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1. Introduction

Over 40% of the disabling medical conditions of persons aged 18 years and over are musculoskeletal related. This number is even higher within the older population (Weinstein, 2000). Surgical treatment for age-, trauma- or cancer-induced critical-size bone loss is particularly challenging. Current grafting material options for scaffold-assisted surgical repair of critical-size bone loss include autogenic bone grafts (autografts), allogenic bone grafts (allografts), and synthetic bone substitutes. Still considered as a golden standard, autografts, retrieved from patients' own skeleton, are used in approximately 50% of all orthopedic bone grafting procedures. Complications arising from possible donor-site morbidity and insufficient grafting materials are major drawbacks of autografting procedures (Bostrom & Seigerman, 2005). In addition, this option is highly limited within the aging population as the elderly are less likely to be qualified for such a procedure due to higher incidences of osteoporosis and metabolic diseases. Allografts, obtained from another human donor or animal cadaver, represent a useful alternate to autografts, and are used in approximately 40% of bone grafting surgeries. However, allografting procedures suffer from risks for rejection and disease transmission, and a significant structural failure rate due to poor tissue integration, both structurally and biochemically (Blokhuis & Lindner, 2008; Bostrom & Seigerman, 2005; Eagan & McAllister, 2009; Goldberg & Stevenson, 1994). These limitations, along with the growing aging population, has led to an increasing need for viable synthetic bone substitute alternatives (Salgado et al., 2004). Current clinically used synthetic bone grafts such as brittle ceramics and weak gel foams are used in only ~10% of all bone grafting procedures (Bostrom & Seigerman, 2005), primarily due to their unstable graft fixation and insufficient tissue-graft interactions (Carson & Bostrom, 2007; Goldberg & Stevenson, 1994; Place et al., 2009; Stevens, 2008). In the past two decades, many new synthetic bone grafts designed to mimic key structural and biochemical properties of bone to enhance osteointegration and graft healing have emerged in literature. This rapidly evolving field has been extensively reviewed by others, including broad overviews of current requirements and techniques for preparing synthetic bone grafts (Burg et al., 2000; Salgado et al., 2004), calcium phosphate–based bone substitutes (De Long et al., 2007), polymeric bone substitutes (Seal et al., 2001), and biomimetic nanocomposite orthopedic biomaterials (Chan et al., 2006; Murugan & Ramakrishna, 2005). This chapter highlights the evolvement of non-metallic orthopedic biomaterials from bioinert,

biodegradable/bioresorbable, bioactive to tissue-responsive, and emphasizes the strategic integration of key structural elements of bone in the design of organic-inorganic composite bone substitutes. Using FlexBone, an easy-to-fabricate elastomeric 3-dimensional hydrogel-nanocrystalline hydroxyapatite (nHA) composite exhibiting excellent structural integration, as an example, we illustrate the feasibility of accomplishing multifaceted functional requirements of a viable synthetic bone substitute by integrating the major bone mineral component with a hydrophilic 3-dimensional hydrogel matrix.

2. Brief overview of the evolvement of synthetic orthopedic biomaterials

Most synthetic polymers traditionally used in orthopedic care, including poly(ethylene terephthalate) (PET) as implant coating, polyetheretherketone (PEEK) as spacers for cervical fusion, maxillofacial defect repair, and hip prostheses (Eschbach, 2000; M. M. Kim, Boahene, & Byrne, 2009; Kulkarni, Hee, & Wong, 2007), poly(methyl methacrylate) (PMMA) as bone cements, ultra high molecular weight polyethylene (UHMWPE) as total joint replacement components, and polysulfone (PSU) as internal fracture fixators (De Long et al., 2007; Eschbach, 2000; Mano et al., 2004; M. Wang, 2003) are considered as bioinert. They are primarily used to provide structural or mechanical support without eliciting significant immune responses. The primary drawback of bioinert implant materials is that they lack the intrinsic ability to promote osteogenesis, thus are unable to structurally or biologically integrate with the host tissue. To overcome such limitations, physical modification (e.g. increasing porosity) or blending bioinert materials with bioceramics or biodegradable polymeric components have been attempted (Aparecida et al., 2008; Fini et al., 2002; Mano et al., 2004; Tan et al., 2003; Tanner 2010; K. Zhang et al., 2002).

