Ex Vivo Modeling of Acute Cartilage Injury to Study the Mechanisms of Post-Traumatic OA

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I have no financial conflicts to disclose
Impact of PTOA in the Hip, Knee & Ankle

Joint injury leads to “post-traumatic OA”

Prevalence

Financial Impact

Financial Impact
~$3B/year direct and
~$12B direct +indirect

Disease Progression

Patients present with severe PTOA at least 10 years earlier

>12% of all OA attributable to PTOA
Estimate ~5.7M people
Ex vivo model cartilage/joint injury in vitro?

For PTOA studies we use fresh human knee and ankle joints collected through the organ bank. Donors of all ages (19 – 90 yo) and both genders are utilized.

Five ex vivo models of cartilage injury have been developed, validated and studied:

1) Cartilage damage created by mechanical impaction using the pneumatic pressure controlled impactor with the transferred impulse of 1Ns. In this case, fresh human normal tali (cartilage with bone attached) are used;

2) Full depth cartilage explants treated with high dose interleukin-1β (IL-1β);

3) Co-culture of damaged (impacted or IL-1β-treated) cartilage with normal intact synovium;

4) Donut-shaped cartilage explants, 10 mm in outer diameter and 6 mm central hole, have been created to model a chondral defect;

5) Co-culture of osteochondral plugs (impacted and/or cytokine-treated) and synovium.
Model 1: **Acute injury to ankle cartilage** leads to cell death and matrix degeneration

- If untreated, chondrocytes death by necrosis and apoptosis propagates horizontally and longitudinally not only through the areas that experienced injury, but also to the one adjacent to the injury site.

Pascual-Garrido et al, OA&C 2009
Model 2: Cartilage Injury (cytokines +/- impact) +/- synovium

Two damage models
- IL-1β (10 ng/ml), added 48 hours before co-culture
- Mechanical impaction (600N within 2 ms)

Cartilage explant (8mm) and grossly normal synovium (8mm) were harvested from human joints

Culture schemas
1. Non-impacted cartilage
   Control/Impacted cartilage
   Harvest Co-culture
2. IL-1β-treated cartilage
   IL-1β 48hr
   Harvest Co-culture

Samples were collected at 0, 2, 14 days

Kang et al. AJSM 2009
Pascual Garrido C. et al. OA&C 2009
Bajaj S. et al. JOT 2010
Rush co-culture model: Cell viability

- Both type of damaged cartilage displayed elevated cell death especially in the superficial layer (red squares).

- In the presence of normal synovium collected from the same joint, chondrocyte survival significantly increased (green squares).
Rush co-culture model: Cartilage Histology

- In the presence of synovium, Mankin score was decreased at 2 and 14 days.

*M P < .05, ** < .01 vs damaged cartilage*
Immediate Cellular Responses to Acute Trauma/Targets for early interventions

- bFGF
- IL-6
- IL-8
- TNF-α
- NF-κB
- Casp.3
- Casp 9
- MMPs
- Agg-ase

Anderson et al, JOR 2009
Chondroprotective Therapy tested in acute ankle cartilage injury model leads to decrease in cell death and thus cartilage protection from degeneration.
RUSH-MIT-Harvard

Human Cartilage / Bone / Synovium

In vitro organ co-culture for drug discovery/delivery:

Slide courtesy of Dr. A. Grodzinsky
Model 4: Studies with Human Cadaveric Cartilage: Modeling of chondral/osteochondral defect

1. 10mm Full Thickness Tissue Explant disc procured from the load bearing area of the joint

2. 5.8mm core taken out from the center of the 10mm disc

3. Chondral phase of Agili-C™ was supplied by CartiHeal

4. The construct was inserted into the 5.8 mm cored area of the 10mm disc and cultured for 60 days in 20% FBS (Fetal Bovine Serum). HA is added to the media.

5. Facedown: The tissue is placed on a microscopic slide with the surface (indicated by the thick black arrow below) touching the slide

In vitro:
- Human donor adult articular cartilage (knees and ankles)
- Agili-C™ implant (the chondral phase) was placed inside cartilage plugs and cultured for up to 60 days +20% FBS +HA
- Face Microscopy
- Chondrocytes viability
- Histology
- PG synthesis/content
- Coll 1,2,10 and Agg gene expression

Chubinskaya et al, KSSTA, 2018
Histology: H&E Staining after 60 days culture

Control w/o Agili-C construct

Cartilage Explant with Agili-C construct

- Empty defect
- Matrix filling the gap
- Round cells
- Flat cells

Progenitor cells?

Chubinskaya et al, KSSTA, 2018

Chondrocytes are able to fill the defect and deposit hyaline-like cartilage. Details are in the referenced paper.
Conclusions

• The results of these studies confirm the utility of ex vivo models for studying human cartilage/joint injuries and the mechanisms of development of PTOA.

• Further, they provide an excellent platform for testing various biologic approaches as potential treatments for preventing PTOA.