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UPDATES IN SPINE SURGERY - TECHNIQUES, BIOLOGICS, AND NON-OPERATIVE MANAGEMENT (W HSU, SECTION EDITOR)

Recent Research Advances in Biologic Bone Graft Materials for Spine Surgery

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Abstract

Purpose of Review Biologic bone graft materials continue to be an important component of various spinal fusion procedures. Given the known risks and morbidity of harvesting iliac crest bone graft, the historical gold standard for spinal fusion, these biologic materials serve the purpose of improving both the efficacy and safety of spinal fusion procedures. Recent advances in biomedical and materials sciences have enabled the design of many novel materials that have shown promise as effective bone graft materials. This review will discuss current research pertaining to several of these materials, including functionalized peptide amphiphiles and other nanocomposites, novel demineralized bone matrix applications, 3D-printed materials, and Hyperelastic Bone®, among others.

Recent Findings Recent investigation has demonstrated that novel technologies, including nanotechnology and 3D printing, can be used to produce biomaterials with significant osteogenic potential. Notably, peptide amphiphile nanomaterials functionalized to bind BMP-2 have demonstrated significant bone regenerative capacity in a pre-clinical rodent posterolateral lumbar fusion (PLF) model. Additionally, 3D-printed Hyperelastic Bone® has demonstrated promising bone regenerative capacity in several in vivo animal models. Composite materials such as TrioMatrix® (demineralized bone matrix, hydroxyapatite, and nanofiber-based collagen scaffold) have also demonstrated significant osteogenic potential in both in vitro and in vivo settings.

Summary Advances in materials science and engineering have allowed for the design and implementation of several novel biologic materials, including nanocomposites, 3D-printed materials, and various biologic composites. These materials provide significant bone regenerative capacity and have the potential to be alternatives to other bone graft materials, such as autograft and BMP-2, which have known complications.

Keywords Biologics · Biomaterials · Spine fusion · Nanotechnology · Bone regeneration

Introduction

The volume of spine fusion procedures performed in the USA has consistently increased over the last 30 years [1–3]. Historically, the gold-standard graft material has been autologous iliac crest bone graft (ICBG). Although the efficacy of ICBG has been proven in numerous studies, the risks of donor-site morbidity and long-term functional impairment are well documented [4–6]. Therefore, there has been significant interest in the design of novel materials that can yield high fusion rates and eliminate the need for highly morbid bone harvesting. Biologic materials are one domain that has gained significant interest recently [7]. Our group and collaborators have focused our efforts on a few of these materials: functionalized peptide amphiphiles and other nanocomposites, novel demineralized bone matrix applications, 3D-printed materials, and Hyperelastic Bone®. Each of these materials have notable advantages and disadvantages for different applications in spine surgery. A number of these materials are still being investigated in ongoing research studies, while others are used clinically. The aim of this review is to provide an overview of

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these biomaterials and summarize recent research findings pertaining to them.

Peptide Amphiphiles

Peptide amphiphiles are lipopeptide molecules that self-assemble into nanofibers due to the polarity of the hydrophilic peptides and the hydrophobic lipid attachments [8]. The basic composition of these molecules can be manipulated to produce supermolecules with specific biochemical properties for different applications [9]. These molecules were first described in 1995 by Tirrell et al. [10]. Since then, peptide amphiphiles have been applied in a wide array of domains, including tissue engineering, regenerative medicine, and drug delivery [11]. These molecules have been shown to promote cell adhesion, differentiation of osteogenic cells, and bone mineralization [12, 13].

The Samuel Stupp lab at Northwestern University has reported the development of peptide amphiphiles (PA) that self-assemble into nanofibers with a diameter of 6–10 nm and a length on the scale of microns [14, 15]. In a previous study, the group had also functionalized PA molecules to form heparin-binding peptide amphiphiles (HBPA) that provide prolonged released of various growth factors, such as fibroblast growth factor 2 (FGF-2) and vascular endothelial growth factor (VEGF) [16]. Additionally, the HBPA-containing material was found to promote growth of vascularized connective tissue in a subcutaneous implant model [17]. In a separate study, the group engineered a functionalized bio-gel containing HBPA that is capable of binding to and facilitating controlled release of BMP-2 [18]. This material was studied in vivo with an established rat femoral defect model and was determined to significantly enhance bone healing [18].

