JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 6 (1995) 813–817

Laser surface modification of polymers to improve biocompatibility

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The effect on neutrophil chemokinesis and fibroblast adhesion of changing surface topography was examined using two polymeric substrates; polycarbonate and polyetherimide, modified by laser treatment to produce pillars of varying dimensions on the surfaces of these materials. The dimensions for the pillars were 7, 25 or 50 μm square, 0.5, 1.5 or 2.5 μm deep. Human neutrophils were isolated, by centrifugation, on ficoll from heparinized whole blood obtained from healthy volunteers. Isolated neutrophils were exposed to the surfaces for 20 min and tracked using image processing and analysis techniques. The mean speed for each cell on each surface was calculated and this data statistically analysed using multivariate analysis of variance to determine any significant effect on speed of movement due to the surface topography. Compared to the potent stimulator FMLP all surfaces did not stimulate significant cell movement, but within the groups some surfaces had more effect on cell movement than others, and were stimulating cells to move faster than on the same untextured surface. Surface topography can stimulate neutrophils to move at different speeds across a surface. L929 fibroblasts were incubated on the surfaces for 48 h and then examined using scanning electron microscopy to study fibroblast position and adhesion with respect to the pillars. No pattern of orientation with respect to the pillars were observed and fibroblasts spread and elongated whether in contact with the pillars or on a smooth area of the material.

1. Introduction

When a biomaterial contacts tissue fluid or blood it is the surface of the material that comes into contact with the physiological environment. Many implantable biomaterials are constructed using polymeric materials and the biocompatibility of these materials is critical to their satisfactory performance. The first physiological process that occurs within the initial stages of exposure is the adsorption of biomolecules onto the surface and this is usually followed by cellular interactions. Both the surface topography and the surface chemistry can significantly affect the type and intensity of these interactions. As a result a considerable amount of research has been devoted to surface modification of biomaterials [1–14]. The aim of this work was to assess the possibilities of using laser treatment on two polymers in order to generate topographic modifications to modify the cellular response.

2. Materials and methods

2.1. Materials

Two polymeric substrates were used for this work. These included polycarbonate (PC, Lexan) and polyetherimide (PEI, ULTEM), injection moulded into plaques of 150 × 150 × 3 mm. These plaques were cut into 100 × 25 mm sizes for laser treatment.

2.2. Laser surface modification

A KrF (wave length = 248 nm) laser (Lambda physik EM6 201) was used for surface modification. The conditions used for laser treatment of PC and PEI are shown in Table I. In order to produce different surface morphologies a masking technique was employed using meshes of various sizes as shown in Table I. The polymeric substrate was covered with the mesh and the sample was then exposed to KrF laser with a range of pulses varying from 1–10. Each laser pulse produced a crater of approximately 0.5 μm deep. The KrF laser created microtextures of 7, 25 and 50 μm square pillar with heights of 0.5, 1.5 or 2.5 μm.

2.3. Neutrophil isolation

Neutrophils were isolated from fresh heparinized human blood obtained from healthy volunteers and mixed 2 volumes blood with 1 volume 6% dextran. After 25–30 min, the supernatant was layered on to lymphocyte separation mixture (Ficoll-Hypaque,

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TABLE I Conditions used for excimer laser treatment of PC and PEI samples

Condition no.	Material	Mask size (μm)	Number of pulses
1	PC	50	1
2	PC	50	3
3	PC	50	5
4	PC	7	1
5	PC	7	3
6	PC	7	5
7	PC	25	1
8	PC	7	10
9	PEI	7	1
10	PEI	7	3
11	PEI	7	5
12	PEI	25	1
13	PEI	50	1
Untreated	PC	–	–
Untreated	PEI	–	–

Pharamcia, UK) and centrifuged at 1200 r.p.m. for 25 min. The pellet was transferred to a fresh conical bottomed test tube and washed once in HBSS-MOPS (hanks balanced salt solution–morpholinopropane sulphonic acids). The remaining red blood cells were lysed by resuspending the pellet in 1 ml sterile distilled water for 30 s, after which 10 ml HBSS-MOPS was added. This provided a population of 95% neutrophils [15].

