



# Novel Growth Factor Amplifies Osteogenesis Via Osteoinductive, Angiogenic, and Mitogenic Proteins

A. Govil<sup>1</sup>, S. Cadotte<sup>1</sup>

<sup>1</sup>Advanced Biologics, Irvine, CA  
agovil@advancedbiologicscorp.com

Advanced Biologics

## 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<sup>TM</sup> process, their affinity to allograft and synthetic scaffolds, and these proteins' effect on mineralization and osteogenesis *in vivo*. OsteoAMP<sup>TM</sup> is an allograft that utilizes the AMP<sup>TM</sup> technology and harnesses the osteoinductivity of autograft tissue. OsteoAMP<sup>TM</sup> contains osteoinductive and angiogenic growth factors at levels higher than those found in other allografts on the market.

## MATERIALS AND METHODS

Bioactive allografts (OsteoAMP<sup>TM</sup> and OsteoAMP<sup>TM</sup> Bone Putty, Advanced Biologics, Irvine, CA) were prepared from tissue using a minimal manipulation process. Briefly, tissue was obtained from a cadaveric donor, and the growth factors were harvested, purified, and either lyophilized into a soluble powder or bound to demineralized cortical bone powder.

For growth factor quantification, the proteins were reconstituted with sterile water prior to testing. BMP-2 concentration was quantified using ELISA (R&D Systems, Minneapolis, MN). TGF- $\beta$ 1, 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- $\beta$ 1 found in demineralized cortical bone powder and platelet rich plasma were used for comparison.

To test BMP-2 binding to a variety of carriers, OsteoAMP<sup>TM</sup> 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<sup>TM</sup>, Orthovita, Malvern, PA), and a collagen/calcium phosphate composite sponge (Mozaik<sup>TM</sup>, Integra OrthoBiologics, Irvine, CA). BMP-2 was then quantified from the depleted solution using ELISA.

*In vivo* osteoinductivity was tested by implanting OsteoAMP<sup>TM</sup> pre-bound to demineralized bone powder and adding OsteoAMP<sup>TM</sup> lyophilized powder to demineralized cortical bone powder prior to implantation in an athymic rat model. Demineralized cortical bone powder (from the same donor) without OsteoAMP<sup>TM</sup> was used as a control. After 28 days, mineralization was quantified by x-ray density and osteogenesis was quantified by histomorphometry.

## RESULTS

*In vitro* characterization of OsteoAMP<sup>TM</sup> demonstrated that soluble levels of BMP-2, TGF- $\beta$ 1, aFGF, FGF-6, ANG-1, and VEGF were all present in significant concentrations (Figure 1). Most notably, BMP-2 and TGF- $\beta$ 1 levels in OsteoAMP<sup>TM</sup> were roughly 170 times and 3 times the levels found in demineralized cortical bone powder, respectively<sup>[1][2]</sup> (Figure 2). Substantial levels of BMP-2 in OsteoAMP<sup>TM</sup> became bound to all carrier materials tested (Figure 3).

*In vivo* histology results after 28 day athymic rat implantation demonstrated amplified osteogenesis for OsteoAMP<sup>TM</sup> and OsteoAMP<sup>TM</sup> Bone Putty compared to demineralized bone powder control (Figure 4). Histomorphometric analysis revealed that OsteoAMP<sup>TM</sup> and OsteoAMP<sup>TM</sup> Bone Putty produced greater than 16 times and 29 times more new bone area respectively, compared to demineralized bone powder (Figure 5). Radiographic analysis demonstrated that both OsteoAMP<sup>TM</sup> and OsteoAMP<sup>TM</sup> Bone Putty resulted in higher mineralization when compared to demineralized bone powder control (Figure 6).

