Original Article

Extraction of Hydroxyapatite Obtained From Human Premolar Teeth Using Smart Dentine Grinder and Calcination Process for Biomedical Application- An In vitro Study

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ABSTRACT

Background: The present study is conducted with an aim to characterize Hydroxyapatite (HA) produced from therapeutically extracted premolar teeth using two different procedures. Hydroxyapatite was produced from premolar teeth using the process of calcination at 8500c and using Smart Dentine Grinder. The process of sample preparation influences its properties, the crystal structure and thermal stability of the produced powder was investigated using X-ray diffraction analysis (XRD) and Fourier transform infrared spectroscopic (FTIR) analysis. The former confirmed the successful production of HA crystals, while the latter confirmed the presence of hydroxyl (OH-) and phosphate (PO4-3) functional groups.

Results: The FTIR and XRD analysis graphs obtained for the samples from the two procedures were compared with graphs of Hydroxyapatite samples from previous studies.

Conclusion: FTIR and XRD analysis used to characterize the samples obtained from two procedures namely calcinations & using Smart Dentin Grinder confirmed the presence of Hydroxyapatite crystals. The procedure used has an effect on the crystalline properties of the Hydroxyapatite.

Keywords: Hydroxyapatite; Fourier transform infrared spectroscopic analysis; X-ray diffraction analysis (XRD); Calcination; Smart Dentine Grinder.

INTRODUCTION

The white spot lesion (WSL), has been defined as “subsurface enamel porosity from carious demineralization” that presents itself as “a milky white opacity, when located on smooth surfaces” [1]. In most cases, white spot lesions and enamel demineralization commonly tends to occur during orthodontic treatment and sometimes even after course of treatment [2]. This phenomenon became a clinical problem in fixed orthodontic therapy since directly bonded orthodontic brackets were introduced [3].This process of decalcification of enamel surface adjacent to the orthodontic appliances is an important and prevalent iatrogenic effect of orthodontic therapy[4]. Bonding of appliances to teeth increases plaque retention sites, since pH of plaque is low, it interferes with process of remineralization and thereby resulting in decalcification of enamel[5]. And as enamel translucency is directly related to the degree of mineralization , the initial demineralization of enamel due to low pH of plaque usually manifests clinically as “white spot lesion (WSL)” [1]. The prevalence of such WSLs is reported to vary from 4.9% to 84 % of tooth surfaces [6,7].

Mitchell,from his longitudinal study found overall prevalence of 18.5% of tooth surfaces , with average accuracy of 1.6% tooth surface area affected [8]. In study by Mizrahi E, there is increase in prevalence of WSLs i.e., from 72.3% to 84% was observed following completion of orthodontic treatment and he concluded that treatment with multibanded appliances contributed to development of new areas of enamel demineralization [7]. Further is it imperative to differentiate between carious and non-carious WSLs and in order to do that a clinician must gently evaluate the consistency and texture of the tooth surface with a periodontal probe . Commonly , carious WSLs are typically found on the buccal surfaces of beneath a thick accumulation of plaque and around the perimeter of orthodontic brackets where performing oral hygiene practice is difficult. Whereas, non-carious WSLs due to fluorosis, developmental enamel hypomineralization, enamel hypoplasia can have genetic and environmental bases [9]. Since, clearly it is the responsibility of orthodontists ’ to be aware about the risk from decalcification, precautions must be taken to avoid or limit their development. Therefore, it is important to prevent formation of WSLs, because once they are established.

It is extremely difficult or even impossible to achieve complete remineralization . And if they left untreated , they may lead to carious cavitation for which restorative treatment is necessary. Thus, treatment of WSLs is crucial to prevent tooth decay and thereby restoring the aesthetics of smile . Hence it is important to adapt measures in order to manage such lesions.

