REGENERATION OF JOINT TISSUES
Cartilage

M. Spector, Ph.D.
Total Knee Replacement Prosthesis

- Co-Cr Alloy
- Bone
- Polyethylene
- Bone
- Bone

Knee Joint
- Bone
- Art. Cart.

Meniscus, Ligament

Medical illustration removed due to copyright restrictions.

Implant photo removed due to copyright restrictions.
INTRAARTICULAR JOINT TISSUES

• What are the unique characteristics of the joint environment?
• Why don’t these tissues heal?
• How are such diverse functions met by only one structural protein - collagen?
INTRAARTICULAR ENVIRONMENT

- Synovial fluid
- High mechanical loads
- Low vascularity
JOINT TISSUES

Limitations to Healing

• Absence of a fibrin clot
  – Absent or low vascularity
  – Dissolution of clot in synovial fluid

• Cell migration restricted by matrix

• Low cell density

• Low mitotic activity

• Mechanical loading disrupts reparative tissue
## TISSUES COMPRISING JOINTS

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Permanent Prosthesis</th>
<th>Regeneration Scaffold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Articular cartilage</td>
<td>No</td>
<td>Yes*</td>
</tr>
<tr>
<td>Meniscus</td>
<td>No</td>
<td>Yes*</td>
</tr>
<tr>
<td>Ligaments</td>
<td>No</td>
<td>Yes*</td>
</tr>
<tr>
<td>Synovium</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

* In the process of being developed
## JOINT TISSUES

<table>
<thead>
<tr>
<th>Loading Type</th>
<th>Tissue Type</th>
<th>Cell Type</th>
<th>Round/ Lac. Coll. PG Vasc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meniscus</td>
<td>C/T Cart.</td>
<td>Fibro- Fibro- Yes I 0/+ 0* 0</td>
<td></td>
</tr>
<tr>
<td>ACL</td>
<td>Tens. Tissue</td>
<td>Fibrous Fibro- No I 0 0** 0</td>
<td></td>
</tr>
</tbody>
</table>

* Inner third
** Mid-substance
Several slides on structure of cartilage removed due to copyright restrictions. (Medical illustrations.)
Micro-fracture

Osteochondral Plug Autograft ("Mosaicplasty")

Autologous chondrocytes injected under a periosteal flap (Genzyme; "Carticel")

Current Clinical Practice

30 years

Arthroscopic Debridement

Figure by MIT OpenCourseWare.
Future Clinical Practice
Implementing Tissue Engineering

Implantation of a cell-seeded matrix

Figure by MIT OpenCourseWare.

Microfraz

Stem cells from bone marrow infiltrate the defect

Implantation of the matrix alone, or supplemented with growth factors or genes for the GFs
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 ~$20,000).

Image removed due to copyright restrictions.

http://content.nejm.org/cgi/content/abstract/331/14/889

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

Image and embedded video removed due to copyright restrictions.
Autologous Chondrocyte Implantation

Image removed due to copyright restrictions.

Membranes

• Prevent the collapse and infiltration of surrounding tissue into the defect.

• **Contain cells in a defect.**

• **Serve as a carrier for cells.**
Autologous Periosteal Flap as a cover on the defect to contain the cells

Image removed due to copyright restrictions.
Fig. 2 in M Russlies, et al.
*Cell and Tiss. Res.* 319:133;2005
Results also showed no difference in the make-up of the cartilaginous reparative.
Membranes

- Prevent the collapse and infiltration of surrounding tissue into the defect.
- Contain cells in a defect.
- Serve as a carrier for cells.
MATRIX-INDUCED AUTOLOGOUS CHONDROCYTE IMPLANTATION

MACI

The defect area is covered with tissue-engineered collagen membrane which is pre-loaded with autologous chondrocytes.
Future Clinical Practice
Implementing Tissue Engineering

Implantation of a **cell-seeded matrix**

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

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

Autologous Chondrocyte Implantation

- Periosteum harvest site
- Cells harvested
- Cells isolated *
- Untreated defects
- Cells injected under periosteum
- Cells cultured *
- Cells suspended *

* by Genzyme Biosurgery
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
EC

Periosteum
Alone
P

Cultured
Autologous
Chondrocytes
CAC

4mm

Suture

Fibrin Glue

Periosteum

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

Diagram removed due to copyright restrictions.

Collagen discs
9 mm diam x 3 mm thick
Defects treated by autologous chondrocyte implantation, 6 months postoperative


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

See H. Breinan, M. Spector et al. JOR 2001;19:482

12 mo. Degraded tissue

6 mo. Art. cart. and fibrocartilage

3 mo. Hyaline cartilage (some articular cartilage), fibrocartilage, and fibrous tissue

1.5 mo. Fibrous tissue

AUTOLOGOUS CHONDROCYTE IMPLANTATION
Implantation of Cells Alone or in a Type II Collagen Matrix

Cultured Autologous Chondrocytes

% Original Defect Area

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

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

No diff. after 12 mo.


