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Research Article

Development of (SS-HAP-Ag) hybrid composite material from naturally extracted hydroxyapatite for orthopedic implants

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Abstract

In present times the HAP is of great interest among researchers because of its similar characteristics similar to human bone. The HAP can be synthesized in two ways, through chemical methods and through extraction from natural resources. The chemical route can be expensive and un-sustained for the environment while as the extraction method from natural resources can be key to make the whole process environmentally friendly. Even though HAP is similar to human bone it lacks the necessary mechanical properties which are essential for bio-implants. It is a well-known fact that Stainless Steel (316L) is suitably bio-compatible, but it also lacks the necessary Anti-bacterial properties which can be improved by addition of an alternative candidate having anti-bacterial properties such as Silver (Ag). This study proposes development of a hybrid composite of (SS316L, HAP, Ag) through powder metallurgy route with improved properties.

Keywords: *Stainless Steel, hybrid composite, HAP etc.*

1. Introduction

A biomaterial is considered as a drug-free material that is used for treatment or replacement of an organ or tissue as well as used to enhance the function in an organism[1] [2] [3]. Natural biomaterials have been recommended to be the most preferable over many reasons; they are biodegradable, having good biocompatibility and are nontoxic[4]. Biomaterials are categorized according to materials i.e; polymers, metals, ceramics and natural materials (obtained by natural sources i.e. plants and animals). [1], [5], [6]. As a single entity the metals are very rarely occurred in nature, mostly occurred in compound or ores as oxides, sulphides, and carbonates excluding the metals such as gold, and platinum. In ancient times the natural metals were used in dental application. Research has revealed that the natural products are source of new bioactive materials for the development in bio-medicine [1], [7], [8]. There are several materials derived from the natural sources such as animal or plant which are considered as biomaterials thus named as natural biomaterials. natural materials for implants are preferred as they have similarity and are compatible to body systems. Problems associated with toxicity are faced by the synthetic materials while natural materials do not cause the toxicity problems that is why the field of biomimetics is rising[9].

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Hydroxyapatite (HAP) having chemical formula (Ca_{10} $(PO_4)_6$ $(OH)_2$) is the ceramic material based of calcium phosphate which is preferably used for orthopedic and dental repairs due to its similar properties to human bone. Synthetic hydroxyapatite is fabricated by various techniques including dry methods (solid-state and mechano-chemical), wet methods (chemical precipitation, hydrolysis, sol-gel, hydrothermal, emulsion, and sono-chemical), and high temperature processes (pyrolysis and combustion) [10]. natural HAp is non-stoichiometric as compared to synthetic HAp, since it contains trace elements such as Na^+, Zn^{2+}, Mg^{2+}, K^+, Si^{2+}, Ba^{2+}, F^-, and CO_3^{2-} which resembles the naturally extracted hydroxyapatite to the chemical composition of human bone[11].

In the modern development, researchers are in attention to HAp due to its outstanding biological properties such as osteo-conductivity and bonding efficiency in body without causing any toxic effects. However, the poor mechanical properties such as fracture stiffness and fragility of pure HAP limits their application in implants so various metal ions(Zn, Ag, Ti, Ce, etc.) and reinforced with various materials such as silica, zirconia, TiO_2, Fe_3O_4 and Stainless steel etc in order to achieve better mechanical strength [5], [6], [7] (Zn, Ag, Ti, Ce, etc.)

