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Calcium hydroxyapatite bioceramics and evaluation of their *In Vitro* biocompatibility

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ABSTRACT

Calcium hydroxyapatite (HAp) ceramics analogous to the mineral component of bones. This study investigated the in vitro biocompatibility study by the effect of calcium hydroxyapatite bioceramics on cell adhesion of sheep bone marrow derived mesenchymal stem cells (MMSCs), were cultured in Dulbecco's modified Eagle medium for 3 and 7 days. The hydroxyapatite bioceramics prepared by utilizing the solution combustion technique. The results demonstrate that, in addition to favorable biocompatibility, the calcium hydroxyapatite ceramics can stimulate cell adhesion of the MMSCs in vitro.
Keywords Bioceramics, MMSCs, Hydroxyapatite, Biocompatibility

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INTRODUCTION

Calcium hydroxyapatite is the most ubiquitous family of bioceramics well known for their use in biological application. There are many phases of hydroxyapatite exhibiting different crystal structures Ca/P ratios which represent resorbable and Non-resorbable ceramics and cements [1-2]. Several forms of this material can be used as scaffolds for tissue engineering, as drug delivery agents, non viral gene carriers, as prosthetic coatings and composites [3-5]. All of these manifestations of hydroxyapatite find applications for bone reconstruction and replacement as bone defect-filling drug carriers and as coating of metal prostheses.

Synthesis of hydroxyapatite (HA) $Ca_{10} (PO_4)_6(OH)_2$ is the powder with a high potential for bone and tooth implants [6-7], due to its excellent biocompatibility [8]. HA ceramics have the drawback of poor mechanical properties and bio – degradability [8]. Basically, all bioceramics which have good mechanical properties and suitable for load bearing application should be bioinert. Hydroxyapatite has high bioactivity and able to promote bone growth and form a direct, strong bond with living bone [9]. Calcium hydroxyapatite was thus the first material of interest for application in implant materials and has received intense focus over the past 25 years [10-11]. Several research groups have developed preparative procedures for hydroxyapatite. Traditionally, two main methods are employed for preparation of HA powders: wet (chemical) method (including precipitation method [12], hydrothermal technique [13], and hydrolysis [14]) and dry (solid state reaction) method [15] differences in the preparative routes leads to variations in morphology, stochiometry, and level of crystallinity. Other methods, such as sol-gel [16-17], spray pyrolysis [18-19], mechano-chemical method [20], have also been developed. On the other hand recently, several researchers proposed the solution combustion method to synthesize simple, mixed oxides [21-22]. Using this method, the heating and evaporation of metal nitrate with an organic compound (such as glycine, urea or citric acid etc.). Results in self firing and generates intense heat by exothermic reaction, this intense heat is used to synthesize the ceramic powders. This novel approach has advantages of inexpensive raw materials relatively simple preparation process and a fine resulting powder with high homogeneity of low cost materials is obtained. On this basis, we prepared pure calcium hydroxyapatite ceramics and investigate the biocompatibility in vitro by using the bone marrow mesenchymal stem cells (MMSCs) of sheep. In this work ceramic powder was synthesized by according to the flow chart show in figure 1.

