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Biomimetic collagen/elastin meshes for ventral hernia repair in a rat model

Silvia Minardi [a,b], Francesca Taraballi [a], Xin Wang [a], Fernando J. Cabrera [a], Jeffrey L. Van Eps [a], Andrew B. Robbins [c], Monica Sandri [b], Michael R. Moreno [a,c,d,e], Bradley K. Weiner [a,e], Ennio Tasciotti [a,e,*]

[a] Center for Biomimetic Medicine, Houston Methodist Research Institute (HMRI), 6670 Bertner Ave., Houston, TX 77030, USA
[b] National Research Council of Italy – Institute of Science and Technology for Ceramics (ISTEC-CNR), Via Granarolo 64, 48018 Faenza, RA, Italy
[c] Department of Biomedical Engineering, Texas A&M University (TAMU), 401 Joe Routt Blvd, College Station, TX 77843, USA
[d] Department of Mechanical Engineering, Texas A&M University (TAMU), 3123 TAMU, College Station, TX 77843, USA
[e] Department of Orthopedics, Houston Methodist Hospital, 6565 Fannin Street, Houston, TX 77030, USA

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ABSTRACT

Ventral hernia repair remains a major clinical need. Herein, we formulated a type I collagen/elastin cross-linked blend (CollE) for the fabrication of biomimetic meshes for ventral hernia repair. To evaluate the effect of architecture on the performance of the implants, CollE was formulated both as flat sheets (CollE Sheets) and porous scaffolds (CollE Scaffolds). The morphology, hydrophylicity and *in vitro* degradation were assessed by SEM, water contact angle and differential scanning calorimetry, respectively. The stiffness of the meshes was determined using a constant stretch rate uniaxial tensile test, and compared to that of native tissue. CollE Sheets and Scaffolds were tested *in vitro* with human bone marrow-derived mesenchymal stem cells (h-BM-MSC), and finally implanted in a rat ventral hernia model. Neovascularization and tissue regeneration within the implants was evaluated at 6 weeks, by histology, immunofluorescence, and q-PCR. It was found that CollE Sheets and Scaffolds were not only biomechanically sturdy enough to provide immediate repair of the hernia defect, but also promoted tissue restoration in only 6 weeks. In fact, the presence of elastin enhanced the neovascularization in both sheets and scaffolds. Overall, CollE Scaffolds displayed mechanical properties more closely resembling those of native tissue, and induced higher gene expression of the entire marker genes tested, associated with *de novo* matrix deposition, angiogenesis, adipogenesis and skeletal muscles, compared to CollE Sheets. Altogether, this data suggests that the improved mechanical properties and bioactivity of CollE Sheets and Scaffolds make them valuable candidates for applications of ventral hernia repair.

Statement of Significance

Due to the elevated annual number of ventral hernia repair in the US, the lack of successful grafts, the design of innovative biomimetic meshes has become a prime focus in tissue engineering, to promote the repair of the abdominal wall, avoid recurrence. Our meshes (CollE Sheets and Scaffolds) not only showed promising mechanical performance, but also allowed for an efficient neovascularization, resulting in new adipose and muscle tissue formation within the implant, in only 6 weeks. In addition, our meshes allowed for the use of the same surgical procedure utilized in clinical practice, with the commercially available grafts. This study represents a significant step in the design of bioactive acellular off-the-shelf biomimetic meshes for ventral hernia repair.

1. Introduction

Ventral hernia is a bulge through an opening in the abdominal muscles [1]. Ventral hernias are currently repaired through the surgical implantation of a multiplicity of grafts used to patch the defect in the abdominal wall [2]. Grafts for ventral hernia repair

* Corresponding author at: Center for Biomimetic Medicine, Houston Methodist Research Institute (HMRI), 6670 Bertner Ave. Suite R10-116, Houston, TX 77030, USA.
E-mail address: etasciotti@houstonmethodist.org (E. Tasciotti).

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remain a major clinical need in the United States, with over 350,000 cases annually, for an approximately $1 Billion market [3,4]. As 2–20% of hernias becomes chronic, these surgeries are one of the most expensive of the United States [5]. Currently, two categories of meshes are used in clinical practice: permanent and resorbable. Since their introduction, non-resorbable synthetic meshes became the standard of care for abdominal wall repair (e.g. Marlex®, Goretex®, Prolene®) [6]. However, their poor biodegradability is associated with several complications, including chronic inflammation, infection, adhesion, extrusion, chronic pain and recurrence [4,7–9], making surgeons reluctant to implant non-resorbable prostheses in complex hernias. More recently, the use of decellularized matrices (e.g. AlloDerm®, Permacol®, Veritas®) represented an effort towards the development of biomimetic grafts, characterized by higher biocompatibility and improved *in vivo* performances [6,10]. To some extent, biologically-derived grafts demonstrated advantageous, by triggering a lower inflammatory response and fewer infections, than synthetic meshes [11,12]. Recent evidence showed that their major drawback remains the low bioactivity, which causes poor vascularization and uneven cellularization of the implant itself, ultimately resulting in a lack of integration to the surrounding tissues and tissue regeneration [13,14]. Thus, these prosthetics have proved useful in proving immediate repair of abdominal wall defects, but are hindered by poor long-term mechanical strength, due to their uncontrolled biological activity and biodegradation mechanisms [15].

