How You Can Use Stem Cells

Dr. Ralph T Bright
Macquarie Cosmetic Medicine
21B Bathurst Street
Liverpool NSW 2170
www.cosmeticmedicine.com.au
(02) 9824 3044

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Stem Cell Transfer

Many of the people in this room have most of the skill sets needed to perform Stem Cell Transfer.

- Liposuction
- Laboratory technology
  - Collagenase digestion
  - Centrifugation
  - Cell counting – number and viability
- Ultrasound guided injection
- Intravenous injection

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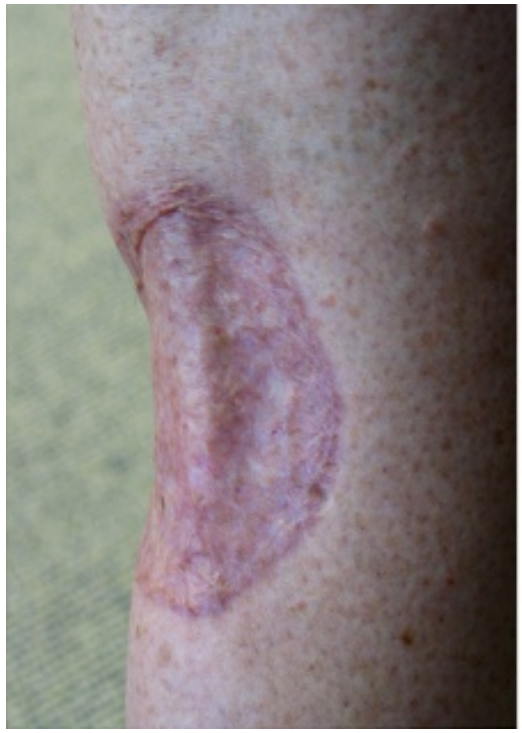
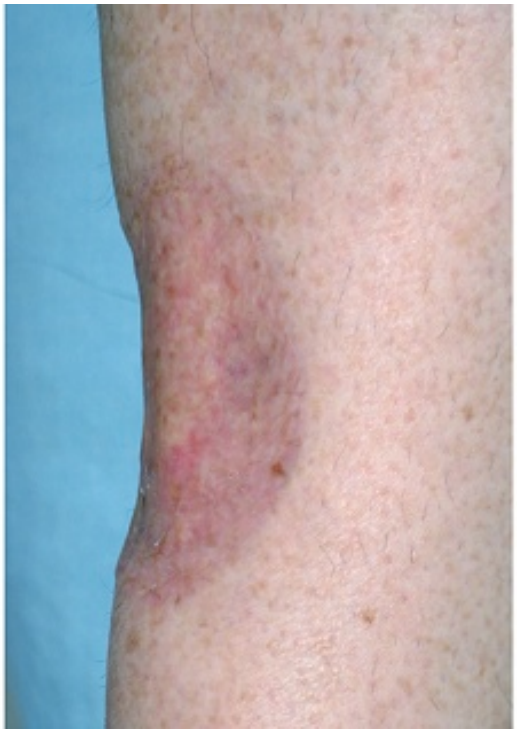
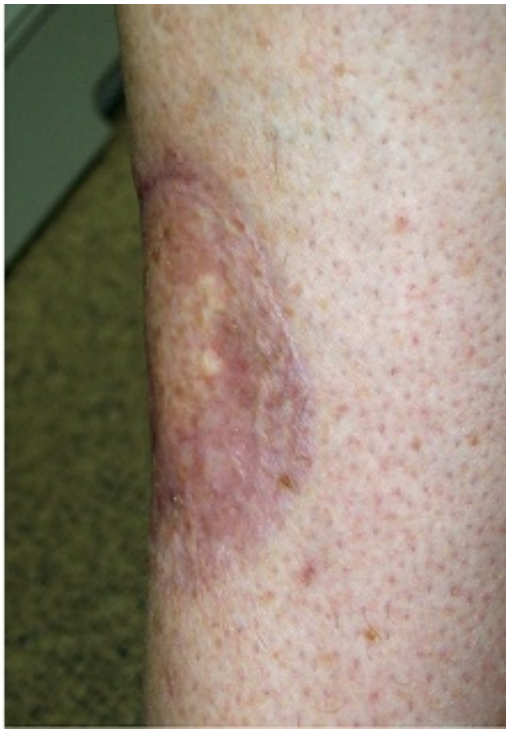
Fat transfer



No feeling



Uncomfortable after first



Needs anaesthesia after third

A Landmark Paper

TISSUE ENGINEERING
Volume 7, Number 2, 2001
Mary Ann Liebert, Inc.

Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies

PATRICIA A. ZUK, Ph.D.,[1,2] MIN ZHU, M.D.,[1,2] HIROSHI MIZUNO, M.D.,[2] JERRY HUANG, B.S.,[2] J. WILLIAM FUTRELL, M.D.,[3] ADAM J. KATZ, M.D.,[3] PROSPER BENHAIM, M.D.,[2] H. PETER LORENZ, M.D.,[2] and MARC H. HEDRICK, M.D.[2]

ABSTRACT

Future cell-based therapies such as tissue engineering will benefit from a source of autologous pluripotent stem cells. For mesodermal tissue engineering, one such source of cells is the bone marrow stroma. The bone marrow compartment contains several cell populations, including mesenchymal stem cells (MSCs) that are capable of differentiating into adipogenic, osteogenic, chondrogenic, and myogenic cells. However, autologous bone marrow procurement has potential limitations. An alternate source of autologous adult stem cells that is obtainable in large quantities, under local anesthesia, with minimal discomfort would be advantageous. In this study, we determined if a population of stem cells could be isolated from human adipose tissue. Human adipose tissue, obtained by suction-assisted lipectomy (*i.e.*, liposuction), was processed to obtain a fibroblast-like population of cells or a processed lipoaspirate (PLA). These PLA cells can be maintained *in vitro* for extended periods with stable population doubling and low levels of senescence. Immunofluorescence and flow cytometry show that the majority of PLA cells are of mesodermal or mesenchymal origin with low levels of contaminating pericytes, endothelial cells, and smooth muscle cells. Finally, PLA cells differentiate *in vitro* into adipogenic, chondrogenic, myogenic, and osteogenic cells in the presence of lineage-specific induction factors. In conclusion, the data support the hypothesis that a human lipoaspirate contains multipotent cells and may represent an alternative stem cell source to bone marrow-derived MSCs.

What Cell Would You Like?

TABLE 1. LINEAGE-SPECIFIC DIFFERENTIATION INDUCED BY MEDIA SUPPLEMENTATION

Medium	*Media*	*Serum*	*Supplementation*
Control	DMEM	10% FBS	none
Adipogenic (AM)	DMEM	10% FBS	0.5 mM isobutyl-methylxanthine (IBMX), 1 μM dexamethasone, 10 μM insulin, 200 μM indomethacin, 1% antibiotic/antimycotic
Osteogenic (OM)	DMDM	10% FBS	0.1 μM dexamethasone, 50 μM ascorbate-2-phosphate, 10 mM β-glycerophosphate, 1% antibiotic/antimycotic
Chondrogenic (CM)	DMEM	1% FBS	6.25 μg/ml insulin, 10 ng/ml TGF-β1, 50 nM ascorbate-2-phosphate, 1% antibiotic/antimycotic
Myogenic (MM)	DMEM	10% FBS, 5% HS	0.1 μM dexamethasone, 50 μM hydrocortisone, 1% antibiotic/antimycotic

Adipose Derived Stromal Cells

- Fat has a connective tissue support structure in which run the blood vessels and nerves.
- The parenchymal cells are fat cells.
- Multipotent stromal cells in adipose tissue are able to differentiate into osteoblasts, chondrocytes, myocytes, adipocytes and beta pancreatic islet cells

Stroma: The supportive framework of an organ (or gland or other structure usually composed of connective tissue. The stroma is distinct from the parenchyma which consists of the key functional elements of that organ. The Greek word "stroma" means "anything spread out for sitting or lying upon," essentially a mat.
The stroma in anatomy is thus the supporting tissue

