Accelerating the Pathway from Bench to Bedside: The Printability of Human Dental Pulp Stem Cells with Physiological Relevance.

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Statement of Purpose: Extrusion-based bioprinting (EBB) provides a foundational in-vitro platform to investigate tissue engineering applications with unprecedented potential for experimental scalability. For early proof-of-concept demonstrations, researchers in the field of regenerative medicine can conduct complex three-dimensional (3D) bench studies to better mimic human tissue environments and determine the ideal niche for stem cell behavior. Although the long-term objective is to develop a standardized protocol for EBB as a translational animal-free model for mesenchymal human dental pulp stem cell (hDPSC) differentiation, the immediate need is the characterization of a versatile ink candidate that informs optimal printing conditions for therapeutic benefit. Methacrylated Gelatin (GelMA) is an ink in the current bioprinting armamentarium with demonstrated tunability, cell-biocompatibility and cell viability. Given the ability to tune the stiffness of GelMA to match matrix stiffness using photopolymerization, we hypothesized that the dynamic rheological properties could be optimized for the EBB of hDPSCs to create a physiologically relevant bench model. The stepwise methodology presented in this work is particularly translatable to commercial dentistry applications, where dental curing devices are commonly used.

Methods: Rheological measurements were conducted with a rotational rheometer (MCR 102 Anton Paar, Germany) operating in oscillatory shear to characterize the viscoelastic properties of commercially available GelMA bioink (Advanced BioMatrix, San Diego, CA) and determine the optimal gelation temperature to limit cell sedimentation during the printing process. Temperature ramps were conducted with a 25 mm parallel plate geometry and a 500 µm gap. Oscillatory measurements were obtained under a strain of 0.1% and frequency of ω= 6.28 rad/sec. To determine the effect of temperature on the viscoelastic properties of the GelMA bioink, cooling temperature ramps were conducted on 5% and 10% (w/v) GelMA samples after loading at 37°C. The Peltier plate was cooled at a rate of 1°C/min from 37°C to 4°C to determine gelation. Heating temperature ramps were conducted at a rate of 1°C/min from 4°C to 37°C to assess the gel-sol transition of 10% (w/v) GelMA bioink. During both directional temperature ramps, the measured torque of the oscillated samples was used to determine the temperature-dependent storage modulus, G', loss modulus, G", and loss factor $tan\delta$, where $tan\delta = G"/G'$. Next, the GelMA was optimized for bioink stability and hDPSC viability by adjusting the photoinitiator concentration, curing distance and ultraviolet (UV) exposure time. Finally, the GelMA stiffness was tuned for human dental pulp tissue mimicry by varying the UV intensity and GelMA concentration using ultraviolet (UV) in-situ rheology.

Results: Figure 1 shows that the gelation temperature of GelMA increases as the ink concentration increases, and that human dental pulp stem cells should be mixed in the bioink between 20-24°C when the tan δ is >1. Although human dental pulp stem cells are viable and printable in 10% (w/v) GelMA, concentrations that are ≥ 10% (w/v) may be both UV intensity and concentration dependent, whereas 7.5 % (w/v) GelMA is less dependent on the UV intensity and matches the reported storage modulus of hDPSCs between 2-7 kPa [1].

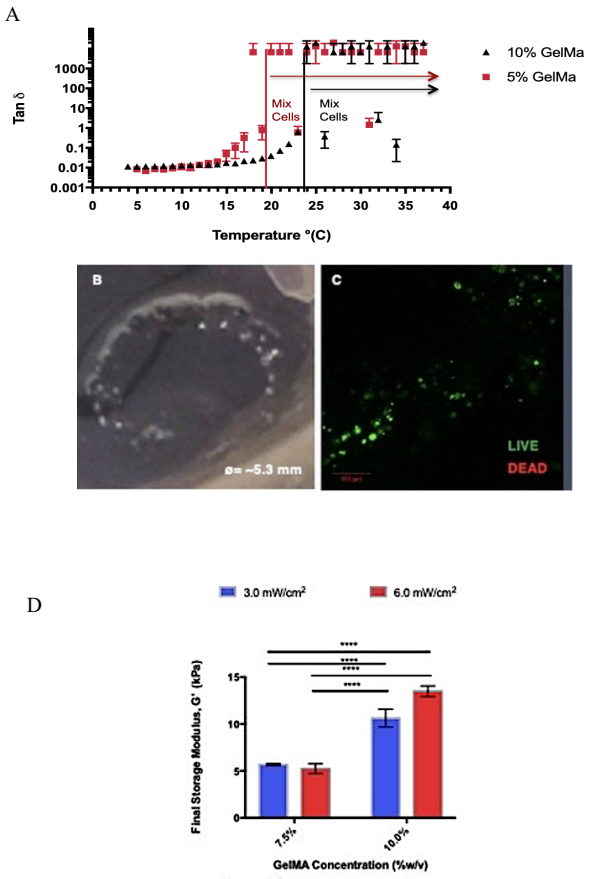

Figure 1A: Effect of temperature on the loss factor, tan δ. 1B: Representative images and viability of bioprinted hDPSC printed in 10% w/v GelMA circular constructs after 900 seconds of UV exposure**.** 1C: Live/Dead viability of bioprinted hDPSC. Magnification = 10x. Scale bar = 100 µm. 1D. The effect of GelMA concentration and UV intensity on the final storage modulus, *G'*. **** $p \leq 0.0001$.

Conclusions: Meeting clinical safety and efficacy regulatory endpoints for commercial applications could be accelerated with the effective design of normal and diseased bioprinted tissue models and could be used to develop preventative therapies and test novel devices.

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References: [1] Erisken C. J Endod. 2015;41(10):1711-7.