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Research Paper

Application of UVA-riboflavin crosslinking to enhance the mechanical properties of extracellular matrix derived hydrogels

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ABSTRACT

Hydrogels derived from extracellular matrix (ECM) have become increasing popular in recent years, particularly for use in tissue engineering. One limitation with ECM hydrogels is that they tend to have poor mechanical properties compared to native tissues they are trying to replicate. To address this problem, a UVA (ultraviolet-A) riboflavin crosslinking technique was applied to ECM hydrogels to determine if it could be used to improve their elastic modulus. Hydrogels fabricated from corneal, cardiac and liver ECM were used in this study. The mechanical properties of the hydrogels were characterized using a spherical indentation technique. The microstructure of the hydrogels and the cytotoxic effect of crosslinking on cell seeded hydrogels were also evaluated. The combination of UVA light and riboflavin solution led to a significant increase in elastic modulus from 6.8 kPa to 24.7 kPa, 1.4 kPa to 6.9 kPa and 0.9 kPa to 1.6 kPa for corneal, cardiac and liver ECM hydrogels respectively. The extent of this increase was dependent on a number of factors including the UVA exposure time and the initial hydrogel concentration. There were also a high percentage of viable cells within the cell seeded hydrogels with 94% of cells remaining viable after 90 min exposure to UVA light. These results suggest that UVA-riboflavin crosslinking is an effective approach for improving the mechanical properties of ECM hydrogels without resulting in a significant reduction of cell viability.

1. Introduction

In recent years, hydrogels have become increasingly popular for many clinical and biomedical research applications including for their use in tissue engineering (Drury and Mooney, 2003), cell encapsulation (Nicodemus and Bryant, 2008), drug delivery (Hoare and Kohane, 2008) and wound healing (Muzzarelli, 2009). Hydrogels consist of a water swollen network of crosslinked polymer chains that can have a wide range of physical properties depending on the composition of

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the polymer and the nature of the crosslinks. They are particularly attractive for medical applications due to their biocompatibility, permeability, viscoelasticity and ability to be remodeled by cells (Ahearne, 2014). However, for tissue engineering and regenerative medicine applications most hydrogels lack essential extracellular matrix (ECM) components found in the native tissue that are required to modulate cell behavior and regenerate tissue.

Recently, hydrogels derived from the ECM of several different types of tissue have been fabricated for use in tissue engineering and regenerative medicine. The main advantage of these hydrogels over conventional hydrogels is that they can retain a variety of ECM components such as proteins, proteoglycans and growth factors that can enhance the restoration of functional tissue. To date this approach has been applied to generate hydrogels from orthopedic tissues (Sawkins et al., 2013; Wu et al., 2015), cardio-vascular tissues (Singelyn et al., 2009; Sonnenberg et al., 2015), nervous tissue (Medberry et al., 2013), ocular tissue (Ahearne and Lynch, 2015), dermal tissue (Wolf et al., 2012), adipose tissue (Young et al., 2011) and liver (Lee et al., 2014). ECM hydrogels derived from a patients own tissue have also been suggested as a method of delivering cells to a defect site that would reduce the chances of an immune response (Shevach et al., 2015).