Calcium phosphate–based bioceramics have long been used clinically as bioactive bone fillers (De Long et al., 2007; Nandi et al., 2010). They are known for good biocompatibility, osteoconductivity and easy surgical handling. However, these bone substitutes suffer from poor mechanical properties such as high brittleness and are often unsuitable for weight-bearing applications (De Long et al., 2007; Tanner, 2010). Their integration with the more compliant polymeric matrices, therefore, has been of intense investigations (Kim et al., 2006; Miranda et al., 2010; Rezwan et al., 2006; M. Wang & Bonfield, 2001).

Biodegradable synthetic polymers have great potential as resorbable orthopedic implants and tissue scaffolds. The *in situ* generated porosity of degradable polymers as a result of hydrolytic degradation is thought to be beneficial to tissue penetration / osteointegration. In addition, the gradual resorption of biodegradable polymer-based orthopedic fixation devices, if timed to match with the tissue integration rate, could ensure adequate mechanical integrity at the site of implantation while potentially eliminating the need for a second surgery for implant retrieval. Among all degradable synthetic polymers, poly(lactic acid) (PLA) (R. Y. Zhang & Ma, 1999), poly(glycolic acid) (PGA), poly(lactic-co-plycolic acid) (PLGA) (Ishaug et al., 1997; Lu et al., 2000; Ma & Choi, 2001; Mooney et al., 1996), polyhydroxybutyrate (PHB) (Y. W. Wang et al., 2004), polycaprolactone (PCL) (Yoshimoto et al., 2003), and their co-polymer blends (Tanner, 2010) have been the most investigated.

Blending biodegradable polyesters with weakly basic osteoconductive minerals such as tri-calcium phosphate (TCP) or hydroxyapatite (HA) have been widely pursued as a strategy for further enhancing scaffold osteoconductivity, drug retention capacity, and for neutralizing acidic degradation products and mitigating inflammatory tissue responses (Liao et al., 2007; Peter et al., 1999; Tanner, 2010; M. Wang, 2003). Achieving adequate

structural integration between the organic matrix and the inorganic minerals, however, remains one of most significant challenges for the clinical translation of these polymer-mineral nanocomposites for orthopedic care as loosely integrated ceramic particles could not only lead to inferior mechanical properties of the composite, but also cause ectopic bone formation in nearby soft tissues. This is because most polyesters are hydrophobic in nature and exhibit an intrinsically low affinity to bioceramics. Recent development of high-affinity HA-surface mineralization strategies applicable to hydrophilic hydrogels such as poly(2-hydroxyethyl methacrylate) (pHEMA) and pHEMA-based copolymers (Song et al., 2005; Song et al., 2003a, 2003b), and identification of novel HA-binding/nucleating ligands, either small molecule-based (Licata, 2005; Yoshinari et al., 2001) or peptide-based (Bertozzi et al., 2006; Chung et al., 2011), could help address this challenge.

The past decade has witnessed an increasingly elaborated trend in the design of bioactive synthetic biomaterials (Bonzani et al., 2006). For bone tissue engineering applications, integrin-binding peptide sequences for promoting cellular adhesion, phosphorylated ligands for promoting HA-mineralization, heparin-mimicking motifs for drug retentions, and degradative enzyme substrate sequences have all been incorporated into multi-modality synthetic scaffold designs (Hartgerink et al., 2001; Jeon et al., 2007; M. P. Lutolf et al., 2003; M. R. Lutolf et al., 2003; Patterson & Hubbell, 2010). Of particular novelty is the design of self-assembling peptide-amphiphile (PA) gels by Stupp and coworkers for simultaneous presentation of cell adhesion peptide sequences, HA-mineral-nucleating sites, reversible crosslinking sites, and other therapeutic agents all within a single PA molecule (Cui et al., 2010; Hartgerink et al., 2001; Palmer et al., 2008; Palmer & Stupp, 2008) that self-assembles and dissembles in response to environmental perturbations. Likely limitations of these unique PA gels are their relatively high manufacturing cost and low mechanical modulus which could limit their use to treating smaller non-weight bearing skeletal lesions. Another innovative concept introduced by Hubbell and coworkers was to induce scaffold degradation by using peptide substrates of the degradative enzymes matrix metalloproteinases (MMPs) as the chemical crosslinker of a non-fouling crosslinked hydrogel system (M. P. Lutolf et al., 2003; M. R. Lutolf et al., 2003). Given the elevated expression of some MMPs within both degenerative bony defects and arthritic knee joints, such a hydrogel system could be useful for bone and cartilage repair as the *in situ* increase of scaffold porosity in response to tissue microenvironment-specific enzymatic degradation could promote cellular infiltration and matrix deposition. The selection of MMP substrates with proper degradation kinetics matching with those of the matrix deposition rate, however, is not a trivial task (Bahney et al., 2011).

