Strong and tough nanofibrous hydrogel composites based on biomimetic principles

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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.

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 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 ($0°/0°/0°/0°$), as (ii) cross-ply, where alternating layers were perpendicular ($0°/90°/0°/90°$), and as (iii) angle-ply, with four different fibre orientations ($0°/45°/90°/-45°$). 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-(dimethyl-aminopropyl) 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 × 20 mm gauge length were stretched at 0.5 mm s^{-1}. For mode I fracture tests (Fig. 2a), specimens with a dimension of 10 × 15 mm containing a 3 mm transverse notch were stretched at 0.5 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 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, 45 and 90 ° 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 ± 24 nm from the static collector and of 99 ± 18 nm from the rotating collector. Gelatin fibres became larger after cross-linking, with the diameter of aligned fibres swelling to 231 ± 49 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 $E = 78 \pm 19$ kPa and $\sigma_f = 19 \pm 9$ kPa, respectively while gelatin hydrogels had $E = 240 \pm 25$ and $\sigma_f = 10 \pm 3$ 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 ± 0.48 ($A \approx 41$) and 2.94 ± 0.30 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
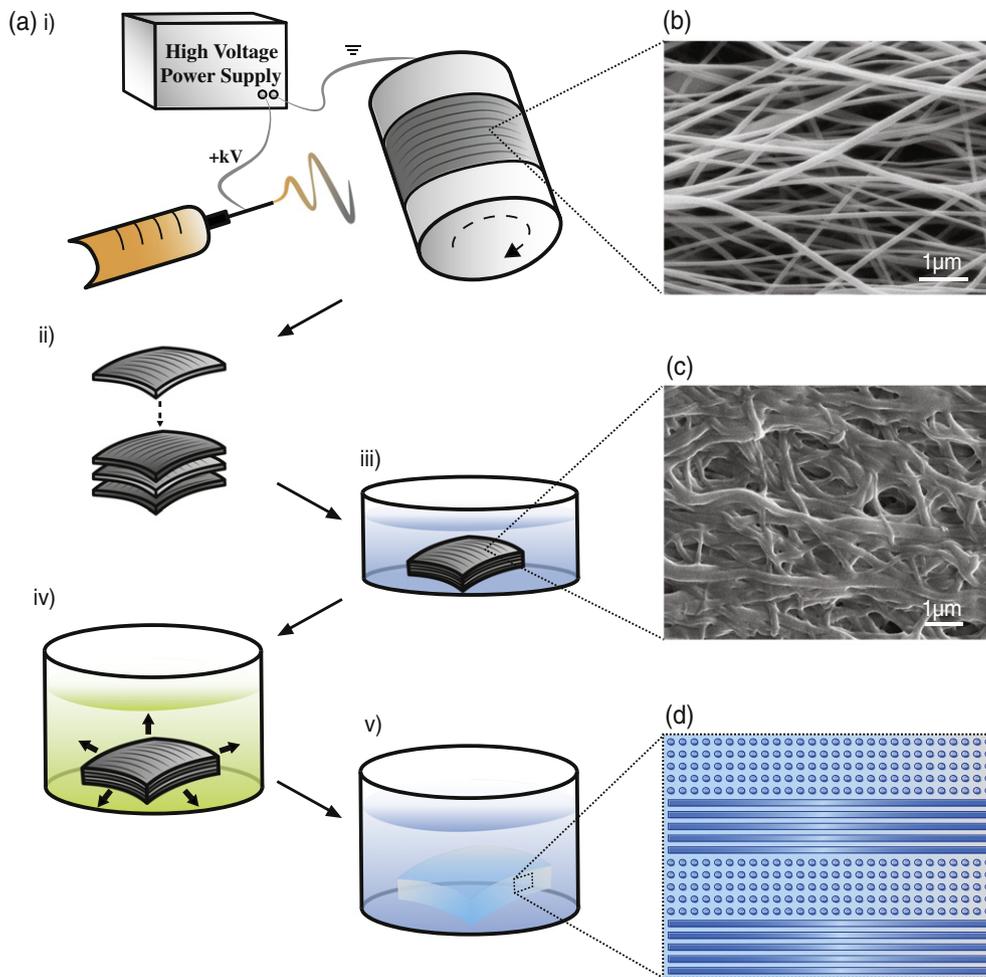
Fig. 1. (a) Schematic illustration of fibre-reinforced hydrogel composite laminate fabrication. i) Electrospun fibres are collected on a rotating mandrel to define the dominant fibre direction, ii) stacked with varying dominant fibre orientation, iii) cross-linked in ethanol solution with EDC/NHS, iv) submerged in a water-alginate solution, and v) gelled in a calcium chloride solution followed by further EDC/NHS cross-linking. (b) SEM of the aligned gelatin nanofibres as collected from the rotating mandrel (a,i). (c) SEM of the electrospun gelatin fibres following the first cross-linking step (a,iii). (d) Schematic of a typical biomimetic (cornea-like) multilayer laminate hydrogel composite (a,v) with four orthogonal layers of aligned gelatin in a cross-ply 0°/90°/0°/90° fibre orientation pattern.