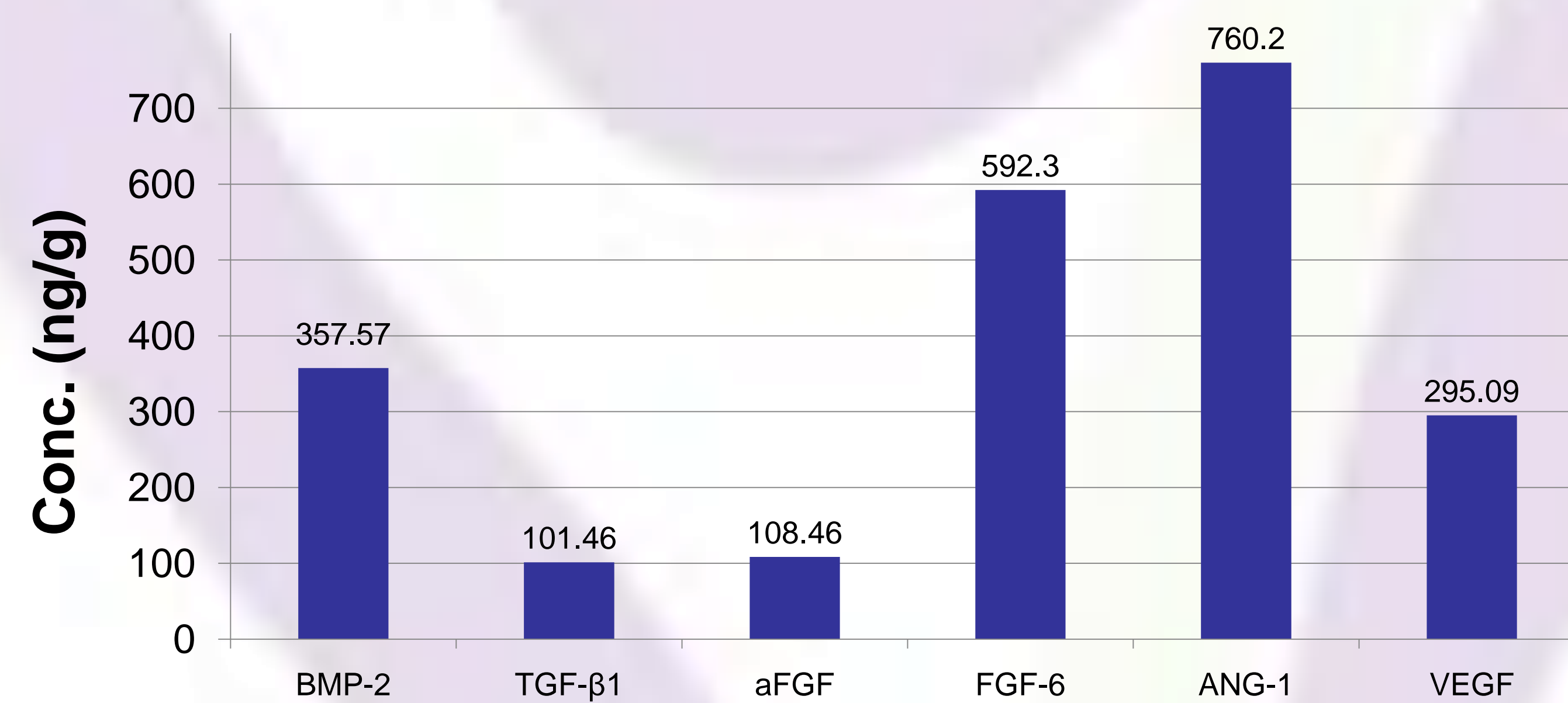


Figure 1. Osteoinductive, angiogenic, and mitogenic growth factor proteins found in OsteoAMP<sup>TM</sup> soluble powder

