The Potential of Adipose Tissue and Adipose-derived Stem Cells

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Discovery of Adipose-derived Stem Cells (ASCs)

• Human “Preadipocytes” were first discovered in the mid-1970’s
  - Stiles et al, 1975; Dardick et al, 1976
• Plasticity of preadipocytes was first published in 2001
• Our laboratory was established in 2002
  – Co-Directors: J. Peter Rubin and Kacey Marra
Adipose Tissue

Fat

Bone

Cartilage

Muscle

Zuk et al.
Tissue Engineering,
2001 7(2) 211.
ASC Isolation: Stromal Vascular Fraction (SVF) vs. Plated ASCs
Basic Science Projects in the Adipose Stem Cell Center

(Both SVF and expanded ASCs)
Four Research Cores

ASC Biology

Soft Tissue Repair

Wound Healing

Nerve Regeneration
ASC
Biology
Basic Biology

• Heterogeneity of stem cell population
  – Sorted 4 subpopulations of cells in the SVF
  – Collaboration with the Donnenbergs

• Identification of adipogenic factors
  – High throughput screening via LOPAC
  – Identification of new agents such as NPY
Basic Biology

- Lentiviral transfection
  - Can label subpopulations with four different colors and track in vivo to determine survival and fate
Basic Biology

• 3D expansion/differentiation
  – Can culture adipose tissue ex vivo for 3 months
  – Collaboration with Jörg Gerlach
  – Healthy vs. Diabetic Fat
Wound Healing
Cell Delivery Systems

- Direct injection into wound
- Encapsulation within hydrogels
- Gene therapy
- Cell sheets
Excisional Wound Model (dorsal aspect)

Mouse

Mouse

Pig
Soft Tissue Repair
Adipose Tissue Engineering

- Controlled drug delivery
- Fat graft retention
- Novel biomaterials
Drug Delivery Systems

• Polymeric microspheres
  – Dexamethasone, Insulin, SFRP1, NPY and VEGF-loaded PLGA microspheres

• Gene Therapy
  – Increased expression of proteins in ASCs that enhance fat retention
Biomaterials

• Silk scaffolds
  – Collaboration with David Kaplan
• HA-based scaffolds: Injectable
• ECM-based scaffolds

= ASCs
Clinical Studies throughout the world are Promising (>80 trials)....

...only ~5 trials in the US
High Profile Cases


ASCs/SVF in Clinical Studies

- Soft tissue augmentation
  - Breast augmentation
  - Facial lipoatrophy
- Wound healing
  - Fistula repair
Breast Augmentation

Cell-Assisted Lipotransfer for Cosmetic Breast Augmentation: Supportive Use of Adipose-Derived Stem/Stromal Cells

Kotaro Yoshimura · Katsujiro Sato · Noriyuki Aoi · Masakazu Kurita · Toshitsugu Hirohi · Kiyonori Harii


http://news.makemeheal.com/plastic-surgeons/interviews/augment-your-breasts-naturally-through-stem-cells
Cell-Assisted Lipotransfer

Aspirated fat (ASC-poor fat) + Freshly isolated SVFs → Aspirated fat is used as a living scaffold

Cell isolation (1.5 h) in Cell-Processing Room

CAL fat (ASC-rich fat)
Promising but questions remain

• SVF vs. ASCs?
• Cell survival?
• Optimal mode of cell delivery?
• ASCs/SVF for back pain, MS or diabetes?
Clinical Translation with Dr. Peter Rubin

Lipoaspirate intended for fat grafting + Freshly isolated SVF = Graft material mixed with autologous adipose stem cells

Portion of lipoaspirate separated and used for stem cell isolation
Pittsburgh Clinical Trials

