Physical And Chemical Properties Of Commercially Available Mineralized Bone Allograft

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ABSTRACT

Bone graft materials are critical to the success of dental implants when there is a need to increase the volume of bone in a defect. The surface properties of these graft materials will have a profound impact on the outcome of the graft procedure. The clinician has many choices of bone graft substitutes when augmenting bony deficits. Allograft bone is the most widely used class of bone graft substitutes. Within this class there are a number of different bone allografts, which are manufactured utilizing widely varying processing techniques. There also appears to be a wide range of results in the published literature across the spectrum of different bone allografts.

This in-vitro study evaluated chemical and surface properties of five different commercially available mineralized bone allografts (Straumann Dental's LifeNet Health cortical and cancellous allograft, Zimmer Dental's *Puros*® cortical and cancellous allograft, and BioHorizons' MinerOss® cortical/cancellous allograft) by analyzing surface area, chemical composition and sample morphology. The results showed that the calcium/phosphate (Ca/P) ratio for the *Puros* bone allografts was closest to that of natural, unprocessed bone mineral (Ca/P = 1.67). *Puros* bone allograft had Ca/P ratios of 1.67 for cortical and 1.66 for cancellous, versus 1.84 for LifeNet Health cortical and 1.52 for LifeNet Health cancellous, and 1.72 for the *MinerOss* material. Furthermore, the surface area of the *Puros* bone allograft was significantly higher than that of the other bone graft materials. The surface area for *Puros* cancellous allograft was 0.81m^2/g, 0.48 m^2/g for *Puros* cortical allograft, 0.18m^2/g for LifeNet Health cancellous allograft, 0.09m^2/g for LifeNet Health cortical allograft, and 0.27m^2/g for the *MinerOss* allograft. Density measurements showed that the cortical bone samples had a slightly higher density than the cancellous samples, which was expected.

The higher surface area presented by the *Puros* bone allograft means that there is more area for early attachment of osteoblasts and for proliferation of cells in the repair process. In addition, the results showed that the Ca/P ratio of the *Puros* allograft was closest to natural human bone, which means that the processing did not alter the chemical nature of the bone. The results, taken together suggest that bone graft processing plays a critical role in the resulting surface properties of these bone allografts. The use of surfactants and antibiotics that could be left on the bone surfaces could have affected the results presented here. It appears that the processing used for the preparation of *Puros* bone allograft results in a bone graft that is the closest in properties to natural human bone.

INTRODUCTION

It has been well established that adequate bone volume is necessary for the successful long-term outcome of dental implants.[1-2] The clinician has a wide variety of treatment options when considering how to obtain sufficient bone for these procedures. Generally some form of bone graft substitute is used to fill an extraction site, augmentation of a mandibular ridge or maxillary sinus in order to regenerate sufficient bone to place an implant. The initial healing response of the body to the bone graft substitute plays a critical role in the ultimate quality and quantity of new bone formed. Therefore, the surface of the bone graft is critical to early successful incorporation of the graft and ultimate bone formation. The vast majority of grafting procedures are performed with particulate bone graft substitutes, although barrier membranes and block allografts are sometimes used.

For particulate bone graft substitutes, there is an ever-increasing number of options available for the practicing clinician. Autografts remain the gold standard[3] 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.[4] 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. Because of these limitations, the development of synthetic and xenogenic biomaterials has increased significantly over the past 10 to 15 years. Calcium phosphate ceramics[5-6] and glasses,[7-8] deproteinized bovine bone[9-10] and a variety of other compositions of materials[11] have been utilized experimentally and clinically instead of autografts. These materials are osteoconductive, and depending on chemistry, morphology and particle size, have been variably successful filling bony defects.[11]

Allogenic bone grafts are perhaps most commonly used to restore adequate bone volume for the placement of dental implants. This type of bone graft processed from donated human bone is commercially available in both demineralized and mineralized forms and in a variety of particle sizes. Both demineralized freeze-dried bone allograft (DFDBA) and mineralized bone allograft are regulated through the Food and Drug Administration to ensure safety (21 CFR 1271). In addition, the American Association of Tissue Banks (AATB) sets guidelines for

tissue procurement, processing and sterilization practices (available online at: http://www.aatb.org/AATB-Standards-for-Tissue-Banking, 9/2012). These stringent regulations ensure a high level of safety for all allogenic grafts.

