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

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9  
10 **Abstract**

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

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

32 **Keywords: Laser processing; Abrasive polishing; Cell adhesion; Cell proliferation; Cell**  
33 **migration**

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## 46 **1 Introduction**

47 Mammalian cells have evolved to interact with their physical environment and this  
48 interaction is crucial for many important cellular behaviours including; adhesion, migration  
49 and proliferation. In vivo, cells depend on an interaction with a 3D scaffold known as the  
50 extracellular matrix (ECM). It is thought that the geometrical organisation and mechanical  
51 compliance of the ECM is extremely important in helping to regulate the aforementioned cell  
52 behaviours {Huttenlocher, 2011 #2497}. As a consequence, there has been a significant  
53 research effort that has focused on the development of cell substrates designed to have both  
54 2D and 3D surface structures that mimic the features of the ECM. This has largely been  
55 achieved through patterning materials to develop ‘functional’ or ‘smart’ surfaces that can be  
56 used to better control cellular responses in vitro.

57 Development of such surfaces has been shown to have significant impact on improving the  
58 integration of prosthetic implants. For example, modification of dental implants to alter  
59 surface roughness properties has been shown to improve implant integration {Le Guehennec,  
60 2007 #2416}. Similarly, enhancing the surface roughness of breast implants has been shown  
61 to increase the surface adhesive properties for fibroblast cells and it has been suggested that  
62 this increased cell adhesion will improve wound healing following implantation, thus limiting  
63 the risk of capsular contracture {Valencia-Lazcano, 2013 #2411}. Therefore, there is a clear  
64 benefit and need to develop such materials for use in biomedical applications.

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66 Much of the work in this area has focused on developing surfaces that have specific features  
67 with defined geometries and sizes on a range of different materials. For example micro and  
68 nano-scale grooves {Reynolds, 2012 #2391}, pillars {Ghibaud, 2009 #56} and pits {Curtis,  
69 2001 #25}. These surfaces have been shown to influence cell adhesion {WojciakStothard,  
70 1996 #11} and proliferation {Dalby, 2002 #12} and migration {Ko, 2013 #2366} of a range

71 of cell types including; fibroblast cells {Curtis, 2001 #25}, osteoblasts {Biggs, 2009 #28},  
72 endothelial cells {Koo, 2014 #2463}, epithelial cells {Andersson, 2003 #79} and neurons {Li,  
73 2015 #2470}.

74 Many methods are available for modifying topography to develop functional surfaces. One of  
75 the most widely used techniques involves the use of a template mask which is placed over the  
76 surface that is due to be processed, thus leaving a predetermined pattern, post processing.  
77 This technique is seen in lithography-based approaches including; electron beam lithography  
78 {Curtis, 2001 #25;Alaerts, 2001 #33;Karuri, 2006 #2377}, colloidal lithography {Wood,  
79 2002 #2393;Dalby, 2005 #18}, photolithography {Clark, 1990 #29;Lee, 2009 #64;Reynolds,  
80 2012 #2391}, Langmuir–Blodgett lithography {Lenhert, 2005 #58} and X-ray lithography  
81 {Karuri, 2004 #2375;Liliensiek, 2006 #2384}. Such methods are advantageous, as they allow  
82 the development of substrates having a range of well-defined geometries; however they often  
83 require expensive equipment and are generally time-consuming processes.

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

92 The aim of this work was to evaluate the use of abrasive polishing for producing nano/micro-  
93 patterned polyurethane substrates that can promote cell adhesion, migration and proliferation.

94 Any material that may be considered as a coating for biomedical applications should promote  
95 cell attachment, flattening, spreading and migration as these steps are important in

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

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## 118 **2.0 Materials and Equipment**

### 119 **2.1 Preparation of stainless steel moulds**

120 All patterns surfaces were generated upon a biocompatible polyurethane polymer (described  
121 in Section 2.4). Patterns were developed on the polymer indirectly, by casting off the flat end  
122 surface of a cylindrical stainless steel mould that had been cut from stainless steel rods (grade  
123 316, cylinder height 13mm, diameter 18mm). To prepare the stainless steel moulds for  
124 patterning, their flat surfaces were first polished to remove all marks caused by the cutting  
125 process. This was achieved using a METASERV universal polisher and silicon carbide sheets  
126 of decreasing grit size (60 to 1200B) followed by a polishing cloth. This resulted in the  
127 stainless steel cylinder having a mirrored surface finish (mean Ra value of approximately  
128 0.02 $\mu$ m) which could then be used for processing.

