

# The Biomechanical and Osteo-Immunomodulatory Profiling of Evoke® Demineralized Bone Matrix (DBM) Putty to Enhance Osteoinductive Potential

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**INTRODUCTION:** Tissue-engineered scaffolds for bone repair/regeneration must support both osteoconductive (OC) and osteoinductive (OI) properties. While demineralized bone matrix (DBM) particulates possess these properties and are often utilized for bone repair, they can be difficult to apply clinically to irregular bone defects and could result in delayed healing.<sup>1,2</sup> In contrast, DBM putties offer moldability for osseous defect conformity.<sup>2</sup> Evoke DBM Putty (Evoke Putty) is a 100% allograft bone composite containing DBM within a bone allograft matrix carrier, enhancing its malleable handling. The demineralization strategies imposed on Evoke Putty's DBM composition can influence both its biomechanical and osteo-immunomodulatory profile to induce OI. Specifically, Evoke Putty supports osteoinductivity driven by endogenous BMPs (i.e., BMP-2,4,7) and osteo-immunomodulatory growth factors/cytokines (i.e., TGF- $\beta$ , TREM-1, Galectin-3, etc.)<sup>3,4,5</sup> while the carrier contributes viscoelasticity and porosity for enhanced handling facilitating OC/OI potential.

**METHODS:** All Evoke Putty test articles (TAs) underwent PTT's proprietary tissue processing methods. A minimum of three (3) TA replicates with multiple donors (male or female) were used. 1) *Scanning Electron Microscopy (SEM) Imaging:* SEM imaging of TAs under standard operating procedures (Nanofiber Solutions, Dublin, OH). 2) *Rheology:* Samples were prepped for loss and storage moduli and complex viscosity (Anton Paar RheoCompass, UT Arlington, Bone-Muscle Research Center, Arlington, TX). 3) *Immunomodulatory Growth Factor (GF) ELISA/Multiplex Quantitation:* Samples digested under GuHCl were assessed via relative quantification (RQ) levels of BMP-2/4/7 through ELISA (Quantikine ELISA, R&D Systems, Minneapolis, MN). TGF- $\beta$  isoforms (TGF- $\beta$ 1/2/3) and 80 plex immuno-GFs were quantified through bead-based antibody multiplexing (Millipore bead panel #TGFBMAG-64K-03 & ProcartaPlex™ Human Immune Response Panel #EPX800-10080-901 respectively). 4) *OI Pathology Analysis:* TAs were implanted into a muscle pouch in the dorsal bilateral region of the lumbar area in an athymic nude rat model under ASTM F2529-13. After 28 days, extracted TAs were prepared for H&E and histopathology was assessed via the Edwards semi-quantitative scoring scale.<sup>6</sup>

**RESULTS:** 1) Figure 1 illustrates that Evoke Putty under SEM contains DBM layered particulates crucial for OC cellular migration conduits and has rheological viscoelastic properties (storage modulus (G')/loss modulus (G'') and complex viscosity ( $\eta^*$ )) over an angular frequency 0.1 – 100 rad/s. 2) Figure 2 maps the osteo-immunomodulatory profile of Evoke Putty over 86 GFs/cytokines related (top 9 highlighted) to M1/M2 polarization/pleiotropy osteo-repair responses. 3) Figure 3 highlights the OI potential of Evoke Putty in which H&E analysis reveals new bone (NB) and new bone marrow (BM) formation from an *in vivo* athymic nude rat model.

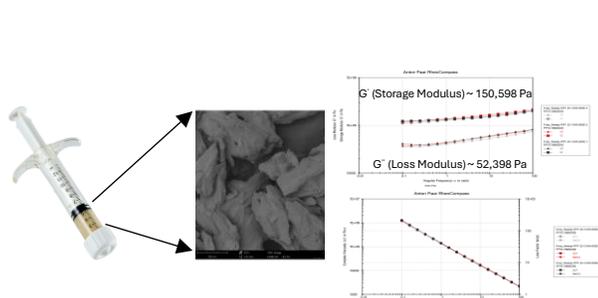
**DISCUSSION:** SEM (Fig. 1) features the biomechanical OC framework via DBM layered particulates providing pore sizes of 100  $\mu$ m+ which are crucial for cellular integration/migration into Evoke Putty.<sup>7</sup> The recorded storage modulus (G') is about 2.87 X higher than the loss modulus (G'') (Fig. 1) illustrating Evoke Putty's viscoelasticity, while the complex viscosity decreases linearly with a constant stable slope suggesting its shear thinning properties (Fig. 1). BMP-2/4/7 are driving OI factors but are further enhanced/supported by the M1/M2 osteo-immunomodulatory factor profile in Fig. 2. The prominent M2 rich environment of Evoke Putty suggests its potential to bolster OI via anti-inflammatory tissue repair pathways. *In vivo* histopathology analysis (Fig. 3) corroborates Fig. 2 and illustrates Evoke Putty producing new bone (NB) and new bone marrow (BM) in an ASTM athymic nude rat model.<sup>8</sup>

**SIGNIFICANCE/CLINICAL RELEVANCE:** In all, the results demonstrate that Evoke Putty exhibits enhanced OI potential not only based on its biomechanical characteristics but also through its osteo-immunomodulatory cytokine/GF (M1/M2) profile. This immunomodulatory signature favors M2 anti-inflammatory, pro-healing pathways, suggesting that Evoke Putty's OI may be driven in part through mechanisms beyond BMPs alone. Collectively, these properties support the clinical applications of Evoke Putty for the repair, replacement, or reconstruction of osseous defects.

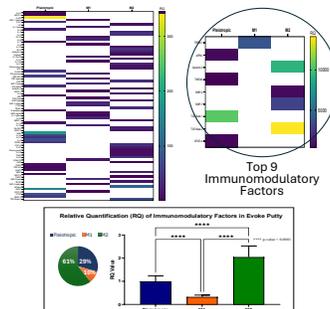
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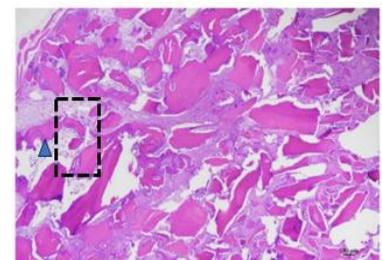
**ACKNOWLEDGEMENTS:** Nanofiber Solutions, LLC, UT-Arlington, IBEX, Scientific Solutions, LLC and Dr. Molly Speltz, DVM, Diplomate, ACVP



**Figure 1.** Left) Phase image of Evoke Putty in a syringe & SEM image at 210X, scale bar = 300  $\mu$ m. Right) Rheology measurements of Evoke Putty TAs; Top - storage (G') and loss modulus (G'') measurements in Pa and Bottom - complex viscosity measurements in Pa\*s.



**Figure 2.** Osteo-Immunomodulatory M1/M2 86 factor profile of Evoke Putty. Top Left & Right) 86 immunomodulatory factor heat map & subsequent top 9 factors. Bottom) Pleiotropic, M1, and M2 statistical profile of the 86 factors.



**Figure 3.** H&E image of implanted TA (triangle) illustrating new bone and/or new bone marrow (dashed rectangle). 40X total mag., scale bar = 100  $\mu$ m.