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PREPARATION, CHARACTERIZATION AND EVALUATION OF SILVER NANOPARTICLES INCORPORATED NANOHYDROXYAPATITE

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ABSTRACT

The aim of the present study is to prepare, characterize and evaluate of silver nanoparticles incorporated nanohydroxyapatite as implants for bone defects and deformation. Hydroxyapatite is a naturally occurring rare mineral, but its most common occurrence is as the main inorganic constituent of natural bone and teeth mineral. It has excellent biocompatibility, bioactivity and osteoconduction properties. In the present study hydroxyapatite was isolated from bovine bone and silver nanoparticles were prepared by chemical reduction methods. The isolated hydroxyapatite was incorporated with the prepared silver nanoparticles by varying the concentrations of binders. The crystallinity of Hydroxyapatite was confirmed by X-ray diffraction. The particle size in the range of 2-5nm is found by Transmission electron microscopy. As the Implant contains both organic and inorganic phases of the bone, with porous nature, the osteoconduction will occur by creeping substitution mechanism, which is a desirable property for an ideal bone graft.

KEY WORDS

Hydroxyapatite, Silver nanoparticles, Implants, Osteoconduction.

INTRODUCTION

The objective of the present work is to prepare silver nanoparticles and incorporated Hydroxyapatite as a bone implant for the bone defect. . Recently, biomaterials are emerging as the most studied area of materials science [1–6]. This is a new interdisciplinary branch set to achieve new and improved materials with biological properties for use in clinical applications Orthopaedic body implants utilization has tremendously increased in the present past because of traumatic condition and deformations like Musculoskeletal disorders (MSDs) resulting due to working environment e.g. computer operator (elbow), sports-tennis (elbow), badminton (el- bow), cricket (shoulder) etc and required reconstructive surgery. Bone is the substance that forms the skeleton of the body. Bones composed of calcium phosphate, calcium carbonate and also serves storage area for minerals, blood cells and calcium, playing a large role in calcium balance in the blood. Bones are largely made up of an organic matrix (osteoid) and the mineral calcium hydroxyapatite, which gives the strength and hardness to the bones. Hydroxyapatite (HAP), $Ca_{10} (PO_4)_6(OH)_2$, is one of most important bioceramics are successfully used as implants as they are chemically similar with the inorganic constituent of biological hard tissue reconstruction and replacement of affected hard tissue. This application is due to the similarity of the composition of this material with that of the mineral part of bone and tooth. [7] It is present in bone, teeth and tendons to give these organs stability, hardness and function. Hydroxyapatite is not only a biocompatible, osteoconductive, non-toxic, noninflammatory and nonimmunogenic agent, but also bioactive, i.e. it has got the ability to form a direct chemical bond with living tissues [8,9].On account of its chemical similarity with the biological calcified tissue it is remarkably biocompatible. Due to the formation of strong bond with the hard tissue, it is widely used in orthopedics or in dental implants.

HAP is also a potential implant material due to its excellent osteoconductive properties and also it stimulates osteoconduction and is a material that can be integrated into bone without provoking an immune reaction. The biological response to HAP implants is influenced by its properties. HA has the capability of stimulate bone formation via direct chemical bonding with natural human bone even-though contact site provides the room to microbe growth which is generally born due to the adsorption of proteins, amino acids and other organic substances on the surface of HA resulting in implant related infections. [10] Once these microbes colony formed, it affects the bonding between biomaterial and natural bone and implanted material gets loosened from the site and requires multiple operations. Hydroxyapatite has to be incorporated with some good antibacterial material to become prominent implant biomaterial.

Silver has been used for centuries for incorporation into wound dressing, burns and various bacterial infections. Silver binds to many cellular components, the interaction with the membrane components probably being more important than that with the nucleic acids. HA has the ability to achieve the substitution of Ca^{2+} ions with other metal ions such as Cu^{2+}, Zn^{2+}, and Ag^{2+}, without changing its initial structure and properties. [11]

MATERIALS AND METHODS

Materials

Silver nitrate, Sodium borohydride were purchased from HI- MEDIA, Poly vinyl pyrrolidine (PVP) were purchased from S.d fine chemicals and the other chemicals are of analytical grade.

Methods

Synthesis of Hydroxyapatite

The fresh bovine thigh bone was obtained from local slaughter house. Using a sharp scrapping knife the adhered tissues was removed. The adhered tissue bone was repeatedly washed with distilled water. The bone was broken and the bone marrow was removed completely. It was chopped into 1×1cm^2 lengths by using prebraker. The broken pieces of bones were immersed in chloroform and methanol in 1:1 ratios in order to remove the fats and washed repeatedly with cold distilled water. It was dried at 50°c for 1hr. It was heated on a hot plate until all the organic matter evaporated until the fumes no more evolved. The resulting charred bone was placed in muffle furnance at 800°c for a period of 5 hrs. After 5hrs the content was cooled and taken out of muffle furnance, crush using a pulvernizer and is sieved using fine mesh to obtain HA nanoparticles.