Despite the many exciting orthopedic biomaterials emerging in the literature, successful clinical translations are rare. The challenge lies in the difficulty in accomplishing the functional sophistication of viable synthetic bone substitutes (e.g. physical properties enabling easy surgical handling and stable graft fixation, structural and biomechanical properties facilitating its osteointegration, biocompatibility ensuring long-term safety) within an easy-to-fabricate biomaterial that can be reproducibly manufactured at low cost. We, as well as some in the orthopedic biomaterials research community, believe that functional sophistication are not synonymous with complicated materials designs (Bonzani et al., 2006; Stevens, 2008). Instead, we believe that the key to meeting this challenge lies in the strategic integration of key structural elements of bone, which play multifaceted roles in defining the unique properties of the native tissue, in a low-cost biocompatible synthetic biomaterial.

3. Key structural elements of bone and their multifaceted functions

From a material's perspective, bone is an organic-inorganic composite comprising two major structural components that are hierarchically organized across various length scales: the calcium apatite crystals (primarily as substituted nanocrystalline hydroxyapatite nHA, but also as crystalline precursors) and the type I collagen matrix (Weiner et al., 1999). The quantity and quality of the hard calcium apatite crystals (crystal size, maturity and structural integration with the collagen matrices) influences the mechanical properties of bone (Tong et al., 2003). For instance, the bending and compression strength of bone is known to positively correlate to bone mineral content (Follet et al., 2004). In addition, bone minerals also support bone cell attachment, and serve as an important reservoir of calcium and phosphate ions, and help retain the secreted factors that are indispensable in defining the biochemical environment of the bony tissue. Thus, HA has long been recognized as an important design element for tissue-engineered bone substitutes (El-Ghannam, 2005). The intrinsic affinity of the dynamic apatite crystal surfaces for many acidic non-collagenous proteins widely found in calcified tissues (George et al., 1996; Gilbert et al., 2000; Stubbs et al., 1997) have also inspired the use of bioceramic scaffolds (Le Nihouannen et al., 2008) or polymer-bioceramics composite scaffolds (Abarrategi et al., 2008; Filion et al., 2011; Xu et al., 2009) to retain and deliver recombinant proteins for therapeutic uses. Overall, HA has been explored for bone tissue engineering applications more as a way to enhance the mechanical strength than as a tool to mediate the biochemical properties of the scaffold (Stevens, 2008; Tanner, 2010). In general, the potential of the large surface areas provided by nHA as opposed to micrometer-sized mineral particles for more efficient therapeutics delivery (e.g. higher retention capacity, more sustained release) has not been exploited to the fullest extent in the design of synthetic bone substitutes.

Type I collagen matrix of bone serves as a compliant template for the structural integration of the calcium apatite crystals, and, along with the mineral component, is responsible for defining the 3-demensional structure as well as the strong, tough, yet relatively compliant mechanical properties of bone (Scharnweber et al., 2004; Weiner et al., 1999). In addition, it also interacts with many non-collagenous proteins and mediates cellular adhesion and functions (Heino, 2000). The Gly-Pro-Hyp (Hyp: hydroxyproline) triplet repeats of type I collagen may also play an important role in template-driven biomineralization. Recent discovery of novel HA-binding oligopeptides using the combinatorial phage display technique reveals a [Pro-(OH)-X] tripeptide pattern (OH: hydroxylated amino acid residues (Ser, Thr, Tyr); X: any amino acid) among the dominant HA-binding motifs (Bertozzi et al., 2006; Chung et al., 2011). Such a hydroxylated tripeptide pattern resembles that of the type I collagen, underscoring the importance of hydroxylated residues in directing ligand-mineral interactions on a molecular level. These oligopeptides were shown to template the nucleation and growth of HA *in vitro* (Bertozzi et al., 2006; Chung et al., 2011), and may be useful in the design of synthetic polymer scaffolds enabling template-driven mineralization of HA or the preparation of bulk organic-inorganic bone-like composites with improved interfacial binding affinity. We also showed earlier that polymeric hydrogels displaying hydroxylated (e.g. pHEMA) and acidic residues could be used to template the surface mineralization of HA with excellent interfacial adhesion strength (Song et al., 2005; Song et al., 2003a, 2003b), further supporting the favorable interaction between the hydroxyls and the calcium ions. The strategy of modifying the surface of polymers or metallic substrates with hydroxylated or anionic coatings has also been pursued to facilitate the nucleation and growth of calcium apatite (Murphy & Mooney, 2002; H. L. Zhang et al., 2006).

