Introduction: There are over 17 demineralized bone matrix based products (DBMs) commercially available as bone graft extenders for fusion procedures. Very few of these DBMs have been evaluated for reliability and fusion efficacy. Recent studies have shown both intra product variability (due to production lots) and inter product variability (product formulations). The purpose of this study is to assess lot-to-lot variability of one DBM product (intra variability) using both in vitro and in vivo assays.

The bone morphogenetic proteins BMP-2 and BMP-7 are known to be osteoinductive, but research on the correlation between BMPs in commercial DBMs and in vivo fusion success is limited. Additionally, in vitro assays for alkaline phosphatase (AP), a marker for osteoblast differentiation, have been used to predict in vivo DBM osteoinductivity, but results have been variable.

To assess lot-to-lot variability and determine whether BMP-2, BMP-7 and/or AP assays can accurately predict the in vivo osteoinductive potential of individual DBM lots from a single vendor.

Materials and Methods: Materials: 10 individual production lots from a commercially available DBM putty in a lecithin carrier (Biomet/EBI: Intergro® DBM Putty, Interpore Cross International, CA) were used.

In vitro methods: 1) BMP-2 and BMP-7 concentrations in each of DBM lots were measured using ELISA. 2) Mouse myoblasts were incubated with each DBM lot. The extent of osteoblast differentiation induced by each lot was detected using an AP assay.

In vivo osteoinductive potential: 40 mature athymic nude female rats were used (137-188, avg.170g, Harlan Sprague Dawley, IN). L4-L5 posterolateral intertransverse process fusion was performed with decortication of only the L4 and L5 transverse processes (lamina and facet joints were left intact without decortication). Wounds were well irrigated. Each of 4 rats was implanted with an aliquot (equal to 0.3 cc/side) from one lot (n = 4 rats per each of 10 lots, for a total of 40 rats). The rats were sacrificed at 8 weeks. High Resolution Radiographs were obtained. The Kappa value was 0.86 indicating excellent agreement between two radiographic coders. Histology was done. Explanted lumbar spines were manually tested for intersegmental motion.

Results: In vivo study: There was significant manual fusion variability across lots (p<0.04) where 23% of the rats were completely manually fused at 8 weeks. While 2 lots almost always promoted fusions, 5 lots consistently failed. 96% of the rats showed de novo bone formation on high resolution radiographs of explanted lumbar after sacrifice at 8 weeks. (Scores from 1-4 were assigned to radiographs based on degree of bone density and remodeling: 1 and 2 = no or little density, while 3 and 4 = massive density with some or good remodeling).

In vitro study: From lowest to highest, there was a five fold difference in amounts of BMP2 and a three fold difference for BMP7 revealing lot-to-lot variability. There was a positive correlation between amount of BMP2 and BMP7 in lots of DBMs (r=0.77, p<0.0001). AP levels did not correlate well with BMP concentrations (p > 0.05) or manual fusion. For some lots, AP was undetectable.

Further, BMP-2 and BMP-7 concentrations positively predicted rate of successful fusions across lots of DBM (BMP2 p < 0.01; BMP7 p<0.009).

Discussion: This is the first of a series of studies to test in vitro predictors of in vivo lot-to-lot variability of Demineralized Bone Matrix (DBM) in one product. The in vivo results demonstrate lot-to-lot differences with consistency within a lot for over half of the DBM lots implanted; several lots demonstrated consistently little bone formation and other lots consistently demonstrated bone formation. ELISA extracted BMPs elucidate sources of variability. Variability accounts for errors in previous DBMs animal studies in which lot variability is not controlled. Yet more importantly, variability may undermine consistent and reliable results when DBM is used clinically.

CONCLUSIONS: There is significant lot-to-lot variability in BMP levels, extent of AP induction, and in vivo fusion rates. BMP-2 and BMP-7 predict spinal fusion success. BMP-2 and BMP-7 levels in DBM are positively correlated.

Levels of AP induction do not correlate well with fusion success. However, AP induction levels may act as a secondary predictor when BMPs are low. Selection for high levels of BMP-2 and BMP-7 in DBM may optimize their use for spinal fusion. BMP-2 and BMP-7 assays may be used to screen DBM lots for osteoinductive potential prior to clinical use. This would improve the predictibility of spinal fusion outcomes.