The bone-forming potential of Natural Matrix Protein® (NMP®) bioimplants compared with cellular, peptide, and growth factor-enhanced bone graft substitutes

Sean Peel, Ph.D.[a,b,c] | Presented at the 38th Annual Canadian Biomaterials Meeting, Halifax, Canada June 14-17, 2023

Introduction

Achieving osteoregeneration is a significant challenge in many cases due to underlying comorbidities (severe trauma, osteoporosis, smoking, etc.) resulting in over 1.6 million procedures annually in the United States and requiring the use of bone grafts to enhance the body's natural osteoregenerative potential.

Autogenous bone graft (ABG) harvested from a donor site (i.e. iliac crest) is considered the gold standard bone graft material, as it provides a scaffold, osteogenic cells and potentially growth factors. However, there are well-recognized problems associated with ABG including increased operating time, blood loss, donor site infection and pain.[1] Further, there is a limited amount of autogenous bone available for grafting. This has led to the use of a number of alternatives to ABG (Table 1). These include several types of grafts that claim to possess enhanced potential due to the presence of growth factors, peptides, or cells including:

Demineralized bone matrix (DBM) comprises a collagenous matrix that contains growth factors including BMP-2, BMP-7, VEGF, TGF-ß and PDGF. DBM is considered to have osteoinductive potential due to the presence of multiple bone morphogenetic proteins (BMPs) within its matrix. However, the growth factors are bound within the matrix and are released very slowly; hence the osteoinductive activity and clinical efficacy of DBM is limited due to the low bioavailability of these growth factors.[2]

OsteoAMP® (Bioventus) is a bone allograft product that has been processed with marrow components in a manner that is reported to enhance the biologic activity of the matrix. To date, there is limited evidence that OsteoAMP is osteoinductive.

OsteoFactor® Allogenic Proteins (Xtant Medical) is a protein extract of growth factors found within the endosteum of allograft bone. It is combined with a scaffold material to enhance the scaffold. Currently there is no evidence as to whether OsteoFactor is osteoinductive or enhances the osteogenic potential of other bone graft substitutes.

Cellular Bone Allografts (CBA) contain viable cells within an osteoconductive bone matrix. These can either be a mixture of cell types including mesenchymal cells (MSCs) (i.e. Trinity Elite®, Orthofix) or can be enriched for committed osteoprogenitor cells (i.e. Vivigen®, J&J). The evidence of clinical efficacy of CBAs is limited and some studies suggest that the presence of viable cells has no effect on the performance of CBAs.[3]

i-FACTOR® (Cerapedics) is a graft that combines bovine anorganic bone matrix with a peptide (P-15) to promote cell adhesion. Studies have indicated that the addition of the peptide increases bone formation following implantation in various animal models. However, in clinical studies, the outcomes are similar to those seen with mineralized osteoconductive grafts that lack peptide enhancement.

INFUSE® Bone Graft (Medtronic) comprises recombinant human BMP-2 (rhBMP-2) soaked onto an acellular collagen sponge (ACS). rhBMP-2 is osteoinductive, stimulating the differentiation of connective tissue progenitor cells into osteoblasts and INFUSE has been shown to be as effective as

(continued pg 2)

Table 1: Types of Bone Grafts

Graft Type	How it Works	Problems
Autograft (local or from donor site)	Scaffold – osteoconduction; Cells - osteogenic cells; growth factors (osteoinductive ?)	Limited supply (local, pediatric) Additional OR time, pain, donor site morbidity
Synthetic, Xenograft, Mineralized allograft	Scaffold;	Limited effectiveness often combined with autograft, bone marrow aspirate (BMA)
Demineralized bone matrix (DBM)	Scaffold; BMPs – osteoinductive Other growth factors	Efficacy dependent on processing and donor variability – clinical efficacy limited
Cellular Bone Allografts (CBA) (bone + cells, +/- DBM)	Scaffold Cells – osteogenic (?) DBM - growth factors (?)	Cell viability concerns, cell types and fates poorly understood, dependent on donor characteristics; increased risk of disease transmission; difficult to store & prepare; Expensive
Peptide Enhanced (P15 on anorganic bone)	Scaffold; Peptide enhances cell adhesion	Limited evidence that peptide provides any benefit, limited approved indications; Expensive
Natural growth factor enhanced (bone/cell extracts)	Scaffold; Tissue extracts – BMPs (?)	Limited evidence that extracts provide any benefit
Recombinant Growth Factor Enhanced	Delivers recombinant human growth factors from a carrier (BMP-2 or PDGF-BB)	Severe adverse events – inflammation, ectopic bone formation, pain, irregular bone formation/resorption Very Expensive; limited indications for use (warning - cervical spine)