4. FlexBone: A scalable functional bone substitute integrating key structural elements of bone

Inspired by the multifaceted roles of type I collagen matrix and nanocrystalline HA (nHA) in defining the unique structural, mechanical and biochemical properties of bone, we have developed a 3-dimensional synthetic bone substitute named FlexBone that integrates hydroxylated biocompatible pHEMA hydrogel with 50 wt% of nHA. This elastomeric structural composite exemplifies how multiple functional requirements for a viable bone substitute could be met with a scalable biomaterial that could be readily prepared at low cost. Here we focus our discussion on how the nHA component and its structural integration with the pHEMA matrix defines FlexBone's physical properties that enables its easy surgical insertion and stable fixation at the site of defect, its ability to retain and release protein therapeutics in a localized and sustained manner, its ability to enrich endogenous protein signals within the microenvironment of the tissue defect, and its ability to enable functional repair of critical long bone defect without exerting negative systemic side effects.

4.1 Preparation, microstructures and compressive behavior of FlexBone

FlexBone was prepared by crosslinking 2-hydroxyethyl methacrylate (HEMA) in the presence of up to 50 wt% nHA (Figs. 1A & B) in molds of any size and shape as previously described (Song et al., 2009). The choice of crosslinked pHEMA as the organic scaffold of FlexBone was inspired by its biocompatibility (Kost, 1987; Montheard et al., 1992), elasticity (enabling convenient surgical handling), potential high-affinity integration with the HA (providing long-term structural stability), and low manufacturing cost, all of which are critical considerations for bench-to-bedside translations. The choice of 50 wt% nHA as the inorganic component of FlexBone was inspired by the osteoconductive mineral content approximating that of human bone (An, 2000; Phelps et al., 2000) and the large surface areas of the nanocrystals enabling better integration with the hydroxylated hydrogel matrix and better retention of both endogenous protein signals and exogenous protein therapeutics.

The as-prepared FlexBone can be cut into any desired configuration matching with that of a potential defect, drilled with channels, and equilibrated with water to thoroughly remove radical initiators (Filion et al., 2011; Song et al., 2009). Upon freeze-drying, FlexBone can be stored long-term at room temperature, making it ideally suited as an "off-the-shelf" synthetic bone substitute for clinical applications.

Scanning electron microscopy (SEM) analysis revealed an even distribution of loose aggregates of nHA within the 3-dimensional pHEMA hydrogel matrix (Figs. 1A & B). As expected, the incorporation of 50 wt% of nHA in pHEMA resulted in an increase of the stiffness of the bulk material (Fig. 1C). Despite its high nHA content, however, FlexBone exhibited elastomeric properties in both as-prepared and fully hydrated states, showing excellent shape recovery after being subjected to repetitive moderate (MPa) compressive loadings under physiological conditions (in water, at body temperature). The excellent structural integration of nHA with the hydroxylated pHEMA matrix was reflected by the ability of freeze-dried FlexBone to withstand hundreds-of-megapascals compressive loadings and >80% compressive strains without exhibiting brittle fractures (Song et al., 2009). SEM examination of the cross-section of a freeze-dried FlexBone after it was being compressed to >80% did not reveal any microfractures (Fig. 1D). Instead, the spherical aggregates of nHA were compressed into plywood-like structures, suggesting that the rearrangement of nHA under the compressive loading provided an effective mechanism for

energy dissipation, thereby contributing to the toughness of FlexBone. Such a fracture-resistant property could not be obtained with composites containing the same weight percentage of micrometer-sized HA particles, where crack propagations within the composite under the same compressive loading was observed (Song et al., 2009). These findings underscore the critical role that nHA plays in defining the microstructural and mechanical properties of FlexBone. The elastomeric and fracture-resistant properties of FlexBone will enable its surgical insertion into an area of defect by convenient press-fitting.