2.4. Neutrophil cell tracking

Isolated neutrophils (1×10^6 cells/ml) were placed inside a small dish (the centre dish of an organ culture dish machined out) then placed on the test surface. The dish was held in place by surface tension, eventually forming a tight seal with the sample due to evaporation of excess buffer. The sample and chamber were placed on a slide and positioned on the stage of a microscope that was equipped with a closed environment incubator at 37 °C. Neutrophils were left to settle for 5 min then an area of sample with cells was observed for 20 min. Within this time period images of the cells were taken using a Hitachi KP140 black and white video camera connected to a Perceptics Instruments frame grabber card (Improvision, Coventry, UK) inside a Macintosh IIfx and stored on optical disc for assessment by image analysis. This procedure was repeated for all surfaces with the two different substrates. FMLP 10^{-8} M (N-formyl-methionine-leucine-phenylalanine), 10% FCS (foetal calf serum) were used as the positive controls and PBS (phosphate buffered saline) was used as the negative control. Cell tracking, image processing and analysis of the stored images was then performed by PRISM software (Analytical Vision Inc, Raleigh, NC, USA) using in house routines written specifically to return data on neutrophil speed and direction. The speed of the cells was compared across samples using a Waller Duncan MANOVA (multivariate analysis of variance) for unbalanced data sets, cross correlating all samples (SAS, ver6.04, SAS Institute Inc, USA).

2.5. Fibroblast *in vitro* culture

Mouse L929 fibroblasts were incubated with the materials at 37 °C, 5%. CO_2 for 48 h in 199 culture medium (Gibco, UK) supplemented with 5% foetal calf serum and antibiotics. After this incubation the cells were fixed with 2% glutaraldehyde for 10 min. The fixed cells were then dehydrated through graded alcohols (70%, 80% and 90%). Finally the samples were adhered to SEM stubs and sputter coated with gold, then examined using Jeol JSM-35C scanning electron microscope.

3. Results and discussion

20 surface/substrate modifications were analysed. The abbreviations are as follows: material PC or PEI (polycarbonate or polyetherimide). Size of pillars 7, 25 or 50. Number of pulses i.e. height 1, 3 or 5 (1 pulse = 0.5 μm) H or P for HSA present or absent respectively. e.g. Sample PC7-3H would be polycarbonate 7 μm pillars, 1.5 μm high in HSA.

None of the samples examined stimulated neutrophils to change shape and move with respect to the weak positive controls of 50:50 and 75:25 FCS/PBS (Tables II and III). Both weak positives stimulated significantly quicker movement than all the laser treated samples. No orientated movement with respect to the edges, corners or grooves in the surfaces were observed. Neutrophils stimulated with FMLP (potent chemotactic stimulator, positive control) in a medium containing HSA on a glass slide moved at a speed of 24.9 μm/min, in real terms therefore the neutrophils on these surfaces had remained stationary. However taken as a group, statistically (Table III) the samples differ in their degree of non-movement. This may demonstrate some effects due to change in surface substrate and the change in surface topography.

TABLE II Neutrophil speed

Sample	Number of cells tracked	Mean speed ± SD
FMLP GLASS	66	24.9 ± 11.0
50/50 FCS/PBS	18	2.2 ± 0.8
75/25 FCS/PBS	24	1.9 ± 0.8
PBS GLASS	51	0.4 ± 0.2
PBS contH	24	0.3 ± 0.2
PBS contP	22	0.2 ± 0.3
PC50-3H	18	1.3 ± 1.0
PC50-3P	18	0.3 ± 0.1
PC50-5H	15	0.4 ± 0.2
PC50-5P	20	0.2 ± 0.1
PC7-1H	10	1.1 ± 0.4
PC7-1P	14	0.7 ± 0.3
PC7-3H	25	0.6 ± 0.3
PC7-3P	20	0.3 ± 0.2
PEI50-1H	20	0.8 ± 0.7
PEI50-1P	13	0.1 ± 0.0
PEI7-5H	15	0.5 ± 0.1
PEI7-5P	17	0.1 ± 0.1
PEI7-3H	19	0.5 ± 0.2
PEI7-3P	16	0.5 ± 0.5
PEI25-1H	9	0.5 ± 0.4
PEI25-1P	23	0.5 ± 0.5

TABLE III MANOVA General linear models procedure

Sample	Duncan grouping	Mean (speed μm/min)
50/50 FCS/PBS	A	2.195
	A	
75/25 FCS/PBS	A	1.899
PC50-3H	B	1.334
	B	
PC7-1H	C B	1.081
	C	
PEI50-1H	C D	0.848
	C D	
PC7-1P	C D E	0.756
	D E	
PC7-3H	F D E	0.610
	F D E	
PEI7-5H	F G D E	0.551
	F G D E	
PEI7-3H	F G D E	0.534
	F G D E	
PEI7-3P	F G D E	0.513
	F G E	
PEI25-1P	F G H E	0.471
	F G H E	
PEI25-1H	I F G H E	0.459
	I F G H E	
PC50-5H	I F G H E	0.457
	I F G H	
PCPBScontH	I F G H	0.305
	I F G H	
PC7-3P	I F G H	0.285
	I F G H	
PC50-3P	I F G H	0.275
	I G H	
PC50-5P	I G H	0.205
	I G H	
PCPBScontP	I G H	0.197
	I H	
PEI7-5P	I H	0.137
	I	
PEI50-1P	I	0.0093

