USING MELT-ELECTROWRITTEN FIBROUS MESH TO ELEVATE FLUID PRESSURISATION IN HYDROGELS

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Introduction

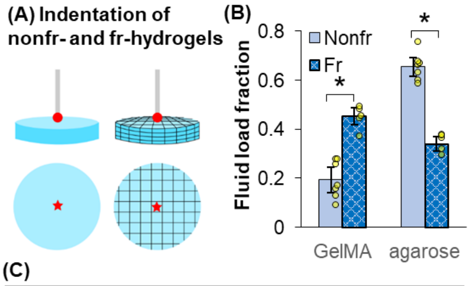
Soft hydrogels play an important role in providing three-dimensional (3D) support for cell growth. Cell-seeded hydrogels are typically cultured under free-swelling unloaded condition. However, such culturing conditions do not always favour protein synthesis of mechano-sensitive cells, such as those found in cartilage and tendon. Mechanical stimuli of various forms have been applied to stimulate cell growth. Fluid pressure is a key mechanical stimulus and has been shown to promote protein production and phenotype retention for cells [1]. Recently, melt-electrowritten (MEW) fibrous mesh was used to enhance mechanical stiffness of the gelatin methacryloyl (GelMA) hydrogels [2], but little is known if the MEW mesh can induce fluid pressurisation in the hydrogels during mechanical loading, which can serve as an additional form of stimulus. The purpose of this study was two-fold: (i) to determine the load-induced fluid pressurisation in two hydrogel systems, GelMA and agarose, using indentation tests coupled to an analytical biphasic material model, and (ii) to determine if MEW fibrous mesh can improve the load-induced fluid pressurisation in these hydrogels. We hypothesised that MEW mesh can elevate the fluid pressurisation in both GelMA and agarose hydrogels.

Methods

Poly-caprolactone (PCL) fibres of 20 µm diameter were MEW-printed in a grid structure on a layer-by-layer manner with 600 µm inter-fibrillar spacing to make a fibrous mesh of ~2mm thickness. 15% w/v GelMA and 4% w/v agarose mesh-free (non-fr) hydrogels of 2mm thickness were fabricated using custom-built Teflon mould. MEW mesh-hydrogel composite (fr-hydrogels) was fabricated by infusing the MEW mesh with hydrogel solution before cross-linking. The hydrogel constructs with and without MEW mesh underwent mechanical indentation by a rigid, spherical indenter of 1 mm in diameter (Fig. 1A). The indentation protocol contained a combination of different indentation depths (50–250 µm, corresponding to 2.5–12.5% strain) applied at different loading rates (0.5–20 µm/s). The resulting force responses were curve-fitted to a biphasic Hertz material model to derive the biphasic mechanical properties of the hydrogels [3], which included the fluid load fraction representing the proportion of applied mechanical load carried by fluid pressure.

Results

The incorporation of MEW fibrous mesh into the hydrogels successfully improved the fluid load fraction for GelMA, but not for agarose (Fig. 1B). The negligible fluid pressure in nonfr-GelMA (~5 kPa) was elevated to as high as 64 kPa after being reinforced by the MEW mesh (Fig. 1C). However, the opposite was true for the agarose (Fig. 1C).



(C)

	Fluid pressure at different indentation depth (kPa)		
	50 µm	150 µm	250 µm
Nonfr-GelMA	4.1	4.0	5.3
Nonfr-agarose	21.7	35.4	45.6
Fr-GelMA	28.7	43.7	63.8
Fr-agarose	20.0	23.8	33.3

*Figure 1: (A) Indentation setup for nonfr and fr-hydrogels. (B) Fluid load fraction, and (C) fluid pressurisation at different indentation depths for nonfr- and fr-hydrogels (GelMA and agarose). * indicates statistical difference between the compared groups.*

Discussion

GelMA was covalently cross-linked whereas agarose was physically cross-linked. Mechanical reinforcement by MEW mesh in terms of fluid pressurisation appears to depend on the cross-linking mechanism of the hydrogels. We show that MEW fibrous mesh can improve the load-induced pressurisation in GelMA, thereby suggesting additional advantages of using the MEW mesh to improve the cell growth through additional stimulus in the form of fluid pressure.

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