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Surface texturing of electrospun fibres by photoembossing using pulsed laser interference holography and its effects on endothelial cell adhesion

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ABSTRACT

Electrospun fibres are nowadays extensively studied for biomedical applications such as tissue engineering and drug delivery. Here, fibres for such applications are electrospun from photopolymer blends using a volatile solvent. The obtained nonwoven fibre mats were textured using a photoembossing technique that uses the interference of two coherent UV laser beams followed by a thermal development step. AFM measurements revealed that the patterned exposure using pulsed laser interference holography resulted in a surface texture on the fibres. The effect of temperature and UV dosage on fibre texturing was studied and was similar to results seen in photoembossed films. Fibronectin and cell adhesion was evaluated on textured fibres of 1 μm diameter with relief structures of 60 nm height and 2 μm pitch. Initial results did not seem to indicate improved cell adhesion for photoembossed fibres compared to their non-embossed counterparts.

1. Introduction

Surface texturing of polymers has been employed in numerous applications including biosensors, cell growth, in-situ cavity fillers, microelectronics and micro-optical display elements [1–4]. Various techniques have been used to create micro- and nano-relief features including hot embossing, cast moulding and lithography [5–7].

Photoembossing is a relatively new technique that creates relief structures in thin films and this technique eliminates the use of a wet-etching step, i.e. the relief structures are formed upon heating [8–12]. Moreover, exposure can be performed using a simple contact mask which is possible because the photo-resist is a non-tacky solid at room temperature. Photoembossing uses a mixture of a multifunctional monomer, a polymeric binder and a photo-initiator. The photoembossing procedure typically involves creating a thin film from the mixture followed by ultra violet (UV) light irradiation through a patterned contact photo-mask. The photo-initiator generates free radicals in the areas exposed to UV-light and the monomers in the exposed areas are polymerised upon heating. Depletion of monomer in the illuminated areas creates a monomer concentration and chemical potential difference between the exposed regions and the non-exposed regions which results in the diffusion of monomer from non-exposed to exposed regions. This mass transport from dark to illuminated areas creates the surface relief structure. Typically, photoembossing is used as a relatively new technique to produce relief structures in thin films which are deposited on flat polymeric or glass substrates. Surface structuring of fibres, especially if relief structures perpendicular to the fibre axis are desired, is not straight forward and cannot be simply generated by adjusting for example die design. To explore the potential of photoembossing in fibre technology, the technique was recently also used to create micro-relief structures on the surface of monofilament fibres [13,14].

Electrospinning is one of the most recognized techniques to make ultrafine polymer fibres. The technique, which uses an electric field to draw charged threads of polymer solutions [15,16], has been readily adopted in the biomedical field as it can be used for the fabrication of porous three-dimensional scaffolds to support cellular in-growth and proliferation in tissue engineering [17–20].

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The extracellular matrix (ECM) is a heterogeneous, connective network composed of fibrous proteins that provide the physical scaffolding necessary for the regeneration and maintenance of tissues and organs [21,22]. Collagen I constitute major parts of the fibrillar ECM, thus providing structural integrity and mechanical resilience. The collagen structure is composed of three polypeptides (α-chains) that are each coiled into a left-handed helical pattern, and then these three chains are wrapped around each other into a right-handed helical pattern that is well organized (quarter stagger) into insoluble fibrils of great structural strength [23]. The quarter stagger arrangement gives the collagen fibrils a textured appearance called D-banding with a characteristic repeat of 67 nm. These fibrils form bundles of collagen fibres with diameters up to 10 μm [24]. Chemical and physical cue on these fibres provide binding sites for cellular receptors which in turn send signals to cell interiors which regulates different cell behaviours, including differentiation and apoptosis. An important interaction site between a cell and its matrix is known as a focal adhesion, mediated by a family of transmembrane proteins called integrins. Integrin α_2 β_1 has been studied as the main receptor for collagen type I [25]. The effect of the D-banding on collagen fibres has been studied by Friedrichs et al. [26] who showed that cells aligned along the fibre axis on the nanopatterned collagen and did not show any alignment on smooth surfaces.

Previously it has been shown that photoembossing of films can improve cell adhesion and alignment on acrylate-based polymers [27–30]. In the current study, photoembossing is used to texture electrospun fibres to simulate D-banded collagen and to study the effect of endothelia cell adhesion on these textured fibres. This should be of interest for tissue engineering applications as endothelia cell adhesion on vascular implants helps prevent thrombogencity and drug delivery.