While the regulation of allografts has assured a high level of safety, different tissue banks utilize varying processing methods to create an easy-to-use (or user-friendly) bone graft substitute. All tissues are recovered using aseptic technique and then shipped to a processing facility. There are limited standards and regulations related to the actual processing of human tissues for transplant. In some instances, tissue banks continue to use aseptic processes throughout the decellularization process.[12] The process of decellularizing the tissue is necessary to remove the natural antigenic materials that would be released from the transplantation of non-autologous cells. Generally, these processes use chemicals, which can vary according to the processing method used. In many cases surfactants and detergents are used to clear cellular debris, bacteria and other organic materials that could cause unfavorable responses to the implanted allograft.[13] Finally, some processors use sterilization techniques such as gamma irradiation to create an allograft that will be sterile upon implantation thus providing an additional level of safety. Gamma irradiation, if properly deployed provides a high level of sterility that is the standard for implantable medical devices, but not all allograft tissue is distributed in a terminally sterilized condition.

Bone is a composite material made up of collagen, other organic molecules and calcium hydroxyapatite. These components may react differently to the various chemicals and processes used to create the final allograft particulate. It is known that calcium phosphate is more soluble in acid conditions, so the use of acidic liquids could have an effect on the mineral content of the bone. Harsh chemicals could denature the collagen and this could affect the initial response of the allograft to the host. Detergents and surfactants could also be left behind on the surface of the bone allograft after processing. Residual materials could cause an inflammatory response that delays or interferes with proper healing.

A variety of techniques can be used to sterilize bone graft substitutes. There are no standards or regulations regarding the sterility of bone graft substitutes. Unfortunately neither are there any standards or regulations for labeling of human tissue grafts related to sterility, this leads to confusion. Examples of some types of labeling used are: (1) Sterile A, (2) USP 71 and (3) Sterile R. Sterile A is aseptic only (which means the processor has procedures to prevent contamination during processing but does not eliminate pathogens which may be inherent to the tissue) and does not employ a physical sterilization method. USP 71 is a culturing method which sets a standard for measuring a sample of the donor for contamination, this culturing method is known to be at best 70% accurate according to CDC and is usually used in concert with Aseptic processing. Gamma irradiation is the most commonly used form of ensuring a terminally sterilized bone graft substitute. It is generally accepted that high-dose gamma sterilization (over 25 kGy) can cause damage to the collagen structure of the bone and cause denaturation of the proteins, including bone morphogenetic proteins (BMP).[14] In order to preserve the characteristics of native bone, gamma irradiation can be performed on dry ice which minimizes temperature rise during the process, and has been shown to minimize or eliminate the amount of tissue damage.[15]

Since various processing techniques can be used, differing bone allograft materials may not perform in the same manner. Since the surface of the graft is the first material that the host responds to, the properties of these surfaces are a major factor in determining the early success of the procedure. Certainly, in the case of demineralized bone powder (DBM), studies have shown widely differing levels of osteoinductivity between DBM from multiple tissue banks and even within various lots from a single tissue bank.[16] While mineralized allograft is said to be only osteoconductive, osteoinductive factors within the mineralized structure may also become available as the material is resorbed and remodeled during the bone regeneration process.[17] The same processing variables that affect the performance of DBM powder may also potentially affect the surfaces and therefore the efficacy of mineralized allografts.

The purpose of this study was to compare and contrast the surface properties and chemical composition of 5 commercially available mineralized allograft materials.

