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A qualitative *in vitro* evaluation of the degradable materials poly(caprolactone), poly(hydroxybutyrate) and a poly(hydroxybutyrate)-(hydroxyvalerate) copolymer

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A qualitative *in vitro* evaluation of poly(caprolactone) (PCL), poly(hydroxybutyrate) (PHB) and a poly(hydroxybutyrate)-(hydroxyvalerate) (PHB-PHV) copolymer was carried out using primary human osteoblasts (HOB) and a human osteosarcoma (HOS) cell line. The cells were grown on films of these polymers and cultured for 2 and 4 days with cells grown on Thermanox as a control. The cells on each of the polymers exhibited different cellular morphologies with different rates of cell proliferation. Results from a preliminary degradation study demonstrated that biodegradable materials can be partially degraded using enzymes such as papain and trypsin. Of the solutions tested, papain caused the greatest degradation, with phosphate-buffered saline (PBS) a physiological buffer having very little effect over a six week period. The bone cells were grown on partially degraded polymers and no differences in the performance of HOS and HOB cells on the materials were observed.

1. Introduction

There has been increasing interest in degradable polymer systems for use in biomedical applications such as drug delivery [1–6], fracture repair [7–10], bone and cartilage remodelling [11–14] and soft tissue implants [15]. Degradable materials have certain advantages that make them desirable for orthopaedic use. Their degradation rates and tensile strengths can be controlled by varying their molecular weights [16–18] and, for copolymers, varying the ratio of the components can also dramatically affect their degradation rates [16, 18, 19].

These materials have been poorly characterized using *in vitro* methods which have simply involved the assessment of fibroblast and some osteoblast and hepatocyte growth on them [20–25]. There has been considerable work done with degradable polymers *in vivo* [7–14] but the mechanisms of cell attachment and proliferation on these polymers has not been investigated. Moreover, the effect of the cellular activity on the degradation of the polymer and the effect of the degradation products on the cells are not well understood.

The effect of degradative enzymes on PHB and PCL was studied to gain information on the degradation characteristics of the materials in order to develop methods to artificially degrade the polymers prior to cell culture. The cellular response of primary human osteoblasts and a human osteosarcoma cell line cultured on the polymers was investigated to produce a system where a polymer can be partially degraded in order to study the effect of the degradation products on the cells and the effect of surface changes caused by the degrading polymer on cell adherence and proliferation.

2. Materials and methods

2.1. Polymer formulation

A 3% solution of PHB (ICI) and PHB–PHV (ICI, PHV content 7%) was made in chloroform and dissolved by refluxing at 70 °C for 4 h. A 7% solution of polycaprolactone (Aldrich) was dissolved in chloroform at 37 °C and did not require refluxing. The solutions were cast on to glass slides and dried under glass petri dishes overnight. These were further allowed to stand in an open container for 7–10 days to allow the chloroform to evaporate.

2.2. Degradation studies

The dried polymers were cut into 1 cm × 1 cm squares and incubated in the following solutions at 37 °C: PBS (PBS, Oxoid); trypsin (Sigma, 0.2% solution in PBS) and papain (Sigma, 0.2% in PBS). The films were exposed to constant roller mixing at 37 °C and at various time points the films were removed, washed in distilled water and allowed to dry in air. These were

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sputter coated with gold and viewed under a scanning electron microscope (SEM). The partially degraded polymers were compared to the "as cast" films.

2.3. Cell culture methods

Both the primary human osteoblasts (HOB) and a commercial human osteosarcoma cell line (HOS), TE-85, ECACC No (87070202) were used at passages 10–15 for the experiments in order to compare the performance of the two cell types on the various materials. The cells were grown in Dulbecco's Modified Eagles Medium (DMEM, Gibco) supplemented with 10% FCS (Gibco), 0.02 M HEPES (Gibco), 2 mM L-Glutamine (Gibco), 150 μg/ml Ascorbate and 1% Penicillin/Streptomycin (Gibco). The HOBS were isolated from femoral heads obtained from patients undergoing surgery for total joint replacement. Trabecular bone fragments were removed, washed in PBS and incubated in supplemented DMEM as above for a period of 4–5 days. The fragments were then digested using collagenase (1000 u/ml in PBS) and trypsin (0.02% in PBS) for 20 min after which the solution was centrifuged and the cell pellet washed twice in complete medium, resuspended and seeded out at an appropriate density. Both HOS and HOB cells were grown to confluency at 37 °C with 5% CO_2 and then removed from the tissue plastic surface using trypsin (0.02% in PBS and HEPES). Both were resuspended in

