Regenerative Medicine: a role in cartilage repair

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Structure of the knee

Front view of right knee

Side View of Knee

Femur

Anterior cruciate ligament

Lateral meniscus

Lateral collateral ligament

Fibula

Medial collateral ligament

Medial meniscus

Posterior cruciate ligament

Tibia

Articular cartilage (on end of bone)

Quadriceps muscle

Prepatella bursa

Patella

Infrapatella bursa

Meniscus
Joint disease & disability

- Cartilage has a poor capacity for self-repair.

- Degenerative joint disease (arthritis) follow 50% of articular cartilage injuries.

- Osteoarthritis and degenerative joint disease affect 3 million people p.a.

- 10,000 patients/year (UK) suffer cartilage damage which needs repair

- No effective pharmacological alternatives to orthopaedic surgery

- Current treatments have limitations

- Huge economic burden (1-2% GDP)
Normal Articular Cartilage

- **Superficial zone**
  - Function: "biological shock absorber".
  - One cell type: chondrocyte.
- **Deep zone**
  - Function dependent on correct ECM structure.
  - No direct blood supply.
  - Poor capacity for self repair.

Calcified cartilage

Gwynn et al. J Microscopy 2000;197:159-172
Articular cartilage repair

- **Problem:**
  - Cartilage lesions
- **Classical Approaches:**
  - Joint washout and debridement
    - Removes loose tissue debris, symptomatic relief.
  - Surface abrasion and microfracture.
    - Pain relief, 'scar tissue' (fibrocartilage) formed which is not durable.
  - Total joint replacement.
    - Prosthetic loosening (5% may need revision surgery).
Revision surgery

Knee revision prosthesis

www.orthogate.org/patient-education
Regenerative medicine

- Emerging interdisciplinary field of research and clinical applications.
- Focussed on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function.
  - Congenital defects, disease, trauma, aging
- Uses a combination of technological approaches
  - Natural and synthetic biomaterials, soluble molecules, gene therapy, cell/tissue transplantation, cellular reprogramming.
Regenerative medicine

Simplistically:

- Implantation of appropriate cells alone.
- Implantation of cells on a biomaterial support
- Implantation of ‘smart’ biomaterial to direct \textit{in vivo} regeneration of tissue.
- Implantation of a ‘neo’ tissue grown in the laboratory.
Knee prosthesis

Current therapies
Surface abrasion, micro-fracture

Regenerative medicine
ACI, MACI
Cartilage transplantation
Tissue Engineering.

Disability

Arthritic disease
Cartilage injury

Trauma

biomaterials
Revision

Expert tissues
The University of Sheffield.
Mosaicplasty (Autologous Cartilage Transplantation)

Removal of healthy, uninvolved cartilage from one site to surgically repair a defect (e.g. mosaicplasty):

Procedure is not without problems and risks (donor site morbidity, inadequate supply, non-integration at site of surgery, etc.).

Autologous Chondrocyte Implantation (ACI):

1. Remove healthy cartilage biopsy (200-300mg).
2. Isolate cartilage cell and expand cell numbers in monolayer culture (15-20x10^6 cells) in the laboratory.
3. Clean and remove damaged cartilage.
4. Suture sheet of periosteum over defect.
5. Introduce chondrocyte suspension to defect.

Rehabilitation with strict regime. Patient not full weight bearing for 10-12 weeks.
Cartilage repair summary

- **Mosaicplasty** restores tissue architecture:
  - limited to small defects, and concerns regarding donor site morbidity.
- **ACI** good clinical outcome in 65-85% patients.
  - dependent on the site and number of lesions. 50% patients form fibrocartilage/scar tissue repair- (not durable).
  - upto 2y needed to obtain ‘mature’ cartilage.
  - expensive:-must be 70-100% more effective than microfracture for QoL benefit at 2y, but only 10-20% more effective if QoL maintained 10y.
  - insufficient clinical trial data for decision by NICE.
Tissue engineering

“The use of biological and engineering principles to construct functional tissues to replace or supplement diseased or defective body parts.”

