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Research Article

Biomimetic Calcium Phosphate Coatings for Polymeric Artificial Spinal Disc Implants

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Abstract Discs of poly carbonate urethane (PCU) were coated with a thin layer of calcium phosphate (CaP) by a biomimetic process at temperatures of 28°C and 37°C. The coating morphology was analyzed by SEM. Contact angle analysis was used to assess surface wettability. Human osteoblasts (HOBs) were cultured on uncoated and CaP-coated PCU discs for up to 14 days. Cell metabolism, structure, and morphology were analyzed and compared with the wettability of each sample group. HOBs proliferated and grew on uncoated and CaP-coated samples, but a greater cell metabolism was observed for uncoated samples at early time points in cell culture. A more even dispersion of HOBs across the disc surface, attributable to the increased surface wettability, was seen for CaP-coated samples.

Keywords calcium phosphate coating(s); CaP; biomimetic; poly carbonate urethane (PCU); apatite; octacalcium phosphate (OCP); human osteoblasts (HOBs); *in vitro*; wettability

1 Introduction

Artificial total spinal disc replacement devices have been used clinically for over fifteen years to treat damaged or herniated intervertebral discs [5]. Even with careful positioning during surgery, artificial spinal disc devices require effective anchoring to ensure that they are not able to migrate from the disc space [5]. Mechanical stabilization of the implant is paramount; however, further steps can be taken to improve the anchoring. Bone ongrowth provides an enhanced level of attachment between artificial disc devices and adjacent vertebrae. In order to facilitate and encourage bone ongrowth, calcium phosphate coatings can be applied to the superior and inferior endplate surfaces of artificial disc devices [5]. For new, innovative polymeric disc devices without metal endplates, high temperature methods of applying calcium phosphate coatings can result in substantial damage to the polymeric substrate during coating and thus are not feasible.

In this study, the application of calcium phosphate coatings to poly carbonate urethane (PCU) endplate material by a biomimetic coating method was explored. The chemical and structural properties of the substrate and calcium phosphate (CaP) coatings were analyzed, and osteoblast cell culture was performed to examine the behavior of bone cells on CaP-coated and uncoated substrates.

2 Materials and methods

Microtextured poly carbonate urethane (PCU) discs of thickness 1 mm and diameter 10 mm were produced by a patented molding technique (Ranier Technology Ltd., Cambridge). PCU sheets were coated in a concentrated coating solution, similar to a concentrated form of simulated body fluid (SBF), by a biomimetic process adapted from the work of Kokubo et al. [1,4] and Tas and Bhaduri [7]. Samples were coated in solution for 5 hours at two different temperatures, 28°C and 37°C, before being washed three times with deionized water and dried at 80°C. The different sample groups are identified in Table 1.

A JEOL 5800-LV scanning electron microscope (SEM) operating at an accelerating voltage of 10–15 kV was used

Sample label	Preparation
Uncoated	Uncoated PCU
T37	PCU coated at 37°C
T28	PCU coated at 28°C

Table 1: Labeling of sample groups.

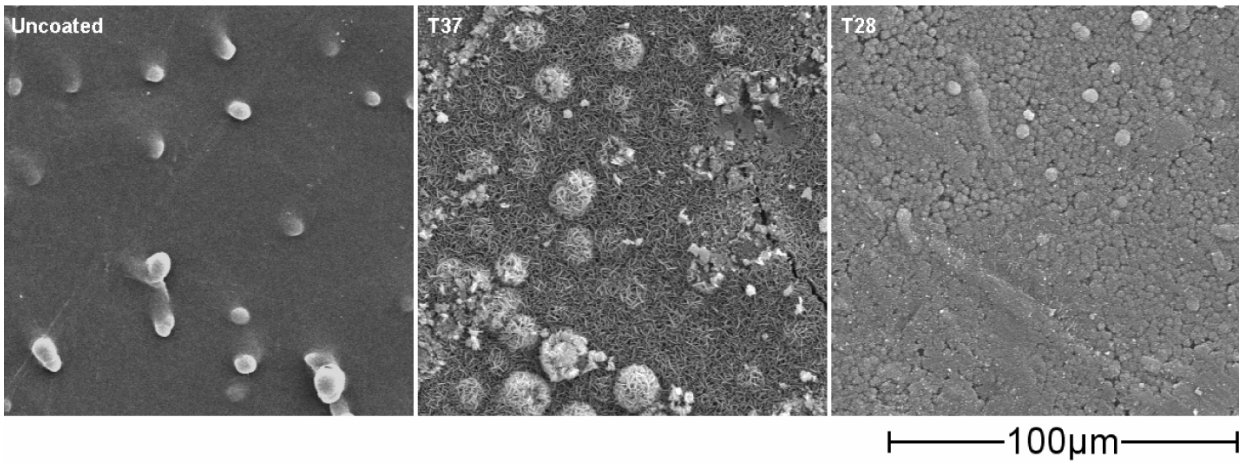

Figure 1: SEM surface micrographs (L to R): Uncoated, T37, and T28.

to study the surface morphology. Samples were coated prior to SEM analysis with a thin layer of palladium.

