Towards Multi-Domain Cellular Models: Bio-fabrication and Rheological Characterization of Extremophile Hydrogels

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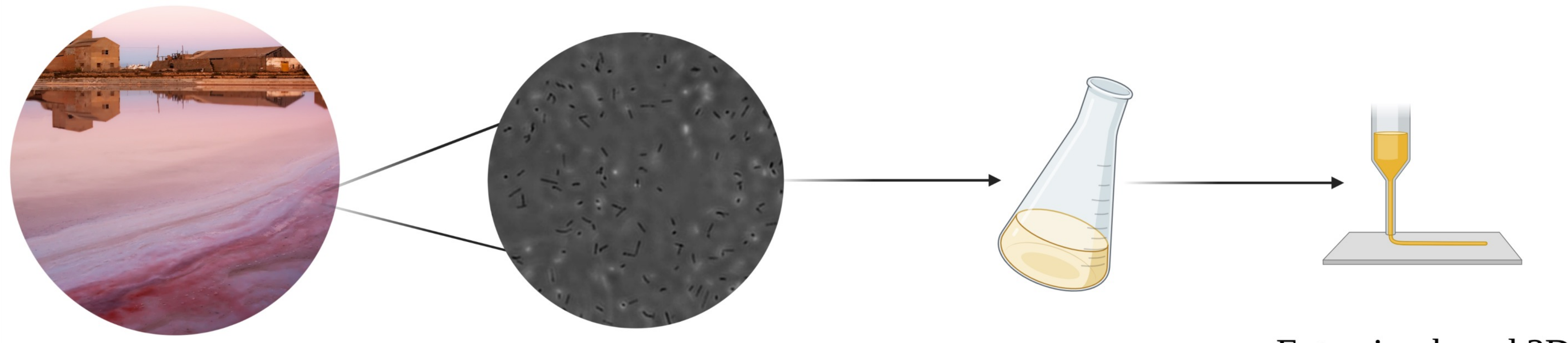
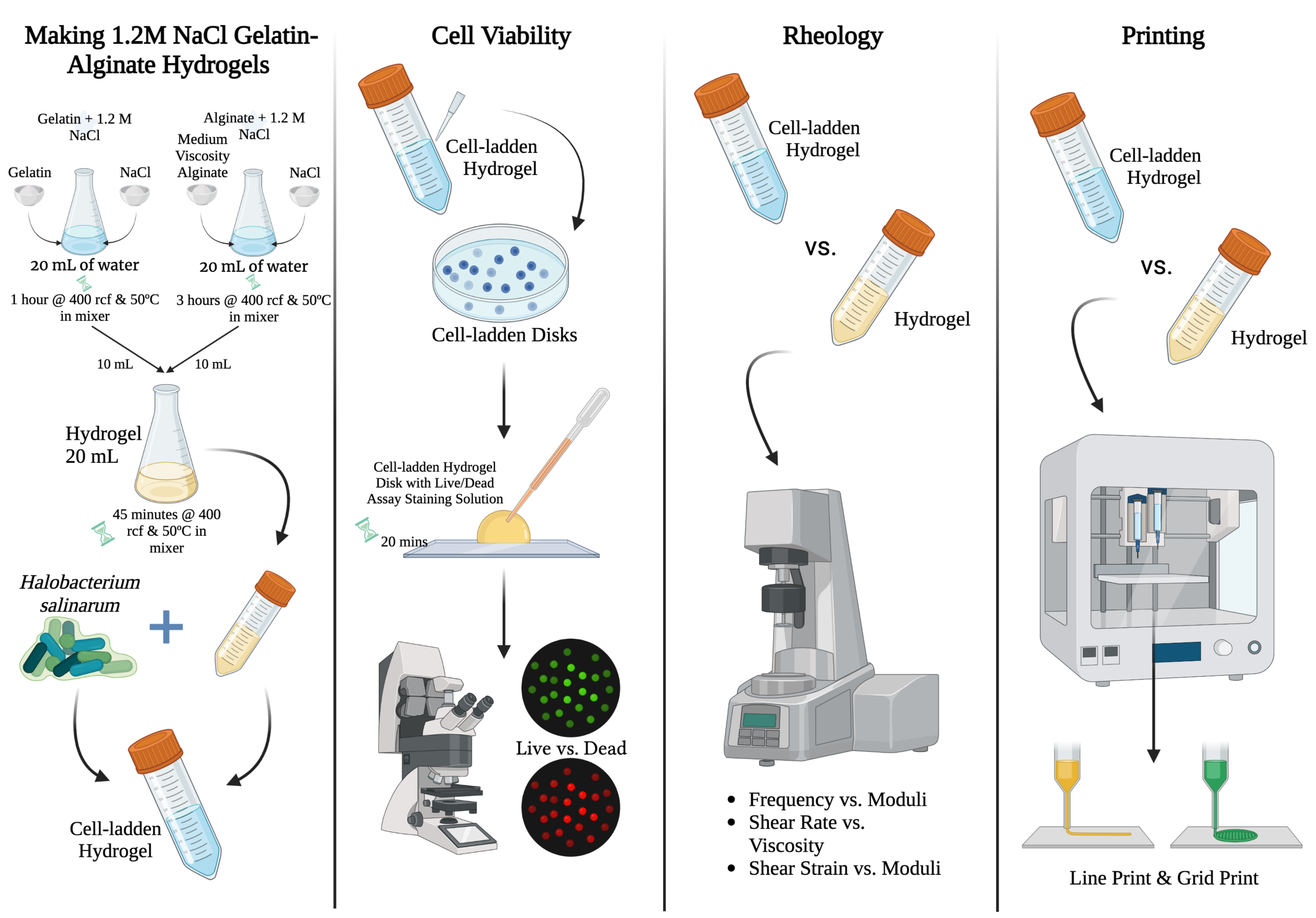
INTRODUCTION

