Regenerative Medicine:-a role in cartilage repair

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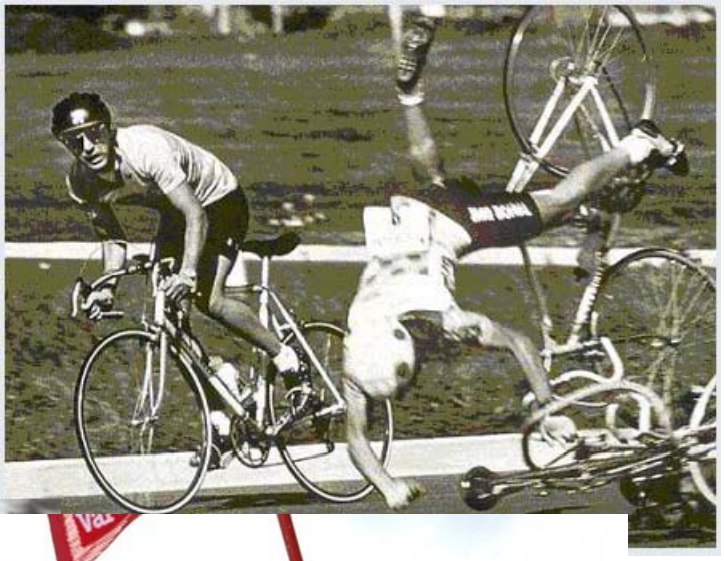
EXPERTISSUES



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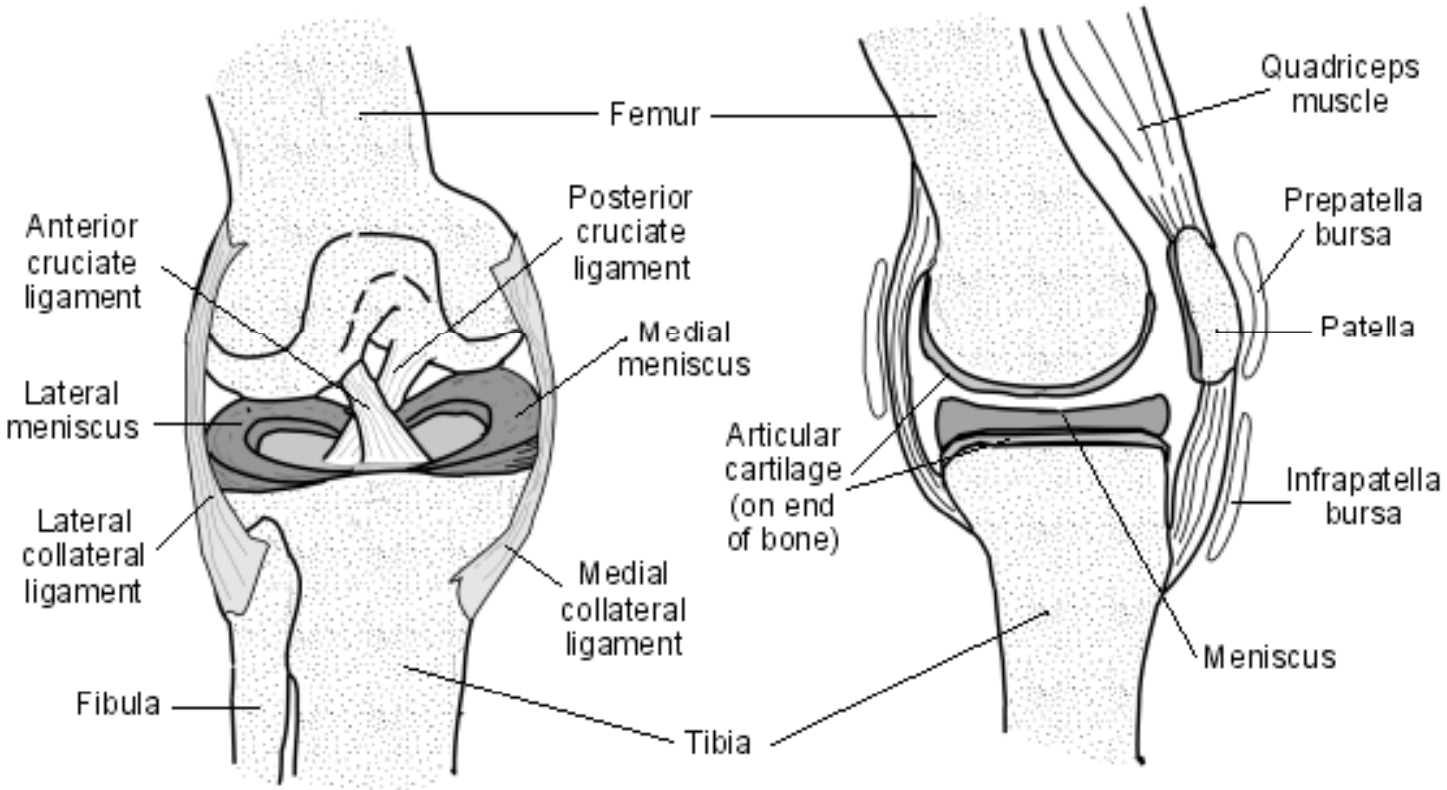
Structure of the knee



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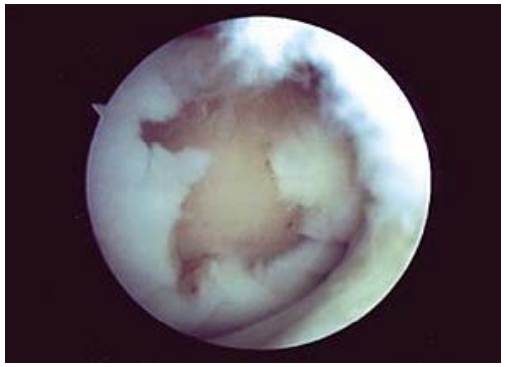
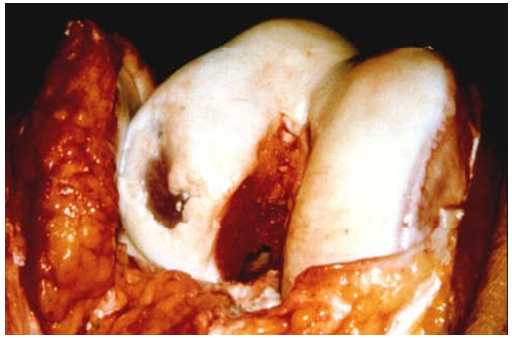
Joint disease & disability





- 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)

