, n=6) and cell-enriched fat injection with hASCs and hPRP (group IV, n=6).

After 10weeks, newly formed tissue were evaluated by biochemical measurement of an adipogenic differentiation marker (glycerol-3-phosphate dehydrogenase, G-3-PDH), Macroscopical histology and immunoflorescence.

Results

This studies showed adipose tissue formation in heterotopic site of nude mice. Macroscopically, after 10 weeks in vivo, layers of adipose tissue accompanied by new vessels were found on group II and IV. The control grafts (group I) and specimen from group III showed minimal fat tissue survival with large oil cysts under a capsule. Vessel ingrowth was also rarely seen in group I and group III. In group II and group IV, specimens showed adipose tissue and a rich vascularization adherent to the fatty mass under a capsule.

In microscopic examination, the control grafts in group I showed capsule and minimal fat tissue with few cells. Tissue from group II and IV showed complete architecture of fat tissue with abundant vascular tissue.

Immunofluorescent analysis for BS-Ⅰ lectin and DiL labeled cells showed definite incorporation of fluorescence-labeled human adipose stem cells into the new vessels.

Analysis of GPDH revealed nine-fold higher activities of GPDH in cells of group IV as compared to other groups.

Conclusions

These findings demonstrate that the positive synergy effect of hASCs and hPRP on fat graft survival in animal experiment. According to this result, it may be necessary to modify routine fat injection procedure by addition of hASCs and hPRP for better graft fat survival. Further investigation may be necessary.

P-20

The role of chemokines in proangiogenic action induced by human adipose tissue-derived mesenchymal stem cells in the murine model of hindlimb ischemia

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Proangiogenic action of human adipose tissue-derived mesenchymal stem cells (hASCs) transplantation has been shown to be mediated by secretory factors. In this study, we determined whether human granulocyte chemotactic protein-2(GCP2) or monocyte chemoattractant protein- 1(MCP1) is involved in the proangiogenic action of hASCs transplantation in the hindlimb ischemia model. hASCs secrete GCP2 and MCP1, of which addition increased tubule formation of hASCs. The downregulation and external addition of GCP2 or MCP1 showed the opposite effects on MCP1 and GCP2 secretion, respectively. GCP2 and MCP1 affects on VEGF secretion. Downregulation of GCP2 and MCP1 expression inhibited hASCs-induced proangiogenic action, and the overexpression of GCP2 increased it. The downregulation of MCP1 or VEGF inhibited GCP2 overexpression-induced increase of blood flow recovery. These data indicate that the proangiogenic action of hASCs transplantation is mediated by the interaction among GCP2, MCP1 and VEGF which are secreted from the transplanted cells.

P-21

Locally-delivered FGF2 enhances the angiogenic efficacy of adiposederived stromal cells transplanted to ischemic limbs

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Introduction

Stem cell therapy offers significant therapeutic promise for ischemic diseases, but poor survival following transplantation to ischemic tissue limits its efficacy. Here we demonstrate that polymer nanosphere-mediated growth factor delivery can enhance the survival of transplanted human adipose-derived stromal cells (hADSCs) and secretion of human angiogenic growth factors per cell, and substantially improve therapeutic efficacy of hADSCs.

Methods

hADSCs were cultured in normoxic conditions (20% O_2), hypoxic (1% O_2) conditions, or hypoxic conditions with daily addition of 50 ng/ml FGF2. Heparin-conjugated PLGA nanospheres (HCPNs) were used to deliver FGF2 locally. One day after femoral arterial dissection, the mice were divided randomly into four groups and treated with either fibrin gel plus HCPNs, fibrin gel plus HCPNs loaded with 25 μg of fibroblast growth factor-2 (FGF2), hADSCs suspended in fibrin gel with HCPNs, or hADSCs suspended in fibrin gel with HCPNs loaded with 25 μg of FGF2 (n=10 per group).

Results

In vitro, in hypoxic (1% oxygen) and serum-deprived conditions which simulate *in vivo* ischemia, FGF2 significantly reduced hADSC apoptosis and enhanced angiogenic growth factor secretion. *In vivo*, hADSCs delivered intramuscularly into ischemic hindlimbs in combination with FGF2 resulted in significant improvements in limb survival and blood perfusion, as well as survival of the transplanted hADSCs and secretion of human angiogenic growth factors (i.e., vascular endothelial growth factor, hepatocyte growth factor, and FGF2). Interestingly, the majority of transplanted hADSCs were localized adjacent to the microvessels rather than being incorporated into them, suggesting that their major contribution to angiogenesis might be to increase paracrine secretion of angiogenic growth factors.

Conclusions

This study demonstrates the potential of hADSCs in combination with growth factors for use in the treatment of ischemia.

P-22

SDF-1alpha pre-conditioning of adipose-derived stromal cells decreases required dose during treatment for ischemic injury

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Intravenous (i.v.) delivery of human adipose-derived stem cells (hASCs) is a promising option for the treatment of ischemia. Following delivery, hASCs that reside in the injured extravascular space aid recovery of tissue perfusion and function, although low rates of incorporation into the target tissue and high rates of lung entrapment currently limit safety and efficacy. We submit that control of hASC trafficking through the microcirculation is possible by targeting adhesion molecules preferentially expressed on njured endothelium. We previously showed the ability of hASCs to firmly adhere VCAM-1 and that preconditioning hASCs with SDF-lalpha significantly enhanced this capability *in vitro*. Here, we investigated the ability of SDF-alpha pre-conditioned hASCs to restore blood flow *in vivo* during hindlimb ischemia. On day 0, femoral artery ligation was performed in nude mice. On day 1, a low- or high-dose of either SDF-lalpha preconditioned hASCs or non-conditioned hASCs were injected i.v. (low dose=100,000 hASCs; high dose=500,000 hASCs). Laser Doppler perfusion imaging was used to assess blood flow in the hindlimb (day 2, 4, 8; % of control limb). All high-dose injections (non-conditioned and pre-conditioned hASCs) restored perfusion to hindlimbs. Conversely, low-dose injections of non-conditioned hASCs failed to restore perfusion above controls (0.44 vs. 0.5), while low-dose injections of SDF-lalpha preconditioned hASCs completely restored perfusion (0.91 vs. 0.5). This represents a 5-fold decrease in required cell-dose. In additional studies, hASC incorporation into the pulmonary microcirculation (secondary capture) and the ischemic mouse spinotrapezius muscle was assessed following i.v. injection, which uniquely allowed assessment of their location in the extravascular space. Collectively, data suggest potential strategies to increase safety during therapy and offers clues to the ultimate fate of hASCs following i.v. injection.

