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Investigation on Citric Acid-based Nano Hydroxyapatite Composite for Dental Bone Graft

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ABSTRACT

Citrate is a well-known kreb cycle product, makes up around 90% of the total content is located in the skeletal system. Hydroxyapatite (HA) is the most widely accepted biomaterial for the repair and reconstruction of bone tissue defects. It is biocompatible, bioactive, osteoconductive, non-toxic, non-inflammatory, and non-immunogenic. Periodontal regeneration is defined as the restoration of lost periodontium or supporting tissues and includes the formation of new alveolar bone, new cementum, and periodontal ligament. Initially, the majority of synthetic materials were based on calcium phosphates, such as HA and beta-tricalcium phosphate, because of their ability to replicate the native mineral constituent of bone tissue. Although biomimetic and osteogenic, their applications are severely limited when fabricated into porous structures due to the inherent brittleness and very slow degradation rates. To improve their utility, the hybridization of bioceramics and biodegradable polymers has been widely adopted to reform the mechanical properties and bioactivity of the resulting materials. Thus, a scaffold was prepared, that is HA was incorporated in the elastomeric polymer (poly-1,8-octanediol), which contains citric acid as the additive factor, to enhance the formation of bone and bone mineral density. This study aims to evaluate citric acid-based nano HA composite graft in the regeneration of supporting bone around the teeth over 1 year.

Key words: *Periodontitis, Bone regeneration, Citric acid.*

1. INTRODUCTION

The normal periodontium provides the support necessary to maintain teeth in function. It consists of four principal components: Gingiva, periodontal ligament, cementum, and alveolar bone.

1.1. Periodontitis

In India, National oral health survey, 2002-2003 conducted by Dental Council of India states that the prevalence of chronic periodontitis in 35-44 years age group was 89.6% and 65-74 years age group was 79.9% [1,2].

Clinical signs of inflammation, such as change in color, contour and consistency, as well as bleeding on probing, may not always be positive indicator of the ongoing attachment loss (Figure 1). This periodontal disease is presented clinically by pus formation and loss of attachment pus. This loss often is accompanied by periodontal pocket formation and change in the density and height of subjacent alveolar bone. This loss of alveolar bone height is called as bone loss or intrabony defects [1].

The treatment of periodontal diseases includes short-term and long-term goals. Short-term goals are the elimination of all infectious and inflammatory processes that cause periodontal and oral problems. This includes scaling and root planning procedures. Long-term goals are the reconstruction of a healthy dentition that fulfill all functional and esthetic requirement [1].

There are various materials used in dentistry for the reconstruction of the alveolar defects is as follows: Autografts, allografts, alloplasts, and xenografts.

Among these, the most widely used are the alloplast bone graft material.

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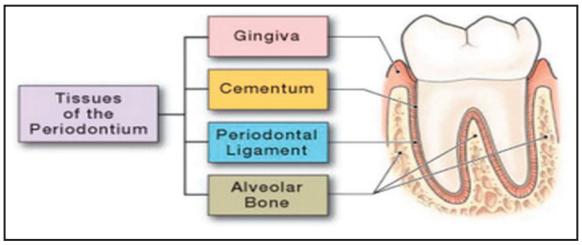

Figure 1: Tissues of periodontium. A graphic representation of the periodontium in cross section.

1.2. Alloplasts
Inorganic synthetic bone grafts; ceramic and polymers.

1.3. Ceramic Materials
Commercially available ceramic-based materials include calcium phosphates (CaPs) (e.g., tricalcium phosphate (TCP) and hydroxyapatite [HA]), calcium sulfate, and bioactive glass.

1.4. Polymers
Natural polymer: Polysaccharides (e.g., agarose, alginate, hyaluronic acid, chitosan) and polypeptides (e.g., collagen and gelatin).

1.5. Synthetic Polymers
Poly(glycolic acid), poly(L-lactic acid), polyorthoester, polyanhydride provide a platform for controlling the biomechanical properties of scaffolds as well as targeting drug delivery in tissue engineering.

In this study, we have made a synthetic bone graft using citric acid and nano HA to evaluate its effect on periodontal regeneration.