Duncan's Multiple Range Test for variable: Speed (μm/min)
Alpha = 0.05 df = 340 MSE = 0.212324
Harmonic Mean of cell sizes = 16.72683
Means with the same letter are not significantly different

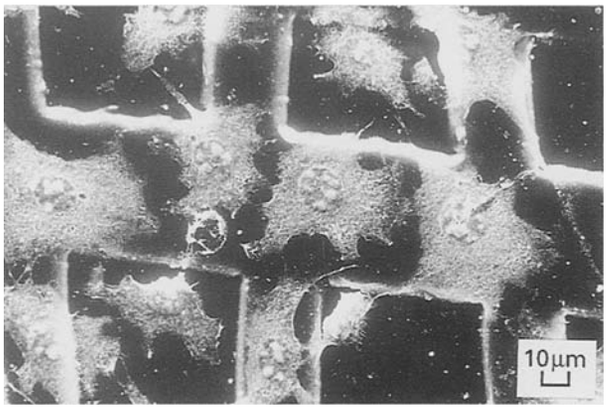

Figure 1 SEM of fibroblasts on PC 50 μm pillars with 5 pulses (2.5 μm deep).

SEM and confocal micrographs from some of the laser treated surfaces with fibroblast cultures are shown in Figs 1–11. Fig. 1 shows that the 50 μm grid with 5 pulses allows the cells to spread. The 7 μm grid with 3 pulses (Figs 2–5) cause the cells to spread and elongate. Figs 6 and 7 demonstrate that the cells spread on the 7 μm grid sample away from the treated surface. Figs 8 and 9 are on the 7 μm grid with 1 pulse, the grid markings are very indistinct and it can be observed that cells spread and were still elongated. Figs 10 and 11 show two typical confocal micrographs. There was again no clear distinction between the cellular responses.

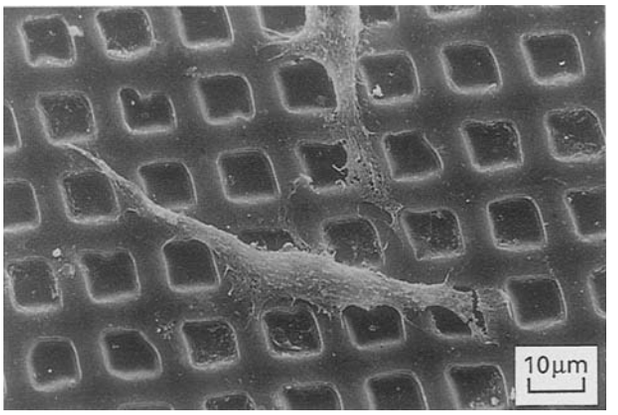

Figure 2 SEM of fibroblasts on PC 7 μm pillars with 3 pulses (1.5 μm deep).

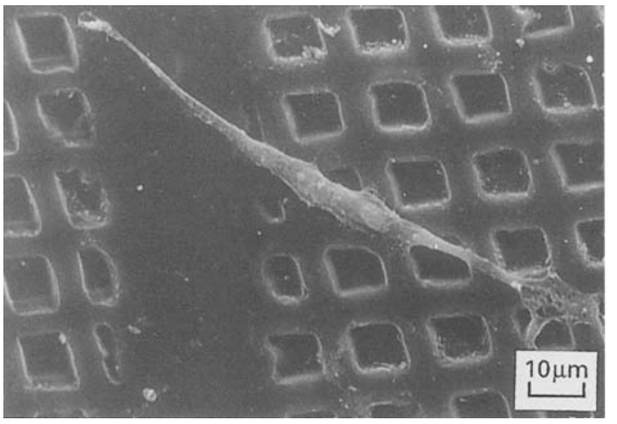

Figure 3 SEM of fibroblasts on PC 7 μm pillars with 3 pulses (1.5 μm deep).

