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Synthesis of Hydroxyapatite Crystals from Egg Shells

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ABSTRACT

The aim of the review is to raise awareness to synthesise hydroxyapatite (HAp) crystals from eggshells. The development of hydroxyapatite crystals shows a systematic approach towards the development in various biomedical applications. The economic approach of the synthesis of crystalline hydroxyapatite crystals from the eggshells is done using ultrasound assisted technology. The study demonstrates the use of biowastes such as egg shells which is a promising technique to develop hydroxyapatite crystals due to its mechanical and osteogenic properties. The hydroxyapatite crystals are synthesised using biowastes such as egg shells which are rich in calcium and ammonium dihydrogen phosphate. This is a viable substitute for bone regeneration and orthodontic appliances due to its properties such as fracture toughness, mechanical strength, elastic modulus, osteoconductivity, bioactivity and differentiation. These minerals are similar to the inorganic content of the bone and are used widely for complete or partial bone augmentation, filling bone and teeth. They are used as coatings in orthopaedics and dental implants. The organic components of the matrix of the egg shells are mainly composed of mucopolysaccharides, proteins, mainly composed of chondroitin sulphate A and B, glucosamine, galactosamine, galactose, mannose, fructose and sialic acid. They are widely used as the matrix fibres have a strong influence on the mechanical strength of the entire egg shells. These crystals are characterised for bone tissue engineering due to the following properties such as biocompatibility, bioactivity and other mechanical properties without getting rejected by the immune system.

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INTRODUCTION

Hydroxyapatite (HAp) crystals is a biomaterial that plays a vital role in hard tissue repair and bone tissue engineering, mainly due to its osteoconductivity and bioactive property (Poinern *et al.*, 2013). Systematic approaches are developed using the advent of nanotechnology. There is an urgency to develop such biomedical devices that are significantly used in bone regeneration implant procedures (Jiang *et al.*, 2013; Poinern *et al.*, 2013). Moreover, they are widely used for various industrial applications such as catalyst support, liquid chromatographic column, lighting materials, powder carrier, sensors, ion conductors, retardent of cancer cell and drug delivery agent etc (Chen *et al.*, 2010a; Kim and Park, 2005). In case of the mineral phase of the bone tissue, about 69% of the total weight is occupied by hydroxyapatite crystals with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. The egg shell is a three layered biowaste, namely cuticle, spongious layer and lamellar layer (Wang *et al.*, 2006). The cuticle constitutes the outer surface and mainly contains proteins while the spongeous and lamellar layer forms a matrix together, which contains protein fibres bonded to calcium carbonate Crystals (Yoshimura and Suda, 2017). Scanning electron microscopy(SEM) analysis, shows that the matrix fibres not only surround the calcite crystals but also pass through the crystals. Hence it is noted that the matrix fibres have a strong influence on the mechanical strength of the entire egg shell (Krishna *et al.*, 2007).

The organic components of the matrix involve complexes such as of mucopolysaccharides, proteins, mainly composed of chondroitin sulphate A and B glucosamine, galactosamine, galactose, mannore fructose and sialic acid (He and Huang, 2007). The egg shells represent a total of 11% of the entire weight of the egg which is composed mainly of calcium carbonate (94%), calcium phosphate organic matter (4%) and magnesium carbonate (1%) (Ozdemir *et al.*, 2013).

Many industries consider the egg shell biowaste as an industrial residue that would contribute to air pollution due to the microbial action on it whereas only some eggshells have been used as a fertilizer because of its high content of calcium and nitrogen. Certain studies have reported about the conversion of eggshells into a man and animal foodstuffs. The procedure involves desiccating the egg shells until they become the final debris and it is heated in a heat chamber at 80°C to minimize the pollution and eliminate most of the organic components (Rivera-Munoz, 2011). In most biological systems, the HAp is represented as the inorganic content of the bones and teeth (Ngiam *et al.*, 2011). Hence, attempts have been made to synthesise such bioactive minerals using novel techniques.

