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Enhancing structural integrity of hydrogels by using highly organised melt electrospun fibre constructs

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ABSTRACT

Applying additive manufacturing technology to the principles of fibre reinforcement of hydrogels, we have fashioned weak hydrogels into mechanically enhanced composites. We combined the extracellular matrix-like structure of gelatin-methacrylamide (GelMA) and GelMA/hyaluronic acid-methacrylamide (HAMA) hydrogels with highly oriented poly(ε-caprolactone) (PCL) fibres fabricated by Melt Electrospinning Writing (MEW) to achieve fibre-reinforced GelMA/HAMA composites with improved compressive properties. Stacked fibres with lay-down patterns of 0°–90° and 0°–60°–120°, and spacing of 400 and 800 μm were prepared by MEW. These defined fibrous structures were infiltrated with hydrogels, namely GelMA (10%) and GelMA/HAMA (0.125%, 0.25% and 0.5%) in custom-made moulds and crosslinked by a reduction–oxidation initiating system (ammonium persulphate/tetramethylethylenediamine). Mechanical properties and deformation characteristics of the constructs were evaluated under uniaxial compression loading conducted at 37 °C in culture media with an integrated camera. Reinforced constructs showed more than a 35-fold-increase of the compressive Young's modulus. However, the compressive Young's moduli were highly strain-rate dependent. The fibre reinforcement has a particular impact on the Poisson's ratio of the composite constructs, decreasing from values of approximately 0.4 to 0.01. The high interfacial surface area between the fibre structure and the hydrogel matrix is believed to be one of the main factors responsible for the significant increase in the mechanical properties of the constructs. In summary, we have found that reinforcement of hydrogels with defined MEW fibre architectures achieves an outstanding increase in the mechanical properties at high strain rates.

1. Introduction

It is a frequently observed phenomenon in nature that tissues are supported by fibres to provide mechanical reinforcement; such is the case of mammalian cells, with the fibrillar actin cytoskeleton, tubulin microtubules and intermediate filaments embedded into the highly aqueous gel-like cytosol [1]; or articular cartilage which is built by stiff and strong collagen fibres intertwined within a very weak matrix of proteoglycans [2]. In living organisms, such examples of

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fibre-reinforced hydrogel constructs are numerous, and this concept of reinforcement has already been successfully applied in the field of materials engineering, such as in the case of carbon fibre- [3], natural fibre- [4] or glass fibre-reinforced materials [5].

As a class of material, hydrogels have been extensively studied as drug delivery vehicles, and a wide spectrum of bioactive molecules have been effectively loaded into their molecular structures [6,7]. Hydrogels are also particularly attractive as bioinks for 3D cell printing [8]. Their high water content and nano- and micro-scale pore sizes allow the diffusion of growth factors and other molecules present in the interstitial extracellular matrix space [9]. Collagens and hyaluronic acids (HA) are both essential components in various tissues and, as such, hydrogels synthesised using either of these molecules have a strong potential to mimic the native tissue environment [10,11]. HA has been shown to play crucial roles in wound healing, cellular proliferation, angiogenesis and cell-receptor interactions [12–14]. Owing to such obvious benefits, many research groups have undertaken methacrylation of the HA molecules to form crosslinkable hydrogels with tuneable physical properties and stiffness [15]. Gelatin is obtained by the denaturation of collagen, which is the most abundant protein in tissues throughout the human body [16]. Gelatin is biocompatible, and has been used in a large number of small molecule delivery and TE applications [17–22]. Similar to HA, gelatin can also be methacrylated to form crosslinkable GelMA hydrogels. While these hydrogels contain cell adhesive functional groups and allow for cell spreading, crosslinked GelMA hydrogels are structurally weaker compared to HA hydrogels [17]. The combination of GelMA and HAMA into hybrid hydrogels brings together the advantages of both, and increased amounts of HAMA have already been reported to enhance the mechanical properties of GelMA gels [15]. However, the outstanding biological performance of hydrogels is often eclipsed by their compromised mechanical properties [23]. Mixtures of fibrous collagen hydrogels with non-fibrillar agarose have been proposed as a strategy to obtain collagen-based matrices of enhanced mechanical properties [11,24,25]. In some instances, nanofibrous, disorganised solution electrospun meshes have been utilised in combination with hydrogels in attempts to enhance their mechanical properties, as recently reviewed in Ref. [26]. Moreover, literature shows recent progresses towards applying solution electrospinning technologies to manufacture aligned fibrous meshes [27–31]. Yet, using conventional solution electrospinning fibres can only be aligned in one main direction, and there is no control over fibre spacing due to the inherent electrical instabilities of the jet associated to the process [32]. Therefore, using this technology it is not possible to investigate in a systematic manner the impact of fibre organisation and porosity on the reinforcement effect of composite hydrogels.

