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# Interaction of $CO_2$ laser-modified nylon with osteoblast cells in relation to wettability

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ABSTRACT

It has been amply demonstrated previously that $CO_2$ lasers hold the ability to surface modify various polymers. In addition, it has been observed that these surface enhancements can augment the biomimetic nature of the laser irradiated materials. This research has employed a $CO_2$ laser marker to produce trench and hatch topographical patterns with peak heights of around 1 μm on the surface of nylon 6,6. The patterns generated have been analysed using white light interferometry, optical microscopy and X-ray photoelectron spectroscopy was employed to determine the surface oxygen content. Contact angle measurements were used to characterize each sample in terms of wettability. Generally, it was seen that as a result of laser processing the contact angle, surface roughness and surface oxygen content increased whilst the apparent polar and total surface energies decreased. The increase in contact angle and reduction in surface energy components was found to be on account of a mixed intermediate state wetting regime owing to the change in roughness due to the induced topographical patterns. To determine the biomimetic nature of the modified and as-received control samples each one was seeded with $2\times10^4$ cells/ml normal human osteoblast cells and observed after periods of 24 h and 4 days using optical microscopy and SEM to determine mean cell cover densities and variations in cell morphology. In addition, a haemocytometer was used to show that the cell count for the laser patterned samples had increased by up to a factor of 1.5 compared to the as-received control sample after 4 days of incubation. Significantly, it was determined that all laser-induced patterns gave rise to better cell response in comparison to the as-received control sample studied due to increased preferential cell growth on those surfaces with increased surface roughness.



## 1. Introduction

It is seen that nylon can be utilized within the biomaterial industry as sutures [1], vascular grafts [2] and other hard tissue implants [3]. By extrapolating from past and current research it is imperative that any biomaterial should be optimized in order for that material to function appropriately and efficiently within the desired biological environment. In numerous instances it is seen that the bulk properties of a biomaterial are decided upon such that the surface properties are compromised [4,5]. In particular, this is seen throughout the use of polymeric biomaterials as they offer excellent bulk properties for biological applications; however, the surface properties they possess do not lend themselves to high performance in regards to biomimetics [6]. On account of this, it is necessary to vary the surface properties of the material without hindering the bulk properties in order to enhance the wettability and bioactivity. In terms of bioactivity a biomaterial can be surface modified both topographically and chemically in order to manipulate the way in which the cells react. That is, cell signaling could be optimized, allowing the filopodia to assess the extracellular matrix (ECM) and substrate so that the largest amount of integrin receptors could be localized around the suitable binding site to improve upon adhesion characteristics [7]. This is of great importance to those cells such as osteoblast cells that are highly dependent on ECM anchorage and as a direct result necessitates adhesion with the biomaterial prior to the initialization of normal cell function [8]. Integrin receptors are crucial to the way in which a biomaterial is accepted into the biological environment insofar as cellular interactions take place through the receptors creating focal adhesions. These focal adhesions are also important as they produce a high density of adhesion transmembrane receptors in areas of cellular adhesion to the biomaterial owed to the fact that they are closely associated with the actin cytoskeleton and other factors which regulate the signaling that takes place as the cell functions [9].

One of the most interesting subject areas in biomaterials surface science is that of wettability, with many workers endeavoring to determine the complex links between surface wetting and bioactivity [10]. Numerous theories have been expressed in order to explain this phenomenon in which two basic regimes have arisen. The first takes the biomimetic properties and attempts to correlate it with the

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surface energy whilst the second involves water solvent properties near the surface in which a correlation between the contact angle and bioactivity is strived for. However, in both cases a fundamental factor dominates in which the surface energy/wetting is generally related somewhat to the biological response [11]. Various approaches have been undertaken as to ascertain quantitative reasoning to bioactivity such as Van Oss et al. [12] by using the 'equation of state' approach to calculate interfacial tensions from previously measured contact angles in order to attempt and predict cell adhesion. Such approaches have been found to fall short for achieving a quantitative theory regarding the bioactivity of a material. Through the available literature it can be seen that extensive research is now being carried out regarding this in the attempt to link wettability and bioactivity of materials [13,14]. Once a quantitative link has been forged between these two parameters one can extrapolate that this will give those throughout the biotechnological industry a means to produce materials which have the ability to either enhance or even hinder the biomimetic nature; for instance, materials produced with surfaces that hinder the growth of bacteria could be widely used throughout the food packaging industry. On the other hand, enhanced biomimetics could be utilized for numerous applications such as biological implants and BioMEMs applications [15].