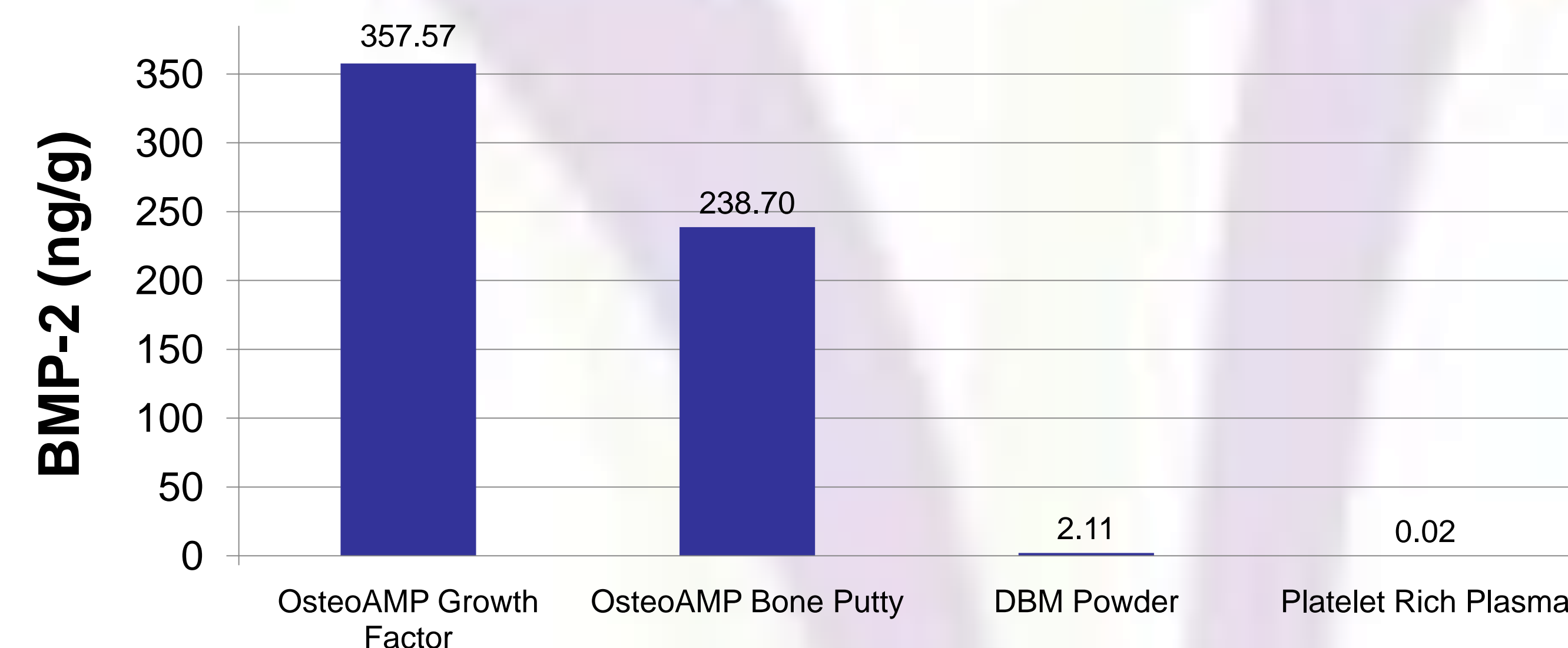


Figure 2. OsteoAMP<sup>TM</sup> and OsteoAMP<sup>TM</sup> Bone Putty BMP-2 content compared to DBM powder and platelet rich plasma

