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Mitigation of hypertrophic scar contraction via an elastomeric biodegradable scaffold

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ABSTRACT

Hypertrophic scar (HSc) occurs in 40–70% of patients treated for third degree burn injuries. Current burn therapies rely upon the use of bioengineered skin equivalents (BSEs), which assist in wound healing but do not prevent HSc contraction. HSc contraction leads to formation of a fixed, inelastic skin deformity. We propose that BSEs should maintain their architecture in the wound bed throughout the remodeling phase of repair to prevent HSc contraction. In this work we study a degradable, elastomeric, randomly oriented, electrospun micro-fibrous scaffold fabricated from the copolymer poly(L-lactide-co-ε-caprolactone) (PLCL). PLCL scaffolds displayed appropriate elastomeric and tensile characteristics for implantation beneath a human skin graft. In vitro analysis using human dermal fibroblasts demonstrated that PLCL scaffolds decreased myofibroblast formation as compared to an in vitro HSc contraction model. Using a validated immune-competent murine HSc contraction model, we found that HSc contraction was significantly greater in animals treated with standard of care, Integra, as compared to those treated with collagen coated-PLCL (ccPLCL) scaffolds. Finally, wounds treated with ccPLCL were significantly less stiff than control wounds at d30 in vivo. Together, these data suggest that scaffolds which persist throughout the remodeling phase of repair may represent a clinically translatable method to prevent HSc contraction.

1. Introduction

Dermal scarring affects more than 80 million people worldwide annually [1]. For example, over 4.4 million people are injured in motor vehicle accidents, thousands of our nation's warriors are wounded in military excursions, and over 2.4 million patients are burned [2,3]. The World Health Organization states that "there is no doubt that the social and medical costs of burns are significant. Economic impact of burns includes lost wages, and the costs related to deformities from burns, in terms of emotional trauma and lost skill." In severe burns (~28,000 patients/year in the United States), the incidence of hypertrophic scar (HSc) is 40–70% [1,4]. HSc are firm, raised, red, itchy scars that are disfiguring and can have a severe impact on quality of life. 70% of HSc occur across joints or other areas of high tension in the body, resulting in a scar contractures that restrict range-of-motion [5]. Current preventative therapies for scar contracture are ineffective, and patients requiring intervention undergo at least four corrective surgeries on average [1,5].

HSc develops during the first 4–8 weeks following injury and continues to mature and contract throughout the remodeling phase of repair for as long as six months post trauma; however, commercially available bioengineered skin equivalents (BSE) degrade and are remodeled in the wound bed within 1–4 weeks [6]. There are currently three BSEs on the market which are approved for

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third degree burn treatment by the Food and Drug Agency as well as the Center for Medicare and Medicaid Services: Epicel, TransCyte, and Integra. Of these, Integra is the most widely used for treatment due to its cost and ease of use. Integra Dermal Regeneration Template (Integra LifeSciences, Plainsboro, NJ) is a bovine collagen-based lyophilized matrix covered by a silicone dressing. The biologic layer remains in the wound bed, while the silicone layer is removed prior to application of skin graft. Previous work studying collagen-based scaffolds for dermal regeneration has shown that prolonging the half-life of the material in the wound bed has a profound impact on minimizing wound contraction; however, the longest scaffold half-life tested in these studies was 2–4 weeks [7]. We hypothesize that to improve mitigation of HSc, the scaffold should be present in the wound bed throughout the remodeling phase of repair when HSc occurs.

In comparison to biologic biomaterials, synthetic biomaterials have advantages in that they possess tunable mechanical properties and biodegradation rates. In searching for a suitable synthetic elastomer for this application, we looked for favorable characteristics of mechanical strength, elasticity, and biodegradability. We selected the copolymer poly(L-lactide-co-ε-caprolactone)s (PLCL), synthesized from a 50:50 ratio of poly(lactic acid) (PLA) and poly(ε-caprolactone) (PCL) [8–12]. The FDA-approved PLA and PCL have been extensively studied for tissue engineering applications [13]. Although neither PLA nor PCL is elastomeric, PLCL displays elastomeric characteristics due to the phase separation of the crystalline PLA and the amorphous PCL segments, creating hard and soft domains somewhat akin to that observed in elastomeric polyurethanes [8–10]. Synthetic materials have additional advantages over biologics with respect to ease of handling, long shelf-life, low cost, and well-defined physicochemical properties.

Application of mechanical load has been shown to initiate HSc formation in mice [14]. Thus, it has been suggested that stress-shielding cells from transmission of mechanical load could assist in mitigating HSc [15]. Mechanical tension and inflammatory cytokines (primarily transforming growth factor-beta (TGFβ)) secreted following dermal injury cause resident fibroblasts to differentiate into myofibroblasts [16]. Myofibroblasts are distinguished from fibroblasts by (1) the presence of a contractile apparatus, similar to that of smooth muscle cells, and (2) the neo-expression of an actin isoform found in vascular smooth muscle cells, α-smooth muscle actin (αSMA) [17]. When de novo αSMA is incorporated into stress fibers, myofibroblasts produce strong contractile force and physically contract the wound or scar bed. To achieve tissue level contraction, single myofibroblasts join stress fibers at sites of adherens junctions, resulting in cytoskeletal alignment and the formation of a coordinated cellular syncytium [18]. This conglomeration allows myofibroblasts to multiply their contractile forces along the axis of cell alignment, and coincides with the alignment of the extracellular matrix (ECM). This is observed in scar tissue as linear arrays of ECM, as compared to randomly oriented ECM in uninjured skin.

