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Tissue Engineered Bone Graft Substitute

S. Sylvester

Timothy L. Norman
Cedarville University, tnorman@cedarville.edu

Nilay Mukherjee

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*Sylvester, S; *Norman, TL; +*Mukherjee, N
*West Virginia University, Morgantown, WV

Introduction: Tissue engineering products are recognized for their potential as treatment alternatives for repairing/regenerating tissue lost due to trauma or degeneration, or to correct congenital malformations. The goal is to restore organ function (1). Our tissue engineering strategy to generate bone follows the paradigm of implanting osteogenic cells in a scaffold material that will temporarily support the cells until they can form bone. In this study we isolated cells from bone marrow of New Zealand white rabbits and tested their osteogenic potential in various culture conditions. We then embedded these cells in a commercially available mineralized collagen matrix (Healos from Orquest, Mountain View, Ca) and assessed the ability of these cells to populate the matrix and form bone like tissue in vitro. The overall goal of this research is to develop alternatives to autologous bone graft substitutes for procedures such as posterolateral spinal fusion. If successful, the findings may eventually lead to enhanced healing and reduce morbidity associated with autologous bone grafting.

Methods: Bone marrow was isolated from adult male New Zealand white rabbit femurs. They were expanded in monolayer culture adapted from standard protocols developed for rat models (2) (3). The fastest growing cells were selected for further experiments.

Well-plate: Approximately $5x10^4$ cells were placed in each treatment well of a 24 well plate and exposed to one of three treatments: Minimum essential medium (MEM), 40ng/mL Dexamethasone and MEM or 50ng/mL BMP-2, 40ng/mL Dexamethasone and MEM. The non-control treatments were given additional mineralizing agents of $2.8x10^{-4}$M concentration of L-ascorbic acid and 10mM concentration of β-glycerophosphate starting on day 7 and every media change thereafter (4) (5). The media was changed every three days.

Wells were stained with alkaline phosphatase staining kit (Sigma, St. Louis, MO) to determine the activity of the alkaline phosphatase enzyme, an early indicator of osteogenesis (6) and von Kossa (7), which indicates mineralization, a late stage of osteogenesis

Healos: Healos matrix was cut into 4mm squares and placed into sterile 24 well plates. $2.5x10^6$ bone marrow derived cells suspended in 0.2ml of MEM media were added to the Healos mesh. After allowing 2 hours for the cells to attach, culture media was added. 40ng/ml Dexamethasone was used as an osteoinductive agent (8). The different groups were: Healos matrix alone and Healos matrix + cells. Media was changed every 3 days. After 1 and 2 weeks in culture, the constructs were fixed in 10% neutral buffered formalin, processed for histology and stained with Hematoxylin and Eosin (H&E) and Von Kossa.

Essential Results:

Well-plate: ANOVA analysis showed that some groups were significantly different from others [F=0.0023 at p=0.05 level] in terms of alkaline phosphatase activity. The trend in the data showed that alkaline phosphatase activity increased over a three-week period with a decline in activity seen at week 4 (Figure 1). Von Kossa staining was not observed at week 1 – 3 sampling time points. Week 4 showed a positive stain for Von Kossa (Figure 2) with the MEM+BMP-2+Dexa treatment having the largest percent area coverage [p=0.001].

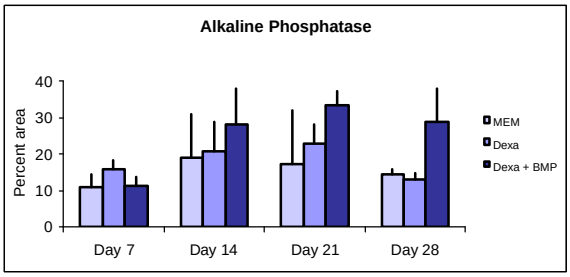

Fig 1: Alkaline Phosphatase enzyme activity increased through day 21 and declined later [F=0.002 at p=0.05 level].

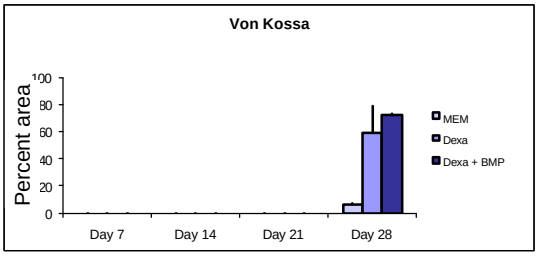

Fig 2: Von Kossa staining was not observed through day 21 but appeared at day 28. Treatment groups showed significantly higher mineralization than controls on day 28 [p=0.001]

Healos: The cells appeared to survive in the Healos mesh. The media changed color signifying that the cells were metabolically active. Some cells seemed to migrate from the mesh and populated the bottom of the dish. The H &E staining showed that at two weeks, many cells attached to the mesh and were able to deposit extra-cellular matrix around them. The Von Kossa staining showed that the matrix had not yet mineralized at 2 weeks, as expected (Figure 3a,b).

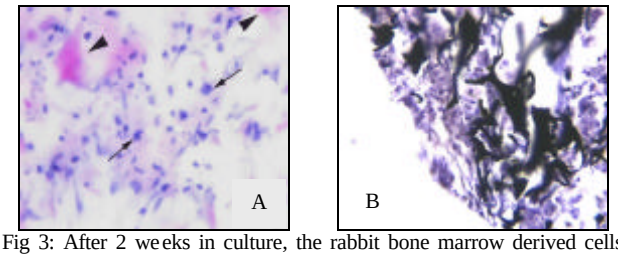

Fig 3: After 2 weeks in culture, the rabbit bone marrow derived cells (nuclei stained blue, indicated by arrows) populated the matrix, which stained pink, indicated by arrow heads (A). The cells are able to lay down matrix around them (faint pink). B) Under Von Kossa staining, the Healos matrix stains black while the extracellular matrix deposited by the cells is apparent but not mineralized.

Discussion: This work showed that we were able to isolate precursor cells from bone marrow that could be induced to differentiate down the osteogenic pathway in vitro, as shown by alkaline phosphatase activity and von Kossa staining. These cells were able to populate the healos mesh and lay down extracellular matrix around them. Since the Healos is bovine collagen coated with hydroxyapatite, it also stained positive for Von Kossa and it will be hard to tell if the cells deposited mineralized matrix at time points greater than 2 weeks. These results have encouraged us to use this tissue engineering construct as a bone graft substitute in a rabbit posterolateral spinal fusion model.

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