fibre direction. Random fibre reinforcement approximately doubled the failure strain ε_f of the unreinforced hydrogels, from 0.32 ± 0.07 to 0.71 ± 0.16, while the aligned fibre-reinforcement under parallel (90°) loading yielded a four-time increase in failure strains to 1.62 ± 0.23.

Reinforcing with either randomly-oriented or aligned gelatin nanofibres resulted in hydrogels with none or only one preferred high-strength direction. However, a large number of applications require hydrogels to sustain multidirectional stresses. Cross-ply (0°/90°/0°/90°) and angle-ply (0°/45°/90°/-45°) laminate composite hydrogels were created to have two or four preferred high-strength directions. Unidirectional (0°/0°/0°/0°) laminated composite hydrogels were created to validate the layered manufacturing technique. Similar to single-layer aligned fibre-reinforced composite hydrogels, the strength of unidirectional laminates was greatest in the fibre direction and was smaller in directions away from the fibre axis (Fig. 3g) Cross-ply laminates exhibited greatest strength, nearly a half of the maximum strength of unidirectional laminates, in directions parallel to the orientation of the two fibre populations (Fig. 3h). Angle-ply laminates showed approximately equal strength, slightly smaller than the maximum strength of cross-ply laminates, in all three testing directions (Fig. 3i). Ductility of the hydrogels was also substantially improved as a result of lamination, as all laminated hydrogels could be stretched to at least double their original lengths.

3.3. Fracture properties of composite hydrogels

Without fibre reinforcement, alginate and gelatin hydrogels exhibited brittle failure at small strains in a mode I single-edge notch test (Fig. 4a). In single layer aligned fibre composites, the fibre reinforcement resulted in crack deflection based on the fibre orientation (Fig. 4b–d). In cross-ply laminates, the crack tips blunted and crack propagation was difficult (Fig. 4e). These qualitative observations are quantified in Fig. 5, which plots the strain to failure ε_f for the mode I notched samples as shown in Fig. 4. The failure strain increased by about an order of magnitude for aligned fibres transverse to the crack (Fig. 5f) or for cross-ply laminates in any orientation (Fig. 5g–i) compared with unreinforced specimens (Fig. 5a–b) or specimens with fibres parallel (Fig. 5d) or at 45°(Fig. 5e) to the notch. Random fibre composites were intermediate (Fig. 5c).