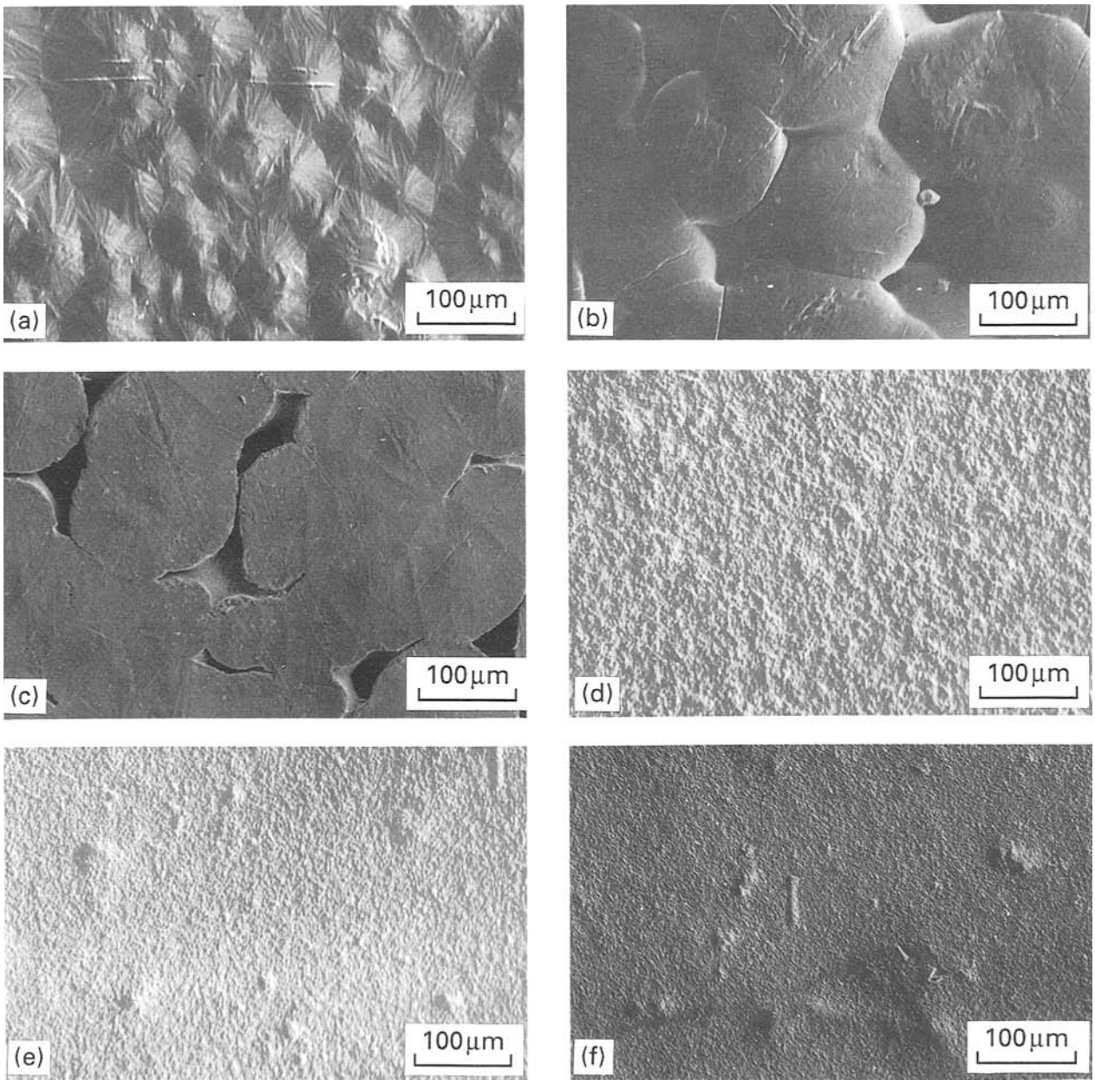

Figure 1 Scanning electron micrographs of the untreated surface of PHB and PCL and surfaces of the polymers after incubation in trypsin and papain solutions over a six week period. (a) PCL untreated; the surface of the polymer is irregular due to the spherulites which extend outwards and vary in size. (b) PCL in trypsin; the spherulites have become enlarged and smooth with small gaps appearing in between the enlarged structures. (c) PCL in papain; the spherulites have been degraded down to a smooth flat surface with large gaps appearing in between the flat surfaces. (d) PHB untreated; the structure is granular with the presence of tiny nodules. (e) PHB in trypsin; the overall granular structure remains, with fewer nodules being present. (f) PHB in papain; the surface appears "smoother" as compared to the untreated PHB but the overall structure remains intact.

cell types to give a final cell concentration of 8 $\times 10^4$/ml.

3. Results

The dry surface of PCL consisted of spherulites which ranged in size from 50–100 μm in diameter (Fig. 1a). Upon incubation with trypsin the spherulites became enlarged, possibly due to water absorption, leading to a smoother surface with the presence of small holes in between the spherulites (Fig. 1b). The effect of papain was even more marked with the spherulites appearing flattened and large holes occurring in between the flat surfaces (Fig. 1c). The effect on PHB by trypsin and papain was less dramatic with very few surface structure changes observed. Both trypsin (Fig. 1e) and papain (Fig. 1f) did not appear to affect the surface of PHB and at most there was a slight smoothing of the granular surface of PHB. HOBs grown on Thermanox after 2 days in culture appeared spread out and exhibited normal cellular morphology (Fig. 2a). At day 4 (Fig. 2b) the cells were nearing confluence due to cell proliferation. On PHB at day 2 (Fig. 2c) and at day 4 (Fig. 2d) the cells were less prevalent compared to the Thermanox control and appeared to aggregate

