Evaluation of a Novel Allograft Derived Growth Factor

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INTRODUCTION

Surgical tissue engineering approaches to repair skeletal defects require the osteobiologic elements of osteoinduction and osteoconduction. Currently available demineralized bone matrix (DBM) products provide some osteoconductivity but offer low growth factor concentrations, little osteoinductivity, and a high degree of variability. While rhBMP-2, manufactured from genetically modified chinese hamster ovary cells (InFuse, Medtronic, Inc., Memphis, TN), has been shown to be highly osteoinductive, complications and price concerns drive the need for an alternative. Advanced methods have recently been developed to better harness the osteoinductivity of allograft tissue. The hypothesis is that these methods will result in a more potent osteoinductive allograft.

This study examined the spectrum of osteoinductive, angiogenic, and mitogenic proteins derived from allograft tissue using the AMP™ process, and these proteins' effect on osteogenesis *in vivo*.

MATERIALS AND METHODS

Allograft growth factor (OsteoAMP™, Advanced Biologics, Irvine, CA) was prepared from tissue using a minimal manipulation process. Briefly, tissue was obtained from a cadaveric donor, and the growth factors were harvested, purified, and lyophilized into a soluble powder. Demineralized allografts (OsteoAMP™ Putty and OsteoAMP™ Sponge, Advanced Biologics, Irvine, CA) were prepared from cadaver tissue utilizing the AMP minimal manipulation process. Briefly, tissue was harvested from a cadaveric donor, demineralized using a proprietary method, and lyophilized into a dehydrated powder or compressible sponge forms.

For growth factor quantification, the growth factor was reconstituted with sterile water prior to testing. BMP-2 concentration was quantified using ELISA (R&D Systems, Minneapolis, MN). TGF-β1, aFGF, FGF-6, ANG-1, and VEGF were quantified using a Quantibody Protein Array (Raybiotech, Norcross, GA). Previously reported concentrations for BMP-2 and TGF- β1 found in demineralized cortical bone powder[1], percent demineralized bone found in DBM product[2], and previously reported BMP-2 concentration found in OsteoSponge™ (Bacterin International, Inc., Belgrade, MT)[3] were used for comparison.

To test BMP-2 binding to a variety of carriers, the growth factor was added to allograft and synthetic carriers. Allograft carriers included cortical, cancellous, demineralized cortical, and demineralized cancellous bone. Synthetic carriers included a collagen sponge (ACS, Medtronic, Memphis, TN), calcium phosphate granules (Vitoss™, Orthovita, Malvern, PA), and a collagen/calcium phosphate composite sponge (Mozaik™, Integra OrthoBiologics, Irvine, CA). BMP-2 was then quantified from the depleted solution using ELISA.

In vivo osteoinductivity was tested by adding OsteoAMP™ to demineralized cortical bone powder prior to implantation in an athymic rat model. Demineralized cortical bone powder (from the same donor) without OsteoAMP™ was used as a control. After 28 days, osteoinductivity was quantified by histomorphometry.

RESULTS

In vitro characterization of OsteoAMP™ demonstrated that soluble levels of BMP-2, TGF-β1, aFGF, FGF-6, ANG-1, and VEGF were all present (Figure 1). Most notably, BMP-2 and TGF- β1 levels in OsteoAMP™ were roughly 170 times and 3 times the levels found in demineralized cortical bone powder, respectively[1] (Figure 2). The data also demonstrated that OsteoAMP™ Putty and OsteoAMP™ Sponge contained significantly higher levels of BMP-2 than previously reported values of demineralized cortical bone powder and demineralized cancellous bone (OsteoSponge™). OsteoAMP™ Bone Putty contained greater than 350 times more BMP-2 than found in DBX Putty (Figure 4). OsteoAMP™ Bone Sponge contained greater than 25 times more BMP-2 than the OsteoSponge™ . Substantial levels of BMP-2 became bound to all carrier materials tested (Figure 3). *In vivo* histology results after 28 day athymic rat implantation demonstrated amplified osteogenesis for OsteoAMP™ compared to demineralized bone powder control (Figure 5). Histomorphometric analysis revealed that OsteoAMP™ produced greater than 16 times more new bone area compared to demineralized bone powder (Figure 6).

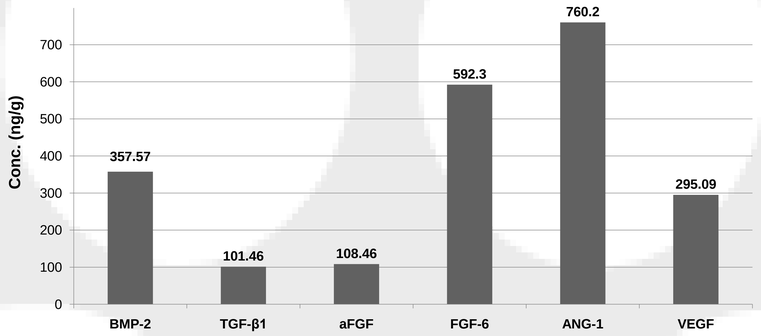

Figure 1. Osteoinductive, angiogenic, and mitogenic growth factor proteins found in OsteoAMP™ soluble powder

Figure 2. OsteoAMP™ and OsteoAMP™ Bone Putty BMP-2 content compared to DBM powder and platelet rich plasma