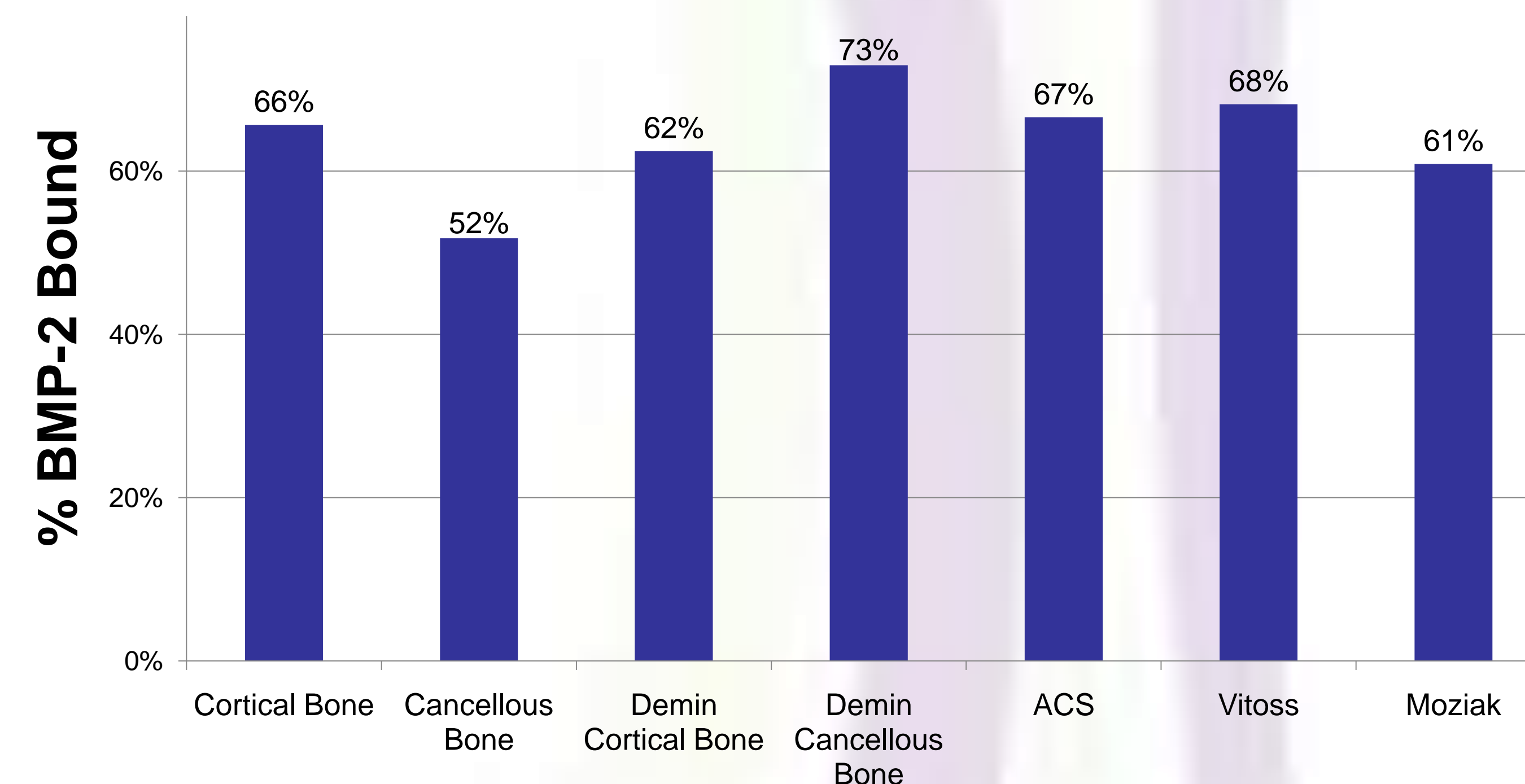


Figure 3. OsteoAMP<sup>TM</sup> BMP-2 binding affinity to carriers.

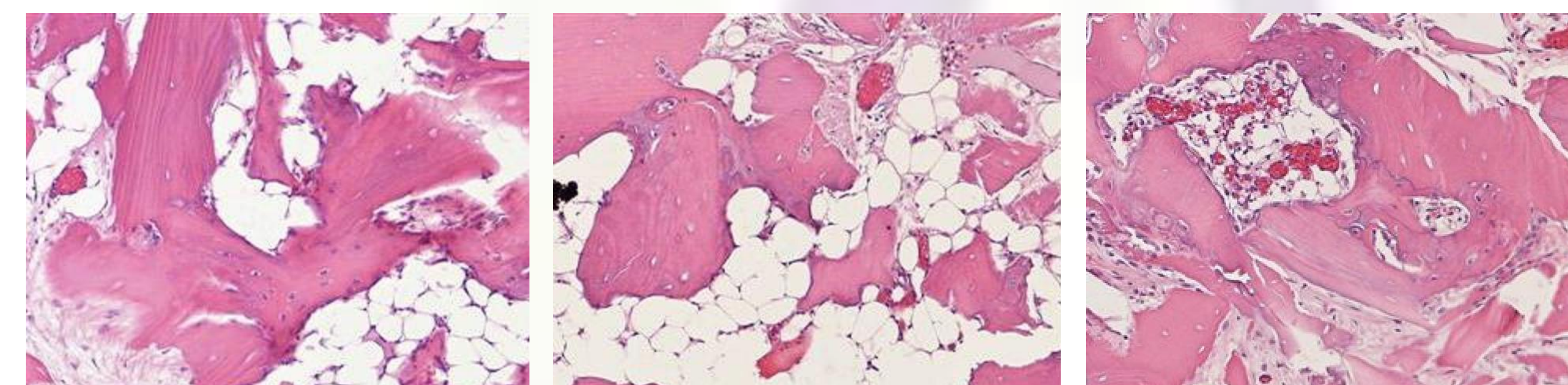


Figure 4. Histology (20x mag.) after 28 day implantation in athymic rat. OsteoAMP<sup>TM</sup> Bone Putty (left), DBM powder (center), OsteoAMP<sup>TM</sup> (right)

