The use of abrasive polishing and laser processing for developing polyurethane surfaces for controlling fibroblast cell behaviour

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Abstract

Studies have shown that surfaces having micro and nano-scale features can be used to control cell behaviours including; cell proliferation, migration and adhesion. The aim of this work was to compare the use of laser processing and abrasive polishing to develop micro/nano-patterned polyurethane substrates for controlling fibroblast cell adhesion, migration and proliferation. Laser processing in a directional manner resulted in polyurethane surfaces having a ploughed field effect with micron-scale features. In contrast, abrasive polishing in a directional and random manner resulted in polyurethane surfaces having sub-micron scale features orientated in a linear or random manner. Results show that when compared with flat (non-patterned) polymer, both the laser processed and abrasive polished surface having randomly organised features, promoted significantly greater cell adhesion, while also enhancing cell proliferation after 72 hours. In contrast, the abrasive polished surface having linear features did not enhance cell adhesion or proliferation when compared to the flat surface. For cell migration, the cells growing on the laser processed and abrasively polished random surface showed decreased levels of migration when compared to the flat surface. This

study shows that both abrasive polishing and laser processing can be used to produce surfaces having features on the nano-scale and micron-scale, respectively. Surfaces produced using both techniques can be used to promote fibroblast cell adhesion and proliferation. Thus both methods offer a viable alternative to using lithographic techniques for developing patterned surfaces. In particular, abrasive polishing is an attractive method due to it being a simple, rapid and inexpensive method that can be used to produce surfaces having features on a comparable scale to more expensive, multi-step methods.

Keywords: Laser processing; Abrasive polishing; Cell adhesion; Cell proliferation; Cell migration

1 Introduction

Mammalian cells have evolved to interact with their physical environment and this interaction is crucial for many important cellular behaviours including; adhesion, migration and proliferation. In vivo, cells depend on an interaction with a 3D scaffold known as the extracellular matrix (ECM). It is thought that the geometrical organisation and mechanical compliance of the ECM is extremely important in helping to regulate the aforementioned cell behaviours {Huttenlocher, 2011 #2497}. As a consequence, there has been a significant research effort that has focused on the development of cell substrates designed to have both 2D and 3D surface structures that mimic the features of the ECM. This has largely been achieved through patterning materials to develop 'functional' or 'smart' surfaces that can be used to better control cellular responses in vitro.

Development of such surfaces has been shown to have significant impact on improving the integration of prosthetic implants. For example, modification of dental implants to alter surface roughness properties has been shown to improve implant integration {Le Guehennec, 2007 #2416}. Similarly, enhancing the surface roughness of breast implants has been shown to increase the surface adhesive properties for fibroblast cells and it has been suggested that this increased cell adhesion will improve wound healing following implantation, thus limiting the risk of capsular contracture {Valencia-Lazcano, 2013 #2411}. Therefore, there is a clear benefit and need to develop such materials for use in biomedical applications.

Much of the work in this area has focused on developing surfaces that have specific features with defined geometries and sizes on a range of different materials. For example micro and nano-scale grooves {Reynolds, 2012 #2391}, pillars {Ghibaudo, 2009 #56} and pits {Curtis, 2001 #25}. These surfaces have been shown to influence cell adhesion {WojciakStothard, 1996 #11} and proliferation {Dalby, 2002 #12} and migration {Ko, 2013 #2366} of a range

of cell types including; fibroblast cells {Curtis, 2001 #25}, osteoblasts {Biggs, 2009 #28}, endothelial cells {Koo, 2014 #2463}, epithelial cells {Andersson, 2003 #79} and neurons {Li, 2015 #2470}.