Synthesis of silver nanoparticles

Silver nanoparticles using 0.001M silver nitrate solution and 0.002M sodium borohydride. 50ml of silver nitrate solution was prepared using 30ml 0.002MSodium borohydride which was cooled and placed in the ice bath until complete addition of silver nitrate. The sodium borohydride acts as the reducing agent to isolate the silver ions. Silver nitrate solution was added dropwise into the sodium borohydride under constant stirring. The addition of silver nitrate was stopped when the solution turned light yellow colour indicating the formation of silver nanoparticles.

Preparation of Hydroxyapatite Incorporated Silver Nanoparticles

The prepared HAp is binded with Ag Np's with the help of poly vinyl pyrrolidine (pvp) as a binding agent in varying concentrations. Known concentration of HAp, pvp was taken to that 1ml of silver Ag Np 's was added and stirred for 15 minutes at 2500rpm.The resulting mixture was poured into petri dish and kept for drying at room temperature at 28°c.

MATERIALS CHARACTERIZATION

1. **Scanning Electron microscopy (SEM)**
   The prepared Ag Np's-HAp samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of gold coat over the samples were done by using an automated sputter coater (Model - JEOL JFC-1600) for 3 minutes and scanned under SEM (Model: JOEL-JSM 5600) at required magnifications.
2. **Transmission Electron Microscopy (TEM)**
   A drop of prepared Ag Np's-HAp sample is placed on the carbon coated copper grid wait for 5-10 minutes. Drain the excess Ag Np's-HAp sample with help of filter paper, washed and stained with 2% uranyl acetate. Then sample was observed under TEM at various magnifications (Model: Hitachi, H-7500).
3. **Fourier Transform Infrared Spectroscopy (FTIR)**
   FTIR spectra data was observed to find out the functional group in the sample. FTIR spectra of samples were obtained by mixing with potassium bromide and converted into pellets by pressing at 1 ton/unit. Spectral scanning was done in the range of 3500-1000^{-1}cm.

RESULTS AND DISCUSSION

1. **Scanning Electron Microscopy (SEM)**
   The morphology of Ag Np's-HAp Fig. No: 1Here, it was focused with the ZEISS equipment to get the surface morphology at different magnifications.

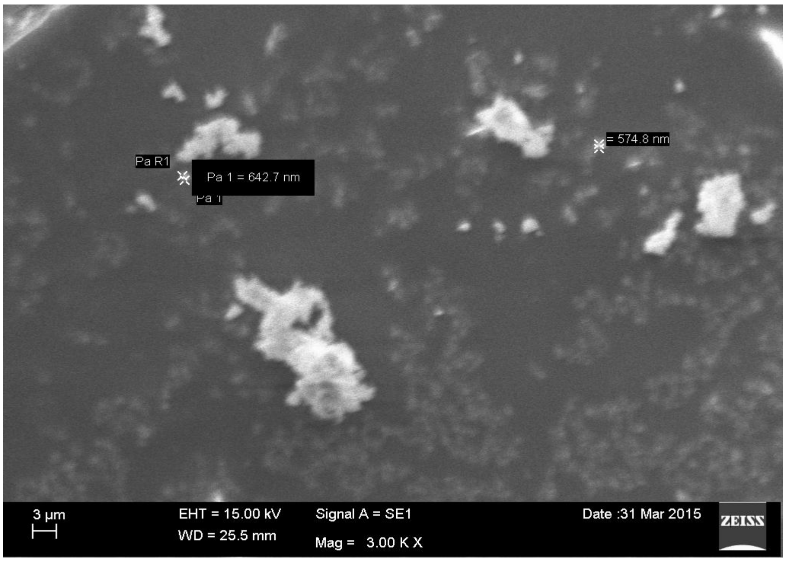

Fig. No: 1 SEM image of Silver nanoparticles incorporated Hydroxyapatite

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Silver nanoparticles incorporated Hydroxyapatite SEM image is given in the Fig. No: 1 which shows the particle size after combination in the range of 500-700nm. Few particles measurement is displayed in the SEM image which shows the particle of 574.8 and 642.7nm.

