VASCULARIZATION OF A DBM CARRIER MASS IN THE LUMBAR POSTEROLATERAL FUSION RABBIT MODEL

INTRODUCTION: Cell-based therapies are increasingly being investigated as a means of enhancing the healing of segmental bone defects and spinal fusion. The goals of these therapies are to provide focal, sustained delivery of specific gene product [1], or the introduction of specialized cells that have unique and/or enhanced reparative capabilities [2]. In most studies to date, the cells have been introduced at the time the segmental bone defect or spinal fusion is performed [1, 3]. However, this may not be the most appropriate time to add cells since surgery disrupts the normal blood supply of the fusion site for a variable period of time. The survival and performance of a cell-based product implanted into a devascularized fusion bed may be suboptimal.

For the past several years we have been using the New Zealand white (NZW) rabbit posterolateral intertransverse fusion model [3] to explore a cell-based therapy aimed at improving spinal fusion rates. This study was performed to determine the sequence and characteristics of vascularization of a fusion site mass containing a guanidine extracted demineralized bone matrix (gDBM) cell carrier. Our ultimate goal is to optimize the timing and thereby the efficacy of cell implantation.

METHODS: Following IACUC approval, single-level lumbar posterolateral arthrodesis were performed in 20 adult NZW rabbits as previously described [3]. 2.5 cc of gDBM, prepared from the long bones of previously sacrificed NZW rabbits [4], was then implanted onto the fusion beds on each side of the L5-L6 level. Four animals were sacrificed at 5, 10, 15, 20 and 30 days post-operatively. After sacrifice, the L5-L6 segment was dissected free, fixed and decalcified. The decalcified fusion mass was split longitudinally on the mid-sagittal plane. 5µm transverse sections were cut from the cranial, mid and caudal regions of the fusion mass, and 5µm sagittal sections were cut from the medial, mid and lateral portion of the right fusion mass. Slides from every region were stained with mouse anti-human endothelial cell antigen (CD: 31; Dako Ltd. UK) at 1:40 dilution. Vessels were easily identified as immunostained oval or circular structures, with or without red blood cells. The stained sections were used to quantify overall vascularized area, and regional vessel ingrowth and density.

Vascularized Area: Two sagittal slides from the mid-portion of the right fusion site mass were analyzed from each animal. The perimeter of the carrier mass (gDBM) and the innermost margin of the vascularization front were outlined with colored markers using an inverted microscope [Fig. 1A]. Slides were scanned at 600dpi. NIH image software was used to directly quantify the area of the carrier mass [Fig. 1B] and the avascular region [Fig. 1C]. The vascularized area was determined by subtracting the avascular area from the total carrier mass areas; the percentage of vascularized area was calculated by dividing the vascular area over the total carrier mass area.

Vascular Ingrowth Distance: Vascular ingrowth distance was quantified on the sagittal sections by measuring the distance from the perimeter of the carrier mass to the vascularization front on lines radiating outward from the center of the carrier mass [Fig. 2A]. To assess regional differences in vascular penetration, ingrowth distance was measured from the transverse processes (TP), outer muscle tissue (OM), central muscle tissue (CM), and intertransverse ligament (ITL) [Fig. 2B].

RESULTS: All of the animals survived the study and were available for analysis at the designated sacrifice timepoints.

Vascularized Area: The gDBM carrier used in this study vascularized at an average rate of about 3% per day, with essentially complete vascularization by day 30 (99±4%). On days five, ten and fifteen, vascularization was 15±7%, 41±3% and 73±6%, respectively.

Vascular Ingrowth Distance: Consistent with the results for vascularized area, vascular ingrowth increased steadily as a function of post-op day (~0.6±0.2 mm/d). The only two timepoints where vessel penetration were similar were post-op days 15 and 20, otherwise it increased from one timepoint to the next (p<0.001). While vessel penetration generally increased with time, there was a significant interaction of region (TP, OM, CM, ITL) and time (p<0.001). Starting at post-op day 15, vessel ingrowth distance from OM was greater than CM and ITL, and by day 30 vessel ingrowth distance from TP was also greater than CM and ITL; before day fifteen, there were no regional differences in vascularization.

Vessel Density: Vessel density also varied as a function of post-op day (p<0.001) and analysis region (p<0.001). Vessel density on post-op days 20 and 30 was greater than that on day 15, which was in turn greater than those on days 5 and 10.

DISCUSSION: This study was performed to characterize the vascularization of a gDBM carrier used for cell implantation in a spinal fusion model. We found that the soft tissues (OM, CM and ITL) actively contribute to the vascularization of the carrier mass. This process is time-dependent, occurring at a rate of ~0.6±0.2 mm, or about 3% of the carrier mass, per day. There are also regional differences in vascularization, with higher vessel penetration rates and vascular densities from the cranial and caudal muscle bed and the decorticated transverse processes. The results of this study should provide guidance for the rational timing and localization of cell placement in cell-based therapeutic approaches.

REFERENCES:

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