P-23

Effect of adipose-derived stem cells on radiofrequency catheter ablated canine 3rd AV node block model

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Introduction

High degree of AV nodal disease evoked by dysfunction of conducting system or uncontrolled injury is irreversible and required an artificial permanent pacemaker for the life. The aim of this study was to investigate the effect of adipose-derived stem cells in canine catheter ablated AV node block model as an alternative potency to recovery conducting system.

Materials and Methods

Four male mongrel dogs weighing 28 to 35 kg were used for this study. Adipose tissue was derived from abdominal cavity of dogs undergoing elective operation. The cells were isolated, cultured and labeled with super paramagnetic iron oxides (SPIO, Feridex/PLL®) to track after transplantation. After preparation of cells, pacemaker implantation (VVIR, St Jude Co, Screw Lead) was performed under cardiac catheterization. At 1 week later, the ablation catheter was implemented via femoral vein guided and complete 3rd AV nodal ablation (1 pulse 40W, 60 ℃, and 60 sec) was performed through intracardiac mapping. Allogenic ASCs (5 x 10^5) were administrated with intravenous infusion 24hours after radiofrequency ablation. ECG and Pacemaker interrogation was taken and recorded during 4 weeks after cell application. SPIO-labeled ASCs were detected by Prussian blue stain and immunohistochemistry performed at the site of ablation and lead pacing.

Results

Continuous ECG and pacemaker interrogation showed success of nodal damage but no significant functional change of conducting system. Histologically, SPIO-labeled ASCs were stained blue color by Prussian blue staining and most of them were founded in ablation sites and especially leads pacing sites of the hearts. In immunohistochemistry, cardiogenic markers including troponin Ⅰ, connexin 43 and α-actinin were expressed in the cytoplasm of Prussian blue stained cells.

Conclusion

This study suggests that radiofrequency catheter ablation induced AV block guided Adipose stem cells can be delivered at ablation site and pacemaker lead site, speculated electrical stimulation. Further functional studies and may allow future implementation of pacemaker function adjacent to the native conduction system.

P-24

Differential induction for *in vitro* adipogenesis bioassay using human adipose-derived stem cells

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Introduction

Adipose-derived stem cells (ASCs) possess the advantage of multipotency, high proliferation rate and easiness to harvest. However, more intensive studies about effect of culture conditions, donor site or cryopreservation on differentiation ability of ASCs are required for clinical trials. Moreover, *in vitro* bioassay system has been developing a valuable application that can evaluate whether ASCs sustain multipotency or not is necessary for development of efficient methods for culture, differentiation or cryopreservation.

Methods

Human ASCs of 3rd passage were seeded grown to confluence and basal media exchanged to adipogenic medium. After 3 weeks, six wells plate was fixed and stained for oil red O. Conditioned media were collected every 3 days and Leptin concentration were measured by ELISA assay. Cells were detached at day 3, 6 and 21 followed by RT-PCR analysis. The experimental groups were divided into control group and cocktail 1 to 5 group. Each adipogenic cocktail was composed with different concentration of insulin, dexamethasone, isobutylmethylxanthine, and indomethacin.

Results

Accumulation of lipid droplets in cytoplasm was started at day 7~11 and different by adipogenic cocktail. Cocktail 1 group started lipid accumulation at day 7 and adipocytes maturation at day 14. After day 17, the number of mature adipocytes was slowly decreased. Cocktail 2 group observed a lipid accumulation at day 9 and have retarded adipocytes maturation. High concentration of insulin and indomehacin on cocktail 5 group induced lipid accumulation at day 7. Leptin concentration and the number of mature adipocyte were suddenly increased to day 14, but sharply decreased as from day 17. Cocktail 4 group had started lipid accumulation at day 9 followed by adipocytes maturation at day 11. Although cocktail 5 group lost mature adipocytes after day 17, cocktail 4 group sustained mature adipocytes until 3 weeks.

Conclusions

We identified the differences in differentiation rate by adipogenic cocktails using marker expression, histological study and biochemical assay. Protein and gene expression profiling from the present approach would be valuable to setting of bioassay system by differentiation condition and purpose.

P-25

The effect of PLA cell on the viability of injected adipose tissue in nude mice

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Introduction

Autotransplantation of human adipose tissue is a widely accepted technique to correct various soft tissue defects.However, plastic surgeons are faced with uncertainty concerning the ultimate outcome because it has limitation of partial absorption. Using Human processed lipoaspirate cell, multipotent stem cell derived human adipose tissue, we studied the effects of PLA (Processed LipoAspilate) cells on fat grafts enhancing angiongenesis and volume maintenance.

Methods

There were 5 athymic nude mice in each group: a study group and a control group. In the study group, PLA cell (5X105per 1ml) was injected into the subcutaneous layer in the back of the mice 24 hours earlier than the fat graft. In the control group, 1ml of normal saline was injected into the subcutaneous layer in the back of the mice 24 hours earlier than the fat graft. After 24hours, one milliliter of fat tissue aspirated from the human abdomen, was injected into each mouse in both groups.

After 16 weeks, the weight and the volume of grafted fat were measured. And histologic examination was performed. Micro vessels were counted and analyzed. The results were as following.

Results

The weight of grafted adipose tissue is 0.4697 ± 0.00231g (study group), 0.4522 ± 0.00676g (control group). The volume of it is 0.9170 ± 0.02312ml (study group), 0.8930 ± 0.01636ml (control group). On histologic examination of grafted adipose tissue, normal nucleus fat cell are 2.31 ± 0.28460 (study group), 2.14 ± 0.15055 (control group). And Cyst and vacuole are 2.09 ± 0.13703 (study group), 1.58 ± 0.13166 (control group). The point of Inflammatory cells is 2.15 ± 0.19579 (study group), 2.34 ± 0.12649 (control group).

First, with regard to the weight and the volume of grafted fat, no significant difference was found between two groups. Second, it was found for micro vessels to be increased more significantly in the study group than in the control group through histologic analysis. Last, regarding the other histologic parameters, no significant difference was investigated.

Conclusions

Although PLA cells enhanced angiogenesis of fat graft, other various factors such as the volume and the weight as well as histologic parameters remained constant. Therefore, further studies to understand fully how grafted fat survives in the body should be conducted.

