

# MODULATION OF OSTEOBLAST CELL RESPONSE THROUGH LASER SURFACE PROCESSING OF NYLON 6,6

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## Abstract

With an ageing population demand on medical facilities is growing, especially for bio-implants. Therefore, there is a need for cheaper, more efficient implants. This paper details how CO<sub>2</sub> and KrF excimer lasers can be employed to modulate osteoblast cell growth on nylon 6,6 in relation to laser-modified wettability characteristics. Through patterning the contact angle,  $\theta$ , increased by up to 19°, indicating the presence of a mixed state wetting regime; whereas  $\theta$  decreased by up to 20° for the whole area irradiative processed samples. After 24 hours and 4 days incubation the cell cover density and cell count was somewhat modulated over the laser-modified samples compared to the as-received sample. A likely increase in surface toxicity gave rise to a hindered cell response for those samples with high energy densities and high incident pulse numbers. No strong correlations were determined for the laser-induced patterned samples which can be attributed to the likely mixed-state wetting regime. Correlative trends were found between the cell response,  $\theta$ , polar component and surface oxygen content for the whole area irradiative processed samples. Thus, allowing one to identify the potential for this technology in regenerative medicine.

## Introduction

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 (1). 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.

It has been realized worldwide within the scientific community and various industries that lasers offer major advantages over alternative techniques for materials processing (2-5). Some of the main advantages of using a laser for materials processing are:

- Relative cleanliness.
- Accurate processing.
- Precise placement of the beam onto the target material.
- Post-processing techniques required are usually minimal.
- Non-contact processing.
- Automation of a laser system 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 (6).

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 (7-9) to surface chemistry modification (10-12) and have given rise to the increased interest in using polymers as biomaterials (13-20). 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 (21). 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 (22). Having said that, the use of nylon 6,6 within this work gives high value experimentally insofar as to 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 biocompatible 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.

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 Figure 1.

## Experimental Technique

### Nylon 6,6 Material

The nylon 6,6 ( $T_m$ : 255°,  $\rho$ : 1.3 gcm<sup>-3</sup>) was sourced in 100 mm<sup>2</sup> 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<sub>2</sub> laser (Everlase S48; Coherent, Ltd).

### CO<sub>2</sub> Laser-Induced Patterning

In order to generate the required marking pattern with the Synrad 10 W CO<sub>2</sub> laser system, Synrad Winmark software version 2.1.0, build 3468 was used. 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 20.5 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 employed 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.

There were four patterns induced onto the surfaces of the nylon 6,6 samples; were trenches with 50 µm spacing (CT50), hatch with 50 µm spacing (CH50), trenches with 100 µm spacing (CT100) and hatch with 100 µm spacing (CH100). In addition, an as-received control sample was used (AR). For each of the irradiated patterns the laser power was set to 70% (7 W) operating at 600 mms<sup>-1</sup>.

### CO<sub>2</sub> Laser Whole Area Irradiative Processing

A cw 100 W CO<sub>2</sub> laser (DLC; Spectron, Ltd) was used to scan a 5mm diameter beam across the target sample with one pass in order to irradiate the test area with an irradiance of 510 Wcm<sup>2</sup>. By using a galvanometer, scanning speeds of 150, 100, 75, 50, 25 and 20 mms<sup>-1</sup> were employed to irradiate six samples with effective fluences of 16.84 (samples CWA17), 25.51 (sample CWA26), 34.18 ( sample CWA34), 51.02 (sample CWA51), 102.04 (sample CWA102) and 127.55 (sample CWA128) Jcm<sup>-2</sup>, respectively. As with the laser-induced patterning experimentation the samples were held in place on a z-variable stage with the whole experiment being carried out in a laser safety cabinet with the ambient gas being air.

### KrF Excimer Laser-Induced Patterning

For the patterned experiments the repetition rate 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<sup>2</sup>. In order to induce the intended pattern a projection imaging system was implemented with a focusing lens of x10 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 mms<sup>-1</sup> and 0.25 mms<sup>-1</sup> 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 effect on the incident laser fluence and laser material processing.

