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Creating polymer hydrogel microfibres with internal alignment via electrical and mechanical stretching

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ABSTRACT

Hydrogels have been widely used for 3-dimensional (3D) cell culture and tissue regeneration due to their tunable biochemical and physicochemical properties as well as their high water content, which resembles the aqueous microenvironment of the natural extracellular matrix. While many properties of natural hydrogel matrices are modifiable, their intrinsic isotropic structure limits the control over cellular organization, which is critical to restore tissue function. Here we report a generic approach to incorporate alignment topography inside the hydrogel matrix using a combination of electrical and mechanical stretching. Hydrogel fibres with uniaxial alignment were prepared from aqueous solutions of natural polymers such as alginate, fibrin, gelatin, and hyaluronic acid under ambient conditions. The unique internal alignment feature drastically enhances the mechanical properties of the hydrogel microfibres. Furthermore, the facile, organic solvent-free processing conditions are amenable to the incorporation of live cells within the hydrogel fibre or on the fibre surface; both approaches effectively induce cellular alignment. This work demonstrates a versatile and scalable strategy to create aligned hydrogel microfibres from various natural polymers.

1. Introduction

Previous studies on hydrogels have primarily focused on exploring their mechanical and biochemical versatility [1–4] and elucidating their impact on cellular activities [5–9]. There is a lack of methodologies for engineering *anisotropic* topographical cues in hydrogels to control the 3D spatial patterns of encapsulated cells. As a result, controlling topographically induced cell alignment and migration has not been readily achieved for hydrogel matrices, even though such cellular manipulation on 2D substrates has been shown to be important in controlling cell organization, tissue microarchitecture, and biological function [10–13]. On the other hand, cellular alignment mediated by 2D electrospun micro- and nano-fibre matrices has been shown to effectively promote stem cell differentiation and cellular functions [14,15]. Dispersing solid polymer nanofibres into the hydrogel matrix has been used to generate a composite scaffold [16], however, controlling alignment of the nanofibres inside a hydrogel matrix is challenging. Recently, Kang et al. have reported a microfluidic-based alginate hydrogel microfibre with a surface alignment feature produced by solution extrusion through a grooved micro-channel, and demonstrated guided neurite outgrowth for neurons cultured on the surface of the microfibers [17,18]. This alignment cue is only confined to the surface of the microfibre. Zhang et al. have generated peptide nanofibre hydrogels with long-range nanofibre alignment through heat-assisted self-assembly of amphiphilic peptide molecules and mechanical shear [19]. The resulting aligned nanofibre “noodles” effectively induced cellular alignment in 3D; nevertheless, this method is only applicable to specific peptide materials.

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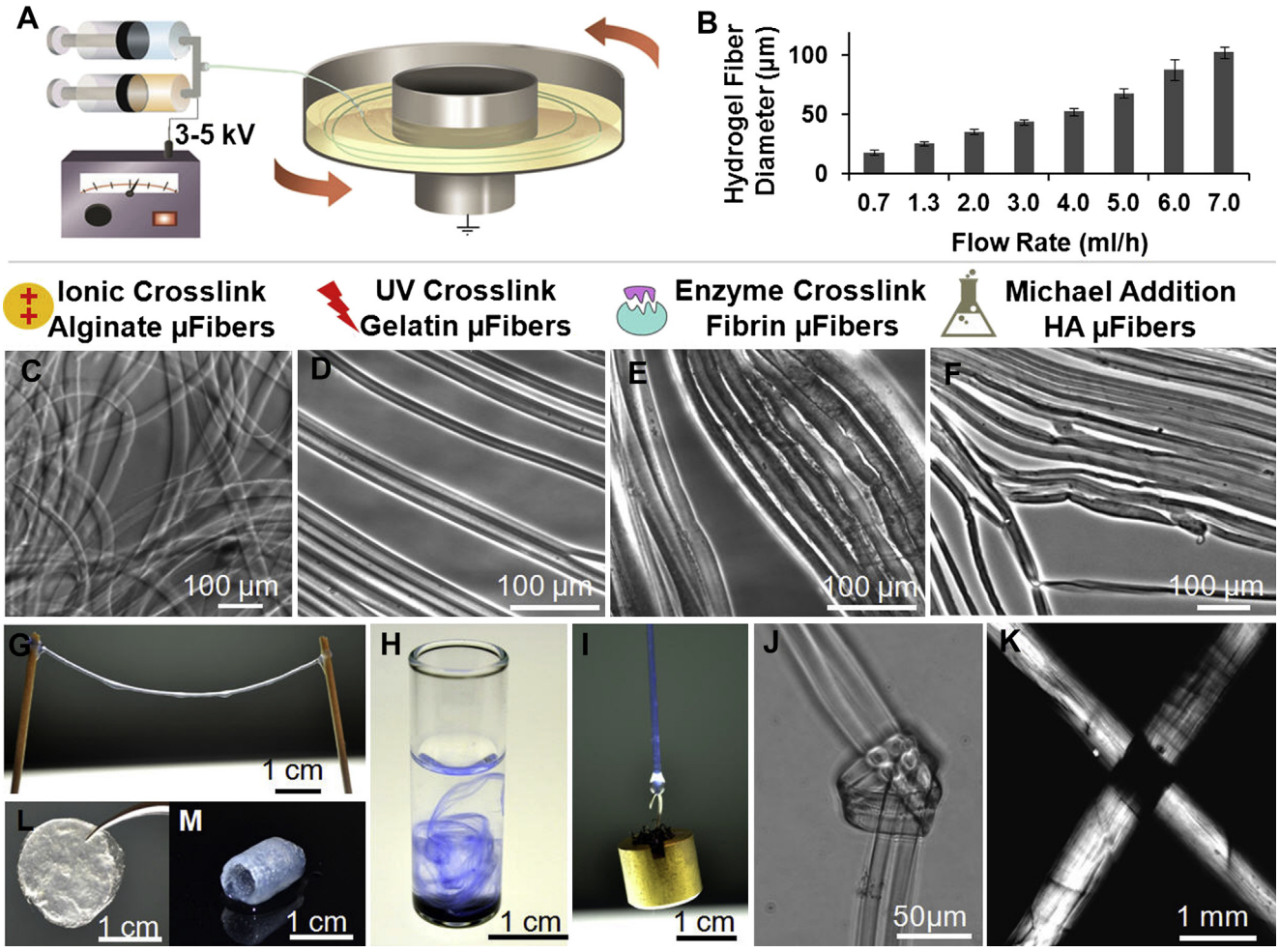