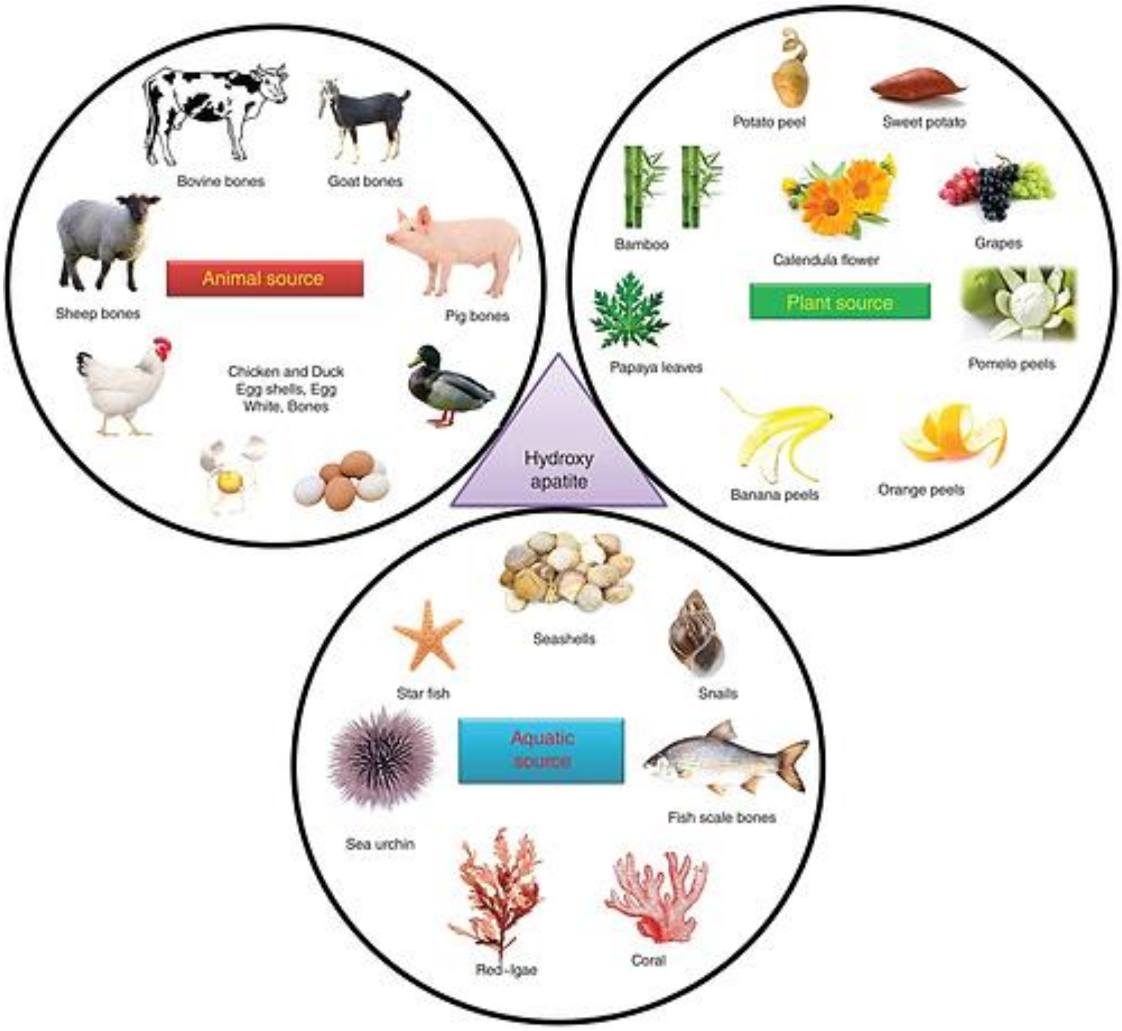

Figure 1: Natural sources for hydroxyapatite (HAp) synthesis: top left (Animal source), top right (Plant source) and bottom (Aquatic source) [12]

Metallic biomaterials are extremely effective for Orthopedic applications i.e, Bone repair ,fracture fixation, etc [13]–[15]. This is because of their improved strength, toughness, and durability[16]–[18]. With the increase in population the demand is increasing for metallic biomaterials with improved mechanical properties [19].

Representative metallic biomaterials are categorized in groups i.e. stainless steel (316L), cobalt and titanium alloys[20]–[23]. The collective demand is that the material should have similar characteristics as human bones [24], [25]. plates, rods, pins and screws are the frequent designs for the implants [32-34]. United States Food and Drug Administration (FDA) approves these metallic biocompatible implants which are to be used in orthopedic practices[26]–[28]. However, the synthetic materials are found inadequate due to their bio-functionality so that the materials with improved biological and mechanical biocompatibility would be mush credible [25], [29]–[31]

The antibacterial activity can be improved by incorporation of supplementary powders within its structure, with promising outcomes in reducing the risks of infections associated with post-implantation [32]–[34], The incorporated silver within the HAP improves its antibacterial properties. Silver is incorporated with different methods to improve antibacterial properties to HAP-based ceramic or composites [35], [36]'. In HAP ceramics, the silver is usually used as dopant (in ionic form) and named as 'silver-doped hydroxyapatite' an alternative term used for these materials is 'silver-substituted hydroxyapatite' as well as 'hydroxyapatite – silver composites'[37], [38],

2. Materials and Methods

Stainless steel (SS-316L), naturally extracted hydroxyapatite and silver powders were used for development of hybrid composite. Natural extraction of Hydroxyapatite was done by Caprine bones purchased from one of the butcher shops at Hyderabad, Sindh. The chemicals Acetone (CH_3COCH_3, 99%), Sodium hydroxide (NaOH, 99.0 %), hydrogen peroxide (H_2O_2, 30.0%), Eosin methylene blue agar (EMB) of analytical grade were purchased from local market. Deionization water (DI) was used during the extraction of hydroxyapatite from caprine bone.

