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Demineralized bone matrix as a vehicle for delivering endogenous and exogenous therapeutics in bone repair ☆

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ABSTRACT

As a unique human bone extract approved for implant use, demineralized bone matrix (DBM) retains substantial amounts of endogenous osteoconductive and osteoinductive proteins. Commercial preparations of DBM represent a clinically accessible, familiar, widely used and degradable bone-filling device, available in composite solid, strip/piece, and semi-solid paste forms. Surgically placed and/or injected, DBM releases its constituent compounds to bone sites with some evidence for inducing new bone formation and accelerating healing. Significantly, DBM also has preclinical history as a drug carrier by direct loading and delivery of several important classes of therapeutics. Exogenous bioactive agents, including small molecule drugs, protein and peptide drugs, nucleic acid drugs and transgenes and therapeutic cells have been formulated within DBM and released to bone sites to enhance DBM's intrinsic biological activity. Local release of these agents from DBM directly to surgical sites in bone provides improved control of dosing and targeting of both endogenous and exogenous bioactivity in the context of bone healing using a clinically familiar product. Given DBM's long clinical track record and commercial accessibility in standard forms and sources, opportunities to formulate DBM as a versatile matrix to deliver therapeutic agents locally to bone sites in orthopedic repair and regenerative medicine contexts are attractive.

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1. Introduction

Demineralized bone matrix (DBM) has a substantial clinical history in skeletal repair with over 100,000 procedures using various DBM-based products performed annually in bone, and a substantial publication record (see accompanying ADDR article on DBM's history of use and bone repair properties in this issue).[1] Since clinical-grade DBM is sourced as a human-derived tissue product, its application in bone

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repair and, importantly, as a protein-based vehicle for delivering bioactive agents depends on bone procurement techniques from human donors, donor age and gender, processing, sterilization and resulting DBM composition and properties [2–5]. Differences in donor bone, preparation and processing methods for bone can impact DBM properties and its consequent clinical performance. These include residual calcium content from demineralization processes, DBM bone-derived particle sizes and distributions, and variable endogenous growth factor contents [1]. Biological bioactivity assays of DBM in vitro and in vivo attempt to provide an "osteoinductive index" (OI) described in reference [1]. Nonetheless, DBM OI variability can be substantial, and this remains a central issue for assessing and predicting DBM 'potency' in osteogenesis in vitro and in vivo. DBM OI values are not a requisite validation for DBM marketing and clinical use and notably are not controlled or consistent from tissue bank sources. Despite the substantial clinical record of use and history, this variability in OI, methods used for its assay, lack of comparisons between in vitro to various preclinical animal healing and osteogenesis models, and human therapeutic experiences make DBM use and efficacy still controversial. Hence, DBM products have variable compositions, OI values and biological properties [1,6], with important resulting implications for clinician users and their patients, and in research on DBM efficacy and uses.

Table 1 shows the typical protein matrix and growth factor components known from DBM extracts: in addition to bone matrix proteins and the predominant protein – type I collagen – numerous notable protein pleiotropic growth factors are present and released from DBM as the implanted DBM matrix degrades.

Type I collagen is the major proteinaceous component in bone and therefore dominates the DBM organic mass balance. Significantly, in bone this collagen has notable contributions to bone growth, structure, function and turnover in homeostasis, particularly in partnership with bone's inorganic calcium hydroxyapatite phases. In DBM, residual collagen contributes essential physical and biological properties to the matrix. Importantly, several bone morphogenetic proteins (BMPs) known to promote bone growth and regeneration are present (i.e., BMP-2, BMP-4, BMP-7) [11,12], but their quantities and potencies also have source- and age-related variations in DBM products argued to affect OI [6].