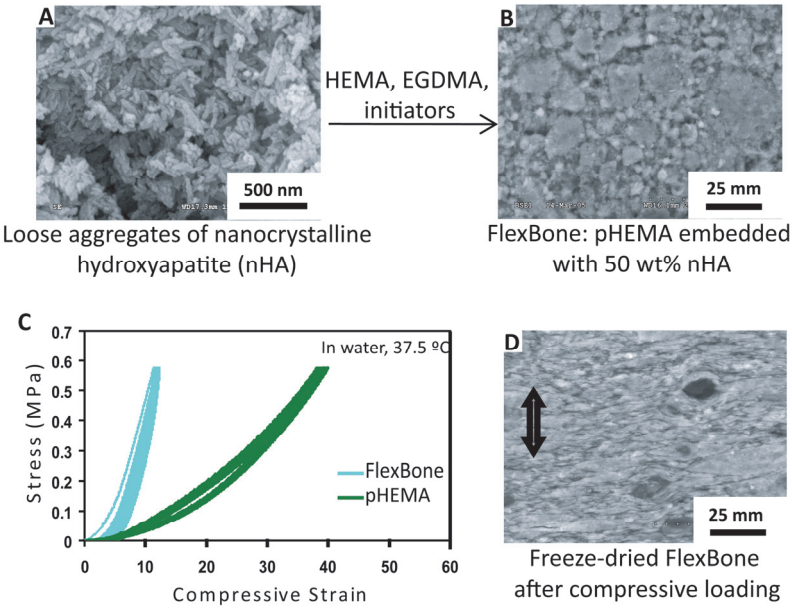

Fig. 1. Structural and mechanical properties of FlexBone. Cross-linking HEMA in the presence of 50 wt% nHA (A) generated FlexBone, where the spherical loose aggregates of nHA were well-distributed throughout the 3-dimensional pHEMA matrix (B) as revealed by SEM micrographs. (C) FlexBone, stiffer than pHEMA, withstood repetitive low-MPa compressive loadings with excellent shape recovery. The stress-strain curves were recorded on a dynamic mechanical analyzer equipped with a submersion compression fixture. Ten consecutive load-controlled loading-unloading cycles (3.0 N/min, 0.01 N to 10.0 N to 0.01 N) were applied to each specimen in water at body temperature. (D) SEM micrograph of the cross-section of a freeze-dried FlexBone after being compressed to >80% compressive strain revealed the rearrangement of nHA into plywood like structures upon compression. Arrow indicates the orientation of the applied compressive loading.

4.2 Retention and localized / sustained release of therapeutics from FlexBone

The large surface area of the nHA component of FlexBone, coupled with its good structural integration with the pHEMA hydrogel matrix, has enabled FlexBone to retain protein therapeutics and small molecule antibiotics and release them in a localized and sustained manner (Xu et al., 2009). Such a feature is attractive for clinical applications where a patient's tissue repair capacity is compromised by either age or metabolic conditions, or where the defect site is prone to infections (Hetrick & Schoenfisch, 2006).