Many methods are available for modifying topography to develop functional surfaces. One of the most widely used techniques involves the use of a template mask which is placed over the surface that is due to be processed, thus leaving a predetermined pattern, post processing. This technique is seen in lithography-based approaches including; electron beam lithography {Curtis, 2001 #25;Alaerts, 2001 #33;Karuri, 2006 #2377}, colloidal lithography {Wood, 2002 #2393;Dalby, 2005 #18}, photolithography {Clark, 1990 #29;Lee, 2009 #64;Reynolds, 2012 #2391}, Langmuir–Blodgett lithography {Lenhert, 2005 #58} and X-ray lithography {Karuri, 2004 #2375;Liliensiek, 2006 #2384}. Such methods are advantageous, as they allow the development of substrates having a range of well-defined geometries; however they often require expensive equipment and are generally time-consuming processes.

Laser processing has been shown to be an effective method for micro-patterning, due to it being a rapid, direct-write and flexible process {Aguilar, 2005 #2402}, which, is also capable of processing relatively large areas {Bolle, 1993 #2399} (e.g. greater than 1 cm^2) by a single exposure {Wu, 2005 #2498}. In contrast, the use of abrasive polishing methods to date has been largely overlooked for developing textured surfaces for manipulating cell behaviour, even though this is a comparatively cheap process and may be used to produce surfaces having features of comparable size to the aforementioned lithographical based methods.

The aim of this work was to evaluate the use of abrasive polishing for producing nano/micro-patterned polyurethane substrates that can promote cell adhesion, migration and proliferation. Any material that may be considered as a coating for biomedical applications should promote cell attachment, flattening, spreading and migration as these steps are important in

determining whether cells will proliferate. Therefore, abrasive polishing was compared with an already established technique, laser processing, for their abilities to produce nano/micro-patterned polyurethane surfaces for promoting cell adhesion, migration and proliferation. We feel that polishing offers a potentially cheaper alternative to more advanced methods for developing patterned surfaces as functional coatings for implant technology.

2.0 Materials and Equipment

2.1 Preparation of stainless steel moulds

All patterns surfaces were generated upon a biocompatible polyurethane polymer (described in Section 2.4). Patterns were developed on the polymer indirectly, by casting off the flat end surface of a cylindrical stainless steel mould that had been cut from stainless steel rods (grade 316, cylinder height 13mm, diameter 18mm). To prepare the stainless steel moulds for patterning, their flat surfaces were first polished to remove all marks caused by the cutting process. This was achieved using a METASERV universal polisher and silicon carbide sheets of decreasing grit size (60 to 1200B) followed by a polishing cloth. This resulted in the stainless steel cylinder having a mirrored surface finish (mean Ra value of approximately 0.02μm) which could then be used for processing.

2.2 Laser patterned surface development

The experiments were performed using an SPI solid state pulsed fibre laser system (G3.0 20W Pulsed Fibre Laser HS Series) with a maximum mean laser power (P) of 20W, wavelength (λ) of 1064 nm and variable pulse duration (τ) of 9-200ns. The laser beam was collimated and expanded up to 10mm through a 5x beam expander. The expanded beam was then propagated to a galvanometer (scanning head, GSI Lightning), which consists of two mirrors that control the laser beam path across the work piece. A translational x-y-z table (Aerotech Inc, UK) was used to accurately position the sample at the focal position of the processing lens. The reflected beam was then focused using a 100mm focal length lens (Linos F-Theta-Ronar 1064 nm+VIS) which produced a focused spot size of 30μm in

diameter. The pattern was generated over an 8mm^2 area. The laser spot was focused onto the stainless steel and programmed to scan in a raster pattern across the surface with a line spacing of 50µm between each pass. The parameters used for processing were: laser power 4W, pulse duration 9ns, frequency 25 kHz, a processing speed of 500mms^{-1} and pass number 20. These parameters resulted in the stainless steel having a grooved surface with features on the micron scale.

2.3 Abrasive patterned surface development

Once polished to achieve an initial mirrored finish (as described above in Section 2.1) the stainless steel cylinder was then polished to achieve a topographical surface patternation through the use of abrasive paper (1200B). By controlling the motion of the stainless steel cylinders relative to the silicon carbide abrasive paper, surfaces having either directional, or random, sub-micron abrasive marks could be produced, termed here as 'nano-scratches'. The directional features are a result of the METASEV universal polisher's spinning motion, while the more random features are the result of manually rubbing the steel mould across the surface in different directions. The surface topography was subsequently characterised by white light interferometry and scanning electron microscopy. These stainless steel cylinders could then be used as master moulds to cast polymer substrates. In total three different patterns were produced; two polished surfaces including the linear polished and randomly polished as well as the single laser patterned surface.

