ATTACHMENT AND PROLIFERATION OF THE SAOS-2 CELL LINE ON CARBON FIBRE-REINFORCED POLYMERS

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Introduction: Carbon fibre-reinforced polymers (CFRP) are being investigated for the next generation of arthroplastic devices. Their mechanical similarities with bone make them ideal for load bearing implants. Previous attempts at using CFRP have failed due to the lack of osseointegration with bone. Treatments to increase surface roughness and topography have shown to affect the attachment and proliferation of osteoblasts on various biomaterials. Better osseointegration may be achieved by altering the surface properties of CFRP. Previous studies on titanium alloys have been ambivalent on the influence of surface characteristics. The majority of literary evidence pointed towards increased cell attachment and proliferation with increases in surface roughness. Nevertheless, there remains significant counter-evidence that indicated smooth surfaces induced improved osteoblastic activity. The purpose of this study is to investigate the attachment and proliferation of Saos-2, an osteoblast-like human osteosarcoma cell line on various surface treatments of a novel CFRP.

Materials and Methods: Cell attachment and proliferation were analysed on four different surface treatments of CFRP; untreated (UT), braded (BR), blasted (BL) and hydroxyapatite-coated (CHA). Their results were compared to that of hydroxyapatite-coated titanium-alloy (TiHA), a commonly used biomaterial combination in arthroplasty. All the materials were manufactured into a disk shape with 14mm diameter and 2mm height. The Saos-2, cell-line, was selected due to extensive literary evidence of it being an idyllic model of osteoblastic behaviours and we have previously done the in vitro and in vivo characterization of the cell line [1]. Environmental scanning electron microscopy (ESEM) was used to assess the surface characteristics of the treatments as well as the morphology of the attached Saos-2 cells. Crystal violet staining of the cell nucleus was performed in 24-well culture plates to appraise proliferation rates at days 4 and 7 with the cells cultured in the wells as a positive control and the materials cultured with the culture medium as a negative control.

Results: The micrographs of ESEM indicated that the surface of the untreated CFRP was smooth. There were some rough areas (scratched) of the braded CFRP and the scratches of the blasted CFRP were deeper. The hydroxyapatite coating on the CFRP was patchy whilst a complete layer of HA particles were noted on the titanium-alloy. All the materials were friendly to the Saos-2 cells. Even the untreated material has cells attached happily on it. The cells preferred to anchor at sites of surface defects such as divots and scratches. The orientations of the attached cells were influenced by the direction of the surface grooves. Saos-2 cells favoured surfaces with high roughness and the existence of hydroxyapatite particles (Figure 1). The proliferating assay showed differences in the optical densities (OD) of the elude dye solutions from the cells on the differently treated surfaces (Table 1 and Figure 2). TiHA showed the highest proliferating rate at day 4 and it reached the level of the plate (positive) control at day 7. Among all CFRP materials CHA presented the highest proliferating rate at both time points and the different with the UT groups were significant. At day 7 both BR and BL demonstrated higher proliferating rates than UT and the BR groups showed better results than the BL. The patterns of the differences among the tested materials at the two time points were significantly correlated (Pearson correlation 0.889, p<0.001; Spearman’s correlation 0.910, p<0.001).

Discussion: Surface treatment of CFRP enhances cell attachment and proliferation. Due to the small number of available specimens, the properties found in this study are not definitive. Nevertheless, these results achieved the purpose of being the foundation to further in vivo and clinical studies of CFRP based arthroplastic devices.


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Table 1. OD values at day 4 and 7 after subtraction by the material controls

<table>
<thead>
<tr>
<th>Day</th>
<th>UT</th>
<th>BR</th>
<th>BL</th>
<th>CHA</th>
<th>TiHA</th>
<th>Culture plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.135</td>
<td>0.065</td>
<td>0.139</td>
<td>0.226</td>
<td>0.279</td>
<td>0.363</td>
</tr>
<tr>
<td>7</td>
<td>0.214</td>
<td>0.409</td>
<td>0.305</td>
<td>0.335</td>
<td>0.342</td>
<td>0.711</td>
</tr>
</tbody>
</table>

Figure 1. Micrographs of ESEM showing the different surface roughness and cell attachment on the tested materials. The bars at the right bottom corner of each picture equals to 300μm except the TiHA which is 100μm.

Figure 2. Optical densities of the eluded crystal violet solutions at the 4 and 7 day time points. The 95% confidence intervals are given by the vertical lines on the top of each bar.

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