Bone Matrix and Demineralized Bone Matrix Incorporated PLGA Matrices for Long-term Bone Repair and Bone Tissue Engineering

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Statement of Purpose: Mineralized and demineralized freeze-dried bone allografts provide osteoinductive (OI) factors such as proteins and growth factors including bone morphogenic proteins, BMPs, a powerful regulator for bone formation. Mineralized allograft consists mineral, hydroxyapatite which provides osteoconductive (OC) properties, and mechanical strength to the bone to bear the loads. More extensive use of DBM powder in clinical applications is limited due to several reasons including difficulties in handling and concerns about particle migration. Even though, there are several forms of demineralized allograft bone products are commercially available, 50% of clinical failure in orthopaedic applications are reported. In this study, we hypothesize that incorporation of BM and DBM powder into poly(lactic-co-glycolic acid) (PLGA) could be function as a controllable release carrier of osteogenic factors at target site for extended time period. We have also evaluated the murine Bone Marrow Stromal Cells (BMSCs) function in BM/PLGA and DBM/PLGA matrices in-vitro for potential use in bone tissue engineering applications.

Methods: Fresh freeze-dried cadaver bone specimens were used to process BM and DBM powder which were prepared according to Glowacki's protocol [1]. BM or DBM particles (approximately 25-75 μm) were added with the weight ratio of PLGA:BM/DBM, 75:25 while PLGA (85/15) in the soluble state. Matrices of BM/PLGA, DBM/PLGA and PLGA were prepared by solvent cast method and dried them thoroughly. Samples were analyzed by scanning electron microscopy (SEM), fourier transform IR (FTIR). Release measurements of OI factors were performed by exposing all types of matrices into PBS, (8 ml) in the glass vials at 37 °C up to 80 days. Murine BMSCs were isolated by flushing the femurs from 6-8 weeks old mice using Dulbecco's modified Eagle's medium (DMEM), with 2% FBS. All matrices were sterilized under UV for 15 min. Murine BMSCs from 1-2 passages were seeded into all types of matrices in the 24 well plates with the cell culture media containing αMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Cell density used for experiments was 50,000 cells/ml and 0.5 ml of media with cells pipetted into 24 well plates. Cell attachment was tested at 30 min, 1 h, 2 h, 4 h and 6 h, after washing unattached cells with PBS and then attached cells were tripsinized and counted using Coulter counter.

Results / Discussion: The release amount of OI factors from BM/PLGA, DBM/PLGA with respect to PLGA controls which were immersed in PBS and incubated at 37 ºC for 80 day period is shown in Fig. 1. Rapid burst release of OI factors was not observed for both BM/PLGA and DBM/PLGA matrices. Both BM/PLGA and DBM/PLGA matrices follow the linear behavior in release of OI factors during the exposure time to PBS. The release amount of OI factors from BM/PLGA and DBM/PLGA matrices were 5-fold and 2-fold higher at 70 days than at 10 days, respectively. In addition, significantly higher amount of OI factors release from BM/PLGA than DBM/PLGA matrices. The BMSCs cells grown in wells containing BM/PLGA and DBM/PLGA demonstrated significantly higher BMSCs attachment at 30 min, 1 h, 2 h, 4 h and 6 h compared to cells grown in wells with controls ($p<0.05$) (Fig. 2).

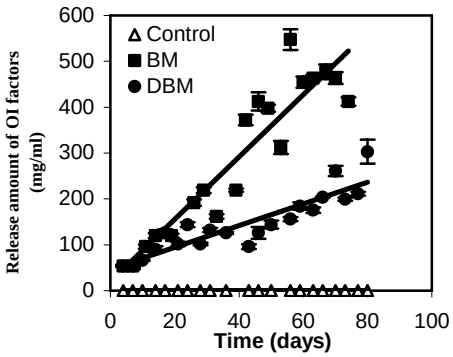

Fig. 1 Amount of OI factors release from matrices exposed to PBS as a function of time

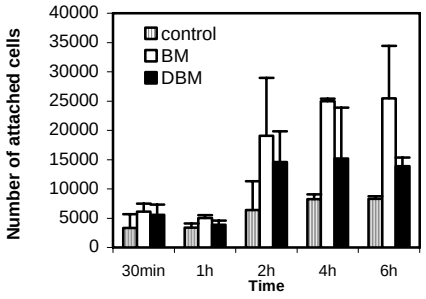

Fig. 2 Number of attached cells on matrices as a function of time

Conclusions: Using PLGA as a carrier for both BM and DBM powder provides controlled release of OI factors. Therefore, BM/PLGA and DBM/PLGA can be used at target sites to release OI factors avoiding particle migration and immediate dispersion with blood serum. Since the PLGA matrix is completely degradable, it could be implanted close to the site where it is needed, such as in the bone to treat bone fracture. This type of matrices can be potentially used for bone tissue engineering applications where BMSCs seeded and cultured in-vitro onto polymer scaffolds prior to implantation at target site. Since synthetic polymer scaffolds are biologically inert, BM or DBM powder can be incorporated into polymer scaffolds in order to achieve OC and OI properties.

References: 1. Glowacki J, Lancet. 1981; 1 (8227):959-62.