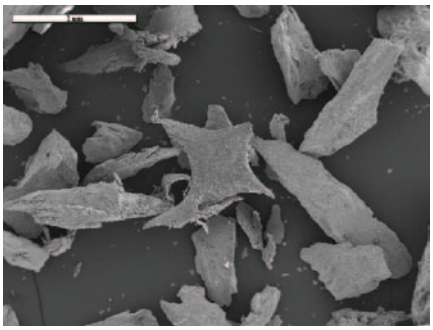
MATERIALS AND METHODS

Bone Allograft

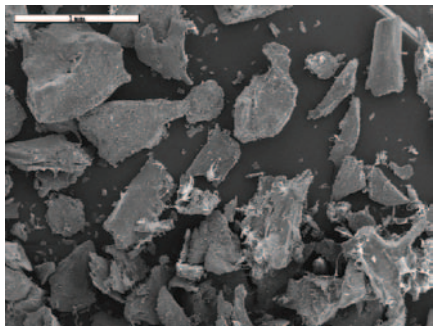
Mineralized particulate allografts were obtained from three different sources: *Puros* Allograft (Zimmer Dental Inc., Carlsbad, CA), LifeNet Health Freeze Dried Allograft (Straumann Dental, Andover, MA), and *MinerOss* Cortical and Cancellous Chips (BioHorizons, Birmingham, AL). Particulate size ranged from 250µm – 750µm (LifeNet Health) and 250µm – 1,000µm (*Puros* Allograft) for cortical and from 250µm – 1,000µm (*Puros* Allograft and LifeNet Health) for cancellous allografts. The combination cortical and cancellous allograft (*MinerOss*) particulates were 600µm – 1,250µm in size. All of the particulate allografts were within their expiration dating. With each particulate allograft, at least 3 different lots were obtained and mixed together to ensure that every analysis consisted of bone allograft from multiple donors. At least 2.5g of each particulate allograft were used in the analyses.

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

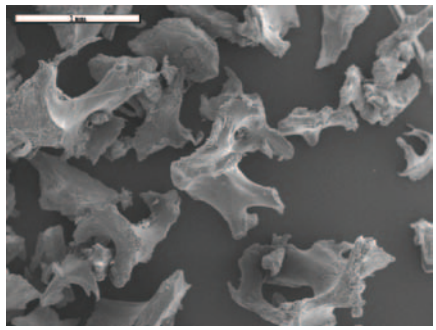
A Teflon spatula was used to gather and sprinkle a small quantity of the mixed graft onto carbon tape placed on an SEM mount. Two samples of each bone allograft particulate were evaluated. One sample was coated with a conductive



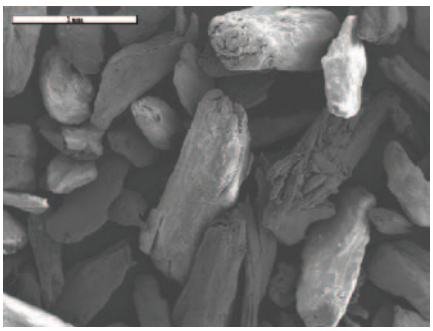
A. *Puros* Cortical



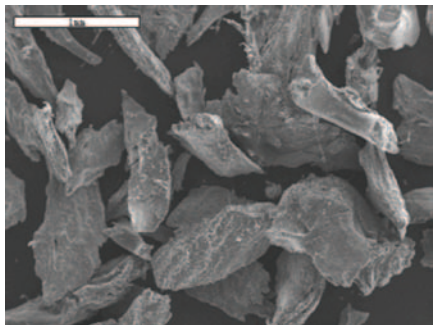
B. *Puros* Cancellous



C. LifeNet Health Cancellous



D. LifeNet Health Cortical



E. *MinerOss* Cortical-Cancellous Mix

Figure 1. Low magnification SEM images of the five materials tested: A) *Puros* Cortical, B) *Puros* Cancellous, C) LifeNet Health Cancellous, D) LifeNet Health Cortical, E) *MinerOss* Cortical-Cancellous mix. All images are 35x magnification.

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 and all spectra were collected at the same working distance. 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).

Density measurements

The apparent density of the samples was obtained using a helium pycnometer (AccuPyc1330, Micrometric, Inc., Sarasota, FL). Samples were weighed and placed in the pycnometer chamber. A minimum of 2g each bone allograft particulate was used for the measurement. An average of 5 separate readings were made. Four stainless steel bearings, 2 mm in diameter, were used to calibrate the system prior to the study measurements.

Surface Area Measurements

Specific surface area measurements of the particulate allograft were obtained using the Brunauer-Emmett-Teller (BET)[18] gas adsorption surface area analyzer (NOVA 1200 Surface Area Analyzer, Quantachrome Corp, Boynton Beach, FL) based on nitrogen absorption.

A minimum of 2.5g of each sample material was weighed to 3 decimal places, placed in a clean 25cc glass sample holder, out-gassed under vacuum at 40°C for 20 hours, and immediately placed in the test station. A six-point nitrogen adsorption program was used to obtain an adsorption isotherm and to calculate the specific surface area which is stated in m^2/g of material.