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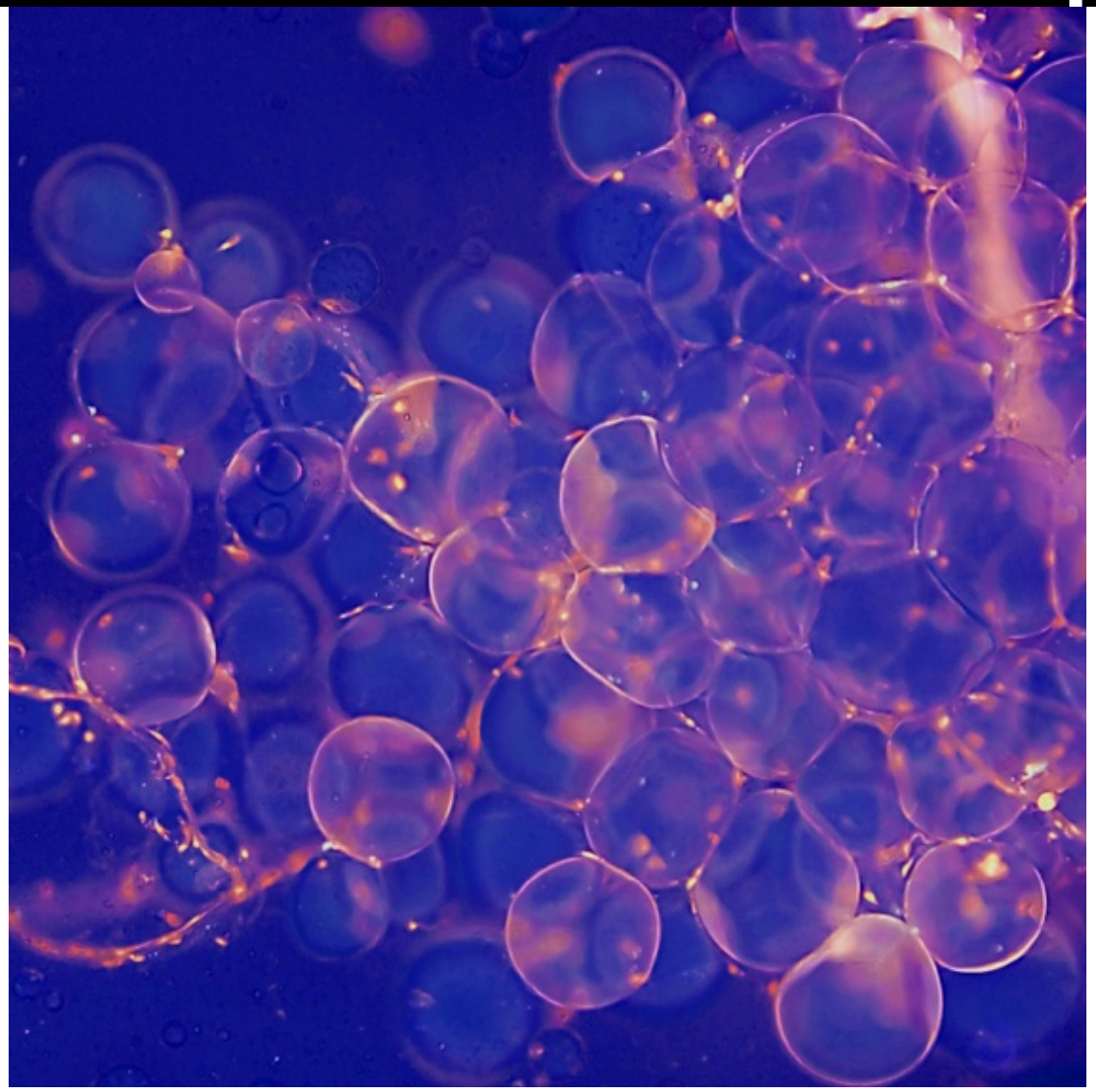
Fat Under The Microscope



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The Power Is In The Pericytes

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Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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A Population of Multipotent CD34-Positive Adipose Stromal Cells Share Pericyte and Mesenchymal Surface Markers, Reside in a Periendothelial Location, and Stabilize Endothelial Networks

Dmitry O. Traktuev, Stephanie Merfeld-Clauss, Jingling Li, Mikhail Kolonin, Wadih Arap, Renata Pasqualini, Brian H. Johnstone and Keith L. March

Circ. Res. 2008;102;77-85; originally published online Oct 25, 2007;
DOI: 10.1161/CIRCRESAHA.107.159475
Circulation Research is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0009-7330. Online ISSN: 1524-4571

Cell Separation

- Fat is harvested using standard tumescent liposuction techniques.
- The connective tissue is dissolved using collagenase and proteinase.
- The fat cells are then separated from the stromal cells using centrifugation.
- The stromal cells are then passed through a flow cytometer to measure viability and count the numbers.

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Collagenase Protocols

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MACS

Special protocol for sample preparation

Preparation of the stromal vascular fraction (SVF) from human lipoaspirate

Contents

1. Description
   - 1.1 Background information
   - 1.2 Principle of SVF preparation from human lipoaspirate
   - 1.3 Reagent and instrument requirements
2. Protocol
3. References
4. Appendix

1. Description

Resolve enzyme at 37 °C in a water bath.

▲ Note: Please see 4. Appendix for a table detailing the conversion of other catalytic units to Wünsch units.

