



# Strong and tough nanofibrous hydrogel composites based on biomimetic principles



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## ARTICLE INFO

### Article history:

Received 17 August 2016

Received in revised form 13 October 2016

Accepted 7 November 2016

Available online 14 November 2016

### Keywords:

Fracture

Toughness

Hydrogel

Nanofibres

Nanocomposite

## ABSTRACT

Mechanically robust hydrogels are required for many tissue engineering applications to serve as cell-supporting structures. Unlike natural tissues, the majority of existing tough hydrogels lack ordered microstructures organized to withstand specific loading conditions. In this work, electrospun gelatin nanofibres, mimicking the collagen network in native tissues, are used to strengthen and resist crack propagation in brittle alginate hydrogels. Aligned nanofibre reinforcement enhances the tensile strength of the hydrogels by up to two orders of magnitude. The nanofibres can be arranged as multilayer laminates with varying orientations, which increases the toughness by two orders of magnitude compared with the unreinforced hydrogel. This work demonstrates a two-part strategy of fibre reinforcement and composite lamination in manufacturing strong and tough hydrogels with flexible microstructures to suit different mechanical and biomedical requirements.

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## 1. Introduction

Hydrogels, water-swollen networks of hydrophilic polymer, are extensively used as scaffolds for tissue engineering since they can provide extracellular matrix (ECM)-like microenvironments and regulate cell fate during tissue regeneration [1–3]. However, using hydrogels as tissue engineering scaffolds to mechanically support cells is challenging, as the large volume fraction of water can result in weakness, compliance and brittleness. The mechanical performance of hydrogel scaffolds is often inferior to that of the native tissues they would replace in tissue engineering applications [4]. Development of mechanically robust hydrogels is thus important to provide sufficient mechanical performance for structural biomedical applications.

Most mammalian ECM has a fibre-reinforced composite design, consisting of a compliant aqueous matrix reinforced with fibrous protein, much of which is fibrillar collagen [5]. The ECMs of different tissues often have diverse microstructures and compositions to efficiently suit different tissue functions [6]. Collagen fibrils in tendons and ligaments are aligned along their axes to resist longitudinal tension, while collagen fibrils in cornea are orthogonally aligned in a laminated structure due to a need for biaxial stiffness to oppose intraocular pressure [7,8]. Further, the ability of fibres to reorient under

deformation not only stiffens and strengthens tissues, but also provides a toughening mechanism to prevent mechanical failure [9–11]. Having a fibrous microstructure generally allows native tissues to simultaneously possess good stiffness, strength and toughness.

A number of tough hydrogels have been developed, including those with hybrid chemical and physical cross-linkers, and double networks of sacrificial short chain and long-chain polymers [12–17]. However, the majority of these hydrogels do not truly mimic the microstructure of the tissues' ECM, lacking fibrillar microstructures organized to withstand specific loading conditions. Fibre reinforced hydrogels have been proposed to mimic soft tissues and to improve the mechanical performance of hydrogels, using 3D printing to make the reinforcement [18–21] or utilizing pre-made woven fibre mats [22–24]. The diameters of such fibres are usually micrometre-scale, an order of magnitude or more greater than the collagen fibre size in natural tissues. In contrast, electrospinning is a simple and commonly used method to produce polymer nanofibres, inspiring recent reviews of electrospun fibres coupled with hydrogels to form nanocomposites [25–27]. The introduction of nanoscale fibres into hydrogels would better replicate the in vivo cellular microenvironment, promote cell attachment and allow for mechanotransduction during tissue regeneration [28].

In the current study, the composite design principles of fibre reinforcement and lamination, often observed in nature and commonly used in conventional composite materials engineering, are applied in hydrogel manufacturing. Nanofibre-reinforced and laminated

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composite hydrogels were produced from gelatin and alginate – abundant and inexpensive naturally-derived polymers – to mimic the microstructure and composition of the ECM of collagenous soft tissue. Gelatin [29] was electrospun [30] into nanofibres and infiltrated with alginate [31] gel. Gelatin and alginate were also used to form single polymer hydrogel controls with no fibre-reinforcement. Composites featured fibres that were in a single layer, either random or aligned, or stacked in multi-layer laminates with specified fibre orientations. Nanocomposite specimens were mechanically tested in uniaxial tension and in fracture modes I and III and found to be simultaneously stronger and tougher than single polymer hydrogels.

## 2. Materials and methods

### 2.1. Fibrous scaffold preparation

All chemicals used in this study were purchased from Sigma Aldrich (Dorset, UK). Nanofibre-reinforced composite hydrogels were manufactured in two main steps: production of gelatin nanofibres and integration of nanofibres into an alginate aqueous matrix (Fig. 1a). Gelatin nanofibres were electrospun from 12 wt% gelatin solution (porcine, 250 g bloom strength) prepared in 90% v/v acetic acid. The solution was discharged through an 18 G needle (BD, Oxford, UK) at  $0.005 \text{ mL min}^{-1}$  with an infusion pump (KR Analytical Ltd., Sandbach, UK). A high voltage power supply (Glassman high voltage Inc., Bramley, UK) was used to apply a voltage difference of 12 kV across the needle and a collector, which were horizontally 10 cm apart. It was previously established that tensile elastic properties of electrospun mats containing aligned gelatin nanofibres could be controlled by varying the speed of a rotating collector [32], up to a point where the properties plateaued. Here, a grounded 5 cm-diameter drum rotating at 3100 rpm was used to collect aligned fibres (Fig. 1b), while a grounded 7 cm-diameter copper plate was used to collect randomly-aligned fibres.