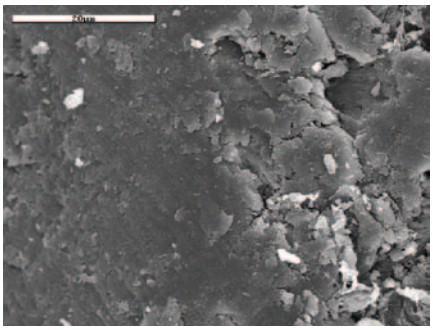
RESULTS

SEM EDX

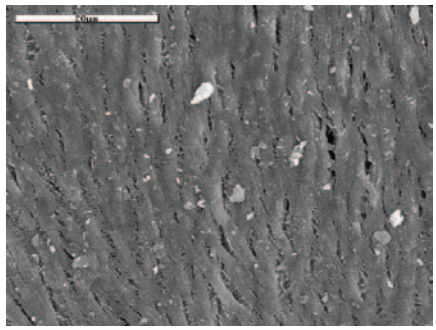
The qualitative SEM results are shown in Figures 1 and 2. These images were taken of randomly selected areas of each allograft material. Samples B (*Puros* Allograft) and C (LifeNet Health) were the cancellous graft materials, while Samples A (*Puros* Allograft) and D (LifeNet Health) were the cortical bone materials. The Sample E (*MinerOss*) material is a mixture of both cortical and cancellous bone.

It is clear from the low magnification images that all of the bone samples have pronounced aspect ratio (length to width). This is to be expected given the standard methods for grinding and the composite and directional nature of bone (alignment of collagen fibers in the direction of stress). It is also clear that some particles have dimensions in the long axis that exceed the stated size of the particles. This is also not unexpected as the process of sieving any particulate material is only a very close approximation, and not absolute.

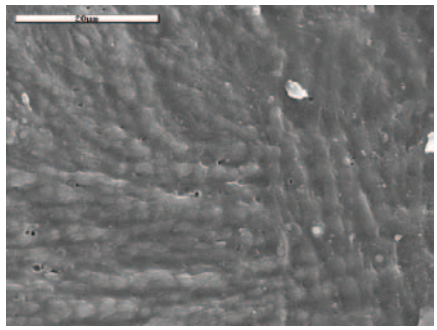
Of the samples analyzed by SEM, the *Puros* and LifeNet Health cancellous bone materials appeared to have very similar cancellous structure at low magnification, with many particles exhibiting a pronounced trabecular structure. The *Puros* cancellous bone sample (Figure 1B)



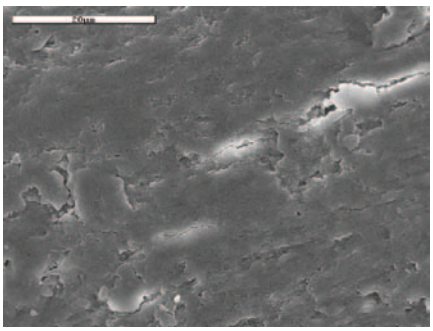
A. *Puros* Cortical



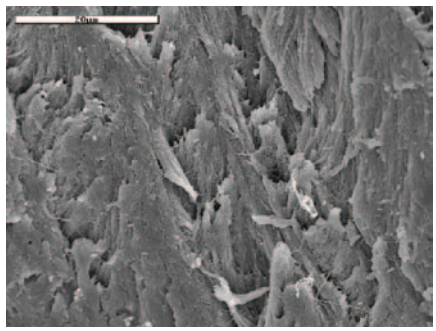
B. *Puros* Cancellous



C. LifeNet Health Cancellous



D. LifeNet Health Cortical



E. *MinerOss* Cortical-Cancellous Mix

Figure 2. High Magnification SEM images of the product surfaces. A) *Puros* Cortical, B) *Puros* Cancellous, C) LifeNet Health Cancellous, D) LifeNet Health Cortical, E) *MinerOss* Cortical-Cancellous mix. All images are at 2000x magnification.

also showed more loose fibrous material that is most likely collagen from its appearance compared to the LifeNet Health sample. The appearance of this material was likely due to the milling process. In addition, small particulates of natural hydroxyapatite crystals could be seen on the surface of the *Puros* material in the higher magnification images (Figure 2B, 35x magnification). The *MinerOss* sample, which was a blend of both cortical and cancellous bone, appeared to have particles with a cancellous structure as well as particles that were blockier in nature, which would suggest cortical bone present.