2.4 Casting polymer substrates

Casting the polymer over the stainless steel moulds produces an inverted pattern on the polymer surface. Employing this indirect processing method ensures that only the surface topography/roughness of the material is altered and not the material chemistry. The polymer used here was polyurethane and was provided by Biomer Technology Ltd. The polymer

substrates were produced using 8% polyurethane in 2:1 Dimethylformamide (DMF) and Tetrehydrofuran (THF). This was poured onto the stainless steel moulds and cured at 60°C for 2 hours. Following this the mould/cast was allowed to cool before peeling off the polymer from the mould following the grain of the pattern. Prior to cell culture all polymer surfaces were sterilised by washing with 70% ethanol then exposing to UV light for 30 minutes. Finally, the polymers were washed with sterilised distilled H_2O.

2.5 White light interferometry for surface characterisation

A Bruker Contour GT-K 3D optical microscope equipped with Vision 64 software was used to image the surfaces of the patterned polymers. This enabled feature heights/widths and roughness to be determined. All images were taken using either ×25 or × 50 magnifications.

2.6 Cell culture

The cells described in this work are human lung fibroblast cells (LL24) which have been purchased from the European Collection of Animal Cell Cultures (ECACC) UK. Fibroblast cells were chosen because they are one of the first cells to encounter foreign implants and are important in biointegration. Also, there is extensive evidence showing that fibroblast cells respond to changes in surface topography both in vitro and *in vivo.* The LL24 cell line was chosen as it is a well characterised, stable, normal human diploid cell line. All cell culture work was carried out under aseptic conditions in a grade II laminar flow cabinet (EBSCO). Cells were maintained at 37°C in a humidified 5% CO_2/ 95% air atmosphere in Dulbecco's Modified Eagles Medium (DMEM) (Sigma-Aldrich, D6429) supplemented with 10% foetal bovine serum (Sigma-Aldrich, 0804) and 1% penicillin-streptomycin. All experiments were carried out using cells at passage number 20-24.

2.7 Cell Adhesion

One of the first responses of cells to a biomaterial is cell adhesion. Therefore in order to determine if the cells had a preference for growing upon any of the processed polymer surfaces, cell adhesion was quantified using the MTT assay. This is a colorimetric assay that relates absorbance of light at a specific wavelength to relative viable cell number. Firstly the polyurethane casts (6mm diameter) were cut using a Biopunch (SLS, UK) and placed in the wells of a 96 well plate. The polymer discs were then sterilised with UV light for 30 minutes. Cells were seeded at a density of 10,000 cells per well and left to incubate for two hours (37°C 5% CO_2). This time was chosen as empirical studies have shown these cells to fully attach to the surface within this time period. After 2 hours the growth medium was removed and the polymer discs were washed gently with PBS. Next, the MTT assay was carried out to determine cell number. Briefly MTT (0.5mg/ml) was added to each well and left to incubate for 3 hours at 37°C in a humidified 5% CO_2/95% air atmosphere. Next, the medium was removed and replaced with DMSO in order to solubilise the formazan crystals. Finally, a 96-well plate reader was used to read the absorbance at 570nm.

2.8 Cell Migration

To determine if the different surfaces affected cell migration, time-lapse imaging and subsequent cell tracking was performed over a four-hour period using a Zeiss LSM 510 confocal microscope. Briefly, the polymer surfaces were sterilised in 70% ethanol, washed in PBS then placed into 35mm cell culture dishes. Next 200,000 cell/cm^2 were seeded onto the polymer surfaces and the dish was placed into the microscope environmental chamber (S-2, PeCon GmbH, Germany). The chamber was maintained at 37°C, 5% CO_2 in a 60-70% humidified air atmosphere using a Temcontrol 37-2 and CTI-controller 3700 (PeCon GmbH, Germany). Images were taken every 15 minutes for 4 hours using a 20× Plan-Apo/0.75 NA DIC objective lens, while scanning using a Helium-Neon (HeNe) laser at 543nm. ImageJ

software (National Institute of Health, NIH) with manual tracking plugin (Institute Curie, France) was used to analyse the data produced from the time-lapse image series.