P-26

Viability and proliferation of human adipose-derived stem cells cultured and cryopreserved as 3-dimensional multicellular aggregates

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Intro

Human adipose-derived stem cells (ASCs) have great potential for multiple tissue repair challenges. ASCs formulated as multicellular aggregates (MAs) may represent a useful approach to the translation of stem cell therapies. The ability to effectively cryopreserve cells prior to use provides significant advantages, and facilitates logistics of repeated dosing, product storage and shipping. The aim of the present study was to determine the effect of different cryopreservation strategies on the viability, adherence, and proliferation capabilities of ASCs isolated from fresh human adipose tissue and formulated as 3-D self-assembling spheres.

Methods

Multicellular aggregates (MAs) of ASCs were frozen in liquid nitrogen for a period of seven days using 8% dimethyl sulfoxide (DMSO), 30% glycerine, 1% pluronic F-68, and Bambanker^{TM} serum-free type cell freezing medium as cryoprotectants. A fifth set of MAs was frozen in standard cell culture media with serum (DMEM10) for the same period of time. A water soluble tetrazolium salt (WST) assay was used to determine the viability of cells following cryopreservation and thaw. In addition, a cell attachment experiment was conducted to determine the adherence and proliferation abilities of MAs following cryopreservation.

Results

Results show that glycerine (75.8% average viability rate) and DMSO (64.0% AVR) act as the most consistent and effective cryoprotectants, while pluronic F-68 (58.4% AVR) and BambankerTM cell freezing medium (57.6% AVR) are not quite as reliable. DMEM10 (30.7% AVR), acting as a negative control, did not effectively preserve cells.

Conclusion

These results suggest that a majority of human ASCs assembled and frozen as 3-dimensional multicellular aggregates can survive the freezing and thawing process, depending on the cryopreservation solution used. Further studies will aim to optimize cell survival as well as further characterize cell function *in vitro* and *in vivo* after thawing.

P-27

Role of reactive oxygen species in adipogenesis of adipose-derived stem cells

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Introduction

Currently, stem cells have received significant attention in scientific and clinical fields. Human adipose tissues have been demonstrated as a good source of stem cells. Many plastic surgeons have researched in autologous tissues (fat, cartilage, bone, etc) that differentiated from adipose-derived stem cells (ADSCs) for clinical trials. There are many attempts to find factors that promote the differentiation of ADSCs, but few correlation studies of ROS (reactive oxygen species) and adipogenesis of ADSCs have been reported. The purpose of this study is to investigate the effect of reactive oxygen species (ROS) on adipogenesis of ADSCs.

Methods

ADSCs were isolated and cultured from the human abdominal adipose tissue. ADSCs (3X10000 cells/well) were cultured in different culture mediums as follows; 1) culture medium (DMEM) as control, 2) adipogenic culture medium 3) adipogenic culture medium with H_2O_2 (Hydrogen peroxide 20 uM / 50 uM) 4) adipogenic culture medium with H2O2 (Hydrogen peroxide 20 uM / 50 uM) and anti-oxidant (Deferoxamine 10 uM / 20 uM). During 20 days, we compared the adipogenesis of these groups by absorbance measurement with Oil red O staining per 5 days.

Results

During 15 days, there were no significant differences. On 20^{th} day, there were significant differences between groups. Absorbance results in adipogenic culture medium with H_2O_2 (20 uM / 50 uM) groups were significantly higher than adipogenic culture medium alone group. And it showed H_2O_2 dose-dependent increase that absorbance results in adipogenic culture medium with 50 uM H_2O_2 group were significantly higher than adipogenic culture medium with 20 uM H_2O_2 group. When anti-oxidant (Deferoxamine 10 uM / 20 uM) were added in adipogenic culture medium with H_2O_2 (20 uM / 50 uM)groups, absorbance results were significantly decreased. But this inhibitory effect of anti-oxidant showed no obvious dose relativity.

Conclusions

Our study suggests that ROS can enhance the adipogenesis of ADSCs. Based on this result, we can extend to further researches that are closely related to clinic such as correlation studies between ROS and obesity or aging.

P-28

Chondrogenic differentiation of human adipose tissue-derived stem cells in micropellet cultures: The effect of oxygen concentration and donor variability

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The impact of available oxygen was investigated to identify optimal conditions for human adipose tissue-derive stem cells (ASCs) chondrogenesis *in vitro*. Four physiologically relevant oxygen concentrations, including 15%, 10%, 5%, and 1%, were compared to ambient air condition, and the ASCs originating from six unrelated donors were subjected to chondrogenic induction in high-density pellet cultures. The qualitative and quantitative assessment of accumulated extracellular matrix and the gene expression analysis revealed marked inter-individual differences. The chondrogenic process was most optimally supported at ambient or 15% oxygen concentrations irrespective of the origin of cells. The histochemical analysis based on alcian blue staining demonstrated that the differentiation took place in a gradient-like fashion, displaying highest levels in regions most often adjacent to the periphery. The two lowest hypoxic conditions at 5% and 1% oxygen seemed to have an inhibitory effect. In summary, the micropellet cultures at ambient or 15% oxygen concentration provided environment most suitable to induce chondrogenesis in ASCs. Furthermore, in light of the fact that the induction appeared in a zone-dependent manner, this format lends itself as a suitable model for further analysis of the relationship between chondrogenic differentiation and the gradient of nutrients.

P-29

Effects of collagen gel contraction with TGF-β,1 and PRP in embedded culture of adipose-derived stem cells or dermal fibroblasts.

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Introduction

The wound-healing process consists of three 3 stages, i.e., inflammation, granulation, and reconstruction. It is effective for wound healing that myofibroblasts lead to wound contraction in the granulation stage.

Aim

Here collagen gel contraction in embedded cultures of adipose-derived stem cells (ASC) and dermal fibroblasts (DF) were examined with TGF-β,1 and platelet-rich plasma (PRP).

Methods

Bell et al. presented cell culture in a three-dimensional collagen matrix as an *in vitro* wound contraction model. Following this method, we produced a collagen gel embedded with ASC and DF, and evaluated gel contraction after adding TGF-β,1 and PRP to the culture supernatant. Moreover, cytochalasin D, an actin-polymerizing agent, was added to the contracted gel to investigate whether it is released.

Results & Discussion

After adding TGF-β,1 and PRP, into the collagen gel-embedded culture of ASC or DF, gel contraction of ASC was similar to that of DF. Cytochalasin D inhibited contraction by depolymerizing actin, suggesting that actin stress fiber formation is involved in cell contraction. In wound contraction in the wound-healing process, not only DF, but also ASC may play a role.

