Characterization and Usability of Decellularized Lung-Based Hydrogels as 3D Printer Bioink

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1. Introduction and objectives

Advancing in lung bioengineering requires a better knowledge of cell-matrix crosstalk. Understanding how stem cells fate and mature cells response are modulated by their 3D mechanical microenvironment is particularly important. Hydrogels based on lung extracellular matrix (L-ECM) are promising scaffolds because, in addition of providing physiological molecular cues to cells, their 3D structure and viscoelasticity can be tuned in a controlled way. However, the mechanical properties of L-ECM hydrogels are still poorly characterized. The aim of this work is to study how the stiffness and 3D printability of L-ECM hydrogels can be modulated by modifying the gel preparation process.

2. Materials

Porcine lungs were decellularized by a detergent-based procedure. The resulting L-ECM was freeze-dried, cryomilled, and the resulting powder was enzymatically digested by pepsin [1] at a concentration of 10mg/mL. Type I Collagen (Col-I) was obtained from rat tail tendons [2] and resuspended at a concentration of 10mg/mL. Hydrogels were prepared by pH neutralization and mixing of different proportions of Col-I and L-ECM (1:1 and 3:1 in volume). After gellification, the resulting structures were chemically crosslinked by using 1mM concentration of genipin for 30'.

3. Methods

The gels were 3D bioprinted (RegenHu, 3DDiscovery) by using F-127 hydrogel as structural and sacrificial layer (Figure 1). The mechanical properties of the resulting structures were measured by applying tensile deformations (Aurora Scientific, 300C-LR) to hydrogel slices and by fitting stress-strain data to the Fung's constitutive model [3], which assumes that the Young modulus increases linearly with stress and the stress increases exponentially with stretch. The elastic modulus at 10% of relative stretch was then computed from the adjusted mathematical model.

4. Results and discussion

Hydrogel stiffness considerably depended on composition and crosslinking (Table I), increasing when varying the Col-I:L-ECM concentration ratio from 1:1 to 3:1, with respect to a 100% Col-I hydrogel, which presented an elastic modulus at 10% relative stretch of 7.3 kPa. In conclusion, hydrogels based on L-ECM exhibit tunable stiffness and thus can be used as 3D bioink to build potentially useful scaffolds for lung tissue engineering.

***Figure 1**. 3D Bioprinting L-ECM:Col-I hydrogels by using F-127 as structural/sacrificial material; resulting 3D structure.*

Col-I:L-ECM	Uncrosslinked	1mM genipin
1:1	2.2 kPa	16.1 kPa
3:1	4.1 kPa	24.9 kPa

***Table 1**. Measured mechanical properties at 10% relative stretch of the bioprinted hydrogels*

References

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