

## Fat-derived Adult Stem Cells and Tissue Engineering Applications

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## What is Tissue Engineering?

- The combination of biodegradable materials, cells and drugs to create new tissue
- Biodegradable materials include polymers, such as stitches that slowly dissolve

## Relevance of TE

- TE is an interdisciplinary field that brings together the principles of the life sciences and medicine with those of engineering.
- Rapid increase in progress in TE is a result of new biomaterials development, improvements in bioreactor design, increased understanding of healing processes, and advancements in stem cell therapies.

*Ann. NY Acad. Sci., 2006, 1068: 352.*

## History of TE

- First report of TE was in 1933: tumor cells were implanted into a pig wrapped in a polymer membrane to protect them from an immune response. *Sischr. Krebsforsch, 1933, 40: 122.*
- Modern era of TE began in the 1980's with the development and clinical use of skin replacements. *Science, 1981, 221: 1052.*

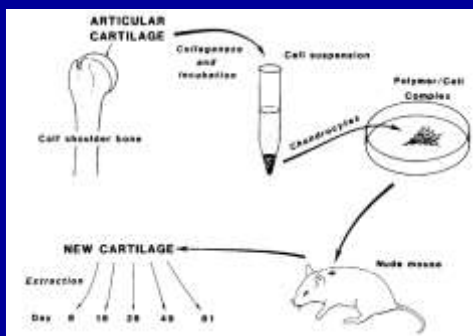
## “Ear on the Back of a Mouse”



Cao et al, *Plastic Recon. Surg.*, 1997, 100(2):297-302

## Tissue Engineering Basic Strategy

- Harvest cells from the patient
- Expand cells in culture
- Seed the cells onto a biodegradable scaffold
- Tissue is grown on the scaffold
- The scaffold resorbs as the new tissue grows



Synthetic Polymers Seeded with Chondrocytes Provide a Template for New Cartilage Formation

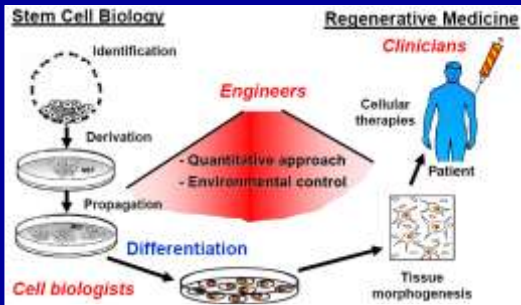
© 1991, Reed, R.D., & Langer, G.S., N. Glick, M.D., and J. P. Francis, M.D.

*Plastic Recon. Surg.*, 1991, 88:5, 753.

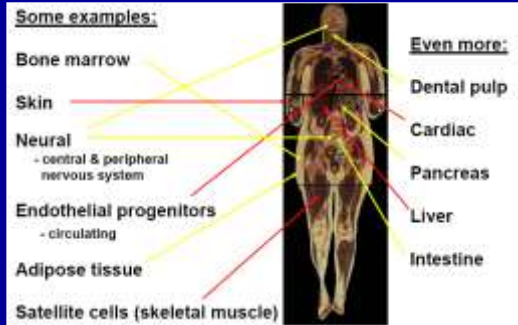
## Examples of Tissue Engineering

- Living skin equivalents
- Autologous cartilage repair
- Bone regeneration therapies
- Are artificial hips an example of TE?
- Are contact lens an example of TE?

## Stem Cell Translation



## Tissue-derived Adult Stem Cells



## Advantages of Adult Stem Cells

- Organ/tissue rejection unlikely if patient receives own stem cells
- Some cells are easy to harvest (e.g., blood stem cells)
- Less coaxing in culture required to stimulate growth of specific cell types

## Disdvantages of Adult Stem Cells

- Limited longevity in cell culture
- Sometimes difficult to isolate and extract
- Limited flexibility or types of cells that can be made
- Typically more scarce with age
- Questionable quality due to age, toxins, and disease

## Logical Sources of Autologous Stem Cells

- Blood
- Bone Marrow
- Adipose = Fat

## What is our Stem Cell source?

## Adipose Tissue



67% of Americans are overweight



## Liposuction Technique



[http://www.plasticsurgery.com/procedure\\_56.htm#liposuction.html](http://www.plasticsurgery.com/procedure_56.htm#liposuction.html)