P-30

Osteogenic potential of adipose derived stem cells in porous polysaccharide-based scaffold for bone tissue engineering

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Introduction

The field of bone engineering is one prominent challenge in the regenerative medicine area. One efficient bone ubstitute could combine osteoprogenitor cells with a 3D matrix to generate bone formation. Recent studies suggest that adipose tissue contains mesenchymal stem cells (human Adipose Derived Stromal Cells: ADSCs) exhibiting the same properties of proliferation and differentiation into multi-lineages phenotypes like human mesenchymal stem cells from bone marrow (hBMSCs). There is a real need to extend these studies to investigate ADSCs behavior within 3D structures. To date, only some polysaccharide based scaffold with biocompatibility suitable for bone engineering are proposed for applications as carriers for transplanted cells to promote regeneration of bone tissue. The aim of this study was to investigate the possibility of using ADSCs as a source of osteoblasts precursors, associated with a new polysaccharide scaffold as a 3D construct to generate bone tissue.

Materials and methods

Porous polysaccharide-based scaffolds were prepared using a mixture of pullulan/dextran 75:25. Human ADSCs were isolated from adipose tissue of healthy patients. ADSCs were seeded on matrices in an inductive IMDM medium, supplemented with 10% SVF and 10-8 M dexamethasone. Human BMSCs are used as control for osteogenic differentiation. Cell behavior into the matrix was followed by time lapse videomicroscopy, and electron scanning microscopy. Constructs were harvested at different time of induction and osteoblastic gene expression or the corresponding protein production was analyzed by Q-PCR and immunostaining respectively. Mineralization was investigated by Von Kossa staining and cytochemical analysis of ALP (alkaline phosphatase) was performed.

Results

Microscopy analysis reveals that cells dispersed within the 3D structure migrate during the first hours of culture and form cell clusters. They are capable of proliferating during at least 15 days and express connexin 43, a gap junction protein known to regulate osteoblastic differentiation. Quantitative PCR and microarray analysis reveal that ADSCs cultured in 3D scaffold express osteoblastic markers as well as with BMSCs. These results were confirmed by analysis of protein and immunostaining. The cell clusters exhibited ALP activity and Von Kossa staining indicative of intense mineralization into the matrix after 15 days of culture.

Discussion

The present findings clearly show that these new macroporous polysaccharide-based scaffolds induce cell cluster formation of human ADSCs, and favor cell-to-cell contact. These porous matrices appear appropriate materials for ADSCs growth, proliferation and osteogenic differentiation. In addition, these matrices stimulate expression of osteoblastic markers. In conclusion, this approach appear as a promising strategy for promoting mineralization and bone regeneration once loaded with ADSCs.

Acknowledgements: This work is supported by Inserm, University Bordeaux 2, Universities Paris 7 and 13, "Délégation Générale de l'Armement" (DGA), and by a specific grant from the French National Research Agency (ANR-07-TecSan-011-01 ITOV).

P-31

Enhanced osteogenic differentiation from adipose-derived stem cells (ADSCs) on BMP-2 loaded photocurable hyaluronan hydrogel

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Objectives

Hyaluronic acid (HA) is a natural polysaccharide in biological tissues and it can be modified to prepare biomaterials. We prepared photocurable HA-hydrogel using aminoethyl methacrylate (AEMA) grafted HA and then loaded different concentrations of Bone Morphogenic Protein-2 (BMP-2) on photocurable hydrogel. It was observation that photocurable hydrogel in different concentration BMP-2 could cell proliferation and osteogenic differentiation of Adipose-derived stem cells (ADSCs) in vitro.

Materials & Methods

HA was dissolved in a MES buffer solution. EDC and NHS were added in the sample solution to activate the carboxylic acid groups of the HA and then AEMA was added to the solution. Methacrylate hyaluronic acid was dissolved in DI water and UV exposure for 5 min at 365nm UV light at x100 μJ/cm2. Different concentrations of BMP-2 (50 ng, 100 ng, and 500 ng per hydrogel disk) were contained photocurable hydrogel. Cell proliferation and osteogenic differentiation of ADSCs were evaluated by the cell viability, Alkaline phosphatase (ALP) activity, western blotting, RT-PCR, and calcium content on the surface culture of the hydrogel.

Results

Cytotoxicity test of HA-hydrogel was determined at 24 h and 48 h. The hydrogels did not affect cell growth because of low cytotoxicity. We observed that 100 ng and 500 ng of BMP-2 loaded HA-hydrogels were sustained release compared with 50 ng of BMP-2 in vitro. And then, ALP activity, western blotting, RT-PCR, and calcium content were measured at 1, 3, 7, and 14 days. We observed that BMP-2 causes a significant induction of ALP activity when differentiated osteogenic from ADSCs. The western blotting and RT-PCR showed that the osteocalcin and osteopontin were increased with increasing BMP-2 concentration. Also, increased BMP-2 concentration observed increasing calcium content. Osteogenic differentiation of ADSCs was upregulated in response to increase BMP-2 concentration.

Conclusion

Photocurable HA-hydrogel were successful carrier for sustained release of BMP-2 in vitro. There results demonstrated that BMP-2 was important osteogenic differentiation factor, and increased BMP-2 concentration promoted more osteogenic differentiation of ADSCs. In conclusion, BMP-2 loaded HA-hydrogel would be valuable for bone tissue regeneration.

P-32

Human adipose tissue-derived stem and regenerative cells for periodontal tissue regeneration

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Objectives

Periodontitis results in the loss of connective tissue and bone support and is a major cause of tooth loss in adults. The ultimate goal of periodontal therapy is to regenerate the periodontal tissues that are lost as a result of periodontitis. The key factors in attaining successful periodontal tissue regeneration are the correct recruitment of cells to the site and the production of a suitable extra cellular matrix consistent with the periodontal tissues. Adipose tissue contains a population of regenerative cells (termed Adipose -Derived Stem and Regenerative Cells [ADRCs]). We have previously shown that syngeneic, culture-expanded cells obtained from ADRCs (termed Adipose-Derived Stem Cells [ADSCs]) induce improved healing in a rat periodontal disease model (Tobita M, et al. Periodontal Tissue Regeneration with Adipose-Derived Stem Cells. Tissue Eng Part A 14: 945-53. 2008). In this study we evaluated human ADSCs and freshly isolated ADRCs in an immunodeficient rat injury model.

Methods

Human ADRCs and ADSCs were isolated from subcutaneous abdominal fat tissue as previously described. Four periodontal tissue defects were made in the upper molars of a nude rat with a dental round bar. ADRCs or ADSCs were implanted using Platelet-Rich Plasma (PRP) as a carrier (n=20 defects each). Defects with non-implant or PRP alone were used as controls (n=20 defects each). Six weeks after implantation histological analysis was performed to evaluate new bone formation and epithelial invasion.

