JRFStimuBlast Demineralized Bone Matrix

Arthrex Research and Development

Introduction

Demineralized bone matrix (DBM) is commonly used as an adjunct to aid in bone healing when a void is present or when the normal healing process is otherwise impaired. Originally used in the dental field, DBMs have been successfully used in orthopaedics for many years including, but not limited to, traumatic fractures/nonunions, fusion procedures, and arthroplasties. The following will be discussed: the origins of DBM; identification of different DBMs on the market; and an explanation of JRFStimuBlast, along with its advantages over other DBM products.

The Definition of DBM

Before DBMs can be discussed, the terms osteoconductive, osteoinductive, and osteogenic need to be defined. These terms are commonplace when it comes to a discussion of bone repair and bone void fillers, whether from natural or synthetic sources. Osteoconductive materials provide a scaffold for bone-forming cells to form new bone and fill in a defect. Osteoinductive materials contain signaling molecules, such as growth factors or cytokines, which stimulate mesenchymal stem cells (MSCs) to differentiate along pathways to foster new bone and fill in a defect. Osteogenic materials add bone-forming cells, such as differentiated stem cells from bone marrow or bone itself, to a defect site to directly promote formation of new bone.

DBM has been used since the late 1800s.[1] However, the reason for its success was only first reported by Marshall Urist in the 1960s.[2] He demonstrated that the action of DBM is established by removing the mineral constituent of cortical bone, which makes the natural bone morphogenetic proteins (BMPs) of bone more accessible. DBM is formed by milling and grinding cortical bone into a powder, soaking the bone powder in an acid decalcifying solution to remove the mineral phase of bone, rinsing, and lyophilizing the resulting powder. The American Association of Tissue Banks (AATB) provides standards that DBM should contain less than 8% residual calcium, but most DBM formulations do not contain any residual minerals.[4] After the cortical bone powder has been decalcified by the acid demineralization solution, BMPs become exposed while leaving the overall collagenous matrix intact. BMPs, as the name indicates, are part of an overall group of growth factors expressed during formation of the nonmineral portion of bone. The most recognized BMPs in DBMs are BMPs-2, -4, and -7.[3] Therefore, DBMs are considered primarily osteoinductive due to the presence of these growth factors. However, since the collagenous matrix remains, DBMs can also be considered somewhat osteoconductive.

DBM products from the major bone graft supplier companies have differing amounts of DBM content in their formulations. DBM is a small particulate, much like the consistency of fine sawdust, so delivery and containment during the first few days post-implantation are important factors in the product's efficacy. The remaining percentage is the carrier used to theoretically prevent the DBM powder from washing away at the defect site. The effectiveness of the carrier substance in delivery of the graft material to the site, and its timely resorption by the body, contributes materially to overall graft efficacy. Some carriers perform this function more effectively than others. AlloMatrix Putty from Wright Medical contains DBM within a calcium sulfate-based carrier. The DBM percentage by volume is listed as 86%;[5] however, this translates to 40% DBM by weight. Because the carrier hardens to a solid mass, only about 5% of the DBM is actually exposed to the defect site. Most DBMs mixed with a carrier have a DBM percentage between 17% and 40% by weight.[6] JRFStimuBlast Putty contains 36% DBM by weight, which makes it one of the higher DBM-containing bone void fillers. Therefore, one is not losing osteoinductivity using JRFStimuBlast compared to other DBM products, but one gains the benefit of the reverse phase medium carrier which will be discussed later in this paper. This carrier material allows for delivery and containment of the JRFStimuBlast, and it is metabolized to expose BMP-containing DBM particles to the bone repair site. Table 1 shows a comparison of the most common DBM products available on the market, and their DBM percentages by weight.

Product - Distributor	Carrier - % DBM (by weight)	Terminal Sterility?	Osteoinduction Assay - Test Every Lot?
[JRF]*StimuBlast Putty - Arthrex*	*Reverse phase medium - 36*	*Yes E-Beam*	*In Vivo* - Yes
AlloMatrix Putty - Wright Medical	Calcium sulfate - 40	Yes E-Beam	*In Vitro* - Yes
DBX Putty - Synthes	Sodium hyaluronate - 32	No	*In Vivo/In Vitro* - Yes
Grafton Putty - Osteotech/Medtronic	Glycerol - 17	No	*In Vivo* - No

Table 1 - DBM Comparison

In order to ensure that DBM is in fact osteoinductive, either an *in vivo* animal implantation assay or a validated *in vitro* assay can be performed for verification. AlloMatrix from Wright Medical is tested with the *in vitro* assay, and every donor/lot of DBM, not the final product with its carrier, is tested to ensure osteoinductivity. Grafton from Osteotech/ Medtronic is tested with the *in vivo* assay; however, not every donor/lot is tested. [JRF]StimuBlast from Arthrex is tested with the *in vivo* assay, and every single donor/lot is tested to ensure osteoinductivity using the classic Urist model. In addition, the final processed [JRF]StimuBlast DBM product is used for the osteoinduction test; many other DBM products test the DBM by itself and not with its carrier.

The *in vivo* assay uses athymic mice implanted with DBM either at an intramuscular or subcutaneous location to verify DBM osteoinductivity.[7,8] When implanted in either nonbony site, DBM induces noncommitted progenitor cells to differentiate into bone forming cells. During the 28-day implantation period, the DBM induces endochondral ossification, which is bone formation with a cartilage intermediate. Histological scoring at 5, 14, and 28 days shows the normal progression of endochondral ossification, from initial cell recruitment to cartilage formation to bone and marrow formation. This method has proven to be consistent and reliable.