Yorkshire Biomaterials Network (2000)

Living cells + biomaterial support or scaffold, combined in vitro with biologically active substances to form functional tissues for subsequent therapeutic application.
Tissue engineering of cartilage grafts

Cells seeded onto scaffolds

Constructs are cultured in chondrogenic medium

Cartilage tissue
Why use a scaffold?

- Provides structural framework
  - Permit accurate “3D moulding/shaping”.

- Cell adhesion

- Exhibit appropriate mechanical properties.
  - For articular cartilage, appropriate mechanical properties could allow early weight bearing without compromising the cartilage graft.

- ‘Smart’-to assist formation of desired tissue:-
  - Surface chemistry/ “bioactivity” can be modified to enhance cell response.
  - Scaffold design.
Requirements for a successful cartilage graft?

- **Cartilage-forming (chondrogenic) cells.**
  - Chondrocytes from cartilage biopsy.
  - Mesenchymal stem cells (MSCs) (e.g. from bone marrow and fat).

- **Suitable support/scaffold for matrix formation.**

- **Synthesis of appropriate tissue matrix.**
  - Component composition
  - Architecture/structure to ensure appropriate mechanical properties
Biodegradable Polymers Used in Tissue Engineering

- **Synthetic:**
  - PLLA-PGA
  - Polyurethanes
  - Polycarbonates
  - Polymers
  - Polycaprolactone

- **Natural:**
  - Collagen
  - Fibrin
  - Chitosan
  - Hyaluronic acid
  - Alginate gels
  - Agarose
  - Silks
Esterified hyaluronan: HYAFF 11®

Hyaluronic acid is a major component of natural cartilage

Commercially sourced from rooster combs.

Carboxyl groups esterified with alcohols to produce biopolymers.

Scaffold types: non-woven fibre, (fibre diameter 10-15 μm) and sponge scaffolds (pore size 150-300 μm, 400-500 μm).
**PolyActive®**

\[
\left(\text{O-CH}_2\text{-CH}_2\right)_n\text{O-} - \left[ \text{O(\text{CH}_2)_4\text{-O-}} \times \right] x \quad \left[ \text{PBT} \right]_y
\]

*Flexible, swelling*  

*Strength*

MW of PEG\_weight\_x/PBT\_weight\_y

- Biodegradable foams, pore size around 200\(\mu\)m.
- 3D fibre deposition method ("printed fibre" scaffolds), pore size around 500\(\mu\)m.
- Properties depend on % weight & MW of PEG.
- Can produce scaffolds with dynamic stiffness and equilibrium modulus similar to native cartilage.
**Silks**

- Proteinaceous filaments
  - Fibroin in silkworm silk
  - Spidroin in spider silks.
- Very resistant to tensile and compressive forces.
- Native silkworm silk is immunogenic needs to be treated to remove protein coating (sericin).
- Ordered β-sheet regions and “disordered regions”
Cartilage-like tissues grown on various polymer scaffolds

- SPCL nanofibres
- Chitosan-PBS
- Polyactive™
- Spider silk
- PGA
- HYAFF 11®
Lubricin Distribution

Control     Lubricin

Native Cartilage

Engineered cartilage
Mechanical properties of TE cartilage

- The frictional response of the TE cartilage was closer to that of native cartilage.
- No damage was observed after completion of the friction test.
- Deformation of the TE cartilage indicated a better retention of the interstitial water.
Matrix composition of Polyactive™ and HYAFF 11®/chondrocyte constructs

![Graph showing the composition of Polyactive and HYAFF 11 constructs.]