- Enzyme stop medium: Dulbecco's Modified Eagles Medium (DMEM, # 130-091-437) containing 20% fetal bovine serum (FBS).
- NH Expansion Medium (# 130-091-680).
- 500 mL Screw Cap Conical Bottom Centrifuge Tubes (Corning # 431123).
- 1 L storage bottles (e.g. Corning # 430518).
- 50 mL conical tubes (e.g. BD Biosciences # 352070).
- 75 cm^2 cell culture flasks (e.g. BD Biosciences # 353136).
- 100 µm cell strainer (e.g. BD Biosciences # 352360)

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What Is In Fat?

Cells

Adipocytes, Pericytes, Fibroblasts, Myoblasts, Osteoblasts, Progenitor Cells, Lymphocytes, Stem Cells, endothelial cells

Cytokines

VEGF	• Vascular Endothelial Growth Factor
EGF	• Epidermal Growth Factor
IGF-1	• Insulin-like Growth Factor -1
bFGF	• Fibroblast Growth Factor Basic
aFGF	• Fibroblast Growth Factor Acidic
TGF-b1	• Transforming Growth Factor Beta 1
Laminin	• Stimulates Basal Lamina Growth for Nerves
Etc, etc	

What Are Stem Cells?

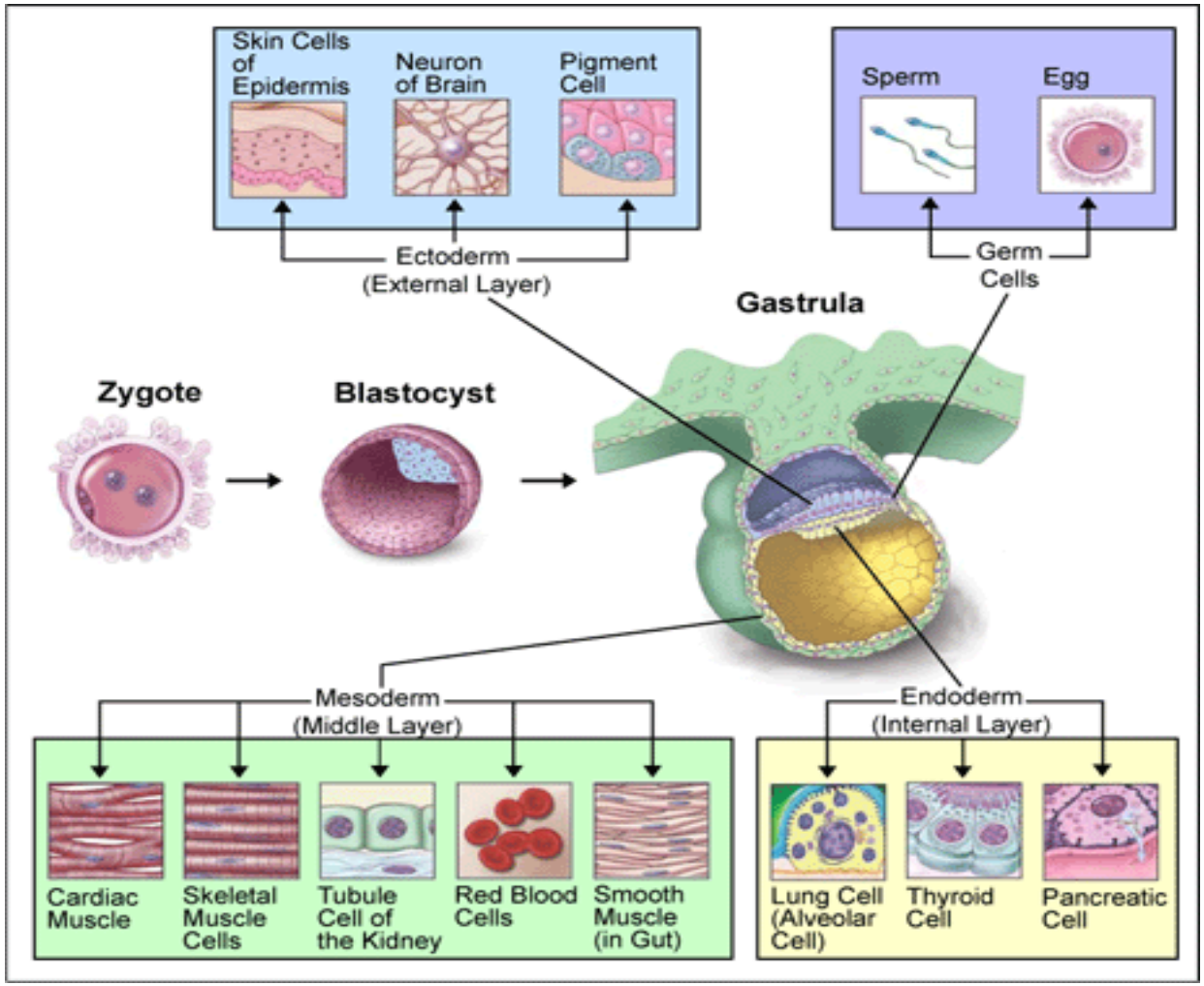
Embryonic Stem Cells

Combining a sperm and an egg allows the creation of every new type of cell in the body.