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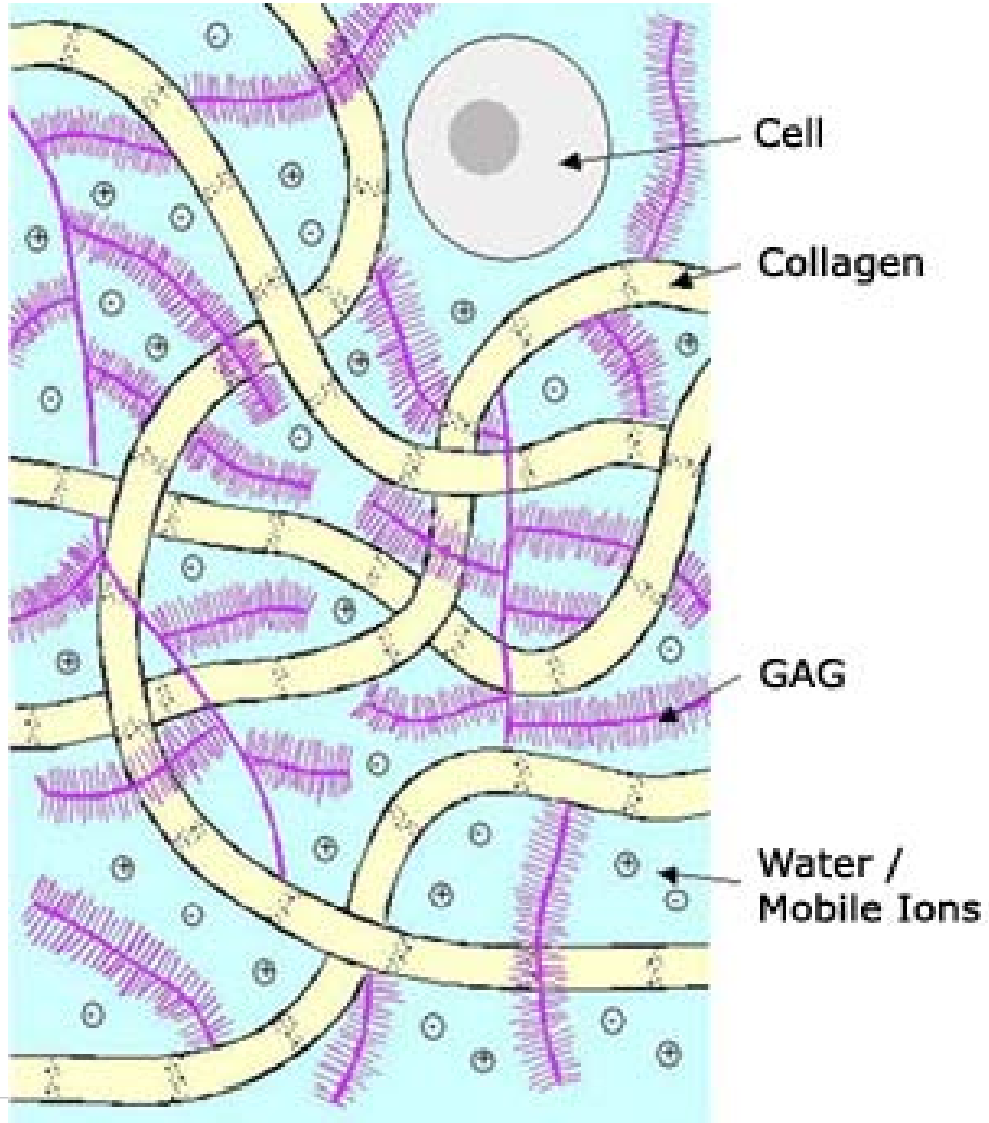
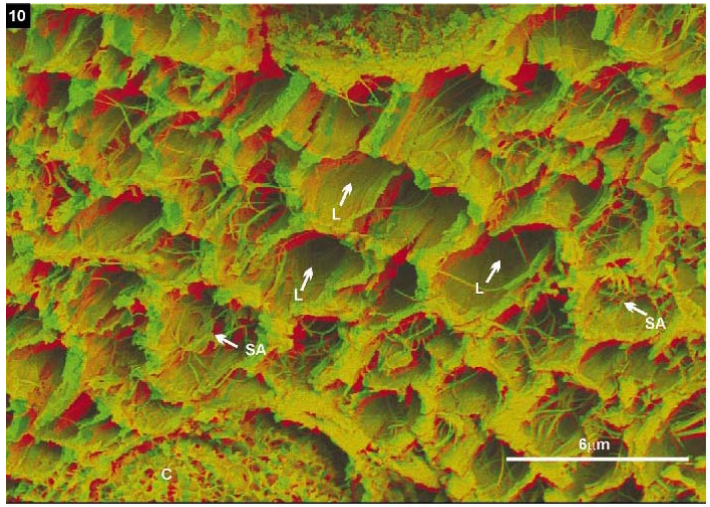
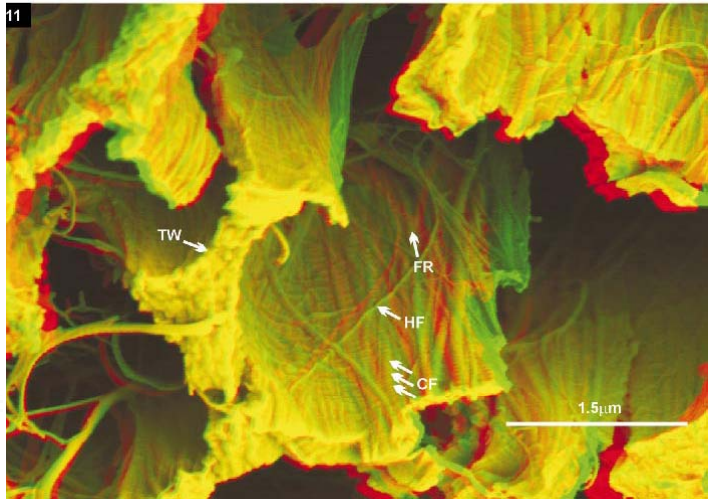
Normal Articular Cartilage







Gwynn et al. J Micoscopy 2000;197:159-172

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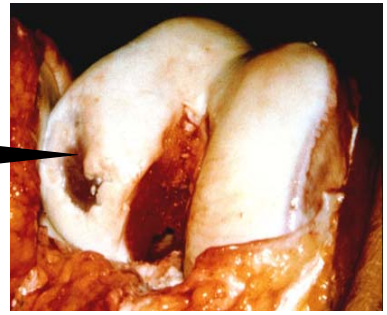
Articular cartilage repair

- Problem:
  - Cartilage lesions

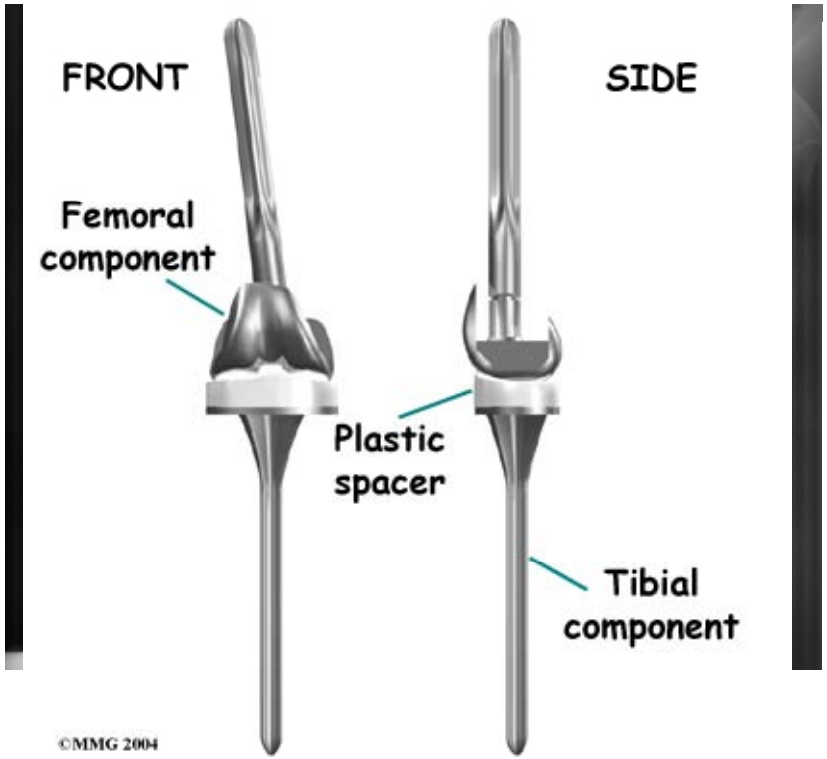


- Classical Approaches:-
  - Joint washout and debridement
    - Removes loose tissue debris, symptomatic relief.
  - Surface abrasion and microfracture.
    - Pain relief, 'scar tissue' (fibrocartilage) formed which not durable.
  - Total joint replacement.
    - Prosthetic loosening (5% may need revision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