Many current research is going on in the dental field with great outcomes. Recent studies have shown that there is an equal risk for the mother and foetus with oral infections due to the lack of oral hygiene (Basha *et al.*, 2018). Certain studies have reported that the anthropometric measurement is the most reliable indicator in determining the width of maxillary anterior teeth (Ariga *et al.*, 2018). Several studies have reported the need to evaluate the non original abutments to implants at the implant abutment junction (Duraisamy, 2019). There is a high requirement for the marginal discrepancy in CAD/CAM processes of the all ceramic complete veneer crowns before luting with resin bonded agents (Ganapathy, 2016). It was reported that marginal discrepancy was more in the incisal region than in cervical regions of the ceramic laminate veneers (Jain *et al.*, 2017). Recent research studies have reported that by wearing removable acrylic resin partial dentures, the periodontal problems are mostly affected when the teeth remain in contact with the resin base (Jyothi *et al.*, 2017; Kannan and Venugopalan, 2018). There are studies on the abutment screw loosening, which has been a common clinical mishap affecting various implant procedures (Ganapathy *et al.*, 2017). Recent advances in various fields of research studies deals with subjects leading to the awareness of the ceramic restorations particularly in the rural regions and the treatment for patients with bone defects respectively (Ashok and Suvitha, 2016; Ashok, 2014). Hydroxyapatite apatite crystals derived from eggshells can also be evaluated and tested in future for various aspects of implant dentistry.

Ceftaroline exhibits low potential and provides microbial resistance, thereby reducing the skin and soft tissue infections (Selvan and Ganapathy, 2016). Several studies had investigated that the aloe vera is widely used to prevent stomatitis, oral submucous fibrosis, obturation and pulpotomy of primary teeth, bleeding and painful gums etc (Subasree *et al.*, 2016). It has been found that there is an increase in the amount of studies dealing with the management of cellulitis caused because of *Streptococcus aureus* (Vijayalakshmi and Ganapathy, 2016). In the field of the facial prosthesis includes the use of silicone elastomers for good esthetics (Venugopalan *et al.*, 2014). Actions of this newly derived hydroxyapatite crystal against various potential oral microorganisms and their clinical effects should be evaluated.

Experimental Procedure

Thermal Technique

This procedure is a novel technique which comprises the synthesis of hydroxyapatite crystals using biowaste for biomedical applications (Ngiam *et al.*, 2011; Kolanthai *et al.*, 2016). After mechanically cleaning the egg shells, they are placed in an oven for a two-stage thermal treatment. In the first stage, the egg shells are heated to 450°C / 2hrs wherein the organic residue is destroyed. In the second stage, the samples are heated to 900 degree

Celsius for 2hrs, wherein the egg shells develop into calcium oxide form by freeing the carbon dioxide according to the following reaction: $CaCO_3$->CO_2+CaO (Kolanthai *et al.*, 2016). Later the CaO obtained is transformed into hydroxyapatite crystals in a phosphate solution. These are later kept in a container designed to produce a moist atmosphere. It is then heated to 1050°C for 3hrs and the solution is filtered and the resultant material is dried overnight at 80°C in an oven (Li *et al.*, 2016).

The concentration of the crystalline HAp through this technique can be improved by optimizing the composition of the phosphate solution. The final product is characterized by X-ray diffraction and SEM. During the X-ray diffraction technique several species with irregular diameter, mechanical strength are seen along with a porous white colour texture of the bio mineral during the thermal processing technique at 1050 degree Celsius.

Ultrasound Method

Nanosized hydroxyapatite crystals were produced using ultrasound mediated chemical techniques. The morphology and the subsequent crystalline structure of the nano-HAp powders were influenced by ultrasonication (Ramesh *et al.*, 2012). It was proven that the presence of ultrasound during the synthesis of HAp promoted the chemical reactions and produced the ultra fine HAp particles. The ultrasound assisted synthesis technique produced nano crystalline hydroxyapatite with the same crystalline structure and morphology (Muralithran and Ramesh, 2000).

