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IN VITRO BIOCOMPATIBILITY TEST OF NANO ALUMINA AND CHARACTERIZATION BY RIETVELD ANALYSIS

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ABSTRACT

Biocompatibility test of nano alumina, has been done by cytotoxicity test method. The crystalline size of nano alumina determined by Rietveld analysis of X ray diffraction studies and Transmission electron microscopy. Cytotoxicity results indicate that cells proliferations has been taken place. So we can conclude that nano alumina is highly biocompatible material. Rietveld analysis of X-ray diffraction and transmission electron microscopy showed that the sample is composed of crystalline and a small fraction of amorphous phases. In view of these, nano alumina may be considered to be future potential for bone substitute material.

KEYWORDS

Nano alumina; Rietveld method; Biocompatibility; Bone materials; TEM

INTRODUCTION

Nanotechnology, more descriptively known as molecular manufacturing, involves the design, modeling, fabrication and manipulation of materials and devices at the atomic scale. Alumina has been used as a biomaterial implants due to its excellent biocompatibility and bioinert nature. Micro alumina is used for orthopaedic and dental implants, and has the ability to be polished to a high surface finish and high hardness. It has been utilized in wear bearing environments such as the total hip arthroplasties (THA) as the femoral head generating reductions in wear particles from ultrahigh molecular weight polyethylene [1]. Total hip replacement (THR) is one of the most successful applications of biomaterials in the short term. However in the long term (>15 years) the current biomaterials, particularly polyethylene, fail because of a concurrent mechanical and biological process wear debris induced osteolysis, which leads to implant failure and to revision surgery [2–8].

Ceramic materials have been recognized as increasingly important for their chemical and physical characteristics, and have progressively attracted interest in the field of biomedicine [9,10]. They were introduced more than 20 years ago in orthopedics for hip implants to solve the critical problems of polyethylene wear [11,12]. Ceramic materials for THR present excellent biocompatibility, low coefficient of friction and high wear resistance [13], and they have good properties of mechanical resistance at high temperatures when metals cannot be used [10,14]. By in vitro studies, the ceramic inserts have significantly reduced wear rate, possibly removing the problem of osteolysis, which represents the major cause of long-term failure in THR. Therefore the ceramic–ceramic coupling is particularly appropriate for young, heavy and active people where the rate of wear is usually higher, besides the ceramic particulate is more tolerated by the organism [15]. In fact, ceramic debris causes a small inflammatory reaction in tissues. Tests performed on synovial tissue at revision or at autopsy with ceramic couplings, have shown less inflammatory reaction and a reduction of necrosis, compared with the ceramic–polyethylene and metal–polyethylene couplings [16,17]. Unlikely with polyethylene liners, it is not the wear that cause loosening in ceramic–ceramic couplings alumina spacers (specifically in revision surgery), knee prosthesis and in the past as polycrystalline and single crystal forms in dental applications as tooth implants. [18] Traditional materials for orthopedic and dental applications have been selected based on their mechanical properties and ability to remain inert in vivo; this selection process has provided materials that satisfied physiological loading conditions but did not duplicate the mechanical chemical and architectural properties of bone. Most importantly, to date, failure of conventional orthopedic and dental implant materials is often due to insufficient bonding to bone. Nanophase materials are new formulations of materials that are composed of grains of the same atoms but with fewer (less than tens of thousands) and smaller (less than 100nm) in diameter atoms than in conventional forms (which contains several billion atoms and have grain sizes of micrometers to millimeters in diameter). Moreover, nanophase ceramics can be synthesized so they possess similar grain size, geometry and micro architecture as that of healthy, physiological bone. Here we used nano-alumina for biomedical application.

EXPERIMENTAL DETAILS

XRD measurements were carried out using Philips PW1840 diffractometer.

The diffraction spectrum was recorded with Cu-K radiation (λ =1.54 Å). For this experiment, 1 o diver-gence slit, 0.2 mm receiving slit, 1 0 scatter slit and 5 0 soller slit system were used. The step scan data (step size 2 = 0.020) for the entire angular range of the experimental samples were recorded. Gaussianity of peaks was estimated using profile fitting technique, pseudo-Voigt function (pV function) for this set up and all the instrumental parameters were incorporated in the Rietveld's analysis software, LS1 [19]. The strongest Bragg reflection (in this case peak at 2Θ = 67.20) is used to calculate the crystallite size . Errors in these parameters are the standard deviation computed by the in-built routine [20]. TEM investigations were made utilizing a JEOL instrument operated at 160 kV. Transmission electron microscopy (TEM) specimens were prepared from ball-milled powders dispersed in doubly distilled acetone and mixed well under ultrasonic vibrator to avoid agglomeration of the fine particles. A drop of this finely dispersed colloidal solution was placed on the 3 mm disc and the disc kept overnight under vacuum to get completely dry and well distributed fine particles over the entire area of the carbon-coated grid.

