Variability Among 10 Production Lots of a Single Demineralized Bone Matrix (DBM) Product

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Introduction: There are over 17 demineralized bone matrix based products (DBMs) commercially available as bone graft extenders for fusion procedures. Few of these “off-the-shelf” 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) [1]. The purpose of this study was to assess lot-to-lot variability of one DBM product (intra-variability) using both in vitro and in vivo assays. In particular, can BMP-2, BMP-7 and/or alkaline phosphatase (AP) assays accurately predict the in vivo osteoinductive potential of individual DBM lots from a single vendor? The bone morphogenetic proteins BMP-2 and BMP-7 are known to be osteoinductive [2], but research on the correlation between BMPs in commercial DBMs and in vivo fusion success is limited. Additionally, in vitro assays for AP, a marker for osteoblast differentiation, have been used to predict in vivo osteoinductivity [3], but results have been variable [4]. The inconsistency of fusion outcomes from previous DBM studies [5] warrants the development of a screening method for ensuring optimal osteoinductivity in clinical settings.

Materials and Methods: 10 individual production lots of a commercially available DBM putty.

In vitro methods: 1) BMP-2 and BMP-7 concentrations in each of 10 DBM lots were measured using ELISA. 2) Mouse myoblasts were incubated with each DBM lot, and the extent of subsequent osteoblast differentiation was detected using an AP assay.

In vivo osteoinductive potential: 40 mature athymic nude female rats were used (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 irrigated. An aliquot from each of 10 DBM lots (0.3 cc per side) was implanted into 4 rats (n = 4 rats / each 10 lots, n=40 rats). Rats were sacrificed at 8 weeks. Radiographs and histology were done. Explanted segments were manually tested for intersegmental motion.

Results: In vivo study: 96% of the rats showed de novo bone formation on high resolution radiographs of explanted lumbar spines after sacrifice at 8 weeks (example radiographs, Fig 1). A Kappa value of 0.86 indicated excellent agreement between two radiographic coders. 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 (Fig 2).

In vitro study: From lowest to highest, there was a five-fold difference in amounts of BMP-2 and a three-fold difference for BMP-7 revealing lot-to-lot variability among the aliquots. There was a positive correlation between amount of BMP-2 and BMP-7 in lots of DBMs (r = 0.77, p<0.0001). Most notably, BMP-2 and BMP-7 concentrations positively predicted the rate of successful manual fusions across lots of DBM (BMP-2 p < 0.01; BMP-7 p<0.009), Fig 3. The same 2 lots that induced the highest fusion rate (75%) also contained the highest concentrations of both BMP-2 and BMP-7.

Discussion: This is the first of a series of studies to test in vitro predictors of in vivo lot-to-lot variability in one product of DBM. There is significant lot-to-lot variability in BMP levels, extent of AP induction, and in vivo fusion rates. Fusion demonstrated lot-to-lot differences with consistency within a lot for over half of the DBM lots implanted; several lots consistently demonstrated low levels of bone formation while others promoted consistently high levels of bone formation and fusion. Levels of BMP-2 and BMP-7 in DBM are positively correlated and predict spinal fusion success. BMP-2 and BMP-7 assays may be used to screen DBM lots for osteoinductive potential prior to clinical use. Future selection for high levels of BMP-2 and BMP-7 may optimize DBMs’ use for spinal fusion procedures.

References: 1) Spine 2006 31(12):1299-306
2) Orthop 2004 (1 Suppl):s161-5
5) Eur Spine J 2007 16(8):1233-40