Table 1: The materials and chemicals used in this research

S.no	Materials/ Chemicals	Purity Level (%)
1	Stainless steel (SS-316L)	99%
2	Silver (Ag)	98%
3	Acetone	99%
4	Hydrogen-peroxide	30%
5	Sodium-Hydroxide	99%
6	Eosin methylene blue agar	-
7	Bones	-
8	DI water	-

Cleaning

Initially, the bones were vigorously cleaned several times with tap water and boiled for 3-5 hours to remove the muscle attachments. For further elimination of attached impurities, these bones were treated with acetone and sodium hydroxide solution.

Alkaline method

For immersion of bones, a solution containing 10 percent of NaOH was prepared. Bones were immersed for 24hours in a solution. then bones were washed with purified water several times to keep their pH as neutral before calcination. Bones were very fragile after chemical treatment and these were processed into powder form without using the method of milling.

Calcination

The bone powder was then calcinated at 1200°C in the electric muffle furnace for 5hours to obtain hydroxyapatite.

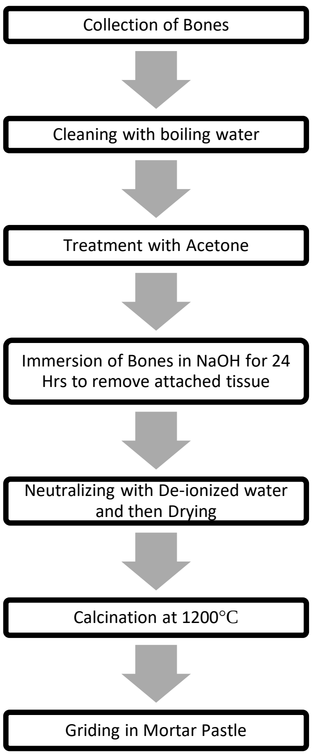

Figure 2: Flow chart of the process of HAP synthesis

Fourier Transform Infrared Spectroscopy (FTIR)

In order to find the existence of functional groups, Fourier transform infrared was performed by (KBr) method. Briefly, the HAP powder was ground with KBr powder and was finally pressed to make the KBr pallet. This prepared pallet is then scanned with the range of 400cm^{-1} to 4000cm^{-1}. The obtained pattern was evaluated to locate the characteristic peaks of the HAP. Figure 3 shows the obtained FTIR pattern. The bands at 1000-1100 cm^{-1} and 560-609 cm^{-1} are attributed to phosphate groups in the said pattern, 1043 and 609 cm^{-1} being the strongest bands of phosphate. The bands observed at 1460-1530cm-1 and 870-880cm-1 are of the carbonate group, out of these the bends at 1417 and 873 cm-1 being the strongest. The broad absorption bends at around 3456-3775 cm-1 are due to the OH groups. After careful investigation of the obtained FTIR spectra it can be said that the HAP has been extracted from natural source successfully.

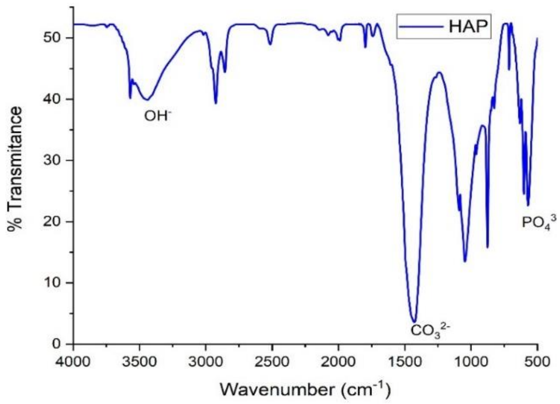

Figure 3: FTIR spectra of synthesized HAP

X-Ray diffraction (XRD)