Results

Histologic analysis revealed that the area of regenerated alveolar bone was significantly greater in ADRC-treated defects (ADRC vs. non-implanted; 0.25 $\pm$ SEM mm^2 vs. 0.129 $\pm$ SEM mm^2; $p<0.001$, ADRC vs. PRP only; 0.25 $\pm$ SEM mm^2 vs. 0.128 $\pm$ SEM mm^2; $p<0.001$). ADRC-treated defects also exhibited significantly reduced epithelial invasion (ADRC vs. non-implanted; 0.29 $\pm$ SEM mm vs. 0.74 $\pm$ SEM mm; $P<0.001$, ADRC vs. PRP only; 0.29 $\pm$ SEM mm vs. 0.43 $\pm$ SEM mm; $P<0.05$). Similar results were observed with ADSC (ADRC vs. ADSC, P=n.s.).

Conclusions

These findings suggest that human ADRCs could improve tissue regeneration in periodontal disease. This method is highly safe and the risk of infections is minimal compared to the risks associated with the allogenic transplantation methods used currently for clinical periodontal tissue regeneration therapy.

P-33

Successful lecithin-cholesterol acyltransferase (LCAT) supplementation in mice by transplantation of human lcat-gene transduced mouse pre-adipocytes.

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Objectives

Adipose tissue has been shown as an excellent source of proliferative cells for clinical treatment. We have been developing protein supplement therapy for inherited or acquired diseases by use of gene-transduced autologous pre-adipocytes. In this study, we have propagated human lcat-gene transduced pre-adipocytes from mouse subcutaneous fat tissue, and assessed the potential of the cells for lecithin-cholesterol acyltransferase (LCAT) protein supplementation. Also we examined the fibrin glue as scaffold for transplantation in comparison to Matrigel.

Methods

Subcutaneous fat from C57BL/6J mice was treated by collagenase and the pre-adipocytes were obtained by ceiling culture technique. After 7 days ceiling culture, the cells were harvested and seeded for subsequent regular culture. Human lcat-gene was transduced into pre-adipocytes by retrovirus vector mediated gene transduction. Transduced cells were transplanted subcutaneously into C57BL/6J or nude mice. The cells were stained by PKH26 prior to transplantation for easy identification by fluorescent microscope. LCAT protein in mouse serum was detected by immunoprecipitation followed by immunoblotting (IP-Western). Cell survival was examined by quantification of transduced lcat cDNA. CD antigen profiles of the cells were examined by FACS analysis.

Results

Propagated mouse pre-adipocytes were positive for CD90, and negative for CD31, CD34 and CD45. They were moderately positive for CD13 and CD105. They secreted human LCAT protein into culture medium. Using IP-Western procedure, LCAT protein was detected up to 1 month in transplanted mice sera. Fibrin glue worked as scaffold equally to Matrigel. Around 40% of the transduced lcat gene was retained at 1 month after transplantation in C57BL/6J mice.

Conclusions

The study using mouse model suggested that the application of autologous lcat-gene transduced pre-adipocytes is feasible for successful LCAT protein supplement therapy. These results shed a light in the application of pre-adipocytes for continuous protein supplementation in variety of inherited and acquired diseases including LCAT deficiency.

P-34

Improved fibrosis and granulation of VICRYL*mesh implantation through the use of adipose derived stem cells

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Background

VICRYL* mesh consists of polyglactin 910, a synthetic absorbable copolymer made from 90 % glycolide and 10% L-lactide. The empirical formula is $(C_2H_2O_2)m(C_3H_4O_2)n$. The mesh is knitted from uncoated and undyed fiber identical in composition to that used in VICRYL* sutures, which has been found to be inert, nonantigenic, nonpyrogenic and to elicit only a mild tissue reaction during absorption. It may be used wherever temporary wound or organ support is required, particularly in instances in which compliant and stretchable support is desired. Successful use is reported for instance as support for the correction of breast deformity. Because a putative potential stem cell population within the adipose tissue has been found to possess therapeutic potentials, we authors sought to determine the effect of adipose tissue-derived stem cells(ADSCs) on VICRYL* mesh implantation.

Methods

VICRYL*mesh was characterized by scanning electron microscopy(SEM). ADSCs of stem cell were isolated from the inguinal fat pads and expanded *ex vivo* for three passages. The morphology of ADSCs attached on VICRYL*mesh was studied using a SEM at 1, 3 and 7 day after *in vitro* culture. Cellular proliferation behavior of ADSCs cultivated on VICRYL*mesh was analyzed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-terazolium bromide(MTT) test at 1, 3 and 7 day after *in vitro* culture. Sprague-Dawley rats were divided into two groups one is control, given the surgery of simple VICRYL* mesh implantation and the other is stem cell group, given the surgery of mesh implantation and ADSCs. (N=12 for each group). Control group received multi-folded VICRYL*mesh implantation to the back of the rats and Stem cell group received the surgery of VICRYL*mesh implantation and ADSCs. After four weeks of operation, matrix formation, capillary density and collagen production were assessed quantatively under the light microscope.

Results

ADSCs led to a statistically significant increase in fibrosis and mature scar formation compared with the control group in histologic examination ($p < 0.05$).

Conclusions

ADSCs was found to be efficacious in promoting fibrosis and scar maturation leading volume replacement on experimental VICRYL* mesh implantation. This study might be used as useful volume replacement with VICRYL* mesh for correcting breast deformity after conservative surgery.

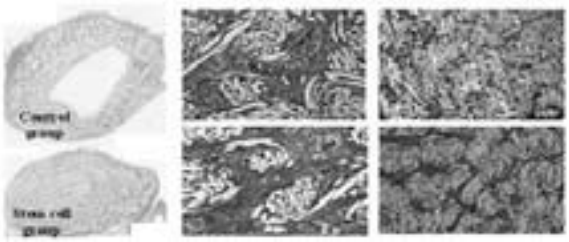

Fig. 1. Obvious differences in matrix formation were observed (H&E stain, X 40)(Left above, below). Fig. 2 Obvious differences in the capillary density, an index of neovascularization, was markedly increased in stem cell group (MT stain, X 100)(Middle above, below). Fig 3. There were significant differences of collagen prodution (MT stain, X100)(Right above, below)

P-35

Effect of allogenic canine adipose stem cells on autoimmune skin disorder in a dog

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Introduction

Recently, many studies indicated transplantation of adipose stem cells (ASC) alleviates tissue damage and can be used to repair and regenerate injured tissues. However, the effect of ASC on autoimmune skin disease was not fully researched.