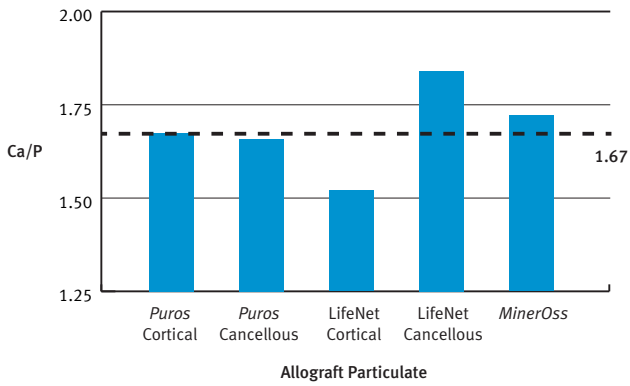
The differences in surface appearance between the various bone allografts are shown in Figure 2. Comparing Figure 2B (*Puros* Cancellous) with Figure 2C (LifeNet Health Cancellous) it appears that there is greater porosity and a more pronounced collagen structure in the *Puros* allograft than in the LifeNet Health sample. One can observe more collagen like fibrous material in the oriented structure in Figure 2B (*Puros* Cancellous) compared with a less porous looking surface in Figure 2C (LifeNet Health Cancellous). In addition, the LifeNet Health particulate allograft has a qualitatively smoother surface compared to the *Puros* allograft. In both Figures 2A and 2B the oriented fine structures are typical of a mineralized collagen network prevalent in cancellous bone.[19] Figures 2A (*Puros* Cortical) and 2D (LifeNet Health Cortical) have similar appearances, with a fairly dense, non-porous surface typical of cortical bone.

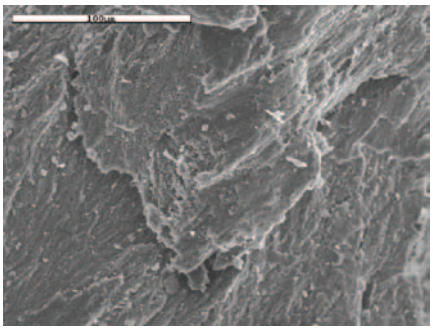
Figure 3 shows representative images taken at 500x that were used to collect all of the EDX spectra. The results of the EDX analyses are shown in Figure 4 and Table 1. The calcium to phosphate ratios (Ca/P) represent the average of 5 separate particle measurements. The standard deviations are

Table 1. EDX Summary Data showing the average of five independent measurements and the standard deviations for each allograft particulate.

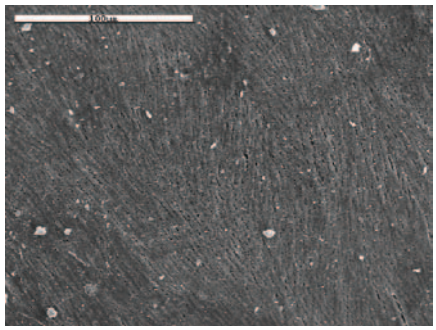
SUMMARY	AVG Ca/P	STD DEV
Puros Cortical	1.67487	0.07229
Puros Cancellous	1.65857	0.16386
LifeNet Health Cancellous	1.52078	0.05156
LifeNet Health Cortical	1.84147	0.19545
MinerOss Cortical-Cancellous Mix	1.72187	0.08364

Figure 4. Plot of Ca/P Ratios for Grafts Tested. Black Line represents Ca/P ratio for human bone (1.667).[29]

