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Research Paper

Cytocompatibility, mechanical and dissolution properties of high strength boron and iron oxide phosphate glass fibre reinforced bioresorbable composites

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ABSTRACT

In this study, Polylactic acid (PLA)/phosphate glass fibres (PGF) composites were prepared by compression moulding. Fibres produced from phosphate based glasses P_2O_5–CaO–MgO–Na_2O (P45B0), P_2O_5–CaO–MgO–Na_2O–B_2O_3 (P45B5), P_2O_5–CaO–MgO–Na_2O–Fe_2O_3 (P45Fe3) and P_2O_5–CaO–MgO–Na_2O–B_2O_3–Fe_2O_3 (P45B5Fe3) were used to reinforce the bioresorbable polymer PLA. Fibre mechanical properties and degradation rate were investigated, along with the mechanical properties, degradation and cytocompatibility of the composites. Retention of the mechanical properties of the composites was evaluated during degradation in PBS at 37 °C for four weeks. The fibre volume fraction in the composite varied from 19 to 23%. The flexural strength values (ranging from 131 to 184 MPa) and modulus values (ranging from 9.95 to 12.29 GPa) obtained for the composites matched those of cortical bone. The highest flexural strength (184 MPa) and modulus (12.29 GPa) were observed for the P45B5Fe3 composite. After 28 days of immersion in PBS at 37 °C, ~35% of the strength profile was maintained for P45B0 and P45B5 composites, while for P45Fe3 and P45B5Fe3 composites ~40% of the initial strength was maintained. However, the overall wet mass change of P45Fe3 and P45B5Fe3 remained significantly lower than that of the P45B0 and P45B5 composites. The pH profile also revealed that the P45B0 and P45B5 composites degraded quicker, correlating well with the degradation profile. From SEM analysis, it could be seen that after 28 days of degradation, the fibres in the fractured surface of P45B5Fe3 composites remain fairly intact as compared to the other formulations. The *in vitro* cell culture studies using MG63 cell lines revealed both P45Fe3 and P45B5Fe3 composites maintained and showed higher cell viability as compared to the P45B0 and P45B5

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composites. This was attributed to the slower degradation rate of the fibres in P45Fe3 and P45B5Fe3 composites as compared with the fibres in P45B0 and P45B5 composites.

1. Introduction

Over the last 30 years a great deal of research has been conducted using different degradable materials as possible biomaterials especially as implants to serve as temporary devices (Barrows, 1986). Since then polymers prepared from glycolic acid and lactic acid as well as some other polymeric materials such as poly (dioxanone), poly (trimethylene carbonate) copolymers, and poly (ε-caprolactone) homopolymers and copolymers have also been investigated as medical devices due to their resorbable nature (Athanasiou et al., 1998). The main advantage of using biodegradable polymers as implants is that it eliminates the necessity of second surgery. In addition a fractured bone, fixed with a rigid, stainless steel plate does not carry sufficient load during the healing process as the load is carried by the stainless steel plate. As a result there is always the possibility that the 'healed' bone could easily re-fracture after plate removal (Pietrzak et al., 1996). An ideal implant should provide strong support during the early stages of implantation and allow gradual transfer of load to the healing bone during the later stages (Pietrzak et al., 1996). Thus, an implant made from a bioresorbable polymer can offer many advantages over stainless steel as it can be engineered to transfer the load slowly to the healing bone *via* controlled resorption over time (Athanasiou et al., 1998; Middleton and Tipton, 2000).

Poly (lactic acid) (PLA) is one of the most widely investigated bioresorbable materials. However, the mechanical properties and or degradation characteristics of PLA alone are not sufficient for load bearing applications (Navarro et al., 2005). One approach to overcome this problem has been the incorporation of an inorganic phase into the polymer matrix to produce composites. Composites made using PLA reinforced with bioactive glass fibres and or hydroxyapatite (HA) have been investigated as potential candidates as the addition of these inorganic phases mostly resulted in an increase of the bioactivity of the material and its mechanical properties. However, the degradation rate of hydroxyapatite or other calcium phosphate ceramics under physiological conditions is very low (Klein et al., 1983; Denissen et al., 1980). As such, alternative composite systems where both phases are completely degradable have been considered. Controlled degradation rates could play a very important role in the field of resorbable materials for bone regeneration (Navarro et al., 2003a). In this respect phosphate glass fibres (PGF) offer a distinct advantage over other materials as their degradation rates can easily be controlled by simply changing the composition to suit the end application (Navarro et al., 2003a; Felfel et al., 2012).

PGF-reinforced composites have previously been investigated as potential bone fracture fixation devices due to their favourable mechanical properties (Ahmed et al., 2008; Han et al., 2013). The mechanical strength and elastic properties of the fibres are very different from the bulk glass with the same composition which have enabled varying applications for these fibres (Otto, 1961). In addition, it has been found that introducing relatively low amounts of modifying oxides (such as, Fe_2O_3, B_2O_3 or TiO_2) to phosphate based glasses (PBGs) was successful for cellular attachment and proliferation (Ahmed et al., 2004; Navarro et al., 2003b; Abou Neel and Knowles, 2008). The success of these *in vitro* studies was linked to their improved durability due to addition of these oxides.

In a recent publication it was reported that addition of B_2O_3 (5–10 mol%) to the P_2O_5–CaO–MgO–Na_2O glass system increased the tensile strength of the PGFs from ~450 MPa to ~1200 MPa (Sharmin et al., 2014), the highest PGF tensile strength ever recorded. It has also been reported that incorporation of B_2O_3 (up to 5 mol%) to the same glass system showed favourable cell metabolic activity, proliferation and morphology (Sharmin et al., 2013). However, previous studies showed that PBG formulations containing Fe_2O_3 showed a much more favourable cytocompatible response as compared to PBG formulations containing only B_2O_3. However, only Fe_2O_3 addition to PBGs did not reveal any significant effects on the mechanical properties of the fibres. Moreover, till to date, the effect of introducing B_2O_3 alone and/or the combined effect of B_2O_3 and Fe_2O_3 on the mechanical properties, degradation and cytocompatibility on bioresorbable composite plates have not been elucidated. Therefore, the main aim of the current study was to investigate the effect of B_2O_3 and Fe_2O_3 on the mechanical properties, degradation and cytocompatibility of PLA reinforced bioresorbable composites.

Composite plates were reinforced with four different fibre formulations (P_2O_5–CaO–MgO–Na_2O (P45B0), P_2O_5–CaO–MgO–Na_2O–B_2O_3 (P45B5), P_2O_5–CaO–MgO–Na_2O–Fe_2O_3 (P45Fe3) and P_2O_5–CaO–MgO–Na_2O–B_2O_3–Fe_2O_3 (P45B5Fe3)) and the effect of B_2O_3 and/or Fe_2O_3 on the initial mechanical properties, degradation and cytocompatibility of the composites were investigated. The composite plates were manufactured using unidirectional fibre mats with alternate PLA film layup. Retention of the composite mechanical properties during immersion in PBS at 37 °C were also evaluated. MG63 osteosarcoma cell line was used to conduct the *in vitro* cytocompatibility studies of the composites.

2. Materials and methodology

2.1. Phosphate glass production and fibre drawing process

Four glass compositions were prepared using the following precursors; sodium dihydrogen phosphate (NaH_2PO_4, Sigma Aldrich, UK, ≥99%), calcium hydrogen phosphate ($CaHPO_4$, Sigma Aldrich, UK, 98–105%), magnesium hydrogen