Archaea, one of the three domains of life, have been found to occupy the human microbiome along with bacteria and eukaryotes. Communities of archaea can be found throughout the human body and have been found to often reside in the gut and within oral cavities [1]. However, in comparison to bacteria, still very little is known about archaea and their behavior in 3D environments that are representative of *in vivo* physiological conditions. Here, we explore the incorporation of a model halophile found in humans, *Halobacterium salinarum*, into alginate-based hydrogels and determine the effects of this organism on the viscoelastic properties required for effective extrusion-based 3D printing. As *H. salinarum* require hypersaline environments, we report printable hydrogels compatible with high salt solutions that are tuned to promote halophile growth and promote proliferation [2].



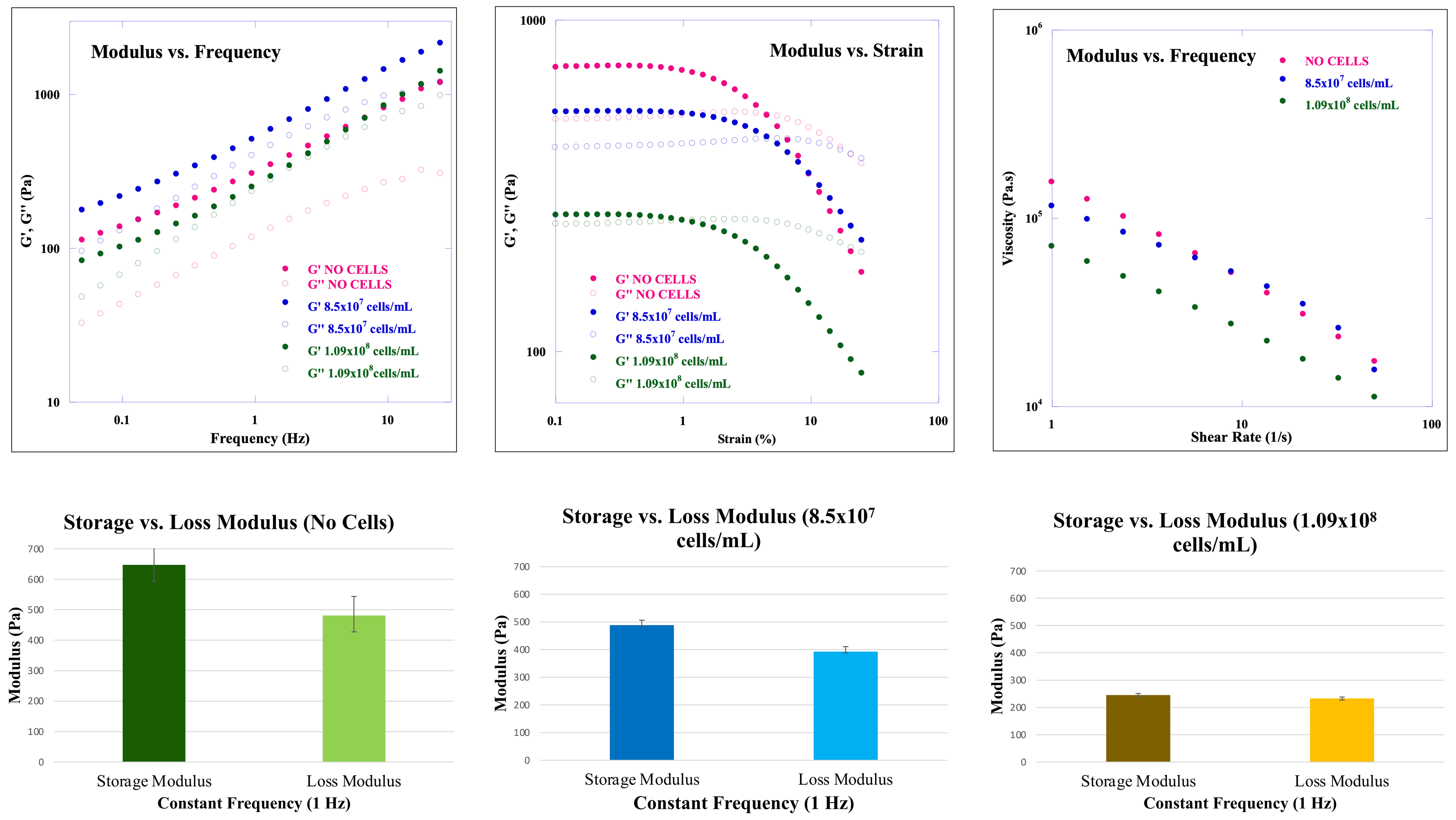
Hypersaline Lake: *Halobacterium salinarum* Photo 1010570 Juan Sevilla, some rights reserved (CC BY-NC-ND)