[a]Red Rock Regeneration Inc, Toronto, Canada [b]University of Toronto, Toronto, Canada
[c]Induce Biologics USA Inc., Irvine, CA

INDUCE BIOLOGICS

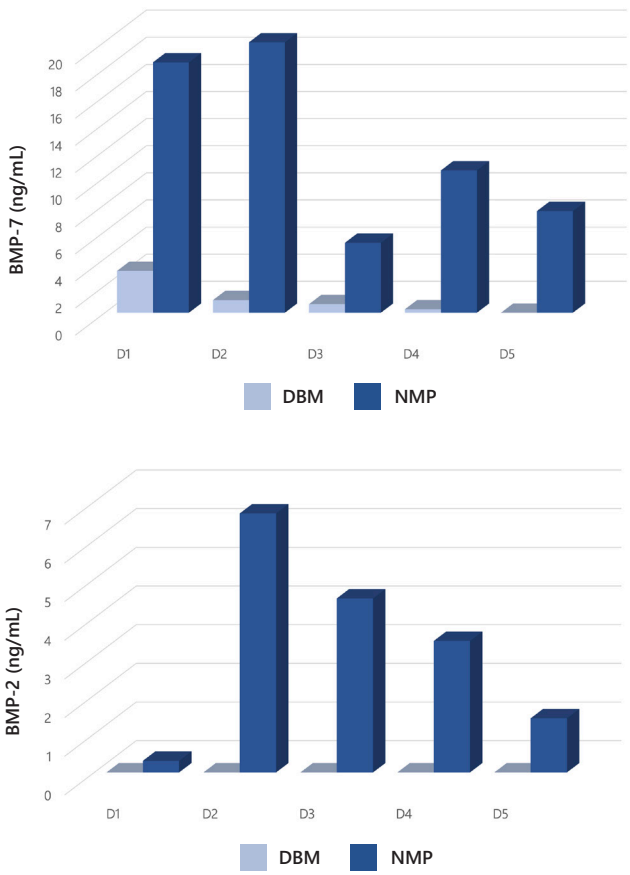
(continued from pg 1)

ABG in some clinical studies, However, INFUSE has been associated with numerous adverse events including ectopic bone formation, severe inflammation, cyst-like voids in the newly formed bone, radiculopathy, retrograde ejaculation, and cancer.[4]

Recently, a novel bone graft substitute **Natural Matrix Protein (NMP; Induce Biologics USA)** bioimplant has been introduced to the market. NMP is derived from bone matrix that has undergone a proprietary processing method that has been shown to consistently increase the bioavailability of growth factors including BMP-2, BMP-7, VEGF, PDGF-BB and TGF-ß1.[5] (Figure 1)

Figure 1

BMP-2 and BMP-7 bioavailability in bone processed into DBM or NMP from 5 different donors



(from Y. KOHEN, S. SHIVANNA, S. PEEL (2022) Evaluation of the Natural Matrix Protein (NMP) bone allograft in vitro and in vivo. JBMR 37:Supplement 1: p342)

The aim of the current study was to compare the bone-forming potential of NMP bioimplants to that of several commercially-available cell, peptide, and growth factor-enhanced bone graft substitutes in the athymic rat muscle pouch model.

Materials & Methods

All test articles were purchased from their manufacturers and were stored and prepared as described in their Instructions for Use (IFUs).

