IV.G. MUSCULOSKELETAL TISSUE ENGINEERING
Cartilage

M. Spector, Ph.D.
Arthroscopic Debridement

“Microfracture”

Osteochondral Plug Autograft ("Mosaicplasty")

Current Clinical Practice

Autologous chondrocytes injected under a periostal flap (Genzyme; “Carticel”)

Total Knee Replacement

30 years

Future Clinical Practice

Implementing Tissue Engineering

Implantation of a cell-seeded matrix

Stem cells from bone marrow infiltrate the defect

Implantation of the matrix alone, or supplemented with growth factors or genes for the GFs

ELEMENTS FOR TISSUE ENGINEERING

Tissue Engineering Triad*

• CELLS
• MATRIX (INSOLUBLE REGULATOR)
  – Porous, absorbable biomaterials
• SOLUBLE REGULATORS
  – Cytokines (Growth Factors)

Environmental Factors

• Mechanical loading

* Used individually or in combination, but probably always best with a matrix (i.e., with a biomaterial)

ARTICULAR CARTILAGE

Limits to Regeneration

• Avascular (and aneural)
• Relatively low cell density
• Cells of low mitotic activity
• Cells cannot freely migrate
Normal Canine Articular Cartilage

Untreated Defect 6 months

What was the source of the cells?

How to improve regeneration?
• Add cells?
• Add scaffold?
• Add growth factors?
TISSUE ENGINEERING

Cells

- Autologous, allogeneic, or xenogeneic
- Differentiated cell of the same tissue type or another tissue type, or stem cell

Autologous Chondrocyte Implantation

This process has been commercialized by Genzyme (for USD$11,500).

Collagen membrane to replace a periosteal tissue graft to contain injected autologous chondrocytes (grown in culture)

Debridement

Implantation of a collagen membrane to contain injected autologous chondrocytes

Autologous Chondrocyte Implantation

Autologous Chondrocyte Implantation

Figure 4. Histologic Section from a Biopsy Specimen Obtained 36 Months after Surgery in a 19-Year-Old Man with a Femoral Condylar Defect (Patient 16).

ROLES OF BIOMATERIALS IN TISSUE REGENERATION

Membranes
- Prevent the collapse and infiltration of surrounding tissue into the defect.
- Contain cells in a defect.
- Serve as a carrier for cells.

**PERIOSTEUM STIMULATES SUBCHONDRAL BONE DENSIFICATION IN AUTOLOGOUS CHONDROCYTE TRANSPLANTATION IN SHEEP**

Results also showed no difference in the make-up of the cartilaginous reparative.

M Russlies, et al., Cell and Tiss. Res. 319:133;2005

**Graph:**
- Comparison of subchondral bone percentage among different treatment groups:
  - Control
  - Chondro-Gide®
  - Periosteum

M Russlies, et al., Cell and Tiss. Res. 319:133;2005
ROLES OF BIOMATERIALS IN TISSUE REGENERATION

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MATRIX-INDUCED AUTOLOGOUS CHONDROCYTE IMPLANTATION

MACI
The defect area is covered with tissue-engineered collagen membrane which is pre-loaded with autologous chondrocytes.

Canine Study
Autologous Chondrocyte Implantation

“Microfracture”:
Stem cells from bone marrow infiltrate the defect

Implantation of the matrix alone,
(or supplemented with growth factors or genes for the GFs)

Implantation of a cell-seeded matrix

Cells injected under periosteum

Cells harvested

Cells isolated *

Cells cultured *

* by Genzyme Biosurgery

Future Clinical Practice
Implementing Tissue Engineering

Implantation of a cell-seeded matrix

Periosteum harvest site

Cells suspended *
CELL-SEEDED COLLAGEN MATRICES

- Chondral defects (to the tidemark)
- Type II (porcine) collagen scaffold
- Seeded with cultured autologous chondrocytes (CAC)

CANINE ACI STUDY TREATMENT GROUPS

- Empty Control
- Periosteum
- Fibrin Glue
- Autologous Chondrocytes

AUTOLOGOUS CHONDROCYTE-SEEDED COLLAGEN MATRIX

- Cells seeded into the matrix 24 hours and 4 weeks prior to implantation

Seeding of Collagen Matrices with CAC

- Harvest Cartilage
- Incubate cells with matrix
- Culture chondrocytes in monolayer

Collagen discs
9 mm diam x 3 mm thick


Chondral defect immediately postoperative. Arrow shows perforation of calcified cartilage and subchondral bone (SCB).

Defects treated by autologous chondrocyte implantation, 6 months postoperative.


1.5 mo. Fibrous tissue
3 mo. Hyaline cartilage (some articular cartilage), fibrocartilage, and fibrous tissue
6 mo. Art. cart. and fibrocartilage
12 mo. Degraded tissue

Tissue that formed after 3 and 6 months did not function longer term. Is the problem a lack of fill or the tissue types comprising the material?

AUTOLOGOUS CHONDROCYTE IMPLANTATION

15 Wks Post-op, Mean, n=5-10

Conclusion: A cell-seeded matrix is better than the current method of ACI.

Implantation of Cells Alone or in a Type II Collagen Matrix

Summary of Results: Canine Model


N. Veilleux

% Original Defect Area

Untreated Control CAC Alone CAC/ Collagen II <12 hr Mean, n=5-10

Conclusion: A cell-seeded matrix is better than the current method of ACI.

% Original Defect Area

Untreated Control CAC Alone CAC/ Collagen II <12 hr CAC/ Collagen II 4 wk

Summary of Results: Canine Model


N. Veilleux

100 µm

+FGF-2

50 µm
P2 Canine Chondrocytes in Type II Collagen Scaffold (carbodiimide x-linked), 2 weeks in culture, Safranin-O Stain for GAG (N. Veilleux) +FGF-2

Saf-O

H&E

P2 Canine Chondrocyte-Seeded Type II Collagen (CD x-linked), 2w +FGF-2

Normal Canine Articular Cartilage

Type II Collagen-GAG (Carbodiimide X-L) Saf O staining

SF+FGF-2

Chondrocytes, 2 wks

SF+TGF-β1

N. Veilleux

S. Vickers

MSCs, 3 wks (SF+IGF-1)

C. Guo

Future Clinical Practice
Implementing Tissue Engineering

Implantation of a cell-seeded matrix

Implantation of the matrix alone, (or supplemented with growth factors or genes for the GFs)

“Microfracture”: Stem cells from bone marrow infiltrate the defect
**Summary of Results: Canine Model**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>% Original Defect Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Control</td>
<td>100%</td>
</tr>
<tr>
<td>CAC Alone</td>
<td>30%</td>
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<tr>
<td>CAC/Collagen II 24 hr</td>
<td>90%</td>
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<tr>
<td>CAC/Collagen II 4 wk</td>
<td>70%</td>
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<td>µfx Alone</td>
<td>80%</td>
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<tr>
<td>µfx/Collagen II</td>
<td>50%</td>
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</tbody>
</table>

**Microfracture**

- The microfracture-treated defect is covered with a collagen membrane.

**Autologous Matrix Induced Chondrogenesis (AMIC)**

The microfracture-treated defect is covered with a collagen membrane.