Patients with perfect oral hygiene and oral health, as the process of bleaching makes the tooth susceptible to formation of caries like lesions [12]. In severe cases , acid microabrasion is recommended , which is a minimally invasive technique based on reactivation of enamel by elimination of the hypermineralized external layer through microabrasion , followed by daily application of casein phosphopeptide-

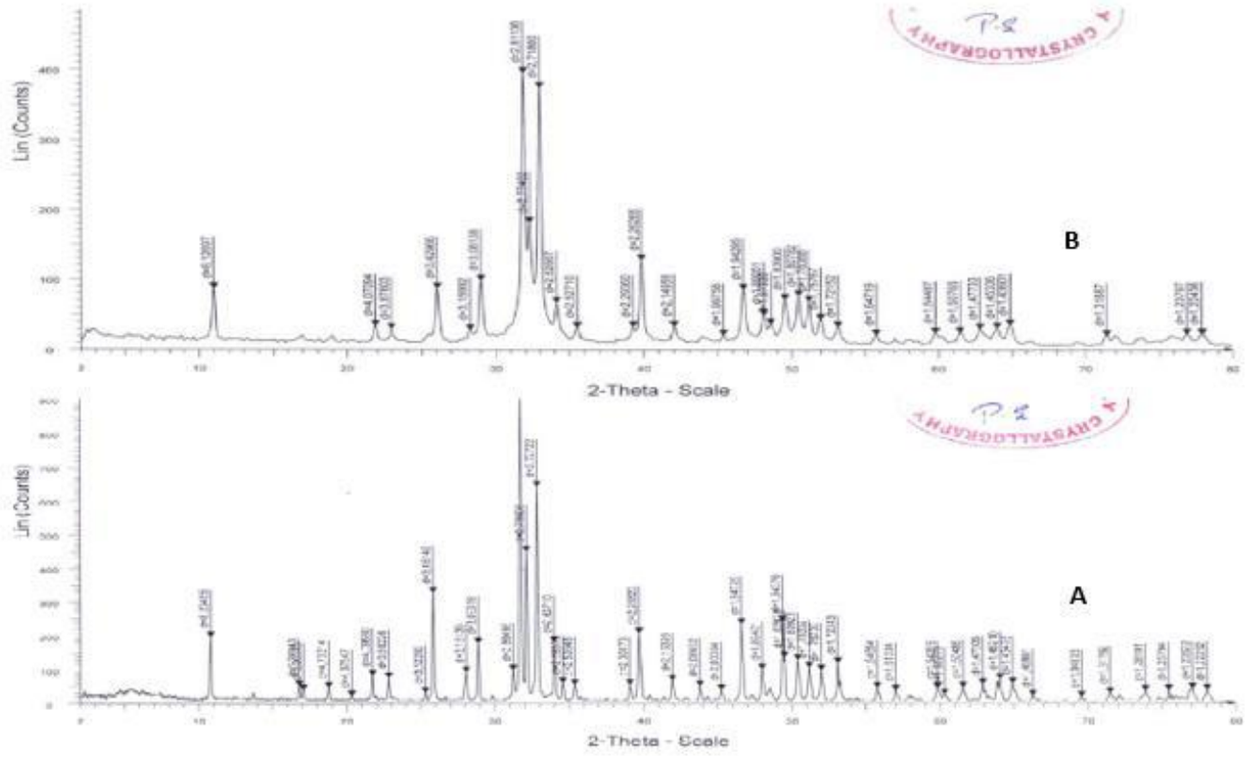

Figure 1: A typical XRD pattern (A) of hydroxyapatite powders prepared by calcination method at 850°C and XRD pattern (B) of hydroxyapatite powders prepared using smart dentine grinder.

