Agarose-Alginate hydrogels as suitable bioprinting materials

Gabriel R López-Marcial[1], Anne Y. Zeng[1], Carlos Osuna[3], Jeannette M. Garcia[1, 2], Grace D. O'Connell[1*]
[1]University of California Berkeley, [2] IBM Almaden Research Center, San Jose, CA, [3]University of California San Diego
*g.oconnell@berkeley.edu

Disclosures: The authors have nothing to disclose.

INTRODUCTION: Osteoarthritis is the leading cause of disability in the United States, accounting for nearly 20% of all disabilities [1]. There have been significant advances in developing biological repair strategies for damaged or osteoarthritic cartilage [2,3], some of which are being evaluated in animal or early stage clinical trials [4]. Casting is the most common fabrication technique used to create engineered cartilage from hydrogels, but casting is not well suited for fast manufacturing of engineered cartilage with patient specific topography. Recent work in 3D printing has been promising for creating patient-specific tissues by printing bioinks (biocompatible materials with cells) [5]. However, it is not clear whether hydrogels that have been ideal for cultivating *de novo* cartilage have ideal printing properties. Therefore, the objective of this study was to evaluate printability of pluronic, agarose, alginate, and agarose-alginate hydrogels. To do this, we evaluated temperature-dependent rheological data (shear thinning properties), bulk gel mechanical properties (yield stress), and assessed shape fidelity of printed lines. Pluronic has been used in 3D printing applications because of its printability, but is not a useful scaffold because of its solubility in aqueous media. Therefore, we hypothesize that the gel with properties closest to Pluronic will be the most effective for printing.

METHODS: All hydrogels were prepared by mixing powder into 0.15 M phosphate buffered saline (PBS). Agarose hydrogels (Type VII) were prepared with final concentrations of 2%, 3%, and 4% weight per volume (w/v; mixed in autoclave: 120 °C for 30 minutes). Pluronic® F-127 hydrogels were prepared at a concentration of 30% w/v, mixed in an ice bath until a homogeneous liquid formed. Alginate-agarose hydrogels were prepared with 3% w/v agarose and 2% w/v sodium alginate. *Mechanics:* Rheology tests were performed by melting samples (65-75 °C for agarose-based constructs and 75-85°C for Pluronic® F-127) before applying compression with an oscillatory shear strain. For temperature ramp tests, samples were equilibrated to 25°C and dynamic mechanical properties (storage modulus, loss modulus, and phase angle) were assessed using an oscillatory stress of 6.64 Pa applied in steps of +5° C/min. Storage modulus, loss modulus and phase angle were calculated. To determine viscosity using flow-rate tests, each sample was equilibrated to 37° C before increasing shear rate from 0.01 to 10.00 Hz. Yield strength was determined as the point where plastic deformation began following a 90% strain ramp in unconfined compression (n = 5 per group). *Printing:* Chondrocytes were acquired from healthy juvenile bovine knees by digesting articular cartilage overnight with collagenase. Cells were encapsulated within the agarose-alginate hydrogels for 3D printing (same final solid concentration; ~0.25M cells/mL). Lines were printed using a BioBot 3D printer and cell viability was assessed using a Live/Dead kit. Z-stack images were acquired using a confocal microscope (10x objective), and a custom algorithm was used to count the total number of cells (MATLAB).

RESULTS: The storage modulus of agarose-based gels decreased nonlinearly as the temperature rose above 40°C, while the storage modulus of Pluronic was stable over the entire range (Fig. 1A – grey dashed line vs colored lines). All hydrogels exhibited shear-thinning behavior with a decrease in viscosity when exposed to higher shear rates (Fig. 1B). The yield strength increased with a higher solid concentration, as expected (*e.g.*, from 2% to 4% w/v agarose, $p < 0.005$ for 4% versus other agarose gels; Fig. 1C). Inclusion of 2% w/v alginate did not significantly alter the yield strength, when compared to the 3% agarose only group ($p > 0.3$; Fig. 1C). The integrity of the print, as observed by the amount of gel spreading on the print surface, was highly dependent on the solid concentration in the gel and material composition (*e.g.*, 3% agarose *vs.* 3% agarose + 2% alginate; Fig. 1D). Cell viability remained high for all printed gel groups with no significant difference between them. (~80%; $p > 0.4$; Fig. 1E).