### KrF Excimer Laser Whole Area Irradiative Processing

For the whole area processing with an excimer laser the raw  $23 \times 12 \text{ mm}^2$  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 were 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  $36 \pm 3$ ,  $54 \pm 5$ ,  $72 \pm 8$  and  $91 \pm 10 \text{ mJcm}^{-2}$ , 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.

### 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  $\times 10$  Mirau lens with a zoom of  $\times 0.5$  and working distance of 7.6 mm. This system also allowed  $S_a$  and  $R_a$  roughness parameters to be determined for each sample.

In accordance with the procedure detailed by Rance (23) the samples were ultrasonically cleaned in isopropanol (Fisher Scientific Ltd.) for 3 minutes 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  $\theta$  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 Instruments, GmbH) to allow the recent advancing and receding  $\theta$  for triply distilled water and the recent advancing angle for diiodomethane to be determined for each sample.

Thereafter the advancing  $\theta$  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  $\theta$ , using two droplets in each instance, were recorded to achieve a mean  $\theta$  for each liquid and surface.

All samples were analysed using x-ray photoelectron spectroscopy (XPS). This allowed any surface modifications in terms of surface oxygen content due to the laser irradiation to be revealed. XPS measurements were performed on a photoelectron spectrometer (Axis Ultra DLD; Kratos Analytical Ltd., UK) employing monochromatic aluminium k-alpha radiation source, operating at 120 W 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  $700 \times 300$  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) analysis software using sensitivity factors supplied by the instrument manufacturer.

### *In Vitro* Experimentation

Prior to any biological testing being carried out the samples were autoclaved (D-Series Bench-Top Autoclave; Systec, GmbH) to ensure that all samples were sterilized. For all biological work undertaken, unless stated, a biological safety cabinet (BSC) (Microflow Class II ABS Cabinet; BioQuell UK, Ltd) was used to create a safe working environment and to provide a clean, sterile environment to manipulate the cells used.

Normal human osteoblast cells (Clonetics CC-2538; Lonza, Inc.) were initially cultured in a T75 (75 ml) flask by suspending the cells in 19 ml culture medium comprising of 90% eagle minimum essential medium (Sigma-Aldrich, Ltd., UK) and 10% foetal bovine serum (FBS) (Sigma-Aldrich Ltd., UK). The flask was then placed in an incubator and left for 24 hrs. After 24 hrs the cells were assessed and the spent media was aspirated before dispensing 15 ml of fresh media and returning the flask to the incubator for three days.

The period of three days allowed the cells to become confluent in the flask providing enough cells for seeding onto the samples. The cells were detached

from the flask using 5 ml Trypsin-EDTA (Sigma-Aldrich Ltd., UK) whilst placed in the incubator for seven minutes. Once all cells had become detached 10 ml culture medium was added to neutralize the Trypsin. In order to aspirate the supernatant the cell culture was centrifuged (U-320R; Boeco, GmbH) for five minutes at 200 g. To ensure the cells were ready for seeding they were resuspended in 10 ml of culture medium and dispensed between the samples in the 6-well plates. This equated to 0.55 ml ( $2 \times 10^4$  cells/ml) for each sample. The well plates were then placed in the incubator for a set time. One plate was removed after 24 hrs and two other plates after four days. A well plate after 24 hrs and 4 days was prepared for the SEM as will be discussed later and the other plate, removed after 4 days, was prepared for counting using an improved Neubauer hemacytometer (Fisher Scientific Ltd., UK) by mixing 10  $\mu$ l of each cell suspension with 10  $\mu$ l of trypan-blue (Sigma-Aldrich Ltd., UK). In order to harvest the cells for counting the cells were detached from the samples using 2 ml Trypsin-EDTA (Sigma-Aldrich Ltd., UK) whilst placed in the incubator for seven minutes. Once all cells had become detached 4 ml culture medium was added to neutralize the Trypsin. In order to aspirate the supernatant the cell culture was centrifuged (U-320R; Boeco, GmbH) for five minutes at 200 g. To ensure the cells were ready for counting they were resuspended in 2 ml of culture medium and 2 ml of the trypan-blue was added.