Management of WSLs is a multifactorial approach, it begins with a good oral hygiene regime and associated with use of fluoride products such as mouthwashes, gels, toothpastes, varnishes, fluoride in bonding agents and fluoride in elastomers. Derks et al. demonstrated that use of higher concentration fluoride toothpastes and gels twice a day exhibited demineralization inhibiting tendency during orthodontic treatment [10]. Even though fluorides proved to be reliable adjuvants for remineralization, it is advised against their usage in higher concentrations as it can arrest the process of remineralization and lead to staining . On other side , remineralization potential of low fluoride concentrations has not been proved in any prospective randomized studies . Apart from fluoridation techniques , external bleaching might be a treatment of option to help camouflage WSLs and thereby obtaining better aesthetic results [11]. But the application of this therapy is restricted to amorphous calcium phosphate complexes (CCP - ACP) [13]. However, the usefulness of this technique in treatment of WSLs is mainly based on clinical case reports and hence , randomized clinical studies are necessary to support these results . In addition to both methods , last treatment of option if the patient is still in need for further aesthetic improvement is aggressive restorative treatment such as a direct or indirect restorative veneer , which is destructive approach of tooth surface.

As the structure of enamel is too complex and remodelling of it is a big challenge due to the fact that basic enamel building blocks are generally hydroxyapatite nanocrystals that are 20- 40 nm [14]. This hydroxyapatite (HA) is mainly responsible for the mechanical behaviour of the dental tissues. Unlike bone, in enamel and dentin, when HA is dissolved, spontaneously it cannot remineralize due to the fact that enamel contain no cells and moreover dentine apposition occurs towards the pulp

tissues. Therefore, the reconstruction of both enamel and dentine is more of a process of prosthetic restoration through application of alloplastic materials [15]. Hydroxyapatite is known as one of vital materials and common use in biomedical field, concentrated in clinical area and has been widely subjected to experiment as bone filler and prosthetic coating due to its biocompatibility and osteoconductivity, bioactivity, direct bonding to bone, etc [16]. The development of HA powder becomes an attractive area in line of research because of simplicity in its production. Liu et al. presented a simple and efficient method for the preparation of HA powder using heating powders of $Ca(OH)_2$, $Ca(H_2PO_4)_2.H_2O$, distilled water and synthesized HA through this method has good crystallinity, good sinterability and also possesses high surface area [17]. Hilmi and co-workers presented a method of obtaining HA from bovine bones and HA produced are highly crystalline with irregular form of particles. They further concluded that processes of defatting and deproteinase using boiling method can be used to extract HA [18]. Despite of its advantage as main synthetic biomaterial as bone filler, its ability to remineralize enamel and dentine, HA is still too expensive to be used in toothpastes and mouth washes. Lately advanced developments such as nanotechnologies opened new opportunities in obtaining cheap HA micro-nano particles by the "bottom-up" methods [19].

The aim of the present study is to apply FTIR and XRD techniques in the assessment of HA powders obtained using two different techniques i.e., HA powder obtained through method of calcination at a temperature of 850^0c and other method is through smart dentine grinder.

MATERIALS AND METHODS

Raw material Preparation: To synthesize hydroxyapatite, a total of 20 freshly extracted premolar teeth for therapeutic reasons were collected. Crown portion of teeth were separated from root and the crown portions were then cleaned and boiled in distilled water for certain amount of time to allow easier removal of organic substances and debris. Following boiling, the teeth were sun- dried for 3 days in order to avoid soot formation in the material during the process of heating [20].Raw teeth impurities found stuck to the teeth were shaved and removed, and then irrigated with a brush in running water, followed by boiling in distilled water for 30 minutes. This process was repeated till it yielded white and clean teeth. The HA powders were then synthesized by two different techniques. The sample was divided into two sets of ten teeth in each.

Calcination: The first set of ten teeth were subjected to the process of calcination in muffle at a temperature of 850^0c for one hour. It was observed that at 450^0c, huge amount of vapours evolved from the sample. To avoid any dehydration in the furnace, the sample was sintered again to 1150^0c for one hour [21].The sintering process ensures that the organics are