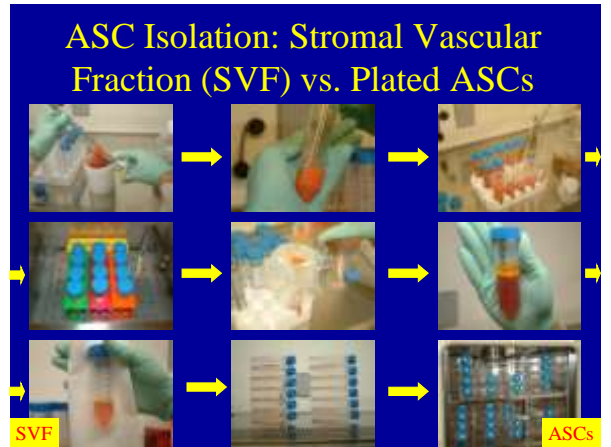
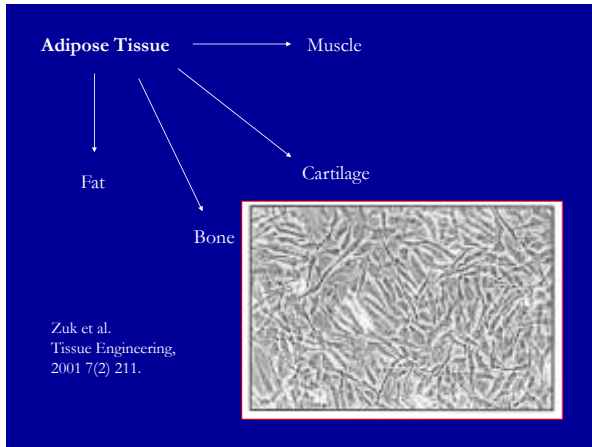
## Tissue Engineering with Adipose-Derived Stem Cells

- Easy to harvest
- Plentiful
- Multipotent adult stem cells



## Discovery of ASCs

- “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
  - Zuk et al, 2001
- Adipose-derived Stem Cells (ASCs)
  - IFATS 2004



**ASCs IN HUMANS??...YES!**

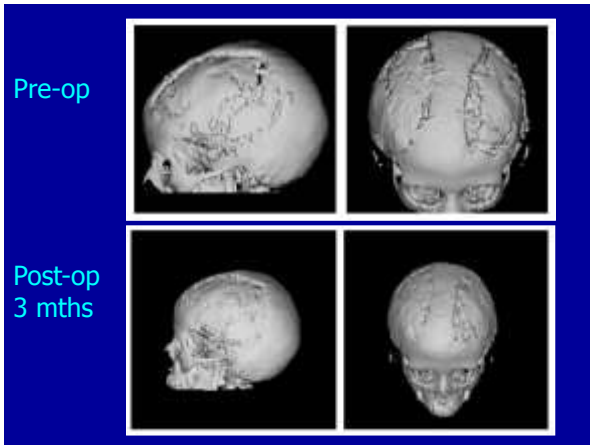
**Fat in Pediatric Skull**

J. Cranio-Maxillofacial Surgery, 2004, 32: 370.

Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report

Stefan LENDECKEL<sup>1</sup>, Andreus DÖRCKE<sup>2</sup>, Petros CHRISTOPHIS<sup>2</sup>, Kathrin HEIDINGER<sup>3</sup>,  
Jan WOLFF<sup>4</sup>, John K. FRASER<sup>5</sup>, Marc H. HEDRICK<sup>3</sup>, Lars BERTHOLD<sup>6</sup>, Hans-Peter HOWALDT<sup>1</sup>

Germany



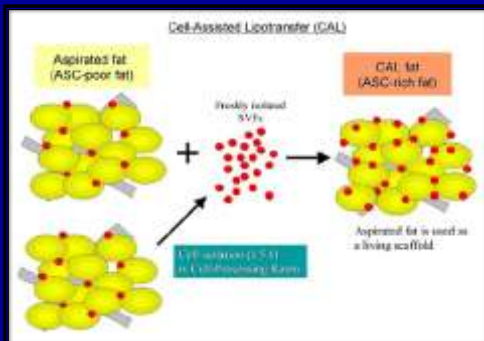
### Cell-Assisted Lipotransfer for Facial Lipoatrophy: Efficacy of Clinical Use of Adipose-Derived Stem Cells

KOTARO YOSHIMURA, MD,<sup>1\*</sup> KAZUHIKO SAKO, MD,<sup>2</sup> NORIYUKI AOKI, MD,<sup>3\*</sup> MASAOKA KUBOTA, MD,<sup>2</sup> KEIICHI ISHIGI, MD,<sup>4\*</sup> HIROTAKA SUGA, MD,<sup>5\*</sup> HITOMI ENO, MD,<sup>6\*</sup> HARIKUNIOKI KATO, MD,<sup>6\*</sup> TOSIYOSHI HIROHI, MD,<sup>1</sup> AND KIYOSHI HARA, MD<sup>2</sup>

