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In vitro cytocompatibility, ageing and wear behavior of ceria stabilized zirconia bioceramic

Ajoy Kumar Pandey[a,*], Koushik Biswas[b]

[a]*Department of Metallurgical and Materials Engineering, National Institute of Technology Warangal, Warangal 506 004, Andhra Pradesh, India*
[b]*Department of Metallurgical and Materials Engineering, Indian Institute of Technology, Kharagpur 721 302, West Bengal, India*

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Abstract

Nano sized ceria stabilized zirconia (CSZ) powders were synthesized by co-precipitation method and then sintered conventionally to near full density. Ageing stability of developed materials was predicted through *in vitro* hydrothermal treatment in the presence of simulated body fluid (SBF). Fretting wear test at different loads was carried out using balls on flat geometry at different intervals of hydrothermal treatment to observe the effects of surface ageing on wear properties. Wear volume, specific wear rate and wear depth were estimated through 3D profile scan of the worn out surface by a surface profiler and it was found that the developed material became more wear resistant with the increase in hydrothermal treatment duration. XRD analysis and hardness study suggest that formation of orthorhombic zirconia during hydrothermal treatment improves the surface hardness (from 946 ± 86 HV20 to 964 ± 39 HV20 after 100 h of hydrothermal treatment) which in turn enhances wear resistance. *In vitro* cytocompatibility of the developed materials was inferred through the formation of hydroxyapatite-like layer on the surface of the material when soaked in SBF at 37.5 °C. Cytocompatibility was further ensured by studding attachment of multilayered human osteoblast cells (MG63) on the surface during cell culture.

Keywords: A. Sintering; C. Wear resistance; D. Apatite; E. Biomedical applications; Osteoconduction

1. Introduction

Excellent technological properties such as chemical inertness, thermal stability, strength, good toughness, wear resistive property and Young's modulus similar to that of stainless steel [1,2] promoted zirconia as one of the leading bioceramic. Zirconia exhibits excellent mechanical properties due to its transformation toughening phenomenon that occurs during propagation of cracks inside partially stabilized zirconia (PSZ) or tetragonal zirconia polycrystals (TZPs) matrix [2–4]. PSZ consists of coherent precipitate of metastable tetragonal zirconia grains in cubic zirconia matrix. On the other hand, TZP consists of 100% tetragonal zirconia grains. The toughening transformation basically depends upon the amount of phase present and their transformability to stable (monoclinic) phase.

While cracks try to propagate through PSZ/TZP matrix, the matrix undergoes phase transformation towards more stable structure (tetragonal → monoclinic). During this process the crack tip loses some energy. This phase transformation is accompanied with some volume expansion (~4%) which results in compressive stress around the crack tip. The compressive stress prevents and eventually stops the crack propagation within the matrix.

In spite of such excellent properties, zirconia as a biomaterial has a controversial history regarding phase metastability with temperature and also *in vitro* conditions. The degradation of material properties due to phase transformation in long term application is termed as ageing. The ageing event resulted in a series of catastrophic failure events of yttria stabilized zirconia (YSZ) during 2002–2003 which forced the researchers to re-think over the

*Corresponding author.
E-mail address: ajoy100@gmail.com (A.K. Pandey).

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reliability of the material for biomedical applications. Typical study on the fracture surface of the failed materials revealed that there was a remarkable increase in monoclinic phase (up to 30%) [5,6] during service.

It is believed that ageing is originated from the reaction between water and Zr–O–Zr bond [7]. The key features of ageing, its mechanism and effect on mechanical properties are described elsewhere [8–11]. The volume increase due to phase transformation during ageing results in surface uplift or grain pullout which increases effective surface roughness, leading to more wear and wear debris. This is detrimental for implants. Hence, development of ageing free or at least ageing free within the lifespan of the implant is in high demand [9].

Keeping prevention of ageing in mind, ceria stabilized zirconia composites were developed. The advantage of ceria over yttria or any other trivalent oxide is that it does not create any oxygen vacancy (defect) during substitution of Zr^{4+}. This phenomenon is well described by our previous work [12]. The ageing of the developed materials in long runs was studied through accelerated ageing tests inside a hydrothermal chamber in the presence of SBF. Literature suggests that 1 h of hydrothermal treatment at 134 °C under 0.2 MPa in the presence of SBF can be extrapolated to 3–4 years equivalence *in vivo* [13–15] condition. Ageing has definite effects on the wear properties. To understand wear response with extent of ageing, fretting wears before and after ageing were carried out.