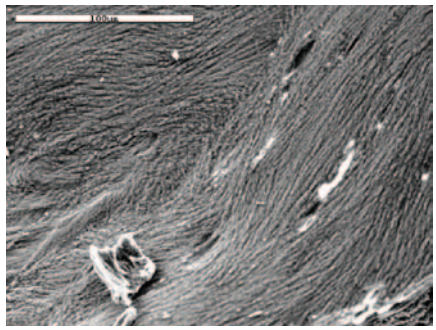




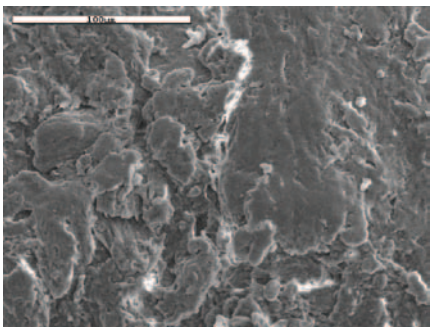
A. *Puros* Cortical



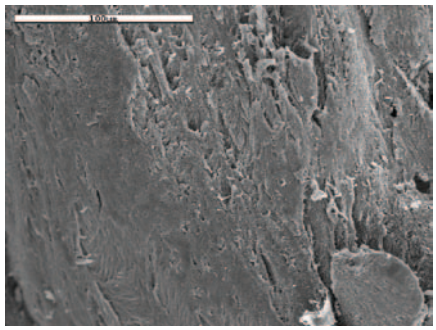
B. *Puros* Cancellous



C. LifeNet Health Cancellous



D. LifeNet Health Cortical



E. *MinerOss* Cortical-Cancellous Mix

Figure 3. Representative 500x images of surfaces used to collect EDX data showing the area used to collect EDX spectra. A) *Puros* Cortical, B) *Puros* Cancellous, C) LifeNet Health Cancellous, D) LifeNet Health Cortical, E) *MinerOss* Cortical-Cancellous.

also included. All spectra were collected at a magnification of 500x. The Ca/P ratios vary from a low of 1.520 to a high of 1.841, demonstrating that there is significant variation in the Ca/P ratios of some of the bone allograft analyzed.

Density Measurements

The helium pycnometer data is presented in Table 2. The densities of the five products tested were similar in value. There was only a 6.5% spread in the measured values by this method. The standard deviation of the measured densities was very low, giving confidence that the measured values were accurate for these samples.

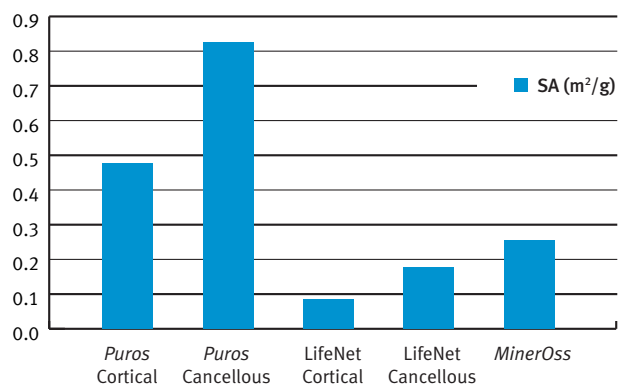
Surface Area

The results from the surface area measurements are presented in Figure 5. Based on the density measurements and preliminary work, it was determined that 2 to 3 g of sample would give enough particulate allograft to measure the surface area using the BET method. The data from the two LifeNet Health samples of particulate allografts showed a correlation coefficient that was much lower than for the other three curves (data not shown). This suggests that the data set was more variable for these samples when compared to the others. In addition, the surface areas for the LifeNet Health allografts were significantly lower than for the *Puros* or *MinerOss* allograft. The surface area for the *MinerOss*, which is a blend of cortical and cancellous bone, was significantly lower than for either of the *Puros* allograft, but higher than the LifeNet Health allograft. Although the weights for each particulate allograft were different, these are normalized with the density measurements and the only effect would be to present additional surfaces on which to adsorb nitrogen gas.

Table 2. Helium Pycnometer Density measurements

SAMPLE	WEIGHT (g)	AVG. DENSITY (g/cc)	STD. DEVIATION
Puros Cortical	2.381	2.134	0.002
Puros Cancellous	1.905	2.163	0.002
LifeNet Health Cancellous	1.505	2.076	0.001
LifeNet Health Cortical	1.368	2.205	0.003
MinerOss Cortical-Cancellous Mix	1.685	2.069	0.002

Figure 5. BET Surface Area Data. Bars represent three independent runs of each sample