2.9 Cell Proliferation

In order to determine the effects of surface topography on cell proliferation the MTT assay was used to determine relative cell density. Cells were seeded onto 6mm sterile polymer casts (placed in 96-well plates) at a density of 10,000 cells per well and left to incubate for 24, 48, or 72 hours (37°C 5% CO_2). At each discrete time point an MTT assay was carried out as described above and absorbance related to relative cell density was determined.

2.10 Statistical analysis

All experiments were repeated 3 times. All statistical analysis was carried out using SPSS. For cell adhesion and proliferation studies, an unpaired, independent two-tailed student's t-test was used to determine whether there was a significant difference between the mean absorbance from the cells cultured on the patterned and unprocessed polymer surfaces. For the migration studies an independent two-tailed student's t-test was used to determine if there was a significant difference between the mean migration distance (μm) of the cells cultured on the patterned polymer and those cultured on the unprocessed surface. All tests were carried out using a 95% confidence limit assuming unequal variances.

3. Results & Discussion

3.1 Development of the laser processed surface

The laser processed surfaces were developed empirically, by keeping the laser power and frequency constant (4W and 25 KHz respectively) while varying the laser speed and the number of passes that the laser makes across the stainless steel surface. The resulting surfaces were then analysed microscopically to determine the level of debris (left on the surface after the laser processing) and general uniformity of feature geometry. This led to the development of an optimum set of laser processing parameters designed to give the cleanest and most uniform surface. The parameters investigated were; speed of processing (200 - 500 mm sec^{-1}, in increments of 100) and the number of passes which ranged between 3 and 10 (in increments of 1) and also between 10 and 50 (in increments of 10). Throughout the surface development process it was noted that surface pitting occurred on each surface, with the pitting effect seen to increase in frequency as the laser speed was reduced and as the pass number increased. Therefore, one of the aims was to reduce this pitting effect. By a process of varying the scan parameters and visual inspection by microscopic imaging, it was determined that using scanning rate of 500mm/s and a pass number of 20 produced surface patterns with the minimum surface debris/pitting and the most uniform features (Figure 1-left). As can be seen from figure 1 (left) the raster scanning resulted in a grooved pattern across the surface. However, 3D imaging using white light interferometry revealed that the laser processed tracks contained height features that rose and fell in an undulating pattern along the track itself (Figure 1-right).

The surface features were measured from peak to valley on both sides of the features, so as to determine the mean height. This was identified to be approximately 1.89µm (+/- 0.72, n=30). The mean width of the feature track was found to be approximately 32.2µm (+/- 0.9, n=30) which is due, in part, to the spot size of the laser and is thus a limiting factor in XY feature size. The approximate width of the structures between the laser processed tracks was found to be approximately 16.5µm (+/-0.88, n=30).

Hence, although the laser processed surfaces seem at first to be strongly directional, they do actually have a significant periodic feature in a direction that is orthogonal to the main grooved surface periodicity.

3.2 Analysis of abrasive polished surfaces

Abrasive polishing allowed the production of surfaces exhibiting surface topographies displaying either a significant degree of directionality or randomness. Figure 2 shows SEM images of the processed stainless steel.

White light interferometry of the stainless surfaces determined the mean abrasive depths to be 0.27µm (+/- 0.1, n=50) and 0.48µm (+/- 0.2, n=50) for the directional and random surfaces, respectively. This difference in mean feature depth (even though the grit size was the same) is likely a result of the manual rubbing process applying greater force applied to the stainless steel, compared to when using the METASEV universal polisher. Thus the increased pressure of the paper on the steel may have increased the depth of abrasions that were produced.