Additionally, our research group demonstrated that bone morphogenetic protein (BMP)-binding PA nanofibers show promising results for bone regeneration in both in vitro and in vivo experiments [19•]. Regarding in vitro investigation, we examined expression of osteogenic genes—Runx2, Osterix (Osx), and osteocalcin (Ocn)—in C2C12 cells exposed to various environments: 1) BMP-2 combined with diluent PA, 2) BMP2-binding PA, 3) diluted BMP-2-binding PA, and 4) heparin (10 μg mL^{-1}) [19•]. The diluted BMP-2-binding PA group had the greatest increase in expression of these three genes, and this same trend was noted when investigating the effects of BMP-2-binding PA nanofibers on alkaline phosphatase (ALP) activity, an established marker of osteoblast differentiation [19•]. These findings are summarized in Fig. 1.

For in vivo assessment, we investigated the efficacy of these biomaterials in an established rat posterolateral lumbar fusion (PLF) model. Three main groups were assessed, each with varying amounts of rhBMP-2: 1) diluted BMP2-binding PA (0, 0.1, or 1 μg of BMP-2), 2) diluent PA (0, 0.1, or 1 of BMP-2), and 3) an absorbable collagen scaffold (0, 0.1, 1, or 10 μg BMP-2) [19•]. The diluted BMP-2-binding PA group outperformed the other groups with regard to mean fusion score and fusion rates (Fig. 2) [19•]. Additionally, microCT bone quantification demonstrated that the diluted BMP-2-binding PA group with 1 μg of BMP-2 yielded the greatest mean new bone volume relative to other groups (Fig. 2) [19•]. These preliminary data are promising because the osteoinductive nature of BMP-2-binding PA nanofibers appears to decrease the required therapeutic dose of rhBMP-2 by 10-fold. Given the known complications of rhBMP-2, particularly at higher doses, the use of nanomaterials like peptide amphiphiles for use in spinal fusion is a promising new area of interest [20].

Demineralized Bone Matrix Applications

Demineralized bone matrix (DBM) is an allograft material produced by treating cadaveric bone in an acidic environment. The majority of DBM is type-1 collagen, which provides the osteoconductive properties of the material [21]. DBM also contains a number of important proteins for osteoinduction, including bone morphogenetic proteins, insulin-like growth factor (IGF), transforming growth factor beta (TGF-β), and fibroblast growth factors (FGF) [21]. Unfortunately, the osteoinductive properties of DBM are limited given that much of the biologic material is lost during processing and sterilization [22]. Additionally, the concentration of BMPs and other growth factors are highly variable across products, which has been attributed to differences in demineralization, sterilization, and processing procedures [21, 23]. DBM is mainly used as a bone graft extender and therefore typically combined with other bone graft substitutes like autologous bone [24].

DBM products are available for use as bone graft extenders, and clinical studies have yielded promising results [24]. Furthermore, there are many novel avenues for improvement of current DBM-based materials. Our lab has specifically investigated novel DBM-based materials for bone regeneration. Hsu et al. demonstrated that a combination biomaterial TrioMatrix®—comprised of DBM, hydroxyapatite, and a nanofiber-based collagen scaffold—yielded 100% fusion rate, determined through manual palpation, in a rat posterolateral spinal fusion model [25]. This fusion rate was significantly higher than a positive control group treated with 10 μg of rhBMP-2 [25]. Additionally, microCT quantification of bone mass volume demonstrated that the TrioMatrix® group had a significantly greater fusion mass volume relative to all other groups, including the rhBMP-2 positive control group (Fig. 3) [25]. Therefore, combining DBM material with other known biologics like

