Comparison Of A Synthetic And Bovine Derived Hydroxyapatite Bone Graft Substitute

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INTRODUCTION

It is well known that the extraction of teeth results in significant dimensional changes in the alveolar bone. The rate of change and the magnitude of these changes have been extensively studied, both in animal models (Araujo 2005a, Araujo 2005b) and in numerous human studies (Devlin 2003, Trombelli 2008, Gholami, 2011). Through these investigations, there is agreement about the key processes that take place immediately after extraction that result in tissue remodeling. These events ultimately lead to an overall reduction in ridge height and width, with significant changes in both the buccal and lingual bone crests. Interestingly, it appears that the buccal crest resorbs more quickly than the lingual crest.

The amount of the structural changes, including vertical and horizontal bone loss that occur have been measured using a wide range of methods, from radiographic assessment (Schropp 2003) to the use of cast models (Johnson 1969) to histological studies in animal models (Araujo 2005b). While the absolute percentage of diminution of the dimensions vary widely depending on the models and methods used, it is clear that the greatest rate of change in dimensions occur within the first three months following extraction. Observable changes continue to occur up to a year post-extraction, and likely beyond that time frame. Recently, multiple comprehensive meta-analyses substantiated these general results (Vignioletti 2012, Ten Heggeler 2011).

The loss of both horizontal and vertical bone height can have an adverse effect on the outcome of ensuing therapies targeted to restore the lost dentition. The consequences of lost bone height and ridge width can make it difficult to get an ideal placement of an implant and can result in compromised aesthetics of the prosthetic restoration. To overcome these potential deficiencies there have been a number of different techniques proposed to preserve the socket and thereby minimize bone loss. These include the immediate placement of implants (Coslyn 2011, Kan 2003), flapless tooth extraction with the goal of an undisturbed site for socket healing (Fickl 2008), and the use of various bone graft materials, with and without membranes (Baldini 2011, Bornstein 2008). Recent reviews of the clinical literature have shown that the use of bone graft substitutes are an effective means of maintaining ridge height and width as well as augmenting bone volume in the maxillary sinus (Klein 2010, Chiapasco 2006).

The possible benefit of using a bone graft material, with or without a covering membrane, has been extensively studied, both in animal models and in human studies. Autologous bone remains the gold standard (Misch 2010), but requires a second surgical site that can result in additional pain and complications, is limited in quantity and increases the cost of the procedure. Allograft bone, either fresh-frozen or demineralized freeze dried bone allograft (DFDBA) has also been used, but the rapid resorption can make it less than ideal for some larger defects. Xenograft materials have been used quite frequently as bone graft substitutes with good success in recent years. Bio-Oss® Bone Substitute (Ed. Geistlich Soehne, Wolhusen, Switzerland) is a bovine bone derivative that undergoes a heat treatment and chemical extraction process by which the organic components are removed but maintains the natural architecture of cancellous bone (Baldini 2011).

There have also been a number of synthetic bone graft substitutes that have been developed for these applications. Most are based on hydroxyapatite or other calcium phosphate minerals, which in many cases are similar to the natural mineral found in human bone. These materials are found either as porous or dense granules of various sizes. As a class of materials, they are osteoconductive, demonstrate very good biocompatibility and are easily sterilized and used in the clinic. The hydroxyapatite materials generally have a low solubility, while calcium phosphate materials generally have a higher solubility and are thought of as resorbable bone graft materials.

The objective of this study is to compare the chemical and physical properties of a bovine derived bone graft material, Bio-Oss Bone Substitute, with a new, highly porous non-biologic hydroxyapatite bone graft material, *IngeniOs*™ HA Synthetic Bone Particles (Zimmer Dental Inc., Carlsbad, CA). This paper will focus on the chemistry, structure and morphology of these two bone graft substitutes.

MATERIALS AND METHODS

IngeniOs HA Synthetic Bone Particles and Bio-Oss Bone Substitute were used in this study and both were obtained from lots within the expiration dates. The stated particle size of both materials is 1 – 2mm. All of the materials used for each product were from the same lot.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDX)

A Teflon® spatula was used to gather and sprinkle a small quantity of each sample onto carbon tape placed on an SEM mount. Two samples of each material were evaluated. One sample was coated with a conductive carbon film (<500nm) to allow for EDX analysis. The second sample was coated with gold-palladium, a highly

conductive coating that allowed for better imaging. Images were obtained using a scanning electron microscope (6400 Scanning Electron Microscope, JEOL Ltd., Tokyo, Japan) at an operating voltage of 15kV. EDX spectra were taken using a microanalysis system (FeatureMax, Oxford Instruments, Abingdon, UK) and EDX software (Link-Isis Semi-Quant Software, Oxford Instruments). All spectra were collected at a magnification of 500x with zero stage tilt for 60 seconds. The data acquisition rates were maintained at 1,400 to 1,500 counts per second for all spectra. One-spectrum each was acquired on 5 different particles of each material. EDX data were then transformed into a semi-quantitative data set using software (Link-Isis ZAF Correction Software Package, Oxford Instruments).