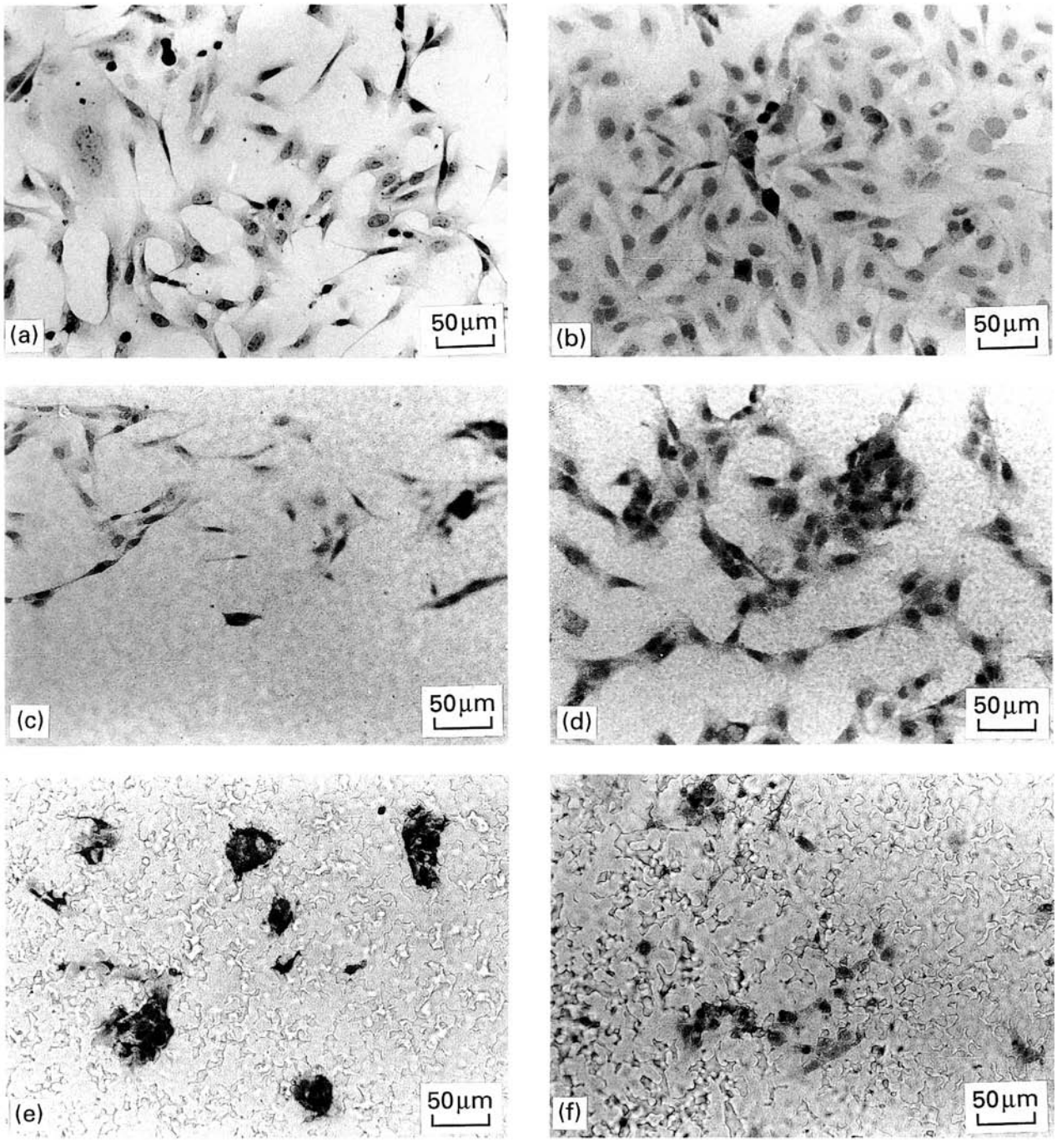

Figure 2 Light micrographs of HOBs on the surfaces of Thermanox, PHB and PHB-PHV copolymer at day 2 and day 4 in culture. (a) On Thermanox at day 2; the cells are viable and subconfluent. (b) On Thermanox at day 4; the cells are almost confluent and retain a normal morphology. (c) On PHB at day 2; the HOBs on the PHB are sparse with very little of the surface area covered. (d) On PHB at day 4; the cells have divided and a larger surface area has been covered by the cells. (e) On PHB–PHV at day 2; the cells are clumped and very few have adhered to the polymer. (f) On PHB–PHV at day 4; the cell clumps have become disaggregated and appear to be invading into the gaps on the polymer surface.

in one area first and then spread outward. The individual cellular morphology appeared normal although the proliferation rate did not appear to be as rapid as that for the control. Cells on PHB-PHV at day 2 (Fig. 2e) were present in clumps which by day 4 (Fig. 2f) had disaggregated but there did not seem to be an increase in cell number. However on PHB-PHV the cells appeared to be invading the holes present on the polymer surface: this will be further investigated using transmission electron microscopy.

4. Discussion

Degradation of polymers is affected by their molecular weights [18, 19, 26], copolymer ratios [18, 25–27], methods of sterilization and formulation [26], crystallinity [28], porosity [29] and their environment [30–32]. In the assessment of biocompatibility of degradable materials it is necessary to examine cellular performance on both the "as cast" surfaces of the materials and partially degraded materials because, during degradation, structural and chemical changes occur within the materials. The effects of these changes on cellular behaviour are not well understood. Williams has demonstrated the effects of hydrolytic enzyme activity on groups of degradable polyesters [33, 34] and more stable polymers [35, 36]. Our results show that there was a significant change in the surface properties of PCL after enzymatic degradation while the only effect of the enzyme solutions on PHB was a slight smoothing of the surfaces. This result supports previous studies which have shown PHB degrades slowly *in vivo* [37].

The results from the cell culture studies show that, although cell attachment and proliferation on the polymers does occur, the extent varies for each polymer type. On Thermanox controls the cells were seen to be more widespread and covered a larger surface area by day 4 compared to cells on either the PHB or PHB-PHV. The morphology of the cells was also different on each of the polymers, with cells on the PHB being more spread out than cells on PHB-PHV which formed clumps. These differences could possibly be explained by the difference in the surface properties of the polymers. Further work involving growth of cells on these polymers which are at different stages of degradation will be carried out and these results should provide information about cell material interactions during polymer degradation.

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