Adult Stem Cells

Once we have every kind of cell that is needed, the major role of stem cells becomes repair and regeneration with replacement of cells that are beyond repair.

Triploblasts: Three Germ Layers



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Potency

Totipotent

(a.k.a omnipotent) stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable, organism. These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent.

Pluripotent

Stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers.

Multipotent

Stem cells can differentiate into a number of cells, but only those of a closely related family of cells.

Oligopotent

Stem cells can differentiate into only a few cells.

Unipotent

Cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).

Ethics

- Embryonic stem cells
  - Kill an embryo
- Induced pluripotent stem cells
  - Can be made from somatic cells - ESCs
  - Myoblasts can make bone
- Adult stem cells
  - Ethically acceptable
  - In clinical use
- Stem cell banks

Fat And Bone Marrow

Fat contains seven times the number of stem cells as are found in Bone Marrow. Fat has the same number of stem cells as cord blood. The volume of fat collected is far greater than what can be harvested from bone marrow or cord blood.

Stem cells from bone marrow need to be cultured to increase the numbers. A typical injection contains 1 to 5 million cells.

Stem cells from fat typically number 20 to 60 million and do not need to be increased in culture.

Fat And Bone Marrow

- Both are mesenchymes derived from all three germ layers.
- Both are capable of making bone, cartilage, muscle and nerves
- Bone marrow cells age, fat stromal cells do not
- Fat is easy to harvest and available in large volumes.
- Harvesting fat improves the appearance of the donor area.
- Harvesting fat improves health for many people by reducing fat volume

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A Useful Article

STEM CELLS®

TISSUE-SPECIFIC STEM CELLS

Concise Review: Adipose Tissue-Derived Stromal Cells—Basic and Clinical Implications for Novel Cell-Based Therapies

ANDREAS SCHÄFFLER, CHRISTA BÜCHLER

Department of Internal Medicine I, University of Regensburg, Regensburg, Germany

Key Words. Adipocyte • Adipose tissue • Mesenchymal stem cell • Tissue engineering • Cell therapy

ABSTRACT

Compared with bone marrow-derived mesenchymal stem cells, adipose tissue-derived stromal cells (ADSC) do have an equal potential to differentiate into cells and tissues of mesodermal origin, such as adipocytes, cartilage, bone, and skeletal muscle. However, the easy and repeatable access to subcutaneous adipose tissue and the simple isolation procedures provide a clear advantage. Since extensive reviews focusing exclusively on ADSC are rare, it is the aim of this review to describe the preparation and isolation procedures for ADSC, to summarize the molecular characterization of ADSC, to describe the differentiation capacity of ADSC, and to discuss the mechanisms and future role of ADSC in cell therapy and tissue engineering. An initial effort has also been made to differentiate ADSC into hepatocytes, endocrine pancreatic cells, neurons, cardiomyocytes, hepatocytes, and endothelial/vascular cells. Whereas the lineage-specific differentiation into cells of mesodermal origin is well understood on a molecular basis, the molecular key events and transcription factors that initially allocate the ADSC to a lineage-specific differentiation are almost completely unknown. Decoding these molecular mechanisms is a prerequisite for developing novel cell therapies. STEM CELLS *2007;25: 818–827*