### 129 **2.2 Laser patterned surface development**

130 The experiments were performed using an SPI solid state pulsed fibre laser system (G3.0  
131 20W Pulsed Fibre Laser HS Series) with a maximum mean laser power (P) of 20W,  
132 wavelength ( $\lambda$ ) of 1064 nm and variable pulse duration ( $\tau$ ) of 9-200ns. The laser beam was  
133 collimated and expanded up to 10mm through a 5x beam expander. The expanded beam was  
134 then propagated to a galvanometer (scanning head, GSI Lightning), which consists of two  
135 mirrors that control the laser beam path across the work piece. A translational x-y-z table  
136 (Aerotech Inc, UK) was used to accurately position the sample at the focal position of the  
137 processing lens. The reflected beam was then focused using a 100mm focal length lens  
138 (Linos F-Theta-Ronar 1064 nm+VIS) which produced a focused spot size of 30 $\mu$ m in

139 diameter. The pattern was generated over an 8mm<sup>2</sup> area. The laser spot was focused onto the  
140 stainless steel and programmed to scan in a raster pattern across the surface with a line  
141 spacing of 50µm between each pass. The parameters used for processing were: laser power  
142 4W, pulse duration 9ns, frequency 25 kHz, a processing speed of 500mms<sup>-1</sup> and pass number  
143 20. These parameters resulted in the stainless steel having a grooved surface with features on  
144 the micron scale.

### 145 **2.3 Abrasive patterned surface development**

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

### 158 **2.4 Casting polymer substrates**

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

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

## 169 **2.5 White light interferometry for surface characterisation**

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

## 173 **2.6 Cell culture**

174 The cells described in this work are human lung fibroblast cells (LL24) which have been  
175 purchased from the European Collection of Animal Cell Cultures (ECACC) UK. Fibroblast  
176 cells were chosen because they are one of the first cells to encounter foreign implants and are  
177 important in biointegration. Also, there is extensive evidence showing that fibroblast cells  
178 respond to changes in surface topography both *in vitro* and *in vivo*. The LL24 cell line was  
179 chosen as it is a well characterised, stable, normal human diploid cell line. All cell culture  
180 work was carried out under aseptic conditions in a grade II laminar flow cabinet (EBSCO).  
181 Cells were maintained at 37°C in a humidified 5% CO<sub>2</sub>/ 95% air atmosphere in Dulbecco's  
182 Modified Eagles Medium (DMEM) (Sigma-Aldrich, D6429) supplemented with 10% foetal  
183 bovine serum (Sigma-Aldrich, 0804 ) and 1% penicillin-streptomycin. All experiments were  
184 carried out using cells at passage number 20-24.

## 185 **2.7 Cell Adhesion**

186 One of the first responses of cells to a biomaterial is cell adhesion. Therefore in order to  
187 determine if the cells had a preference for growing upon any of the processed polymer  
188 surfaces, cell adhesion was quantified using the MTT assay. This is a colorimetric assay that  
189 relates absorbance of light at a specific wavelength to relative viable cell number. Firstly the  
190 polyurethane casts (6mm diameter) were cut using a Biopunch (SLS, UK) and placed in the  
191 wells of a 96 well plate. The polymer discs were then sterilised with UV light for 30 minutes.  
192 Cells were seeded at a density of 10,000 cells per well and left to incubate for two hours  
193 (37°C 5% CO<sub>2</sub>). This time was chosen as empirical studies have shown these cells to fully  
194 attach to the surface within this time period. After 2 hours the growth medium was removed  
195 and the polymer discs were washed gently with PBS. Next, the MTT assay was carried out to  
196 determine cell number. Briefly MTT (0.5mg/ml) was added to each well and left to incubate  
197 for 3 hours at 37°C in a humidified 5% CO<sub>2</sub>/95% air atmosphere. Next, the medium was  
198 removed and replaced with DMSO in order to solubilise the formazan crystals. Finally, a 96-  
199 well plate reader was used to read the absorbance at 570nm.

## 200 **2.8 Cell Migration**

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

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

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## 213 **2.9 Cell Proliferation**

214 In order to determine the effects of surface topography on cell proliferation the MTT assay  
215 was used to determine relative cell density. Cells were seeded onto 6mm sterile polymer casts  
216 (placed in 96-well plates) at a density of 10,000 cells per well and left to incubate for 24, 48,  
217 or 72 hours (37°C 5% CO<sub>2</sub>). At each discrete time point an MTT assay was carried out as  
218 described above and absorbance related to relative cell density was determined.

## 219 **2.10 Statistical analysis**

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

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### **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<sup>-1</sup>, 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).