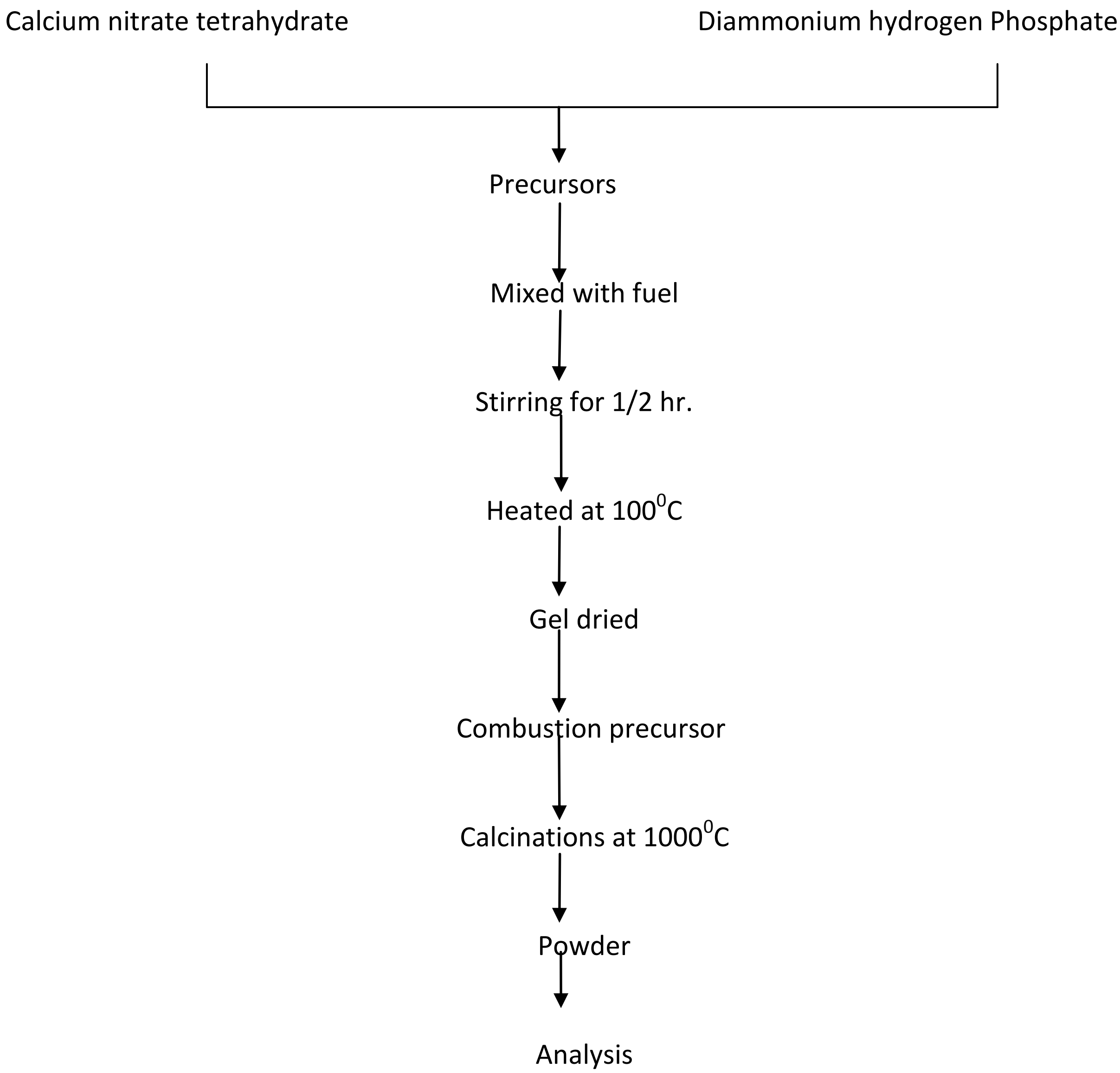
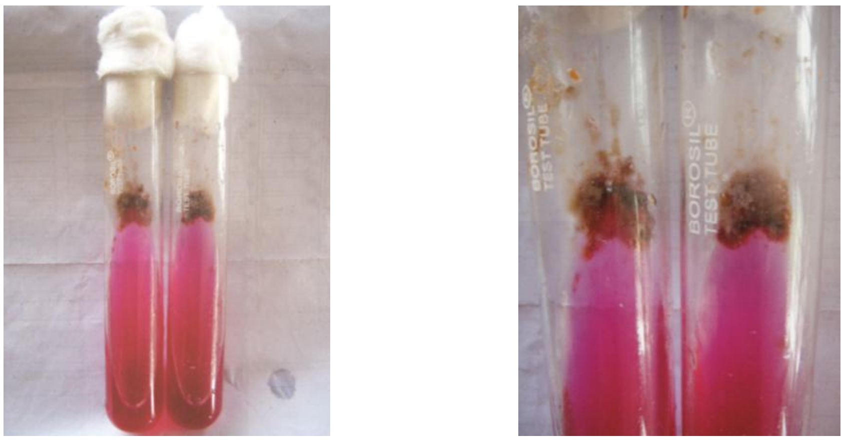


Figure 1 Flow chart for synthesis of calcium hydroxyapatite bioceramics by combustion method.