Following the casting process images were obtained of the polymer surfaces using white light interferometry (figure 3).

Analysis of these images was used to determine surface roughness parameters (Ra, Rt and Rz) for all polyurethane surfaces. As can be seen from Table 1, laser processing produced

surfaces having significantly greater surface roughness values compared to the surfaces produced by polishing (as indicated by the higher R values). Abrasive polishing in a directional manner resulted in surfaces having the least overall surface roughness.

When casting polymers from the steel surfaces the resulting surface have inverse features of the steel mould. These features are comparable in size to collagen fibrils found within the ECM {Davies, 2001 #2481} and also to those produced by more advanced techniques such as electrospinning {Heath, 2010 #2480}. Measurements of the feature height and surface roughness of the polymers compare favourably with those of the stainless steel moulds.

3.3 Cell Adhesion

In order to determine if the processed surfaces affected cell adhesion an MTT assay (colorimetric assay that relates absorbance to viable relative cell density) was carried out 2-hours post cell seeding. As can be seen from Figure 4, both the laser processed surface and the randomly polished surface were found to promote very similar levels of cell adhesion as indicated by absorbance values (absorbance values for the laser processed surface of 0.39 versus 0.4 for the polished surface, respectively). An independent students t-test found the level of cell adhesion on both surfaces to be significantly greater compared to the cells growing on the unprocessed surface ($p<0.05$).

Analysis of surface roughness properties (Table 1) found the mean surface roughness (Ra) for the laser processed surface to be almost twice that of the randomly polished surface. Similarly, the mean maximum height value (Rt) and mean maximum depth value (Rz) for the laser processed surface, were found to be greater than the polished surface by factors of 2.7 and 2.6 respectively.

The laser processed surface was produced using a directional scan pattern to try and produce a ploughed-field effect. Therefore, one might expect the Ra values to be lower compared to the random surface. However, this was not the case, as the laser surface was found to have a Ra value approximately twice of that of the randomly organised polymer surface (Table 1). Upon closer inspection of the laser processed surfaces, it becomes apparent that the processed areas are not uniform and display an undulating pattern along the processed areas (see Figure 1 - right). This seems to have resulted in the laser processed surface having a less ordered surface than may be expected and which may have contributed to the enhanced roughness and cell adhesion.

The unprocessed surface and the polished-directional surfaces were found to promote the least cell adhesion. The MTT assay revealed the absorbance values to be very similar for these two surfaces (0.23 versus 0.27, respectively), thus indicating a similar level of cell retention on these surfaces after 2 hours (Figure 4). Analysis of the surface roughness values for these two surfaces revealed them to have a similar mean surface roughness (Ra), however the values for Rt and Rz were markedly different between the two surfaces, which would be expected (i.e. greater for castings from the abrasively polished surface compared to those from the smooth blank).

As mentioned previously, studies have shown that enhancing the surface roughness can enhance cell adhesion. Clearly, this is not the case with the directionally polished surface compared to the unprocessed surface. It may be that the size of the surface topographical features was too small and the frequency of the features was too high to be recognised by the cells; which may be reflected by the similar Ra values between the unprocessed surface and the directionally polished surface. To explore this further, we prepared directionally polished surfaces using larger grit sizes than the original super fine 1200B silicon carbide abrasive paper, now ranging between the coarser 120-600 grit sizes. This resulted in significantly

larger feature sizes (although still sub-micron) and also larger values for Ra (e.g. Ra 1.2 for grit size 120 - data not shown). However, these coarser surfaces did not enhance cell adhesion when compared to polishing with the original finest 1200B grit size (cell adhesion was similar). Also, the directional polymer surfaces developed using polishing paper with a greater grit size than 1200, were found to have much greater surface roughness compared to the randomly organised polymer surface. However, they still promoted less cell adhesion than was the case for the random surface. In vivo, the ECM is randomly organised in three-dimensions {Wang, 2011 #2500} and thus has greater similarity with the randomly polished surface compared to the more uniform topography of the other surfaces. Therefore, these results suggest that for the surfaces described here, feature directionality may be more important than surface roughness in promoting cell adhesion. This suggested effect of directionally ordered surfaces having a significant effect upon cell adhesion agrees with the previous work of others. For example, Biggs et al {Biggs, 2007 #24} used electron beam lithography and a polymer injection moulding process to generate arrays of nano-pits having varying degrees of order and found that highly ordered symmetry reduced adhesion, when compared to more randomly ordered surfaces. Similarly, Curtis et al {Curtis, 2001 #25} used electron beam lithography, followed by dry etching, to generate ordered and random arrays of micro-pillars and micro-pits on fused silica and found that ordered topography reduces fibroblast cell adhesion very markedly.