Stryker's Partial Knee Resurfacing Implant

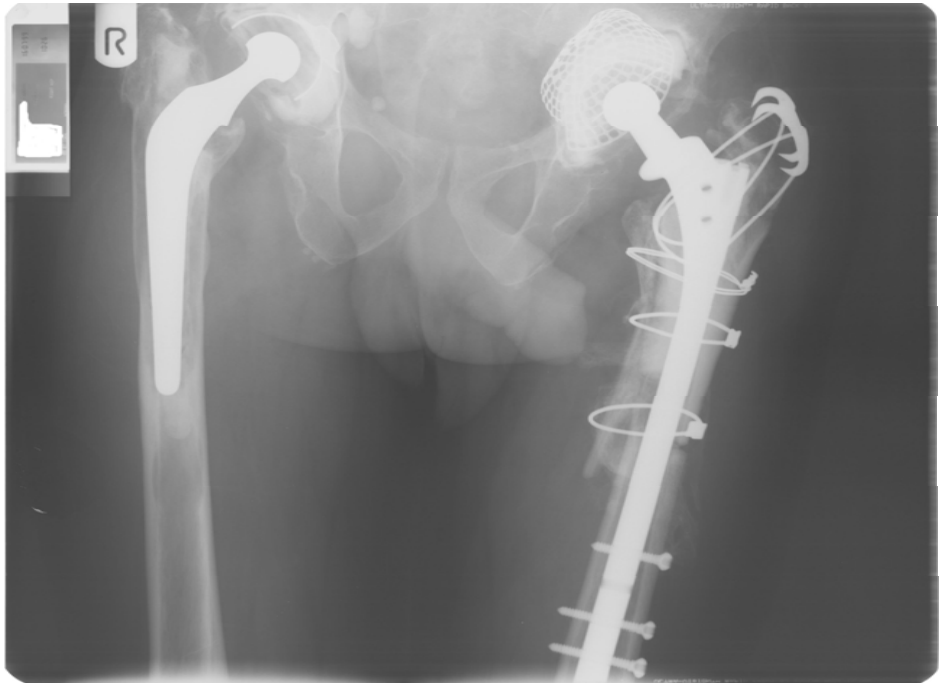
Stryker's Total Knee Implant

Revision surgery



Knee revision prosthesis

www.orthogate.org/patient-education



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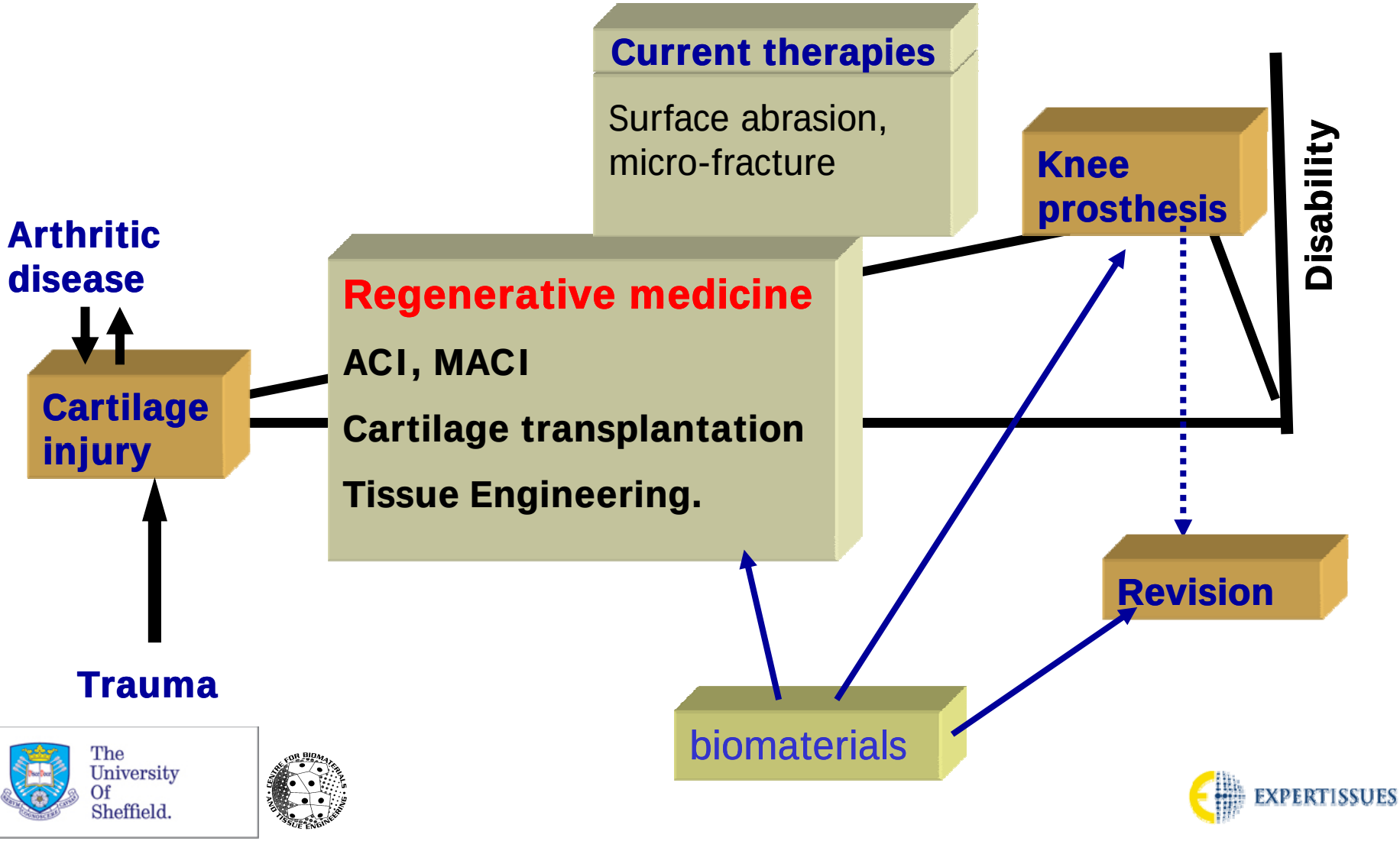
Regenerative medicine

- 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 *in vivo* regeneration of tissue.
- Implantation of a 'neo' tissue grown in the laboratory.


Current therapies
Surface abrasion, micro-fracture
Knee prosthesis
Disability
Arthritic disease
Regenerative medicine
ACI, MACI
Cartilage transplantation
Tissue Engineering.
Cartilage injury
Revision
Trauma
biomaterials
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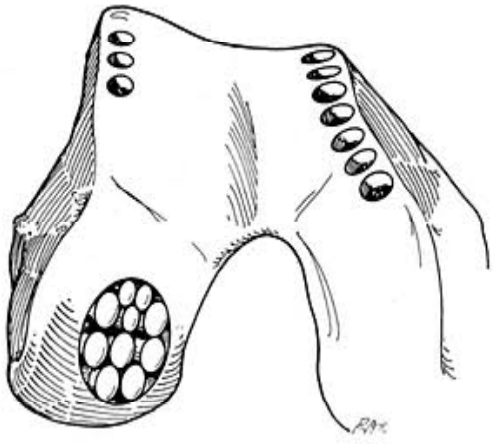
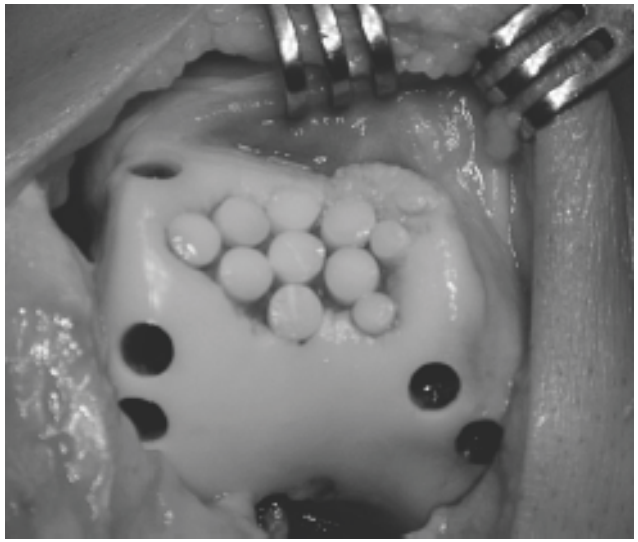
Mosaicplasty (Autologous Cartilage Transplantation)

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





Szerb I *et al.* Bull Hosp Jt Dis. 2005; 63:54-62

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

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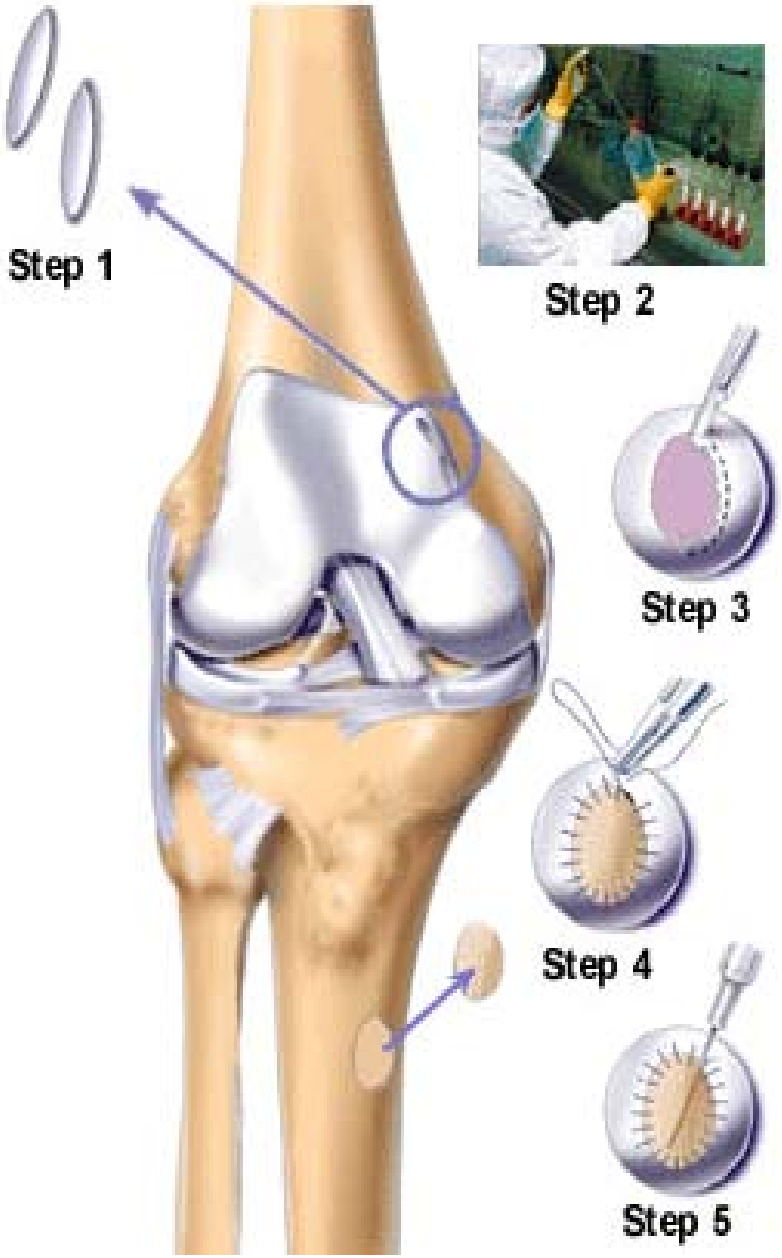

Autologous Chondrocyte Implantation (ACI):

1. Remove healthy cartilage biopsy (200-300mg).
2. Isolate cartilage cell and expand cell numbers in monolayer culture (15-20x10^6cells) 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.

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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.

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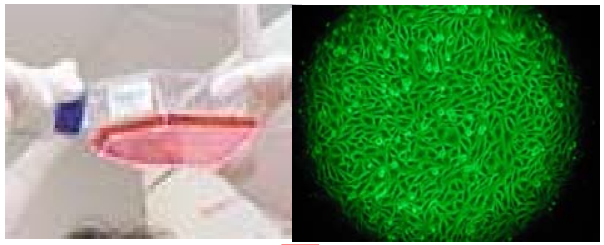
Tissue engineering of cartilage grafts



Cells seeded onto scaffolds

Constructs are cultured in chondrogenic medium

Cartilage tissue

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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.

