Applied Surface Science 257 (2011) 8798–8812

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Wettability and osteoblast cell response modulation through UV laser processing of nylon 6,6

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ARTICLE INFO

Article history:
Received 13 January 2011
Received in revised form 7 April 2011
Accepted 7 April 2011
Available online 13 June 2011

Keywords:
Excimer laser
Nylon 6,6
Wettability
Osteoblast cells
Bioactivity
Regenerative medicine

ABSTRACT

With an ageing population the demand for cheap, efficient implants is ever increasing. Laser surface treatment offers a unique means of varying biomimetic properties to determine generic parameters to predict cell responses. This paper details how a KrF excimer laser can be employed for both laser-induced patterning and whole area irradiative processing to modulate the wettability characteristics and osteoblast cell response following 24 h and 4 day incubation. Through white light interferometry (WLI) it was found that the surface roughness had considerably increased by up to 1.5 μm for the laser-induced patterned samples and remained somewhat constant at around 0.1 μm for the whole area irradiative processed samples. A sessile drop device determined that the wettability characteristics differed between the surface treatments. For the patterned samples the contact angle, θ, increased by up to 25° which can be attributed to a mixed-state wetting regime. For the whole area irradiative processed samples θ decreased owed to an increase in polar component, γ^{P}. For all samples θ was a decreasing function of the surface energy. The laser whole area irradiative processed samples gave rise to a distinct correlative trend between the cell response, θ and γ^{P}. However, no strong relationship was determined for the laser-induced patterned samples due to the mixed-state wetting regime. As a result, owed to the relationships and evidence of cell differentiation one can deduce that laser whole area irradiative processing is an attractive technology for employment within regenerative medicine to meet the demands of an ageing population.

1. Introduction

It has been realized worldwide within the scientific community and various industries that lasers offer major advantages over alternative techniques for materials processing [1–4]. Some of the main advantages of using a laser for materials processing are:

- Relative cleanliness.
- Accurate processing.
  - Allows much control over the Heat Affected Zone (HAZ) due to the ability of relative precise control over the thermal profile and thermal penetration/absorption.
- Precise placement of the beam onto the target material allowing user specified areas of the target material to be processed.
- Post-processing techniques required are usually minimal.
- Non-contact processing.
- Automation (repeatability) of the various processing techniques using a laser is relatively easy to implement.

With a large number of different lasers now commercially available it is possible for one to deduce that almost all materials can be processed using a laser due to the wide range of laser parameters that can be utilized. With the many benefits of using lasers for materials processing it has been found that the interest in laser-induced surface treatment has grown, especially within the biomedical industry. This is due to the fact that lasers offer the user a highly selective, rapid technique to induce surface modification in both organic and inorganic materials [5].

In order to modify the surface properties of polymers for use in biological environments and enhance the cell response by improving upon the adhesion characteristics, a vast number of techniques and methods have been developed. These techniques range from surface topography modification [6–8] to surface chemistry modification [9–11] and have given rise to the increased interest in using polymers as biomaterials [12–19]. Nylon 6,6, the strongest and most abrasive resistant unreinforced nylon, has been used for such biological applications as sutures, tracheal tubes and gastrointestinal segments [20]. With regards to orthopedic applications it can be seen that nylon is not commonly employed due to the hygroscopic nature having a large affect on the mechanical properties over long periods of time [21]. Having said that, the use of nylon 6,6 within this work gives high value experimentally insofar as to

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doi:10.1016/j.apsusc.2011.04.044

ascertain generic factors for polymeric materials which could be used to predict the osteoblast cell response. Also, by modifying the surface of polymeric materials it may be possible to identify other biological applications as this may enhance the osteoblast cell response and biocompatibile properties. On account of nylon 6,6 being a relatively inexpensive polymer when compared to other polymer types, by identifying other applications for this material the biological industry would benefit by being able to implement cheaper, more economic bio-implant materials.

With numerous advances in medicine it has been seen that the population is ageing both within the U.K. and within other major countries around the world. On account of people living longer, a number of institutions have developed a focus on bioengineering to meet the ever growing demands on medical facilities [22]. Furthermore, it is possible for one to realize that with an ageing population there is an ever increasing demand for biological implants. As a result, these needs of the ageing population need to be met more economically and efficiently so that costs and the need for unnecessary surgery are considerably reduced. Therefore, it is imperative for the biomedical industry to devise a way to manufacture cheap implants which can be used in confidence to ensure a dramatic reduction in failure rates. This can be met by the laser surface treatment of polymeric materials to enhance their biomimetic properties.

On account of the many benefits offered by laser materials processing to life sciences this paper is a contribution to the endeavour of enhancing polymer surface properties to determine generic parameters which give rise to the level of biofunctionality. That is, this work undertakes an approach by which the laser modified surface parameters, wettability characteristics and osteoblast cell response are discussed in order to ascertain the variables which link the factors together as shown in Fig. 1.

