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A UV-cured nanofibrous membrane of vinylbenzylated gelatin-poly(ε-caprolactone) dimethacrylate co-network by scalable free surface electrospinning

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ABSTRACT

Electrospun nanofibrous membranes of natural polymers, such as gelatin, are fundamental in the design of regenerative devices. Crosslinking of electrospun fibres from gelatin is required to prevent dissolution in water, to retain the original nanofibre morphology after immersion in water, and to improve the thermal and mechanical properties, although this is still challenging to accomplish in a controlled fashion. In this study, we have investigated the scalable manufacture and structural stability in aqueous environment of a UV-cured nanofibrous membrane fabricated by free surface electrospinning (FSES) of aqueous solutions containing vinylbenzylated gelatin and poly(ε-caprolactone) dimethacrylate (PCL-DMA). Vinylbenzylated gelatin was obtained via chemical functionalisation with photopolymerisable 4-vinylbenzyl chloride (4VBC) groups, so that the gelatin and PCL phase in electrospun fibres were integrated in a covalent UV-cured co-network at the molecular scale, rather than being simply physically mixed. Aqueous solutions of acetic acid (90 vol%) were employed at room temperature to dissolve gelatin-4VBC (G-4VBC) and PCL-DMA with two molar ratios between 4VBC and DMA functions, whilst viscosity, surface tension and electrical conductivity of resulting electrospinning solutions were characterised. Following successful FSES, electrospun nanofibrous samples were UV-cured using Irgacure I2959 as radical photo-initiator and 1-Heptanol as water-immiscible photo-initiator carrier, resulting in the formation of a water-insoluble, gelatin/PCL covalent co-network. Scanning electron microscopy (SEM), attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, differential scanning calorimetry (DSC), tensile test, as well as liquid contact angle and swelling measurements were carried out to explore the surface morphology, chemical composition, thermal and mechanical properties, wettability and water holding capacity of the nanofibrous membranes, respectively. UV-cured nanofibrous membranes did not dissolve in water and showed enhanced thermal and mechanical properties, with respect to as-spun samples, indicating the effectiveness of the photo-crosslinking reaction. In addition, UV-cured gelatin/PCL membranes displayed increased structural stability in water with respect to PCL-free samples and were highly tolerated by G292 osteosarcoma cells. These results therefore support the use of PCL-DMA as hydrophobic, biodegradable crosslinker and provide new insight on the scalable design of water-insoluble, mechanical-competent gelatin membranes for healthcare applications.

1. Introduction

In the past decades, electrospun nanofibrous membranes have attracted great attention due to the small size fibres with fine interconnected pores, extremely large surface area to volume ratio and the versatility of polymers, polymer blends and organic-inorganic composite materials that can be smoothly electrospun [1–6]. Electrospun material products have been successfully commercialised as e.g. hernia mesh or vascular access graft, whilst extensive research has been carried towards the design of electrospun therapeutic devices for regenerative medicine [7], tissue engineering, and chronic wound management [8]. Whilst electrospinning is an effective technique for producing nanofibrous membranes, resulting yield of fibre production is typically restricted to 0.1–1.0 $g \cdot h^{-1}$ for a single spinneret [9], whereby the throughput of the polymer solution in the range of 0.1 to 10 $ml \cdot h^{-1}$ [10]. Consequently, electrospinning can hardly meet the

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needs of industrial scale nanofibre manufacture compared with currently available microfibre spinning technologies, enabling the collection of nanofibre nonwoven fabric area of up to 25 cm^2 [11,12]. To overcome this limitation and increase the yield of fibre formation, a great deal of attention has been put towards the development of needle-free electrospinning apparatus, e.g. by Formhals et al. [13] and Jirsak et al. [14,15]. Whereby high production of nanofibrous webs could be achieved via the rotation of a roller surface in a polymer solution. Most recently, Elmarco Co. (Elmarco, Liberec, Czech Republic) introduced world's first industrial nanofibre free surface electrospinning set-up i.e. Nanospider® [16]. Here, the polymer solution is electrospun from either wire-based or roller electrodes, so that nanofibre production rate can be conveniently adjusted depending on the electrode width, the linear speed of the wires/roller and the number of spinning heads placed in series [13,16,17]. With nanofibrous nonwoven membranes obtained with 50–500 nm nanofibre diameter at a production rate of 1.5 $g{\cdot}min^{-1}$ per meter of roller length [16], this mechanism enables high scalability, low cost, as well as easy operation in comparison with nonwoven membranes electrospun from single spinneret.