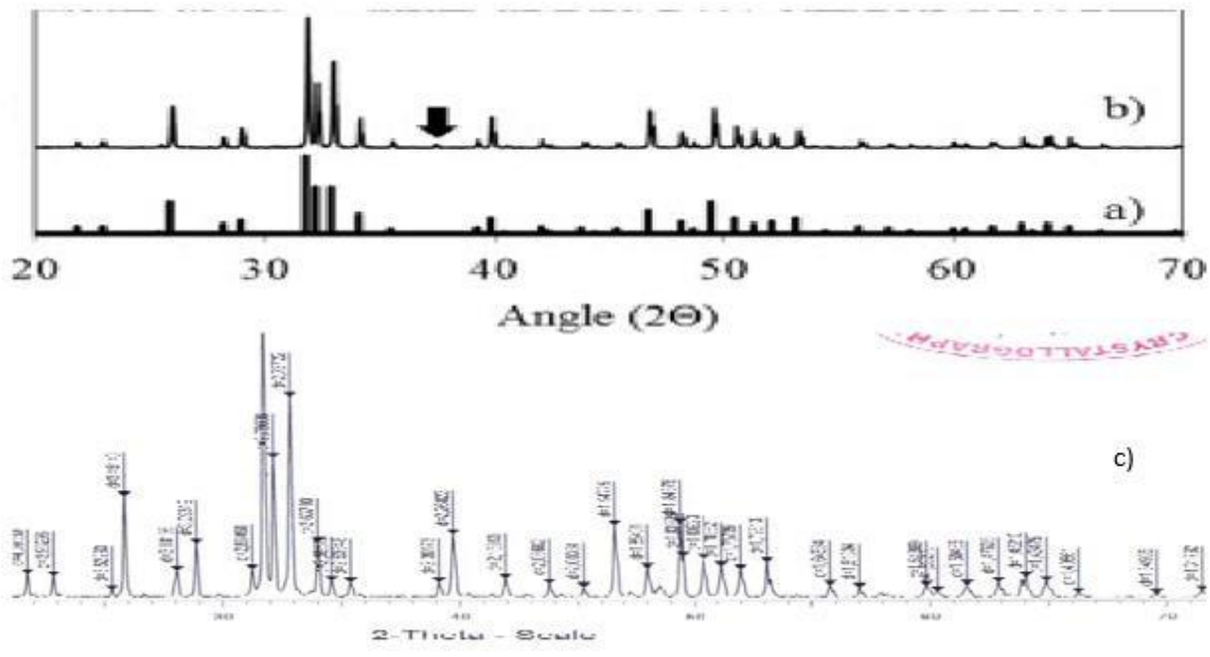

Figure 2: X-Ray diffraction patterns of (a) HA reference (JCPDS#:9-432), (b) pure HA, (c) HA from calcination at 850^0c.

completely removed and the material is safe, to avoid any microbial contamination[22, 23]. To ensure HA purification, the process was repeated till the pH of the sample in water is equal to the water pH [24].

measured between 2^0 and 80^0, with a 2-theta step of 0.0054^0. A CCD solid- state detector in scan mode was used and this mode allows to make a single scan in 16 minutes averaging 10.8 seconds per point. The powder X-ray diffraction patterns were