#### Scanning Electron Microscopy and Cell Cover Density of *In Vitro* Samples

In order to view the attached cells using SEM it was necessary to undertake a procedure to produce a sample that was dehydrated ready for Au coating. The samples were initially rinsed with phosphate-buffered saline (PBS) (Sigma-Aldrich, UK) to remove any unattached cells and then adherent cells were fixed using 1.2% glutaraldehyde in water (Sigma-Aldrich, UK) at room temperature for one hour within the BSC. After an hour the glutaraldehyde solution was removed and the fixed cells were washed with PBS prior to carrying out a graded series of ethanol/distilled water mixtures of 50/50, 80/20, 90/10, 95/5, 98/2 and 100/0. Each sample was left in these mixtures for 10 min to ensure dehydration. Once this procedure was carried out, the samples were mounted and sputter coated with Au so that SEM micrographs could be obtained. In order to produce the best images possible each image was manipulated in terms of brightness, contrast and gamma by using ImagePro Version 5.0.0.39 for Windows XP/Professional software (Media Cybernetics Inc., USA).

In addition to the cell count described in Section 3.5, the cell cover density was determined following both 24 hrs and 4 day incubation. This was done by analysing the cell coverage on each sample using SEM and optical micrographs with the ImagePro software. The optical micrographs were obtained using an upright optical microscope (Flash 200 Smartscope; OGP, Ltd) with magnifications varying between  $\times 20$  and  $\times 100$ .

## Results and Discussion

### Topography – CO<sub>2</sub> and KrF Excimer Laser Processing

Topography modification through the different laser surface treatments has been discussed previously (21,24).

It was evident that a CO<sub>2</sub> laser can be employed to modify significantly the surface topography of nylon 6,6 if the laser-material interaction threshold in terms of fluence is exceeded. The CO<sub>2</sub> laser whole area irradiated processed samples at large fluencies (sample CWA102 and sample CWA28) gave rise to an order of magnitude increase in Sa. It was also found that the topographies of the CO<sub>2</sub> laser-induced patterned samples were somewhat periodic with steep, sharp gradients when considering the peak heights; whereas, the CO<sub>2</sub> laser whole area irradiative processed samples gave rise to a more cratered surface. This was on account of more melting taking place which in turn gave rise to evolved gases being able to break to the surface. As a result of this, the maximum peak heights achieved for the CO<sub>2</sub> laser whole area processed were found to be considerably larger by up to 13  $\mu$ m but were less densely situated when compared to the CO<sub>2</sub> laser patterned samples which had maximum peak heights of approximately 2 to 3  $\mu$ m and were more periodic.

For the CO<sub>2</sub> whole area irradiative processed samples it was found that in terms of surface topography the first three samples (samples CWA17, CWA26 and CWA34) gave rise to negligible modifications when compared to the as-received sample (AR) while all of the CO<sub>2</sub> laser-induced patterned samples gave rise to a noteworthy variation in the surface topography of the nylon 6,6. This can be on account of samples CWA17, CWA26 and CWA34 having been irradiated with fluences below or around the threshold fluence. On account of this, one can then deduce that all other samples gave rise to significant modifications in the surface topography as the fluences used to irradiate the other samples were considerably higher. As such, one can reasonably state that above the threshold, both CO<sub>2</sub>

whole area processing and laser-patterning gave rise to considerable modifications in terms of topography of the nylon 6,6 samples.

It has been observed that KrF excimer laser-induced patterning can significantly modify the topography of nylon 6,6 insofar as the roughness had increased and distinct periodic patterns had been induced into the material surface. In contrast, the KrF excimer whole area irradiative processed samples gave rise to a negligible variation in surface parameters when compared to the as-received sample such that the Sa remained around 0.1 to 0.2  $\mu\text{m}$  and Ra around 0.03  $\mu\text{m}$ .

#### Wettability – CO<sub>2</sub> and KrF Excimer Laser Processing

The wettability results have been discussed in more depth previously (21,24).