*Dermatol Surg* 2008;34:1178-1183

Japan

### Experimental Protocol



### Patient Data

TABLE 1. Summary of Patients' Data

	Method					
	Non-CAL		CAL			
Case	1	2	3	1	2	3
Sex	Male	Female	Female	Male	Female	Female
Age (year)	26	42	42	36	33	40
Diagnosis	LEP	LEP	LEP	PHS	LEP	LEP
Severity grading	3	5	2	4	5	4
Postoperative oral corticosteroids	—	+	—	—	+	—
Donor site	Abdomen	Abdomen	Abdomen	Abdomen	Abdomen	Abdomen
Volume of injection (mL)	100	250	50	110	80	100
Volume of fat (mL)	10	9	9	13	9	9
Clinical results	Fair	Good	Fair	Excellent	Good	Good
Complications	—	Adipose necrosis	—	—	—	—

LEP, lipoatrophy; PHS, Parry-Romberg syndrome.

Excellent: >80%; Good: 60-80%; Fair: 40-60%; Poor <40%

### Results: non-CAL



### Results: CAL



### Conclusions

- CAL vs non-CAL: All patients obtained improvement in facial contour, but the CAL group had a better clinical improvement score than did the non-CAL patients
- No adverse side effects or complications except for one case of lupus erythematosus profundus in the non-CAL group who took oral corticosteroids preoperatively

### Multiple Sclerosis

**Journal of Translational Medicine** Wolters Kluwer

Review

[Open Access](#)

**Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis**

Neil H Ricordan<sup>1</sup>, Thomas E Ichim<sup>1,2</sup>, Wei-Ping Min<sup>2</sup>, Hao Wang<sup>2</sup>, Fabio Solano<sup>3</sup>, Fabian Lara<sup>4</sup>, Miguel Alfaro<sup>5</sup>, Jorge Paz Rodriguez<sup>6</sup>, Robert J Harman<sup>7</sup>, Amit N Patel<sup>2</sup>, Michael P Murphy<sup>8</sup>, Roland H Lee<sup>9,10</sup> and Boris Minny<sup>1,2,7</sup>

Journal of Translational Medicine 2009, 7:29

Costa Rica

## Study Design

- 3 Male Patients with Relapsing-remitting MS:
  - 50 yo (d. 2005)
  - 32 yo (d. 2001)
  - Unreported age (d. 2003)
- Cell treatments:
  - 28, 25 or 75 million SVF cells (2 infusions); multiple intrathecal (injection into spinal canal) and intravenous infusions of allogeneic CD34+ and MSC cells (cord blood)
  - Infusions over 9-10 days and well tolerated

## Patient 1: no changes in plaques

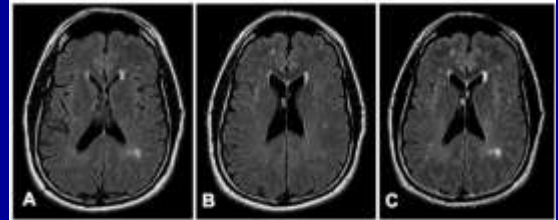


Figure 1  
MRI images obtained before (Panels A and B), and six months after (Panel C) the stem cell treatment of patient 1. Panels A and B: Consecutive axial Fluid-Attenuated Inversion-Recovery (FLAIR) images through the lateral ventricles show multiple small foci of bright signal in the periventricular and subcortical white matter, consistent with plaques of multiple sclerosis. Panel C: Axial FLAIR image shows no significant change in the multiple periventricular and subcortical white-matter plaques. (For the comparison, note that this slice is positioned between those in A and B, and at slightly different scanning-angle, so it includes lesions of both those slices, as well as others slightly out-of their plane.)

## Patient 2: no changes in plaques

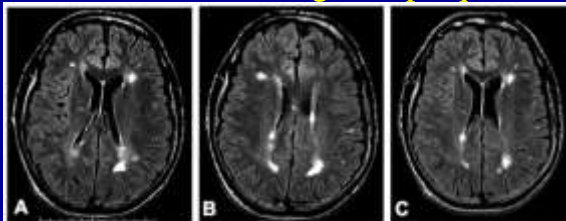


Figure 2  
MRI images obtained before (Panels A and B), and seven months after (Panel C) the stem cell treatment of patient 2. Panels A and B: Consecutive axial Fluid-Attenuated Inversion-Recovery (FLAIR) images through the lateral ventricles show multiple small patches of bright signal in the periventricular and subcortical white matter, consistent with plaques of multiple sclerosis. Panel C: Axial FLAIR image shows no significant change in the multiple periventricular and subcortical white-matter plaques. (For the comparison, note that this slice is positioned similar to slice A but at slightly different scanning-angle, so it includes lesions of both slices A and B.)