In addition, DBM products consist of bone-derived particle sizes and particle size ranges, even protein fibers. Different DBM particle surface geometries may impact host cellular interactions as well as release rates of DBM-resident biological molecules and agents such as bone morphogenetic proteins (BMPs) or loaded drugs and cells both in and out of DBM. Finally, the composition of various biomaterials and device carriers combined with DBM, and DBM compositions and designs with various bioactive substances will influence its clinical and research performance. DBM raw material powders are formulated into a diverse array of DBM products used clinically. The number of different DBM product formulations, biomaterial physical forms and device compositions is diverse [1]. Soft, moldable DBM putty is preferred surgically for bone defect repair, made by mixing DBM powder solids with solutions of water-soluble polymers including sodium hyaluronate or carboxymethylcellulose, or anhydrous water-miscible solvents (e.g., glycerol). Other commercial DBM-containing products include pastes, sheets, strips, and moldable, conforming soft solids. Bone generating efficacy for these diverse formulation designs using DBM carriers are largely empirically assessed in arbitrary test beds that are often not comparable. Hence, direct comparisons of performance for DBM products in bone applications are difficult. Standardization of DBM OI and validation of methods for reporting OI for the various DBM products has been emphasized as an important need to better understand DBM's value in bone regeneration [1].

Table 1
Endogenous protein concentration per gram of DBM (excluding type I collagen)[a].

DBM proteins/growth factors	ng/g	Ref.
Bone sialoprotein (BSP)	40,000	[7]
Osteopontin (OPN)	20,000	[7]
Bone morphogenetic protein-2 (BMP-2)	3800	[8]
Bone morphogenetic protein-7 (BMP-7)	84.1	[9]
Insulin-like growth factor-I (IGF-I)	22	[8]
Bone morphogenetic protein-2 (BMP-2)	21.4	[9]
Transforming growth factor-β (TGF-β)	18	[8]
Bone morphogenetic protein-4 (BMP-4)	5.45	[9]
Acidic fibroblast growth factor (FGFa)	2	[8]
Vascular endothelial growth factor (VEGF)	1.9	[8]
Platelet-derived growth factor (PDGF)	0.1	[8]
Noggin (NOG)	Not known	[10]

[a] Note: DMB protein concentrations vary with donors and vendors [6].

2. Formulating DBM with therapeutic agents for local release to bone

As DBM is surgically placed or injected directly into bone sites, usually to repair defects and act as a bone filler (i.e., regulated as a device) to promote new bone growth, it can conveniently serve a secondary purpose as a controlled drug delivery matrix to administer doses of bioactive agents locally to the same surgical site. Incorporated into resorbable biopolymer putty, paste, sheet or strips containing DBM's endogenous growth factors (Table 1), DBM alone and its presence in implanted biomaterials provide a controlled delivery matrix, degrading under host site proteolysis and hydrolysis in vivo to release its diverse protein growth factor content endogenously bound within DBM. The duration and magnitude of these released growth factors are related to the DBM OI value; they are variable, depending on DBM commercial source, lot and processing [6]. For example, a recent study compared the bone cell stimulatory activity of DBM containing bone morphogenetic protein (BMP) against recombinant human BMP (rhBMP) protein alone [13]. Bone cell markers were up-regulated for 5 days by rhBMP alone, while DBM induced the markers for 14 days, indicating an intrinsic slow release profile of BMP from DBM. Currently, no standard guidelines govern either the OI values or DBM potency in products because DBM is classified as a tissue-sourced medical device (i.e., "intended to affect the structure or function of the body"). When a less than expected outcome is observed from DBM administration, the speculation is a consequence of endogenous DBM-released growth factors that are insufficient in either their dose or duration of release (i.e., DBM pharmacokinetic profile). This clinically variable performance and lack of DBM OI consistency is not part of the device labeling approval. It is however a primary problem with its clinical reliability for regenerating bone.

Augmentation of DBM with exogenously loaded agents addresses two primary therapeutic goals:

1. Some improved and rational capability to 'standardize' variable DBM OI and potency by supplementing recombinant growth factors for bone into DBM, and
2. Crafting more versatile drug delivery from DBM using custom selections of therapeutic drugs, bioactive agents, and cells to enhance DBM's own properties and exploit its clinical use as local bone delivery matrix.

To mitigate potential therapeutic insufficiencies, DBM may be amended with pharmaceuticals of many classes, including biologicals and living cells of specific phenotypes prior to implantation to produce a *combination medical device*: an implant with medical device function (bone filling/augmentation) as its primary mode of action and drug delivery as its secondary mode of action [14].

Consequently, DBM intrinsically possesses many of the compelling properties necessary for a drug delivery vehicle, including:

- Clinical history of use and diverse commercial sourcing
- Convenient capabilities to load and formulate varieties of different drugs within the matrix