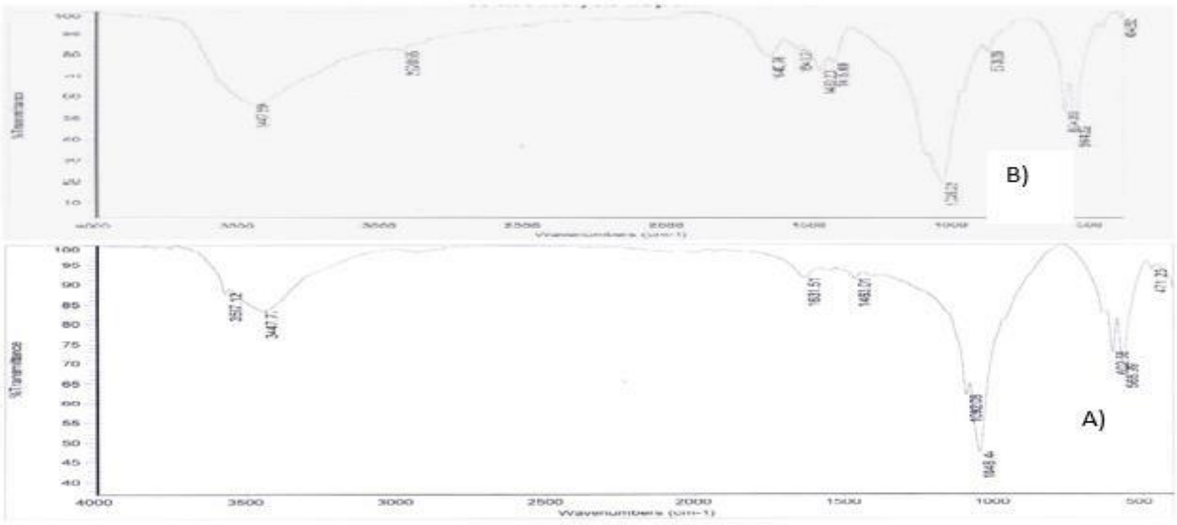

Figure 3: A typical FTIR pattern (A) of hydroxyapatite powders prepared by calcination method at 850°C and FTIR pattern (B) of hydroxyapatite powders prepared using smart dentine grinder.

Smart dentine grinder: The clean and dry tooth, mostly dentin, is grinded using a specially designed 'Smart Dentin Grinder'. It is capable to grind the tooth structure in 3 seconds and then by vibrating movement of the grinding chamber, the particles sieve to another chamber. The dentin particulate of 300-1200 µm is sieved through a special sorting system. The sorted particulate dentin is immersed in basic alcohol cleanser in a sterile container to dissolve all organic debris and bacteria. Then, the particulate is washed by sterile saline. Then this bacteria-free particulate dentin is ready for further utilization [25].

Characterization: The followed tests were performed on the sintered HA powdered particles of size less than 45 µm. The X-ray diffraction (XRD) pattern of the powders from two methods were obtained with a diffractometer with a monochromated CuKα radiation. XRD is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The average bulk composition of the material which is finely ground and homogenized, is determined. Using XRD pattern, one can determine what crystalline phases are in a mixture and how much of each crystalline phase is in the mixture. The diffraction intensity as a function of the angle 2- theta was measured with a diffractometer using the Bragg-Brentano 0 - 0 geometry with Ni-filtered CuKα radiation and a 1-dimensional position sensitive silicon strip detector [26]. The diffraction intensity as a function of the angle 2-theta was measured between 2^0 and 79.99^0, with a 2-theta step of 0.0054^0, for 13.6s per point.

Fourier- transform infrared spectroscope (FTIR) was used to determine the chemical function by the wavelength range up to 4000 cm^{-1} versus transmittance, the KBr is used as a reference to prevent fluctuations in the output [27]. FTIR is a very sensitive technique for determining phase composition and is comparatively quick and easy. It provides information about location of peaks, their intensity, width and shape in the required wave number range.

RESULTS & DISCUSSION

Enamel is the hardest mineral structure in human body and its structure can resist various environmental insults, mechanical injuries, abrasion, chemical attacks etc. However, unlike other mineralized tissues, it lacks proteins as the matrix proteins of enamel are cleaved by proteinases during tooth formation[28]. As oral hygiene is difficult to maintain in patients with fixed orthodontic appliances, decalcification of enamel surface occurs adjacent to them and are manifested as white spot lesions, which in turn progress to carious cavitations and rises aesthetic

problems [4]. The crystal phase and molecular structure of the typical sample were characterized by XRD and FTIR.

(Figure 1) showed that the HA produced from calcinations at 850^0c is only found in high crystalline phase product. The XRD pattern of HA powder through (A) calcination and (B) smart dentine grinder were shown and compared, within the 2-theta range from 20^0 to 90^0. The Bragg peaks at 11, 17, 19, 22, 23, 26, 28, 29, 32, 33, 34, 35, 40, 45, 47, 48, 49, 50, 51, 52, 54, 60, 62, 63, 65 up to 79^0 observed for HA powder produced from calcination at 850^0c. For smart dentine grinder, Bragg peaks were observed at 11, 22, 23, 26, 29, 31, 32, 33, 35, 40, 47, 48, 49, 50, 51, 52, 53, 56, 62, up to 78^0 respectively. XRD analysis also indicated the absence of secondary phases, such as TCP or calcium oxide (CaO). The peaks evident from the diffraction patterns of calcinations at 850^0c resulted in high crystallinity than HA from smart dentine grinder, indicated that the material from former method is pure HA. The XRD pattern of the HA powder from calcination process, showed more number of peaks than HA powder from smart dentine grinder. Moreover, the lower intensity in the latter indicates a poor crystalline HA phase or reduced crystallite dimension.

