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Strength and histology of a nanofiber scaffold in rats

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ABSTRACT

Background: Full-thickness soft tissue defects from congenital absence or traumatic loss are difficult to surgically manage. Healing requires cell migration, organization of an extracellular matrix, inflammation, and wound coverage. PLCL (70:30 lactide:caprolactone, Purac), poly(propylene glycol) nanofibrous scaffolds enhance cell infiltration *in vitro*. This study compares strength and tissue ingrowth of aligned and unaligned nanofibrous scaffolds to absorbable and permanent meshes. We hypothesize that PLCL nanofibrous grafts will provide strength necessary for physiological function while serving as a scaffold to guide native tissue regeneration *in vivo*.

Materials and methods: Abdominal wall defects were created in 126 rats followed by underlay implantation of Vicryl, Gore-Tex, aligned, or unaligned PLCL Nanofiber mesh. Specimens were harvested at 2, 6, and 12 wk for strength testing and 2, 12, and 24 wk for histopathologic evaluation. Specimens were graded for cellular infiltration, multinucleated giant cells (MNG), vascularity, and tissue organization. Mean scores were compared and analyzed with non-parametric testing.

Results: The PLCL grafts maintained structural integrity until at least 12 wk and exhibited substantial tissue replacement at 24 wk. At 12 wk, only the aligned PLCL had persistent cellular infiltration of the graft, whereas both aligned and unaligned PLCL grafts showed the presence of MNG. The presence of MNGs decreased in the aligned PLCL graft by 24 wk.

Conclusions: The aligned PLCL nanofiber mesh offers early strength comparable to Gore-Tex but breaks down and is replaced with cellular ingrowth creating a favorable option in management of complex surgical wounds or native soft tissue defects.

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Introduction

Full-thickness soft tissue defects arise from a variety of causes including traumatic tissue loss, congenital defects, necrotizing fasciitis, neoplasm resections, and large hernia defects. These wounds pose significant challenges in management and frequently have poor long-term outcomes. Formerly, fatal gunshot wounds, shrapnel injuries, and particularly blast injuries have become commonplace survivable injuries on the battlefield and in civilian trauma.[1-3] Severe orthopedic injuries combining soft tissue, bone, and vascular trauma are common, and their difficult wound management situations present significant challenges to trauma surgeons. Skin grafts are frequently required in these cases; any complementary therapies that could improve graft survival, speed wound healing, or reduce the incidence of side effects such as hematoma, infection, and wound dehiscence would be beneficial and potentially life changing to the patient.[4] Current management strategies include the use of artificial or biologic meshes and matrices to facilitate native tissue ingrowth.[5-7] As these measures degrade and fail to incorporate into the native tissue, a weak, disorganized scar is formed resulting in contractures, wound break down, hernias, and other potentially difficult complications. Nanofibrous scaffolds are emerging as a potential solution to challenges with wound healing and poor tissue strength as they facilitate a more organized, natural wound healing process.[8-12]

Poly(L-lactide) (PLLA) nanofibrous scaffolds (Lactel Absorbable Polymers, Pelham, AL) are designed to mimic the body's natural extracellular matrix.[8,9] PLLA mesh is fabricated using an electric spinning technology to create an artificial nanofibrous scaffold with fiber diameter <1 micron.[8,10,13-15] NanoNerve, Inc. developed a similar polymer solution composed of PLCL (70:30 lactide:caprolactone, Purac), poly(-propylene glycol) (MW 425, Sigma), and sodium acetate (Sigma) dissolved in hexafluoroisopropanol (Matrix Scientific) at 15%, 1%, and 0.05% w/v concentrations, respectively. PLCL is more elastic and flexible than PLLA, making it easier to implant and allowing it to conform to soft tissue defects. The diameter of the fibers can be controlled so as to mimic the native biologic collagen fibrils, which serve as the substrate for organized cellular migration, proliferation, and differentiation during the wound healing process. Electrospinning creates a large surface area to volume ratio which facilitates native tissue ingrowth. This electrode spinning can be adjusted to create either randomly oriented nanofibers or aligned nanofibers.[14-18] Initial modes using dermal fibroblasts on PLLA nanofibers showed that cellular migration was enhanced in the direction parallel to nanofiber orientation.[19,20] Previous studies have established nanofiber matrices as potential substrates for skin regeneration and wound healing.[21-23] Additional research has demonstrated enhanced cellular infiltration in aligned nanofibers in short-term *in vitro* and *in vivo* models when compared to unaligned nanofibers.[19] Strength, tissue ingrowth, and scar formation have not been analyzed *in vivo* models with full-thickness tissue defects for PLCL.

The objective of this study is to compare aligned and unaligned PLCL nanofiber mesh to currently available absorbable and nonabsorbable artificial meshes[24-27] in long-term strength and organization of tissue ingrowth. We postulate that the nanofiber mesh will stimulate improved tissue ingrowth with less scar formation and similar strength when compared to these other artificial meshes.

Materials and methods

At total of 126 *Rattus norvegicus* males were used as subjects for the experimentation. They were confirmed to be free of overt signs of disease to include respiratory infection, diarrhea, and external parasites. The subjects underwent induction and maintenance of anesthesia with isoflurane. They were placed supine, the ventral abdominal wall hair was clipped, chlorhexidine solution and sterile drapes were used to prepare the operative site. Preoperative and perioperative antibiotics were deferred. A 2.5-cm vertical incision was made in the midline superior to the umbilicus. Underlying soft tissue was dissected free from the rectus musculature, and tissue flaps were raised bilaterally. A 2 cm × 1 cm full-thickness fascial defect was created for mesh placement.

Four meshes were used in the abdominal wall repair. Vicryl (Ethicon, Inc. 12″ × 12″ Knitted Mesh, Polygalactin 910, undyed) is an absorbable mesh, and Gore-Tex (W.L. Gore & Associates, PRECLUDE Vessel Guard 6.0 cm × 10.0 cm × 0.3 mm) is a nonabsorbable mesh; these were chosen as controls for their current and vast use in tissue defect repair. Aligned and Unaligned Nanofiber PLCL mesh (Nanonerve, Inc. 200 micron thickness) were the experimental meshes. Electrospun membrane-shaped grafts were fabricated using a customized electrospinning setup. The electrospinning polymer solution was composed of PLCL (70:30 lactide:caprolactone, Purac), poly(propylene glycol) (MW 425, Sigma), and sodium acetate (Sigma) dissolved in hexafluoroisopropanol (Matrix Scientific) at 15%, 1%, and 0.05% w/v concentrations, respectively. The bi-layered grafts were manufactured by first electrospinning a monolayer of diametrically patterned fibers, followed by a bulk layer of randomly oriented fibers until a total thickness of 200 microns was reached (Fig. 1). The unaligned grafts were

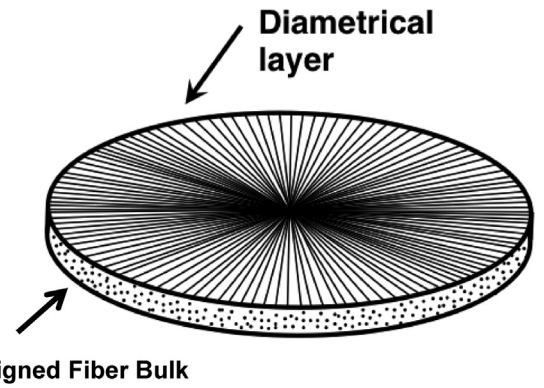

Fig. 1 – The bi-layered graft with electrospun monolayer of diametrically patterned fibers followed by a bulk layer of randomly oriented fibers for a total mesh thickness of 200 microns.