Using a bone morphogenetic protein (BMP)-induced osteogenic trans-differentiation of myoblast C2C12 cell culture model (Katagiri et al., 1994), the ability of FlexBone to retain and release recombinant human bone morphogenetic protein-2/7 heterodimer (rhBMP-2/7) *in vitro* was evaluated. The pHEMA matrix enabled FlexBone to readily absorb an aqueous solution of rhBMP-2/7 and stably sequester these proteins, presumably on the surfaces of the nHA component. As shown in Figure 2, when a FlexBone carrier pre-absorbed with a single low dose of 40 ng of rhBMP-2/7 was placed in the culture of C2C12 cells, trans-differentiation of the myoblasts to alkaline phosphatase (ALP)-expressing (stained in red) osteoblastic cells was observed by 2-4 days. The highly localized ALP staining suggested that the rhBMP-2/7 was released from the FlexBone carrier in a highly localized manner. Further, when the FlexBone carrier retrieved from the 4-day culture was placed in a fresh culture of C2C12 cells, the continually released rhBMP-2 was able to induce osteogenic differentiation in 3.5 days, suggesting that the release was sustained over a period of >7 days. It is worth noting that the 40-ng/graft loading dose of rhBMP-2/7 on FlexBone for inducing trans-differentiation of C2C12 cells was 3 orders of magnitude lower than required for BMP-2 using a TCP-chitosan carrier (Abarrategi et al., 2008). This is likely a result of both the increased rhBMP-2/7 retention on the nHA surfaces and the relatively higher osteogenic potency (~10 fold) of rhBMP-2/7 as compared to rhBMP-2 (Israel et al., 1996; Zheng et al., 2010).

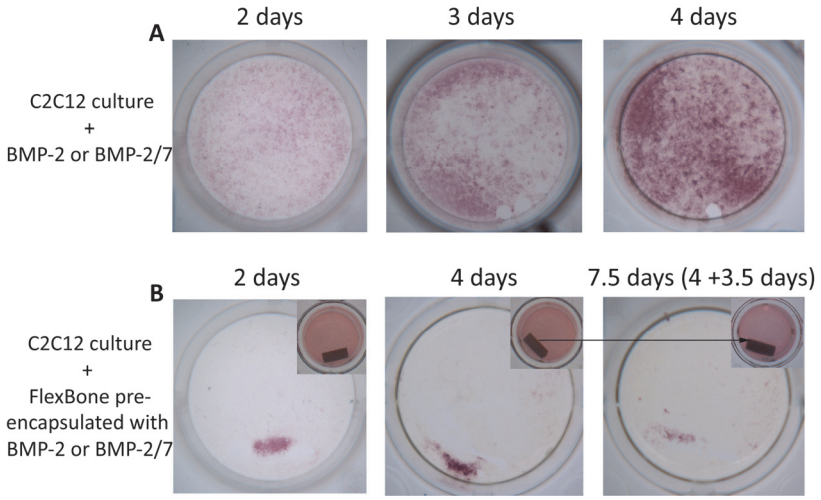

Fig. 2. FlexBone released pre-absorbed recombinant human bone morphogenetic proteins and induced osteogenic trans-differentiation of myoblast C2C12 cells in a localized and sustained manner. (A) Direct supplement of rhBMP-2/7 (40 ng/mL) or rhBMP-2 (300 ng/mL) in C2C12 culture media resulted in osteogenic trans-differentiation of C2C12 cells by 2-4 days as indicated by positive (red) ALP stains. (B) ALP stains were detected in C2C12 cultures in areas immediately adjacent to where the FlexBone carrier pre-absorbed with rhBMP-2/7 (40 ng/carrier) (Xu et al., 2009) or rhBMP-2 (300 ng/carrier) (Li et al., 2011) was placed. Continued detection of ALP stains induced by the BMPs released from FlexBone over 7 days suggests that the release was accomplished in a sustained manner. C2C12 seeding density: 10,000 cells/cm^2.

Similarly, FlexBone was also able to release pre-absorbed receptor activator of nuclear factor kappa-B ligand (RANKL, 10-ng/carrier) in a sustained manner over 7 days and induce the osteoclastogenesis of murine macrophage RAW264.7 cells in culture (Xu et al., 2009). By contrast, the un-mineralized pHEMA control carrier pre-loaded with 10-ng RANKL exhibited a burst-release of RANKL within the first 2 days of culture, and was not able to successfully induce the osteoclastogenesis of RAW264.7 cells over the course of 1 week under identical culture conditions. This observation underscores the critical role of nHA in achieving effective retention and sustained release of the recombinant protein. The low effective loading dose of 10-ng RANKL per carrier for inducing osteoclastogenesis of RAW264.7 accomplished with FlexBone was also significantly lower than that required using literature brushite cement carrier (600–800 ng RANKL per carrier required) using an identical cell culture model (Le Nihouannen et al., 2008). Overall, these findings suggest that FlexBone may be used to deliver protein therapeutics with significantly reduced loading doses that could lead to enhanced safety and reduced cost of growth factor-mediated clinical treatment of skeletal lesions.