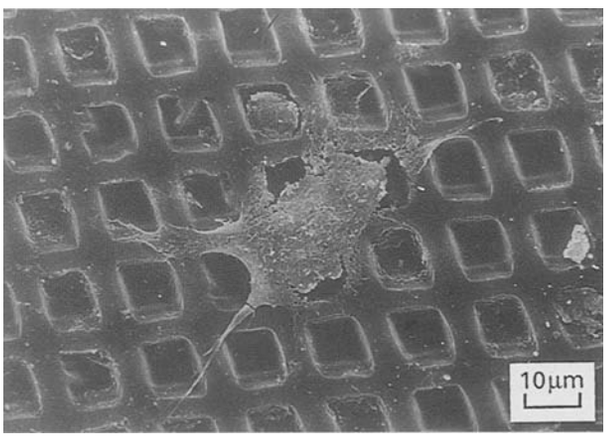

Figure 4 SEM of fibroblasts on PC 7 μm pillars with 3 pulses (1.5 μm deep).

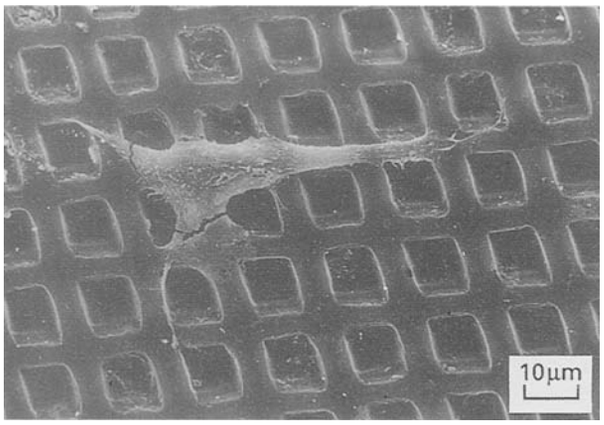

Figure 5 SEM of fibroblasts on PC 7 μm pillars with 3 pulses (1.5 μm deep).

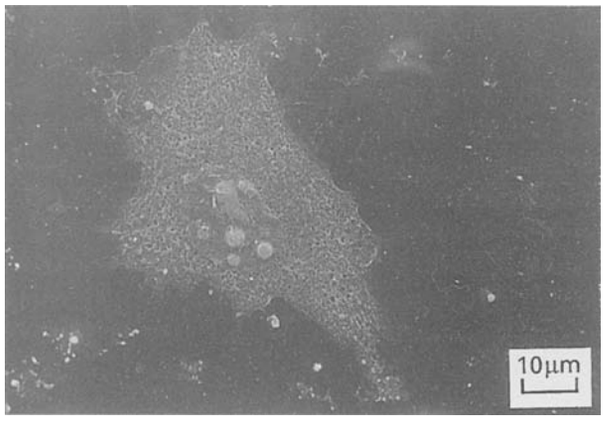

Figure 6 SEM of fibroblasts on PEI 7 μm pillars with 5 pulses (2.5 μm deep).

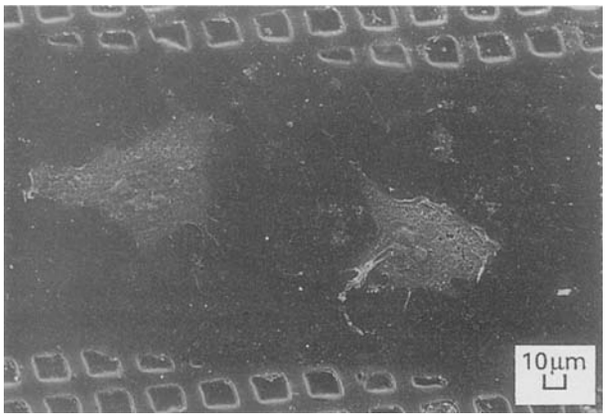

Figure 7 SEM of fibroblasts on PEI 7 μm pillars with 5 pulses (2.5 μm deep).

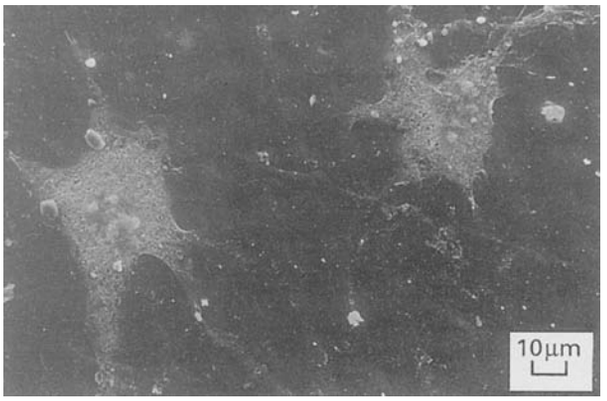

Figure 8 SEM of fibroblasts on PEI 7 μm pillars with 1 pulse (0.5 μm deep).