Owed to the fact that the CO<sub>2</sub> laser-induced patterned samples induced a more hydrophobic response and a more hydrophilic response was observed for the CO<sub>2</sub> whole area irradiated patterns it is possible to deduce that surface oxygen content may not be the main

driving force for wettability as the oxygen content increased in all instances by up to 2 %at. for the patterned samples and up to 5 %at. for the whole area irradiated samples (see Table 1). This is owed to the thermolytical interaction between the nylon 6,6 material and the CO<sub>2</sub> laser light that gives rise to melting of the nylon surface allowing oxidation to take place.

Upon analysing the CO<sub>2</sub> laser-induced patterned surfaces it was found that the  $\gamma^T$  and  $\gamma^P$  had decreased by up to 7  $\text{mJm}^{-2}$  and the surface roughness had considerably increased. It was found that  $\gamma^P$  and  $\gamma^T$  for the samples studied were both a decreasing function of the  $\theta$ , which correlates with current theory; however, current theory states that  $\theta$  for a hydrophilic surface should decrease upon increasing surface roughness which has not been seen throughout this experimentation.

Table 2 allows one to identify that there was some significant differences between the as-received (AR), KrF excimer laser-induced patterned and KrF excimer laser whole area processed nylon 6,6 samples in terms of surface parameters and wettability characteristics.

Table 1 – Results summary for samples showing roughness parameters, surface oxygen content and wettability characteristics following CO<sub>2</sub> laser processing of nylon 6,6.

Sample ID	Sa ( $\mu\text{m}$ )	Ra ( $\mu\text{m}$ )	Polar Component, $\gamma^P$ ( $\text{mJm}^{-2}$ )	Dispersive Component, $\gamma^D$ ( $\text{mJm}^{-2}$ )	Total Surface Energy, $\gamma^T$ ( $\text{mJm}^{-2}$ )	Surface Oxygen Content (%at.)	Contact Angle ( $^\circ$ )
AR	0.126	0.029	17.69	29.66	47.34	13.26	56.4 $\pm$ 1.2
CO <sub>2</sub> Laser-Induced Patterned Samples							
CT50	0.636	0.148	12.24	28.63	40.87	14.33	66.0 $\pm$ 4.0
CT100	0.297	0.185	16.86	29.83	46.69	14.05	57.5 $\pm$ 2.4
CH50	0.423	0.103	10.93	31.64	42.58	14.99	65.8 $\pm$ 2.9
CH100	0.326	0.155	13.63	30.37	44.00	14.84	62.2 $\pm$ 2.3
CO <sub>2</sub> Whole Area Irradiative Processed Samples							
CWA17	0.111	0.060	18.36	28.75	47.11	13.56	55.8 $\pm$ 2.7
CWA26	0.100	0.158	19.67	28.35	48.02	13.86	54.6 $\pm$ 3.5
CWA34	0.101	0.092	14.89	38.55	53.43	14.34	56.4 $\pm$ 3.5
CWA51	0.341	0.139	10.66	38.59	49.26	15.45	64.1 $\pm$ 2.2
CWA102	4.356	1.236	28.49	23.82	52.31	16.77	46.6 $\pm$ 4.7
CWA128	3.201	1.335	31.98	22.78	54.76	18.93	43.0 $\pm$ 3.7

It was observed that for the KrF laser-induced patterned samples  $\theta$  increased even though there was a significant increase in surface roughness. On the other hand, it was found that on account of an increase in  $\gamma^P$  and  $\gamma^T$ ,  $\theta$  decreased for the KrF excimer laser whole area irradiative processed nylon 6,6 samples. It was observed that for all KrF excimer laser processed samples the surface oxygen content increased by up to 5 %at. when compared to the as-received sample (AR). This increase in surface oxygen content indicates that this may have given rise to the observed reduction in  $\theta$  for the laser whole area processed samples. Still, this does not seem to be the case as a reduction in  $\theta$  was not observed for the KrF excimer laser-induced patterned samples.

