

Response to Cellular Doping of Highly Porosity Alumina with P, Mg, and Si

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Perspective

Porous alumina could be used as a porous ceramic biomedical implant. Alumina is the first commercially significant bioceramic. It has been used in biomedical applications that have required hardness, low friction and for chemical stability, for example- dental implants and acetabular cup replacement in total for hip prostheses. Other ceramics such as calcium phosphate do not have the sufficient compressive strength in that porous form. The success of a porous implant depends upon its ability to provide the functional balance between the mechanical strength, pore size, interconnectivity of the porous structure and properties of the osteoconductivity. Mechanical strength is known to be as reduced by porosity. The author has previously published a method to produce the porous alumina by foaming. Porous alumina can be synthesised using in situ evolution of gases from the calcining blends of ammonium sulphate and aluminium sulphate with varying ammonium mole fraction (AMF). High levels of the porosity 94%-96% are achieved with the acceptable mechanical strength of 380 kPa which is comparable to the other biomaterials of similar porosity levels. To make the bioinert alumina material bioactive and thus the potential more suitable for the biomedical application, doping of the porous alumina have considered. For the study, doping with calcium (Ca), phosphate (P), Silica (Si) and Magnesium (Mg) and combinations of low and high concentrations were trialled. Calcium and phosphate coatings are known to be as bioactive and therefore doping with Ca and P has trialled. Incorporation of the magnesium ion in an alumina implant was previously been shown to improve bone cell adhesion. Silica doping of hydroxyapatite and its improved cellular response has been vastly reported.

Silica doping of alumina tubes with small amounts of Si was previously been shown to improve the tissue ingrowth, differentiation and osteogenesis in vivo. The combination of the foamed alumina containing high porosity, pore size, pore interconnectivity and strength when it doped to improve bioactivity can uniquely combine the properties are required for biomedical applications. The key focus of this study is to take an optimal product from the foaming method used to be produce a high porosity (94%) alumina, average pore size of 300 μm , and with a degree of pore interconnectivity and high compressive strength, dope it with Ca, P, Si, Mg and test the cellular response compared with the control foamed alumina. All the alumina specimens was sterilized in 70% ethanol for 30 min and exposed to the ultraviolet (UV) light prior for being introduced into 96 well culture plates. An alumina specimen of 4 mm diameter was attached to that culture plates with Vaseline. MG3 cells was seeded into the specimens along with Dulbecco's Modified Eagle Medium (DMEM) containing calcium (Ca^{2+}), supplemented with 10% foetal bovine serum (FBS) at the density of 15,000 cells/well. The plated specimen has been incubated at 37°C in the humidified atmosphere of 95% air and 5% carbon dioxide for a

period of 3 days. Cell viability of the synthesised porous alumina was assessed by the number of healthy cells in the seed specimens. The MG63 cells are rinsed with the Phosphate Buffered Saline solution (PBS) and trypsinised for 8 min at 37°C. Once cells are detached from the surface, the solution was neutralised with 50 μL of FBS followed by staining using 100 μL of Trypan Blue. The cell suspension has flushed several times for even cell distribution before pipetting 10 μL into the haemocytometer for counting under the light microscope. Cell viability has done in triplicate. Cell morphology indicates that the health of the cell and how well it responds to the chemistry and topography for the underlying substrate. Cultured cells are rinsed with pre-warmed PBS solution and fixed onto that porous specimens using 2% glutaraldehyde at the room temperature for 1 h followed by the dehydrating in the series of graded ethanol solutions (50%, 70%, 80%, 90%, 95% and 100%) for 10 minutes. The fixated cells are then stained with osmium before being critical point dried with the Bal-Tec CPD 030 Critical Point Dryer (CPD). The prepared alumina specimens with fixated cells are then sputter coated with gold to a thickness of 10 nm before being observed through the scanning electron microscopy (SEM). The microstructure of the synthesised porous alumina has observed with the Philips XL30 SEM (FEI, Eindhoven, and The Netherlands). A high energy beam of electrons, with the average accelerating current of 15 kV interacts with the atoms on that specimen surface. Pore size and cell wall thickness was measured with inbuilt measurement software. To prevent the accumulation of the electrostatic charge on the surface, specimens are sputter coated with gold before imaging.

Doping

Observation through SEM and EDS has done for foamed porous alumina samples doped with Ca, P, Mg with dilute (10% concentration) or saturated (100% concentration) and alumina samples doped with Si concentration of 100%. Porous alumina doped with 100% and 10% solutions of calcium (Ca), phosphorous (P), magnesium (Mg), Ca + P and Ca + P + Mg are immersed in concentrated solution for 24 h before drying. Precipitates can be found distributed on the surfaces treated with saturated concentrations of dopants. There are minimal precipitates visible on the surfaces with dilute concentrations of dopants. Silica (Si) doped porous alumina showed homogeneous surface distribution. SEM micrographs of treated surfaces can be seen also. Doping of foamed porous alumina using saturated (100%) and dilute (10%) concentrated solutions of calcium (Ca), phosphorous (P), magnesium (Mg), Ca + P and Ca + P + Mg was done. The results indicated that doping of the foamed alumina can be done and that soaking in the saturated solutions produced more dopant adhering to that surface. Precipitates are observed on the surface of the samples and thus surface modification of the porous alumina has achieved. Precipitates can be found homogenously distributed on the surfaces treated in the saturated solutions of dopants. There are minimal precipitates visible on the surfaces treated in the dilute solutions of dopants. Precipitates are in the size range of approximately 10 μm . Silica (Si) doped porous alumina are showing homogeneous surface distribution. Si doping produced a visually thicker and denser layer.

EDS results is not numerically definitive, suggests dopant was present. The surface effect of doping the porous alumina was further discussed in the relation to the cellular response. To test the biocompatibility of that foamed alumina as a control and the impact of the doping foamed alumina, cell culture

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using MG63 cells was done. Cell viability results showed positive growth rates of the cultured cells on both Si and P doped samples. Cell morphology was seen in the SEM micrographs and also it showed large cells.

Foamed alumina was previously synthesised by the direct foaming of the sulphate salt blends varying AMF, foaming heating rate and sintering temperature. The optimal product was produced with 0.33 AMF, foaming at 100°C/h and sintering at 1600°C. This product attained the high porosity of 94.39%, large average pore size of 300 μm and the highest strength of 384 kPa. This study doped that the foamed alumina with some different concentrations (10% and 100%) of Ca, P, Mg, CaP and CaP + Mg and Si. Doping could be successfully done and results were presented with EDS and SEM. Cellular response was tested with the cell culture of MG63 osteosarcoma cells with the control alumina as well as the doped surfaces. Cellular response to the Si and P doped samples was positive with high cell populations and cell layer formation as seen with cell viability and cell morphology studies [1-5].

In this conclusion, the impact of the doping with phosphate produced a result not previously reported and the cellular response showed that the both Si and P doping were improved the biocompatibility of the foamed alumina.

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