One of the limitations with many hydrogels derived from ECM or natural polymers is that their mechanical properties are inferior to the native tissues (Zhu and Marchant, 2011). For hydrogels implanted at load bearing sites, this could lead to failure of the implant. While several crosslinking chemicals are routinely used to improve the mechanical properties of hydrogels such as glutaraldehyde or N-(3-dimethylaminopropyl)-N' -ethylcarbodiimide, these would have a negative impact on the viability of any encapsulated cells. UVA-riboflavin crosslinking has been used on collagen hydrogels to increase the hydrogel stiffness (Ahearne et al., 2008; Mi et al., 2011; Heo et al., 2015; Rich et al., 2014) and has been applied clinically to treat conditions such as keratoconus (Wollensak et al., 2003; Iovieno et al., 2008; Coskunseven et al., 2009), a condition that results in thinning and weakening of the cornea. In the presence of UVA light, riboflavin produces free-radicals and oxidative species that react with collagen molecules resulting in covalent bonds being formed (Dalle Carbonare and Pathak, 1992; Fawzy et al., 2013). While this form of crosslinking has been successful in increase the stiffness and strength of type I collagen, its influence on many other ECM components is unknown. Cornea, which has been the most extensively tested UVA-riboflavin crosslinked material, principally consists of collagen type I with lesser amounts of proteoglycans and other collagens. Dentin, which has high percentage of hydroxyapatite, has been shown to increase in strength and modulus after UVA riboflavin crosslinking (Liu et al., 2015). While in these cases the presence of non-collagen ECM components did not impede the crosslinking process, this may not be the case for all tissues and therefore requires investigation.

The aim of this study was to determine if the elastic modulus of ECM hydrogels could be increased using UVA-riboflavin crosslinking. ECM hydrogels fabricated from several different tissues were examined. Several different crosslinking parameters such as crosslinking time or riboflavin concentration were also investigated. The effect of UVA crosslinking on the microstructure of the hydrogels and on cell viability within the hydrogels was also assessed.

2. Materials and methods

2.1. *Sample preparation*

All chemicals were purchased form Sigma-Aldrich unless otherwise stated. For this study ECM hydrogels were fabricated from porcine cornea unless otherwise stated. Hydrogels were also prepared using ovine liver or ovine heart to examine if the crosslinking process could be successfully applied to hydrogels derived from ECM of different tissue types. The tissues were removed and washed in deionized water. The tissues were then frozen at −30 °C for 1 h followed by freeze drying at −10 °C, 200 mbar overnight to remove any water from the samples. To form an ECM powder, the samples were milled using a SPEX SamplePrep Freeze/Mill. The powder was dissolved in a 1 mg/ml pepsin solution in 0.1 M hydrochloric acid over 72 h. The concentration of ECM in pepsin solution was 20 mg/ml. Hydrogels were fabricated by combining the ECM in pepsin with a 10% volume 10x Dulbeccos modified eagle medium (DMEM), sodium hydroxide to neutralize the pH and dH_2O. Prior to setting, 500 μl of hydrogel solution was poured inside a filter paper ring of inner diameter 20 mm as shown (Fig. 1A). A second filter paper ring was positioned on top of each hydrogel to provide additional support and the hydrogels were allowed to set at 37 °C for 30 min. Hydrogels with ECM concentrations of 15,

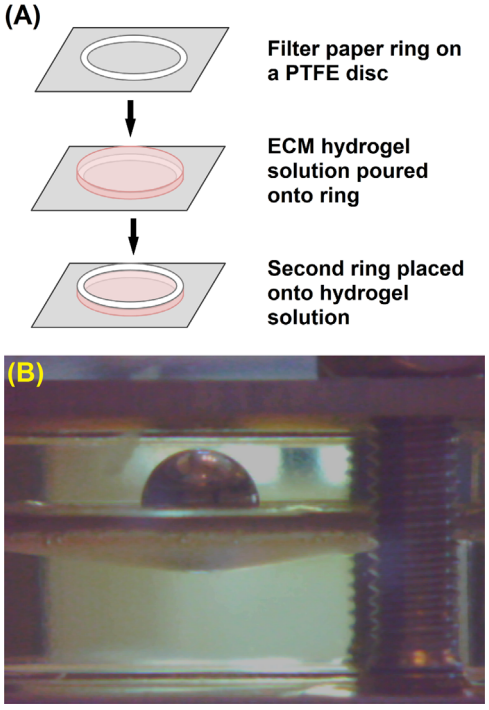

Fig. 1 – (A) Fabrication process for ECM hydrogel; (B) image recorded of an ECM hydrogel under spherical deformation recorded by digital microscope.