METHODS

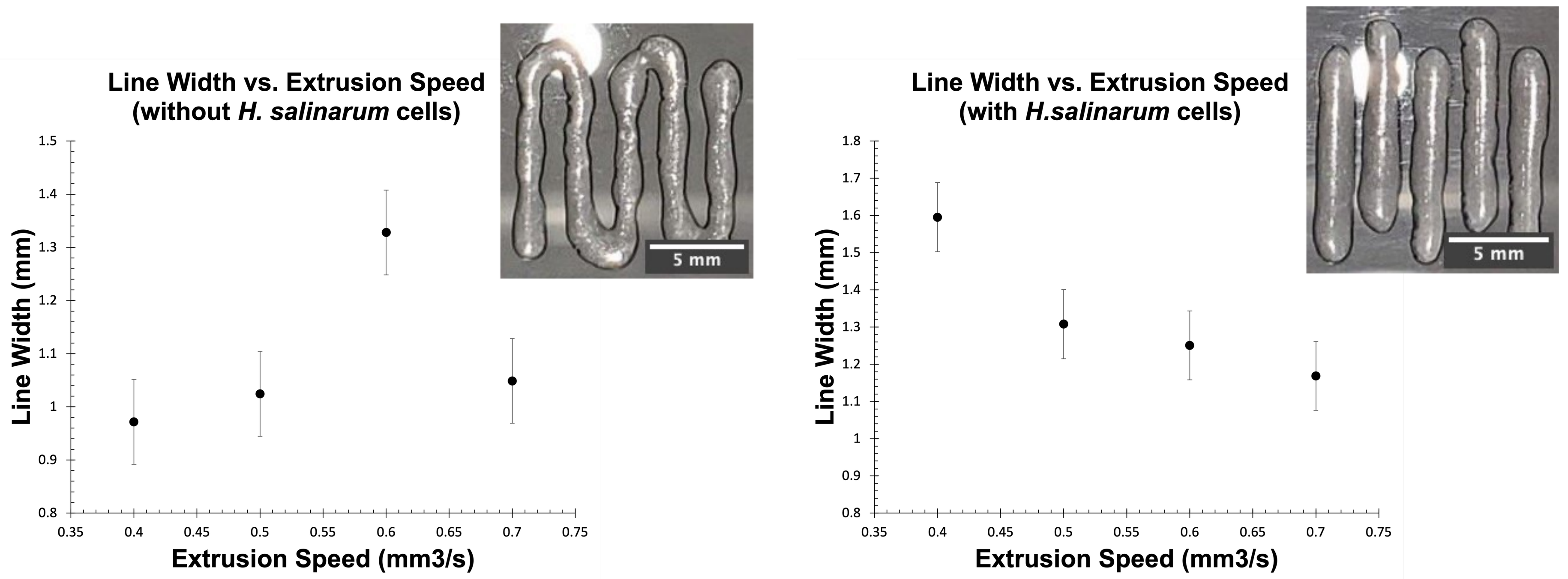


Overview of methods: (1) Hydrogel Fabrication and Cell Culture (2) Viability Assay (3) Bulk Rheology (4) 3D Bioprinting.