Fracture toughness was quantified using the mode III trouser tear test (Fig. 6) due to its insensitivity to specimen geometry [38]. Reinforcing alginate hydrogels with randomly-oriented or aligned fibres enhanced the tear toughness of single-network hydrogels, with $A = 6$ for random fibres and $2.25 \leq A \leq 4.75$ depending on orientation of aligned fibres (Fig. 6c,d). Cross-ply and angle-ply laminations provided two orders of magnitude improvement, enhancing toughness to the order of kJ m^{-2} (Fig. 6e,f), comparable to soft collagenous tissues, from tens of J m^{-2} for the unreinforced hydrogels (Fig. 6a,b).
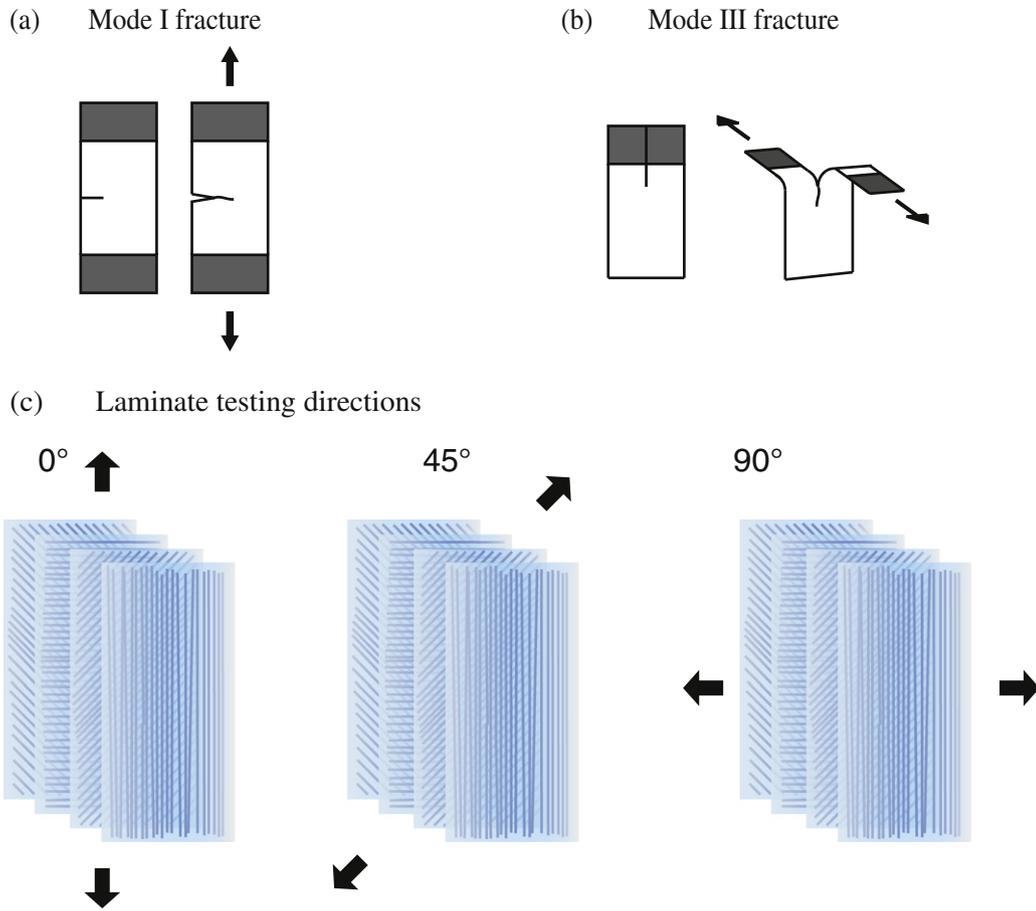
Fig. 2. Specimen geometry and loading schematics for (a) mode I fracture, (b) mode III fracture, and (c) loading directions relative to the orientation of the top (surface) fibre orientation for stacked multilayer laminated composites.

Cross-ply laminates showed the greatest toughness when the initial crack was diagonal to fibres, with $A = 99$ versus $A = 43$, 48 in the parallel and perpendicular orientations (Fig. 6e). Angle-ply laminates also showed excellent toughness, and their fracture behaviour was nearer to isotropic (Fig. 6f).