In photoembossing, generally a patterned light exposure is performed using a mask. This technology was also used in our previous studies on photoembossing of fibres [13,14]. Here, photopolymer fibres were exposed to UV-light through a lithographic mask (grating), which was placed on top of the fibres. This lithographic masks consisted of eight gratings with a pitch varying from 10 to 2000 μm. The height of the surface relief structures created was approximately 30 and 50 μm and was achieved by using relatively large photopolymer fibres with diameters of 100 μm and 500 μm, respectively [13]. In a subsequent study the grating method was used to texture a photopolymeric coating on the surface of a conventional (~200 μm) PET fibre [14]. Bicomponent monofilaments with grating structures perpendicular to the fibre axis were produced with a pitch of 1 and 8 μm and a typical height of 60–110 nm and 900–1300 nm, respectively. Although these studies successfully demonstrated the potential of photoembossing to produce surface textures on fibres, the grating method using a photomask in its current form, is performed as a discontinuous process with static substrates. This limits production speeds and its use in continuous processes like roll-to-roll, extrusion or fibre spinning. In order to create a technology that could potentially be integrated in continuous processes like electrospinning of nonwoven fibre mats, we use here for the first time a photoembossing technique in combination with pulsed laser interference holography to generate patterning in electrospun fibres [31]. In the simplest case, a linearly polarized laser beam is split in two coherent laser beams with the same intensity. These are made to interfere at a certain angle (2θ) in the sample region. As a result, a line interference pattern emerges with a period of λ/2sinθ, λ being the wavelength of light [32–34]. This patterning technique enables to achieve sub-micron periodicities of the light patterns provided that the wavelength of the laser light can be selected in the UV region [34]. Here, we use interference holography with a nanosecond pulsed laser for the texturing of the electrospun photopolymer fibres. The effect of surface texture in these photopolymer fibres on fibronectin and cell adhesion was evaluated in an exploratory study.

2. Experimental

2.1. Materials

Poly(methyl methacrylate) (PMMA) with molecular weights (M_w) of 120 kg/mol and 350 kg/mol were used as received from Sigma Aldrich (UK). The monomer (trimethylolpropane ethoxylate triacrylate; TPETA), photo-initiator 2-Benzyl-2-(dimethylamino)-4′-morpholinobutyrophenone and dimethyl formamide (DMF) were all used as received from Sigma Aldrich (UK). Dapi, phalloidin-TRITC, mouse anti-vinculin, rabbit vinculin antibody, anti-rabbit antibody (594-conjugate), fibronectin and polyllysine polyethylene gylycol (PLL-PEG) were obtained from SuSoS (Germany). Phosphate buffer saline (PBS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), paraformaldehyde, TritonC and gelatine were all obtained from Sigma Aldrich (UK).

2.2. Electrospinning and film coating

A 60:40 wt% ratio of PMMA (120 kg/mol) and TPETA were dissolved in 70 wt% of dimethylformamide (DMF). Irgacure 369 was dissolved at 10 wt% in the monomer. This homogeneous solution was electrospun using a single nozzle with a 1 mm diameter at a collector distance of 20 cm. The applied voltage was 20 kV with a flow rate of 0.8 ml/h. The fibres obtained had a diameter of 2 ± 0.5 μm. Polymer films with a thickness of 20 μm were also prepared on glass slides by wire-bar coating this solution as a control to compare texturing of films and fibres following procedures reported in a previous study [30].

To obtain fibres with a diameter of 1 μm, PMMA with a molecular weight of 350 kg/mol was blended with TPETA at a ratio of 60:40 wt% of PMMA and TPETA, respectively. The monomer and polymer were dissolved in 82 wt% DMF. An initiator was added at 10 wt% to the monomer. Fibres of 1 μm diameter were achieved by using a solution flow rate of 1 ml/h. PMMA on its own was dissolved in 82 wt% DMF and was electrospun as a control.

2.3. Photoembossing

A first set of experiments was carried out to study the effect of UV dosage and processing temperature on 2 μm diameter electrospun fibres. The fibres were placed on a substrate and photoembossed following our previously reported method [31]. A Nd:Yag pulsed laser coupled to second and third harmonic modules was used to produce 4 ns pulses of 355 nm linearly polarized light (with vertical polarization) at a frequency of 10 Hz. The light was split into two beams of equal intensity made to interfere on the substrate. The pitch of the line interference pattern is governed by Bragg's law and can be calculated by $p = \lambda/n.2\sin\theta$, where p is pitch, λ is the wavelength, n is the refractive index of air and θ is the angle between the interfering beams. In this experiment the angle between the two interfering beams was adjusted to produce an interference pattern with a pitch of 10 μm (Fig. 1).

The intensity of the source was varied between 0 and 150 mJ/cm^2. Following UV exposure, a thermal development step was perfomed at 120 °C in air. After the thermal development step the fibres were exposed to UV-light using an Omnicure S2000® (Jenton UV, UK) at 120 °C in air to cure residual monomer. Wire-bar coated films on glass cover slips with a thickness of 20 ± 5 μm were used as positive controls for the photoembossing process. The height of the