Methods

Herein, we report a case of 8 year-old, female, mixed dog with clinical symptoms of systemic autoimmune skin disorder including severe pruritus, peripheral ulceration, superficial pyoderma and systemic seborrheic eczema. The symptoms have relapsed for 5 years and many clinical veterinarians failed to treat the systemic skin disorder. Finally we administrated canine allogenic adipose stem cells three times intravenously with cell number of 1×10^7, 5×10^6 and 2×10^6. Allogenic ASCs were isolated from canine abdominal fat tissue and labeled using feridex labeling.

Results

Microscopical examination via skin biopsy showed the infiltration of numerous neutrophils spreading from dermal layer to epidermal layer. Some part of the spinous layer exhibited spongiosis caused by neutrophil infiltrations, which was diagnosed as pemphigus foliaceous. After stem cell injection, the dog showed mild remission of skin disorder. Histopathologically, we observed therapeutic effect of ASC therapy showing mild alleviation of neutrophilic inflammation.

Conclusion

The present case is the first case showing therapeutic application of ASC in skin disorder of a dog and suggests a potential possibility of stem cell therapy for the veterinary dermatologic therapy. Moreover, we detected improved the autoimmune skin lesions histopathologically, which suggests that ASCs might play an important role in immune modulation.

P-36

The influence of human adipose-derived stromal cells (hASCs) on Wnt signaling in organotypic skin culture

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Introduction

Human adipose-derived stromal cells (hASCs) produce various cytokines such as vascular endothelial growth factor (VEGF), hepatocyte growth factor, and transforming growth factor-β, (TGF-β,). Also, there is a growing opinion that a large proportion of the useful effects of cell therapy may be attributable to the secretion of cytokines. Several reports suggested beneficial effects of hASCs on skin. These include antioxidant activity, accelerated wound healing, whitening effects, and anti-aging. We investigated the effect of hASCs on skin Wnt signaling, which is associated with skin regeneration and differentiation.

Methods

Pieces of human skin were co-cultured with hASCs, and two chambered transwell culture plates were used to prevent direct contact between hASCs and skin. In the control group, pieces of skin were cultured without hASCs. They were harvested at 2, 5, 7, 10^{th} culture day, and canonical Wnt signaling was analyzed. Wnt1, Axin2, TCF1, LEF1, DKK1 mRNA expressions were quantitatively assessed using realtime PCR. The expression levels of β,-catenin were compared using Western blot and immunohistochemical analyses.

Results

The Wnt1 mRNA expression of cultured skin was positively influenced by presence of hASCs in culture medium ($p<0.05$). LEF1 mRNA expression was increased in hASC co-culture group at day 7 ($p<0.05$). The real time PCR results of Axin2, TCF1 showed increased expression in the hASC co-culture group at day 7, but this was not statistically significant. The total β-catenin protein level in hASC co-cultured skin was higher than that of the control group. Immunohistochemical staining showed that the β-catenin-stained area of dermis was larger in the hASC co-cultured group than in the control group, and most of the positively stained cells in the dermis were fibroblasts.

Conclusions

The results of the present study showed that hASCs promoted canonical Wnt signaling in organotypic skin culture through paracrine effects, and the increased Wnt signaling was mainly due to dermal fibroblasts.

P-37

Acceleration of wound healing on adipose derived stem cell therapy with platelet-rich fibrin membrane: Comparison study between platelet-rich plasma(PRP) vs platelet-rich fibrin(PRF)

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Introduction

There has been a recent report that adipose derived stem cell improves wound healing through interacting with platelet-rich plasma(PRP). While the platelets within platelet-rich plasma does promote the action of adipose derived stem cell through secretion of various growth factors like TGF-1b, PDGF, and VEGF, the effect is very short-lived lasting only about a few hours. This shortcoming can be overcome by the arrival of plateletrich fibrin with platelets incorporated within the three dimensional matrix, which in turn releases growth factors for a stable period of time. This study sought to elucidate the longlasting effect of platelet-rich fibrin(PRF) combined with adipose derived stem cell on wound healing and create a protocol for concurrent chronic wound treatment.

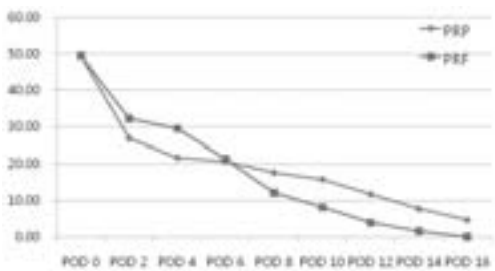
Methods

Ten full-thickness skin wound model was used for wound healing on five nude mice. Two symmetric full thickness skin defects were made on the dorsum of each mouse using 8-mm punch. Human adipose-derived stem cells were isolated and applied to the each wound, combined with either platelet rich plasma(PRP) or platelet rich fibrin(PRF). The platelet-rich fibrin was obtained by centrifuging 7 cc of blood without the addition of anticoagulants at 3000 rpm for 12 minutes and subsequently removing the upper layer of platelet-poor plasma(PPP). It was then compressed between two layers of 4 x 4 gauze and the fibrin sheet was shaped into circular form using an 8 mm punch The experimental group comprised the combination of 1 x 10^6 adipose-derived stem cells and platelet-rich fibrin, at the left side wound of the mice. The control group comprised the combination of the same amount of ASCs and platelet-rich plasma which was derived from the same amount of blood as the platelet-rich fibrin, at the right side wound. The wound area was measured over 14 days. By Day 7 &14, the wound was harvested and histologic analysis was done including blood vessel counting & vessel area measuring.

Results

Healing rate was more accelerated in the platelet-rich plasma group in the first 5 days ($p < 0.05$). But the platelet rich fibrin group surpassed the platelet-rich plasma group after the 6 days ($p < 0.05$). (Fig 1) The average number of blood vessels was 2.22 ± 1.27 in PRP group and 3.56 ± 1.43 in PRF group. (X100 high power field) Conclusion : Platelet-rich plasma accelerated the wound healing during the initial few days, but the wound healing activity of platelet-rich fibrin group dominant over that of platelet-rich plasma group starting on day 4, eventually shortening the time to complete healing. This points to the evidence that platelet-rich fibrin exerts a slow yet pervasive influence over the two-week course of wound healing process. Thus, platelet-rich fibrin is probably more beneficial for promoting the activity of adipose derived stem cell for a sustained period of time.