This technique was found to be the most appropriate procedure as the biomaterial obtained had the following properties like compression strength, elastic modulus, fracture toughness (Kannan *et al.*, 2006). It is also used in the synthesis of such biomaterials in the liquid phase. During the procedure, microjets are created which promote chemical reactions and physical effects including the generation of large numbers of cavitation in the liquid phase to show a decreased level of agglomeration (Akram *et al.*, 2014).

Enamel and Carbonated Calcium Hydroxyapatite Crystals

According to the studies, the economic approach of synthesising nanocrystalline HAp using ultrasound assisted technique using recycled egg shell is referred to as EHAp. While the ultrasound mediated chemical synthesis of HAp using commercially viable calcium hydroxide and ammonium dihydrogen organo-phosphate is referred to as CHAp (Krishna *et al.*, 2007).

The nanocrystals of HAp of the EHAp and the CHAp were observed to have spherical morphology with uniform size distribution (Sun *et al.*, 2007; Fujishiro *et al.*, 2007). According to the studies, the in vitro bioactivity and the biocompatibility study of the EHAp and the CHAp proved that the scaffolds facilitate the cell attachment and proliferation. The research articles showed that the EHAp and the CHAp promote high levels of bioactivity, osteoconductivity and cell differentiation. They don't affect the shape and growth of the cells suggesting that they can be widely used for biomedical application (Li *et al.*, 2015; He *et al.*, 2015).

Biomedical Approach

The synthesis of crystalline hydroxyapatite crystals in a novel technique has led to the development of many biomedical applications (Salgado *et al.*, 2004; Gómez-Morales *et al.*, 2011). The biomaterials synthesised from bio waste such as egg shell waste are used to mimic various biomedical appliances (Gómez-Morales *et al.*, 2011).

In Bioceramics, these bioactive materials have been widely termed for regenerating the bone tissues directly when placed in apposition without intervening the fibrous tissues of the body. They are commonly used as bone grafts, fillers and as coatings for metal implants. They are characterised for bone tissue engineering due to the properties such as biocompatibility, bioactivity and other mechanical properties without getting rejected by the immune systems (Ingole *et al.*, 2016).

They are non-toxic, targeted drug delivery systems and simultaneously eliminate toxic and harmful side effects of the healthy tissues (Dorozhkin, 2010; Nudelman and Sommerdijk, 2012). The hydroxyapatite crystals is a vital component for the vertebrate bones and teeth (Chen *et al.*, 2010b; Dorozhkin, 2010). They are widely used for bone substitutes or replacement substitutes including complete/partial bone augmentation, filling bones and teeth or used for coatings in orthopaedics and dental implants (Roul *et al.*, 2013; Uskoković and Desai, 2014).

CONCLUSION

This review could highlight the various methods used in the synthesis of hydroxyapatite crystals from natural egg shells and its biomedical application in dentistry. Hydroxyapatite crystals are bioactive and biocompatible biomaterial, which is synthesised through a novel approach by utilising biowastes such as egg shells waste which is a high source of calcium and phosphate. They are widely used as substitutes for hard tissue repair and work efficiently as a novel drug delivery system. It has a white crystalline, porous structure with defining properties such as osteoconductive, osseointegration, cell differentiation, mechanical strength, compression strength, elastic modulus, fracture toughness and biocompatible. The hydroxyapatite mineral is similar to the inorganic content of the bone. In recent advances they are used widely in the complete or partial bone augmentation, filling bones and teeth, also used as coatings in orthopaedics and dental implants. The utilisation of the biowaste for the synthesis of the hydroxyapatite minerals helps in revitalising the Earth, thereby using the recycled mass in large quantities for a sustainable future. The novel biomedical approach to synthesise hydroxyapatite crystals is by using complex, comprehensive techniques such as ultrasound mediated chemically synthesised nano HAp powders and the thermal technique which requires high energy mechanochemical activation for the synthesis of hydroxyapatite crystals. The systematic approach to develop hydroxyapatite crystals using recyclable wastes has remarkably produced a positive impact on the sustainable environment. Therefore awareness has to be provided for the novel synthesis of such biomaterials for enhancing various substitutes for biomedical applications.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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