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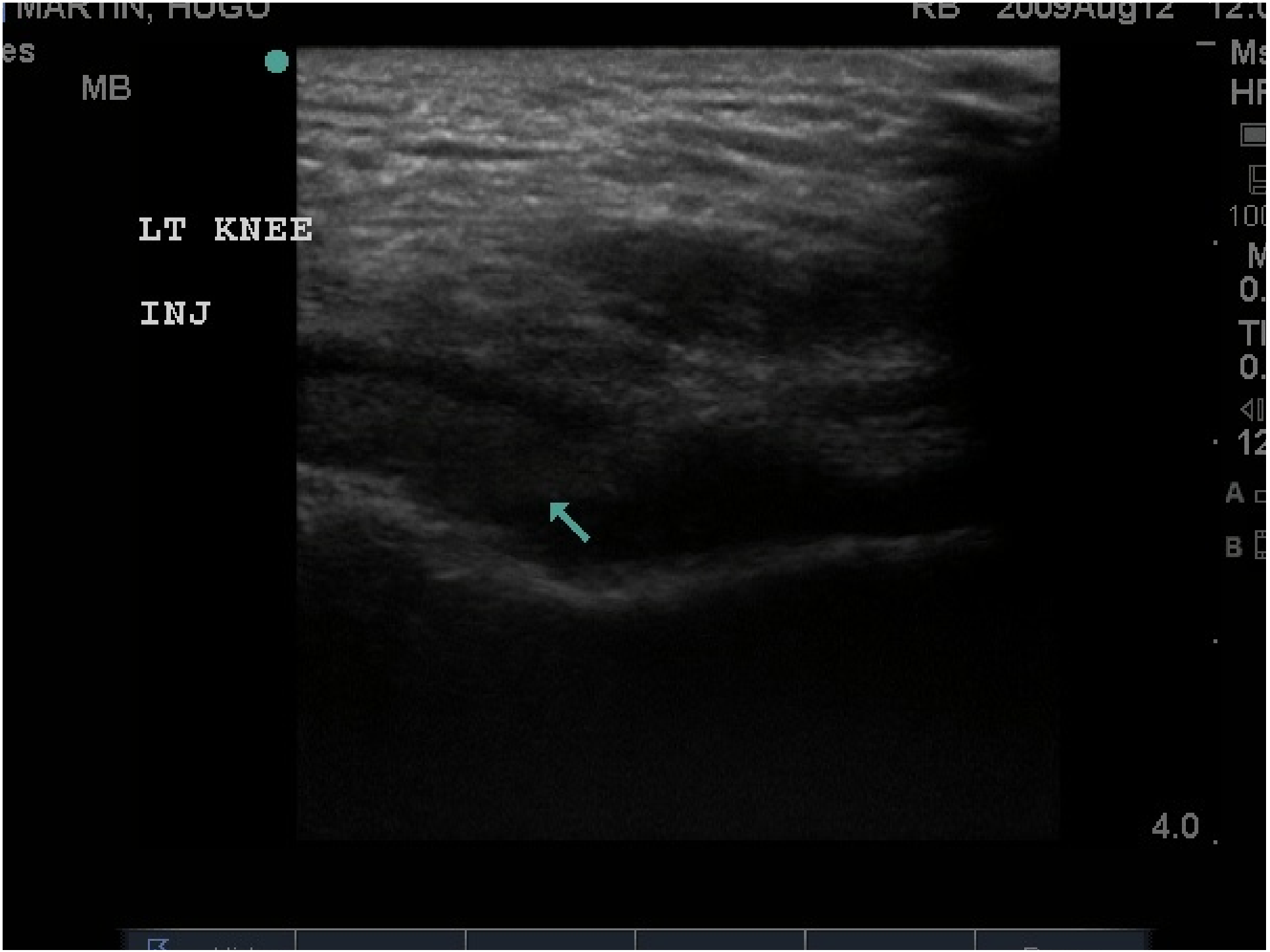
Cells vs No Cells Intra Articular

- Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees.

S. Wakitani*, K. Imoto†, T. Yamamoto†, M. Saito†, N. Murata† and M. Yoneda‡

- Although the clinical improvement was not significantly different, the arthroscopic and histological grading score was better in the cell-transplanted group than in the cell-free control group.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- On the 17th of April 2009 we performed our first stem cell transfer
- We believe this is the first person in Australia to be treated with adipose derived stem cells
- We have now treated eleven people. All have improved. None have had any significant adverse events

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Adverse And Other Effects

- Two patients had transient joint swelling lasting up to 2 weeks
- All spine pain improved some totally
- Asthma in remission
- Rh. arthritis in remission
- Tendonitis pain free
- Depression resolved