### 2.2. Composite preparation

Five different types of composite hydrogels were formed, as characterized by the fibre arrangement: (1) fibres that were random in the plane; (2) a single layer of aligned fibres; (3) laminated fibres designated as (i) unidirectional, where four layers of fibres in the same orientation were stacked ( $0^\circ/0^\circ/0^\circ/0^\circ$ ), as (ii) cross-ply, where alternating layers were perpendicular ( $0^\circ/90^\circ/0^\circ/90^\circ$ ), and as (iii) angle-ply, with four different fibre orientations ( $0^\circ/45^\circ/90^\circ/-45^\circ$ ). Three wt% alginate and 12 wt% gelatin slab-cast hydrogels were used as single polymer hydrogel controls.

Single electrospun mats, or stacks of alternately oriented mats for lamination, were chemically cross-linked (Fig. 1c) in an ethanol-water (7:3) solution containing 25 mM of 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 10 mM of N-hydroxyl succinimide (NHS) at room temperature for 2 h to increase their stability in water [33,34]. Electrospun mats or laminate stacks were then dried for 24 h in a desiccator.

The dehydrated cross-linked gelatin mats were immersed in 3 wt% alginate solution for 4 h before being ionically gelled with divalent calcium ions via a 120 mM calcium chloride solution for 2 h (Fig. 1d) [35]. The composite hydrogels were then cross-linked in the EDC/NHS cross-linking solution for 2 h to induce further covalent bond formation between fibres and within the fibres. The fibres also likely formed covalent bonds to the ionically bonded alginate matrix [31,33,36]. The resulting hydrogels were stored dehydrated and were fully rehydrated prior to mechanical testing.

### 2.3. Mechanical characterization

All mechanical tests were performed with a universal testing machine (Instron model 5544, Canton, MA, USA) equipped with a

500 N load cell. For monotonic strain-to-failure tests, specimens with dimensions of 5 mm wide  $\times$  20 mm gauge length were stretched at  $0.5 \text{ mm s}^{-1}$ . For mode I fracture tests (Fig. 2a), specimens with a dimension of  $10 \times 15 \text{ mm}$  containing a 3 mm transverse notch were stretched at  $0.5 \text{ mm s}^{-1}$ . For mode III trouser tear tests (Fig. 2b), a 3 mm longitudinal notch was introduced and the specimens were torn at  $0.5 \text{ mm s}^{-1}$ . For all tests, at least six specimens were analyzed to calculate average and standard deviation values of corresponding mechanical properties. Single layer aligned composites and multi-layer laminates were tested in three orientations, at  $0^\circ$ ,  $45^\circ$  and  $90^\circ$  to the fibre direction in the top (or only) layer of the laminate (Fig. 2c).

### 2.4. Microscopy

The morphology of fibres in the electrospun mats was characterized by scanning electron microscopy (SEM, Carl Zeiss, Cambridge, UK) at an accelerating voltage of 15 kV. Prior to SEM, samples were coated with a thin layer of gold to produce a conductive surface. The diameter and directionality of the fibres were analyzed from SEM images using ImageJ (NIH, Bethesda, USA). A minimum of  $n = 30$  (10 fibres from each of three images) were used for calculations of the fibre diameter.

## 3. Results

### 3.1. Manufacture of composite hydrogels

Both randomly-oriented and aligned gelatin nanofibres were produced, with fibre diameters of  $133 \pm 24 \text{ nm}$  from the static collector and of  $99 \pm 18 \text{ nm}$  from the rotating collector. Gelatin fibres became larger after cross-linking, with the diameter of aligned fibres swelling to  $231 \pm 49 \text{ nm}$  (Fig. 1b–c). Cross-linked gelatin fibres gradually swelled in alginate solution while maintaining their original shape; without cross-linking the gelatin fibres were extremely hydrophilic and swelled rapidly to the point of being destroyed by the strong surface tension of the alginate solution [37]. The white cross-linked gelatin fibre mats also changed over time to become transparent when rehydrated and infiltrated with alginate.

### 3.2. Elastic properties of composite hydrogels

Single polymer hydrogels of alginate or gelatin were compliant and weak: alginate hydrogels had tensile elastic modulus and tensile strength of  $E = 78 \pm 19 \text{ kPa}$  and  $\sigma_f = 19 \pm 9 \text{ kPa}$ , respectively while gelatin hydrogels had  $E = 240 \pm 25$  and  $\sigma_f = 10 \pm 3 \text{ kPa}$ . (Strength data are shown in Fig. 3; elastic modulus data are not shown but followed similar trends.) However, when gelatin was electrospun into nanofibres and combined with alginate in the form of fibre-reinforced composite hydrogels, elastic properties of the alginate hydrogels could be enhanced by up to two orders of magnitude. An amplification factor,  $A$ , is used here to quantify the improvement in mechanical properties due to the effect of reinforcement, by normalising composite properties to those of the homogenous alginate matrix [26].

Aligned fibre reinforcement yielded the greatest improvement in stiffness and strength, enhancing the elastic modulus and the strength of the hydrogels in the longitudinal direction to  $3.21 \pm 0.48$  ( $A \approx 41$ ) and  $2.94 \pm 0.30 \text{ MPa}$  ( $A \approx 155$ ). As expected, mechanical behaviour of such composite hydrogels were inherently anisotropic. The strength in the longitudinal direction was five or seven times greater than the strength in the diagonal and transverse directions, respectively (Fig. 3d–f). In contrast, reinforcing hydrogels with randomly-oriented fibres resulted in more isotropic characteristics due to the relatively even distribution of the fibres in the plane. The strength of these hydrogels were approximately three times smaller ( $A \approx 49$ ) than those reinforced with aligned fibres and tested in the

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