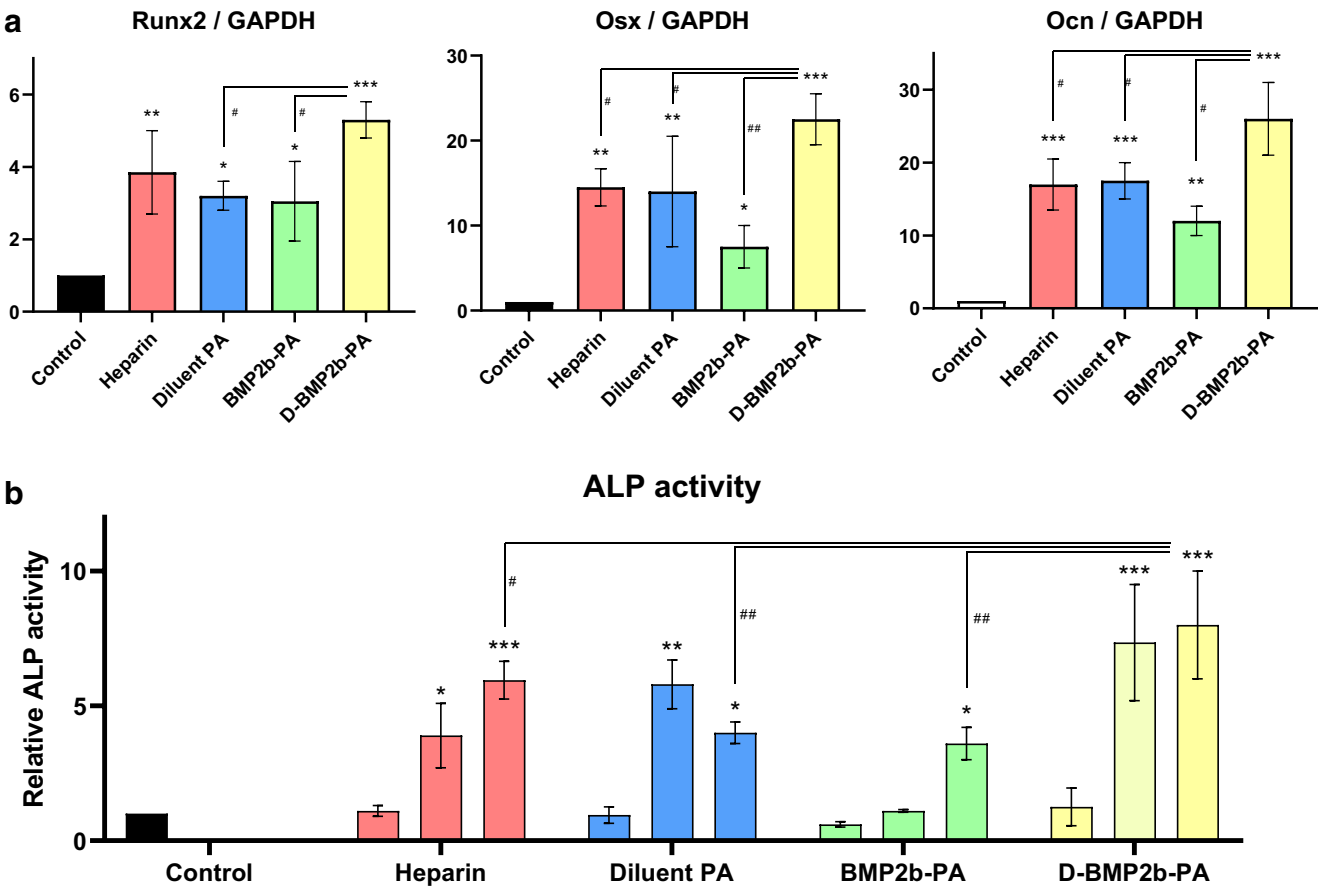

Fig. 1 Markers of BMP-2-induced osteogenesis and osteoblast differentiation are significantly elevated by BMP-2-binding PA nanofibers in C2C12 cell cultures in vitro. **a** mRNA expression levels of three osteogenic markers—Runx2, Osterix (Osx), and Osteocalcin (Ocn)—at 48 h after exposure to various treatment groups. The D-BMP2b-PA group had significantly elevated levels of all three markers relative to the other treatment groups at 48 h. All groups—including the control, heparin, and diluent PA groups—were treated with 10 μg/mL of BMP-2. All expression levels were normalized to GAPDH. The reported values were normalized to the control group (BMP-2 treatment alone). **b** ALP activity measured at 4 days in different treatment groups with three different concentrations of BMP-2 (0, 0.1, 1.0, 10 μg/mL). All expression levels were normalized relative to their individual DNA content. The reported values were normalized to the control group (BMP-2 treatment alone)

hydroxyapatite and nanofiber materials appears to provide significant osteogenic potential. These findings are promising, because they suggest that the osteogenic potential of DBM-based bone graft substitutes can be enhanced with biologics other than autograft.