The XRD pattern of the HA powder from calcination is then compared with X-ray diffraction patterns of HA reference (JCPDS #:9-432) and pure HA (Figure 2). showed the XRD patterns of reference patterns and pattern from HA through calcination process. It was clearly observed that all the samples had specific peaks of HA when compared with HA reference pattern . Xiaoying et al. conducted a study to find out the effective method to prepare natural HA from different sources like pig bones , pig teeth and natural human teeth . From the spectrum of the prepared material , obtained by means of XRD produced consistent results with JCPDS index of HA and confirmed that material prepared is HA indeed. Moreover, they also concluded that the natural HA obtained by calcining at 850^0 c showed desired results [29]. The findings in our study further confirms that calcination at 850^0 c is a suitable preparing temperature for the production of natural HA.

The spectrum of FTIR of calcined product shows characteristic peaks of HA that generally indicates the existence of phosphate (PO_4^{-3}) ions, hydroxyl (OH^-) and carbonate (CO_3^{-2}) ions [30]. (Figure 3 showed the IR spectrum of the samples produced by calcination as well as smart dentine grinder. The FTIR spectrum of standard HA shows peaks for OH^- ions ($3570cm^{-1}$ and $634cm^{-1}$), peaks for PO_4^{-3} ($1090cm^{-1}$, $1040cm^{-1}$, $960cm^{-1}$, $603cm^{-1}$, $568cm^{-1}$) respectively [29].The bands in the FTIR spectra from calcining at 850^0c showed absorption peaks at $3447cm^{-1}$ and $3567cm^{-1}$ can be due to the presence of OH^- ions. These spectra also indicated the presence of phosphate and calcium ions in heated premolar teeth. The observed low intensity bands within $1463cm^{-1}$ and $471cm^{-1}$ are associated with the stretching vibration of CO_3^{-2} of premolar teeth. In human premolar teeth HA, the hydroxyl bond is both shown in $602.58cm^{-1}$ and $3447.77cm^{-1}$, which is similar to that of HA- 200 i.e., $3425.58cm^{-1}$. Almost similar condition of FTIR spectrum was observed for HA obtained from smart dentine grinder. The vibrating band at about $3447.59cm^{-1}$ shows the presence of hydroxyl group (Figure 3). The highest intensity of phosphate group indicated by the vibration bending and stretching of P-O at $1035.22cm^{-1}$. The absence of peaks at 1450 dan $1700cm^{-1}$ shows that Ca-O impurities in HA are not found in this material obtained from calcining at 850^0c and from smart dentine grinder. Hence, 850^0c can be chosen as reliable calcining temperature and the material produced can be used in future investigations such as treatment of WSLs. Further investigations are needed to improve the mechanical properties of obtained HA.

CONCLUSION

The present investigation demonstrated that the calcining method with high temperatures up to 850^0c is an easy, cost effective and reproducible method for the production of HA from human premolar teeth. The results of FTIR and XRD showed that the chemical components of the prepared materials are hydroxyapatite, the process of calcination with high temperature is effective, and shows better characteristics as compared to HA produced by Smart Dentine Grinder.

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LIST OF ABBREVIATIONS

1. HA: Hydroxyapatite
2. WSL: White Spot Lesion
3. XRD: X-Ray Diffraction Analysis
4. FTIR: Fourier transform infrared spectroscopic

CCP- ACP: Casein Phosphopeptide- Amorphous Calcium Phosphate