258 The surface features were measured from peak to valley on both sides of the features, so as to  
259 determine the mean height. This was identified to be approximately  $1.89\mu\text{m}$  ( $\pm 0.72$ ,  $n=30$ ).  
260 The mean width of the feature track was found to be approximately  $32.2\mu\text{m}$  ( $\pm 0.9$ ,  $n=30$ )  
261 which is due, in part, to the spot size of the laser and is thus a limiting factor in XY feature  
262 size. The approximate width of the structures between the laser processed tracks was found to  
263 be approximately  $16.5\mu\text{m}$  ( $\pm 0.88$ ,  $n=30$ ).

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

### 267 **3.2 Analysis of abrasive polished surfaces**

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

271 White light interferometry of the stainless surfaces determined the mean abrasive depths to be  
272  $0.27\mu\text{m}$  ( $\pm 0.1$ ,  $n=50$ ) and  $0.48\mu\text{m}$  ( $\pm 0.2$ ,  $n=50$ ) for the directional and random surfaces,  
273 respectively. This difference in mean feature depth (even though the grit size was the same) is  
274 likely a result of the manual rubbing process applying greater force applied to the stainless  
275 steel, compared to when using the METASEV universal polisher. Thus the increased pressure  
276 of the paper on the steel may have increased the depth of abrasions that were produced.

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

279 Analysis of these images was used to determine surface roughness parameters ( $R_a$ ,  $R_t$  and  $R_z$ )  
280 for all polyurethane surfaces. As can be seen from Table 1, laser processing produced

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

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

### 289 **3.3 Cell Adhesion**

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

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

303 The laser processed surface was produced using a directional scan pattern to try and produce  
304 a ploughed-field effect. Therefore, one might expect the Ra values to be lower compared to  
305 the random surface. However, this was not the case, as the laser surface was found to have a  
306 Ra value approximately twice of that of the randomly organised polymer surface (Table 1).  
307 Upon closer inspection of the laser processed surfaces, it becomes apparent that the processed  
308 areas are not uniform and display an undulating pattern along the processed areas (see Figure  
309 1 - right). This seems to have resulted in the laser processed surface having a less ordered  
310 surface than may be expected and which may have contributed to the enhanced roughness  
311 and cell adhesion.

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

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

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

### 347 **3.4 Cell Proliferation**

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

353 hours, cell growth was greatest on the randomly polished surface, followed by the laser  
354 processed surface, unprocessed surface and the directionally polished surface respectively.  
355 This trend was the same after 72 hours, however the differences were more pronounced  
356 (figure 5). These results mimic the cell adhesion results, in that the surfaces that produced the  
357 greatest adhesion (random-polish and laser processed) also produced the greatest level of cell  
358 proliferation. This would be expected, as one of the first biological responses of a cell to a  
359 surface is adhesion. This followed by cell flattening, elongation, migration and proliferation.  
360 Therefore, modulating cell adhesion also modulates cell proliferation. It has been reported  
361 that micro-roughness can have a negative effect on cell proliferation compared to flatter  
362 surfaces {Kim, 2005 #2495;Sader, 2005 #2496}. This was not the case here, as the laser  
363 processed surface which had characteristic micro-roughness, encouraged a greater level of  
364 adhesion and proliferation compared to the directionally processed which had nano-  
365 roughness characteristics. It should be noted however that the studies of Kim et al {Kim,  
366 2005 #2495} and Sader et al {Sader, 2005 #2496} used a different cell type, namely  
367 osteoblast-like cells growing on treated titanium rather than polyurethane as used here, thus  
368 highlighting the response of different cells to different surface having different materials  
369 properties. The trend presented in figure 5 was found to be repeatable however; statistical  
370 analysis found the difference in the mean absorbance of the cell growing on the unprocessed  
371 surface, not to be significant when compared to that of the cells growing on the surfaces  
372 produced by laser and abrasive polishing ( $p>0.05$ ).

### 373 **3.5 Cell Migration**

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

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

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

393 It should also be noted that cells growing on the laser processed surface had a different  
394 morphology than those growing on both the flat and directional polymer surfaces; exhibiting  
395 less spread. Similarly, cells growing on the randomly organised polished surface were also  
396 observed to move less freely when compared to those cells growing on both the flat and  
397 directionally polished polymer surfaces (see video files). Thus it can be concluded that the  
398 limitation imposed upon the direction of cell movement here has resulted in a reduced cell  
399 migration distance. This work agrees with similar work in this area. For example, Hamilton et  
400 al{Hamilton, 2005 #27} generated grooves on fused silica via photolithography. These  
401 grooves were designed to vary in depth from between 80nm - 9 $\mu$ m and vary in width from 2 -  
402 20 $\mu$ m. It was found that chondrocyte cells did not spread appreciably on any groove size.