2. Experimental technique

2.1. Nylon 6,6 material

The nylon 6,6 (T_m: 255°, ρ: 1.3 g cm^{-3}) was sourced in 100 mm^2 sheets with a thickness of 5 mm (Goodfellow Cambridge, Ltd.). To obtain a conveniently sized sample for experimentation the as-received nylon sheet was cut into 20 mm diameter discs using a 1 kW continuous wave (cw) CO_2 laser (Everlase S48; Coherent, Ltd.). No discernible heat affected zone (HAZ) was observed under optical microscopic examination.

2.2. KrF excimer laser-induced patterning

For the patterned experiments the repetition rate of the KrF excimer laser (LPX 200i; Lambda Physik, Inc.) was kept constant at 25 Hz, with a number of 10 pulses per site and used Aerotech CNC programming to induce the required pattern. A constant laser energy of 80 ± 7 mJ was used with the attenuator set to 0.3 (30%) giving a measured energy at the target sample of 23.67 ± 2.5 mJ, resulting in a fluence of 858 ± 91 mJ cm^{-2}. In order to induce the intended pattern a projection imaging system was implemented with a focusing lens of ×10 demagnification. The patterns induced using this technique were 50 μm trench (ET50), 100 μm trench (ET100), 50 μm hatch (EH50) and 100 μm (EH100). Two non-contact masks were used for both dimensioned patterns which included a brass mask with six 1 mm diameter holes spaced by 2 mm, centre to centre, for the 100 μm dimensions and a SS316 foil (Laser Micromachining Ltd., UK) with five 0.5 mm diameter holes spaced by 1.5 mm, 'centre to centre', for the 50 μm dimensions. To keep the constant 10 pulses per site it should also be noted here that scanning velocities of 0.125 mm s^{-1} and 0.25 mm s^{-1} were used for the 50 μm and 100 μm dimensioned patterns, respectively.

No processing gases were used throughout the experimentation and all laser processing was carried out in an enclosure in which the ambient gas was air. Also, for all laser processing no homogenizer was implemented meaning that the raw beam was used which would have given rise to energy spikes pulse to pulse, having some possible affect on the incident laser fluence and laser material processing.

2.3. KrF excimer laser whole area irradiative processing

For the whole area processing with the KrF excimer laser (LPX 200i; Lambda Physik, Inc.) the raw 23 mm × 12 mm beam was used to irradiate a large section of each sample at a time. In order to hold the sample normal to the beam a bracket on the optical train was used. For the large area processing experiments 6 samples where studied; these being 100 pulses at 100 mJ (EWA100), 100 pulses at 150 mJ (EWA150), 100 pulses at 200 mJ (EWA200), 100 pulses at 250 mJ (EWA250), 500 pulses at 250 mJ (EWA250_500) and 1000 pulses at 250 mJ (EWA250_1000). This gave fluences of 36 ± 3, 54 ± 5, 72 ± 8 and 91 ± 10 mJ cm^{-2}, respectively for the different energies used. Throughout the whole area excimer experiments the repetition rate was kept constant at 25 Hz and Aerotech CNC programming ensured that the correct number of pulses was applied to each sample.

2.4. Topography, wettability characteristics and surface chemistry analysis

After laser irradiation the nylon 6,6 samples were analysed using a number of techniques. The surface profiles were determined using a white light interferometer (WLI) (NewView 500; Zygo, Ltd.) with MetroPro and TalyMap Gold Software. The WLI was set-up using a ×10 Mirau lens with a zoom of ×0.5 and working distance of 7.6 mm. This system also allowed Sa and Ra roughness parameters to be determined for each sample. Where Ra can be defined as the arithmetic average of the absolute values along a single specified direction and Sa the arithmetic average of the absolute values over the whole of the laser surface treated area.

In accordance with the procedure detailed by Rance [23] the samples were ultrasonically cleaned in isoproponal (Fisher Scientific Ltd.) for 3 min at room temperature before using a sessile drop device to determine various wettability characteristics. This was to allow for a relatively clean surface prior to any contact angle, θ, measurements being taken. To ensure that the sample surfaces were dry a specimen dryer (Metaserv, Ltd.) was employed to blow ambient air across the samples. A sessile drop device (OCA20; Dataphysics Instruments, GmbH) was used with relevant software (SCA20; Dataphysics Intrsuments, GmbH) to allow the recent advancing and receding θ for triply distilled water and the recent advancing angle for diiodomethane to be determined for each sample. By starting with a droplet with a volume of 8 μl then the advancing θ were achieved by adding and removing 0.5 μl, respectively, for each measurement. Thereafter the advancing θ for the two liquids were used by the software to draw an OWRK plot to determine the surface energy of the samples. For the two reference liquids the SCA20 software used the Ström et al. technique (triply distilled water–SFT(total:72.80), SFT(D:21.80), SFT(P:51.00); diiodomethane–SFT(total:50.80), SFT(D:50.80), SFT(P:0.00)) to calculate the surface energy of the material. It should be noted here that ten θ, using two droplets in each instance, were recorded to achieve a mean θ for each liquid and surface.

All samples were analysed using X-ray photoelectron spectroscopy (XPS). This allowed any surface modifications in terms of