This suggests that a significant variation in surface topography of the nylon 6,6 may bring about the change in wettability regime; whereas when there is no significant variation in topography it is possible that a variation in wettability is brought about by other parameters such as  $\gamma^P$  and  $\gamma^T$ . In addition, even though there was a change in wetting regime for the KrF excimer laser-induced patterned samples to account for the increase of Sa and Ra, it was still found that  $\theta$  remained strongly linked to variations in  $\gamma^P$  and  $\gamma^T$ .

In terms of collating the  $\gamma^P$  and  $\gamma^T$  results for the entire KrF excimer laser processed samples and the effects thereof on  $\theta$ , Figure 7.7 shows that there was a strong inverse function correlation between  $\theta$  and the surface energy parameters regardless of the processing technique used. This indicates that  $\gamma^P$  and  $\gamma^T$  could be the main driving parameters determining the wettability of the nylon 6,6 samples in this instance. As a result it may be possible to use these parameters as indicators of how nylon 6,6 will wet as long as the results are repeatable. Having said that, as discussed in Section 7.3.2, similar values of  $\gamma^P$  and  $\gamma^T$  give rise to equivalent  $\theta$  suggesting that this process is repeatable.

### Cell Cover Density and Cell Count

The osteoblast cell response, in terms of morphology, has been discussed in more depth previously (24,25).

Figure 1 allows one to see that overall the laser surface treated samples did not have a correlative trend between the cell cover density, cell count and  $\theta$ . This is on account of the laser-induced patterned samples not correlating with the trend observed for the laser whole area irradiative processed samples which can be attributed to the transition in wetting regime for the laser-induced patterned samples giving erroneous results.

Table 2 – Results summary for samples showing roughness parameters, surface oxygen content and wettability characteristics for KrF processed nylon 6,6.

Sample ID	Sa ( $\mu\text{m}$ )	Ra ( $\mu\text{m}$ )	Polar Component, $\gamma^P$ ( $\text{mJm}^{-2}$ )	Dispersive Component, $\gamma^D$ ( $\text{mJm}^{-2}$ )	Total Surface Free Energy, $\gamma^T$ ( $\text{mJm}^{-2}$ )	Surface Oxygen Content (%at.)	Contact Angle ( $^\circ$ )
AR	0.126	0.029	17.69	29.66	47.34	13.26	56.4 $\pm$ 1.2
Laser-Induced Patterned Samples							
ET50	0.281	0.312	15.48	28.74	44.22	13.87	60.6 $\pm$ 3.0
ET100	1.320	0.679	6.52	23.54	30.06	13.53	80.8 $\pm$ 5.2
EH50	0.739	0.548	12.94	17.24	30.17	14.15	75.1 $\pm$ 3.9
EH100	1.530	1.032	17.06	13.55	30.61	14.83	73.0 $\pm$ 3.7
Whole Area Irradiative Processed Samples							
EWA100	0.119	0.029	21.72	27.08	48.79	13.11	52.8 $\pm$ 1.1
EWA150	0.092	0.039	25.98	24.76	50.74	13.03	49.1 $\pm$ 4.7
EWA200	0.108	0.030	25.26	25.86	51.12	13.28	48.9 $\pm$ 2.6
EWA250	0.107	0.031	22.37	28.66	51.03	13.24	50.3 $\pm$ 1.5
EWA250_500	0.164	0.039	33.31	21.40	54.71	15.32	42.9 $\pm$ 1.6
EWA250_1000	0.198	0.036	37.56	20.89	58.46	18.36	37.6 $\pm$ 3.2

For the laser whole area irradiative processed samples, with the exception of sample CWA102 and sample CWA128, the cell cover density and cell count were an increasing function of  $\theta$ . It should be noted here that sample CWA102 and sample CWA128 did not follow the trend on account of the toxicity dominating.