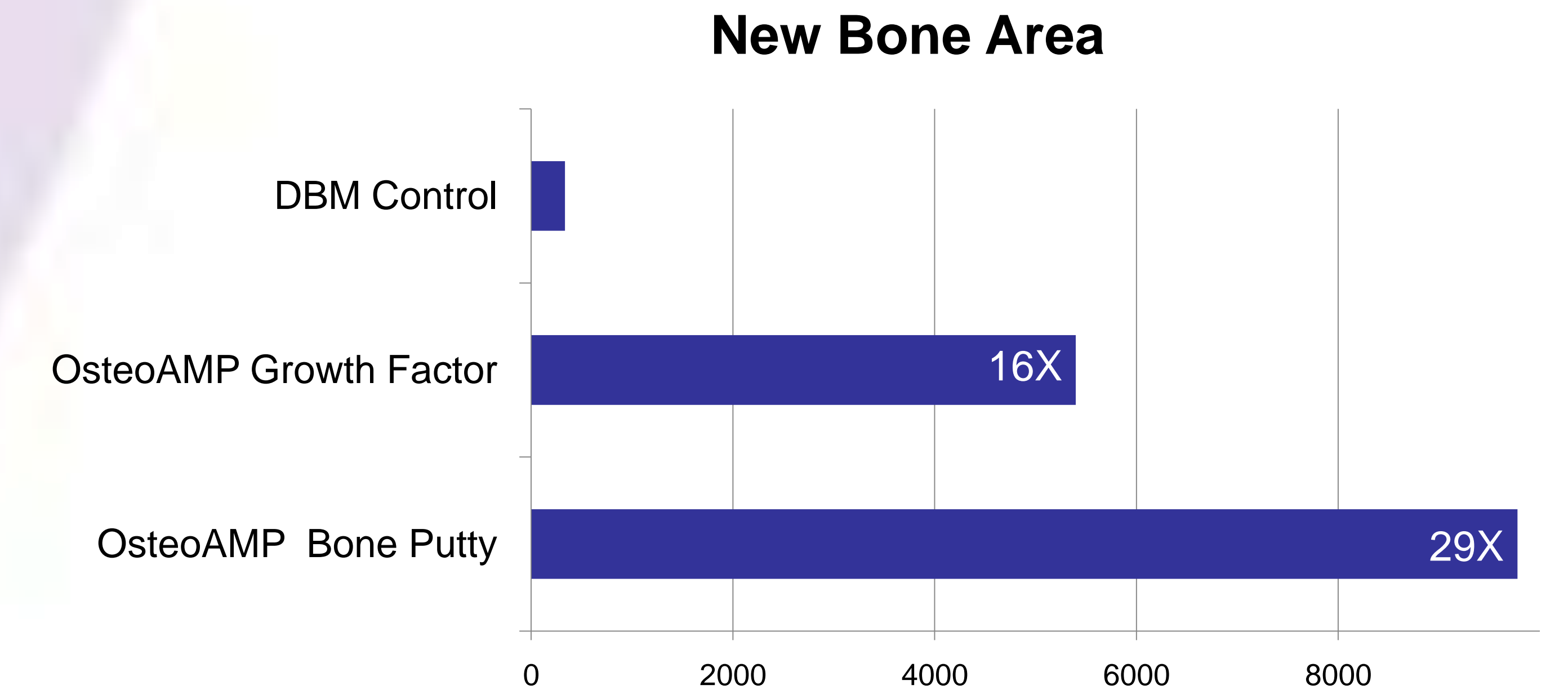


Figure 5. Histomorphometric comparison of osteogenesis of OsteoAMP<sup>TM</sup> and OsteoAMP<sup>TM</sup> Bone Putty compared with demineralized cortical bone powder.

