Bioprinting Cartilage

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Bioprinting of Fibrocartilage & Hyaline Cartilage

Biofabrication

PAPER

A comparison of different bioinks for 3D bioprinting of fibrocartilage and hyaline cartilage

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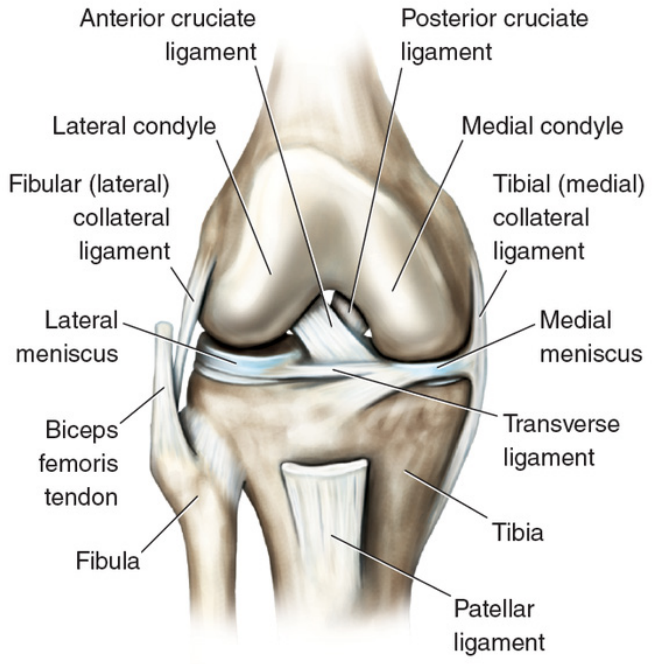
Consider Knee Joint Cartilage



Behnke, R. Kinetic Anatomy, 3rd edition. Human Kinetics 2012.

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 collage.)

Engineered therapies for repair of focal articular cartilage lesions and damaged menisci are helpful as self-repair is limited.

Commercial Bioprinters…

Bioprinter and manufacturer	Fabrication technique	Specified resolution	Recommended materials
3Dn300TE, NScrypt	Extrusion-based	Line widths 20–100 μm	Not specified (viscosity range: 0.001–1000 Pa s)
3D-Bioplotter®, Envisiontec[a]	Extrusion-based	Minimum strand diameter 100 μm	Hydrogels, ceramic, metal pastes, thermoplasts
Bioscaffolder®, Gesim[a]	Extrusion-based	Not specified	Hydrogels, biopolymers (collagen, alginate) bone, cement paste, biocompatible silicones and metling polymers (CPL, PLA)
Biobot 1, Biobots[a]	Extrusion-based	Layer resolution 100 μm	Hydrogels, biopolymers (viscosity range: 100–10^4 Pa s, see table 3 for more details)
Inkredible+, Cellink[a]	Extrusion-based	Layer resolution 50–100 μm	Hydrogels (see table 3)
~~Biofactory®, RegenHU[a]~~	~~Extrusion-based~~ Inkjet	~~Not specified~~	~~Bioink, Osteoink (see table 3 for more details)~~
~~Revolution, Ourobotics~~	~~Extrusion-based~~	~~Not specified~~	~~Collagen, gelatin, alginates, chitosan~~
Bio3D Explorers, Bio3D technologies[a]	Extrusion-based	Not specified	Not specified
CellJet Cell Printer, Digilab		Droplet size 20 nl–4 μl	Water-based, hydrogels, alginate, polyethylene glycol
BioAssemblyBot, advanced solutions	Extrusion-based	Not specified	Not specified
Regenova, Cyfuse	Spheroid assembly	Related to spheroid diameter	Cells only (scaffold/biomaterial-free approach)
NovoGen MMX, Organovo[b]	Inkjet	20 μm	Cellular hydrogels
Dimatix Materials Printer, Fujifilm	Inkjet	20 μm	Water-based, solvent, acidic or basic fluids
Poietis[b]	LIFT	20 μm	Not specified

Holzl, K., S. M. Lin, L. Tytgat, S. Van Vlierberghe, L. X. Gu, and A. Ovsianikov. "Bioink Properties before, During and after 3d Bioprinting." *Biofabrication* 8, no. 3 (Sep 2016).

