Introduction: Demineralized bone matrix (DBM) derived from allograft donor tissue is used clinically for osseous repair. It is believed that the osteoinductive behavior of DBM is derived from the presence of bone morphogenetic proteins (BMPs), especially BMP-2, -4, and -7, associated with the bone matrix [1,2]. Osteoinductive potential (OP) has traditionally been measured by the expensive and time-consuming method of subcutaneous or intramuscular implantation of an aliquot of DBM into athymic mice or rats, typically for 28 days, followed by histomorphometric analysis of new bone formation [3-5]. Such studies have shown a wide variance in the osteoinductivity of DBM derived from different donors [3-5]. Consequently, there is value in rapidly and inexpensively assay each lot of human DBM.

One simple, rapid, and inexpensive test involves the chemical extraction of BMPs from DBM and their quantification utilizing sandwich enzyme-linked immunosorbent assay (ELISA). While not specifically a test for OP, such analysis, coupled to the traditional test method, can be used to correlate the levels of key BMPs in individual donor tissue to the in vivo response [4]. This could provide a rationale for donor screening based on BMP profile. The purpose of this study was to investigate the variability in the levels of BMP-2, -4, and -7 in DBM extracts derived from a population of human DBM donors that had successfully passed screening procedures required by the United States Food and Drug Administration and the American Association of Tissue Banks.

Materials and Methods: Cortical bone was obtained from twenty human donors (eleven female, nine male), ranging in age from 17-65 years (mean 46.8 years). The bone was denuded of soft tissue and processed with Allowash™ (Lifgenet, Virginia Beach, VA). The bone was then lyophilized, milled, and sieved to a size range of <710 µm. Demineralization was performed by extraction in a stirred, 0.5 N HCl, room temperature bath, with 50 mL of acid used per gram of bone powder. After demineralization, the DBM particles were sequentially washed in purified water, pH 7.4 phosphate buffer, and purified water, then lyophilized.

The BMPs were extracted from the DBM following modification of the procedure of Sampath and Reddi [6]. Approximately 0.25 g of DBM powder was stirred with 5 mL of 4 M GuHCl/0.05 M Tris-HCl at 4°C for 24 hours, followed by centrifugation and removal of the supernatant. The pellet was then resuspended in 5 mL of fresh GuHCl solution and stirred for an additional 5 hours, followed by a final centrifugation and removal of the supernatant. The supernatants (10 mL total) obtained from the two extractions were combined and dialyzed against 500 mL of 0.05 M Tris-HCl, using a 12 KDa molecular weight cutoff membrane, for 15 hours at 4°C. The Tris-HCl was then refreshed with an equivalent volume of fresh buffer and dialysis continued for an additional 10 hours.

Commercially available kits (human BMP-4 and BMP-7 DuoSet Kits and BMP-2 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN) were used to quantitatively assay the concentrations of BMP present in the DBM extracts. All samples were assayed in duplicate and reported as the average ± one standard deviation. A student’s t-test was utilized to determine statistical significance (α=0.05).

Results: The measured levels of the various BMPs, per gram of DBM, were BMP-2: 22.4±12.1 ng (range 1.90-43.3 ng), BMP-4: 5.45±2.05 ng (range 2.28-10.3 ng), and BMP-7: 85.1±43.6 ng (range 28.2-174.4 ng). A student’s t-test showed that BMP-7 content was significantly greater than either BMP-2 or BMP-4, and that BMP-2 content was significantly greater than BMP-4. For each donor, the ratios of the BMPs were also calculated. The average ratios were BMP-7 to BMP-2: 5.38±3.95 (range 2.07-16.87), BMP-7 to BMP-4: 16.56±7.30 (range 5.64-31.65), and BMP-2 to BMP-4: 4.15±2.50 (range 0.84-9.55).

There was no apparent correlation between donor age and the levels of the three BMPs (Fig. 1). The amount of both BMP-2 and BMP-7 were found to correlate with gender, while BMP-4 did not (Fig. 2). Females had an average of 52% more BMP-7 (p=0.012) and an average of 79% more BMP-2 (p=0.012) than males.

Discussion: Among the 20 donors examined, there was a 23-fold, a 4.5-fold, and a 6-fold range in the measured concentrations of BMP-2, -4, and -7, respectively. This variation in osteogenic protein levels could translate to variations in OP. Indeed, Honsawek et al demonstrated a 74% linear correlation between BMP-4 levels from DBM extracts and percent new bone formation in vivo [4]. While the extent to which OP is dependent upon the concentration of each individual BMP is unknown, it is likely that the OP response results from synergy among the many BMPs known to be present in bone matrix. Measurement of the levels of multiple BMPs, as performed in this study, may therefore have significant clinical relevance. Also of interest are the ratios of the BMPs to one another. As with the concentrations of the individual BMPs, the ratios between the BMPs exhibited large variability from donor to donor.

Previous studies have shown varied results as to the role of donor age or gender on the osteoinductive potential of DBM [4,5]. In this study, we found no relationship between donor age and BMP levels. Females, however, exhibited increased BMP-2 and BMP-7 content. Future work should be directed toward measuring the levels of these three BMPs in a larger donor population to improve the ability to observe trends with basic donor characteristics. Testing should also be performed to correlate the multiple BMP profile with in vivo bone formation.

References
4. Honsawek S et al. 49th Meeting of the ORS. 2003; Paper # 0535
5. Traimunedes K et al. 49th Meeting of the ORS. 2003; Paper # 0535