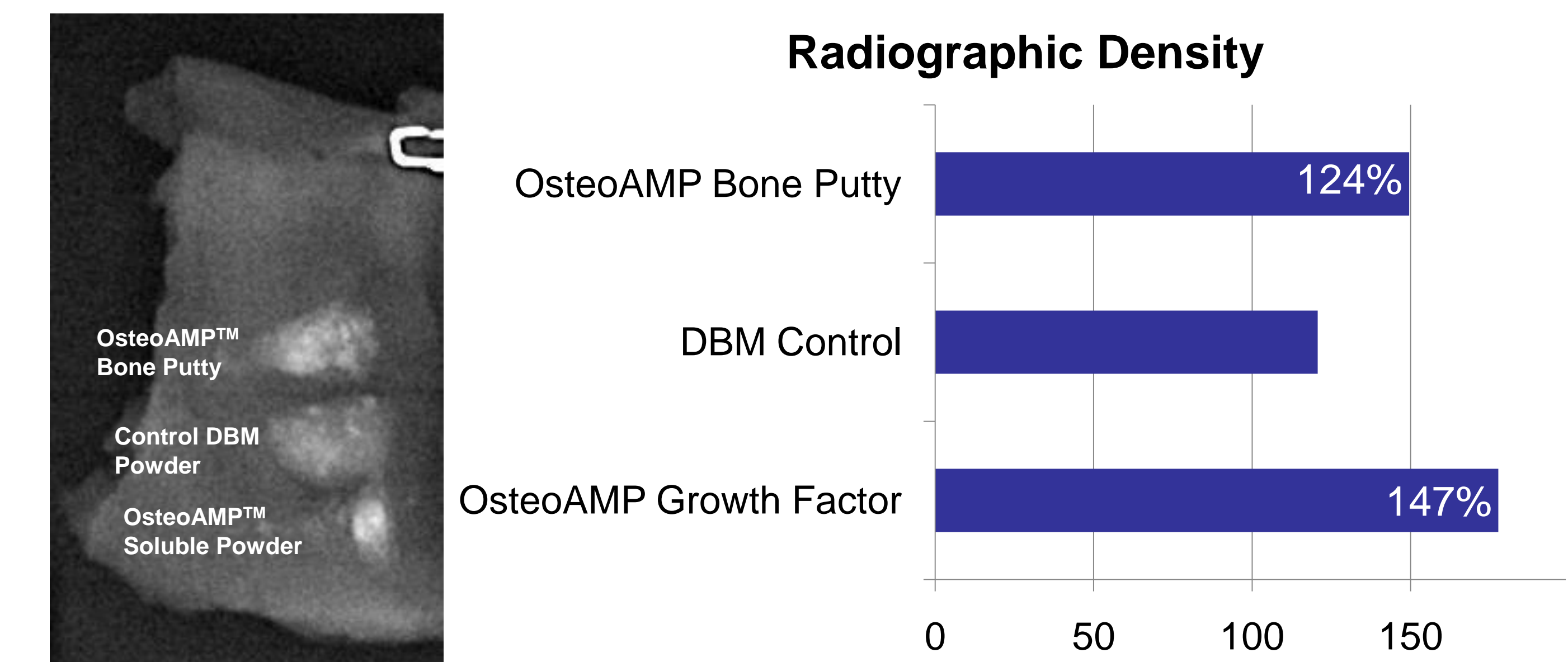


Figure 6. X-ray density after 28 day implantation in athymic rat.

## DISCUSSION

Based on *in vitro* and *in vivo* data, OsteoAMP<sup>TM</sup> and OsteoAMP<sup>TM</sup> Bone Putty provide osteoinductive, angiogenic, and mitogenic proteins resulting in increased osteogenesis. OsteoAMP<sup>TM</sup> and OsteoAMP<sup>TM</sup> Bone Putty 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

1. Blum, B et al. Orthopedics 2004, 27; S161 – S165
2. Kalen, A et al. Biochem and Biophys Res Com 2008, 375; 261 - 265