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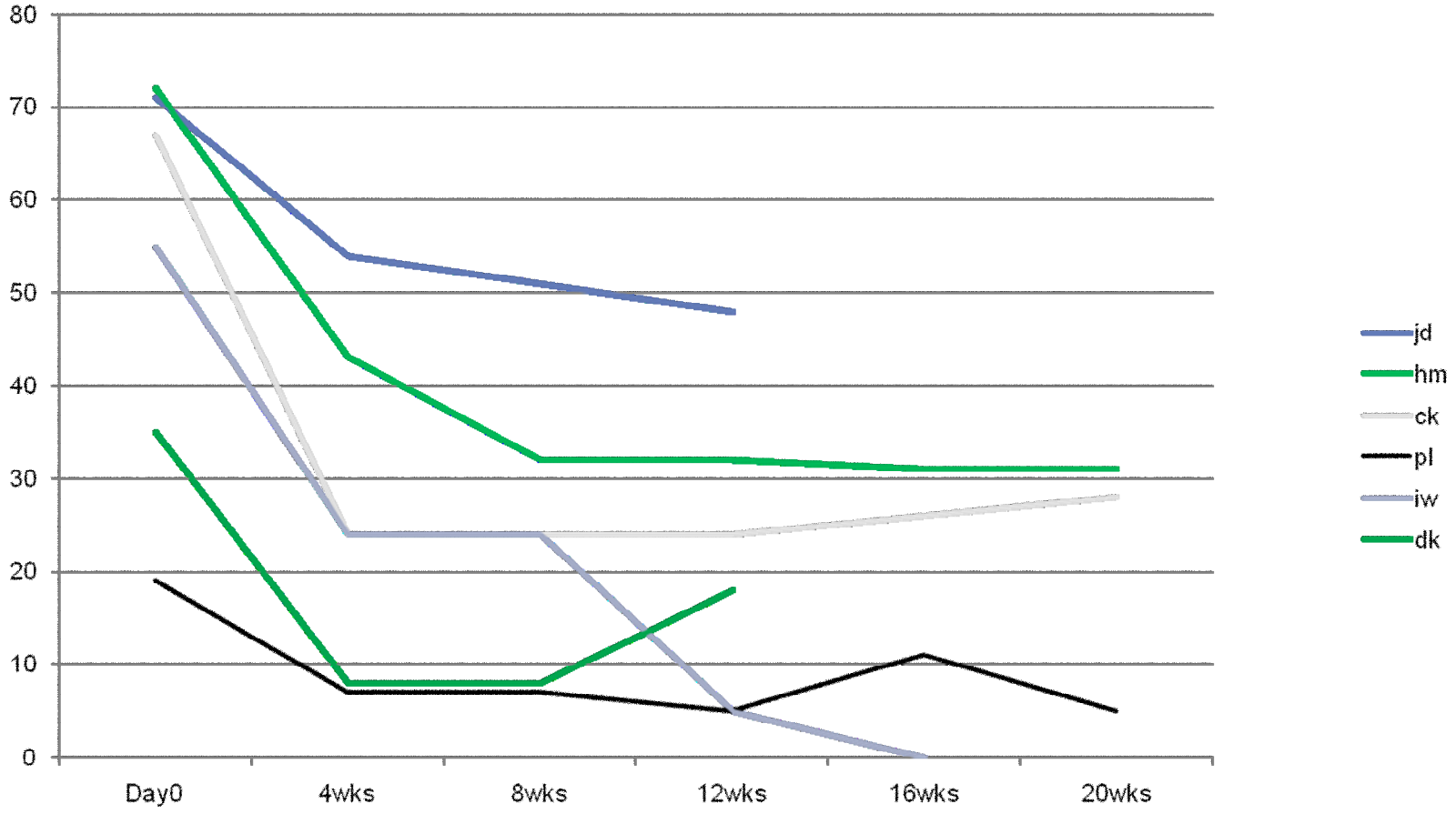
Womac