Citrate, a naturally occurring Kreb's cycle product, is highly conserved in native bone with over 90% of the body's total citrate content being located in the skeletal system. Recent research has suggested that citrate plays significant roles in bone anatomy, physiology, and orthopedic biomaterial development. Citric acid has been found as the integral part of the bone nanocomposite [3-5].

For synthetic biomaterials, research in the field has witnessed a shift from the use of permanent, inert metals toward tissue-engineered biodegradable composites designed to mimic the native composition of bone. Initially, the majority of synthetic materials were based off CaPs, such as HA and beta TCP, because of their ability to replicate the native mineral constituent of bone tissue. Although biomimetic and osteogenic, their applications are severely limited when fabricated into porous structures due to the inherent brittleness and very slow degradation rates [5] To improve their utility, the hybridization of bioceramics and biodegradable polymers has been widely adopted to reform the mechanical properties and bioactivity of the resulting materials for orthopedic applications. However, the current composite materials still suffer from several significant problems such as unsatisfactory mechanical strength, inefficient bone regeneration, poor bone integration and inability to mimic native bone chemical composition, which is composed of 60-65 wt.% HA embedded in a collagen matrix [6]. To address these limitations, we have focused on the development of citric acid-based composite materials for periodontal tissue regeneration.

2. EXPERIMENTAL
2.1. Material
Sybograft, particle size 200-300 µm, Eucare Pharmaceuticals (P) Ltd., citric acid monohydrate-ACS reagent, >99.0% (Anmol Chemicals, Mumbai) and 1,8-octanediol (Fluka).

2.2. Method
The preparation of poly(1,8-octanediol-co-citric acid) (POC): Equimolar amounts of citric acid and 1,8-octanediol were added to a 250 ml three-neck round-bottom flask fitted with an inlet and outlet adapter. Therefore, in the final product each vial contained 7.7 g of material. The mixture was melted under nitrogen gas purge by stirring at 160-165°C and then the temperature of the system was lowered to 140°C. The mixture was stirred for another hour at 140°C to create the pre-polymer solution [7].

The POC prepolymer was mixed with various amounts of HA particles to obtain composites of 65 wt.% HA by weight. Briefly, POC prepolymer was mixed with the desired amount of HA powder. The POC-Nano HA mixture was stirred until it became a uniform mass [8].

This POC-Nano HA is place in separate 10 vials, and these vials were sent for gamma radiation.

2.3. Sterilization
The samples were exposed to Co-60 gamma radiation using gamma chamber (GC)-1200 under laboratory conditions. The GC was calibrated with standard Co-60 source and the samples were kept at the middle of the sample chamber in order to get uniform dose. The dose rate of GC-1200 was 9.0 kGy/h and the samples were exposed in the total doses range from 25 to 27 kGy.

2.4. Sterility Testing
The bone graft, which was prepared, was sterilized according to the standard protocol. The sterility test to check the bacterial contamination, fluid thioglycollate media was used and for the fungal contamination soya bean casein digest was used.

2.5. Preparation of the Fluid Thioglycollate Digest and Soya Bean Casein Medium
A round bottom flask was taken and 200 ml solution of fluid thioglycollate digest and soya bean casein

medium was made (6 g of fluid thioglycollate digest and 200 ml of distilled water, 5.95 g of soya bean casein medium and 200 ml of distilled water). This solution was transferred to 6 screw capped culture tubes (3 for fluid thioglycollate digest and 3 for soya bean casein medium, 50 ml in each tube) and autoclaved at 121°C for 15 min.

2.6. Inoculations of the Bacteria and Fungus

The first tube with fluid thioglycollate digest was inoculated with *Escherichia coli*, second tube was inoculated with the sterile bone graft and third was uninoculated. This was kept at 37°C for 24 h similarly for soya bean casein medium; first tube was inoculated with candida species, second tube was inoculated with the sterile bone graft, and the third was uninoculated. This was kept at 25°C for 24 h.

3. RESULTS AND DISCUSSION

The screw capped culture tube inoculated with the *E. coli* was turbid and the one with sterile bone graft and control was clear, similar results were found for the candida species. Thus, these findings clearly show that material prepared was sterile.

3.1. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The FTIR spectroscopy is used to characterize HA and citric acid based bone graft. The frequencies and intensities of the IR bands provide relevant information on the nature of the molecular bonds, their environment, molecular conformations, and their relative content in the material being analyze [9].