As an index of biocompatibility, an artificial implant material requires formation of bone-like apatite layer on its surface when immersed in SBF. The degree of bioactivity of a material depends upon the formation of bond to living bone through apatite layer formation on the surface [16]. It is already reported that the *in vivo* apatite formability can be anticipated through *in vitro* study in the presence of SBF [17,18]. Hence, the biocompatibility of developed materials was studied *in vitro* by soaking the specimens under SBF to extrapolate the degree of cytocompatibility *in vivo*. *In vitro* cytocompatibility was further ensured through human osteoblast like cell culture on the surface of developed materials.

2. Materials and methods

2.1. Material development

Fourteen mol% ceria doped zirconia powder was synthesized by co-precipitation method using precursor salt zirconyl nitrate and cerous nitrate (Loba Chemie, India, > 99.5% pure) in proportionate quantity, as described in our earlier communication [12]. Both the salts were dissolved in distilled water and then ammonium solution was added dropwise to get the precipitate. For complete precipitation, pH level in the range of 9–10 was maintained and vigorous stirring was done by an ultrasonic stirrer during precipitation. After completion of precipitation, the precipitate was filtered and washed several times and dried at 110 °C for 20 h. The dried precipitated powder thus obtained was calcined at 600, 800, 1000 and 1200 °C for 2 h. The powders were then uni-axially compacted at 600 MPa to pellets with $\Phi = 10$ mm and $t = 3$ mm. A two steps sintering schedule (1600 °C for 1 h and then 1500 °C for 2 h) in a conventional electrical heating furnace (Bysakh & Co, India) was followed to achieve maximum density with a controlled grain size [12].

2.2. In vitro ageing study (hydrothermal treatment)

In vitro ageing studies of developed materials were carried out by hydrothermal treatment in the presence of SBF. The SBF was prepared according to Tadakama et al. [19]. Metallographically polished and ultrasonically cleaned (in the presence of acetone) samples were placed into the stream of SBF in a hydrothermal chamber. The test was carried out at 134 °C under 0.2 MPa for a total duration of 100 h. After every 10 h of hydrothermal treatment, XRD patterns were obtained from the samples and the SBF solution of hydrothermal chamber was replaced in order to maintain a constant ion concentration. Before characterizing, the samples were thoroughly washed with distilled water to ensure abesence of salt deposition on the surface.

2.3. In vitro cytocompatibility study

(i) Osteoconduction study

Polished and ultrasonically cleaned samples were dipped in SBF inside a beaker and the beaker was placed inside a water bath (half dipped in water) maintained at a constant temperature of 37.5 °C. Beakers were covered with aluminum foil to avoid mixing of evaporated water from the water bath. The test was carried out for a total duration of 28 days. After every 3rd day, the SBF in the beaker was replaced with fresh SBF. One sample was taken out after every week for characterization using a scanning electron microscope (SEM: Supra 40, Carl Zeiss SMT, Germany) attached with an energy dispersive X-ray spectrometer (EDX: Oxford Instruments Ltd., UK).

(ii) Cell culture on ceramic discs

Frozen stock of human osteoblast-like MG63 cells was thawed and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 4 mM L-glutamine, 2 mM Na-pyruvate and 1 μl ml^{-1} penicillin–streptomycin (A002A, Himedia, Mumbai, India) in a standard incubator (37 °C, 5% CO_2 atmosphere and 100% relative humidity). The cells were subcultured when they reached 90% confluence. The cells (with cell density 10^4 cells/well) were seeded on a 6-well culture plate fixed with ceramic discs. The culture medium was changed every alternate day. The cells were allowed to attach to the discs for 3 and 7 days after seeding. The density of attached cells on the discs was assayed by the standard method of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, or MTT assay. The adherent cell morphology proliferated on the discs was examined using SEM. To fix the cells with the ceramic discs for SEM analysis, the samples were soaked in 2.5% glutaraldehyde in PBS solution for 4 h. This was followed by a sequential dehydration in an ascending series of ethanol