Figure 2 shows that there was no correlative relationship between  $\gamma^P$ ,  $\gamma^T$  and osteoblast cell response for the laser surface treated nylon 6,6 samples when taken as a whole. Similar to  $\theta$  (see Figure 1), this can be accounted for by the laser-induced patterned samples which gave rise to somewhat erroneous results on account of the likely transition in wetting regime. The relationship between cell cover density, cell count and  $\gamma^P$  for the entire laser whole area irradiative processed samples can be seen in Figure 2 in that the enhancement of osteoblast cell response was an increasing function of  $\gamma^P$ . However, sample CWA102 and sample CWA128 did not correspond with the trend identified and can be explained by the increase in surface toxicity. Leading on, it can be seen from Figure 2 that for none of the samples there did not appear to be any correlative relationship between  $\gamma^T$  and the osteoblast cell response, indicating that  $\gamma^T$  was not a dominant parameter. Even though there was no overall trend observed for  $\theta$  and  $\gamma^P$  it can still be said within reason that if a surface toxicity was low enough as to not dominate the osteoblast cell response, then it appears likely that  $\theta$  and  $\gamma^P$  play an important role in determining the osteoblast cell response to the laser whole area irradiative processed nylon 6,6.

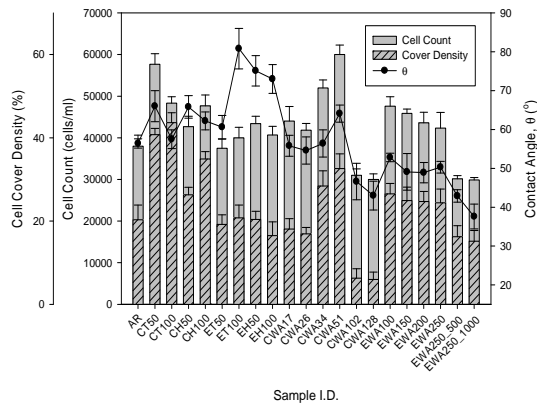


Figure 1 – Histogram showing cell count and cell cover density in relation to  $\theta$ .

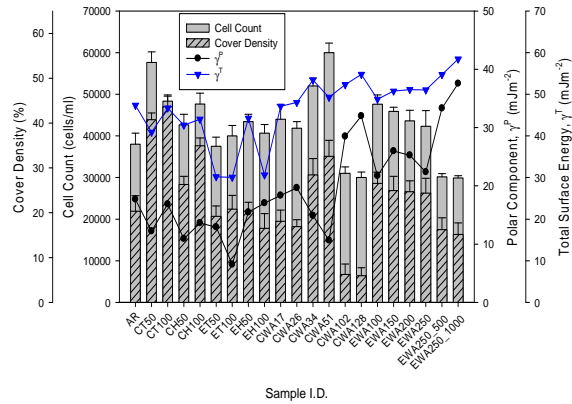


Figure 2 – Histogram showing cell count and cell cover density in relation to  $\gamma^P$  and  $\gamma^T$ .

It can be seen from Figure 3 that overall, the surface roughness in terms of Ra and Sa did not appear to have a distinct relationship with the osteoblast cell response with regards to cell cover density and cell count. Having said that, for those samples with the largest values of Sa and Ra (sample CWA102 and sample CWA128) the surface roughness could be indirectly indicative of the bioactive nature of the CO<sub>2</sub> laser whole area irradiative processed nylon 6,6 samples. This is due to the fact that the melting which gave rise to the proposed increase in toxicity, which dominated the osteoblast cell response, also gave rise to the significant increase in Ra and Sa.

When taking into account all samples with respect to the relationship between the surface oxygen content and osteoblast cell response, it can be seen from Figure 4 that there was no overall trend which could be implemented to predict the osteoblast cell response to the laser surface treated nylon 6,6. Having said that, there was two different observed trends for the laser whole area irradiative processed samples which could potentially be used as an indirect method to estimate whether enhanced osteoblast cell response can be brought about by the nylon 6,6 samples. This is accounted for by the likelihood of the nylon 6,6 samples becoming more toxic upon more laser-induced melting even though the surface will also oxidize, giving rise to an increase in surface oxygen content.