The IR spectra of the HA and HA bone graft are shown in Figure 2. The characteristic band around 568 cm^{-1} correspond to O-P-O bending mode, whereas in the bone graft a characteristic band at 601 cm^{-1}, this shift in the band is due to interaction between prepolymer and HA. The doublet in the wave number range 1020 cm^{-1} was assigned to P=O asymmetric stretching mode. These bands indicate the characteristic molecular structures of the polyhedrons of PO_4^3 - in the apatite lattice. The characteristic peaks at 1722 cm^{-1} correspond to the C=O present in both HA and bone graft. Further, peak at 3426 cm^{-1} is due to −OH group present in HA, whereas in the HA based bone graft –OH band is shifted to 3458 cm^{-1}, this shift in the absorption band can be attributed for the interaction between oxygen of prepolymer and hydrogen atom of HA.

4. CONCLUSIONS

Thus, this citric acid-based nano HA composite bone graft prepared by the above-mentioned method been used for the clinical study in dentistry on humans to evaluate the regeneration of the lost periodontium over the period of 1-year.

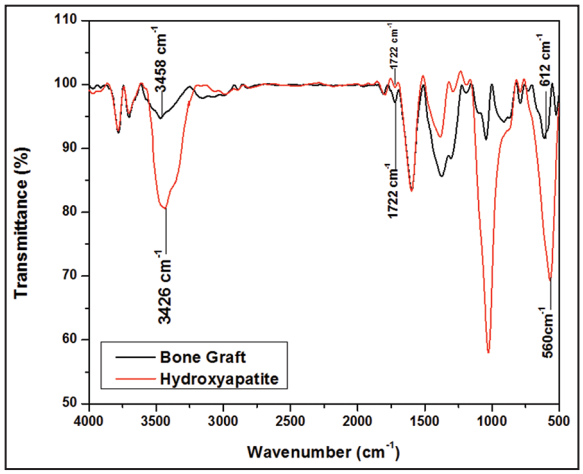

Figure 2: Fourier transform infrared spectrum of hydroxyapatite (HA) and HA bone graft.

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6. REFERENCES

1. M. G. Newman, H. Takei, P. R. Klokkevold, F. A. Carranza. (2012) Carranza's Clinical Periodontology Expert Consult, 11th ed. St Louis: Saunders/Elsevier.
2. P. S. Jacob, R. M. Zade, M. Das, (2011) Prevalence of periodontitis in the Indian population: A literature review, ***Journal of Indian Society of Periodontology,*** **15(1):** 29.
3. Y. Y. Hu, A. Rawal, K. Schmidt-Rohr, (2010) Strongly bound citrate stabilizes the apatite nanocrystals in bone, ***Proceeding of the National Academy of Science USA,*** **107:** 22425-22429.
4. R. T. Tran, (2013) Synthesis and characterization of biomimetic citrate-based biodegradable composites, ***Journal of Biomedical Materials and Research A,*** **102(8):** 2521-2532.
5. L. C. Costello, R. B. Franklin, M. A. Reynolds, M. Chellaiah, (2012) The important role of osteoblasts and citrate production in bone formation: Osteoblast citration as a new concept for an old relationship. ***Open Bone Journal,*** **2014:** 4. DOI:10.2174/1876525401204010027.
6. D. Sun, Y. Chen, T. Richard, (2014) Citric acid-based hydroxyapatite composite scaffolds enhance calvarial regeneration, ***Scientific Reports,*** **4:** 6912.
7. J. Yang, A. R. Webb, G. A. Ameer, (2004) Novel citric acid-based biodegradable elastomers for Tissue engineering, ***Advanced Materials,*** **16:** 6.

8. H. Qiu, J. Yang, P. Kodali, J. Koh, G. A. Ameer, (2006) A citric acid-based hydroxyapatite composite for orthopedic implants. **Biomaterials, 27:** 5845-5854.

9. R. A. Nyquist, C. L. Putzig, M. A. Leugers, (1997) ***The Handbook of Infrared and Raman Spectra of Inorganic Compounds and Organic Salts,*** Vol. 1 and 4. San Diego, USA: Academic Press.