## Conclusions

- 3 compassionate use patients tolerated cell infusions
- No significant improvements previously seen with cord blood/MSC treatments
- These patients reported improvements in memory, energy levels, balance, coordination, and gait (~ 6-7 months)
- Future studies are warranted

## Diabetes

### Human Adipose Tissue-Derived Mesenchymal Stem Cells Combined With Hematopoietic Stem Cell Transplantation Synthesize Insulin

H.L. Trivedi, A.V. Venkat, U. Thakker, A. Froze, S.D. Datta, C.N. Patel, J.V. Patel, A.B. Bhargava, and V. Stankovic

Transplantation Proceedings, 40, 1135-1139 (2008)

India

## Study Design

Table 1. Patient Demographics and Cell Infusion Details

Patient (age/sex)	Diabetes Duration	FBS (mg/dL)	PPBS (mg/dL)	Source No. of Relatives	Infused Cell No. (x10 <sup>6</sup> )	CD34 <sup>+</sup> (%)	CD45 <sup>+</sup> (%)	CD45 <sup>+</sup> (x10 <sup>6</sup> )	CD34 <sup>+</sup> (x10 <sup>6</sup> )
FP 15/F	0 mos	114	205	Mother (2)	125	1	91.84	12.73	12.73
HS 14/F	6 y	188	210	Father (1)	85	0.82	41.1	27.94	27.94
BM 55/F	10 y	180	170	Sister (2)	181	0.8	25.52	28.16	28.16
MD 19/M	1 y	240	407	Mother (1)	36	0.27	16.52	14.8	14.8
PJ 26/M	0 y	99	456	Aunt (2)	122	1.36	—	—	—

In patient 11, the CD45RO measurements could not be performed.  
FBS, fasting blood sugar; PPBS, postprandial blood sugar.

5 patients (M:F 2:3, <30 yo): Type I diabetes (autoimmune)  
Intraportal administration of allogeneic ASCs (from relatives) + Bone marrow  
(Injections in the venous bed through recanalized umbilical vessels)

## ASC culture

- 2 g fat isolated from subcutaneous abdominal region of donor
- Cells expanded for 10 days
- In vitro differentiation for 3 days (HGF)
- Bone marrow aspirated and expanded for 3 passages
- All cells mixed together for infusion into patient

## Study Results

Table 2. Results Following Cell Infusion

Patient	Follow-up (mo)	FW Blood Sugar	Insulin Requirement (units/kg)		Hemoglobin Levels (g/dL)		Glucose Level (%)		Weight (kg)	
			Pre-Infusion	Post-Infusion	Pre-Infusion	Post-Infusion	Pre-Infusion	Post-Infusion	Pre-Infusion	Post-Infusion
FP	3.5	198	44	18	0.06	0.28	8.08	9	45	49
HS	3.5	259	18	7	0.02	0.1	9.91	8.9	24.9	25
BM	3	129	19	40	0.06	0.57	8.35	7.8	62.3	82
MD	3	178	36	9	0.00	1.58	7.38	9.8	54	59
PJ	1.5	162	36	30	0.21	1.37	6.8	6.2	45	46

Insulin requirement

30-50% decrease, ~ 3 mo follow-up

## Conclusions

- All patients were successfully infused CBM plus h-AD-MSC without any untoward effects and showed 30% to 50% decreased insulin requirements with 4- to 26-fold increased serum c-peptide levels, with a mean follow-up of 2.9 months
- This report describes safe and effective treatment of insulinopenic diabetics using insulin-producing h-AD-MSC plus BM without xenogeneic materials

## Clinical Studies throughout the world are Promising....

...still optimizing our therapies in animal models in USA

## High Profile Cases



<http://www.governor.park.edu/~labatovich/History/Beagles/Peter/Manning.jpg>



<http://img.stanmark.com/images/mag2/Files/1000602/2011/06/2011-06-12-11-52-42-1-4/The-governor-nick-perry-is-wed-to-be-iran.jpg>

## 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 1970 in Washington County, PA
- Knew since 5<sup>th</sup> 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<sup>st</sup> 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<sup>nd</sup> child in 1999
- Moved to Pitt in 2002 for tenure-track assistant professor position at Pitt
- Had 3<sup>rd</sup> child in 2006
- Received tenure in 2010
- Continuing research at Pitt and loving it!

## Acknowledgements

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- Anne Argenta, MD
- Harry Nayar, BS
- Patrick Emelife, BS
- Jacqueline Bbley, BS
- Sunny Oh, BS
- Jon Bechtel
- Russell Kling, BS
- Blair Suter
- Arielle Richter
- Michael Lang, BA