Commercially Available Bioinks

Company	Bioink	Material	Features
Bioink Solutions, Inc.	Gel4Cell®	Gelatin-based	UV-crosslinkable Cell viability >90%
	Gel4Cell®-BMP Gel4Cell®-VEGF Gel4Cell®-TGF	Conjugated with different growth factors	Osteoinductive Angiogenic Chondrogenic
CELLINK	CELLINK	Nano-cellulose/alginate mixture	Shear thinning Fast crosslinking For soft tissue engineering
RegenHU	BioInk®	PEG/gelatin/hyaluronic acid-based	Good cell adhesion properties Biodegradable Mimics the natural ECM Possible combination with Osteoink™
	Osteoink™	Calcium phosphate paste	Osteoconductive Chemical composition similar to human bone For hard tissue engineering
Biobot	Bio127	Pluronic F127-based	Gels at room temperature Dissolves when cooled
	BioGel	Gelatin Methacrylate based	When combined with GelKey it Covalently crosslinks when exposed to light

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Common Materials used in Bioinks

Table 1 Common Materials Used in Bioinks and Mechanism of Gel Formation

Compound	Mechanism gel formation	Chemical structure
Agar	Thermal	Polysaccharide
Collagen	Spontaneous gelation/ photoinitiation	Protein
Alginate	Ionic	Polysaccharide
PLGA-g-PEG	Thermal	Poly(lactic-*co*-glycolic acid)
PEGDMA	Thermal/chemical	Poly(ethylene glycol) dimethacrylate
Pluronic	Thermal	Poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol)
Agarose	Thermal	Polysaccharide
Carageenen	Thermal	Polysaccharide
Fibrin	Spontaneous gelation	Protein
Elastin	Photoinitiation	Protein
Silk	Photoinitiation	Protein
Chitosan	Chemical	Polysaccharide
Hyaluronic acid	Chemical	Glycosoaminoglycan
NIPAAM	Thermal	N-isopropyl acrylamide/N-t-butyl acrylamide copolymer

Williams, S. K., and J. B. Hoying. *Bioinks for Bioprinting*. Bioprinting in Regenerative Medicine. Edited by K. Turksen 2015. doi:10.1007/978-3-319-21386-6_1.

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 bodies 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.

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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 1.
- 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.

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RegenHU's 3DDiscovery + Biofactory

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3D Cartilage Printing…

- 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, GelMA (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.

Daly, A. C., S. E. Critchley, E. M. Rencsok, and D. J. Kelly. "A Comparison of Different Bioinks for 3d Bioprinting of Fibrocartilage and Hyaline Cartilage." *Biofabrication* 8, no. 4 (Dec 2016).

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.

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Workflow

- 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.

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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.

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Processing Parameters for each Bioink

	Alginate	Agarose	PEGMA BioINK	GelMA
Printing Temperature	21˚	37˚	21˚	28˚
Polymer Concentration	3.5% Alginate, 60 mMCaCl 2 (Mixed 7:3)	2%		10% GelMA, 0.05% Irgacure
Post Cross-Linking Mechanism	Calcium chloride 50 mM bath (15 min)	Physical (Temperature) 15 min	UV Light 15 min	UV Light 15 min
Extrusion Pressure	0.2 MPa	0.2 MPa	0.14 MPa	0.06 MPa

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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.