Other groups have investigated novel DBM-based materials for similar applications. Specifically, Rodriguez et al. demonstrated that DBM fibers can be engineered into a custom-shaped implant that recruits mesenchymal stem cells and stimulates osteogenesis [26]. Enzyme linked immunosorbent assays and mass spectrometry confirmed that the scaffold contained osteogenic cytokines (bone morphogenetic proteins, insulin-like growth factor-1), therefore providing both osteoinductive and osteoconductive properties [26]. In another recent study, Alom et al. produced hydrogels isolated from demineralized and decellularized bovine bone [27]. They demonstrated that this hydrogel material resulted in a 3.6- to 13.4-fold increase in osteopontin expression and 15.7- to 27.1-fold increase in osteocalcin expression in cultured mouse primary calvarial cells (mPCs) [27].

Regarding future directions, our group and associated collaborators believe that a bone graft substitute consisting of functionalized PAs with both BMP-2 and DBM can yield a promising combination therapy. Combining both BMP-2 and DBM within a PA-based architecture has potential for a highly osteoinductive and osteogenic bone graft substitute. This hybrid material could eliminate the need for autograft or allograft bone, hence changing a DBM-based material from a bone graft extender into an actual bone graft substitute. Given our previous investigation of biogels with functionalized PAs and local BMP-2 release, we hypothesize that the amount of BMP-2 required for successful outcomes with this novel hybrid biomaterial would also be much less than what is currently used clinically and could theoretically minimize the known complications of high-dose BMP-2 [20].

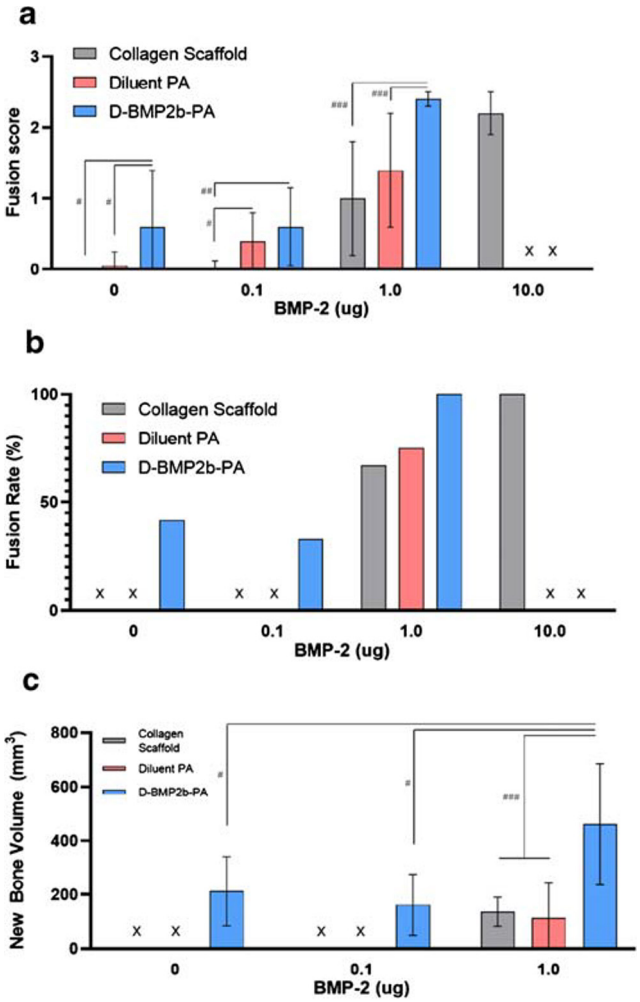

Fig. 2 In vivo assessment of BMP-2-binding PA gel in a rat posterolateral lumbar fusion model. The same graft material was implanted bilaterally onto the L4–L5 transverse processes to mediate fusion in each animal. The different graft materials were an absorbable collagen sponge, diluent PA gel, and diluted BMP-2-binding PA gel, with different doses of BMP-2 (0, 0.1, 1.0, and 10.0 μg/mL). **a** Fusion scores determined by blinded manual palpation of harvest spines at 8 weeks post-operation. A score of 0 indicates no bone bridging, 1 indicates unilateral bone bridging, 2 indicates bilateral bone bridging, and 3 indicates bilateral bone bridging with abundant bone formation. **b** Successful fusion rate (%), determined by a manual palpation score of at least 1.0, calculated at 8 weeks post-operation. **c** New bone volume (mm^3) was determined through microcomputed tomography (microCT) imaging analysis of harvested specimens at 8 weeks post-operation