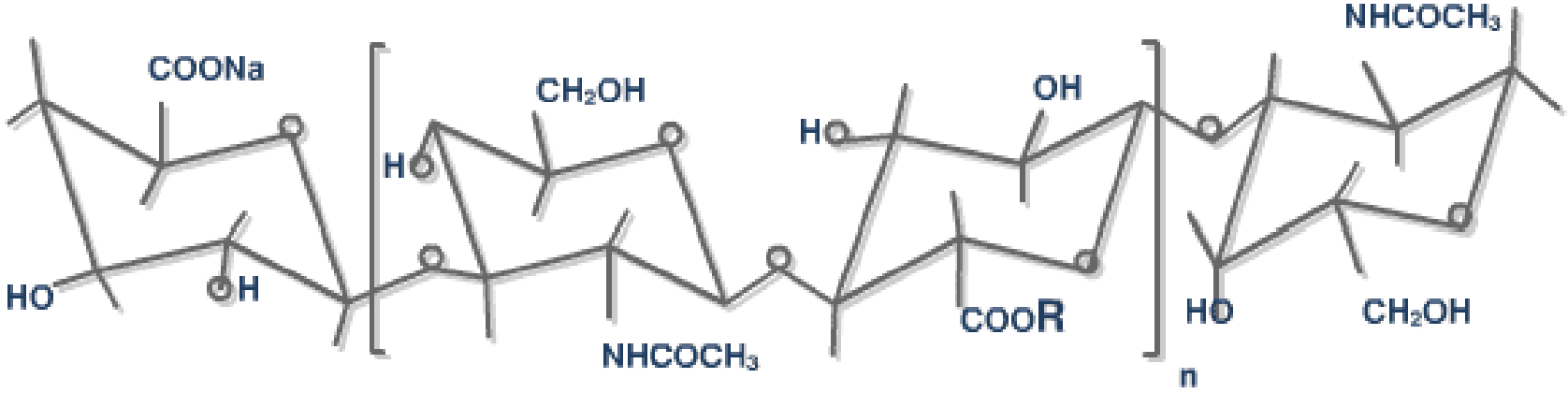
Requirements for a successful cartilage graft?

- 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
  - Polyfumarates
  - PEGT-PBT block co-polymers
  - Polycaprolactone
- ***Natural:***
  - Collagen
  - Fibrin
  - Chitosan
  - Hyaluronic acid
  - Alginate gels
  - Agarose
  - Silks

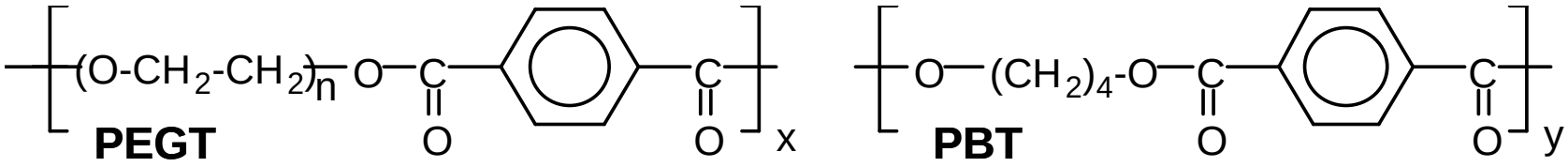
Esterified hyaluronan:-HYAFF 11®



R= alcohol residue

- 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®



Flexible, swelling *Strength*

MW of PEG$PEGT_{weight\%x}/PBT_{weight\%y}$

- Biodegradable foams, pore size around 200μm.
- 3D fibre deposition method ("printed fibre" scaffolds), pore size around 500μm.
- 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"

Bombyx mori

Nephila edulis

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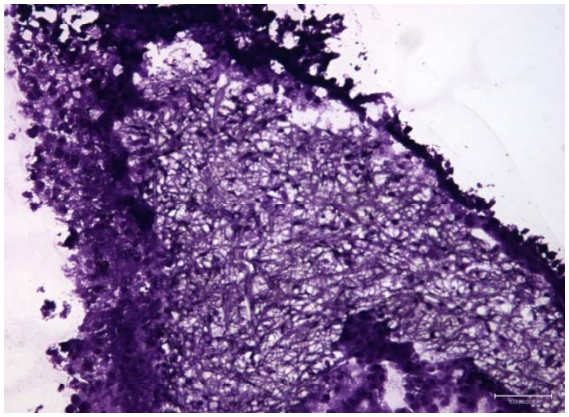
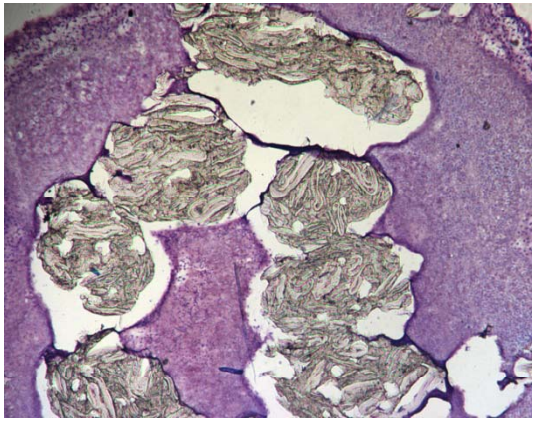
Cartilage-like tissues grown on various polymer scaffolds

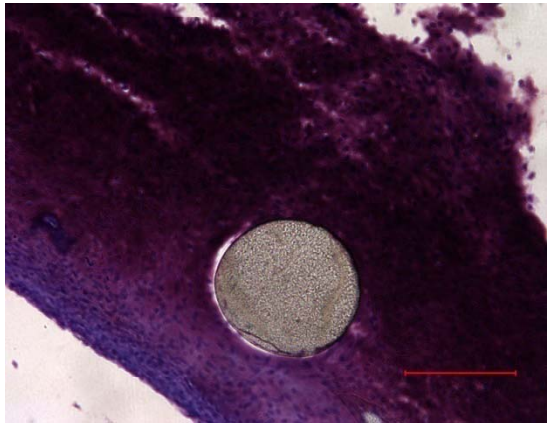
SPCL nanofibres



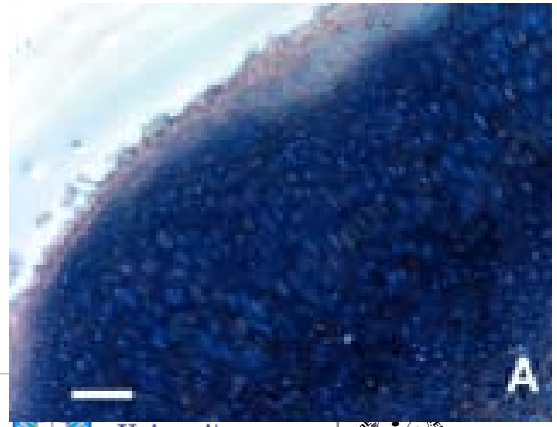
Chitosan-PBS



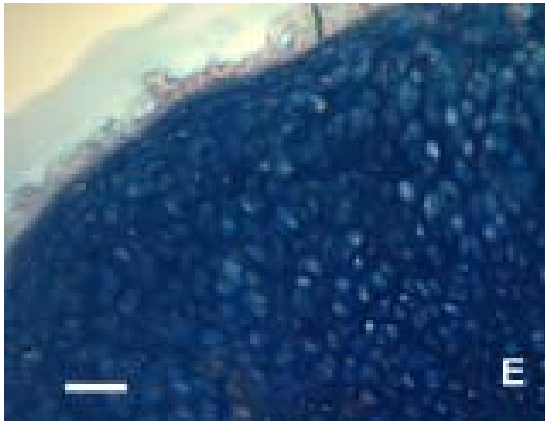
Polyactive™



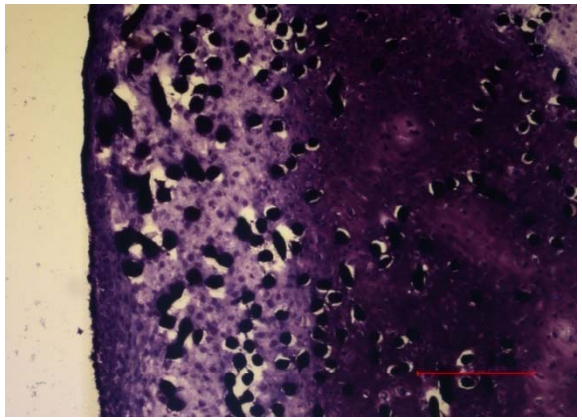
Spider silk



PGA



HYAFF 11®



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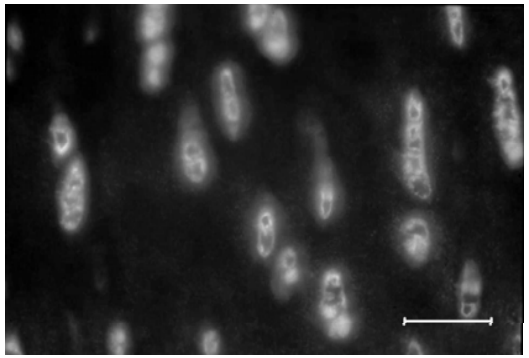
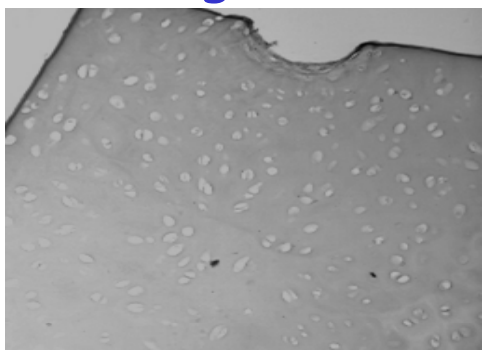
Collagen VI