X-ray Diffraction (XRD)

Approximately one gram of each material was ground into a fine powder using a clean agate mortar and pestle. The samples were then loaded into a standard sample holder. The crystal structure of each powder sample was analyzed using a Phillips (Phillips Electronics, N.V.) x-ray diffractometer at 30kV and 20mA. Data were collected over the 2q range 10-60° at a scan speed of 1.2°/min with the step size 0.02° with Cu Ka radiation. The diffraction patterns of HA were indexed by comparing them with International Powder Diffraction File database.

Fourier Transform Infrared Spectroscopy (FT-IR)

A one gram portion of each material was ground to a fine powder using an agate mortar and pestle and then pressed into a KBr pellet for analysis. A Perkin-Elmer Fourier transform infrared spectroscopy (Perkin-Elmer Corp., Waltham, MA, USA) was used to indentify chemical structure by interpreting the infrared absorption spectrum via the chemical bonds in a molecule. All spectra were collected at room temperature at a nominal resolution of 4.00 and number of sample scans equal to 1000. The FT-IR spectra were recorded in a range of 500-4000 cm-1.

Statistical Analysis

Data for the Ca/P ratios are presented as the mean of 5 independent spectra with a standard deviation. Statistical comparisons were performed using a two-sided Student's t-test. A difference was considered significant at $P<0.05$.

RESULTS

The SEM analysis of the two graft materials showed strong similarities in both the particle size and the morphology and structure of the particles. Figure 1a is a 20x, low magnification image of the Bio-Oss material, and it is clear that the material has retained much of the cancellous nature of the original bone. Figure 1b is an SEM image of *IngeniOs* HA Synthetic Particles taken at the same magnification. The porous nature of this material is very similar to that of Bio-Oss Bone Substitute. The porosity appears to be on the same scale as that of the natural bone mineral and in similar proportions to the Bio-Oss material.

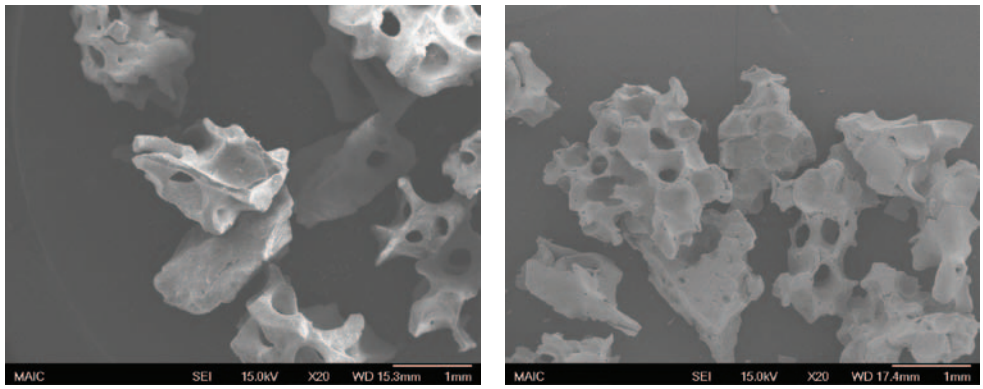

Figure 1. SEM images of Bio-Oss Bone Substitute and *IngeniOs* HA Synthetic Bone Particles at a 20x magnification. The figure clearly shows the macro-porous nature of the two graft materials and the similar structures.

The results of the EDX analysis revealed very similar results in terms of composition and ratio of Ca/P which was 1.58 ±0.15 for Bio-Oss Bone Substitute and 1.62 ± 0.09 for *IngeniOs* HA Synthetic Bone Particles. The Ca/P ratios were close to that of human bone for both materials. There was no statistical difference in the Ca/P ratios between the two materials. Typical EDX plots are shown in Figures 2a and 2b for these materials. There were consistent trace amounts of Na and Mg in all of the Bio-Oss material spectra. Because this material is of natural bovine origin, this is not an unusual finding. The hydroxyapatite mineral in bone is known to be highly substituted.

Figure 3 shows the results of the X-ray Diffraction analysis. The spectra both are very close to the reference hydroxyapatite spectra, (ICDD – PDF no. 9-432). The *IngeniOs* HA spectrum appears to have sharper peaks than the Bio-Oss material spectrum.