Other Nanocomposite Materials

Nanocomposites are materials that incorporate nanoscale particles into a standard material [28, 29]. In general, nanocomposite materials can be engineered to create an environment suitable for cellular ingrowth and differentiation of stem cells to specific lineages [28, 29]. These materials are typically in the form of gels, colloids, or copolymers [28, 29]. In addition to nanocomposites, there are several other nanomaterial

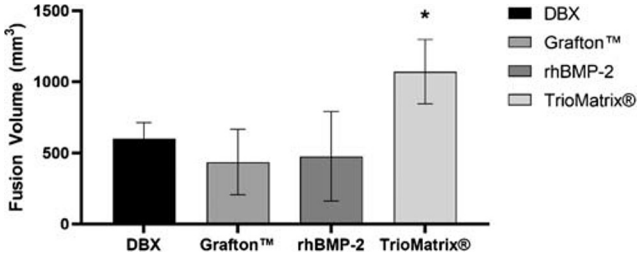

Fig. 3 Fusion mass volume (mm^3) was determined with microcomputed tomography (microCT) imaging analysis of the harvested spine samples. The TrioMatrix®-treated spines had a significantly greater new bone volume than the DBX, Grafton™, and rhBMP-2 groups

structures that have been designed and fabricated for bone regeneration [20].

Other groups have also investigated the osteogenic potential of nanocomposite materials. Recently, Gandimathi et al. demonstrated that an electrospun-electrosprayed technique can be used to produce nanostructured hydroxyapatite structure that stimulate osteogenic differentiation of mesenchymal stem cells in an in vitro model [30]. Liu et al. created a shape memory, porous scaffold that contained chemically cross-linked poly(ε-caprolactone), hydroxyapatite nanoparticles, and rhBMP-2 [31]. MicroCT and histological analysis demonstrated the capacity for substantial bone regeneration when tested in a rabbit mandibular bone defect model [31]. Lee et al. designed a nanocomposite containing reduced graphene oxide and hydroxyapatite, which promoted osteogenesis in a rabbit calvarial defect model [32]. Nanocomposites are a relatively new discovery, and they have not been widely established for orthopedic applications. However, the osteogenic capacity of these unique materials provides an interesting area for future investigation.

3D-Printed Biomaterials

3D-printing applications have gained significant attention in the area of spine surgery over the last decade [33•]. The uses of 3D printing are diverse, including both macroscale and microscale applications. A recent systematic review illustrated that the majority of 3D-printing applications in spine surgery have been for surgical planning, intra-operative surgical guides, and custom implants [33•]. Pre-operative 3D-printed models can provide a detailed understanding of individual anatomy and may allow surgeons to more accurately plan surgical approach. For example, 3D-printed surgical guides have shown promise in improving the safety and accuracy of pedicle screw placement [34].

One macroscale application of 3D printing is customized implants that can be rapidly printed for use in the operating room. 3D printing provides an efficient and cost-effective means for altering the dimensions and mechanical properties of various implant designs. In one

recent study, Rodriguez et al. designed both pre-formed DBM implants and custom 3D-printed DBM implants for bone regenerative applications [26]. In this study, a proof-of-concept computer model of the acetabulum was created based on CT scans of the human pelvis and femoral head [26]. The resulting segmentation model was used to 3D print the custom designed acetabulum mold in acrylonitrile butadiene styrene (ABS). This polymer mold was then filled with demineralized, wet DBM fibers and processed to create a final mold that reflected the complex anatomy of a patient's acetabulum. This same study determined through a series of in vitro and in vivo studies that this type of implant provides osteoinductive and osteoconductive potential [26]. Therefore, it is hypothesized that similar customized 3D-printed implants could be utilized for human spine fusion and osseous defect applications.

Several other groups have utilized 3D-printing technology to create custom implants. Wu et al. summarized six recent studies that have assessed customized 3D-printed macroscale implants for various applications in spine surgery [33•]. Four of these studies assessed fixation devices—a C1/2 posterior fixation device for facet joint arthropathy (Phan et al.) [35], a vertebral body device for C2 Ewing sarcoma (Xu et al.) [36], an occipitocervical fixation device and hemivertebrae prosthetic for C1/2 chordoma and congenital L5 hemivertebrae (Mobbs et al.) [37], and an axial vertebral body fixation device for T9 primary bone tumor (Choy et al.) [38]. Two of these studies assessed entire prosthetic devices: a sacrum replacement prosthetic for sacral chordoma (Wei et al.) [39] and a hemisacrum prosthetic device for sacral osteosarcoma (Kim et al.) [40]. As 3D-printing technology continues to improve, novel designs for spine implants will likely develop as well.