Table 2: Storage and Preparation of Test Articles

Graft Type	Form	Storage Temp	Preparation
OsteoAMP	Granules	11 to 25°C	Rehydrate with saline for 10+ mins
OsteoFactor	Vial of powder	11 to 30°C	Rehydrate powder and apply to scaffold, allow to soak for 15+ mins
i-FACTOR	Putty in syringe	Ambient	n/a
Vivigen	Bone particles in cryoprotectant	<-70°C	Thaw (~15 minutes)
Trinity Elite	Bone particles in cryoprotectant	-70 to -80°C	Thaw, decant, rinse, decant (~20 mins)
INFUSE	Vial powder, vial water, ACS	15 to 30°C	Solubilize GF, apply to ACS soak for 15+ mins
NMP	Granules	Ambient	Rehydrate with saline ~ 5 mins

In Vivo Evaluation of Test Articles

Thirty-five male athymic rats were divided into 7 groups of 5 animals. Each group had 0.2cc of test article implanted in muscle pouches formed in each hind leg (n=10 implants per group).[6]

The test articles are summarized in Table 3 below.

For the INFUSE implants, 0.7mL of the 1.5mg/mL BMP-2 solution was added to the 1.25 x 5.08 x 0.3cm collagen sponge. After allowing the sponge to stand for at least 15 minutes, the sponge was cut into 10 strips each, approximately 0.2mL in volume and containing 0.105mg of rhBMP-2. These strips were then placed in muscle pouches.

The other test articles were prepared according to their IFUs and then packed into an open-barreled 1cc syringe to prepare 0.2mL implants, which were then placed in the muscle pouches.

After 28 days, the rats were euthanized and the implants were recovered, fixed in 10% neutral buffered formalin and evaluated by microCT. Following microCT analysis, the implants were decalcified and processed for light microscopy.

Table 3: Test Articles

Test Article	Bone Graft Type	N	Implant Volume (mL)
OsteoAMP (AMP)	Processed allograft with marrow	10	0.2
OsteoAMP + OsteoFactor (AMP+OF)	Processed allograft with endosteal tissue	10	0.2
i-FACTOR	Peptide enhanced	10	0.2
Vivigen	CBA (committed)	10	0.2
Trinity	CBA (mixed cells)	10	0.2
INFUSE	Recombinant growth factor enhanced	10	0.105mg rhBMP-2 (0.2ccACS)
NMP	Enhanced growth factor bioavailability	10*	0.2

**2 separate lots of NMP were used*

Evaluation of Unimplanted Test Articles

With the exception of INFUSE, a prepared but unimplanted sample of each test article was fixed with 10% neutral buffered formalin and analyzed by microCT. Following microCT analysis, the unimplanted samples were decalcified and processed for light microscopy.

Results

MicroCT Analysis of Unimplanted Test Articles

The OsteoAMP, OsteoAMP+OsteoFactor, Trinity, Vivigen and i-FACTOR unimplanted samples all contained mineralized tissue that confounded the assessment of bone formation in the recovered implants. No mineralized tissue was present in the unimplanted NMP. (Figure 2)

MicroCT Analysis of Recovered Implants

The OsteoAMP and OsteoAMP+OsteoFactor recovered implants appeared similar to the unimplanted samples. Large, irregular, well-defined chips of highly-mineralized tissue were seen throughout the implant. Some smaller fiber-like material was also seen. There was no sign of new bone formation. (Figure 3)

The i-FACTOR recovered implants also appeared similar to the unimplanted sample with small, well-defined, high-intensity "granules" present throughout the recovered implant and no sign of new bone formation.

Vivigen and Trinity both appeared to have small chips and fibers of high intensity with some thin, fibrous areas of moderate intensity. One or two small ossicle-like structures (moderate-intensity mineralized ring surrounding non-mineralized core) were seen in some Vivigen implants. These were more common and larger in the Trinity implants (Figure 3 red arrow).

The recovered implants of INFUSE were visibly smaller than those of the other test articles and microCT showed a single, well-defined ossicle with a moderately intense ring of newly-formed tissue surrounding an unmineralized core.

The recovered NMP implant had a newly-formed moderate-intensity punctate appearance throughout the recovered implant volume, which was suggestive of new bone formation throughout the implant.