Fibroblast L929 Cells in direct contact with nano alumina, Cells (1x 10 3 cells) were seeded in 24 well tissue culture polystyrene plates enriched with Dulbecco's Minimum Essential Medium (DMEM) with Earl's salt supplemented with 10% calf serum and containing 100U/100 μg/ml of penicillin and streptomycin, respectively and incubated at 37º C in humid atmosphere and 5% CO2 in air for 24 hours. When the cells attained confluncy, the cleaned and sterilized samples were placed indirect with the cells in the wells and left for 24 hours under the same condition. Thereafter, the cells were observed for any change in morphology or any other cytotoxic effect.

RESULTS & DISCUSSION

The crystalline size as determined by Rietveld analysis [20] of XRD (Fig1) studies was 20 nm. TEM photograph was shown in fig 2 and Fig3. XRD and electron diffraction studies revealed that nano alumina sample is composed of crystalline and amorphous phase. As received sample consists of 25 % amorphous phase as it calculated from Rietveld analysis (LS1) of XRD pattern . This may be beneficial in many ways, e.g., in preventing grain "pull out "(causing implant loosening and inflammation) which is a problem with the sintered alumina used today [21,22,23,24]. Nano alumina did not show any signs of toxicity embarrassment after 24 h in contact with L929 cells in culture. The ultra structure of the monolayer of fibroblast cells was observed as cells lying parallel in contact with each other by short pseudopodia. Even at ultra structural level no cytotoxic effect was observed in the cells after contact with the nano alumina (Fig4). Compared with negative (high density polyethylene) and positive (copper) control (Fig5). These results indicate that cells proliferations were taken place. In view of these, nano alumina may be considered to be future potential for bone substitute material.

CONCLUSIONS

The management of traumatic defect in long bones and joints has become an increasing problem in surgery of trauma or by accident. This can also be applied to defects caused by malignant diseases. So the repair of bone defect has resulted in growing need for a suitable bone or joint replacement biomaterials. These results indicate that cells proliferations were taken place. Nano structural ceramics provide alternatives not yet fully explored for orthopedic and dental implant-applications; the improved mechanical property of these novel ceramic formulations, in addition to their established exceptional biocompatibility, constitute characteristics that promise improved orthopedic and dental efficacy. Undoubtedly, nanophase ceramics have great potential to be come the next generation of choice proactive biomaterials for innovative biotechnology and biomedical applications that could have profound clinical impact. So we can conclude that nano alumina is highly biocompatible material. In view of these the nano alumina may be considered to be future potential for bone substitute material.

Figure Captions:

Fig.1. XRD pattern of nanoAl2O3 along with the calculated pattern.
Fig2. Transmission electron micrograph of nanoAl2O3 powder.
Fig.3. Electron diffraction pattern of the same sample.
Fig.4 Cytotoxicity test of Nano Al2O3
Fig. 5 Cytotoxicity test of positive (copper) control

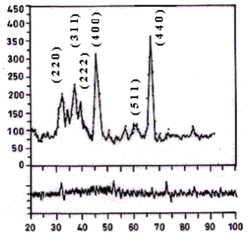

Fig.1

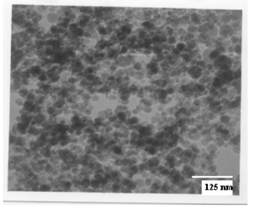

Fig2

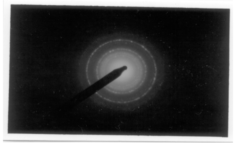

Fig.3.

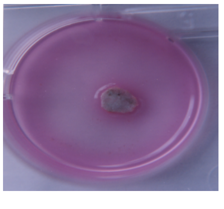

Fig.4

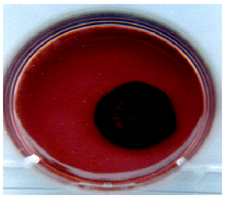

Fig. 5

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