DISCUSSION

It is most important to achieve early healing after any surgical procedure to ensure the best clinical outcome. The introduction of a bone allograft into a surgical site can influence the healing cascade, either positively or negatively. Bone allograft remains the most widely used bone graft substitute. While DFDBA powder has been successfully used in treating bone loss in periodontal defects, and its osteoinductive properties have been extensively documented[20-22] to enhance bone regeneration, its rapid resorption and variability in performance make it less than ideal for larger defects such as extraction sites or for augmentation of the maxillary sinus and mandibular ridge.[23] In contrast to DFDBA, mineralized bone has a more physiologically acceptable resorption profile, and will maintain the defect volume during healing compared with demineralized bone allograft.[23] Because of the osteoinductive potential of DFDBA, much of the published literature on allograft has focused on this material, although more recently a number of clinical articles have demonstrated the effectiveness of using mineralized allograft for bone regeneration prior to the placement of implants.[23-25]

Different processing techniques for cleaning and sterilizing allograft can have an effect on the properties of bone. Because particle surfaces are first to interact with the host tissue, it is important to understand how various processing techniques can affect and perhaps alter the natural structure of the surface of mineralized allografts. Altering the natural structure and chemical composition of the surface can have a deleterious effect on initial healing events. For example, researchers have found that surface topography and surface energy, which are related to the surface chemistry and structure, have a pronounced effect on the rate of bone apposition to implant materials.[26,27] The LifeNet Health and *MinerOss* allograft packaging inserts disclose the use of detergents, surfactants and other chemicals, along with pressure as part of the decellularization and delipidization processes when preparing bone allograft.[28] The *Puros* particulate allograft was manufactured via a proprietary process (Tutoplast® Process, RTI Biologics, Alachua, FL) that utilized delipidization to remove lipids and red and white blood cells; osmotic treatment to disrupt cell membranes to allow easier removal of cellular components; oxidative treatment to remove immunogenic structures, enveloped and non-enveloped viruses; solvent treatment to preserve the natural tissue matrix and allow for a longer shelf life; and low-dose irradiation to produce a terminally sterile graft while preserving structural integrity. According to the respective packaging inserts of all the bone allografts tested in this study, only *Puros* and LifeNet Health bone allografts were terminally sterilized using gamma irradiation.

Under SEM analysis, all tested samples appeared somewhat similar. In Figures 1A and 1B, *Puros* cortical and cancellous samples appeared similar in gross morphology, and Figure 1B shows some fibrillar material that is most likely collagen, especially around the edges of the particles that are consistent with fractured cancellous bone. In addition, many small hydroxyapatite particles can be seen adherent to the *Puros* surface, as determined by EDX analysis (data not shown). This is in contrast to the LifeNet Health cancellous surface which doesn't show many of these particles. The small hydroxyapatite particles that appear on the surface of the *Puros* product (see Figure 1B and 2A and 2B) are likely heldthrough electrostatic interactions which is suggestive of a very clean, pure surface. The LifeNet Health samples in Figures 1C and 1D did have very different gross structural appearances. The cancellous particulate (Figure 1C) clearly showed more typical cancellous trabeculae than the cortical bone particulate shown in Figure 1D. The *MinerOss* sample (Figure 1E) appeared to be all cortical bone from this analysis versus a cortical and cancellous mixture. It was clear from a comparison of the high magnification images in Figures 2B and 2C that the *Puros* cancellous structure appeared more porous than the LifeNet Health graft. When taken together with the surface area results in Figure 5, this strongly suggests that the processing had some effect on the surface structure of the LifeNet Health allograft.

The spectral analysis by EDX showed quite a variation in the Ca/P ratios between the different allograft particulates. The *Puros* samples were both almost identical and very close in value to the known Ca/P ratio of calcium hydroxyapatite (Ca/P = 1.67)[29], which is bone mineral. In contrast, the spectra for the LifeNet Health allograft varied widely, from 1.52 for cancellous bone to 1.84 for cortical bone. The *MinerOss* samples also had a higher Ca/P ratio than the value for bone mineral, although the difference was likely not statistically significant. Because each study sample was a blend of at least 3 donors from different lots (as stated on the outer package) of graft mixed together, it is not likely that the differences in Ca/P ratio could be ascribed to some type of bone deficiency. Based on the results of the graft surface area analyses and the visual differences in the allograft materials, it is possible that some kind of chemical interaction used in tissue processing may have removed or perhaps preferentially deposited Ca or P onto the LifeNet Health and *MinerOss* materials.