Figure 2

MicroCT of unimplanted test articles

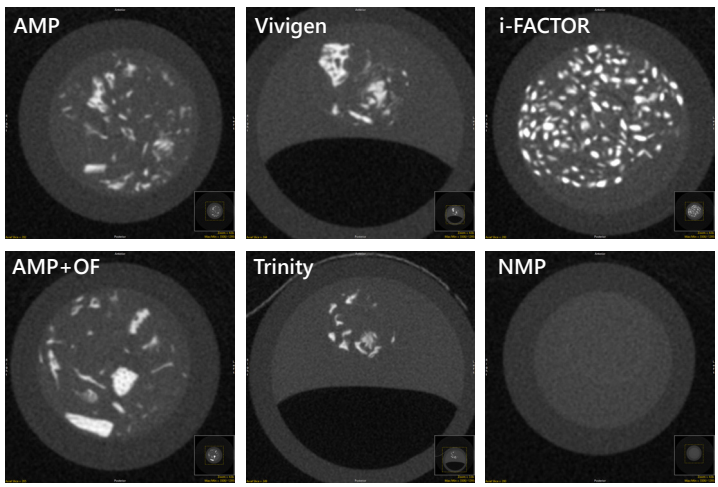
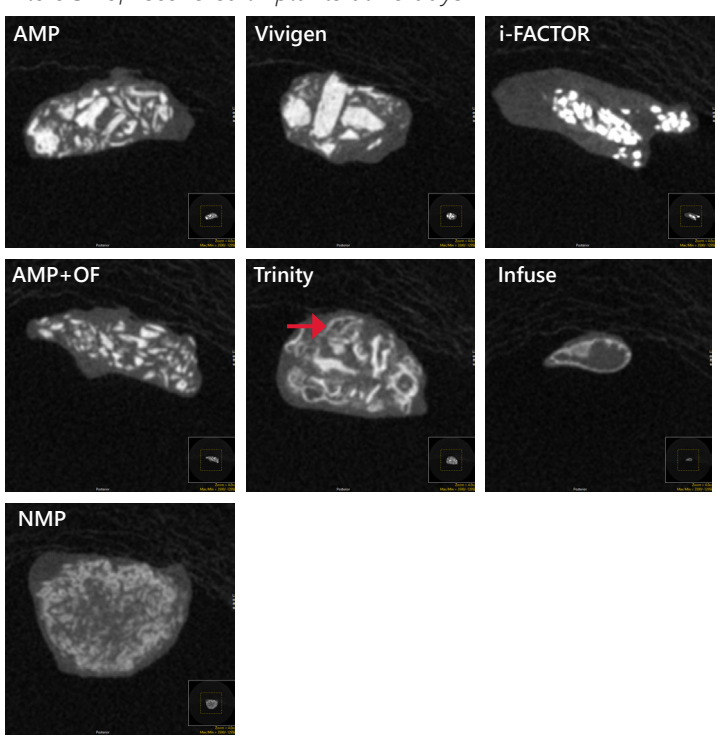


Figure 3

MicroCT of recovered implants at 28 days



Histological Analysis

OsteoAMP and OsteoAMP+OsteoFactor implants had large, non-vital bone chips (NVB) surrounded by a dense cellular infiltrate (*). Smaller, non-vital bone fragments are also visible. No new bone was seen in any implant.

i-FACTOR implants had a dense cellular infiltrate (*) surrounding the anorganic bone matrix (ABM) granules. Small fragments of ABM were also seen (F). No new bone was seen in any implant.

Vivigen implants comprised large, non-vital bone chips (NVB) with loose stromal tissue (ST) within gaps in the large chips that were surrounded by a cellular infiltrate (*). Thin, elongated fragments of non-vital bone were also seen throughout the implant. Five of the ten implants had areas of new bone (NB).

Trinity implants comprised non-vital bone chips (NVB) surrounded by loose cellular infiltrate (*). One or more small to medium-sized ossicles were observed in each of the 10 implants where new bone (NB) formed on the NVB chips and surrounding bone marrow (BM)

All 10 INFUSE implants comprised a single ossicle with a thin shell of new bone (NB) surrounding bone marrow (BM) and residual collagen sponge.

