Collagen Fiber Rebar Leads to Improved Shape Fidelity and Cellular Functionality in Bioinks

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INTRODUCTION

A key challenge in protein-based bioinks is the requirement to balance the rheological properties of the ink with the shape retention for the resulting construct [1],[2]. Increasing the protein concentration of the bioink is a common approach to increase shape retention, but this results in increased viscosity which can complicate printing [3] and increased stiffness of the resulting construct which reduces cellular viability [4]. This has greatly increased the use of carbohydrates and synthetic polymers as an alternate to proteins due to ease of handling and improved mechanical durability [4], but these materials have poor cellular performance and rely on the addition of functional peptides such as RGD to support cellular growth [5]. Another popular approach is to use biopolymers or synthetic materials as additives in protein-based bioinks to improve their mechanical durability. Cellulose nanofibers (CNFs) are widely used to improve the mechanical durability of bioprinted constructs due to their lack of cytotoxicity, bioinert nature, and low cost [6], however CNFs do not drive cell attachment or functionality, and therefore do not contribute to a physiologically relevant cellular environment [7]. To overcome these challenges, 3D BioFibR has developed a collagen microfiber (µCollaFibR). Similar to CNFs, µCollaFibR acts like rebar and greatly improves the mechanical durability of bioinks, while also providing a physiologically relevant site for cell attachment, improving cellular viability and functionality within bioprinted constructs.

MATERIALS AND METHODS

3D BioFibR's dry-spinning approach was used to spin collagen fibers with 1-5 µm diameters, and 30-50 cm lengths. Briefly, this technique involves pulling a spinning solution of Type I collagen, a scaffolding polymer, and 0.20 mM acetic acid between a reservoir and a pin array, and then collecting the fibers over a substrate [8],[9]. These fibers were then suspended in PBS which removes the scaffolding polymer and then cut using a homogenizer to produce microfibers of 30-70 µm lengths. The µCollaFibR was resuspended in GelMA-based, and alginate-based bioinks, and the resulting constructs were tested for extensional and compressive mechanical properties using a Mark-10 uniaxial force tester. Shear viscosity of the bioinks was characterized using a parallel-plate rheometer, allowing analysis of the rheological properties of the bioinks at various concentrations of µCollaFibR. The shape retention, viability, and functionality of cell-laden constructs were tested over 28 days, using immunocytochemistry and microscopy.

RESULTS AND DISCUSSION

At concentrations of µCollaFibR as low as 1.25 mg/mL, the mechanical strength of GelMA-based and alginate-based hydrogels increased by up to 116%. For cell-laden constructs, this translated to only 8% gel-compaction over 28 days in culture, whereas the non-µCollaFibR conditions saw 25% compaction. Corresponding rheological measurements showed only a 1.2x increase in the shear viscosity (at 60 s^{-1}), compared to >6.5x when using the same concentration of CNFs. This allows bioprinter users to maintain their printability and use similar printing parameters to process the µCollaFibR containing bioinks. Over the 28 days in culture, µCollaFibR-containing hydrogels show improved cellular functionality and proliferation. Interestingly, in alginate hydrogels, cells showed high degrees of proliferation and functionality, and were able to attach to the µCollaFibR within the construct, eliminating the need for additional additives such as RGD.

CONCLUSIONS

µCollaFibR can be universally resuspended in natural and synthetic bioinks to provide an enriched collagen environment for 3D bioprinting. The collagen fibers act as a mechanical rebar within the bioprinted construct, increasing the mechanical strength of the construct, which can reduce the compaction within the hydrogel by 68%. This reduction in compaction better maintains the porosity within the construct, increasing the cellular viability and functionality. Additionally, µCollaFibR replicates the structure of natural collagen fibers, providing integrin-binding sites which drive cell adhesion, proliferation, and alignment, providing an alternative to additives such as RGD, and improving the physiological relevance of the bioprinted construct.

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