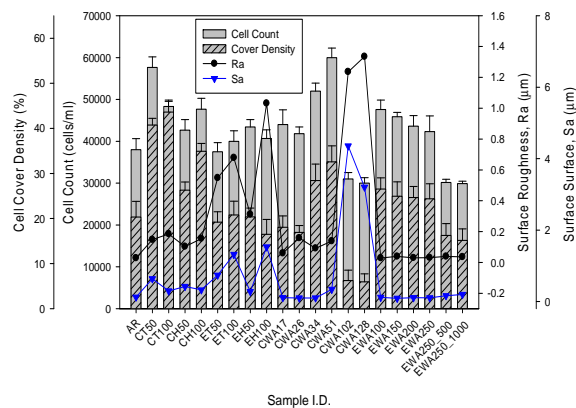


Figure 3 – (a) Histogram showing cell count and cell cover density in relation to Ra and Sa.

In terms of cell cover density and cell count it has been seen that below a potential toxicity threshold  $\theta$  and  $\gamma^p$  appear to dominate the bioactive nature of nylon 6,6 which has undergone laser whole area irradiative processing regardless of the laser type used. With this in mind, one can extrapolate that  $\theta$  and  $\gamma^p$  can be implemented to predict how osteoblast cells will react upon laser whole area irradiative processed nylon 6,6.

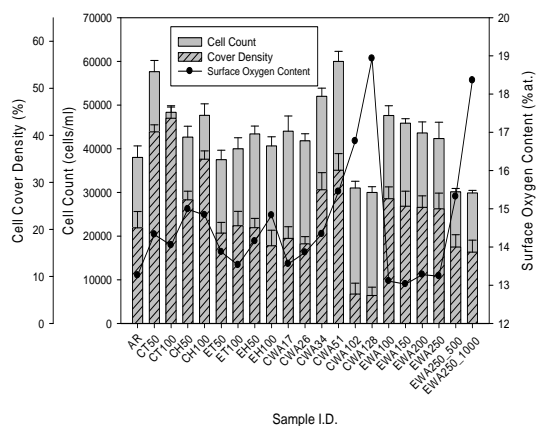


Figure 4 – (a) Histogram showing cell count and cell cover density in relation to surface oxygen content.

### Conclusions

Throughout this research it has been further demonstrated that lasers offer an efficient and convenient means of surface modifying materials - in this case nylon 6,6, both chemically and topographically.

Upon analysing all of the laser processed surfaces it was determined that a number of surface parameters

can be modified. These surface modifications brought about variations in the wettability characteristics of the nylon 6,6 samples. It was found that total surface energy,  $\gamma^T$ , and polar component,  $\gamma^p$ , had decreased by along with an increase in surface roughness. It was also determined that  $\gamma^p$  and  $\gamma^T$  for the samples studied were both a decreasing function of  $\theta$ , which correlated with current theory; however, current theory also states that  $\theta$  for a hydrophilic surface should decrease upon increasing surface roughness which has not been seen throughout this experimentation. This can be attributed to an intermediate mixed Cassie-Baxter/Wenzel regime, in which both Wenzel and Cassie-Baxter regimes arise at the solid-liquid interface as a result of the formation of the water droplet on the various laser modified surface topographies.

With regards to the bioactive nature of the nylon 6,6 samples, the laser-induced patterned samples did not give rise to any identifiable trends between the laser-modified surface parameters and the cell response. This was attributed to the mixed-state wetting regime having a large influence on cell response. On the other hand, it was found for the laser whole area processed samples that  $\theta$  and  $\gamma^p$  had strong trends with the osteoblast cell count and cover density. On account of this, one can see that laser whole area processing, regardless of the wavelength, offers a convenient means to tailor materials to enhance osteoblast cell growth.

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### Meet the Author

Dr. David Waugh completed his Ph.D. under the supervision of Dr. Jonathan Lawrence in July 2010. Dr. Waugh is now a Lecturer at the newly established School of Engineering at the University of Lincoln, UK. He is also part of the Lincoln Laser Engineering Group in which he primarily focuses on the application of laser surface treatment to life sciences. He obtained his MPhys Hons. in Physics with Lasers and Photonics and MSc in Laser Applications in Micro-Machining and Processing from the University of Hull, UK.