The meniscus is a fibrocartilaginous tissue primarily of type 1 collagen fibers. (In contrast to hyaline cartilage on articulating surfaces consisting primarily of type 2 collagen.) Engineered therapies for repair of focal articular cartilage lesions and damaged menisci are helpful as self-repair is limited.
Prof. Steven S. Saliterman


Collagen
- Most abundant protein in the body – 28 Types have been described.
- Type I is found in skin, scars, tendon, vascular ligature, organs, & bone.
  - Accounts for over 90% of the body’s collagen.
  - Most common type used for gel formation.
    - Undergoes fibrillar collagen formation at 37°C and neutral pH.
    - The collagen gel will maintain its structure based on the concentration of collagen in the initial solution.
    - Functionality is derived from various constituents including ions, peptides, proteins and the extracellular matrix proteins.
    - Typically isolated from limited proteolytic treatment of raw material including rat tail, calf skin or human placenta.

Collagen...
- Type II: Main collagenous component of cartilage.
- Type III: “Young” collagen found throughout the interstitium in young individuals. This collagen is replaced by the stiffer collagen type I during maturation. Reticulate, commonly found alongside type I.
- Type IV: Forms basal lamina, the cell–secreted layer of the basement membrane. Non–fibrillar.
- Type V: Found on many cell surfaces, hair and placenta.
- Type X: Non–fibrillar, short chain expressed by hypertrophic chondrocytes during endochondral ossification.

RegenHU’s 3DDiscovery + Biofactory
Daly et al. demonstrate that it is possible to engineer mechanically reinforced hydrogels with high cell viability by co-depositing a hydrogel bioink with polycaprolactone (PCL) filaments, generating composites with bulk compressive moduli comparable to articular cartilage.

They compared a range of commonly used hydrogel bioinks—agarose, alginate, CellMA (gelatin methacryloyl hydrogels) and BioINK™ (aPEGMA based hydrogel)—for their printing properties and capacity to support the development of either hyaline cartilage or fibrocartilage in vitro.

Cartilage is a dense connective tissue with a highly organized extracellular matrix (ECM) consisting predominately of proteoglycans (GAG) and collagens. Mesenchymal stem cell (MSC) laden hydrogels are commonly used for fibrocartilage and articular cartilage tissue engineering. When implanting MSCs as part of a tissue engineered construct, the supporting biomaterial should ideally provide clues to direct their differentiation towards specific cell types and thereby enable the development of specialized tissues such as articular cartilage or meniscal fibrocartilage.

Cell Isolation

Bone marrow derived mesenchymal stem cells (BMSCs) were obtained from the femur of a 4 month old porcine donor.

Materials preparation and cell encapsulation

Each gel had a seeding concentration of 20 million BMSC cells ml$^{-1}$

Biochemical analysis

DNA content was quantified using the Hoechst Bisbenzimide 33258 dye assay.

Proteoglycan content was estimated by quantifying the amount of sGAG in each hydrogel using the dimethyl methylene blue dye binding assay.

Histological and immunohistochemical evaluation

Collagen types I, II, and X were evaluated.

Bioinks for 3D Cartilage Bioprinting

Cartilage is a dense connective tissue with a highly organized extracellular matrix (ECM) consisting predominately of proteoglycans (GAG) and collagens. Mesenchymal stem cell (MSC) laden hydrogels are commonly used for fibrocartilage and articular cartilage tissue engineering. When implanting MSCs as part of a tissue engineered construct, the supporting biomaterial should ideally provide clues to direct their differentiation towards specific cell types and thereby enable the development of specialized tissues such as articular cartilage or meniscal fibrocartilage.
Workflow...

- Mechanical testing
  - Single column Zwick (Zwick, Roell, Germany) with a 100 KN load cell
- Live/dead cell assay
  - Cell viability was established using a live/dead assay kit (Invitrogen, BioScience).
- 3D bioprinting
  - 3D bioplotter from RegenHU (3D Discovery).
  - Polycaprolactone (PCL), Mw=45 000, (Sigma-Aldrich) was melted at 70° in the printing chamber. A screw driven piston (25 rev/min), screw diameter 1 cm extruded the PCL onto a coverslip at a pressure of 0.45 MPa.
- Statistics
  - GraphPad Prism. One way ANOVA was used for analysis of variance with Bonferroni post-tests to compare between groups.

### Processing Parameters for each Bioink

<table>
<thead>
<tr>
<th></th>
<th>Alginate</th>
<th>Agarose</th>
<th>PEGMA BioINK</th>
<th>GelMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Printing Temperature</td>
<td>21°</td>
<td>37°</td>
<td>21°</td>
<td>28°</td>
</tr>
<tr>
<td>Polymer Concentration</td>
<td>3.5% Alginate, 60 mM CaCl₂ (Mixed 7:3)</td>
<td>2%</td>
<td>2%</td>
<td>1% GelMA, 0.05% Irgacure</td>
</tr>
<tr>
<td>Post Cross-Linking Mechanism</td>
<td>Calcium chloride bath (15 min)</td>
<td>Physical (Temperature) 15 min</td>
<td>UV Light 15 min</td>
<td>UV Light 15 min</td>
</tr>
<tr>
<td>Extrusion Pressure</td>
<td>0.2 MPa</td>
<td>0.2 MPa</td>
<td>0.14 MPa</td>
<td>0.06 MPa</td>
</tr>
</tbody>
</table>

### Results

- Histological and immunohistochemical staining at the end of a 4 week culture period demonstrated that the different hydrogels could support the synthesis of either hyaline or fibrocartilage-like tissue components.
- All bioinks supported high levels of cell viability.
- GelMA and BioINK™ supported the development of a more fibrocartilage-like tissue, as evident by the development of a tissue containing both type I and type II collagen.
- Alginate and agarose bioinks were found to support the development of a hyaline-like cartilage tissue.
(A) Histological and immunohistochemical analysis of MSC laden hydrogels following 4 weeks of in vitro culture. Aldehyde fuchsin/alcan blue for sulphated glycosaminoglycans (sGAG) synthesis and immunohistochemical staining for collagen II, collagen type I, collagen type X. Images 10X with 4X inset, scale bar is 100 μm and 1 mm, respectively. Biochemical analysis of all hydrogel after 4 weeks of in vitro culture (n=3–4, ANOVA, P<0.05, Mean ±SD).

(B) Total DNA Content (ng) per whole construct (volume 60 mm3)

(C) sGAG/DNA(D) sGAG (%WW). Significance p<0.05, (a) versus alginate at the same time point, (b) versus agarose at the same time point, (c) versus PEGMA at the same time point, (d) versus GelMA at the same time point, (d*) versus GelMA at day 0.

Summary

- Need for engineered cartilage.
- Collagen types.
- Comparison of bioinks for cartilage printing.
- Workflow
- Different hydrogels can preferentially support the synthesis of either hyaline or fibrocartilage-like tissue components.
  - Mechanically reinforced hydrogels with high cell viability was achieved by co-depositing a hydrogel bioink with polycaprolactone (PCL) filaments
  - GelMA and BioINK™ supported the development of a more fibrocartilage-like tissue, as evident by the development of a tissue containing both type I and type II collagen.