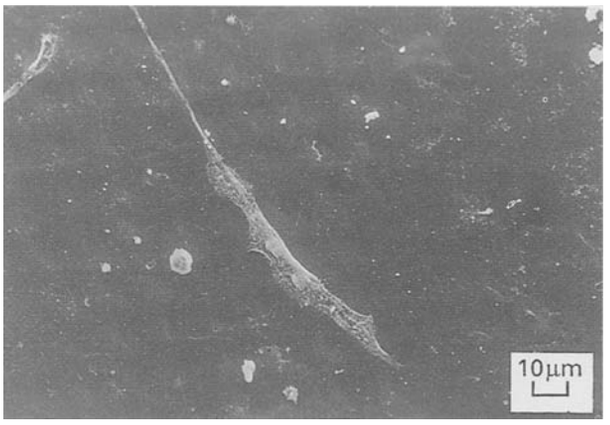

Figure 9 SEM of fibroblasts on PEI 7 μm pillars with 1 pulse (0.5 μm deep).

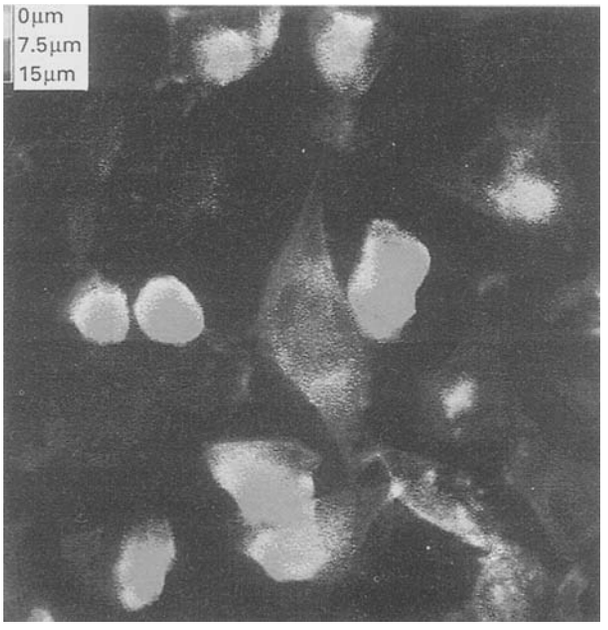

Figure 10 Confocal image of fibroblasts on PC 50 μm pillars with 5 pulses (2.5 μm deep).

4. Conclusions

None of the surface microtextures produced by the laser treatment stimulated comparable neutrophil chemotaxis when compared to the potent chemotactic stimulant FMLP. There was a range of speed of movement when the surfaces were compared against each other and the negative control PBS. The low level of stimulation of the neutrophils by these surface treatments could be because the pillars present too much untextured surface, i.e. not enough edges. Further work is planned to create a laser treated surface with more edges in contact with the neutrophils. The results with the fibroblasts demonstrate that the poly-

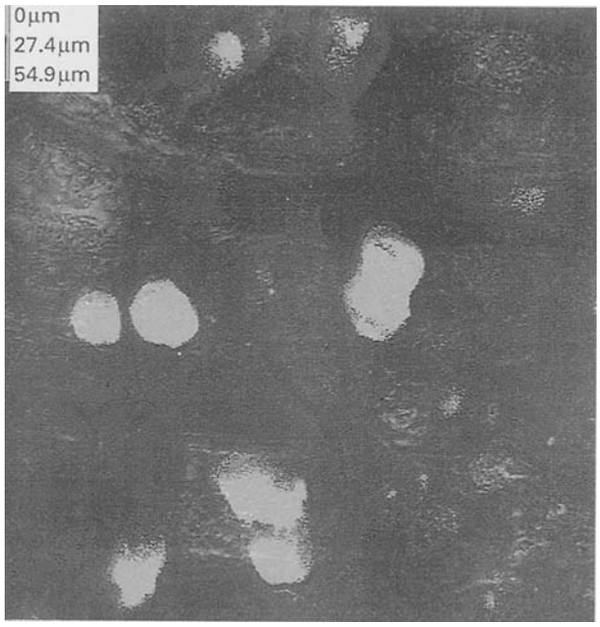

Figure 11 Confocal image of fibroblasts on PC 50 μm pillars with 5 pulses (2.5 μm deep), with material topography visible from reflection microscopy.

mer surfaces showed good cytocompatibility and seemed to confirm the lack of cell recognition of the surface microtexture. Further work is planned to attempt to create surfaces which do influence the cellular interactions.

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Received 29 June
and accepted 4 July 1995