Fig. 1. Wound area measurement



P-38

Hypoxia-enhanced wound-healing function of adipose-derived stem cells: Increase in stem cell proliferation and up-regulation of VEGF and bFGF

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Introduction

Tissue regeneration using the body's own stem cells and growth factors is a good strategy for damaged tissue (e.g.,during and after inflammation). Because of few drawbacks such as ethnic consideration, adult stem cells are becoming a major candidate in stem cell medicine. Indeed, applications of adipose-derived stem cells (ADSCs) for compensation of skin defects have shown some satisfactory results. Not only can A DSCs reconstruct a tissue, but they also have the ability to control or cu re other cells and can reconstruct an integrated function. For example, we have previously demonstrated that A DSCs accelerated wound healing by secreting growth factors. In that study, a conditioned medium of A DSCs (ADSC-CM) activated collagen synthesis and migration of human dermal fibroblasts (HDFs), thus accelerating wound-healing. In addition, ADSCs exhibited an antioxidant effect and protected HDFs from oxidative stress, suggesting that A DSCs play a unique role in tissue protection in hypoxic/oxidative stress. However, little is known regarding the function of ADSCs under hypoxia. To better understand the mechanism of A DSCs-induced tissue protection from wound healing, it is important to clarify this issue because inflammation and oxidative stress near the wound area induce an oxygen deficit.

Methods

We cultured A DSCs in hypoxia and then evaluated their proliferation and survival and compared the data with those of normoxia. In addition, ADSCs-mediated wound-healing function was studied in the conditioned medium collected under a hypoxic or a normoxic condition. Quantitative reverse transcription polymerase chain reaction (Q-RT-PCR) was performed to measure mRNA expression of hypoxic A DSCs, and an enzyme-linked immunosorbent assay (ELISA) was used to quantify the secreted protein levels in the ADSC-CM. Finally, we studied the effect of the neutralizing antibodies against the vascular endothelial growth factor (VEGF) and the basic fibroblast growth factor (bFGF) in the animal study.

Results

Effect of hy poxia on the proliferation of A DSCs was first examined that hypoxia (2% O 2) enhanced the proliferation of A DSCs in either the presence of serum or in the absence of serum. The ADSC-CM harvested under hypoxia (hypoCM) significantly promoted collagen synthes is and the migration of human dermal fibroblasts, compared with that in normoxia (norCM). In the animal studies, hypoCM significantly reduced the wound area compared with norCM. Furthermore, mRNA and protein measurements showed that hypoxia up - regulated growth factors such as VEGF and bFGF. Inhibition of VEGF and bFGF using neutralizing antibodies reversed the migration of the wounded HDFs and the healing of wounds in animal experiment. Collectively, these results suggest that hypoxia increases the proliferation of ADSCs and enhances the wound ?healing function of ADSCs, at least partly, by up-regulating the secretion of VEGF and bFGF.

Conclusions

Hypoxia increased the proliferation of ADSCs and enhanced the wound-healing function of ADSC-CM in *in vitro* and animal studies. The mechanism may be related to the hypoxia up -regulation of the specific growth factors such as VEGF and bFGF in ADSCs.

P-39

Possibility of myofibroblast differentiation of human adipose-derived stem cell

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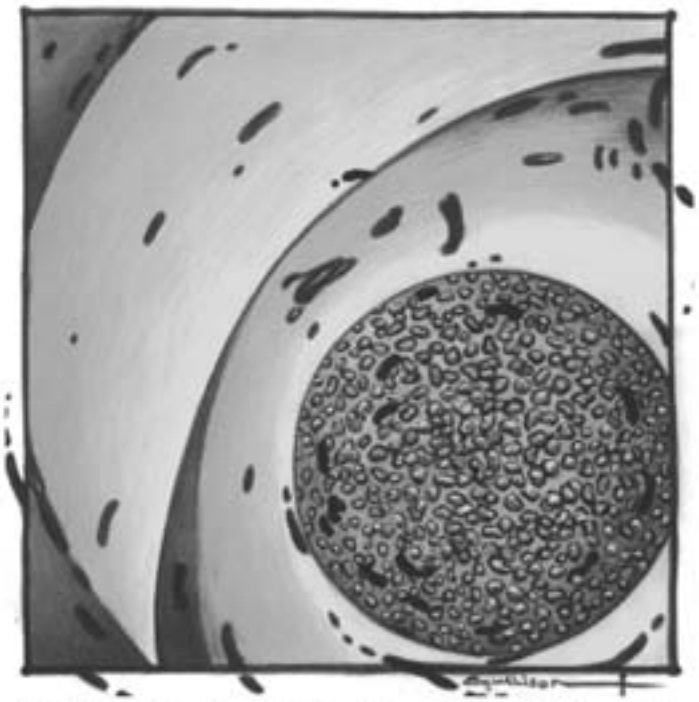
Human adipose-derived stem cells (ASC) are adult pluripotent stem cells, which have multi-differentiation ability into fat, cartilage, bone, and nerves to be applied in tissue engineering. On the other hand, at the point of wound healing, in 1971, Gabbiani reported that granulation tissue contracted after stimulation, and its contraction involved cells showing the characteristics of smooth muscle cells rich in actin bundles as stress fibers, terming these cells myofibroblasts. Myofibroblasts play a primary role in wound contraction in the wound healing process, in which cell skeleton proteins such as α-SMA are positive, and which are able to cell contract.

In this time, we evaluated the possible differentiation of ASC into myofibroblasts. After making up the monolayer culture of ASC, various factors such as TGF-β, were added to the culture supernatant. Fluorescent phalloidin staining was performed in the control and addition groups, to observe actin filaments (F-actin) in cells. α-SMA was fluorescent immunostained to clarify its localization in the cell.

As those results, α-SMA expression in the addition group was serially confirmed by real-time RT-PCR. The addition group showed marked cell contraction. These results suggested the differentiation potential of ASC into myofibroblasts.



Artist, John Cullison, drew his interpretation of Stem Cells after receiving treatment for his rheumatoid and osteoarthritis. John suffered from the disease for 15 years and was unable to use his hands to paint. After he was fully treated using his own fat derived stem cells, he regained full use of his hands.