3.4 Cell Proliferation

In order to compare the effects of the different surfaces upon cell proliferation an MTT test was performed after 24, 48 and 72 hours. Figure 5 shows that after 24, 48 and 72 hours, cells were found to proliferate steadily on all surfaces. After 24 hours the level of cell proliferation was similar for the unprocessed, laser processed and randomly polished surfaces, while cells growing on the directional polished surface displayed the least proliferation. Following 48

hours, cell growth was greatest on the randomly polished surface, followed by the laser processed surface, unprocessed surface and the directionally polished surface respectively. This trend was the same after 72 hours, however the differences were more pronounced (figure 5). These results mimic the cell adhesion results, in that the surfaces that produced the greatest adhesion (random-polish and laser processed) also produced the greatest level of cell proliferation. This would be expected, as one of the first biological responses of a cell to a surface is adhesion. This followed by cell flattening, elongation, migration and proliferation. Therefore, modulating cell adhesion also modulates cell proliferation. It has been reported that micro-roughness can have a negative effect on cell proliferation compared to flatter surfaces {Kim, 2005 #2495;Sader, 2005 #2496}. This was not the case here, as the laser processed surface which had characteristic micro-roughness, encouraged a greater level of adhesion and proliferation compared to the directionally processed which had nano-roughness characteristics. It should be noted however that the studies of Kim et al {Kim, 2005 #2495} and Sader et al {Sader, 2005 #2496} used a different cell type, namely osteoblast-like cells growing on treated titanium rather than polyurethane as used here, thus highlighting the response of different cells to different surface having different materials properties. The trend presented in figure 5 was found to be repeatable however; statistical analysis found the difference in the mean absorbance of the cell growing on the unprocessed surface, not to be significant when compared to that of the cells growing on the surfaces produced by laser and abrasive polishing ($p>0.05$).

3.5 Cell Migration

To compare the effects of the different surfaces on cell migration, fibroblast cells were seeded onto the various surfaces and left for 2 hours. They were then imaged every fifteen minutes over a 4-hour period. This allowed the mean migration distance to be determined for the different populations of cells (n=90 for each surface, with 30 cells tracked from 3 separate

experiments). Interestingly, those surfaces which promoted the greatest adhesion and proliferation (i.e. those with the greatest surface roughness) were found to limit the migration distance. Overall, the directionally polished surface produced the greatest mean cell migration distance, followed by the unprocessed surface, randomly polished and laser processed surface respectively (Figure 6). This basic trend was found to be repeatable. However, when compared to the unprocessed surface mean cell migration distance was found not to be statistically significant ($P<0.05$) .

Upon visual inspection of the time-lapse videos, it was observed that those cells growing on both the unprocessed (video file 1), directionally polished (video file 2) and randomly polished surfaces (video file 3) migrated in a more random manner. In contrast, those cells growing on the laser produced surface (video file 4) were found mainly to be confined to either the laser processed grooves, or to the unprocessed areas and were observed to mainly migrate either along the groove, or along the unprocessed area. In contrast, relatively few cells were seen to migrate in a direction running perpendicualr to the grooves, which may be due to the feature heights being too large for the cells to navigate across.