To encourage random cell alignment, and hence disordered ECM deposition, we electrospun PLCL into a randomly oriented microfibrous scaffold [19–21]. We first studied the behavior of human dermal fibroblasts on this scaffold using the fibroblast populated collagen lattice (FPCL) assay (22), which has been used to model HSc contraction [16,22,23]. This was followed by in vivo evaluation using a murine HSc contraction model recently established in our laboratory; our model is the only immune-competent murine model that mirrors the human condition in terms of causality [24]. Together our in vitro and in vivo data suggest that slowly degrading electrospun scaffolds prevent myofibroblast activation associated with HSc, and provide the necessary longevity to prevent HSc contraction in vivo.

2. Materials and methods

2.1. Synthesis of PLCL

PLCL (50% LA, 50% CL) was synthesized as described elsewhere [8]. Briefly, L-lactide (100 mmol; Purac; Lincolnshire, IL, USA) and ε-caprolactone (100 mmol; Sigma; St. Louis, MO, USA) were polymerized at 150 °C for 24 h in the presence of stannous octoate (1 mmol, Sigma) as a catalyst. After being dissolved in chloroform, the polymer was precipitated in methanol, then dried under a vacuum for 72 h and stored in vacuum pack at −20 °C.

2.2. Fabrication & analysis of electrospun PLCL

PLCL scaffolds were fabricated using continuous single fiber electrospinning to deposit a 3D matrix of fibers on a rotating grounded mandrel using a custom spinning apparatus. PLCL was dissolved 14% (w/w) overnight in dichloromethane. Random fibers were spun at a flow rate of 3 ml/h with a voltage of 8 kV at a distance of 13 cm from the mandrel, which was rotating at ~70 revolutions per minute. Ambient temperature was 22 °C with 43% humidity. Following spinning, fibers were removed from the mandrel and residual solvent was removed by air drying for 72 h. Fiber characteristics and scaffold thickness were analyzed using scanning electron microscopy (FEI XL30 SEM-FEG, Hillsboro, OR, USA).

2.3. Oxygen plasma treatment & collagen coating methods

Samples were placed inside of a plasma asher (Emitech K-1050X, Montigny-le-Bretonneu, France) and treated with reactive oxygen plasma for 45 s at 100 W to improve hydrophilicity prior to cell culture and prepare for covalent collagen coating. Following treatment, samples were immediately immersed in sterile water and subsequently sterilized in 70% ethanol for 20 min. Samples were rinsed thoroughly with water following sterilization. Covalent collagen coating was performed by EDC/NHS chemistry as previously described [25]. This well-characterized method is biocompatible, non-cytotoxic, and does not include a linker-arm. Carbodiimide is not incorporated into the covalent-linkage, allowing the collagen to directly coat scaffold. This method does not modify scaffold morphology and generates a uniform collagen coating covering fibers throughout the depth of the scaffold. Scaffolds in collagen coated PLCL (ccPLCL) groups were covalently coated with bovine type-1 collagen (Nutragen, Advanced Biomatrix, San Diego, CA, USA) prior to in vivo implantation. Contact angle analysis before oxygen plasma treatment, after oxygen plasma treatment, and after collagen coating treatment was carried out on PLCL films using a goniometer as previously described [26].

2.4. Permeability measurement

In order to examine the impact of collagen coating on scaffold permeability, effective hydraulic permeability of PLCL and ccPLCL scaffolds was measured according to ASTM protocol F2952 using a custom-built flowmeter as previously described [27]. In brief, a "scaffold sandwich" was constructed between two silicone gaskets using a 100 μm scaffold and a fine stainless steel mesh. The "sandwich" was placed between mount pieces and a watertight seal was formed by applying light pressure using a threaded screw housing unit. A 50 mL pipette was suspended horizontally 32 cm above the flow chamber and connected to the specimen mount using tubing. Phosphate buffered saline (PBS) fluid flow was equilibrated for 15 min before timing, and was measured at 1 mL intervals for the first 5 mL, then 5 mL intervals for the next 10 mL in order to ensure consistent flow. The total duration of flow (15 mL) was measured and used to obtain the Darcy coefficient, τ, and average pore diameters as previously described [27].

2.5. Static tensile testing of electrospun scaffolds as compared to skin and scar tissues

Static tensile testing was carried out as described in ASTM D3822-07. Human and murine skin and scar tissues were tested as previously described [24]. In breif, scar and uninjured skin samples were gathered from human and murine donors. Uninjured murine tissue samples were collected from the dorsum of 10–12 week-old C57BL/6 mice. Murine scar tissue was taken from contracted d30 skin grafted mice. Tissue from five murine donors was used, with three biological replicates per donor. Human skin samples were donated from discarded human tissue from Duke Hospital operating rooms under exemption by Duke institutional review board. Uninjured human skin was gathered from breast resection, while scar tissue was taken from excised keloid, radiated forearm, and rejected skin graft. Tissue from three human donors was used with 3–5 biological replicates per donor. All human and murine tissues were kept moist on damp gauze between collection and mechanical testing, and analyzed within 1–5 h of collection. Prior to testing, underlying tissue was removed and samples were cut into uniform strips. PLCL, ccPLCL, Integra, and tissue samples were cut to 5 cm × 5 mm strips using a scalpel and surgical scissors and loaded with a 5 mm gap between clamps. Samples were analyzed on a microstrain analyzer (MSA) (RSA II, TA Instruments, New Castle, DE, USA) at a rate of 0.1 mm/s at room temperature (23 °C) until failure. The initial elastic modulus (within the first 0–200% strain) was analyzed for each sample. The lower elastic modulus was selected for analysis because this strain range best mimics strains that