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Biologic Scaffold Remodeling in a Dog Model of Complex Musculoskeletal Injury

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Background. **Current treatment principles for muscle injuries with volumetric loss have been largely derived from empirical observations. Differences in severity or anatomic location have determinant effects on the tissue remodeling outcome. Biologic scaffolds composed of extracellular matrix (ECM) have been successfully used to restore vascularized, innervated, and contractile skeletal muscle in animal models but limited anatomic locations have been evaluated. The aim of this study was to determine the ability of a xenogeneic ECM scaffold to restore functional skeletal muscle in a canine model of a complex quadriceps injury involving bone, tendon, and muscle.**

Materials and Methods. **Sixteen dogs were subjected to unilateral resection of the distal third of the vastus lateralis and medial half of the distal third of the vastus medialis muscles including the proximal half of their associated quadriceps tendon. This defect was replaced with a biologic scaffold composed of small intestinal submucosa extracellular matrix (SIS-ECM) and the remodeling response was evaluated at 1, 2, 3, and 6 mo ($N = 4$ per group).**

Results. **The initial remodeling process followed a similar pattern to other studies of ECM-mediated muscle repair with rapid vascularization and migration of myoblasts into the defect site. However, over time the remodeling response resulted in the formation of dense collagenous tissue with islands of muscle in the segments of the scaffold not in contact with bone, and foci of bone and cartilage in the segments that were adjacent to the underlying bone.**

Conclusions. **SIS-ECM was not successful at restoring functional muscle tissue in this model. However, the results also suggest that SIS-ECM may have potential to promote integration of soft and boney tissues when implanted in close apposition to bone.**

Key Words: **skeletal muscle; extracellular matrix; quadriceps; constructive remodeling; muscle injury.**

INTRODUCTION

Skeletal muscle comprises 40% to 50% of the body mass [1] and injuries to the musculoskeletal system are common. In sporting activities, muscle injuries account for up to 55% of all sustained injuries with over 90% of these being either contusions or strains [2–5]. Traumatic injuries to the extremities and limb salvage procedures for extremity tumors can result in volumetric muscle loss. While muscle flaps and tissue transposition are commonly used procedures for the restoration of function following muscle loss, there are few clinical outcome studies on the treatment of volumetric muscle loss. Heterogeneity in the anatomic location and severity of injury make retrospective studies difficult and interpretation of results questionable. Consequently, the current treatment principles for muscle injuries with volumetric loss have been largely derived from empirical observations.

Following traumatic injury without significant loss of tissue, skeletal muscle has a robust capacity for regeneration [2, 5]. The muscle repair process relies in a large part upon the presence of myogenic satellite cells [6]. When injury is associated with volumetric muscle loss, the ability of the muscle to repair diminishes,

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and it is generally accepted that if more than 20% of the muscle mass is lost, the regeneration process will fail [7–11] with the subsequent accumulation of scar tissue, denervation of the muscle distal to the defect, and loss of function.

Biologic scaffold materials composed of extracellular matrix (ECM) have been successfully used to repair or replace a variety of diseased or damaged tissues including cardiac [12, 13], esophageal [14], urinary bladder [15], and musculotendinous tissues [16, 17]. In many of these applications, such ECM-scaffold materials, promoted the formation of site-appropriate muscle tissue, including cardiac, smooth and skeletal muscle. Non-crosslinked ECM derived from porcine small intestinal submucosa (SIS) has been shown to restore functional, innervated and contractile skeletal muscle in animal models of volumetric muscle loss [16, 17]. However, it remains to be shown whether similar volumetric muscle loss in other body sites, including anatomic sites in direct contact with bone, would show similar constructive remodeling outcomes. Microenvironmental differences inherent in different anatomic sites, particularly at complex injury sites involving bone, muscle, and tendon, can markedly effect ECM-tissue remodeling. For example, in muscle injuries adjacent to bone, particularly in the anterior thigh and quadriceps femoris muscle, the skeletal muscle response to injury can include calcification [18–20]. Myositis ossificans (i.e., heterotopic ossification) can effect up to 20% of patients who receive a contusion injury [21]. In cases of traumatic volumetric muscle loss, myositis ossificans is more common, affecting up to 65% of patients [22, 23].

The objective of the present study was to determine the ability of a xenogeneic ECM scaffold to restore functional skeletal muscle following volumetric loss in a canine model of a complex quadriceps injury site involving muscle, tendon and in close apposition to bone.