- **Polyactive**
  - Total GAG
  - Total Collagen

- **HYAFF 11**
  - Total GAG
  - Total Collagen

- Collagen II (% of total collagen)

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The University Of Sheffield.
Pericellular matrix distribution in non-woven HYAFF 11® and Polyactive™

HYAFF 11®

Polyactive™

Green staining: -collagen VI, pericellular matrix
Blue staining: -DAPI, cell nucleii
Scaffold morphologies
Extracellular matrix production on foam and fibre forms of HYAFF 11®
Pericellular matrix (collagen VI) distribution in cartilage constructs

- HYAFF 11® felt (x200)
- HYAFF 11® Sponge (x200)
- Non specific staining (x100)
Matrix Composition of human articular chondrocyte/HYAFF 11® constructs

<table>
<thead>
<tr>
<th></th>
<th>Total collagen (%dry wt.)</th>
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<tbody>
<tr>
<td>Non-woven HYAFF 11^R (fibre)</td>
<td>16.02 (2.16)</td>
</tr>
<tr>
<td>HYAFF 11^R 400-500(\mu)m (Sponge)</td>
<td>31.18 (2.35)</td>
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</tbody>
</table>
Cell sources for cartilage regeneration

- Biopsy of native cartilage
  - Used for ACI, MACI.
  - Risks of donor site morbidity and tissue arthritic.
  - Cells lose their phenotype (“identity”) during culture period to increase cell numbers.
Cell sources for cartilage regeneration

- **Mesenchymal progenitor/stem cells.**
  - **Sources:**
    - Adult tissues-various
    - Early stage embryos
  - **Good proliferative and chondrogenic potential, non-arthritic.**
  - **Stem cells need to be “matured”/differentiated into cartilage-forming cells.**
Stem cell sources for cartilage regeneration

- **Bone marrow**
  - currently one of most researched sources.
  - reported to hypertrophy and mineralise *in vivo* (Pelttari et al. Arthritis and Rheum 2006).
  - subchondral drilling to release BM-MSCs yields fibrocartilage.

- **Joint Tissues**
  - articular cartilage
Stem cells from joint fluid (synovial fluid)

Adipogenic
[Oil Red O]

Osteogenic
[Alcian Red]

Arthritis cartilage
[Oil Red O]
[Alcian Red]

5 10 15 20
Sublingual

SF
BM

[Alcian Blue]
Collagen II

Chondrogenic

Population doublings

Days in culture

Comparison of joint fluid, bone marrow MSCs and chondrocytes

<table>
<thead>
<tr>
<th>Chondrogenic media</th>
<th>Osteogenic media</th>
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<tbody>
<tr>
<td>Control</td>
<td>Alkaline Phos</td>
</tr>
<tr>
<td>Collagen II</td>
<td>Alizarin Red</td>
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**SF-MSC Constructs**

**Matched**

**BM-MSC Constructs**

Proteoglycan content:
- **SF-MSCs**: 84-111 µg
- **Chondrocytes**: 507-801 µg

Alkaline phosphatase:
- **SF-MSCs**: ND
- **Chondrocytes**: ND

Scale bars = 200 µm
Challenge to Regenerative Medicine Therapies

- Deliver safe, effective, affordable therapies to patients within a reasonable time frame.
- Involves handling/processing living cells aseptically to produce a living cell/tissue product.
- Current technology takes days-weeks.
- Need for 'smart' scaffolds to aid cell binding, maturation, tissue formation.
Acknowledgements

SHEFFIELD
Ana McIntosh
Astrid Frazer
Sarah Fraser
Jen Mundy
Marie Plainfosse
Paul Hatton

DUBLIN
Danny Kelly (IT).
Mary Waller
Patrick Prendergast

BASEL
Ivan Martin

OXFORD
Fritz Volrath
David Knight
Christine Ortlepp

UPPSALA
Wilhelm Engstrom

FUNDING:- European Commission under the 5th Framework Programme
(SCAFCART project GRDR1-1999-00050, SPIDERMAN project GRDR1-2001-40464).
6th Framework Programme (Network of Excellence “EXPERTISSUES” project).

INDUSTRIAL PARTNERS
Fidia Advanced Biopolymers
IsoTis BV (NV).
SPINOX (UK)
Smith & Nephew (UK)