Novel Collagen-Based Inks for Tissue Engineering

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Visual Abstract

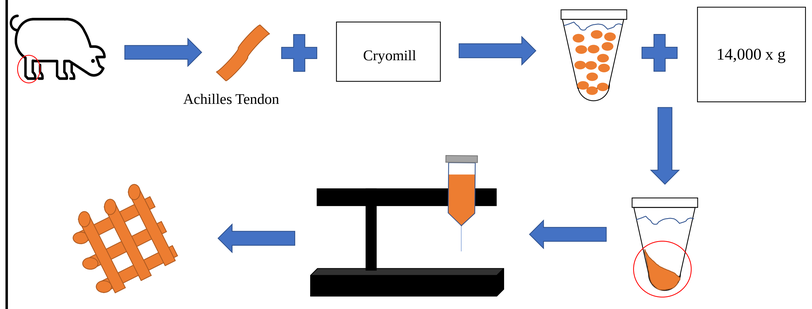

Fig. 1: Matrix preparation procedure.

Introduction

Applying collagen-based inks for 3D-printing extracellular matrices (ECMs) or tissue equivalent implants (TEIs) allows for the precise control of material distribution within ECMs or TEIs where the traditional macroscopic technologies (1) are lacking. However, collagen's unique physical-chemical properties have precluded the development of suitable collagen-based inks for many printing applications (2). As an example, collagen molecules or small collagen molecular aggregates are soluble in acidic solutions only at about 10mg/ml, a concentration too low for practical printing purposes. They also precipitate (coacervate) near neutral pH, at body temperature, or in the presence of neutral salts (e.g., NaCl) forming fibers which prevent the flow of collagen through printer nozzles. To meet the above challenges, we have developed native collagen microfiber-based inks for 3D bioprinting which can better simulate the collagen content in various native tissues for tissue engineering applications.

Materials

Type I collagen fibers were purified from porcine Achilles tendon according to the methods described previously (1). Type II collagen fibers were purified from the articular cartilage of bovine femoral condyles according to the method of Steven and Thomas (3).

Methods

Preparation of Collagen Microfiber-Based Inks: Collagen microfiber-based inks were prepared with a solid content from 20mg/ml to 200mg/ml. Purified collagen fibers were first milled at liquid nitrogen temperature, then sieved to isolate microfiber size groups ranging from smaller than 50µm to 150-175µm. Next, a fixed weight of a microfiber size group (e.g., 125-150µm) was hydrated at either pH 2.5 (0.05M lactic acid), pH 4.6 (acetate buffer), or pH 7.2 (phosphate buffer). Upon hydration at equilibrium, the excess solution was removed via centrifugation (3,000-14,000 x g's) and the final density calculated as weight of collagen (mg) per ml of the remaining solution (mg/ml). The hydrated collagen microfibers were directly applied to 3D printing as the ink.

Printing of 3D ECM Matrices: A mechanical extrusion-based printer (Hyrel3D ESR) was used to prepare matrices using 16G, 19G, and 22G blunt-tip needles. After preparation, collagen microfiber-based inks were extruded in a crosshatch pattern with 1mm between filaments for either 2, 4, or 8 layers at room temperature. Printing speed and flow rate ranged from 100-1600mm/min and 1.2-42.9µl/second respectively depending on the ink and nozzle size.

Morphology & Pore Structure: Surface and cross-sectional morphology and pore structure of freeze-dried matrices were observed via SEM (Hitachi S-4800).

Mechanical Testing: Matrices were prepared as described previously using 110mg/ml of 75-100µm Type I collagen microfibers at pH 7.2, and a 19G nozzle with 1mm between filaments. After printing, matrices were lyophilized, formaldehyde crosslinked, and rinsed. Matrices were hydrated prior to testing with a Chatillon CS2-225 mechanical tester at a crosshead speed of 0.5mm/min for all tests. Unconfined compression to 15% strain on 10mm×10mm×3mm matrices, tensile testing to failure on 20mm×10mm×3mm matrices, and 3-point bending to failure on 20mm×10mm×6mm matrices were performed.

Results

Fig. 2 shows the range of densities obtained for each Type I collagen microfiber size-range hydrated at each pH using the method described previously. Due to the significant solid content, the inks can be printed at room temperature for multiple layers and then immediately removed from the printer for further processing without extra stabilization steps (e.g., utilizing support hydrogels or polymerization at 37ºC) (2). Fig. 3 shows examples of printed microfiber-based ECM matrices using 200mg/ml of 150-175µm Type I collagen microfibers at pH 4.6 and a 16G nozzle (left) and using 143mg/ml of Type II collagen microfibers smaller than 50µm at pH 7.2 and a 22G nozzle (right). Fig. 4 demonstrates multilayer-printed matrices using 110mg/ml of 75-100µm Type I collagen microfibers at pH 7.2 and a 19G nozzle. Fig. 5 shows SEM images of the surface and cross-section of a freeze-dried ECM matrix containing 97.7mg/ml of Type I collagen microfibers smaller than 50µm at pH 4.6, showing pore sizes appropriate for cell infiltration into the intra-filament space. Values in Table 1 indicate that after crosslinking, matrices provide tensile and compressive strength while remaining flexible.