Gelatin is a natural biopolymer derived from partial hydrolysis of collagen, mostly composed of randomly-oriented polypeptide chains [18,19]. Aiming to mimic the nanofibrous architecture of the extracellular matrix (ECM) of biological tissues, gelatin has been successfully electrospun into nanofibrous membranes. In light of its non-toxicity, biodegradability, biocompatibility, formability and low-cost commercial availability [20], gelatin has been excessively used as building block for the design of smart wound dressing and healing materials [21,22], pharmaceuticals [23], personal care [24] and food industry products [25], as well as drug delivery systems [26–28] and scaffolds for tissue engineering [29]. However, electrospun gelatin typically present uncontrollable water-induced swelling and dissolution, and display weak mechanical strength in the hydrated state, which substantially limit long-term fibre performance [30]. In light of the presence of amine and carboxylic groups along gelatin backbones, various chemical treatments have been proposed to introduce covalent crosslinks lost following collagen extraction and denaturation ex vivo, so that micro-/macroscopic structural features and mechanical properties of hydrated gelatin nanofibre membranes could be controlled [31]. Crosslinking strategies have been pursued by carbodiimide chemistry [29,32–34], bifunctional compounds such as glutaraldehyde (GTA) [33,35–42] and genipin [30,43,44], silanisation [41], dehydrothermal [46] and plasma treatments [47], as well as via ultraviolet (UV) light [30,48–50]. Although chemical crosslinking is the most widely used method, crosslinking agents are often associated with risks of cytotoxicity and calcification in host polymer scaffolds [51–53], are unable to ensure fibrous retention and minimal membrane dissolution in aqueous media [30,48,54], may cause thermal degradation of gelatin [55,56], or may lead to side reactions, resulting in hardly-controllable process-structure-property relationships. Recently, functionalisation of ECM-derived proteins with photoactive compounds, e.g. 4-vinylbenzyl chloride, has proved to lead to the prompt formation of water-stable, mechanically-competent, UV-cured covalently-crosslinked networks [57], whose preclinical performance has been successfully evaluated in diabetic mice [58]. Together with crosslinking strategies, the use of co-polymers, such as polyvinyl alcohol (PVA) [59,60], alginate [61], chitosan [62], poly(D,L-lactide-*co*-glycolide) (PLGA) [63] and poly(ε-caprolactone) (PCL) [64–66], has also been pursued as additional means to control gelatin swellability in physiological media [64], with only partial success. Among the different biodegradable polymers investigated, PCL has shown promises as gelatin-stabilising building block, in light of its hydrophobicity, biocompatibility, hydrolytic degradability, whilst it is also FDA-approved for use as degradable suture [45]. At the same time, simple formation of composites made of physically-interacting polymer building blocks is associated with concerns related to the homogeneous material degradability, inevitably leading to material instability in vitro and in vivo [44,67]. Here, we explored whether the formation of covalent linkages between the natural and synthetic polymer chains can be achieved to obtain electrospun systems with retained fibrous architecture and enhanced mechanical properties in physiological conditions.

The objective of the study was to investigate whether scalable, geometrically-stable electrospun nanofibrous membranes could be achieved via sequential free surface electrospinning and UV-cured co-polymer network formation in the fibrous state. Vinylbenzylated gelatin was employed as suitable photoactive biomimetic building block and dissolved with PCL-DMA as hydrophobic, hydrolytically-degradable crosslinker. Electrospun membranes were UV-cured aiming to retain their three dimensional and nanofibrous architecture in physiological media via the introduction of covalent crosslinks between polymer chains. The presence of PCL-DMA as additional photoactive phase in the electrospun fibre was expected to mediate the photocrosslinking reaction between distant gelatin chains, thereby leading to enhanced yields of network formation as well as thermal and mechanical properties which respect to electrospun, UV-cured, gelatin controls. The synthesis of the covalent gelatin-PCL co-network was carried out in the fibrous state via UV-initiated radical crosslinking reaction in the presence of (2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone) (I2959) as water-soluble, UV-compatible photoinitiator [68–71]. I2959 was selected since no toxic response was detected during cell culture with either bovine chondrocytes, human fetal osteoblasts or osteosarcoma cells with up to 0.5 $mg{\cdot}ml^{-1}$ I2959 [72–74]. To establish defined process-structure relationships, viscosity, surface tension, and electrical conductivity of the electrospinning solutions were measured and linked to fibre characteristics. Scanning electron microscopy (SEM) was pursued to investigate the effect of UV-cured co-network formation on fibres morphology following contact with water, whilst the effect of the solvent employed during UV-curing was also addressed. Differential scanning calorimetry (DSC)-based thermal analysis, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) as well as contact angle, tensile and swelling tests were also carried out aiming to identify the structure-property relationships of the electrospun system.

2. Materials and methods

2.1. Materials

Acetic Acid (AcOH), PCL-DMA (M_n: 4000 $g{\cdot}mol^{-1}$), I2959, 4VBC, 1-heptanol (HpOH), methylene iodide (MI), trimethylamine (TEA), 2,4,6-Trinitrobenzenesulfonic acid solution (5% w/v, TNBS), Tween-20, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from (Sigma-Aldrich-UK). Phosphate buffered solution (PBS) was purchased from (Lonza-UK).

2.2. Synthesis of vinylbenzylated gelatin

Vinylbenzylated gelatin was obtained via reaction of gelatin with 4VBC. Gelatin from porcine skin (type A, high gel strength, Sigma-Aldrich) was dissolved in phosphate buffered solution (PBS, 0.01 M, pH 7.4) via magnetic stirring at 50.0 °C, prior to addition of Tween-20 (1 wt% of the solution weight). 4VBC was applied to the reaction mixture with a 25 M ratio with respect to the molar content of gelatin lysines, as determined via (2,4,6)-trinitrobenzenesulfonic acid (TNBS) assay ($3{\cdot}10^{-4}$ mol of lysine per gram of gelatin) along with an equimolar amount of TEA ([TEA] = [4VBC]). Following 5-h reaction, the mixture was precipitated in 10-volume excess of pure ethanol; ethanol-precipitated reacted gelatin product was recovered by centrifugation, re-dissolved in PBS and re-precipitated in ethanol, prior to centrifugation and air-drying. The degree of 4VBC-mediated gelatin functionalisation was equal to 40 mol% of gelatin lysines, as confirmed via 2, 4, 6-trinitrobenzenesulfonic acid (TNBS) [75].