EXPERIMENTAL

For preparation of hydroxyapatite analytical grade, calcium nitrate tetrahydrate (Ca $(NO_3)_2.4H_2O$) and diammonium hydrogen phosphate $[(NH_4)_2HPO_4]$ (A.R grade) were used as calcium and phosphorus precursors, respectively. 20gm glycine ($C_2H_5NO_2$) was dissolved in 50ml of de ionized water in to this aqueous solution 10gm of calcium nitrate tetra hydrate and 3.5gm ammonium dihydrogen orthophosphate was added and stirred till all the components was completely dissolved. The solution was then heated on hot plate till the contents turned in to homogeneous paste, heating continued till it turned into white mass. Complex precursor was then heat at temperatures of 1000^{0}C for 2 hr. from above experiment, it was found that calcium nitrate tetrahydrate possesses hydroscopicity, the reactant mixture easily absorbed moisture from air to become a white slurry mixture, which was heated on hot plate and thoroughly dehydrated. The dried mixture (after termed as precursor) possesses the characteristics of combustion and can be ignited to start combustion reaction using muffle furnace.

Biocompatibility in vitro



2a 2b

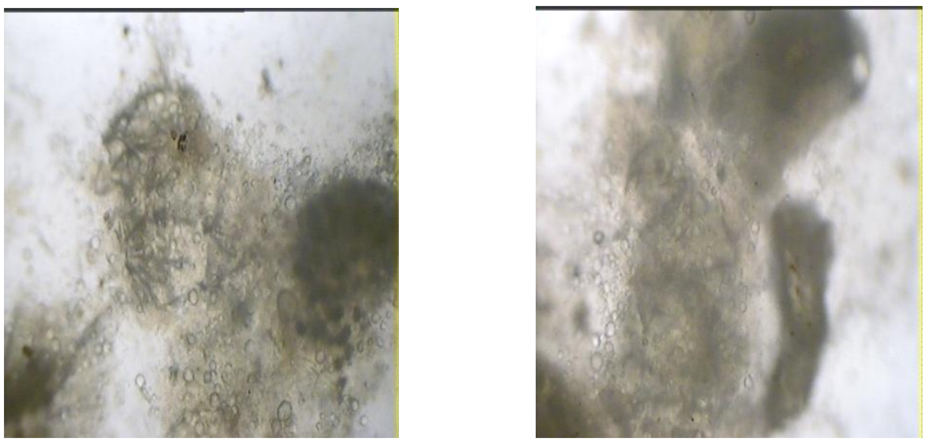
Figure 2 (a-b) Photograph of bone marrow mesenchymal stem cells implanted on calcium Hydroxyapatite ceramics.

The biocompatibility in vitro of the sintered calcium hydroxyapatite (HAp) ceramics was evaluated using microscopic methods. It is necessary to examine whether the HAp is able to support morphology of the MMSCs. Briefly, Primary bone marrow mesenchymal stem cells (MMSCs) were aspirated from the bone marrow of sheep and plated in to the 50 ml test tube. The cells were partially immersed in Dulbecco's modified Eagle medium supplemented with10% serum of same animal, 100U/mL penicillin G, 100µg/mL streptomycin (invitrogen) and 15mg hydroxyapatite (sintered powder) added on the surface of cultured bone marrow cells. The culture was maintained at 37^{O}C in humidified atmosphere of 5% CO_2 for 7 days respectively. Figure 2 (a-b) shows the bone marrow mesenchymal stem cells implanted with hydroxyapatite ceramics. At the selected time points, surface layer of culture medium was removed for observation under 40X magnification microscope. (Coslab, Model HL -9 ISO 9001:2000). Culture medium of the MMSCs without spreading the hydroxyapatite powder considered as positive control.