Fig. 1. Electrostretching setup and features of hydrogel microfibres. (A) Illustration of the electrostretching setup. To prepare a dual component microfibres, two syringes (A & B) are used (e.g. sodium alginate solution in syringe A and fibrinogen solution in B) to mix the solutions prior to extrusion; a voltage of 3–5 kV was charged between the spinning solution and the collection bath. Typically only one syringe is needed for single solution spinning. (B) Effect of alginate solution feeding rate on the diameter of hydrogel microfibres. Alginate hydrogel microfibres with an average of 17–116 μm were prepared with a solution containing 2% sodium alginate and 0.2% PEO fed at a flow rate ranging from 0.7 to 7.0 ml/h. (C–F) Various crosslinking mechanisms have been employed to crosslink alginate, gelatin, fibrin and hyaluronic acid hydrogel microfibres. The crosslinking of the fibres was initiated with a fast calcium gelation of alginate, followed by additional crosslinking of the second component polymer with UV-initiated, enzymatic, or the Michael-addition reaction for methylated gelatin, fibrin and hyaluronic acid, respectively. (G) Using this method, hydrogel microfibres of any desired length can be prepared. (H) When dispersed in water, alginate hydrogel fibres formed a loose network of hydrogel fibres. Trypan blue was used to stain the fibres and enhance observation. (I) A 10-g metal pillar was lifted with an alginate hydrogel microfibre bundle. (J) A micro-knot was made with two alginate hydrogel microfibres. (F) Under a cross polarized light microscope, light extinction was observed at the crossover point of two hydrogel microfibre bundles, indicating uniform alignment in both fibres. (L–M) Beyond microfibre bundles, these hydrogel microfibres can also be fabricated into other forms like fibrous films (L) and self-supporting hydrogel tubes (M).

Since polymer chain alignment can be induced during electrospinning, we hypothesized that similar chain alignment can be induced by electrospinning of an aqueous polymer solution and such an alignment can be fixed by crosslinking, thus generating hydrogel fibres with internal alignment. To enhance polymer chain alignment through mechanical shear, we collected the spinning polymer solution jet on a rotating bath containing crosslinking agents. In this study, we examined feasibility of generating internally aligned hydrogel fibres by combined electrospinning and mechanical stretching (electrostretching) approach, and characterized the internal alignment by X-ray scattering analysis and its effect on the mechanical properties of these hydrogel fibres. We tested the versatility of this method by generating hydrogel microfibres from various water-soluble natural polymers using different crosslinking mechanisms. We then investigated the effect of the alignment cue of these hydrogel fibres on the morphology of cells seeded on fibre surface or encapsulated within the hydrogel microfibres.

2. Materials & methods

2.1. Materials and reagents

Sodium alginate from brown algae (the viscosity of 2% solution at 25 °C is ~250 cps), fibrinogen from bovine plasma and poly(ethylene glycol) (PEO, average Mv *ca.* 4000 kDa) were purchased from Sigma Aldrich. Methacrylated gelatin was prepared according to a previously reported protocol [21]. Thiol-modified hyaluronic acid (HA) and PEG-diacrylate (PEGDA) was purchased from Glycosan BioSystems Inc. Photo initiator Irgacure 2959 was from CIBA Specialty Chemicals. High voltage power supply was from Gamma High Voltage Research. Direct current permanent magnet motor was from Leeson Electric Corp. (Grafton). UV source used was Mineralight Lamp UVGL-25 from UVP LLC.

2.2. Generating hydrogel microfibres

To produce alginate hydrogel microfibers using the device shown in Fig. 1A, 2–5 kV voltage, 1.5–3.0 wt% alginate and 0.1–0.6 wt% PEO solution were used. In a circular collection bath, 20–100 mM $CaCl_2$ served to stabilize these alginate hydrogel fibres. With similar flow rates and applied voltages, fibrin, gelatin and HA hydrogel fibre bundles were also prepared. Typically, fibrin hydrogel fibres were produced using an aqueous solution that contains 0.67 wt% fibrinogen, 1.0 wt% sodium alginate and 0.1 wt% PEO. Upon collection, the hydrogel fibres were crosslinked in