Enzyme Responsive Peptide Gels for Regenerative Medicine

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INTRODUCTION:

Spontaneous formation of macroscopic hydrogels from small molecule building blocks via self-assembly provides a route toward designed functional biomaterials. We will show that a number of small peptide amphiphiles, consisting of (mixtures of) dipeptides linked to fluorenylmethoxycarbonyl (Fmoc) spontaneously form fibrous hydrogels in physiological conditions. The self-assembly process is driven by π-stacking of the conjugated fluorenyl moieties and formation of helical conformations as is demonstrated by circular dichroism and fluorescence spectroscopy. The amino acid sequence within the Fmoc-dipeptide building blocks controls the architecture and the physical properties of the assembled structures. Combinations of Fmoc-dipeptides were identified that formed fibrous hydrogels that were i) stable under cell culture conditions, ii) of similar dimensions to the fibrous components of the extracellular matrix and iii) capable of supporting cell culture of chondrocytes in 3D.[1] We demonstrate that peptide gel formation can be triggered selectively by using proteases. [2]

METHODS: Characterisation of hydrogels: circular dichroism, fluorescence spectroscopy, cryo-scanning electron microscopy, rheology. Chondrocyte cell culture in 2D and 3D, collagen antibody staining, fluorescence microscopy, environmental scanning electron microscopy (ESEM).

RESULTS & DISCUSSION: We found that a number of peptide gels could be formed that were transparent and stable at pH7 in tissue culture medium. The physical properties and fibrous morphologies of these gels were strongly dependant on the nature of the amino acid side chains (see Figure 1). These peptide gels supported metabolically active chondrocyte cells for periods of up to two weeks (Figure 2). Peptide gels could undergo sol to gel transitions in response to changes in temperature and ionic strength, which is useful for minimal invasive surgery strategies.

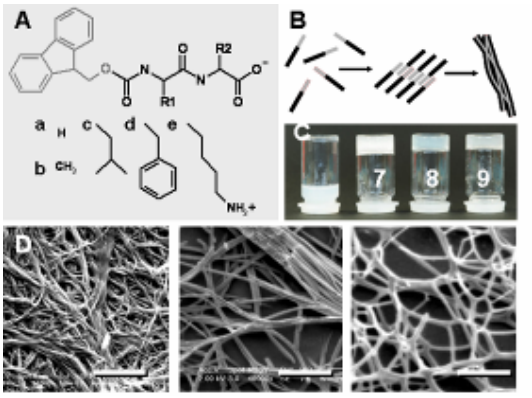

Figure 1: A: Molecular structure of Fmoc-dipeptides. The R groups are for amino acids Gly (**a**), Ala (**b**), Leu (**c**), Phe (**d**) or Lys (**e**). B: Proposed self-assembly mechanism. D: Cryo-SEM micrographs of nanofibrous materials.

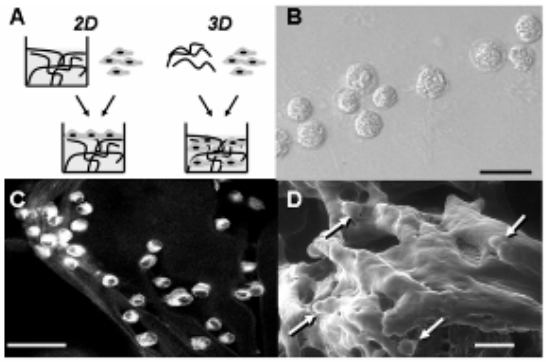

Figure 2: Chondrocyte cell culture on peptide hydrogel scaffolds. A: 2D and 3D cell culture. B: chondrocytes on hydrogel surface. C: DAPI stained chondrocytes in 3D culture. D: ESEM study of hydrated gel with chondrocytes (arrows).

CONCLUSIONS: We demonstrate that short peptide amphiphiles can form extracellular matrix mimics that support chondrocyte cell culture in 2D and 3D.

REFERENCES: **(**1) Jayawarna V, Ali M, Jowitt TA, Miller AF, Saiani A, Gough JE, Ulijn RV. *Adv. Mater.* **2006**, 5, *in press.* (2) Toledano, S.; Williams, R. J.; Jayawarna, V.; Ulijn, R. V. *J. Am. Chem. Soc*. **2006**; 128(4); 1070-1071.