METHODS

Overview of Experimental Design

Sixteen female dogs, randomly assigned to four equal groups were subjected to unilateral resection of the distal third of the vastus lateralis muscle and the medial half of the distal third of the vastus medialis with the proximal half of their associated quadriceps tendon. This experimentally created defect was replaced with a biologic scaffold material composed of small intestinal submucosa extracellular matrix (SIS-ECM). The time points for evaluation were 1, 2, 3, and 6 mo ($n = 3$ at each time point). The remaining four animals were assigned as no-treatment controls ($n = 1$ per time point). Intramuscular electromyography and muscle contractility tests were performed immediately prior to euthanasia to evaluate muscle function within the site of SIS-ECM placement. Microscopic analysis included histochemistry and immunolabeling to examine skeletal muscle and tendon regeneration, vascularization, and innervation.

Scaffold Design and Creation

Porcine small intestine, harvested from market weight pigs (240–280 lbs), was mechanically delaminated to remove the tunica muscularis externa and the majority of the tunica mucosa. The remaining tunica submucosa, muscularis mucosa, and basilar portion of the lamina propria, now termed SIS, was then disinfected and decellularized in a 0.1% peracetic acid solution followed by two rinses each in phosphate-buffered saline and deionized water. This process yielded an acellular material, which was then lyophilized and the resulting dried material milled to produce particulate SIS with dimensions of either 2 mm × 15 mm, 850 um^2 or 250 um^2. These particulate SIS materials were combined in a ratio of 2:1:1, respectively, and vacuum pressed to form two constructs 25 mm × 12.5 mm × 3 mm and 12.5 mm × 12.5 mm × 3 mm. These constructs were then encapsulated between four layers of SIS to form a "pillow" of particulate SIS (Fig. 1). In addition a 10-layer multi-laminate of SIS 5 cm × 5cm, configured in a longitudinal fashion to maximize mechanical strength, was fabricated by vacuum pressing [24]. Terminal sterilization was accomplished by exposure to 2.5 Mrad gamma radiation.

Surgical Procedure

Approval was obtained from the University of Pittsburgh Institutional Animal Care and Use Committee. Sixteen female dogs (weight 18–25 kg), randomly divided into four groups of three based on survival time, with the remaining four animals used as no-treatment controls ($n = 1$ per time point), were used in this study. Each dog was anesthetized by intravenous administration of 2% thiopental sodium followed by intubation. Inhalant isoflurane was used to maintain a surgical plane of anesthesia. After aseptic preparation of the surgical site and injection of 40 mg/kg Cephalothin the quadriceps muscle and the associated patellar tendon were exposed. The distal third of the vastus lateralis and medial half of the distal third of the vastus medialis muscles and proximal half of the associated patellar tendon bundle were resected leaving the femur exposed. Hemorrhage was controlled with cautery. The resected tissue was replaced with the SIS construct and sutured in place with resorbable Vycril suture.

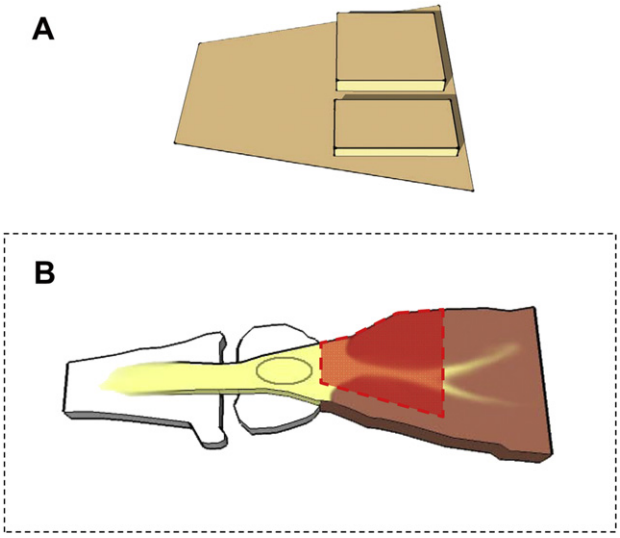

FIG. 1. Diagram of the SIS-ECM implant and surgical site. The SIS-ECM scaffold (A) was composed of a 10-layer ECM sheet (5 × 5 cm) containing two encapsulated particulate SIS-ECM constructs (one 25 × 25 × 3 mm and one 12.5 × 25 × 3 mm) at the proximal end. This was implanted into a surgical defect (B) that consisted of the distal third of the vastus lateralis and the medial half of the distal third of the vastus medialis. (Color version of figure is available online.)