Microscale applications of 3D printing are another growing area of clinical and research interests. 3D printing allows for fine control of the mechanical and biologic properties of the implant material to optimize cell adhesion, proliferation, and degradation [41]. Several groups have developed techniques for 3D printing of ceramics, namely calcium phosphate and hydroxyapatite, given the osteoinductive properties of these materials. Bergmann et al. demonstrated that 3D printing can be used to print implants composed of calcium phosphate ceramic and bioactive glasses [42]. Cox et al. also utilized 3D printing for purposes of creating individual porous hydroxyapatite scaffolds [43]. Additionally, Inzana et al. demonstrated that composite calcium phosphate and collagen scaffolds can also be 3D printed for bone regeneration applications [44]. Additionally, different groups have investigated the efficacy of 3D-printed implants for clinical use. Recently, McGilvray et al. designed a 3D-printed porous titanium alloy interbody cage material that had success in an ovine lumbar fusion model [45•]. This 3D-printed implant provided porosity for purposes of promoting bony ingrowth and is currently commercially available for clinical use. This particular implant yielded significantly greater total bone volume on microCT imaging and reduced flexion-extension range of motion relative to a classic polyetheretherketone (PEEK) cage at 8 weeks and 16 weeks post-operatively [45•]. As novel techniques are developed, 3D printing may offer an efficient and cost-effective means for controlling the osteoinductive and osteoconductive properties of implanted materials.

Hyperelastic Bone® Material

Hyperelastic Bone® (Dimension Inx, Chicago, IL) is a bioceramic composed of > 90% hydroxyapatite, and either polycaprolactone or poly(lactic-co-glycolic acid) [46•]. The material mimics the ceramic composition of human bone but is also elastic and flexible. Therefore, the material can be manipulated to have the optimal shape and dimensions for various applications. Additionally, this material can be 3D printed quickly (up to 275 cm^3/h) at room temperature and deployed in the operating room to produce customized implants for different applications [46•]. Hyperelastic Bone® has been marketed for spine fusion, in addition to other orthopedic and dental applications, such as bone void fillers, ligament and tendon sleeves, and cleft fillers, among others.

Our group and associated collaborators previously demonstrated that the material is highly absorbent (50% material porosity), exhibits significant mechanical elasticity (~ 32 to 67% strain to failure, ~ 4 to 11 MPa elastic modulus), supports cell viability and proliferation, and induces osteogenic differentiation of bone marrow-derived human mesenchymal stem cells in vivo without the addition of any osteoinductive factors [46•]. For in vivo experimentation, this material was tested in a mouse subcutaneous implant model for biocompatibility, in a rat PLF model for bone formation and in a large, non-human primate calvarial defect case study [46•]. Overall, Hyperelastic Bone® did not elicit a significant immune response, was vascularized and integrated within surrounding tissues, and rapidly ossified and supported new bone growth [46•]. Regarding the rat PLF model study, the Hyperelastic Bone® scaffold was compared with an absorbable collagen sponge (ACS) control, DBM, hydroxyapatite granules, and a Hyperelastic Bone® with 1.5 μg of rhBMP-2 [46•]. The mean fusion score and fusion rate of the Hyperelastic Bone® group were significantly greater than the ACS and groups and equivalent to the DBM group [46•]. Additionally, the combination of Hyperelastic Bone® with rhBMP-2 led to significantly greater mean fusion score and fusion rates [46•]. MicroCT imaging was used to quantify new bone volume in the L4–L5 fusion bed. The Hyperelastic Bone® group induced considerable new bone formation, and the Hyperelastic Bone®

with rhBMP-2 induced significantly more bone than Hyperelastic Bone® alone (Hyperelastic Bone® group 19.5 ± 6.3 mm^3 and Hyperelastic Bone® with rhBMP-2 38.9 ± 11.4 mm^3) [46•]. Therefore, this material provides osteogenic potential that is comparable to DBM, and its efficacy can be optimized with the addition of known osteogenic growth factors (e.g., rhBMP-2).