Conclusion: A cell-seeded matrix is better than the current method of ACI
Summary of Results: Canine Model


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

% Original Defect Area

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

Hyaline  Fibrocartilage  Fibrous

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

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

Normal Canine Articular Cartilage
Type II Collagen-GAG (Carbodiimide X-L) Safranin O staining

SF+FGF-2
Chondrocytes, 2 wks
N. Veilleux

SF+TGF-β1
S. Vickers

MSCs, 3 wks (SF+IGF-1 )
C. Guo

6mm
Future Clinical Practice
Implementing Tissue Engineering

Implantation of a cell-seeded matrix

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

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

**CANINE MICROFRACTURE STUDY**

**TREATMENT GROUPS**

- Microfracture
- Microfracture + Collagen Implant

4 mm

- AC
- Bone
- Collagen (II) film
- Collagen (II) sponge

Summary of Results: Canine Model

- Untreated
- Control
- CAC Alone
- CAC/ Collagen II 24 hr
- CAC/ Collagen II 4 wk
- μfx Alone
- μfx/ Collagen II

15 Wks Post-op, Mean, n=6

★ Procedures currently used
Autologous Matrix Induced Chondrogenesis

AMIC

The microfracture-treated defect is covered with a collagen membrane.
Several slides removed due to copyright restrictions.

- Medical illustrations of knee joint, with focus on cartilage surfaces and ligaments.
- Meniscus collagen architecture (cutaway diagram)
- Mechanical force analysis for femoral and tibial surfaces.
- Directional properties of meniscus (stress vs. strain graph)
- Histology photos of meniscus tissues: vascularity, fibrochondrocytes, Transmission Electron Microscopy and Polarized Light Microscopy
- Diagram of typical meniscal tear patterns, and arthroscopic view of a complex posterior horn meniscal tear (see http://www.orthoassociates.com/SP11B39/)
Regeneration of Meniscal Cartilage with Use of a Collagen Scaffold. Prelim. Data

• Collagen scaffold as a template for the regeneration of meniscal cartilage

• 10 patients in a clinical feasibility trial (FDA-approved)
  – The goal of the study was to evaluate the implantability and safety of the scaffold as well as its ability to support tissue ingrowth.
  – The study based on in vitro and in vivo investigations in dogs that demonstrated cellular ingrowth and tissue regeneration through the scaffold.
  – Nine patients remained in the study for at least thirty-six months.
Photograph of the collagen meniscal implant.

Images removed due to copyright restrictions.

Scanning electron micrograph of a cross section of the collagen meniscal implant.

The sizes and shapes of the meniscal lesions as well as the menisci after placement of the collagen meniscal implant.

Photo removed due to copyright restrictions.
Drawings showing insertion and suturing of the collagen meniscal implant.

Two drawings removed due to copyright restrictions.

The collagen scaffold was implantable and safe over 3-yrs.

Histologically, it supported regeneration of tissue in meniscal defects of various sizes.

No adverse immunological reactions were noted.

At 3 or 6 months after implantation, gross and histological evaluation revealed newly formed tissue replacing the implant as it was resorbed.
Regeneration of Meniscal Cartilage with Use of a Collagen Scaffold. Prelim. Data

• At 3 yrs., the 9 pts. reported a decrease in symptoms.
• A scale assigned 1 point for strenuous activity and 5 points for an inability to perform sports activity
  – The average score was 1.5 points before the injury
  – 3.0 points after the injury and before the operation
  – 2.4 points at six months postoperatively
  – 2.2 points at twelve months
  – 2.0 points at twenty-four months
  – 1.9 points at thirty-six months.
• Scale assigned 0 points for no pain and 3 points for severe pain
  – The average pain score was 2.2 points preoperatively
  – 0.6 point 3- yrs. postoperatively.
Regeneration of Meniscal Cartilage with Use of a Collagen Scaffold. Prelim. Data

- One patient, who had had a repair of a bucket-handle tear of the medial meniscus and augmentation with the collagen scaffold, had retearing of the cartilage nineteen months after implantation. Another patient had debridement because of an irregular area of regeneration at the scaffold-meniscus interface twenty-one months after implantation.

- Magnetic resonance imaging scans demonstrated progressive maturation of the signal within the regenerated meniscus at three, six, twelve, and thirty-six months. These findings suggest that regeneration of meniscal cartilage through a collagen scaffold is possible. Additional studies are needed to determine long-term efficacy.
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