RESULTS

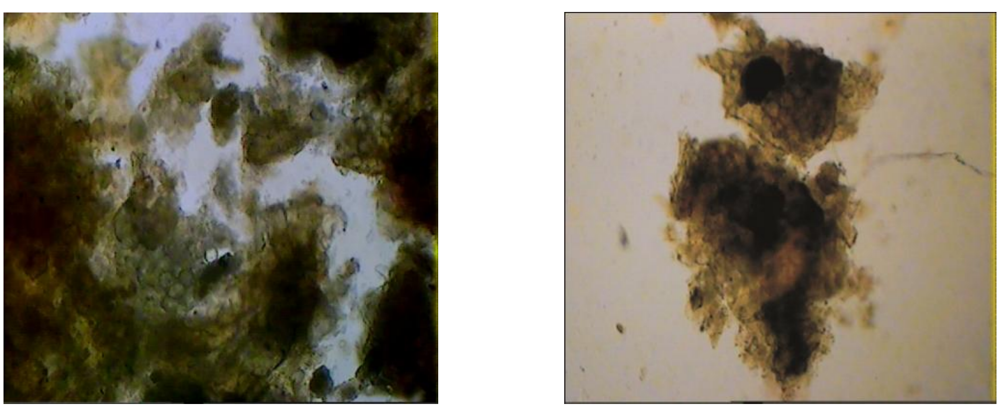
The glycine – calcium nitrate, phosphate solution on heating turned in to a uniform gel which later charred in to a black powdery precursor mass with evaluations of large amount of gases on continued heating. The yellow fumes appearing during the charring stage indicated decomposition of nitrate from the gel. Most of the nitrate sepsis from calcium nitrate turned in to oxide along with glycine ion leaving a non hygroscopic, amorphous precursor material. When the charring was not allowed to complete, the resultant product was a hygroscopic one due to incomplete decomposition of nitrate species

Biocompatibility in vitro



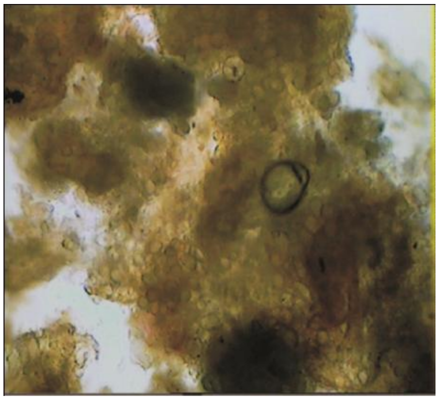
3a 3b

Figure 3: (a-b) The morphological behavior of MMSCs cells in culture medium as a positive control.

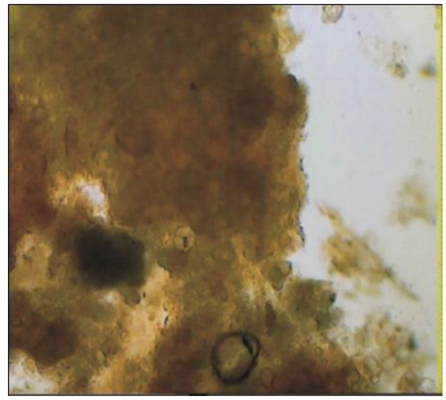


4a 4b

Figure 4. (a-b) The morphology of the MMSCs adhering and spreading on the calcium hydroxyapatite ceramics after 3 day



5c



5d

Figure5: (c-d) The morphology of the MMSCs adhering and spreading on the calcium hydroxyapatite ceramics. after 7 days.

Biocompatibility of the calcium hydroxyapatite ceramics was evaluated in vitro by observing the behavior of the MMSCs cells after incubation of 7 days. Figure 4a-4b and 5c-5d shows the micrographs of cell attachment on to the ceramics after 3 and 7 days cell culture. From the micrographs, we can see that after 3 days culture, large amount of cells adheres to the surface of sintered ceramics. After 7 days, the population of the MMSCs increases manifestly. Obviously, the hydroxyapatite ceramics constructs appeared to have no negative effect on cell morphology. The morphology of the MMSCs attached on to the ceramics was evaluated using microscope. The result indicates that the hydroxyapatite ceramic supported MMSCs adhesion which providing an indication of calciumhydroxyapatite biocompatibility property. From figure 3a and 3b shows the culture medium of the MMSCs without spreading the hydroxyapatite powder considered as a positive control, shows that no significant change occurs in concern with adherence.

CONCLUSION

Calcium hydroxyapatite bioceramics powders successfully synthesized by combustion route and in vitro biocompatibility test investigated by the effect of calcium hydroxyapatite ceramics on cell adhesion of the MMSCs. The MMSCs treated with the hydroxyapatite powder which exerts cell adhesion responses, providing an indication of hydroxyapatite biocompatibility property. These results indicate that the hydroxyapatite ceramics fulfill the basic requirement of bone tissue engineering scaffold and have the potential to be applied in orthopedics.

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