Naturally-derived Hydrogels with Tunable Mechanical Properties for 3D Culture of Lung Adenocarcinome Cells

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1. Introduction and objectives

In vitro models do not generally reflect the complexity of the cellular microenvironment found *in vivo*. Naturally derived materials, like Type I Collagen (Col-I), can be used to form hydrogels for three-dimensional cell cultures, which is a step further towards mimicking the entire native environment. Hydrogel mechanical properties can be tuned by physical and chemical crosslinking. The aim of this work is to study the influence of the mechanical properties of Col-I hydrogels on the 3D culture of human lung adenocarcinome cells.

2. Materials

Human adenocarcinome alveolar epithelial cells expressing GFP were cultured with Ham`s F12 and GlutaMax-1 medium supplemented with 10% FBS. Col-I was obtained from rat tails following an established protocol [1]. Pre-gel was pH-stabilized and then mixed with $8x10^5$ cells suspended in 1ml of culture media for a final Col-I concentration of 7.5mg/ml. The cell-laden hydrogel was physically crosslinked by incubation at 37ºC for 30'. Additional chemical crosslinking was performed by using 2mM genipin for 30'.

3. Methods

The mechanical properties of acellular structures were measured by applying tensile deformations (Aurora Scientific, 300C-LR) to hydrogel slices (approximately $10x2x1mm^3$) and by fitting stress-strain data to the Fung's constitutive model [2], which assumes that the Young's modulus increases linearly with stress and the stress increases exponentially with stretch. The elastic modulus at 10% of relative stretch was computed from the adjusted model. 3D cultures were imaged by confocal microscopy (Nikon D-Eclipse C1) at days 1, 5 and 7. Live/Dead assays were performed at day 2. TUNNEL assays were performed at day 5. Images were computed with ImageJ software by using a thresholding algorithm [3].

4. Results and discussion

Young's moduli of the measured acellular structures were 7.3 kPa and 41.3 kPa for the physical-only and physical+chemical crosslinked structures, respectively. Live/Dead analysis showed more than 80% cell viability at day 2 and TUNNEL assays showed approximately 50% cell viability at day 5 in both cases. Results on the proliferation of the cells (Table 1) showed a trend in the number of cells when increasing the elastic modulus of the 3D structure. In conclusion, tuning the mechanical properties of the 3D structure is a potential factor for modulating adenocarcinoma cell proliferation.

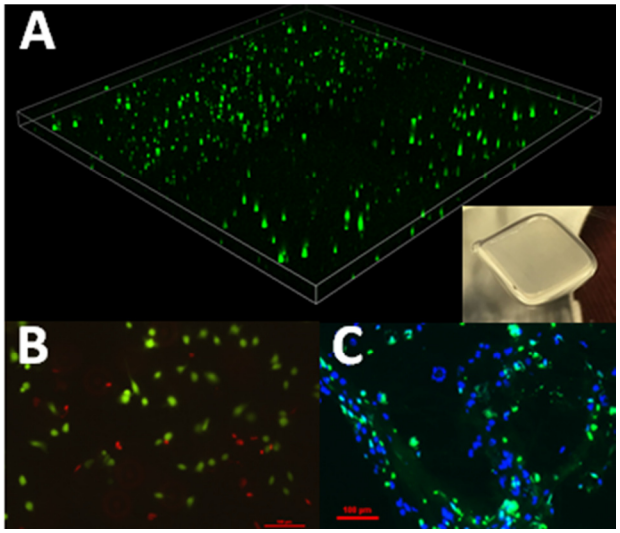

***Figure 1**. Representative images of 3D confocal imaging (a), Live/Dead (b) and TUNNEL (c) assays.*

	Physical	Physical+chemical
Day 5	0.85	0.76
Day 7	1.40	1.59

***Table 1**. Fold-change of the proliferation of cells in physical and physical+chemical crosslinking 3D Col-I hydrogels*

References

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