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Functionally graded hydroxyapatite-alumina-zirconia biocomposite: Synergy of toughness and biocompatibility

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ABSTRACT

Functionally Gradient Materials (FGM) are considered as a novel concept to implement graded functionality that otherwise cannot be achieved by conventional homogeneous materials. For biomedical applications, an ideal combination of bioactivity on the material surface as well as good physical property (strength/toughness/hardness) of the bulk is required in a designed FGM structure. In this perspective, the present work aims at providing a smooth gradation of functionality (enhanced toughening of the bulk, and retained biocompatibility of the surface) in a spark plasma processed hydroxyapatite-alumina-zirconia (HAp-Al_2O_3-YSZ) FGM bio-composite. In the current work HAp (fracture toughness ~1.5 MPa.m$^{1/2}$) and YSZ (fracture toughness ~6.2 MPa.m$^{1/2}$) are coupled with a transition layer of Al_2O_3 allowing minimum gradient of mechanical properties (especially the fracture toughness ~3.5 MPa.m$^{1/2}$). The *in vitro* cyto-compatibilty of HAp-Al_2O_3-YSZ FGM was evaluated using L929 fibroblast cells and Saos-2 Osteoblast cells for their adhesion and growth. From analysis of the cell viability data, it is evident that FGM supports good cell proliferation after 2, 3, 4 days culture. The measured variation in hardness, fracture toughness and cellular adhesion across the cross section confirmed the smooth transition achieved for the FGM (HAp-Al_2O_3-YSZ) nanocomposite, i.e. enhanced bulk toughness combined with unrestricted surface bioactivity. Therefore, such designed biomaterials can serve as potential bone implants.

1. Introduction

The development of new biomaterials with enhanced mechanical properties and biocompatibility has become a major challenge in biomaterials community. HAp is widely recognized as a potential material for tissue replacement and repair, as it supports new bone formation, necessary for implant osseointegration [1]. Moreover, HAp is a good osteoconductive material, that supports cell fate process [2]. In view of such favorable properties, HAp has been used as an orthopedic implant [3,4]. But, it cannot be used for large segmental bone defect owing to its low fracture strength (<120 MPa), and poor fracture toughness (0.8–1.2 MPam$^{1/2}$) in comparison to that of human bone (fracture strength ~150 MPa and fracture toughness 2–12 MPam$^{1/2}$) [5–9]. Therefore, HAp alone cannot be implanted as monolithic material for load bearing implants, such as: tooth and artificial bones [10–12]. As a result, a large number of studies have been devoted to enhance the mechanical properties of HAp material. To amend the mechanical properties, HAp has been doped with different metals (Ti or Ti-6Al-4V alloys) [5,13–15], ceramics (ZrO_2, Al_2O_3, B_2O_3, glass etc.) [16,17], polymers (LDPE, UHMWPE etc.) [18] and carbon nanotubes (CNTs) [19]. Especially zirconia has been extensively employed for such applications attributing to its bio-inertness and high fracture toughness (6–10 MPam$^{1/2}$) [20–22]. Another possible application of HAp could be a coating on metallic implants [23]. One way of altering mechanical properties is to enforce HAp on a substrate of higher toughness. Gandhi et al., concluded that HAp coating is not a good alternative if the material is to be used for the load bearing applications as these coatings may result in weak interfacial bonding [24]. Plasma spraying has emerged as one of the prime techniques for deposition of ceramics (such as YSZ, HAp, Al_2O_3, etc.), and with controlled powder treatment and process parameter selection, toughness enhancement can reach as high as 57% with 8 wt.% CNT reinforcement in Al_2O_3 matrix [19,25–29]. Although, plasma spraying can enable to achieve good interfacial bonding, but high plasma spraying temperature (excess of 5000 °C) can result in decomposition of HAp phase [19,23,25,30].

Functionally graded material can mimic the bone properties without compromising on the mechanical properties of the material. For example, a gradation in fracture toughness can reduce the danger of

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having sudden change in the toughness along the material interface, which might result in easy crack propagation along the interface [31]. A number of studies are undertaken on HAp/metal FGMs for example HAp/Ti FGM [14,32]. But such FGMs may have a problem of poor interfacial bonding at ceramic/metal interface. FGM of ceramic/ceramic (HAp/Zirconia) have been developed by Guo et al. [31]. Due to large difference in fracture strength of HAp and ZrO_2 there is a chance of crack propagation along HAp-ZrO_2 interface. Such a graded structure involving only HAp and ZrO_2 requires a large number of intermediate layers and an undesirable reaction can occur between HAp and ZrO_2 to form TCP and fully stabilized ZrO_2 [33]. The idea of FGM also mimics the bone, since the cross section of bone elicits a complex and gradient structure.

In the past two decades, spark plasma sintering (SPS) has emerged as one of the most significant and effective sintering method, which enables processing of powder to fabricate fully dense compact at relatively lower sintering temperature and time [34,35]. SPS is a pressure assisted sintering route in which high voltage and pulsed current is applied across the powder compact and sintering is accomplished due to joule heating at the powder particle interfaces, where highly dense and homogenous microstructures can be obtained at relatively lower temperatures [34,35]. Recently, SPS has been widely utilized for the processing of ZrO_2, Al_2O_3 and HAp based ceramic composites [12,34,36]. The usage of SPS is widely increasing in developing functionally gradient materials (FGM), intermetallics, fiber reinforced matrix, and metal-ceramics matrix, where it is difficult to sinter with conventional techniques [37]. Interestingly, SPS of FGM provides an easy route of achieving graded structure while obtaining near theoretical densities of the biocomposites [38].