Collagen II

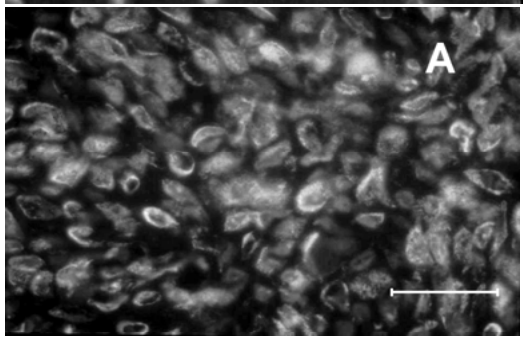
Native

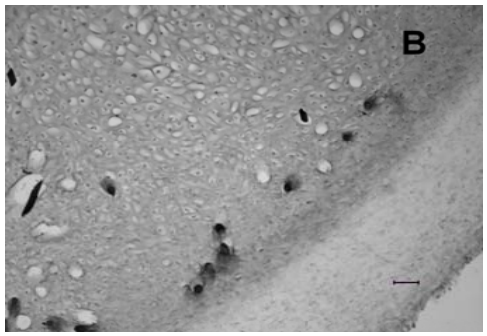




Native

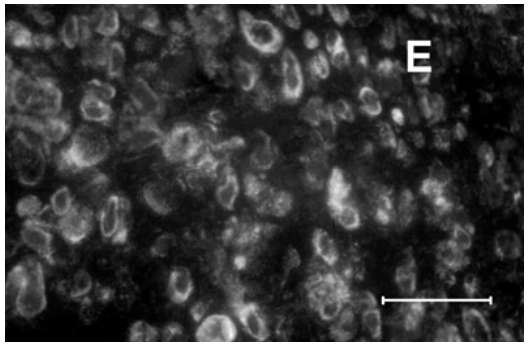
Construct
(bovine cells)

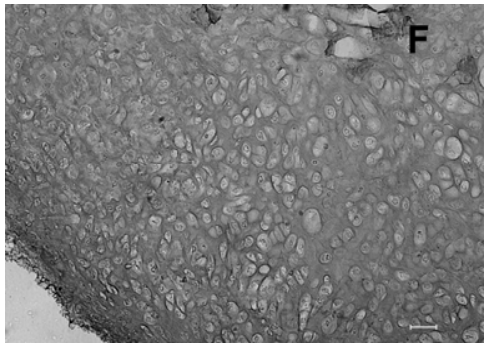




Construct
(bovine cells)

Construct
(human cells)





Construct
(human cells)

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Scale bars=25μm

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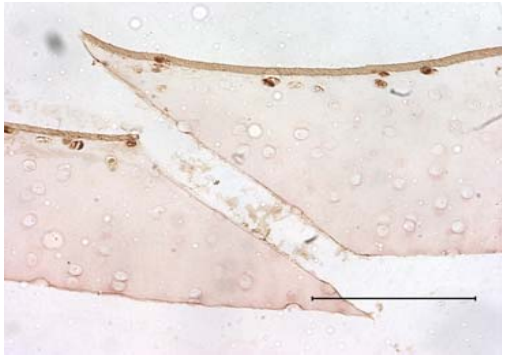
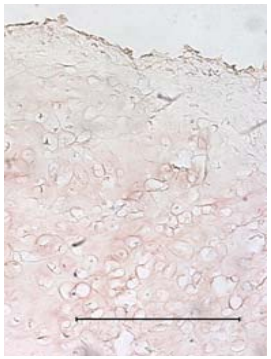
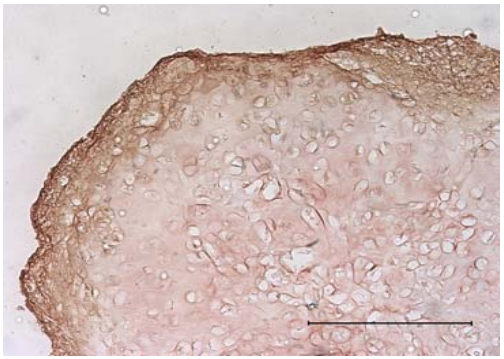
Lubricin Distribution

	Control	Lubricin
Native Cartilage		
Engineered cartilage		

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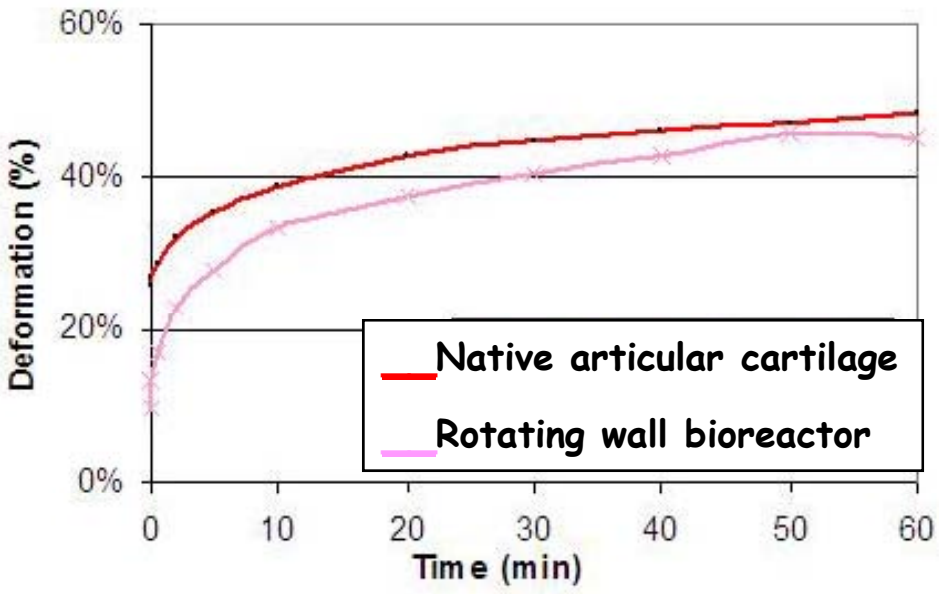
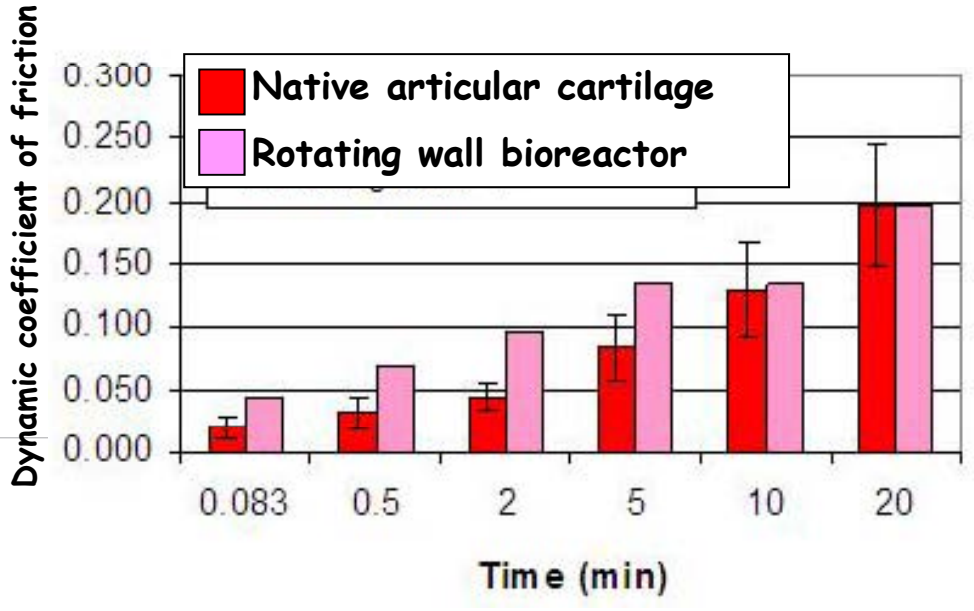
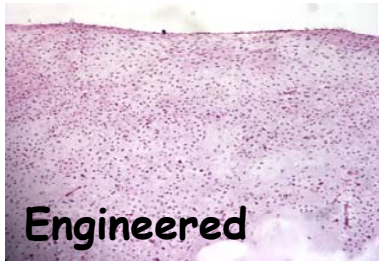
Mechanical properties of TE cartilage