In another study, Alluri et al. combined gene therapy with 3D-printed Hyperelastic Bone® material to produce a novel biologic for bone regeneration [47•]. Specifically, this group loaded a 3D-printed Hyperelastic Bone® material with human adipose-derived stem cells (ADSCs) transduced with a lentiviral (LV) vector to overexpress bone morphogenetic protein-2 (rhBMP-2). In a mouse hindlimb muscle implant model, it was determined that the LV-BMP2 with and the Hyperelastic Bone® group had significantly greater bone growth when compared with other groups, including and Hyperelastic Bone® alone [47•]. This study shows promise for gene therapy as an alternative to rhBMP-2-loaded materials. Further design and investigation of combination materials that incorporate the unique properties of Hyperelastic Bone® may be a fruitful area of research.

Conclusion

Biologic bone graft materials in spine surgery continue to be of significant clinical and research interest. In addition to biologic products with proven clinical efficacy, there is a wide range of novel biomaterials currently being investigated. Current technologies, such as nanotechnology and 3D printing, have facilitated the design of novel biologic bone graft materials. The materials discussed in this review—functionalized peptide amphiphiles and other nanocomposites, novel demineralized bone matrix applications, 3D-printed materials, and Hyperelastic Bone®—have demonstrated significant bone regenerative capacity in both in vitro and in vivo applications. These materials have the potential to be safer alternatives to other bone graft materials such as autograft and BMP-2, which have known complications. Bone graft technologies continue to be an exciting and diverse area of research and a promising tool for improving the efficacy and safety of spinal fusion procedures.

Compliance with Ethical Standards

Conflict of Interest Mark Plantz declares that he has no conflict of interest.

Dr. Wellington Hsu declares the following conflicts of interest:

Personal fees from Stryker, personal fees from Relievant, personal fees from Bacterin, personal fees from CeramTec, personal fees from Graftys, personal fees from Globus, personal fees from AONA, personal fees from Medtronic, personal fees from Bioventus, other support from AONA, other support from Stryker, other support from Pioneer Surgical, other support from Medtronic, other support from Bioventus, other support from AONA, and grants from Medtronic, outside the submitted work.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards. No information that identifies any particular individual was included in this review article.

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- •• Of major importance

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45.• KC MG, Easley J, Seim HB, Regan D, Berven SH, Hsu WK, et al. Bony ingrowth potential of 3D-printed porous titanium alloy: a direct comparison of interbody cage materials in an in vivo ovine lumbar fusion model. Spine J. 2018;18(7):1250–60 **This study compared three different interbody implants—polyetheretherketone (PEEK), plasma sprayed porous titanium-coated PEEK (PSP), and 3D-printed porous titanium alloy cage (PTA)—in an ovine interbody lumbar fusion model. The 3D-printed porous titanium alloy cage (PTA) yielded superior results, including a significant reduction in range-of-motion, increased stiffness, and increased bony ingrowth relative to the other materials.**
46.• Jakus AE, Rutz AL, Jordan SW, Kannan A, Mitchell SM, Yun C, et al. Hyperelastic "bone": a highly versatile, growth factor–free, osteoregenerative, scalable, and surgically friendly biomaterial. Sci Transl Med. 2016;8(358):358ra127-358ra127 **This study assessed the bone regenerative capacity of Hyperelastic Bone® in both *in vitro* and *in vivo* models. The material was assessed in several models, including a mouse subcutaneous implant model, a rat posterolateral lumbar fusion (PLF) model, and a non-human**

primate, calvarial defect model. The material was found to quickly integrate with nearby tissue without a negative immune response, vascularize, ossify and facilitate bone growth without the need for growth factors.

47.• Alluri R, Jakus A, Bougioukli S, Pannell W, Sugiyama O, Tang A, et al. 3D printed hyperelastic "bone" scaffolds and regional gene therapy: a novel approach to bone healing. J Biomed Mater Res A. 2018;106(4):1104–10 **This study evaluated the osteogenic potential of Hyperelastic Bone® loaded with human adipose-derived stem cells (ADSCs) transduced with lentiviral (LV) vector to overexpress bone morphogenetic protein-2 (BMP-2). This combination therapy was assessed in both *in vitro* and *in vivo* (hindlimb muscle pouch model) models. The Hyperelastic Bone® group loaded with transduced ADSCs demonstrated ectopic bone formation *in vivo*, which was not evident in the other groups [Hyperelastic Bone® loaded with a) LV-green fluorescent protein, b) ADSCs alone, and c) scaffold alone]. This study demonstrated that combining gene therapy with materials engineering is a promising new area of research with regards to bone graft design.**