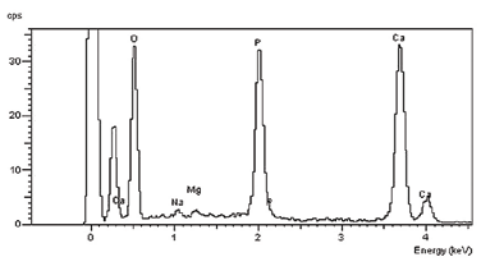

Figure 2a. EDX: Bio-Oss Bone Substitute

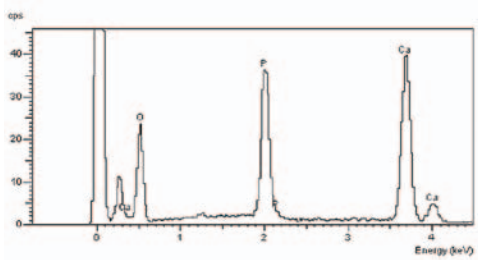

Figure 2b. EDX: *IngeniOs* HA Synthetic Bone Particles

Figure 2. Energy Dispersive X-Ray analysis (EDX) of Bio-Oss Bone Substitute (Fig. 2a) and *IngeniOs* HA Bone Particles (Fig 2b). The spectra are very similar with the exception that there are small trace amounts of sodium and magnesium in the Bio-Oss material spectra.

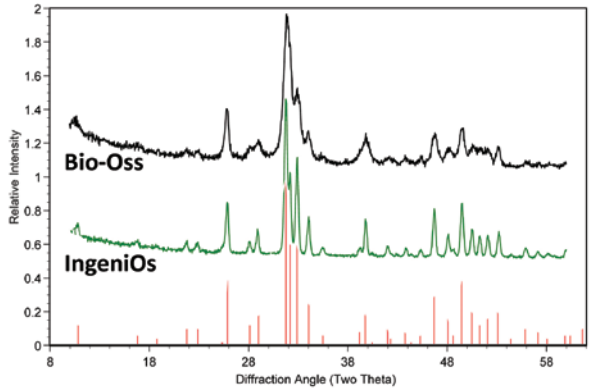

Figure 3. X-Ray Diffraction analysis of Bio-Oss Bone Substitute and *IngeniOs* HA Synthetic Bone Particles. The reference spectra is the stick pattern in red.

Figure 4 shows the FT-IR spectra for both Bio-Oss Bone Substitute and *IngeniOs* HA Synthetic Bone Particles. Note that both products show the typical PO4-3 double peak at 550 cm-1 and 600 cm-1 as well as the P-O stretching vibration at 1038 cm-1. In addition, the Bio-Oss material sample shows a peak that is likely associated with a CO3-2 vibration. The area of the Bio-Oss material spectra from about 1300 cm-1 to about 1500 cm-1 also appears to be higher intensity than the spectra from the *IngeniOs* HA sample.

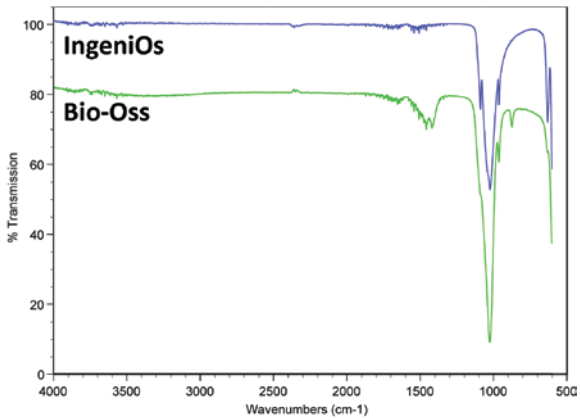

Figure 4. FT-IR spectra of Bio-Oss Bone Substitute (green) and IngeniOs HA Synthetic Bone Particles (blue).

DISCUSSION

The objective of this study was to compare the chemical and physical properties of a novel, highly porous synthetic hydroxyapatite material (*IngeniOs* HA Synthetic Bone Particles) with a widely used bovine derived bone graft substitute (Bio-Oss Bone Substitute). These two materials have rather similar chemical, physical and structural properties. Both materials are indicated for a wide range of bone grafting procedures including augmentation of the alveolar ridge and maxillary sinus augmentations.