• Craniofacial Fat Grafting
  – Fat Only
  – Fat + SVF
  – Fat + Higher dose SVF

• Amputee Fat Grafting

• Pedal Fat Grafting
Phase I: Fat Grafting Only
Example Patient

Preop

1 mo PO

3 mo PO

9 mo PO
Example patient

Preop  PO 1 mo  PO 9 mo
Preop  PO 1 mo  PO 9 mo
Preop  PO 1 mo  PO 9 mo
Preop  PO 1 mo  PO 9 mo
In humans, volume retention of the fat grafts are calculated with analysis of 3D CTs that are performed at 2 weeks, 3 months and 9 months.
RESULTS

BASIC SCIENCE
For each patient, lipoaspirate is processed into a stromal vascular fraction using our lab’s GLP collagenase isolation protocol. SVF is then subjected to flow cytometry.
The flanks of athymic nude mice are injected subcutaneously with 1mL of fat graft processed via the Coleman method. Fat grafts are explanted at 8 weeks and volumes are measured using a gas pycnometer.
Phase Two

- Part I - to establish baseline data on standard fat grafting
- Part II – Enrich fat grafts with concentrated ASCs and determine effects on volume retention
Human Trial Examining Efficacy of Including ASCs

Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial

Lancet 2013; 382: 1113–20

Fat graft vs Fat graft + 20 x 10^6 ASCs/mL fat
Study Design

Baseline

Day -14
- 13 patients had clinical evaluation and gave informed consent
- 13 patients had minor liposuction

Day -1
- 13 patients had ASC isolation and expansion for 14 days
- 11 patients had first MRI*

Day 0
- 11 patients had liposuction
- 11 patients had graft preparation and implantation

Day 121
- 10 patients had third MRI†
- 10 patients had graft removal
MRI Results

- Baseline
- Day 0
- Day 121
Volume Retention

80% (ASCs) vs 16% (Control) *p<0.0001
Additional Data

A. Adipose tissue
B. Newly formed connective tissue
C. Necrotic tissue
D. Existing connective tissue
E. Vessel density

- Adipose tissue: p=0.0109
- Newly formed connective tissue: p=0.0115
- Necrotic tissue: p=0.0105
- Existing connective tissue: p=0.0060
- Vessel density: p=0.39
Commentary

• "These therapies could revolutionize breast reconstruction after cancer and reconstruction of deformities after trauma, for example.

• A crucial open question that will affect use of this therapy is whether there is an optimum cell dose for efficacy, and/or if there is a critical threshold cell dose for therapeutic effect…

• Another unresolved issue for this therapy is whether high concentrations of stem cells can stimulate the growth of residual breast cancer cells…

• This issue will be best addressed in large clinical trials."

Summary

• Highly collaborative research projects focusing on:
  – Stem cell biology
  – Small and large animal models
  – Gene therapy
  – Novel biomaterials
  – Controlled drug delivery
  – Clinical translation
Relationship to High School Education

• Combine chemistry, biology, engineering and medicine on a daily basis
• Chemistry is utilized in synthesis of novel biomaterials
• Biology is relevant for adult stem cell culture
• Engineering results in improved and optimized devices or systems
• Medicine provides the clinical motivation
• ROHSS: Research Opportunities for High School Students
My background

• Born in Washington County, PA
• Knew since 5th grade I wanted to be a scientist
• Graduated 1988 Chartiers-Houston High School
• Attended Pitt and earned a BS in chemistry 1992
• Stayed at Pitt for graduate school and earned a PhD in organic chemistry in 1996
• Got married during graduate school
• Moved to Atlanta for a post-doctoral fellowship in biomedical engineering in 1996
My background, cont

- Had 1\textsuperscript{st} child in Atlanta in 1997
- Moved back to Pittsburgh in 1997 for another post-doctoral fellowship at Carnegie Mellon University
- Promoted to Research Scientist at CMU in 1998
- Had 2\textsuperscript{nd} child in 1999
- Moved to Pitt in 2002 for tenure-track assistant professor position at Pitt
- Had 3\textsuperscript{rd} child in 2006
- Received tenure in 2010
- Continuing research at Pitt and loving it!
Find and follow your passion....

Success is not the key to happiness. Happiness is the key to success. If you love what you are doing, you will be successful.

—Albert Schweitzer
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