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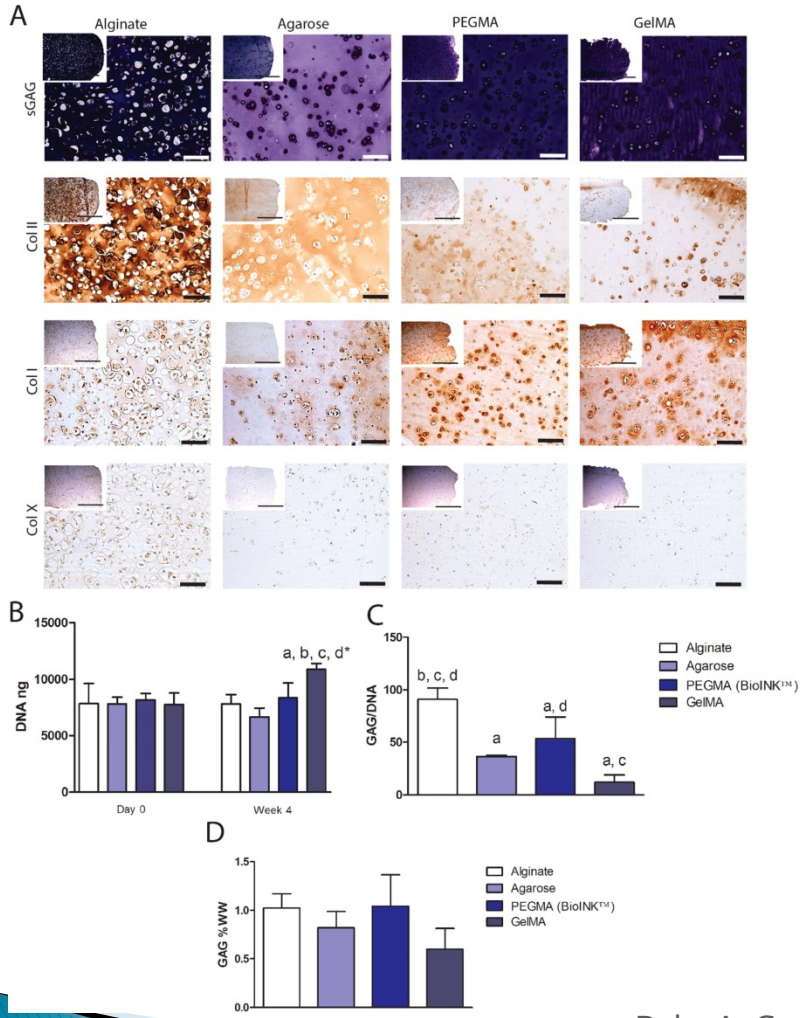

(A) Histological and immunohistochemical analysis of MSC laden hydrogels following 4 weeks of in vitro culture. Aldehyde fuschin/alcian blue for sulphated glycosaminoglycans (sGAG) synthesis and immunohistochemical staining for collagen II, collagen type I, collagen type X. Images 10X with 4X inset, 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 $\pm$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.

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Comparison of Hydrogel Printability