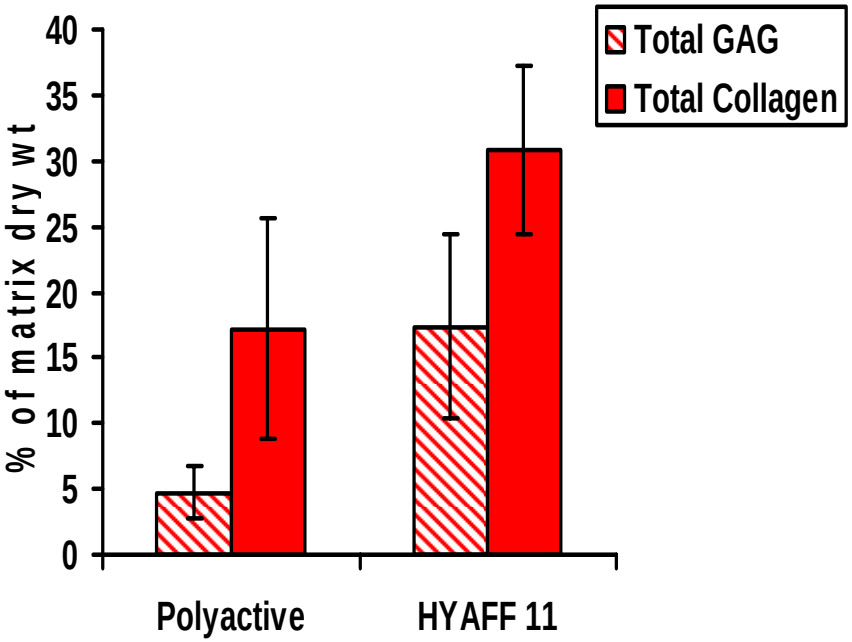
After testing

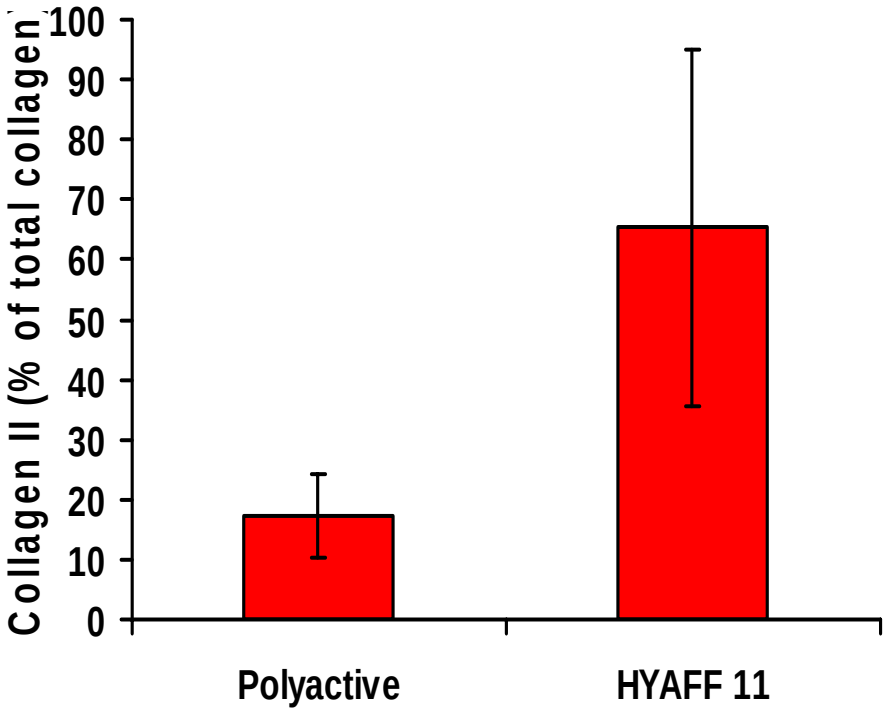


- **The frictional response of the TE cartilage was closer to that of native cartilage.**
- **No damage was observed after completion of the friction test.**
- **Deformation of the TE cartilage indicated a better retention of the interstitial water.**

EXPERTISSUES

Matrix composition of Polyactive™ and HYAFF 11®/chondrocyte constructs





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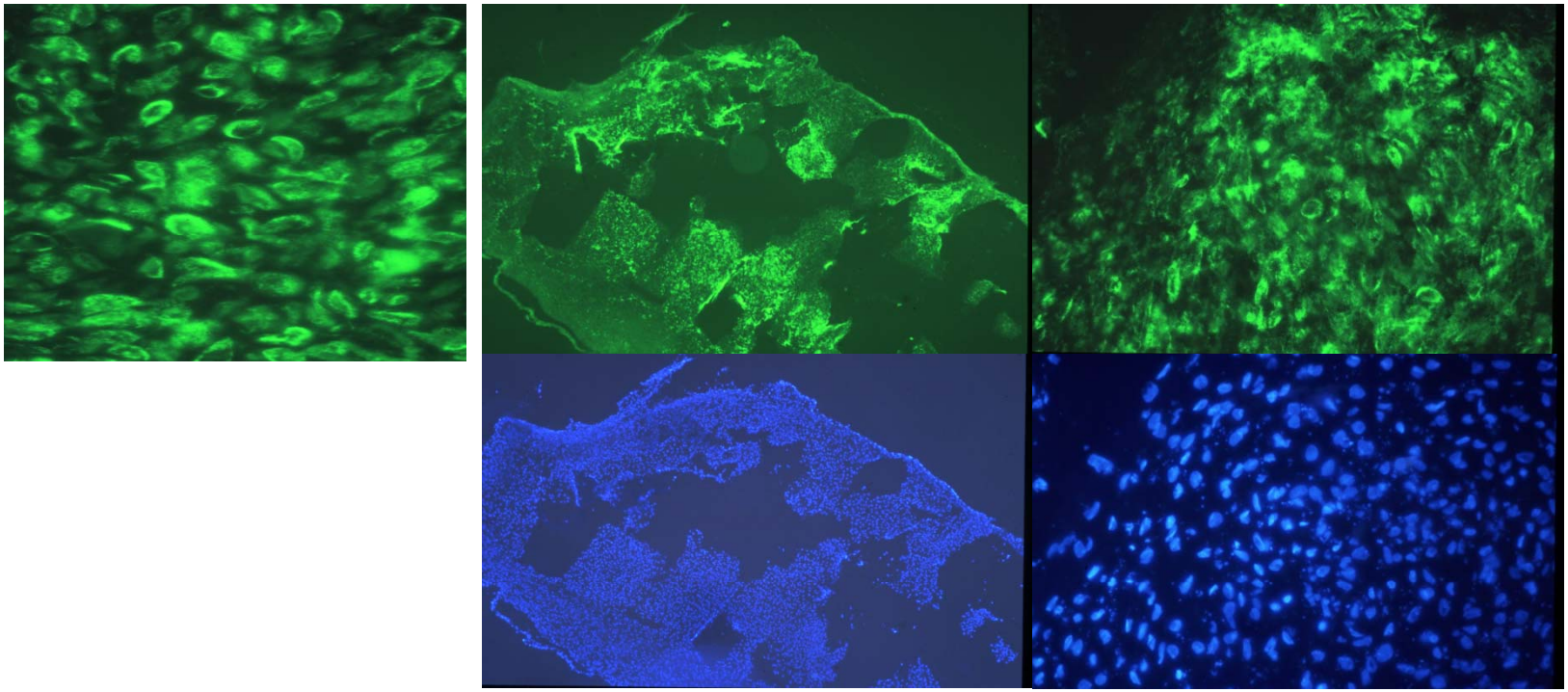
Pericellular matrix distribution in non-woven HYAFF 11® and Polyactive™