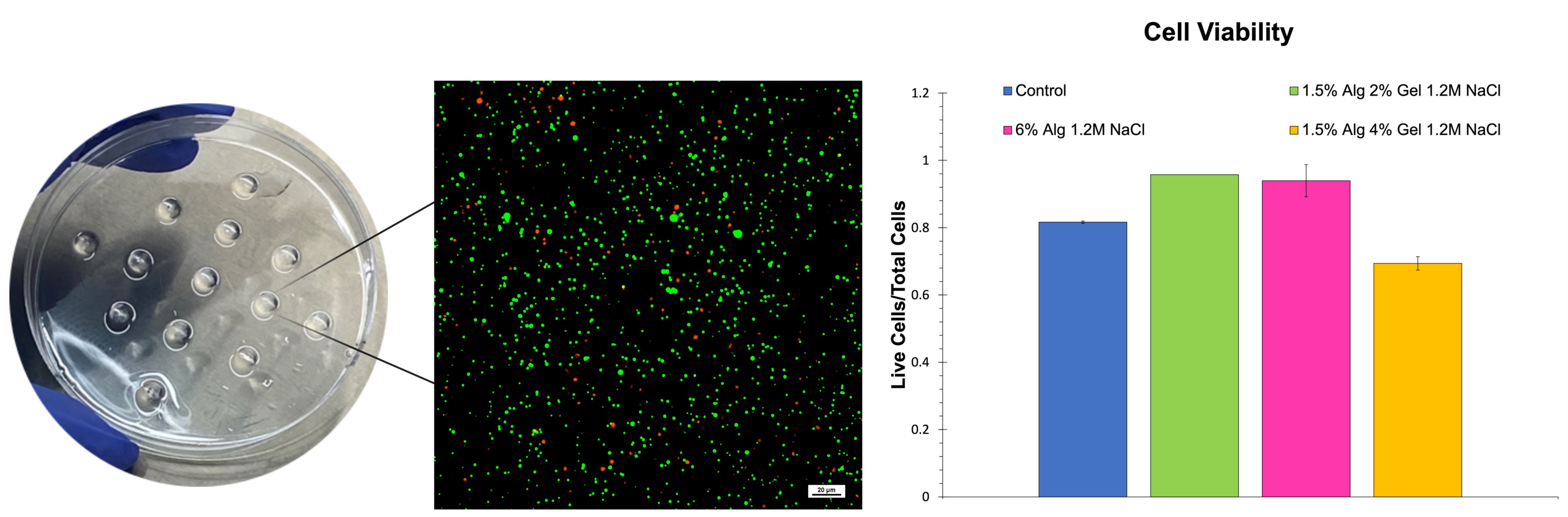
RESULTS AND DISCUSSION



Rheology of Hypersaline Hydrogels for *H. salinarum* Culture. Hydrogels consist of 6%wt./vol. 773kDA Sodium Alginate with in a 1.2 M NaCl solution.



Printing and Line Spreading Analysis of *H. salinarum*-laden hydrogels. Hydrogels loaded with 8.5X10^7 cell/mL of *H. salinarum* are shown to reduce line spreading with increasing extrusion rate versus hydrogels lacking cells.



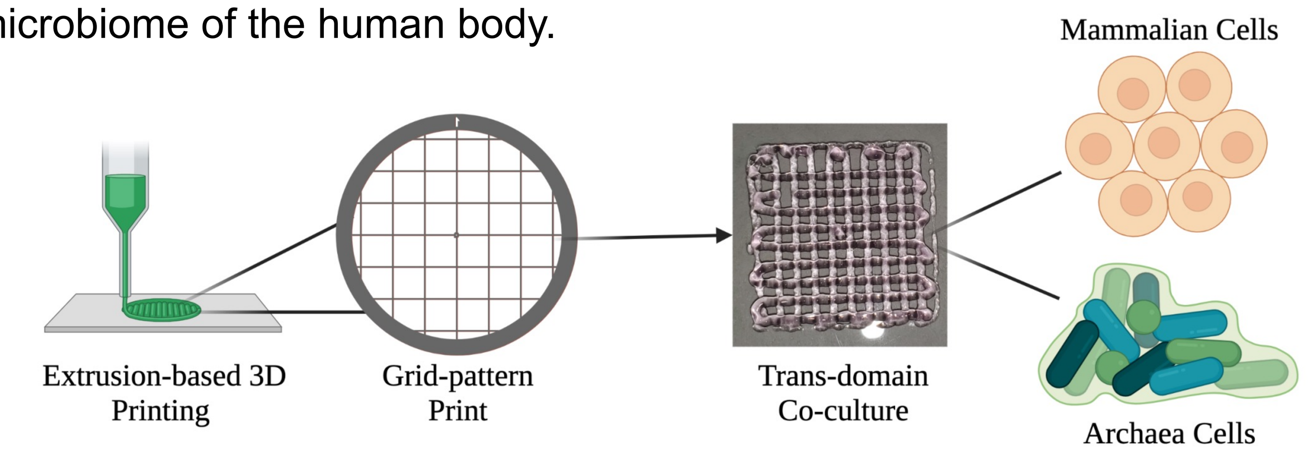
Cell Viability on Alginate and Alginate-Gelatin Hydrogels. Cell viability of three different types of gelatin and alginate hydrogels to a control group from a culture of *H. salinarum* cells in broth.

CONCLUSION

- The 6% alginate and 1.2M NaCl hydrogel were more solid-like at low shear rates, as observed by a greater storage modulus vs. loss modulus. Hydrogel decreases in stiffness with greater cell density.
- Halophiles can survive in hydrogels with salt concentrations below normal culturing conditions. The 6% alginate hydrogel with 1.2M NaCl concentration showed viability of *H. salinarum* greater than 90%.
- Line spreading decreases with the addition of *H. salinarum*.

FUTURE WORK

- Modify hydrogels to find optimal biopolymer compositions with the purpose of obtaining better printability and higher cell viability.
- Modify conditions of hydrogel to incorporate mammalian cells and *H. salinarum* cells in a co-culture to produce tissues that closely represent the microbiome of the human body.



ACKNOWLEDGEMENTS

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