In the present study, the analysis of the physical, chemical and structural properties of the two graft materials demonstrated that the materials are very similar. The SEM images show that the materials appear to have the same particle size, shape and pore structure. According to the manufacturer's data, the porosity of the Bio-Oss material is around 67%, while the porosity of the *IngeniOs* HA material is between 70% and 80%. In addition, the energy dispersive X-ray analysis showed that the Ca/P ratio, which for human bone is 1.67 (LeGeros 1991), were essentially the same for both products (Ca/P =1.62 for *IngeniOs* HA material and ca/P=1.58 for Bio-Oss material). It is interesting to note that the standard deviation was greater in the Bio-Oss material, which is perhaps due to the natural variability in the actual mineral content of the source of the bone. However, these differences were not statistically significant. There also were some trace elements, mainly sodium and magnesium that were found in the EDX spectra of the Bio-Oss material. This too is to be expected since natural bone is not pure calcium hydroxyapatite, but a substituted HA.

The XRD data (Fig 3) showed that the primary crystalline phase of both materials is a calcium hydroxyapatite. It should be noted that the appearance of the two spectra are somewhat different. The peaks associated with the HA crystalline structure are sharper for *IngeniOs* HA Synthetic Bone Particles, which suggests a more regular crystalline structure and higher percent of crystallinity. While the high crystallinity of both products indicates a low level of solubility, the very high crystallinity of *IngeniOs* HA Synthetic Bone Particles suggests an even slower resorption rate of the material. While this result is purely qualitative, if one considers the route of production of each product, this makes sense. The *IngeniOs* HA material is made from high purity, reagent grade chemicals in a very tightly controlled process. While the process for producing Bio-Oss Bone Substitute is likely to be just as well controlled, the starting material (native bovine bone) will have natural variations in mineral content and crystal size. These factors will then contribute to the broadened XRD peaks shown in Figure 3.

The FT-IR data (Fig. 4) also shows slight differences in the structure of these materials. Both have the signature PO4-3 double peak at 550 cm-1 and 605 cm-1, although the double peak is much more pronounced in *IngeniOs* HA Synthetic Bone Particles. This is consistent with the broadened XRD spectra and suggests that the crystal structure is more uniform and more crystalline in the *IngeniOs* HA material when compared to the Bio-Oss product. In addition, there is clearly a carbonate peak in the Bio-Oss Bone Substitute (CO3-2) that is most likely a remnant of the original material, or possibly a result of the processing and thermal treatment of the material. This peak is totally absent in the *IngeniOs* HA material.

A recently published cell culture study using an osteoblast cell line compared the adhesion of the cells and proliferation of the cells on both *IngeniOs* HA material (formerly Osbone® Synthetic Bone Substitute) and Bio-Oss Bone Substitute (Bernhardt 2010). In this study, the bone cells were cultured in

the presence of granules of each material for various time periods up to 28 days. The results showed better adhesion and better proliferation on the *IngeniOs* HA material when compared with the Bio-Oss material. The authors ascribe the differences seen to the different processing parameters used in generating the two graft materials. The authors conclude that these differences make *IngeniOs* HA material a promising candidate for bone grafting applications.

Autografts remain the gold standard (Donos 2005) because they contain the requisite osteoinductive, osteogenic and osteoconductive properties necessary to regenerate bone for implant placement. However, there are drawbacks to harvesting autograft, including increased operating time, potential complications and morbidity of the harvest site and limitations in available bone quantity. In addition, the type of bone available (cortical or cancellous), the quality of the bone (density) and the ultimate amount (quantity) of the harvested bone can also make the use of autografts problematic. Due to these limitations there has been significant effort placed on the development of various categories of bone graft substitutes, including calcium phosphate and hydroxyapatite materials, bioactive glasses, as well as allograft and xenograft materials.

Both synthetic and xenograft-based hydroxyapatite bone graft materials have been relatively successful in many dental applications. Studies in various animal models have compared deproteinized bovine bone to a wide range of synthetic calcium phosphate materials (Santos 2010, Kruse 2011). These studies have shown equivalence of bone regenerative capacity amongst all of these materials. Although more limited in number, human comparative studies have nonetheless demonstrated equivalence of the synthetic bone grafts compared with the xenograft material (Mardas 2011).

CONCLUSIONS

This study has demonstrated that *IngeniOs* HA material and Bio-Oss Bone Substitute are hydroxyapatite materials with a very similar chemical and structural nature. Differences between the two materials do exist however, in crystallinity, trace elements and porosity. In addition, the cell culture study referenced here showed an excellent response of the cells to *IngeniOs* HA material. Thus, *IngeniOs* HA Synthetic Bone Particles should be considered as a non-biologic grafting alternative for augmentation of the alveolar process due to loss of dentition or for augmentation of the maxillary sinus, particularly when a minimally-resorbing, space-maintaining graft is desired.

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