HYAFF 11®

Polyactive™



Green staining:-collagen VI, pericellular matrix

Blue staining:-DAPI, cell nucleii

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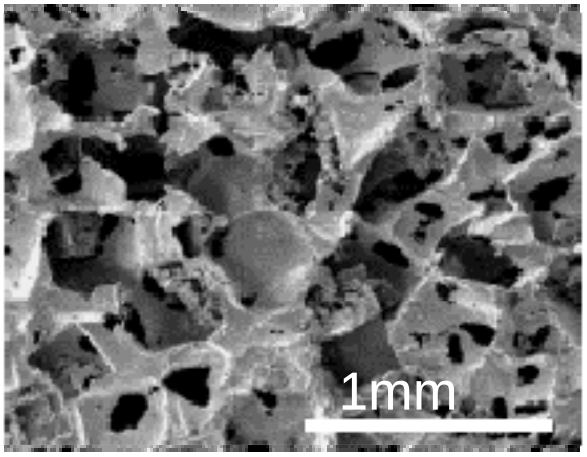
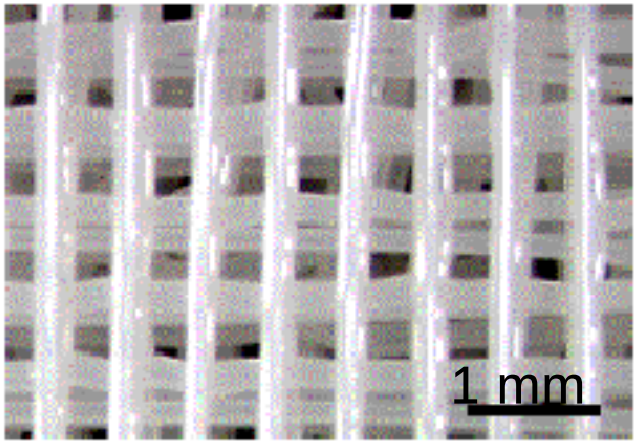
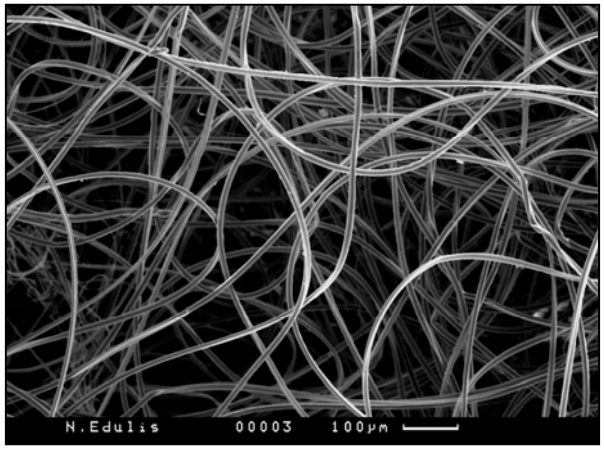
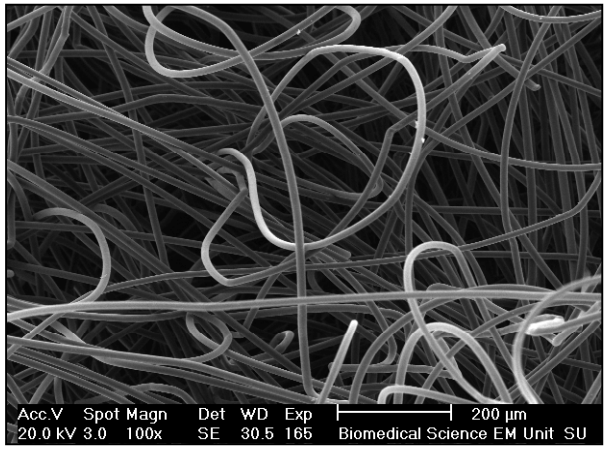
Scaffold morphologies









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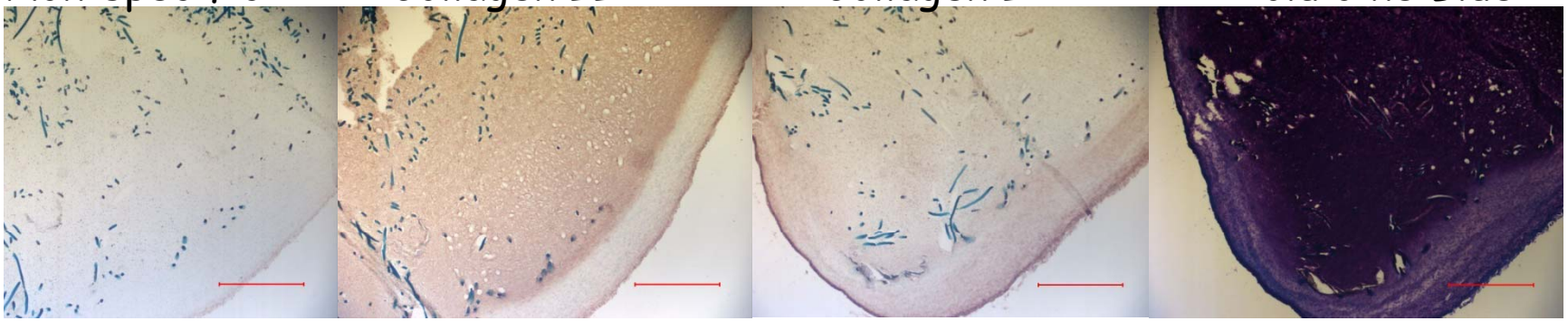
EXPERTISSUES

Extracellular matrix production on foam and fibre forms of HYAFF 11®

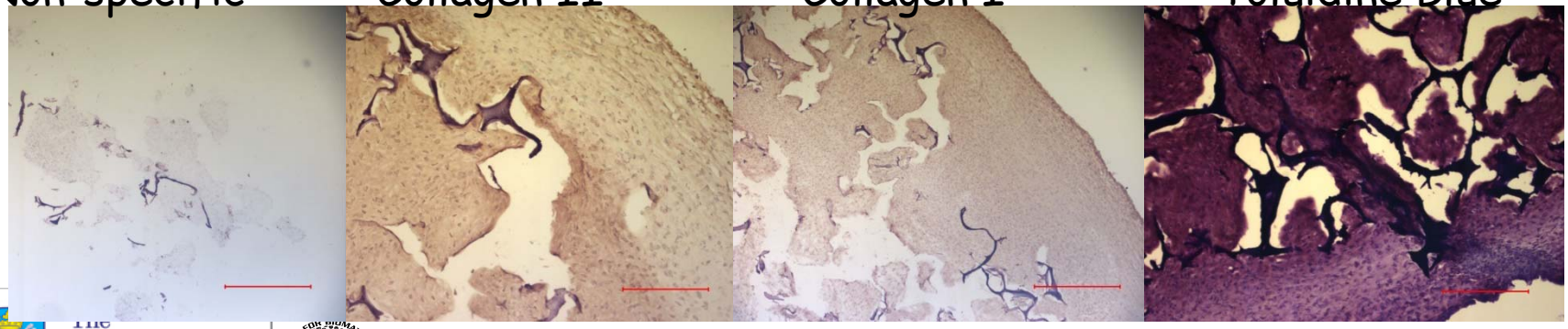
Fibre scaffold

Non-specific | Collagen II | Collagen I | Toluidine Blue



Foam scaffold

Non-specific | Collagen II | Collagen I | Toluidine Blue



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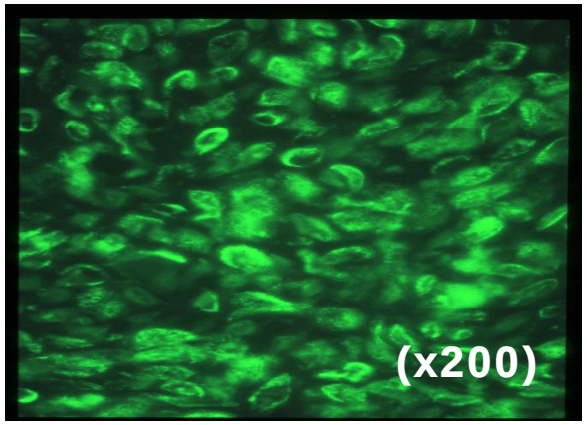
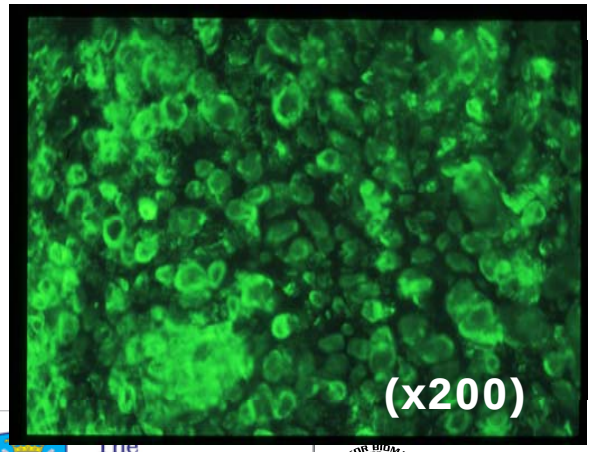
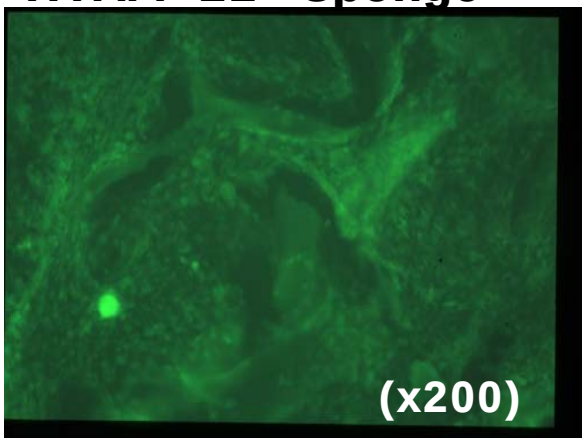
Pericellular matrix (collagen VI) distribution in cartilage constructs