To evaluate the phase and crystallinity of synthesized HAP, X-Ray diffraction (Model: Bruker-X8) was used. In this study, the XRD source of Cu K(alpha) radiation with 0.154 nm wavelength was used to analyze the geometry. Samples were scanned using a current of 40 kV at diffraction angle of 10-45° with 1°/minute scanning speed. The obtained diffraction pattern was compared with the standard diffraction pattern file 00-009-0432 for recognition of HAP phases. The XRD pattern of prepared hydroxyapatite are shown in Figure 4. Characteristic peaks of Hydroxyapatite are clearly visible in the pattern. The obtained pattern reveals that the HAP spectrum has close similarities with the standard XRD spectrum known as "JCPDS card No.09-0432". The major sharp intensity peaks detected at 31.8°, 32.2° and 32.9° 2theta respectively are attributed as hkl (211), (300) and (112) are recognized as hydroxyapatite, verified by the Powder Diffraction File (PDF) standard pattern 09-0432. It is possible to relate the low amplitude of the peaks to evolving crystallization and even to small particle size.

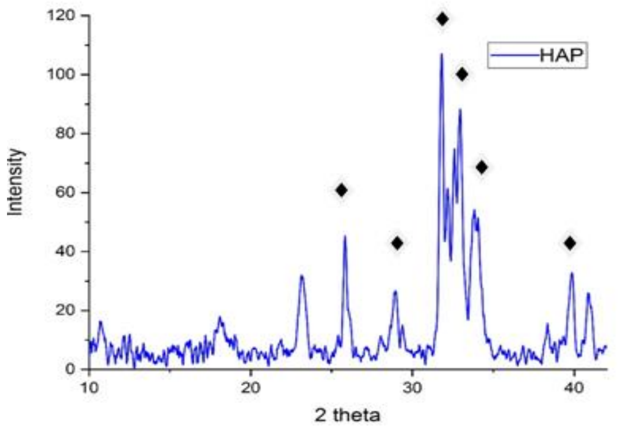

Figure 4: XRD pattern of synthesized HAP

Hardness

The prepared composite samples were analyzed with Shimadzu HMV-G31S micro-vicker's hardness tester. The obtained micro-vicker hardness are summarized in the table below:

Table 2: Shows the micro-vicker hardness values of the different prepared composites

Sr. No.	Sample	HMV
1	10% HAP	231
2	15% HAP	216
3	10% HAP + 1% Silver (Ag)	243
4	15% Hap + 1% Silver (Ag)	238

Antibacterial studies

Using the disc diffusion process, the anti-bacterial behavior of the developed (SS-HAP-Ag) hybrid composite disc was investigated. Following the standard practices, EMB (Eosin methylene blue) agar was poured for micro - organisms cultivation and dispersed uniformly into ultrasonically cleaned glass petri dishes. similarly Escherichia Coli, (E.Coli) KCCM 40880, gram-negative bacteria was distributed onto plates containing nutrient by spread plate process, and was incubated in Trypticase soy broth (TSB) for 24 hours at 30°C. A hydraulic press was used to prepare 2 disc samples with a diameter of 10 mm. The developed HAP discs were put inside the Petri dish and incubated for 24 hours at 30°C. After that, A ruler was used to study, and weighed the transparent region around the disc.

Zone of inhibition (Anti- bacterial response)

The anti-bacterial activity of the prepared composite is shown in Figure 5 below. The figure shows a transparent region around the hybrid composite disc which is evidence of antibacterial activity. The clear-area region's diameter was measured by a ruler[39]. The transparent region around the disc had a diameter of about 23mm.

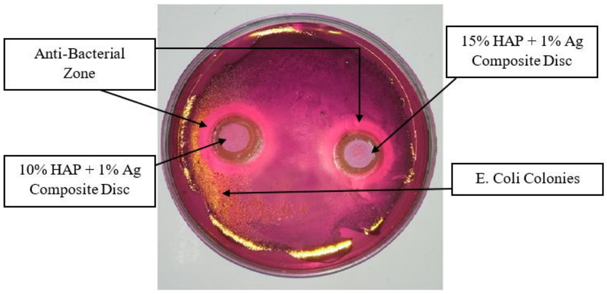

Figure 5: Zone Inhibition

Conclusion:

Naturally extracted hydroxyapatite derived from caprine bone was confirmed by FTIR and XRD. Stainless steel was utilized in as received condition. Three compositions of hydroxyapatite and stainless steel were developed with 5%, 10% and 15% of hydroxyapatite while remaining included stainless-steel grade 316L. hardness of the samples was carried out with micro vicker testing machine. It has been observed that increasing hydroxyapatite was increasing the porosity due to that the ratio of hydroxyapatite effected the hardness of the composite.

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