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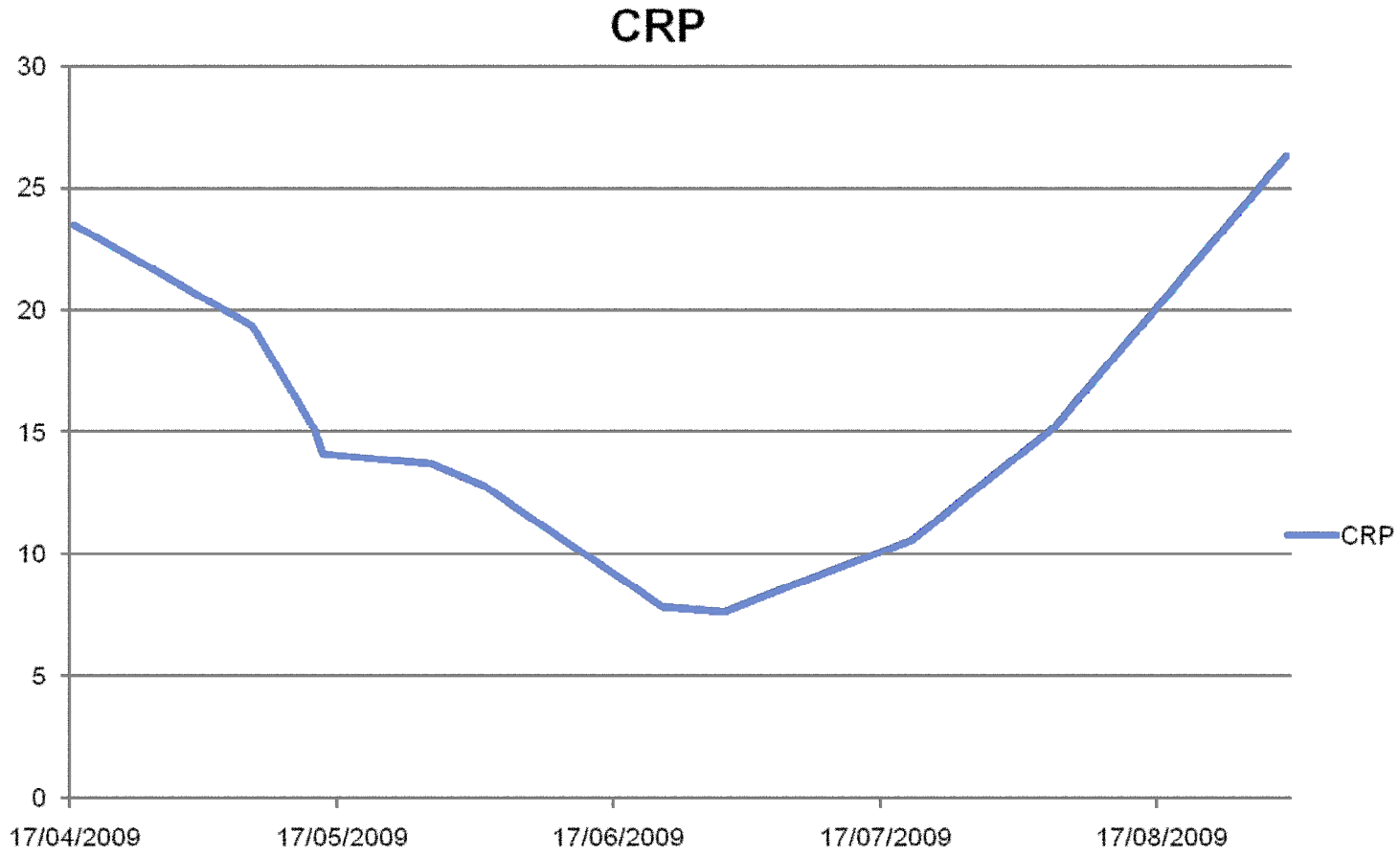
Change In Systemic Inflammation



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What We Can Do Now?

- We are able to isolate all the cells from fat
- It is safe to inject these isolated cells into joints
- Joints with cartilage cover can become pain free and fully mobile
- Distant joints can become fully mobile and pain free
- Systemic illness (Rheumatoid, Asthma) can improve

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What Next?

- I need to simplify the cell separation procedure
- Streamlining the process will make it less expensive
- Other applications need exploration
- Other doctors need to be shown that it is safe and effective and how to use these cells.

Uses So Far

Osteoarthritis	– very successful unless bone on bone
Rheumatoid Arthritis	– some improvement
Asthma	– good response
Rotator cuff syndrome	– good response
Multiple Sclerosis	– Bristol study
	– Ben Lahey, Canberra
Diabetes	– India
Parkinsons	– Xcell centre
Cerebral Palsy	– Xcell centre, 70

Our Future As Stem Cell Doctors

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Our Future?

As a physician or patient, what does the future look like because of the ICMS? The year is 2011, hundreds of physicians around the country have opened safe, adult stem cell labs as part of their medical practices. These labs are credentialed through the ICMS. Because of our efforts, adult stem cell therapy is now commonly available and patients with hundreds of diseases can consult with physicians they know and trust. ICMS initiatives are working with insurers and payors to establish guidelines and reimbursement.

The alternative? Big pharma controls stem cell therapy, not your doctor. Patients wait 5-10 years while the drug approval process grinds out for each medical indication. This approval process for the patient's own adult stem cells doesn't increase safety, only bureaucratic red tape. If the patient happens to have a disease where a business model isn't likely to be profitable, no stem cell therapy is on the horizon. Your doctor's hands are tied by the system. What kind of future do you choose?

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The Future For Stem Cell Therapy

Can we inject the spine?

Should we inject into a vein?

Should we inject into an artery?

Many questions will be answered

How many stem cells do we need?

What is the role of the associated cytokines?

What other diseases will respond?

Should we manipulate these cells before injection?

Can we donate stem cells?

Etc, etc

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Double Edged Sword?

- Cortisone was the miracle drug of the 70's
- Stem cells are the miracle treatment of this decade
- So far all promise with no drawbacks

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What Can You Do?

- Fat is easy to collect
- Collagenase dissociation is a short learning curve
- Fluorescent microscopy
- Subcutaneous injection
- Ultrasound guided joint injection
- IV injection

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