4. Discussion

Nanofibre reinforcement of hydrogels has been demonstrated to be a flexible mechanism for controlling gel fracture and strength. Depending on the application, fibres can be randomly oriented, in
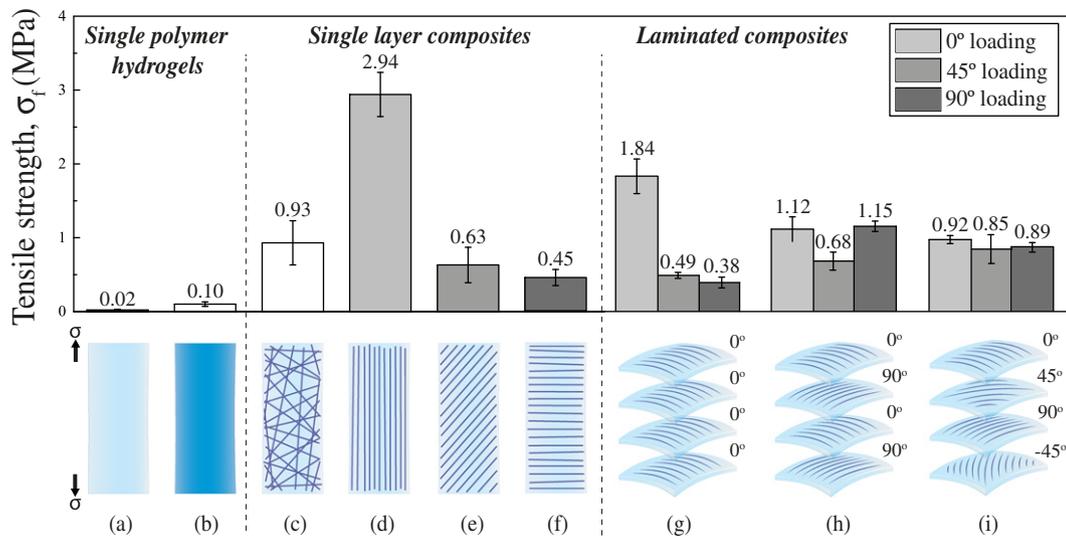

Fig. 3. Tensile failure strength σ_f for single polymer hydrogels (a) alginate and (b) gelatin; for single layer hydrogel-nanofibre composites with (c) random fibres, (d) fibres aligned in the loading direction, (e) 45° aligned fibres, (f) 90° aligned fibres; and for four layer laminate stack composites with a (g) uniaxial 0°/0°/0°/0° fibre orientation pattern, a (h) cross-ply 0°/90°/0°/90° fibre orientation pattern, or an (i) angle-ply 0°/45°/90°/-45° fibre orientation pattern.
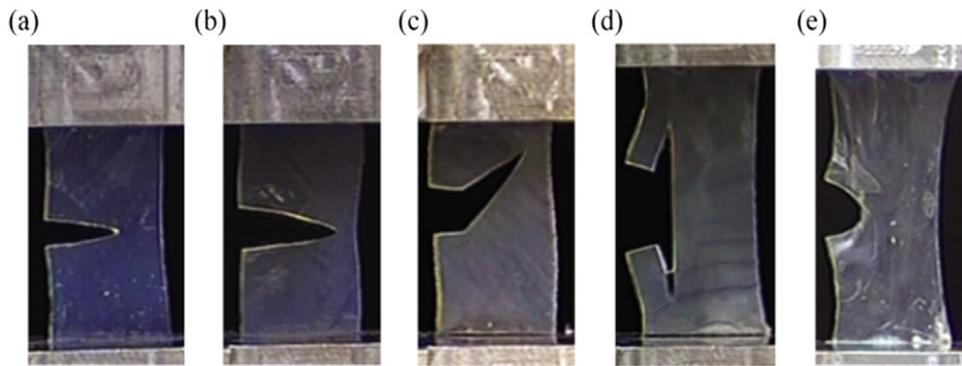
Fig. 4. Crack patterns in alginate hydrogels with (a) no fibres, (b) fibres aligned 90° to the loading direction, (c) fibres aligned 45° to the loading direction, (d) fibres aligned in the loading direction, and (e) cross-ply 0°/90°/0°/90° fibre laminates.

aligned layers in a chosen orientation relative to the loading direction (or to a crack or notch), or in multilayer laminates with varying layer orientation. Both the strength and the toughness of the reinforced materials are increased relative to the base alginate hydrogel matrix, particularly when both the fibre-reinforcement and lamination strategies are used together.