To overcome the problem of drastic change in the fracture toughness from HAp to YSZ, in the present work, alumina is incorporated as an interlayer to achieve a gradual transition in the fracture toughness from HAp to YSZ region. The top surface of HAp can provide enhanced bioactivity, whereas the bottom surface of pure YSZ can provide good strength/toughness. This approach can be extended to developing a tough bone-implant material because of thick YSZ core (acting as structural material) with surface of highly bio-compatible HAp (to achieve enhanced cell-activity on surface) while providing a gradual transition of interlayer (Al_2O_3-YSZ) to resist damage around articulating surfaces. Thus, the work aims at the synthesis of HAp-Al_2O_3-YSZ FGM via SPS processing route and to study its mechanical properties followed by *in vitro* studies in order to explore the excellent capabilities of such materials to be successful in applications such as bone implant materials.

In this study, L929 Fibroblast and Saos2 Osteosarcoma cell lines have been used to evaluate the biocompatibility of the processed FGM [39–41]. The motivation of using two different cell lines (connective tissue and bone cell) is to confirm the application of FGM for the bone tissue replacement application. Fibroblast cells (L929) are cells of connective tissues. Important properties of fibroblast are that they are least specialized and adherent cell line, which is helpful for the assessment of cellular adhesion and cytotoxicity of the material. Whereas, Saos2 cells are osteoblast like cells, derived primarily from the cancerous bone tissue. The present study aims for a promising biomaterial which can be used for bone implants. Therefore, a cell culture study with osteoblast cells is necessary to ensure the biocompatibility of FGM for bone implantation. In the above perspective, we report spark plasma sintering of FGM (HAp-Al_2O_3-YSZ) and we demonstrate how a gradual transition in physical and biocompatibility property can be achieved in such compositionally designed biomaterials.

2. Experimental details

2.1. Materials and methods

Hydroxyapatite (HAp) powders (size 80–100 nm, suspension precipitation synthesis [42]), 3% (mole fraction) yttria stabilized zirconia (YSZ) (particle size – 80 nm, Allied Hi-Tech Product Inc U.S.A) and α-Alumina (Al_2O_3) powders (mean particle size – 50 nm, purity – 99.5%, Cenogen Materials Pvt. Ltd) were used in this study. The initial powder mixtures (Table 1) were wet ball milled with a ball-to-powder mass ratio of 10:1 using ethanol. Milling was done in the agate jar using agate balls with a rotation velocity of 250 rpm for 10 h. Subsequently the powder mixtures were dried overnight in an oven at 100 °C.

2.2. Processing of functionally graded material via spark plasma sintering

The spark plasma sintering (SPS) apparatus (Dr. Sinter 511S SPS) with ON–OFF pulse duration of 12:2 ms was used. The dried powders were placed in a graphite die (outside diameter 30 mm; inside diameter 15 mm). The samples were placed in a die maintained at vacuum of 6 Pa (to assure the absence of CO_2), and sintered for 5 min under uniaxial mechanical pressure of 30 MPa. The temperature was measured with an optical pyrometer focused on the surface of the graphite die.

To have an idea for optimum parameters for sintering FGM, individual pellets of the powder mixtures were sintered at various temperatures (Table 1). The sintering time of 5 min was maintained for all the samples, and the rate of heating was optimized to 50 °C/minute. Final temperature of synthesizing FGM were selected at 1200 °C on the basis that at this temperature fully dense YSZ can be obtained to serve as structural material, whereas other layers – HAp-Al_2O_3 support cell growth due to presence of HAp and Al_2O_3-YSZ layer act as a transition layer between HAp-Al_2O_3 and YSZ layers.

2.3. Phase and microstructural characterization of processed functionally graded YSZ-Al_2O_3-HAp

The density of samples was measured via Archimedes' water immersion principle. The microstructural characteristics of the powders and the sintered compacts were studied using a scanning electron microscope (CARL ZEISS EVO 50). The phase analysis using X-ray diffraction technique (XRD), (model: *ISO Debyeflex-2002*) with Cu Kα radiation, $\lambda = 0.15418$ nm was carried out. XRD analysis on the individual layers of FGM was not viable; therefore XRD analysis was performed both on the powder samples and on individual pellets sintered at same parameters as that of FGM. Raman spectroscopy (WITec GmbH, Germany, alpha 300 series microscope) was performed on the top and bottom surfaces of FGM to provide complimentary information on phase analysis.

2.4. Mechanical property evaluation of YSZ-Al_2O_3-HAp

The hardness (*HV*) of FGM was evaluated using Vickers indentation technique at a load of 100 g for HAp-Al_2O_3 layer and 500 g for Al_2O_3-YSZ layer and YSZ layer. For evaluating the fracture toughness, a load of 500 g was applied on HAp-Al_2O_3 and 2 kg load on Al_2O_3-YSZ layer and YSZ layer, owing to the difference in hardness. Correspondingly,

Table 1
Nomenclature of the spark plasma sintered pellets.

Notation	Composition	Sintering temperature (°C)	Sintering time (min)	Pressure (MPa)
HAp-Al_2O_3	HAp + 20% (weight fraction) Al_2O_3	1100	5	30
Al_2O_3-YSZ	Al_2O_3 + 20% (weight fraction) Yttria stabilized zirconia	1250	5	30
YSZ	3 mol.% Yttria stabilized zirconia	1200	5	30
FGM	Above three compositions stacked together	1200	5	30