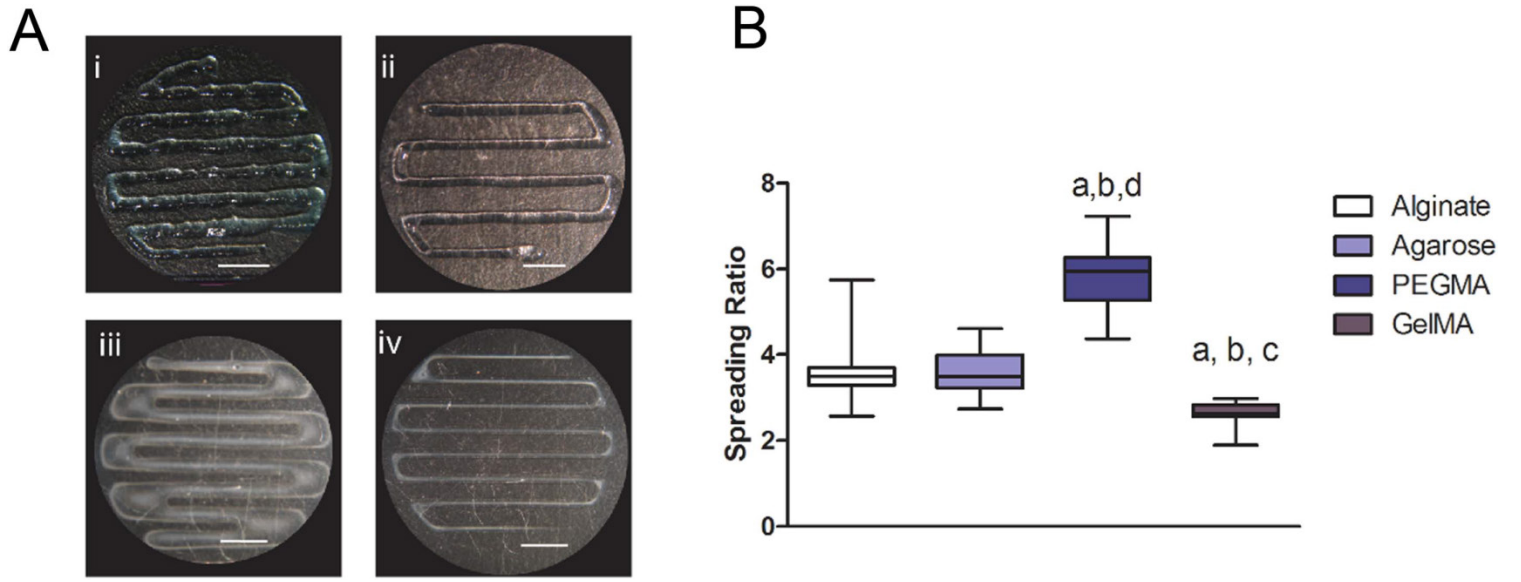

Figure 2. Comparison of hydrogel printability, (A) printed hydrogel patterns generated using (i) Alginate, (ii) Agarose (iii) PEGMA (BioINKTM) and (iv) GelMA, scale bar 2 mm. (B) Post printing spreading ratio (filament diameter/needle diameter), (ANOVA, $p < 0.05$, Mean $\pm$ SD): (a) versus alginate, (b) versus agarose, (c) versus PEGMA, (d) versus GelMA.

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Influence of PCL Co-Deposition on Cell Viability

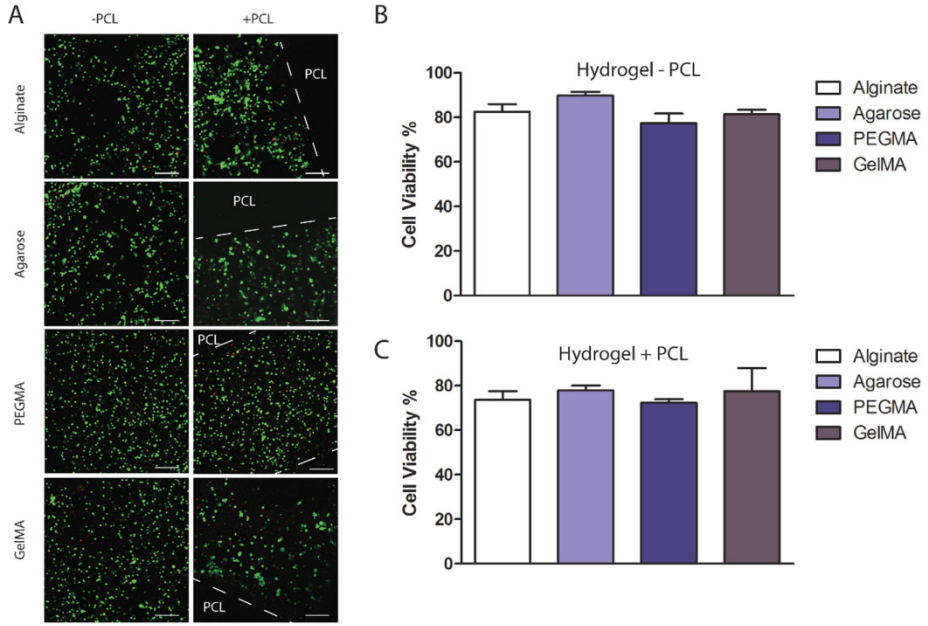

Figure 3. Influence of PCL co-deposition on cell viability. (A) Representative 10x live dead staining of cells within printed filaments of hydrogel adjacent to (+PCL) and not adjacent to (−PCL) PCL filaments. Quantification of cell viability both with (B) and without (C) the incorporation of PCL into the printed structure ($n = 4$, ANOVA, $P < 0.05$, Mean ± SD).

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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.