Finally, 5 wt% of antibiotic tetracycline could be encapsulated in FlexBone without compromising the structural and compressive properties of FlexBone (Xu et al., 2009). The pre-encapsulated tetracycline was slowly released from FlexBone, with >80% of the tetracycline still retained on FlexBone after 1 week. By contrast, the un-mineralized pHEMA control released pre-encapsulated tetracycline much more rapidly, with only ~40% of tetracycline retained on the hydrogel scaffold after 7 days. We have also recently shown that the encapsulation and sustained release of vancomycin could also be accomplished using FlexBone as a carrier (Li et al., 2011).

4.3 FlexBone-mediated functional repair of rat critical-size femoral defects

Inspired by the elastomeric and fracture-resistant properties of FlexBone as well as its ability to deliver therapeutic agents in a localized and sustained manner, we recently evaluated the efficacy of FlexBone as a synthetic bone graft in mediating the repair of 5-mm critical-size femoral defects in rats with or without a single dose of 400-ng rhBMP-2/7 (Filion et al., 2011). The 5-mm femoral defects were stably press-fit with the elastomeric FlexBone with or without the absorption of 400-ng rhBMP-2/7 (Fig. 3). The grafts were predrilled with intersecting orthogonal drill holes to permit bone marrow access to expedite callus formations both within and surrounding the graft.

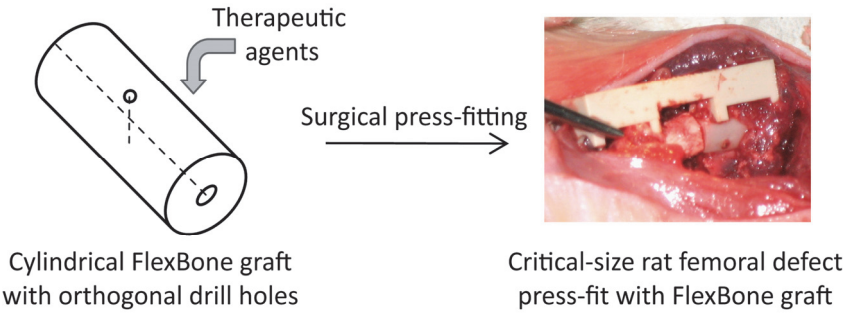

Fig. 3. FlexBone pre-drilled with intersecting channels can be pre-absorbed with osteogenic growth factors and stably press-fit into 5-mm femoral segmental defects in rats.

Histology, polarized light microscopy and microcomputed tomography (microCT) analyses showed that FlexBone enabled partial healing of the defect by 12 weeks in the absence of any exogenous growth factors (Filion et al., 2011). By 4 days post-operation, an internal callus emerged within the drill holes of FlexBone, which continued to mature and was recanalized by 6 weeks. The external callus bridging over the defect started to be mineralized at 2 weeks via the endochondral ossification mechanism. The partially mineralized external callus was matured and recanalized by 12 weeks, although it did not completely bridge over the entire defect. In a subset of experiments, we demonstrated by immunohistochemical staining that the partial healing enabled by FlexBone in the absence of any exogenous factors could be attributed to the ability of the nHA component to retain/sequester the endogenous protein signals present in the defect microenvironment. Specifically, FlexBone grafts retrieved from the surgical implantation site at different time points over the course of the first week of implantation revealed retained endogenously secreted transforming growth factor β (TGFβ), interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), vascular endothelial growth