403 However, cells were found to show accelerated movement on grooves having a depth of  
404 750nm when compared to flat surfaces {Hamilton, 2005 #27}. This suggests that surface  
405 feature size does indeed have an effect cell migration, specifically that submicron scale  
406 features promote the migration rate, whereas micron-sized features inhibit cell migration.  
407 Overall the laser processed surface used here, which has the largest feature size, produced the  
408 lowest level of cell migration, which would support the work of Hamilton et al.

409 Video files to appear here.

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

426

427 **4.0 Conclusion**

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

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

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450 **References**

- 451 1. Huttenlocher, A. and A.R. Horwitz, *Integrins in Cell Migration*. Cold Spring Harbor  
452 Perspectives in Biology, 2011. **3**(9).
- 453 2. Le Guehennec, L., et al., *Surface treatments of titanium dental implants for rapid*  
454 *osseointegration*. Dental Materials, 2007. **23**(7): p. 844-854.
- 455 3. Valencia-Lazcano, A.A., T. Alonso-Rasgado, and A. Bayat, *Characterisation of breast implant*  
456 *surfaces and correlation with fibroblast adhesion*. J Mech Behav Biomed Mater, 2013. **21**: p.  
457 133-148.
- 458 4. Reynolds, P.M., et al., *A Dual Gradient Assay for the Parametric Analysis of Cell-Surface*  
459 *Interactions*. Small, 2012. **8**(16): p. 2541-2547.
- 460 5. Ghibaudo, M., et al., *Substrate Topography Induces a Crossover from 2D to 3D Behavior in*  
461 *Fibroblast Migration*. Biophysical Journal, 2009. **97**(1): p. 357-368.
- 462 6. Curtis, A.S.G., et al., *Substratum nanotopography and the adhesion of biological cells. Are*  
463 *symmetry or regularity of nanotopography important?* Biophysical Chemistry, 2001. **94**(3): p.  
464 275-283.
- 465 7. WojciakStothard, B., et al., *Guidance and activation of murine macrophages by nanometric*  
466 *scale topography*. Experimental Cell Research, 1996. **223**(2): p. 426-435.
- 467 8. Dalby, M.J., et al., *In vitro reaction of endothelial cells to polymer demixed nanotopography*.  
468 Biomaterials, 2002. **23**(14): p. 2945-2954.
- 469 9. Ko, Y.G., C.C. Co, and C.C. Ho, *Directing cell migration in continuous microchannels by*  
470 *topographical amplification of natural directional persistence*. Biomaterials, 2013. **34**(2): p.  
471 353-360.

- 472 10. Biggs, M.J.P., et al., *The use of nanoscale topography to modulate the dynamics of adhesion*  
473 *formation in primary osteoblasts and ERK/MAPK signalling in STRO-1+enriched skeletal stem*  
474 *cells*. Biomaterials, 2009. **30**(28): p. 5094-5103.
- 475 11. Koo, S., et al., *Micro- and nanotopography with extracellular matrix coating modulate*  
476 *human corneal endothelial cell behavior*. Acta Biomater, 2014. **10**(5): p. 1975-84.
- 477 12. Andersson, A.S., et al., *The effects of continuous and discontinuous groove edges on cell*  
478 *shape and alignment*. Experimental Cell Research, 2003. **288**(1): p. 177-188.
- 479 13. Li, S.F., et al., *Microtopographical features generated by photopolymerization recruit*  
480 *RhoA/ROCK through TRPV1 to direct cell and neurite growth*. Biomaterials, 2015. **53**: p. 95-  
481 106.
- 482 14. Alaerts, J.A., et al., *Surface characterization of poly(methyl methacrylate) microgrooved for*  
483 *contact guidance of mammalian cells*. Biomaterials, 2001. **22**(12): p. 1635-1642.
- 484 15. Karuri, N.W., et al., *Nano- and microscale holes modulate cell-substrate adhesion,*  
485 *cytoskeletal organization, and -beta1 integrin localization in SV40 human corneal epithelial*  
486 *cells*. IEEE Trans Nanobioscience, 2006. **5**(4): p. 273-80.
- 487 16. Wood, M.A., D.O. Meredith, and G.R. Owen, *Steps toward a model nanotopography*. IEEE  
488 Trans Nanobioscience, 2002. **1**(4): p. 133-40.
- 489 17. Dalby, M.J., et al., *Morphological and microarray analysis of human fibroblasts cultured on*  
490 *nanocolumns produced by colloidal lithography*. Eur Cell Mater, 2005. **9**: p. 1-8; discussion 8.
- 491 18. Clark, P., et al., *Topographical control of cell behaviour: II. Multiple grooved substrata*.  
492 Development, 1990. **108**(4): p. 635-44.
- 493 19. Lee, S.W., et al., *Influence of etched microgrooves of uniform dimension on in vitro responses*  
494 *of human gingival fibroblasts*. Clin Oral Implants Res, 2009. **20**(5): p. 458-66.
- 495 20. Lenhert, S., et al., *Osteoblast alignment, elongation and migration on grooved polystyrene*  
496 *surfaces patterned by Langmuir-Blodgett lithography*. Biomaterials, 2005. **26**(5): p. 563-70.