DISCUSSION: 3D bioprinting has rapidly become a promising approach for producing constructs and scaffolds for tissue engineering and regeneration. All of the hydrogels evaluated exhibited shear-thinning qualities, suggesting that the materials are printable. However, we did not observe a clear relationship between shear thinning properties and the ability to maintain shape fidelity during printing. That is, agarose-only gels often exhibited too much spreading or clumping during printing. After multiple agarose-alginate mixtures, the 3% agarose + 2% alginate mixture proved effective for providing smooth printed structures. Importantly, the printing process in air (~10 min) did not significantly impact cell viability compared to casted gels, but additional research is needed to evaluate matrix production with extended culture times. Future work will also evaluate whether shape fidelity of printed agarose-based hydrogels can be improved using the FRESH method [6], where hydrogels are printed within a sacrificial hydrogel (*e.g.*, Pluronic). In conclusion, the findings from this study suggest that materials that are ideal for cartilage tissue engineering can be 3D printed, allowing researchers to create complex patient-specific shapes and geometries.

SIGNIFICANCE: Advancements in 3D printing with hydrogels with ideal properties for tissue engineering and regeneration will allow researchers to decrease production time for cultivating patient specific tissues. Furthermore, the findings from this study demonstrate that a mixture of two well-established materials (agarose and alginate) for cartilage engineering is printable and maintains print integrity.

REFERENCES: [1] Health, US, 2017, http://www.cdc.gov ; [2] Stanton M.+, *Lab Chip*, 2015; [3] Ragunath, J. +, *Biotech Appl*, 2007; [4] Huang, B.+, *Biomat*, 2016; [5] O'Connell +, *ACS Biomat*, 2017; [6] Hinton, TJ. +, *Sci Adv*, 2015.

ACKNOWLEDGEMENTS: This study was funded by the National Science Foundation (GRLM, Graduate Fellowship) and the National Academy of Engineers (GDO & JMG, Grainger Foundation).

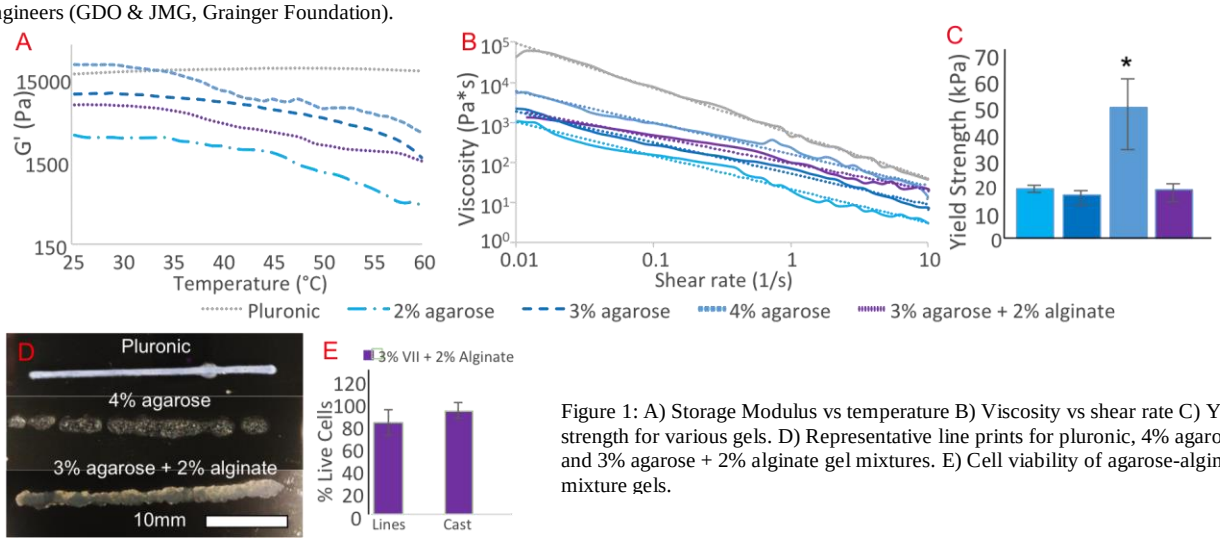

Figure 1: A) Storage Modulus vs temperature B) Viscosity vs shear rate C) Yield strength for various gels. D) Representative line prints for pluronic, 4% agarose, and 3% agarose + 2% alginate gel mixtures. E) Cell viability of agarose-alginate mixture gels.