Further evidence that tissue processing had some effect on the surfaces of these graft particulates was reflected in the surface area measurements (Figure 5). These data were generated using the BET method, which consisted of degassing the graft samples in order to create a clean surface; one that is free of oils, water, etc. This was achieved under high vacuum at 40°C for 20 hours, followed by cooling the material with liquid nitrogen and adsorbing nitrogen gas onto the surface. The gas molecule has a known cross-sectional area, and from the density of the material (measured in Table 2), the weight of the graft and the amount of nitrogen that was adsorbed, a specific surface area could be calculated. The surface area for the *Puros* cancellous graft was double that of the cortical material. Given the difference in the architecture of the two different materials, this result affirmed the different

natures of the two types of allograft. However, this was not the case for the LifeNet Health allograft. The correlation coefficient for the LifeNet Health allograft was very low compared with both the *Puros* and *MinerOss* samples (data not shown), which strongly suggested that something had interfered or interacted with the adsorption of nitrogen gas. The smoother appearance (Figure 2) in the SEM images of the LifeNet Health material compared with the *Puros* material might be the result of the particular processing techniques and chemicals used in the LifeNet Health process. The combination of the smoother appearance in the SEM and the lower surface area means that there is less surface for the early colonization of mesenchymal stem cells (MSC's) and osteoblasts that are required for robust, early bone formation.

The effects of adsorbed organics are known to affect the adsorption of nitrogen in the BET process and therefore can influence surface area measurements by reducing the available surface for nitrogen adsorption.[18] Generally, the higher the correlation coefficient (greater than 0.98 for the BET method) of the isotherm measured in this technique, the more reproducible the results. The low correlation coefficient of the LifeNet Cortical Allograft (data not shown) may be the result of adsorbed material on the surface or simply large variations in the material. Furthermore, the specific surface area of the LifeNet Health processed allograft was significantly less than that of the *Puros* and *MinerOss* allograft. One possible reason for the difficulty in obtaining good surface area data for the LifeNet Health processed graft could be that there were residual chemicals on the surface. As mentioned above, the packaging inserts for the LifeNet Health and *MinerOss* allograft particulates clearly stated that there may be some residual chemicals, detergents, surfactants or antibiotics remaining on the graft. Because all of the bone allograft tested had to go through the rigorous screening processes to ensure good quality tissue, and because the chemical properties and surface properties of natural human bone tissue are relatively consistent, it is likely that the processing of the tissue is at least partially responsible for the SEM, EDX and surface area results.

In contrast to both the *MinerOss* and LifeNet Health processes, the Tutoplast process was described as a gentle process that required a number of steps to produce a safe and sterile graft without damaging the natural collagen or mineral structure of bone.[30] The study data suggested that the Ca/P ratio of the *Puros* allograft was similar to that reported for bone mineral. The increased surface area of the cancellous versus the cortical particulate was expected, and the density of the products was consistent. Taken together, these data suggest that the *Puros* allograft was closer in surface composition and surface properties to natural human bone than the other allografts tested.

It is known that the initial surface of a dental implant material presented to the surgical site is critical for good healing and tissue regeneration.[24] Studies have shown that implant topography as well as chemistry play a major role in cellular response. In a recent study comparing Tutoplast-processed allograft (*Puros* Allograft) with five synthetic bone grafts in a cell culture study using mesenchymal stem cells, the results showed that the *Puros* allograft produced better initial cell attachment that resulted in earlier and greater gene expression from these cells compared with the other calcium phosphate bone graft materials.[31] Recent clinical studies have also shown excellent results in bone regeneration specifically with *Puros* allograft when compared with bovine xenograft material and autologous bone.[32] Taken together, these results demonstrate that *Puros* bone allograft presents consistent surface topography and chemistry for early cellular attachment, proliferation and differentiation of MSC's and osteoblasts. These steps are critical in the early healing and ultimate bone regeneration in bone graft sites.

CONCLUSIONS

Within the limits of this study, the data presented here demonstrates that the process, used to prepare *Puros* allograft material, produced a mineralized bone allograft that visually very closely resembled natural human bone and that was consistent across multiple donors. It was also evident that the surfaces of the Tutoplast processed allograft were closer to that of natural human bone tissue in chemistry and surface properties than any of the other materials tested. These surface properties are likely responsible for the excellent clinical results of the *Puros* allograft as a bone graft compared with other bone graft materials. Additional studies should be conducted to further elucidate the effects of processing on the surface properties of these materials and the resulting bone regeneration.

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