- 497 21. Karuri, N.W., et al., *Biological length scale topography enhances cell-substratum adhesion of*  
498 *human corneal epithelial cells*. J Cell Sci, 2004. **117**(Pt 15): p. 3153-64.
- 499 22. Liliensiek, S.J., et al., *The scale of substratum topographic features modulates proliferation of*  
500 *corneal epithelial cells and corneal fibroblasts*. Journal of Biomedical Materials Research Part  
501 A, 2006. **79**(1): p. 185-92.
- 502 23. Aguilar, C.A., et al., *Direct micro-patterning of biodegradable polymers using ultraviolet and*  
503 *femtosecond lasers*. Biomaterials, 2005. **26**(36): p. 7642-7649.
- 504 24. Bolle, M. and S. Lazare, *Characterization of Submicrometer Periodic Structures Produced on*  
505 *Polymer Surfaces with Low-Fluence Ultraviolet-Laser Radiation*. Journal of Applied Physics,  
506 1993. **73**(7): p. 3516-3524.
- 507 25. Wu, L.J., et al., *Fabrication of large area two- and three-dimensional polymer photonic*  
508 *crystals using single refracting prism holographic lithography*. Applied Physics Letters, 2005.  
509 **86**(24).
- 510 26. Davies, J., *Extracellular Matrix*. 2001.
- 511 27. Heath, D.E., J.J. Lannutti, and S.L. Cooper, *Electrospun scaffold topography affects*  
512 *endothelial cell proliferation, metabolic activity, and morphology*. J Biomed Mater Res A,  
513 2010. **94**(4): p. 1195-204.
- 514 28. Wang, L. and R.L. Carrier, *Biomimetic topography: bioinspired cell culture substrates and*  
515 *scaffolds*. 2011: INTECH Open Access Publisher.
- 516 29. Biggs, M.J.P., et al., *Regulation of implant surface cell adhesion: Characterization and*  
517 *quantification of S-phase primary osteoblast adhesions on biomimetic nanoscale substrates*.  
518 Journal of Orthopaedic Research, 2007. **25**(2): p. 273-282.
- 519 30. Kim, H.J., et al., *Varying Ti-6Al-4V surface roughness induces different early morphologic and*  
520 *molecular responses in MG63 osteoblast-like cells*. Journal of Biomedical Materials Research  
521 Part A, 2005. **74A**(3): p. 366-373.

- 522 31. Sader, M.S., et al., *Effect of three distinct treatments of titanium surface on osteoblast*  
523 *attachment, proliferation, and differentiation*. Clinical Oral Implants Research, 2005. **16**(6): p.  
524 667-675.
- 525 32. Hamilton, D.W., et al., *The response of primary articular chondrocytes to micrometric surface*  
526 *topography and sulphated hyaluronic acid-based matrices*. Cell Biology International, 2005. **29**(8): p.  
527 605-615.
- 528 33. Lincks J, Boyan BD, Blanchard CR, Lohmann CH, Liu Y, Cochran DL, et al. Response of MG63  
529 osteoblast-like cells to titanium and titanium alloy is dependent on surface roughness and  
530 composition. Biomaterials. 1998;19(23):2219-32.

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### 533 **Figure Legends**

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

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

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

538 Figure 4 MTT assay results for cell adhesion showing absorbance for cells attached to the  
539 different surfaces. Error bars represent standard error of the mean (\* denotes significance at  
540 95% confidence limits,  $P < 0.05$ ).

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

543 Figure 6 Mean cell migration distance ( $\mu\text{m}$ ) for LL24 cells (n=90) growing on the different  
544 surfaces over a 4-hour period. Error bars represent standard deviation.

545 **Table Legends**

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

548

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

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