2. Transmission Electron microscopy (TEM)

The Transmission Electron microscopy of silver nanoparticles incorporated Hydroxyapatite

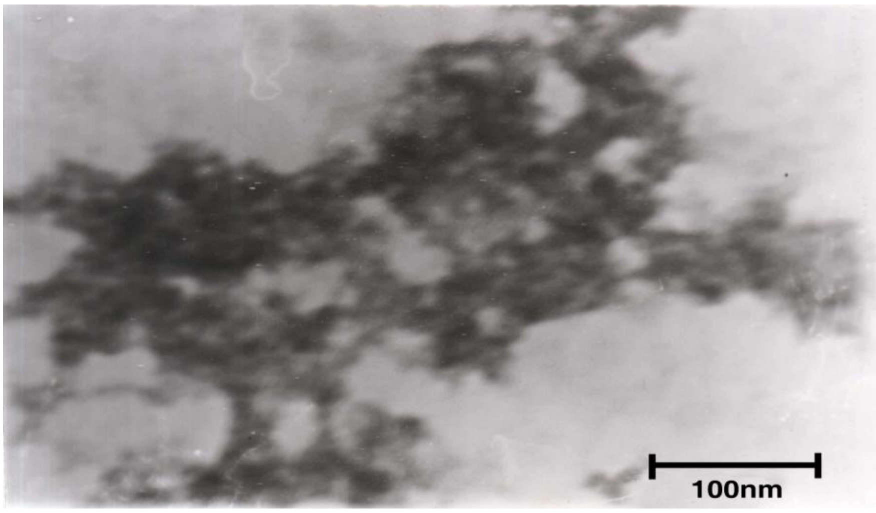

Fig. No: 2 TEM image of silver nanoparticles incorporated Hydroxyapatite

The silver nanoparticles incorporated Hydroxyapatite TEM image in Fig. No: 2 show the presence of aggregated particles in the size of about 100nm.

3. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The Fourier transform infrared (FTIR) spectra of Hydroxyapatite binded with silver nanoparticles were recorded using KBr pellet method.

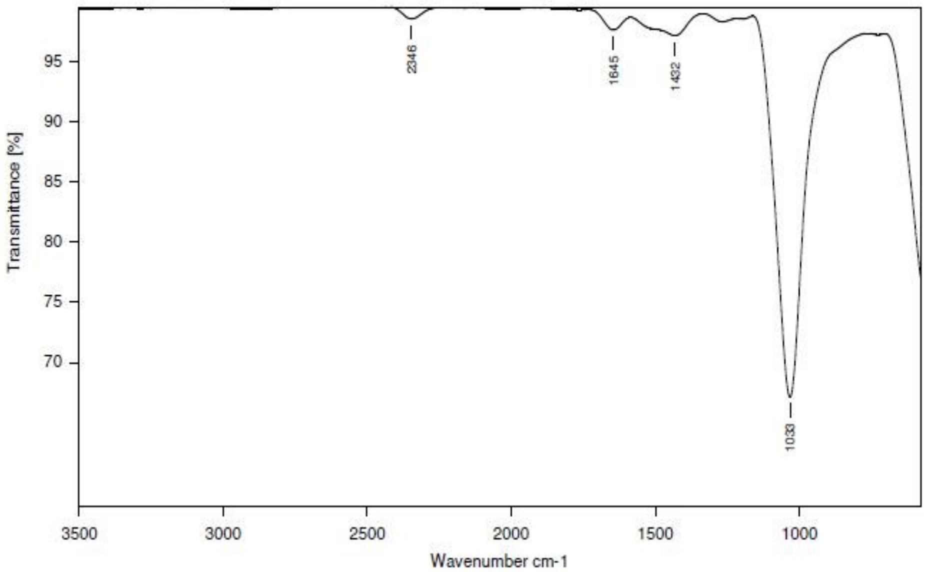

Fig. No: 3 FTIR Analysis of Hydroxyapatite- pvp- silver nanoparticles

The FTIR spectrum of pvp embedded silver nanoparticles is shown Fig. No: 3. it is observed at the absorption peak at 1658cm^{-1} represents the functional unit C = O present in pvp. A shift to 1645cm^{-1} show a decrease in the wave number of C =O bond may occur due to the bond weakening as a result of the partial bond formation with the surface silver atoms which eventually passivate the silver nanoparticles. Moreover the peaks at 1033cm^{-1} is due to C-N shift. The peak shifting corresponding to C-N bonds towards higher wave number may be attributed due to chemical coordination with silver nanoparticle due to C-N.

CONCLUSION

Bovine bone discarded as waste in the slaughter house is utilized to isolate the inorganic part as nano hydroxyapatite by heat sintering processing at 800°c for a period of 5 hours. The resultant nanohydroxyapatite was bounded with silver nanoparticles synthesized by chemical reduction method and characterized by SEM, TEM and FTIR. With the characterized results it was evident the silver nanoparticles presence can prevent infection of micro- organisms when Hydroxyapatite is used as bone filler or bone implant material.

A successful attempt for the preparation of silver nanoparticles incorporated into nanohydroxyapatite for the applications in orthopaedic and dental as implants.

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