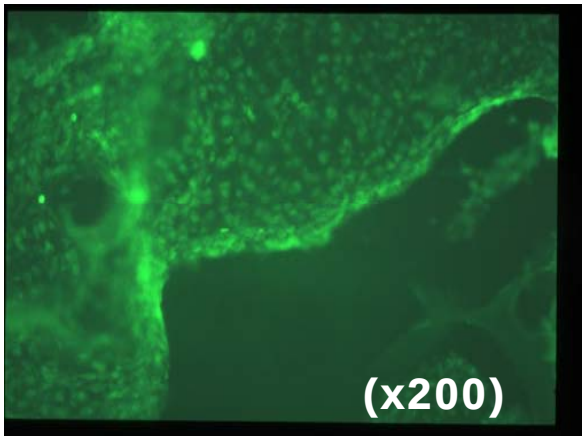
HYAFF 11® felt



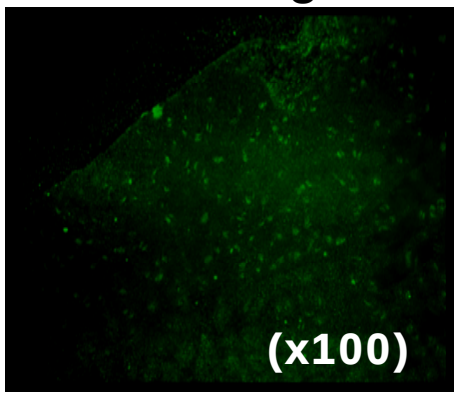


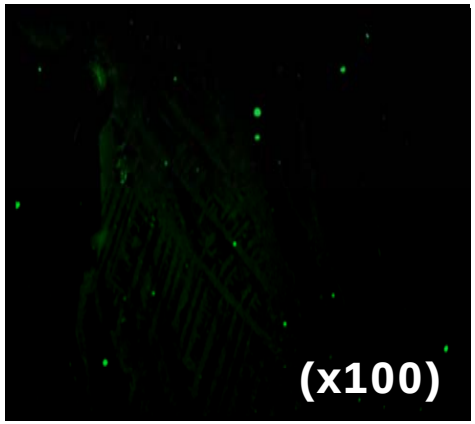
HYAFF 11® Sponge





Non specific staining





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EXPERTISSUES

Matrix Composition of human articular chondrocyte/ HYAFF 11® constructs

	Total collagen (%dry.wt.)
Non-woven HYAFF 11R (fibre)	16.02 (2.16)
HYAFF 11R 400-500μm (Sponge)	31.18 (2.35)

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EXPERTISSUES

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.

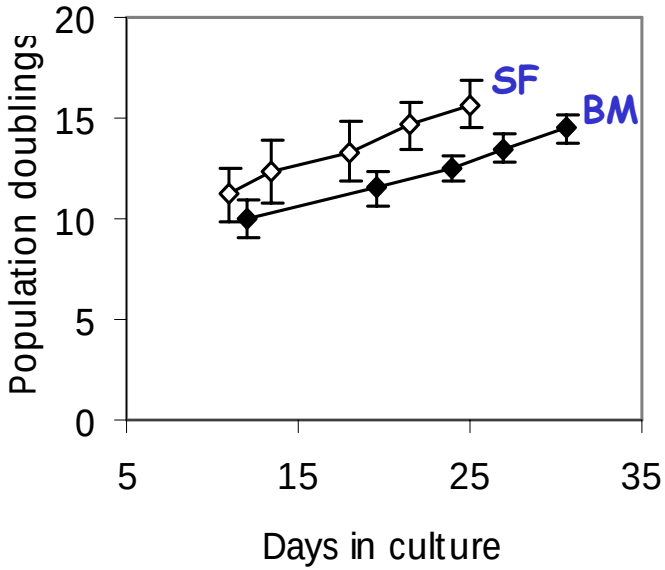
Cell sources for cartilage regeneration

- 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

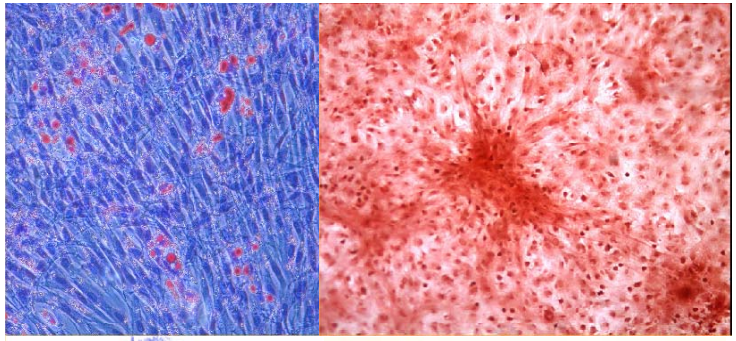
- Bone marrow
  - currently one of most researched sources.
  - reported to hypertrophy and mineralise *in vivo* (Pelttari et al. Arthritis and Rheum 2006).
  - subchondral drilling to release BM-MSCs yields fibrocartilage.
- Joint Tissues
  - articular cartilage
  - joint fluid (synovial fluid) (Jones et al Arthritis & Rheum 2004).

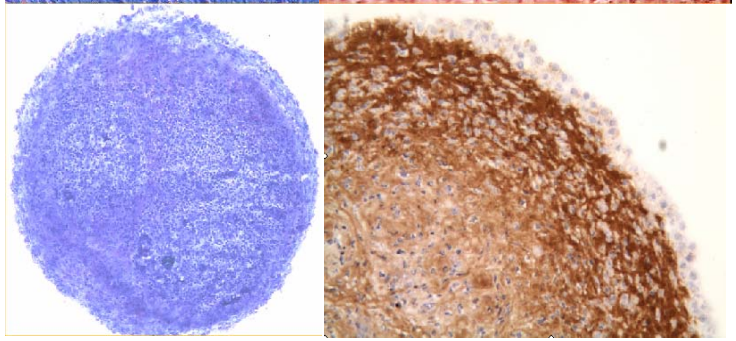
Stem cells from joint fluid (synovial fluid)



Adipogenic [Oil Red O]

Osteogenic [Alcian Red]





[Alcian Blue] Collagen II

Chondrogenic

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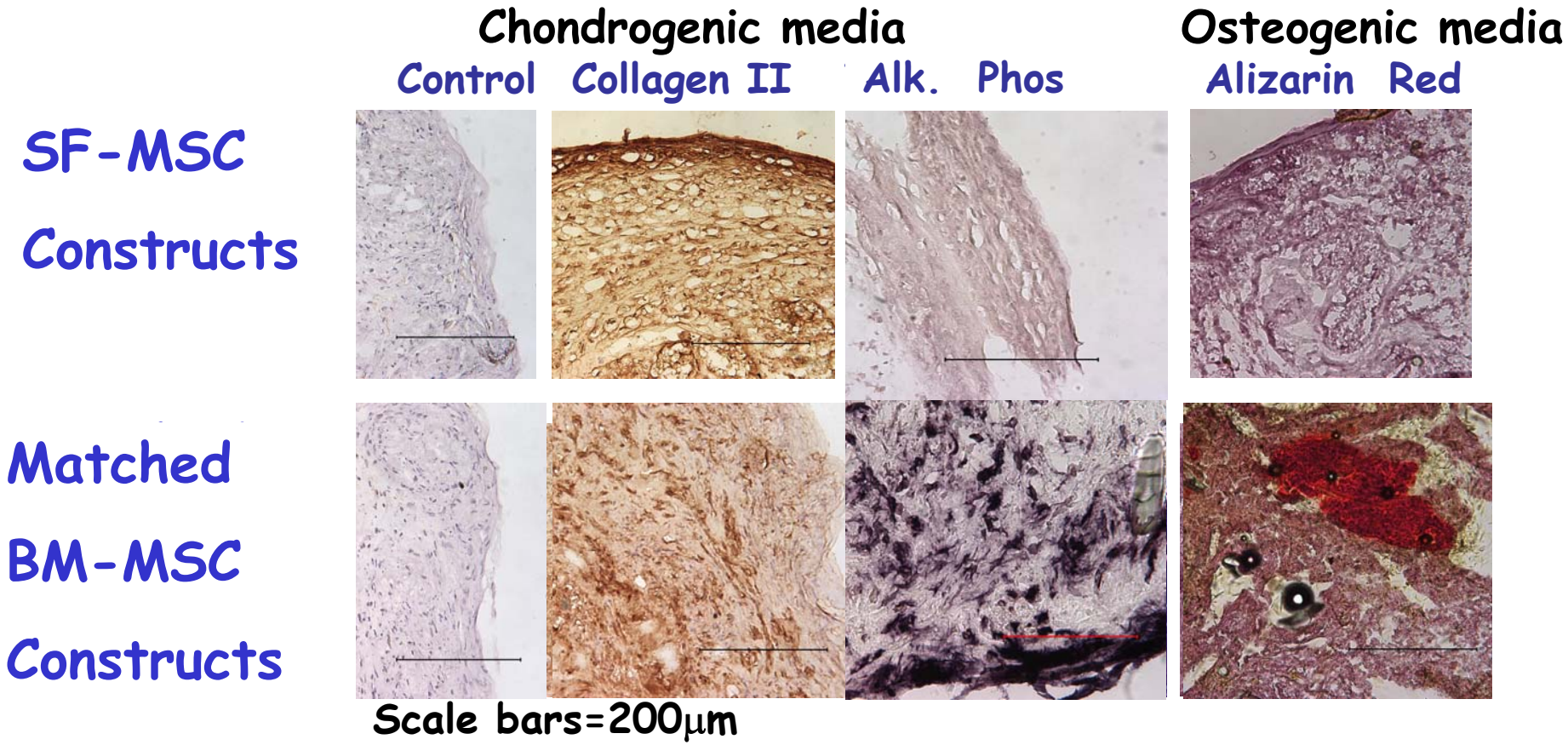
Comparison of joint fluid, bone marrow MSCs and chondrocytes



	SF-MSCs	Chondrocytes
Proteoglycan content:	84-111μg	507-801μg
Alkaline phosphatase:	ND	ND

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.

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