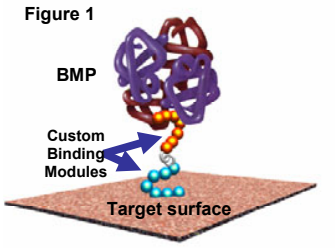
High Affinity Binding Peptides for Bone Morphogenic Protein

+*Hamilton, PT; *Orgambide, G; *Benson, RE; *Dey, M; *Solan, A; *Hyde-DeRuyscher, R; *Beyer, W; *Chen, Y; *Hodges, JA; **Cook, SD
+*Affinergy, Inc., RTP, NC, **Tulane Medical Center, New Orleans, LA

Statement of Purpose: Bone grafts that combine growth factors such as bone morphogenic protein (BMP) and biomaterial carriers have shown great promise. However, they still face limitations. For example, high initial doses of BMP are required to maintain physiological levels during the interval required for healing. This makes recombinant therapies more expensive and may lead to detrimental side effects such as ectopic bone formation, allergic reactions, or production of neutralizing antibodies. We have developed a generalized approach to create target-specific modular peptides termed "interfacial biomaterials" (IFBM's, **Figure 1**) that bind biologic agents (growth factors, cells, etc.) to synthetic surfaces (polymers, metals, etc.) that can address these limitations. Coating bone grafts with these linker peptides has the potential to improve their ability to retain exogenously delivered BMP and also potentially promote the attachment of endogenous growth factors on their surface at a site of healing. The objective of this study was to demonstrate that a collagen:BMP IFBM improves retention of BMP-2 on a collagen matrix.

Figure 1



Methods and Results: Biotinylated rhBMP-2 was immobilized onto streptavidin-coated microtiter plates and subjected to 3 rounds of phage display selections. Monoclonal phage were propagated on *E. coli* overnight. The cells were removed by centrifugation and 10 µl of the phage containing supernatant was added to the wells containing BMP-2 or to control wells containing buffer. After incubation and washing, phage were detected using an HRP-conjugated, anti-M13 antibody. The DNA from phage displaying peptides that bound to BMP-2 was analyzed and the peptide sequence deduced (**Table 1**). The peptides that bind to BMP-2 fall into two different sequence clusters. The first cluster of peptide sequences contains the motif W-X-X-F-X-X-L and the second cluster contains the motif L-X-F-P-L-K-G-X-X-V (where X can be any amino acid (AA)). A series of biotinylated synthetic peptides were generated and mixed with rhBMP-2. Based on titrations of peptides + rhBMP-2, an estimate of the affinity of the peptides for BMP-2 was calculated (**Table 1**).

We have also identified a series of type I collagen-binding peptides through phage display selections on Type I collagen. The BMP-binding peptides were synthetically linked to collagen-binding peptides. These collagen:BMP IFBM's were tested for their ability to increase the amount of BMP-2 bound and retained on a collagen sponge. Two collagen:BMP IFBM's (7010 & 7049), a collagen-binding peptide (0016), and no peptide solutions were mixed with varying concentrations of BMP-2 and loaded onto collagen matrix. The sponges were washed and the amount of BMP retained was determined using an anti-BMP-2 antibody. Compared to the untreated collagen matrix, the 7010 and 7049 treated matrix binds and retains 5 to 8 fold more BMP-2 (**Figure 2**). The addition of saturating concentrations of BMP binding peptides to rhBMP-2 also does not decrease the activity of rhBMP-2 measured by induction of alkaline phosphatase activity in the mesenchymal cell line C3H10T1/2. Finally, we have initiated evaluation of the collagen:BMP-2 IFBM for improving healing of critical size defects in the canine ulna. At the lowest dose of rhBMP-2 tested (18.5 µg/ml), the defect treated with BMP-2 + IFBM showed more bone formation compared to the defect treated with BMP-2 alone at 12 weeks (**Figure 3**).

Conclusion: Short bi-functional linker peptides (IFBM's) can be used to promote the attachment and retention of rhBMP-2 on a collagen sponge. In the future, these modular peptide linkers may help promote bone formation and osseointegration on a variety of natural and synthetic materials.

Table 1 - BMP-2 Binding Peptides

Phage	Peptide	Peptide Sequence	Aff. (nM)
1-1F	AFF2011	ssDWGVVASAWDAFEALDAsr	55
1-3A	AFF2006	srSSDSAWSSFSALEGSVVsr	21
6-3D	AFF2007	srGGEAAAGAWVSFSALESsr	79
4-5D	AFF2008	ssWEVFSSLESGSVGAGAGsr	99
6-2B	AFF2012	ssSVDLYFPLKGDVVsr	9
3-3E	AFF2009	ssFEPLRFPLKGVPVsr	10

Figure 2: Peptides promote BMP-2 retention on a collagen sponge.

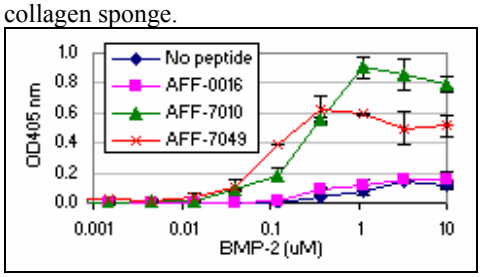


Figure 3: H&E staining of bone defects treated with BMP-2 (18.5 µg/ml) without (left) and with (right) peptide at 12 weeks.