The *in vitro* assay uses processed DBM incubated with cells that can differentiate in the presence of active BMPs to verify DBM osteoinductivity.[9] Myoblasts, for example, have the potential to convert to osteoblasts in the presence of DBM that has retained its osteoinductive capabilities. Therefore, this is the *in vitro* version of the intramuscular implantation assay. After incubation, the cells are measured for alkaline phosphatase activity, which is a hallmark of osteoblast formation. This cell culture assay correlates with the above mentioned *in vivo* assay, but it does not produce actual bone as what is produced by the previously mentioned mouse model.[10]

As indicated in Table 1, not all DBM products are sterile. [JRF]StimuBlast is sterilized using terminal electron beam (E-Beam) sterilization. This process ensures a Sterility Assurance Level (SAL) of 10^{-6} according to the United States Pharmacopeia (USP). E-Beam has been shown not to be harmful to DBM or its bioactivity.[11] Other DBM products such as Grafton and DBX are processed aseptically, but are not terminally sterilized.

Carriers for DBM

There are many different carriers available for DBM powder, which when placed in a defect can be prone to wash-out after application or be contained too long a period of time by the carrier during the critical period when osteoinductivity would be most needed. Both synthetic and biologically-derived carriers have been used. In addition to the carriers listed in Table 1, other carriers include lecithin for Biomet's InterGro DBM and porcine gelatin for Regeneration Technologies' Osteofil DBM.

The carrier for [JRF]StimuBlast DBM is a Reverse Phase Medium (RPM). This material is flowable and moldable at room temperature, but thickens and is more viscous at body temperature. This is opposite to the behavior of most polymers, which are more viscous at room temperature and less viscous at higher temperatures. The polymer material class that RPM belongs to is called a poloxamer. It is a nonionic material composed of hydrophobic polypropylene oxide (PPO) surrounded on either side by hydrophilic polyethylene oxide (PEO). Poloxamers have been well studied and used for many medical applications, such as for drug release within pharmaceutical formulations for many years.[12,13] They are considered "inert" by the FDA.

Preclinical Studies

In vitro cell culture studies have been performed using poloxamers such as RPM as a carrier for DBM. One such study showed that osteoblasts from the human SaOS osteoblast-like cell line cultured on RPM retained their ability to release alkaline phosphatase and calcium at 7 and 14 days.[14] The osteoblasts did not lose any functionality while on the poloxamer. The phosphorous content was also examined to look at the formation of hydroxyapatite (HA) mineral in culture, which is determined by the calcium/phosphorous ratio. These ratios were between 1.81 and 2.06, which is near the average 1.67 ratio for HA mineral within bone. Another study examined the viability of osteoblasts from the human MG-63 osteoblast-like cell line in RPM, cultured with and without platelet-rich plasma (PRP).[15] Cell viability dropped from 100% to 60% of control in six days without PRP, but increased to 117% of control in six days with PRP treatment. There were also similar constant amounts of released alkaline phosphatase and collagen I from zero to six days, both with and without PRP.

As mentioned above, Marshall Urist first described why DBM performed well with "induction of new bone formation" – demineralization of bone effectively exposes naturally occurring BMPs. This effect was initially tested in ectopic sites in various animals[2] and gave rise to the use of the athymic rodent model as a determinant of inductive potential. Urist also found that using a poloxamer with reverse phase characteristics as a carrier for DBMs and BMPs was much

more effective in ectopic new bone formation intramuscularly in mice than other carriers such as collagen and HA.[16]

Other studies have focused on more clinical applications of DBM within a reverse phase poloxamer carrier. In rabbit calvarial defects, the DBM/RPM mixture promoted a greater amount of new bone formation than ceramic materials such as calcium sulfate and calcium phosphate cements.[17] In another rabbit calvarial defect study, statistically similar amounts of new bone formation were seen at 12 weeks, whether the DBM/poloxamer mixture was used alone or with calcium hydroxide.[18] The same DBM/poloxamer mixture was used in a rat calvarial defect as well, which showed improved bony incorporation and fusion.[19] In rabbit spinal fusion studies, the DBM/poloxamer combination was shown to perform as well as autograft.[20,21]

Clinical Studies

There have also been many clinical studies comparing DBM mixed with reverse phase poloxamer carrier to other DBM products with differing carriers, as well as autograft. Two studies found excellent new bone ingrowth using the DBM/poloxamer combination in dental applications.[22,23] Two other studies demonstrated this type of DBM having a higher overall healing rate compared to Grafton in nonunions, along with metaphyseal and periarticular defects.[24,25] Lastly, DBM/poloxamer has been used successfully in foot and ankle fusions, both in comparison to Grafton,[26] as well as autograft.[27]

Conclusion

[JRF]StimuBlast DBM has the same percentage of DBM and RPM as mixtures used in the previous studies described. Therefore, [JRF]StimuBlast has been shown to perform well in preclinical studies, as well as in many clinical applications. Its unique reverse phase medium carrier, RPM, allows [JRF]StimuBlast DBM to be clinically useful to fill voids for traumatic fractures, small joint applications (hand, wrist, foot and ankle), ACL tunnels, and around high tibial osteotomy (HTO) wedges. In addition, this carrier makes the [JRF]StimuBlast product superior to its competitors when being used in a bone void filling environment; it will not wash away under direct fluid pressure. If desiring a product with additional macroscopic scaffold properties, [JRF]StimuBlast CB provides this benefit with the addition of cancellous chips. When seeking a DBM product for sports medicine and small joint bone void filling applications, [JRF]StimuBlast and [JRF]StimuBlast CB provide ideal delivery, containment and carrier bioresorption properties allowing for bone repair and augmentation.

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