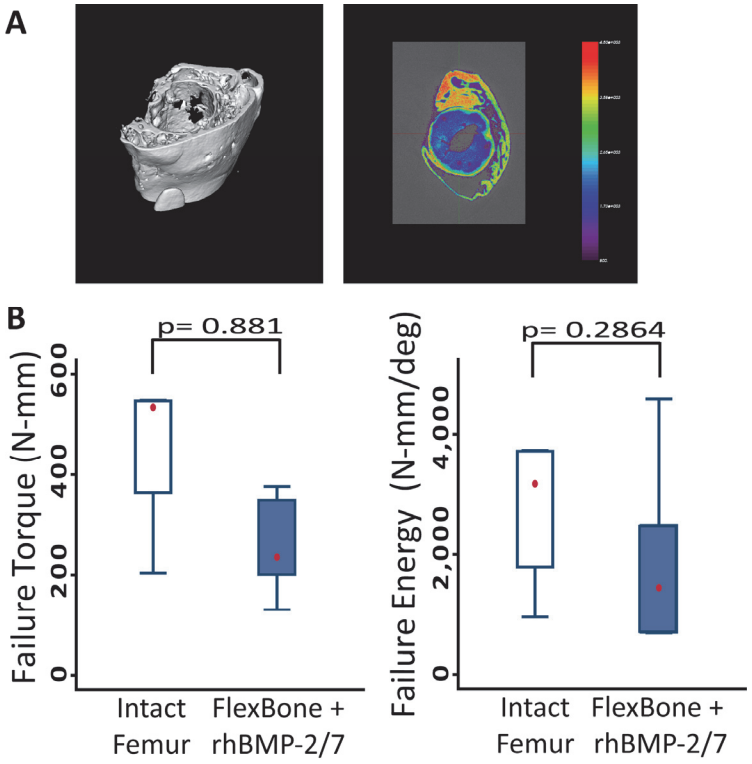

Fig. 4. FlexBone pre-absorbed with 400-ng rhBMP-2/7 led to functional repair of 5-mm segmental defects in rats as supported by the microCT analyses (A) and torsion tests (B) of 12-week explants. Effective voxel size of 18×18×18 μm^3 was applied to the reconstructed 3-D isosurface image and the 2-D color map of the center slice of the explant (red representing a higher degree of mineralization). No statistically significant difference was observed between the 12-week explant and the age-matched un-operated control femur.

factor (VEGF), RANKL, BMP-2, BMP-7 and stromal cell-derived factor-1 (SDF-1) in a temporally defined manner. These factors are known to play critical roles in initiating the inflammation / graft healing cascade and the recruitment of stem cells (Einhorn, 1998; Ito et al., 2005; Kitaori et al., 2009; Lieberman et al., 1999; Schindeler et al., 2008). The effective sequestering of these signals within FlexBone, but not the un-mineralized pHEMA control graft, supported the critical role of nHA in enabling the consequent partial repair of the defect.

With the absorption of a single dose of 400-ng rhBMP-2/7, FlexBone was able to enable the functional repair of the defect in 8-12 weeks (Filion et al., 2011). By 8 weeks, mature and recanalized external bony callus completely bridged over the defect as indicated by both histology and microCT analyses (Fig. 4A). More importantly, biomechanical testing revealed that the torsional strength of the repaired defects was restored to the level of age-matched un-operated femur controls (Fig. 4B). It is worth noting that such a functional repair of the defect was accomplished by FlexBone with a single low dose of rhBMP-2/7 that was 1-2 orders of magnitude lower than what has been required for treating similar defects using other scaffolds in combination with rhBMP-2 (Abarrategi et al., 2008; Kirker-Head et al., 2007). Such a feature of FlexBone could both reduce the cost and minimize negative systemic side-effects of scaffold-assisted BMP therapies. Indeed, the vital organs collected from the rats 12 weeks after receiving FlexBone-rhBMP-2/7 implants were pathologically indistinguishable from the age-matched un-operated controls.

In summary, FlexBone combines some of the best features of structural allografts (osteoconductivity and dimensional stability), desirable surgical compressibility, and the scalability of an easy-to-prepare synthetic biomaterial. The ability of FlexBone to locally deliver biological therapeutics in a significantly reduced effective dose to enable expedited functional repair of the critical defect opens the door to engineer the biochemical properties of the synthetic bone substitute based on individual patients' needs.

5. Conclusions

Many exciting orthopedic biomaterials have emerged in literature in the past 20 years, illustrating the shift of the focus in materials design from bioinert, biodegradable/bioresorbable, to bioactive and tissue-responsive. By recapitulating the multifaceted roles that key extracellular matrix components of bone play in defining bone-specific structural and biochemical properties, we show that easy-to-prepare biomaterials can be designed to facilitate the functional repair of critical-size bony defects. Our work supports the notion that functional sophistication of synthetic tissue grafts is not synonymous with complicated chemical/engineering designs.

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