4.1. Stiffness and strength of composite hydrogels

Similar to other fibre-reinforced composite materials, elastic properties of the composite hydrogels were largely influenced by the fibre orientation and the loading direction [39]. The greater the fraction of fibres in the direction of the applied load, the stiffer and stronger the composite hydrogels were. The significant decrease in strength when aligned fibrous composites were loaded in the diagonal and the transverse directions corresponds to a decreasing fraction of fibres that can transmit strain in the loading direction. Composites with randomly-oreinted fibres displayed a more isotropic strength, approximately three times smaller than composites reinforced with aligned fibres. This experimental ratio of strengths agrees well to the theoretical value since aligned fibre reinforcement typically provides about 2.7 times greater reinforcement efficiency than reinforcement with randomly-oriented fibres [39]. Unidirectional, cross-ply and angle-ply laminates also showed strengths proportional to the fraction of fibres in the direction of the applied load.

Unidirectional laminated composite hydrogels were weaker than the single-layer aligned fibre-reinforced composite hydrogels despite the similar fibre orientation in their through thickness structures. This could be due to a larger swelling ratio, or hydration level, of the laminated hydrogels. Stacked gelatin sheets had greater surface area to volume ratio of the fibres exposed to alginate solution than a single gelatin sheet, absorbing water and resulting in greater swelling ratios of the resulting hydrogels. The laminated hydrogels had a swelling ratio of 80%, while the single-layer fibre-reinforced hydrogels had a swelling ratio of 70%. An increase in swelling ratio of the composite hydrogels leads to an increase in volume fraction of the compliant matrix phase and a decrease in strength of the fibres, both of which diminish the mechanical properties of the hydrogels.

4.2. Toughening mechanisms in composite hydrogels

Here, the nanofibrous reinforced alginate gels showed increased toughness and altered fracture behaviour due to the presence of fibres. Previous work has demonstrated that randomly-oriented electrospun PCL microfibres reoriented in the direction of an external load during a single-edge notch (mode I) fracture test [40]. In contrast, randomly-oriented dry gelatin nanofibres fractured without any apparent reorientation [41]. In the current work, it is unclear if the fibres are undergoing reorientation within the hydrogel matrix and whether this represents an active toughening mechanism in the hydrogel composites. Fibre reorientation could be restricted due to
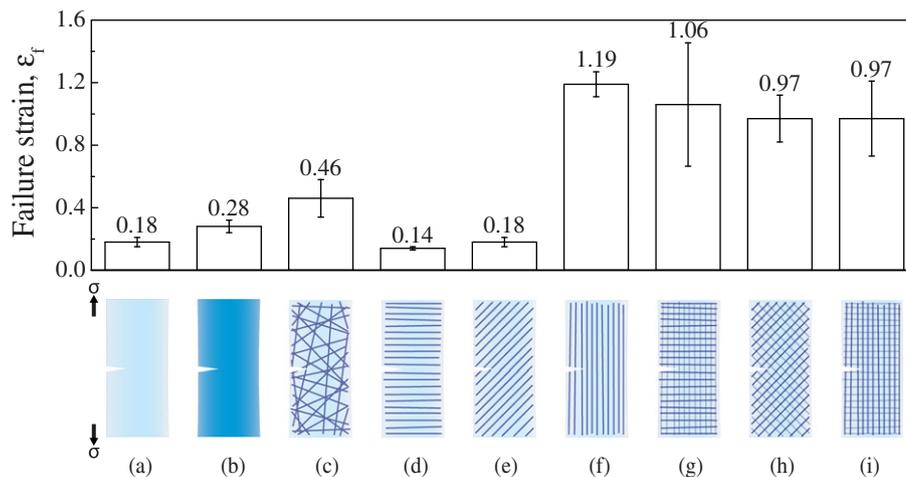