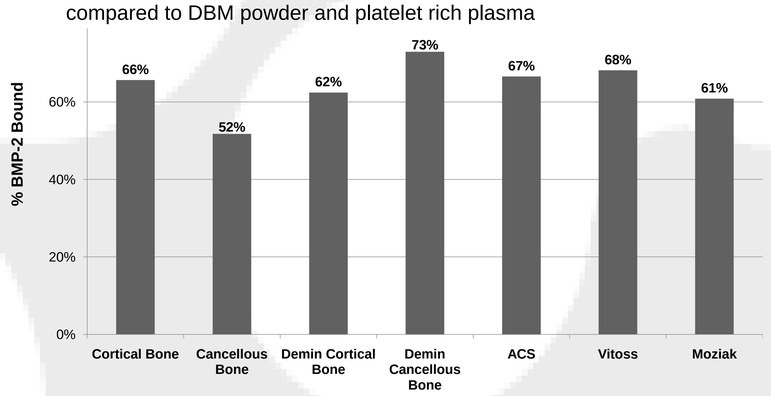

Figure 3. OsteoAMP™ BMP-2 binding affinity to carriers.

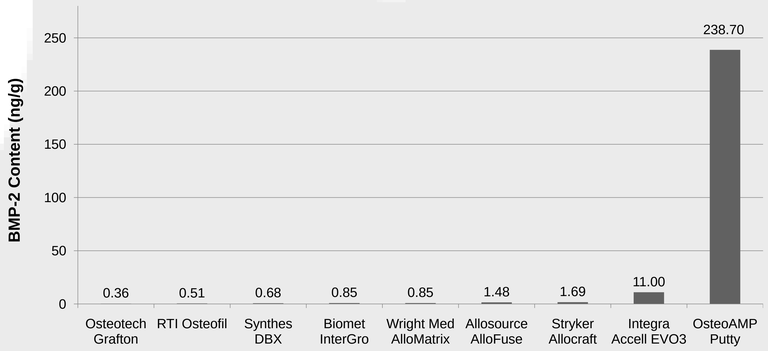

Figure 4. Levels of BMP-2 found in various DBM products compared with OsteoAMP™ Bone Putty

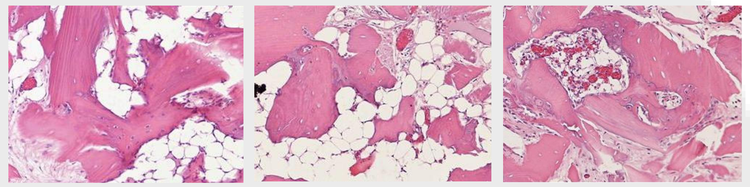

Figure 5. Histology (20x mag.) after 28 day implantation in athymic rat. OsteoAMP™ Bone Putty (left), DBM powder (center), OsteoAMP™ (right)

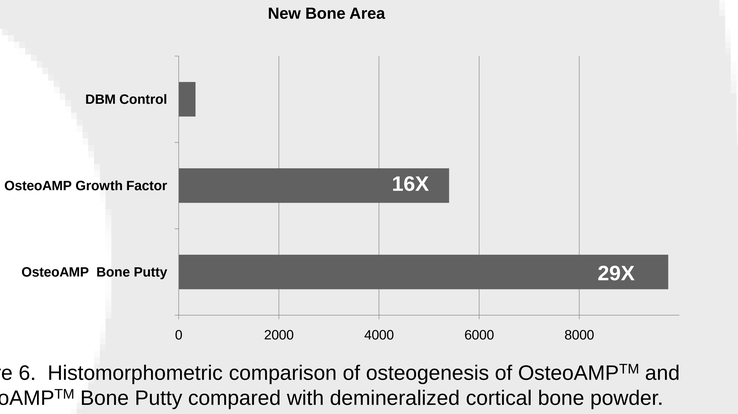

Figure 6. Histomorphometric comparison of osteogenesis of OsteoAMP™ and OsteoAMP™ Bone Putty compared with demineralized cortical bone powder.

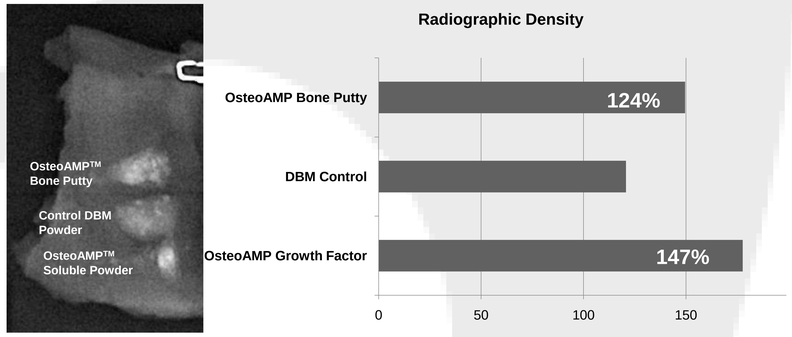

Figure 7. X-ray density after 28 day implantation in athymic rat.

DISCUSSION

Based on *in vitro* and *in vivo* data, OsteoAMP™ is an osteoinductive growth factor that provides osteoinductive, angiogenic, and mitogenic proteins to increase osteogenesis. OsteoAMP™ may be effective in orthopedic applications when rhBMP-2 is not approved for usage and greater levels of osteoinductivity are required than demineralized bone products can provide.

REFERENCES

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2. Kay JF, Vaughan LM. AW0204.1 2004
3. Bacterin OsteoSponge Marketing Brochure 5041A 2006

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