Numerous techniques have been developed that have the ability to modify the surfaces of different materials [16]. Some of these methods are radiation grafting [17], plasma surface modification [18,19] and using various coatings [20]. Another method which has the ability to produce surface modifications is that of laser treatment [21,22] and offers a number of benefits such as accurate, precise, non-contact and clean processing. One other major advantage that lasers offer over other competing techniques is that they can produce micro and nano scale topographical and surface chemical variations with negligible effect to the bulk properties of the material. Also, it should be noted that as most laser systems are now automated this technique holds the ability to be used for large area processing. Being able to produce these topographical and surface chemical variations can be seen to be of great advantage when applying laser surface modification to fields such as biomimetics especially as previous work has shown a very high dependence of micro and nano scale topography and surface chemistry on the cell–material interaction [7–9,15,16,22,23].

On account of the numerous advantages laser materials processing has to offer for laser surface treatment it is necessary that considerable research is undertaken to ascertain how this technique can be employed in such fields as biotechnology. Such research will lead to the ability of assessing the plausibility and reliability for using lasers to produce surface modifications to aid in the enhancement of the biomimetic nature of biomaterials. On account of the required significant research in this field the first ever initial study into how $CO_2$ laser generated surface patterns influence both the wettability characteristics of nylon 6,6 and normal human osteoblast cell response, when studied *in vitro*, has been carried out. This investigation has taken place in order to study how the biomimetic nature of laser surface patterned nylon 6,6 can be affected by surface properties such as characteristic recently advancing contact angle, surface roughness, surface oxygen content and the surface energy.

## 2. Experimental techniques

### 2.1. Laser irradiation procedure

The nylon 6,6 was sourced in $100 \times 100$ 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 30 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.

In order to generate the required marking pattern with the 10.6 μm Synrad cw 10 W $CO_2$ laser system Synrad Winmark software version 2.1.0, build 3468 was used. In addition, the software was capable of using images saved as .dxf files which can be produced by using CAD programs such as, in this case, Licom AutoCaM. The nylon 6,6 samples were placed into the laser system onto a stage in which they were held in place using a bracket with a 30.5 mm diameter hole cut into the centre of the bracket. The surface of the sample was set to be 250 mm away from the output facet of the laser system to obtain focus and the system utilized a galvanometer scanner to scan the 95 μm spot size beam directly across the stationary target material. It should be noted that the target material and laser system was held in a laser safety cabinet in which the ambient gas was air and an extraction system was used to remove any fumes produced during laser processing.

20 samples were irradiated altogether to produce 4 identical 6-well plates with each corresponding well having the same pattern. These were named plates 1A, 1, 2 and 3. There were four patterns induced onto the surfaces of the nylon 6,6 samples which were trenches with 50 μm spacing (A3), hatch with 50 μm spacing (B1), trenches with 100 μm spacing (B2) and hatch with 100 μm spacing (B3). In addition, an as-received control sample was used (A1). For each of the irradiated patterns the laser power was set to 70% (7 W) operating at 600 mm s$^{-1}$.

### 2.2. Topography, wettability characteristics and surface chemistry analysis

After the laser irradiation of the nylon 6,6 samples plate 1A was analysed using a number of techniques. An optical microscope (Flash 200 Smartscope; OGP, Ltd) was used to obtain optical micrographs of the samples. The surface profiles were determined using a white light interferometer (WLI) (NewView 500; Zygo, Ltd) with MetroPro and TalyMap Gold Software. The Zygo WLI was setup 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.

In accordance with the procedure detailed by Rance [24] the samples were ultrasonically cleaned in isopropanol (Fisher Scientific Ltd., UK) 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 measurements being taken. To ensure that the sample surfaces were dry a specimen dryer (Metaserv, UK) was utilized 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 contact angles for triply distilled water and the recent advancing angle for diiodomethane to be determined for each sample. From the measured advancing and receding contact angles the hysteresis for the system was established. Thereafter the advancing contact angles for the two liquids were used by the software to draw an Owens, Wendt, Rabel and Kaeble (OWRK) plot to determine the surface energy of the samples. For the two reference liquids the SCA20 software used the Ström et al. technique to calculate the surface energy of the material. It should be noted here that ten contact angles, using two droplets, in each instance was recorded to achieve a mean contact angle for each liquid and surface.

Selected samples were analysed using X-ray photoelectron spectroscopy (XPS) analysis. This allowed any surface modifications in terms of surface oxygen content due to the laser irradiation to be revealed. These samples were selected in terms of contact angle; the as-received reference sample, laser patterned sample with the lowest contact angle and the laser surface patterned sample with the highest contact angle was used. XPS measurements were performed on a Kratos Axis Ultra DLD photoelectron spectrometer employing monochromatic aluminium k-alpha radiation source, operating at 120W power and an associated photon energy of 1486.6 eV. To test the reproducibility of the surface, two sections of each sample were analysed; the analysis area on each sample was 700x300 microns. The spectrometer was run in its Hybrid mode and spectra were acquired at pass energies of 20 eV (for the high resolution scans) and 160 eV for the survey scans. All data was analysed through CasaXPS (v2.3.14)