Fig. 5. Tensile strain to failure ε_f for mode I notched specimens of single polymer hydrogels (a) alginate and (b) gelatin; for single layer hydrogel-nanofibre composites with (c) random fibres, (d) 90° aligned fibres, (e) 45° aligned fibres, (f) 0° aligned fibres; and for cross-ply 0°/90°/0°/90° laminates tested with the surface fibres at (g) 90°, (h) 45° and (i) 0° orientation relative to the loading direction.
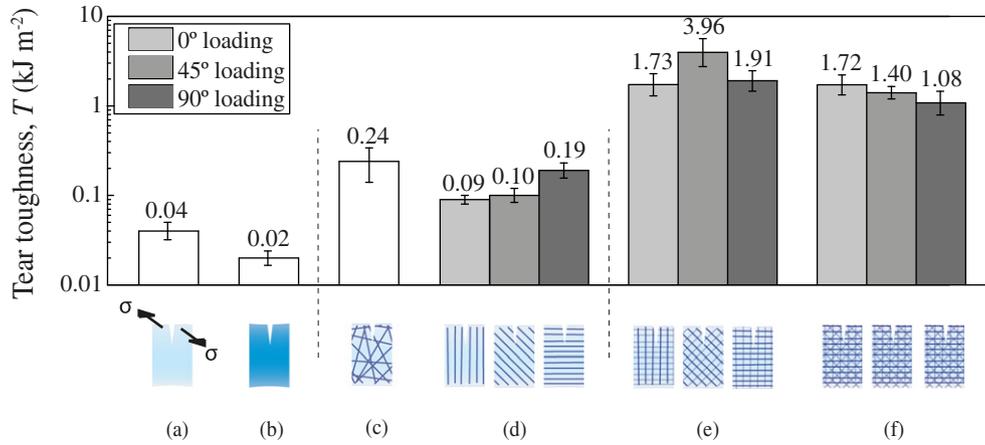
Fig. 6. Mode III tear toughness T for single polymer hydrogels (a) alginate and (b) gelatin; for single layer hydrogel-nanofibre composites with (c) random fibres, with aligned fibres (d) parallel to the tearing direction, (e) at 45° to the tearing direction, (f) perpendicular to the tearing direction; and for (e) cross-ply 0°/90°/0°/90° laminates in three orientations relative to the tear direction and for (f) angle-ply 0°/45°/90°/-45° laminates in three orientations relative to the tear direction.

crosslinking between fibres before the alginate is added (Fig. 1a, iii), as well as resistance from the alginate matrix.

It is well-established that fibres transverse to a notch or crack result in substantial increases in the fracture resistance of composite materials. Aligned fibre reinforcement can cause cracks to turn, and propagate along the fibre direction. Here, a crack normal to the direction of fibre alignment caused interfacial (fibre-matrix) debonding, and this was also seen with fibres at a 45° angle to the loading direction. This behaviour has also been seen in tendon, whose ECM contains dense parallel collagen fibres [42]. This crack-deflecting mechanism dissipates energy and locally reduces stresses [43,44], inhibiting cracking and increasing the toughness of the composite hydrogels.

The intra-laminar failure mechanism for cross-ply and angle-ply laminated hydrogels typically involved a considerable degree of interfacial debonding. Additionally, matrix delamination, an inter-laminar failure mechanism, also occurred as the stress increased. These energy dissipating mechanisms likely caused the significant increase in the toughness compared to single layer composites, whether with aligned or non-aligned fibres. The three combined observations in this section suggest that multiple mechanisms are at work in increasing the toughness of nanofibre composite hydrogels, depending on microstructure.