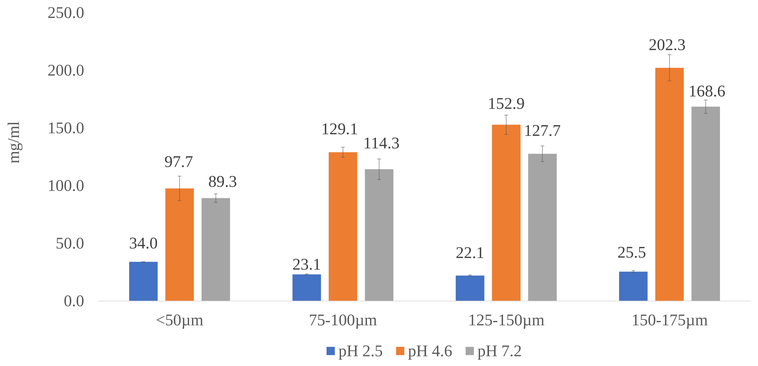

Fig. 2: Range of densities achieved for Type I collagen microfibers.

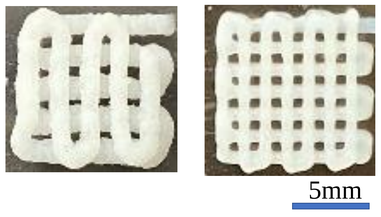

Fig. 3: 2-layer matrices of 200mg/ml Type I collagen microfiber ink (left) and 143mg/ml Type II collagen microfiber ink (right).

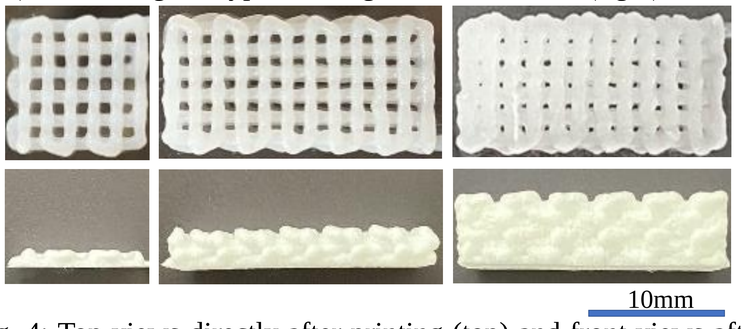

Fig. 4: Top views directly after printing (top) and front views after lyophilizing (bottom) of 2,4,and 8-layer matrices consisting of Type I collagen microfibers at 110mg/ml.

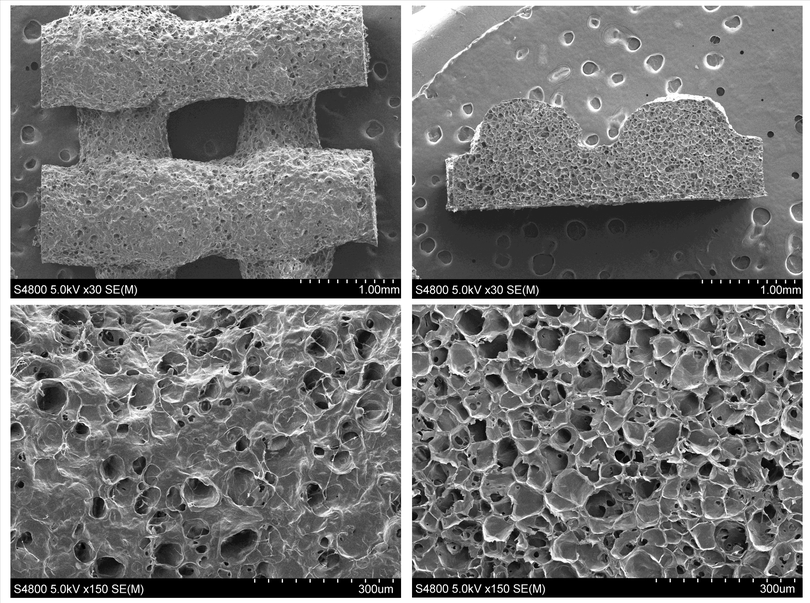

Fig. 5: SEM images of the surface and cross-section of a 3D-printed matrix consisting of 97.7mg/ml of Type I collagen microfibers.

Compressive Modulus	Tensile Modulus	Flexural Modulus
15 ± 1.7 N/cm^2	105.2 ± 11.9 N/cm^2	6.8 ± 1.9 N/cm^2

Table 1: Properties of crosslinked matrices containing 110mg/ml of Type I collagen microfibers.

Discussion

The uniqueness of these inks is multifold. First, we maintained collagens in their native fiber structure to preserve their innate biological properties for *in vivo* functions. Second, the weight of collagen in the ink can range from 20mg/ml to at least 200mg/ml to better match the native collagen content of various tissues. Third, decellularized ECMs can be processed according to the present method to include as much as possible the biologically active components for *in vivo* function. Fourth, multiple collagens can be simultaneously mixed into a single ink if needed to improve the *in vivo* function. Lastly, selective macromolecules and cells can potentially be incorporated into the ink at pH 7.2 to more closely simulate the structure of a particular tissue or organ.

Significance

The goal of this research is to advance the current state of collagen-based inks for 3D bioprinting of extracellular and tissue-equivalent matrices. A more physiologically relevant bioink can better support the development of tissue engineering solutions via 3D bioprinting of collagen-based inks.

Acknowledgements

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References

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