Fused Deposition Modelling (FDM) is a different polymer processing technology that led additive manufacturing (AM)-based scaffold technologies for many years [33,34], fabricating filamentous scaffolds made from thermoplastic polymers such as poly(ε-caprolactone) (PCL). Controlled scaffold architectures have already been successfully applied in challenging applications such as load-bearing long bone defect models using FDM manufacturing technologies [35,36]. However, FDM scaffolds contain quite large fibre diameters (>200 μm), which lead to constructs with very low surface areas compared to those of solution electrospun scaffolds.

More recently, melt electrospinning, an innovative successor of solution electrospinning, has enabled the deposition of highly ordered fibres through direct writing, referred to as Melt Electrospinning Writing (MEW). Hereby, polymer melts are used instead of solutions as a way to avoid both mechanical and electrical coiling of the fibres as well as cell toxicity issues due to the use of toxic solvents [37]. In comparison to other contemporary scaffold manufacturing technologies, MEW can produce highly organised fibrous 3D structures in the micron scale, which are built layer-by-layer similarly to other AM-based 3D printing technologies [38]. Our group has published several articles describing the working principles of MEW and highlighting the potential applications of scaffolds produced by this innovative technology [39–41].

Recently, we have used MEW to design and fabricate significantly-enhanced material and structural properties to hydrogels [42]. Encouraged by the positive results obtained, this research is a systematic study of the impact of hydrogel composition and fibre architecture on the holistic mechanical properties of fibre-reinforced hydrogels. Therefore, we prepared tunable hybrid gelatin methacrylamide (GelMA) and hyaluronic acid methacrylate (HAMA) hydrogels with a variety of compositions and initiator concentrations in combination with PCL fibres collected in well-ordered 3D architectures. We hypothesise that crosslinking these hydrogels around tailored melt electrospun fibre architectures will generate an outstanding increase in the mechanical properties of the composite material, and that the reinforcement effect will depend on the surface area, lay-down pattern and fibre spacing. Our results confirm that reinforcing a hydrogel with highly organised, micron-size fibres results in significantly enhanced mechanical properties.

2. Materials and methods

2.1. Fabrication of melt-electrospun fibre reinforcements

An in-house built MEW device was used to manufacture defined fibrous reinforcements [38], as is schematically shown in Fig. 1a. Briefly, a 5.0 ml glass syringe (Gastight® #1005, Hamilton Co., USA) with an attached 23G Luer Lock metal needle was filled with PCL pellets (Purasorb® PC 12, Purac Biomaterials, The Netherlands) and heated up to 100 °C. A syringe pump (AL-1000, World Precision Instruments Inc., USA) enabled the extrusion of the molten polymer with a constant flow rate of 15 μl/h. The distance between the collector and needle tip was kept at 15 mm while a high voltage between 11.5 and 12 kV (DX250R, EMCO High Voltage Co., USA) was applied to the molten polymer through the metal needle to obtain an optimum polymer jet. The extruded fibres were collected in a controlled, layer-by-layer manner, on a grounded aluminium plate