4.3. Strength versus toughness

Strength and fracture toughness are both important mechanical properties to be considered when designing hydrogels for tissue engineering scaffolds [45]. Since scaffolds have to sustain cyclic fatigue-type loading and provide structural support for biological cells until functional tissues develop, the initial mechanical properties will determine the performance and lifetime of the scaffolds and the success of tissue regeneration. The mechanical performance of nanofibre-reinforced and laminated composite hydrogels in relation to other materials is illustrated in Fig. 7, as an "Ashby plot" [46], where the strengths of different classes of materials are plotted against their fracture toughness. For reference, included in the plot are well-known tough hydrogels developed by others [14–16] utilizing different toughening strategies than those employed in the current work. Soft collagenous tissues normally have strength σ_f in the range of 1–10 MPa and fracture toughness T in the range of 1–10 kJ m^{-2}, comparable to engineering rubbers [38,47–54].

Alginate and gelatin single polymer hydrogels have strength and toughness of around tens of kPa and tens of J m^{-2}, respectively. Utilizing one or two toughening strategies, fibre-reinforcement and fibre alignment within single fibre populations, there are large increases in the material strength but not substantial increases in
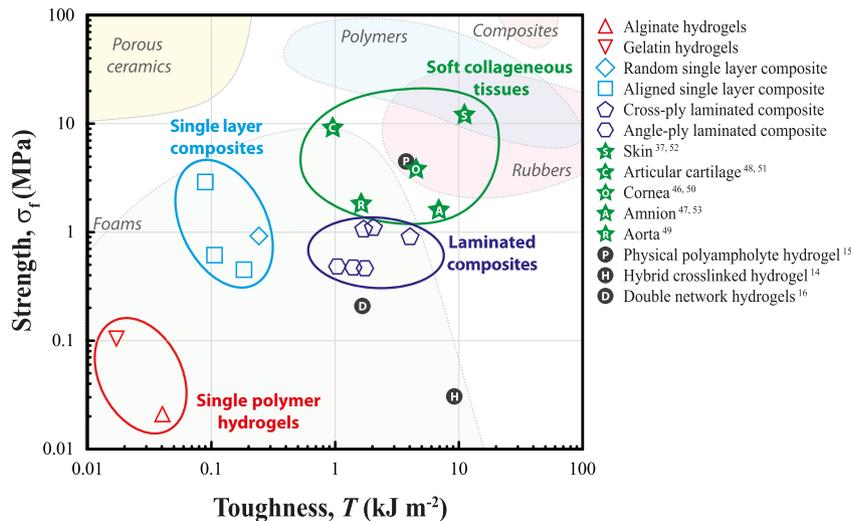

Fig. 7. "Ashby plot" [46] of failure strength σ_f versus tear toughness T for single polymer hydrogels, single layer hydrogel composites and laminated composites from the current study (open symbols) compared with collagenous soft tissue and other hydrogel data taken from the literature (closed, lettered symbols).

the fracture toughness. However, then adding a second strategy, that of lamination with stacked layers of different orientation, results in large increases in toughness with no further change in strength. By combining the multiple strategies, the properties of soft biological tissues are approached in a biomimetic system. The attraction of these reinforced biomimetic materials is the flexibility in design; they can be customized to have a wide range of microstructures with tailored near-isotropic or anisotropic properties to suit diverse biological and engineering applications. Therefore, the concept of fibre reinforced and laminated composites provides a promising and systematic way to design mechanically robust hydrogels, and can be applied to a variety of different fibre-matrix material systems.

5. Conclusion

In this work, novel nanocomposite structures were generated using laminated and non-laminated electrospun gelatin nanofibres in an alginate hydrogel matrix. The composites were fabricated with a wide range of microstructures and mechanically tested for both strength and fracture resistance. Nanofibre reinforcement with aligned fibres increased the tensile strength of the hydrogels by up to two orders of magnitude without significantly improving the toughness. However, arranging the nanofibres as multilayer laminates increased 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. Further, this approach utilized a generic nanocomposite structural strategy equally applicable across a range of fibre and matrix materials.

Disclosures

The authors declare no competing financial interests.

Acknowledgments

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