3D Printing of Highly Viscous Cellulose Nanofiber-enhanced Gelatin Methacryloyl for Tissue Engineering

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Introduction: In recent years, 3D printing has been increasingly investigated to produce diverse tissue engineering scaffolds with precise dimensions and structures. Hydrogels are appealing materials for 3D printing in tissue engineering due to their excellent biocompatibility, biodegradability and similarity to extracellular matrix. Gelatin methacryloyl (GelMA), synthesized by modifying gelatin with methacrylate group, has Arg-Gly-Asp (RGD) motifs (which are favourable for cell adhesion) and can be crosslinked by UV light, which makes GelMA one of the commonly used hydrogels for 3D printing [Gao Q, *et al*., *Biofabrication*., 2019, 11(3): 035006]. However, the poor printability of GelMA prevents it from making 3D printed complex structures with good shape fidelity and accuracy. Cellulose nanofiber (CNF) is a viscosity enhancer for 3D printing in tissue engineering [Markstedt K, et al. *Biomacromolecules*, 2015, 16(5): 1489-1496]. The addition of CNF in hydrogel may significantly improve the viscosity of GelMA hydrogels. In this study, CNF was added to GelMA to formulate an ink with desired printability for 3D printing. The viscosity enhancement and printability of CNF-incorporated GelMA were investigated.

Methods: GelMA was synthesized based on Van Den Bulcke *et al*.'s method [Van Den Bulcke AI, *et al*. *Biomacromolecules*, 2000, 1(1): 11-38]. Three inks were prepared: 10% (w/v) GelMA, 5% (w/v) CNF, 10% (w/v) GelMA added with 5% (w/v) CNF (CNF/GelMA). Briefly, a certain amount of synthesized GelMA was dissolved in deionized (DI) water at 60 °C under vigorous stirring. A photoinitiator (2-Hydroxy-2-methylpropiophenone) was then added in the solution to obtain GelMA ink. 5% (w/v) CNF was prepared by adding CNF powder in DI water and ultra-sonification for 30 minutes to homogenously disperse CNF in the suspension. To prepare CNF/GelMA ink, 20% (w/v) GelMA and 10% (w/v) CNF were prepared separately. These two were then mixed thoroughly at a volume ratio of 1:1 at 60 °C to obtain the CNF/GelMA ink. To investigate the effect of addition of CNF on hydrogels, the shear viscosities of GelMA, CNF and CNF/GelMA hydrogels were measured using a rotational rheometer (MCR302, Anton Paar, Austria) equipped with a 25 mm parallel plate and at a 0.55 mm measurement gap. One-layer and multi-layer grids were printed from the inks using a 3D bioprinter (3D DiscoveryTM Evolution, regenHU Ltd, Switzerland) to study the printability of these three inks.

Results: In the shear viscosity tests, it was observed that viscosities of all three inks decreased with an increase in shear rate ranging from 0.1 to 500 s^{-1} (Fig.1a). This shear thinning behavior is a critical factor for successful printing of hydrogels in extrusion-based 3D printing [Jungst T, *et al*., *Chem. Rev.*, 2016, 116(3): 1496-539]. More specifically, pure GelMA had the lowest viscosity across the whole range of shear rates applied, while CNF had much higher viscosity than pure GelMA. Noticeably, GelMA/CNF blend ink exhibited the highest viscosity, indicating that addition of CNF greatly enhanced the viscosity of the GelMA hydrogel. To evaluate the printing resolution of these three inks, one layer of small grid (8×8 mm^2, line spacing of 2 mm), as shown in Fig.1b, was printed. The line width of each grid was measured using the ImageJ software. It was found that 10% (w/v) GelMA had the largest line width (about 800 μm) when printed and GelMA/CNF had the smallest (about 400 μm). The addition of CNF had greatly increased the viscosity of GelMA hydrogel and hence allowed it to be printed into structures with high printing resolution and excellent shape fidelity.

Printing hydrogels still poses challenges since they often have insufficient strength to prevent structural collapse during and after 3D printing. In 3D printing, vertical height and shape fidelity directly reflect the stackability and printability of the hydrogel. Fig.2 shows 3D printed multi-layered structures using GelMA, CNF and GelMA/CNF ink, respectively. These printed structures had a layer thickness of 0.3 mm with interspace of 1 mm and contained 15 layers. It was observed that the shape of pure GelMA structure was not stable and the designed internal porous structure failed to form during the printing process. On the other hand, the 3D printed GelMA/CNF structure exhibited good internal porous structure (as viewed from the top) and maintained a stable shape (as viewed from the side), suggesting excellent printability of the GelMA/CNF hydrogel.

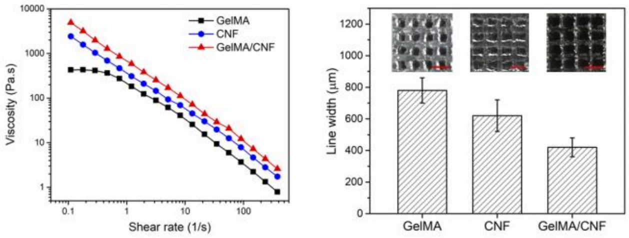

Fig. 1. (a) Shear viscosity of GelMA, CNF and GelMA/CNF inks, and (b) corresponding printed one-layer grid and line width results (Scale bar: 2 mm).

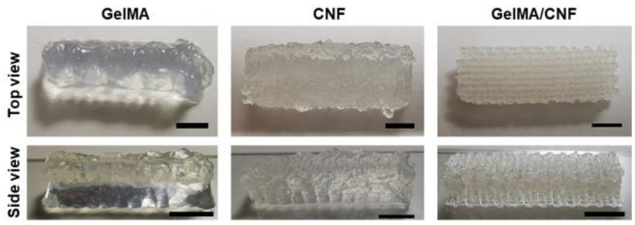

Fig. 2. Photographs of 3D printed scaffolds from three inks (GelMA, CNF and GelMA/CNF) with top views and side views (Scale bar: 5 mm).

Conclusions: A 3D printable hydrogel was formulated based on GelMA and CNF. The addition of CNF greatly increased the viscosity of GelMA hydrogel and hence improved its printability. The shape of 3D printed GelMA/CNF structures was stable during and after printing, and the designed internal porous structure was produced. These results showed that the developed GelMA/CNF hydrogel has the high potential for making, via 3D printing, complex tissue engineering scaffolds with high precision.

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