It should also be noted that cells growing on the laser processed surface had a different morphology than those growing on both the flat and directional polymer surfaces; exhibiting less spread. Similarly, cells growing on the randomly organised polished surface were also observed to move less freely when compared to those cells growing on both the flat and directionally polished polymer surfaces (see video files). Thus it can be concluded that the limitation imposed upon the direction of cell movement here has resulted in a reduced cell migration distance. This work agrees with similar work in this area. For example, Hamilton et al{Hamilton, 2005 #27} generated grooves on fused silica via photolithography. These grooves were designed to vary in depth from between 80nm - 9µm and vary in width from 2 - 20µm. It was found that chondrocyte cells did not spread appreciably on any groove size.

However, cells were found to show accelerated movement on grooves having a depth of 750nm when compared to flat surfaces {Hamilton, 2005 #27}. This suggests that surface feature size does indeed have an effect cell migration, specifically that submicron scale features promote the migration rate, whereas micron-sized features inhibit cell migration. Overall the laser processed surface used here, which has the largest feature size, produced the lowest level of cell migration, which would support the work of Hamilton et al.

Video files to appear here.

This work has shown that completely different methods i.e. laser processing and abrasive polishing can generate surfaces that promote cell adhesion through enhancing surface roughness. It is unclear at this stage from a biological point of view as to why cell adhesion was enhanced. However, one may speculate based on the literature. Others have shown that for cells of mesenchymal origin, an increase in surface roughness results in an increase in cell adhesion. For example, it has been shown that cell adhesion on rougher surfaces is associated with an increase in protein expression, particularly those associated with cell adhesion e.g. collagen and TGF-Beta (24). It is likely that the enhanced adhesion has resulted from an increase in the expression of proteins associated with cell adhesion i.e. those found within the focal adhesion complexes e.g. vinculin, however, more work would be needed to clarify this. Such work was beyond the scope of this research. With respect to enhanced proliferation it known that if cells are allowed to attached to a surface then they can spread more rapidly, form mature focal adhesions and proceed through the cell cycle. Given that the rougher surfaces (i.e. laser processed and abrasive polished in a random manner) promoted greater cell adhesion it is not surprising that these surfaces would also promote greater cell proliferation.

4.0 Conclusion

This paper set out to compare the use of laser processing and abrasive polishing for developing patterned polyurethane surfaces for use as cell substrates for controlling cell adhesion, migration and proliferation. The results show that abrasive polishing can be used to produce surfaces having ordered, or random, nano-scale features. The abrasive surfaces are similar to those produced by the more expensive lithography based methods, whilst laser processing can produce surfaces having micron-sized features. Both techniques can be used for controlling cell behaviour with the results summarised in table 2. In particular, both the random orientation abrasive polishing method and the laser processed surfaces were found to enhance fibroblast cell adhesion and proliferation compared to the unprocessed surface. In contrast, the directionally abrasively polished surface was found to promote a similar level of cell adhesion and migration compared to the unprocessed surface.

This work therefore presents a cost effective method (polishing) of producing functional polyurethane surfaces having directional or random nano-scale features and which may be used to enhance cell adhesion and proliferation.

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Figure Legends

Figure 1 SEM image of laser processed steel (left) and white light interferometer image (right)

Figure 2 SEM images of randomly polished (left) and linearly polished (right) stainless steel.

Figure 3 White light interferometry images of randomly polished (left) and linearly polished (right) polyurethane casts.

Figure 4 MTT assay results for cell adhesion showing absorbance for cells attached to the different surfaces. Error bars represent standard error of the mean (* denotes significance at 95% confidence limits, $P<0.05$).

Figure 5 Proliferation assay results showing mean absorbance versus time (hours). Error bars represent standard error of the mean.

Figure 6 Mean cell migration distance (μm) for LL24 cells (n=90) growing on the different surfaces over a 4-hour period. Error bars represent standard deviation.

Table Legends

Table 1 Mean surface roughness measurements (Ra, Rt and Rz) for the polymer casts generated via white light interferometry (N=5 from each surface)

Table 2 Effects of the patterned polymer surfaces on cell behaviour compared to the unprocessed polymer surface (* denotes statistical significance at 95% confidence limit, $P<0.05$)