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Hair / Hip augmentation / Severe Scar / Anti-aging

Filler Geller System

Patent KFDA

Filler Geller System(Medical cutting mill)
Scaffold Filler Fat Gel & Other licensed material
System to make scaffold filler on the site of operation room depending on individual conditions of the patient,
A new type of tissue replacement,

Fat Gel
- increased stem cell density
- With natural filler, which are made of fat, your fine and smooth contour can be best emphasized,
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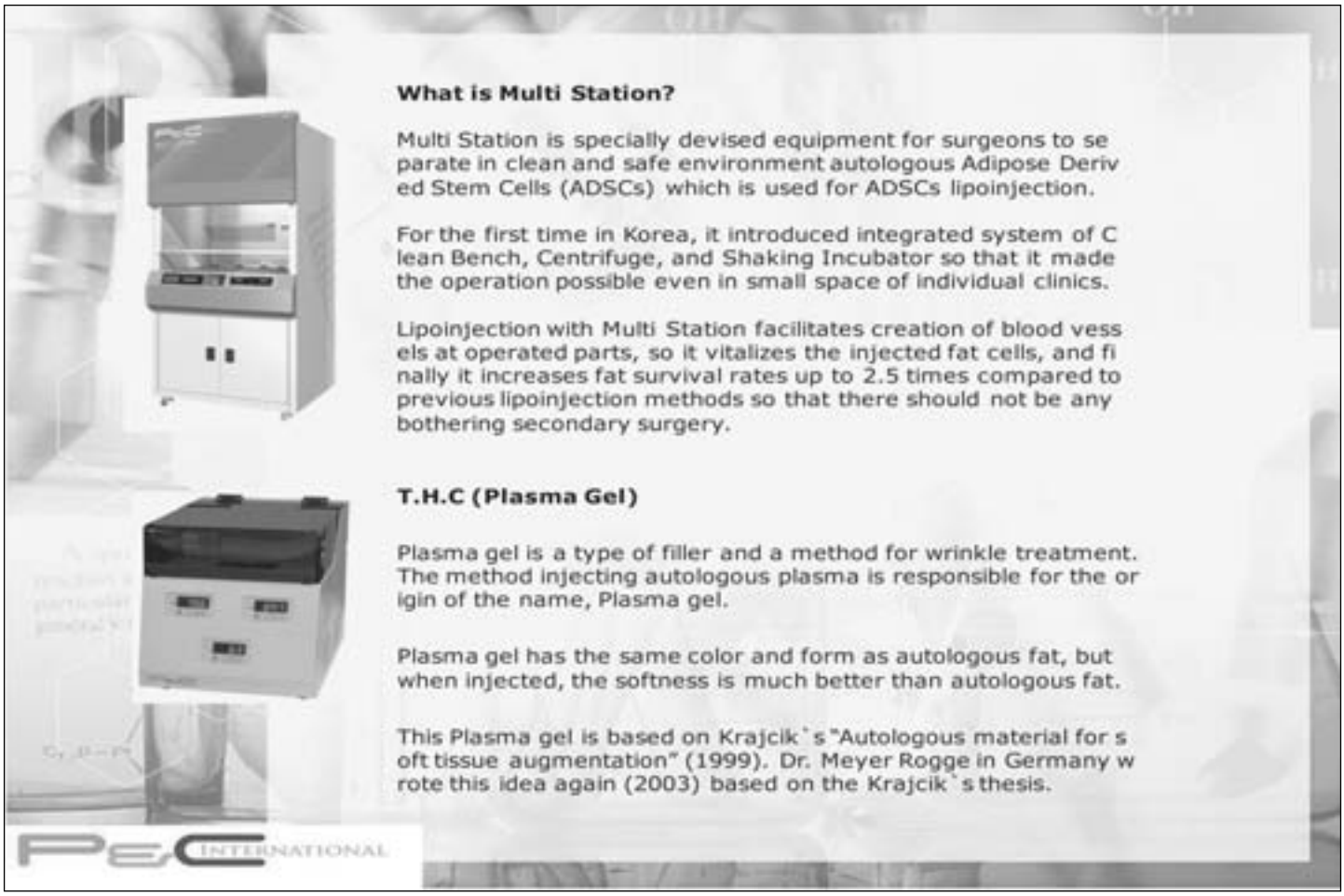

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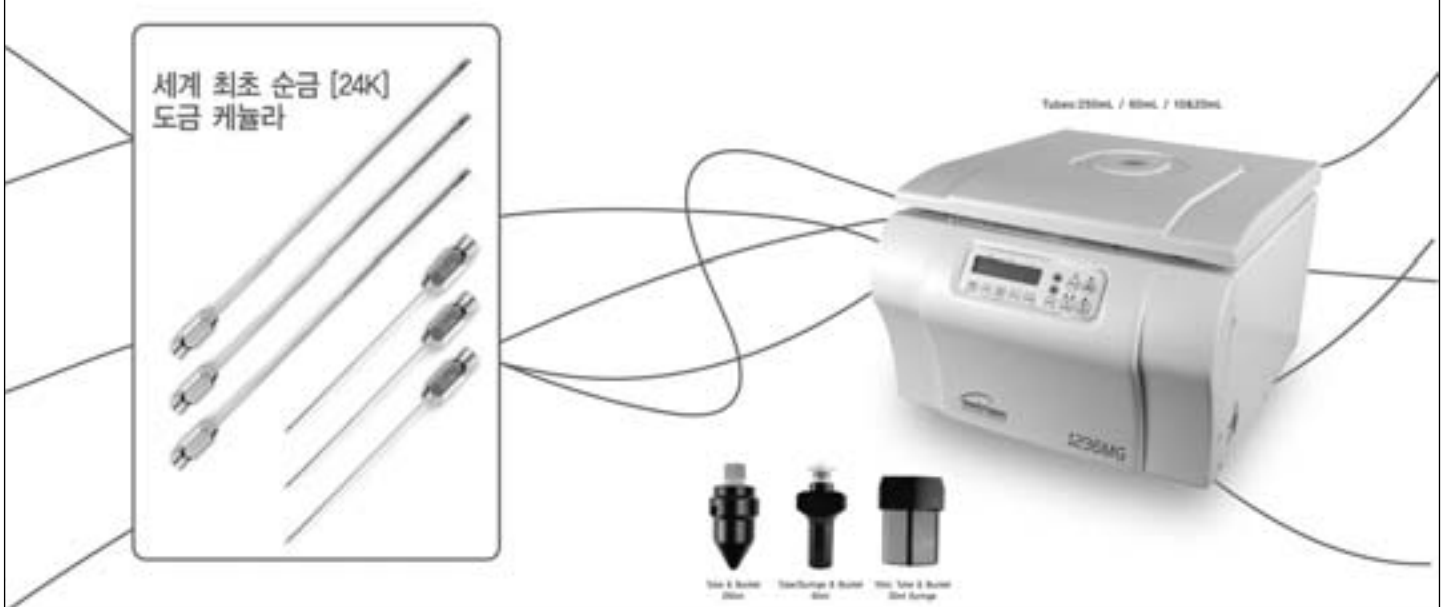

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