A CAM 200 optical contact angle and surface tension goniometer (KSV Instruments) were used to assess surface wettability. A 1.0 μL drop of deionized water was dropped onto the surface of each sample in order to measure the static contact angle. At least five measurements were made on at least two different samples for each sample group. Previous studies have shown that wettable surfaces, that is, surfaces with narrow acute contact angles, demonstrate enhanced protein adsorption and, as a result, improved cell attachment [3,6].

Human osteoblasts (HOBs) were extracted by enzymatic digestion of trabecular bone from femoral heads donated by hip replacement patients following informed consent (Orthopaedic Research Unit, Cambridge) and cultured in McCoy's 5A medium (GIBCO, Invitrogen) containing 10% fetal bovine serum (GIBCO, Invitrogen), 1% penicillin-streptomycin-glutamine (Invitrogen), and 30 μg/mL L-ascorbic acid phosphate Mg salt n-hydrate (Wako Chemicals GmbH). Disc samples were prepared for *in vitro* cell culture by ultrasonication in deionized water for 15 seconds, followed by a 20 minute wash in 70% ethanol and a further 20 minute wash in 100% ethanol.

HOBs were seeded on disc samples at concentrations of approximately 15,000 cells/cm^2. Samples were incubated at 37°C and 5% CO_2 in air throughout the study. Cell proliferation was measured by using alamarBlue^{TM} assay (Invitrogen) at days 1, 4, 7, and 14. AlamarBlue^{TM} solution was diluted to 10% with culture medium; 1 mL of this solution was put in each sample well (six per sample group) and in one blank well. Samples were incubated for four hours before their fluorescence was measured, in triplicate, with a fluorometer (Fluostar, BMG Labtech) at 590 nm wavelength. To study cell morphology in SEM, HOBs were fixed in 4% glutaraldehyde and critical-point dried at days 1, 4, 7, and 14. Confocal light microscopy was used to examine cell cytoskeletons and nuclei. For this, HOBs were fixed with 4% paraformaldehyde and stained with Texas red phalloidin-TRITC for the actin cytoskeleton and DAPI (Vectashield) for the nuclear DNA.

3 Results and discussion

Representative micrographs of the surface microstructure of each of the three sample groups are shown in Figure 1. The asperities on the surface of the uncoated substrate were from the microtexture applied during molding of the polymer. The other sample groups show contrasting calcium phosphate coating morphologies; T37 samples have a coating microstructure representative of octacalcium phosphate [2], while apatite crystals can be seen on the surface of T28 samples.

The surface wettability measurements are shown in Figure 2 and Table 2.

The uncoated substrate is significantly more hydrophobic than either of the CaP-coated substrates. T37 and T28 samples were both hydrophilic; however, the T37 samples showed a significantly higher hydrophilicity, which is likely to be attributable to both greater coating thickness and different coating morphology.

AlamarBlue^{TM} assay provides a measure of the metabolic activity of cells, which can be used to assess cell proliferation. The alamarBlue^{TM} results are shown in

Sample group	Contact angle	Surface area covered
Uncoated	$88.2° \pm 5.0°$	30.3 mm^2
T37	$10.1° \pm 3.2°$	78.5 mm^2 (full disc area)
T28	$35.0° \pm 9.4°$	75.3 mm^2

Table 2: Contact angle measurements.

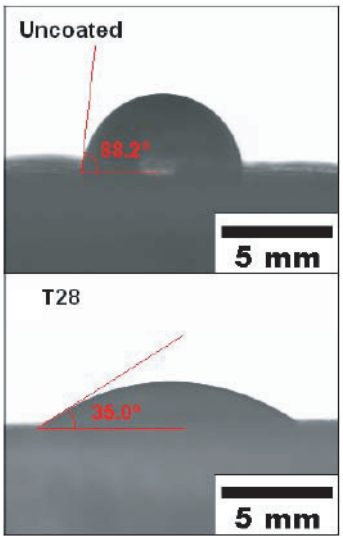

Figure 2: Photographs of 1.0 μL drops of deionized water on sample surfaces.

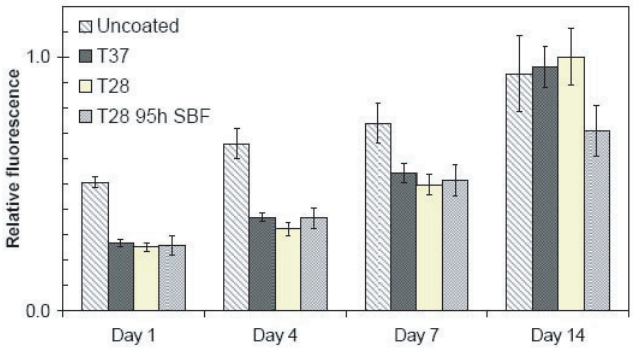

Figure 3: AlamarBlue™ results showing levels of cell metabolism. T28 95h SBF samples were soaked in 1x SBF for 95 hours & washed in deionized water before cell culture.