All 10 NMP implants contained multiple ossicles of new bone (NB) fused to the NMP granules (NMP) that surrounded bone marrow (BM). Occasional islands of cartilage were also seen.

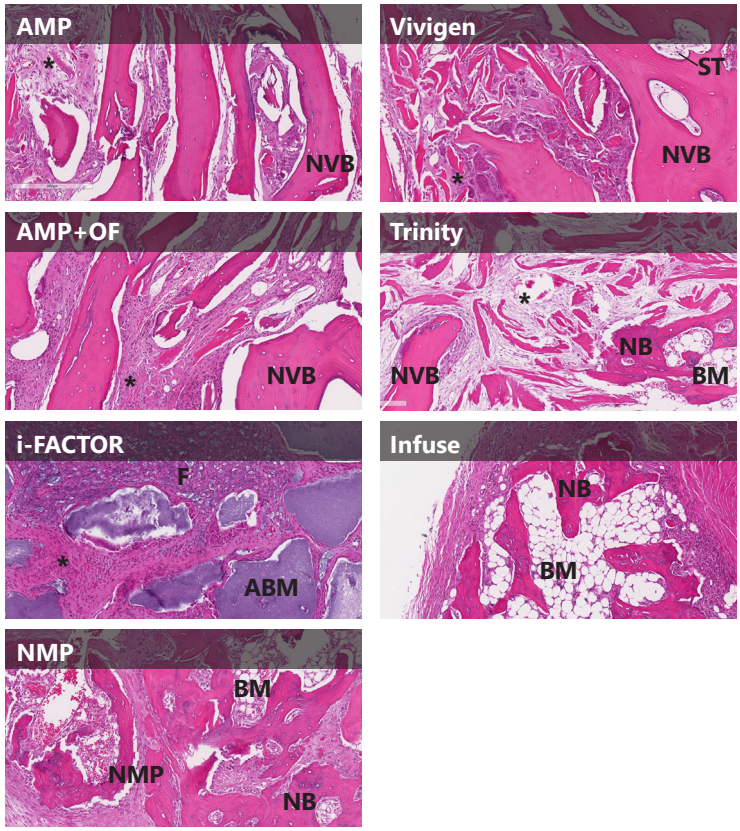
Conclusions

The NMP bioimplant was osteoinductive and consistently produced new bone throughout the implant in the athymic rat muscle pouch model. INFUSE produced significantly less bone than NMP and formed large cyst-like ossicles.

OsteoAMP and i-FACTOR did not demonstrate any osteoinductive or osteogenic potential. The addition of OsteoFactor to OsteoAMP did not stimulate bone formation.

The osteogenic potential of the cellular bone grafts was variable with Trinity being more osteogenic than Vivigen. These grafts were the most challenging to store and prepare.

Figure 4
Histological appearance of implants



References

[1] Arrington et al. Complications of iliac crest bone graft harvesting. Clin Orthop Relat Res. 1996; 329:300-9.

[2] Gruskin et al. Demineralized bone matrix in bone repair: History and use. Advanced Drug Delivery Reviews 2012; 64: 1063-1077

[3] Russell et al. *In vivo* Performance of Seven Commercially Available Demineralized Bone Matrix Fiber and Putty Products in a Rat Posterolateral Fusion Model. Front Surg 2020; 7: 10

[4] Mroz et al. Complications related to osteobiologics use in spine surgery: a systematic review. Spine 2010; 35: S86-104

[5] Kohen Y., Shivanna S., Peel S. (2022) Evaluation of the Natural Matrix Protein (NMP) bone allograft *in vitro* and *in vivo*. JBMR 37:Supplement 1: p342

[6] ASTM F2529-13 "Standard Guide for *in vivo* Evaluation of Osteoinductive Potential for Materials Containing Demineralized Bone (DBM)"

Disclosures

Performance *in vitro* & *in vivo* are not necessarily indicative of human clinical outcome.

NMP grafts are intended for use as a bone void filler for filling voids and gaps in the skeletal system that are not intrinsic to the stability of the bony structure.

Please see the NMP IFU for a complete list of indications, contraindications, warnings, precautions, and other important medical information.

NMP grafts are marketed by Induce Biologics USA https://www.inducebiologics.com.