Figure 3. Human osteoblasts (HOBs) seeded on uncoated samples showed a heightened cell metabolism at days 1, 4, and 7, in comparison to HOBs seeded on CaP-coated samples. However, by day 14, the measured cell metabolism of HOBs on T37 and T28 samples was at least as great as the cell metabolism of HOBs on uncoated samples. A further test showed that the soaking of samples for 95 hours in 1x SBF prior to cell culture did not improve the response of HOBs to the surface, despite increasing the wettability of the surface, shown in Figure 3 as 'T28 95h SBF'.

For CaP-coated samples, it is possible that regions of the coating dissolved over time, and thus HOBs attached to these regions would be released. With fewer cells attached to the surface, the measured cell metabolism would be lower. Samples soaked for 95 hours in SBF had thicker, more soluble coatings, which would further reduce the measured cell metabolism.

The wettability provides further explanation for differences in cell metabolism between CaP-coated and uncoated PCU samples at days 1, 4, and 7; because the drop of culture medium containing HOBs spreads out considerably less on a hydrophobic uncoated sample, the cell seeding density on the surface of the sample is greatly increased. With the HOBs in closer proximity and at greater density, the rate of cell proliferation is likely to be increased, and the cells will reach confluence more rapidly than those seeded at lower density.

The surface area calculated from the volume and contact angle for drops of culture medium containing HOBs are shown in Table 2 for each sample group. HOBs covered a significantly smaller area when seeded on uncoated instead of CaP-coated discs. Despite the relative hydrophobicity of uncoated discs, water was found to adhere strongly to the surface, which was measured by suspending deionized water drops on the surface upside-down.

Secondary electron images and confocal microscopy at day 14, shown in Figure 4, indicate that after two weeks, human osteoblasts seeded on the uncoated samples were confluent in the center of the disc but were much sparser at the edge. HOBs seeded on CaP-coated PCU discs were confluent in the central regions and relatively dense at the extremities of the disc. These micrographs are consistent with observations during contact angle analysis, in that cells adhered at a higher density in the central region of uncoated PCU discs, but did not spread across the disc. CaP-coated discs showed a more even dispersion of HOBs across the disc surface. The cell metabolism for HOBs showed a marked increase on uncoated compared to CaP-coated PCU discs at early time points; at higher cell seeding densities, cells come out of the lag phase more rapidly and thus show a marked increase in cell metabolism.

4 Conclusions

The wettability of a surface was shown not to be the sole factor in determining the *in vitro* response of human osteoblasts to surfaces. The results show that HOBs can proliferate and grow both on hydrophilic calcium phosphate-coated substrates and on relatively hydrophobic uncoated PCU substrates. HOBs adhered centrally to uncoated PCU discs and reached confluence more rapidly than on calcium phosphate-coated discs, where the HOBs were spread more evenly across the disc surface. A thicker coating, formed by a second soaking in 1x SBF, did not improve the cell response (results not shown here).

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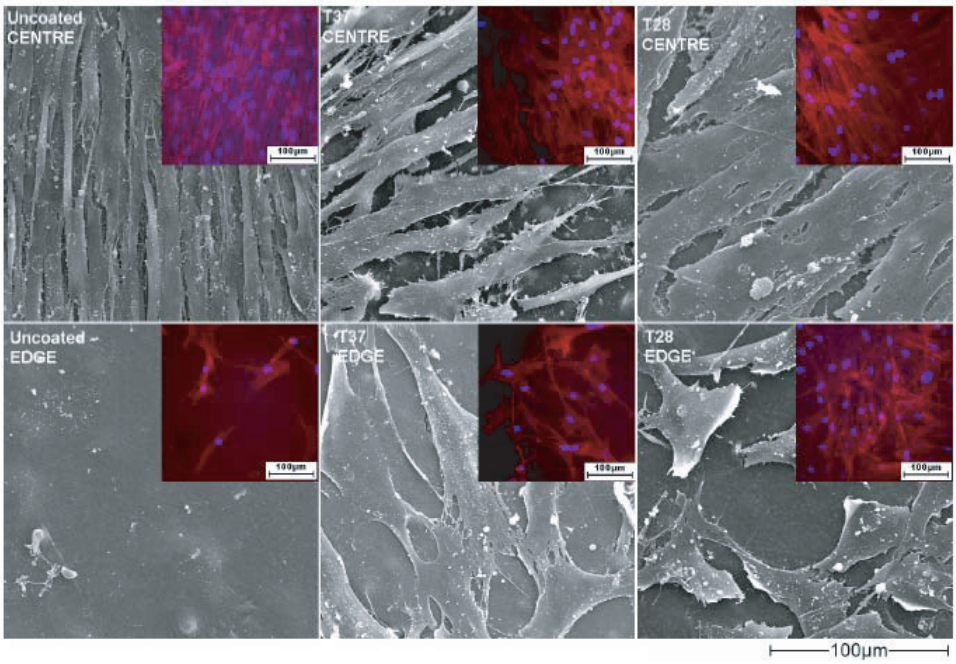

Figure 4: SEM surface micrographs (main image) and confocal microscopy images (inset) at Day 14 of cell culture. Images were taken in the centre (top) and edge (bottom) regions of uncoated (left), T37 (middle) and T28 (right) samples.

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