Following implantation, a graft undergoes one of the following fates: (i) neovascularization and new ECM deposition; (ii) fibrotic encapsulation; (iii) resorption/degradation [16]. Thus, the ideal graft should be conducive for early neo-vascularization, fully biocompatible and biomimetic to promote a regenerative immunological response, rather than inflammation that could cause fibrotic encapsulation [17,18]. Furthermore, it should allow for the recruitment of stem cells and adult tissue-specific cells to ultimately recover tissue function [19]. Towards this end, biologically inspired approaches to the design of biomimetic meshes for abdominal wall reconstruction are currently been investigated [20,21].

Based on this evidence, we addressed this clinical need using the overarching principles of biomimicry to create materials that better resemble the composition, architecture, and mechanical properties of the target tissue. Collagen and elastin are the most abundant components of the extracellular matrix (ECM) of almost every tissue in the body, that provides essential cues for cell attachment, migration and organization [22]. Collagen is essential in maintaining tissue architecture, while elastin provides resilience and deformability to tissues. The properties of elastin are critical to specific tissue functions (e.g. dermis, vessels, muscles) [23]. Furthermore, several evidences suggest a role of soluble elastin in favoring tissue regeneration by promoting angiogenesis [24,25]. Collagen and elastin are also the two main components of the ECM of the abdominal wall [26], and changes in their ratio or metabolism have been associated with hernia occurrence [27,28]. It is established that the pathological changes in the collagen of the abdominal wall set the stage for the development of hernias [29]. Thus, we have formulated a type I collagen/elastin crosslinked blend (CollE) for the fabrication of meshes with enhanced mechanical properties and bioactivity, to improve hernia repair. The porosity of biomaterials also plays a crucial role in their interaction with cells and newly formed tissues [30]. For this reason, the CollE was molded in two different architectures: as flat sheets (CollE Sheets) and porous scaffolds (CollE Scaffolds), to evaluate the effect of porosity on the performance of CollE, in a rat ventral hernia model. Collagen type I sheets and scaffolds were used as controls (Coll Sheet, Coll Scaffold).

2. Materials and methods

2.1. Fabrication of collagen and collagen/elastin Sheets and Scaffolds

For the fabrication of the collagen sheets (Coll Sheets) and the porous collagen scaffolds (Coll Scaffolds) a 1 g of type I collagen (Nitta Casings Inc.) was dissolved in an acetate buffer (pH 3.5) to reach the desired concentration (20 mg/mL). The collagen suspension was precipitated by the addition of sodium hydroxide (0.1 M) solution at pH 5.5. The collagen was washed three times with DI water. The resulting collagen slurry was cross-linked through incubation in a 1,4-butanediol diglycidyl ether (BDDGE) (Sigma-Aldrich) aqueous solution (2.5 mM) for 48 h, setting up the BDDGE/collagen ratio to 1 wt%, as previously optimized [31]. The cross-linked collagen was washed 3 times in DI water. To fabricate Coll Sheet, the slurry was molded in metal racks, at a thickness of 5 mm and air dried under a fume hood for 5 days (final thickness: 0.3 mm). On the contrary, to fabricate the Coll Scaffolds, the slurry was molded into metal racks, at a thickness of 5 mm thick and freeze dried. For the fabrication of the collagen/elastin sheets (CollE Sheets) and scaffolds (CollE Scaffolds) the same procedure to prepare the collagen slurry was followed. Then, the elastin was added (10 wt%) to the collagen slurry, and blended. The resulting collagen/elastin slurry was cross-linked as previously described. After the washings, the collagen/elastin slurry was casted as previously described, to fabricated either the CollE Sheets or CollE Scaffolds.

2.2. Scanning electron microscopy

The morphology of sheets and scaffolds was evaluated by scanning electron microscopy (SEM) (Quanta 600 FEG, FEI Company, Hillsboro, OR). Freeze dried samples were sputter coated with 10 nm of Pt/Pd and imaged at a voltage of 10 kV. Also, scaffolds seeded with h-BM-MSC were images after 1 and 3 weeks in culture. Rat ventral fascia was also imaged. Prior to SEM imaging samples were dehydrated according to an established protocol for the preparation of fresh tissues for SEM imaging, as reported elsewhere [32].

2.3. Contact angle

The contact angle measurements were conducted using a Ramé-Hart 200-F1 goniometer. The contact angles were measured by dropping a 2 μL water drop onto the surface of Coll Sheets, Coll Scaffolds, CollE Sheets and CollE Scaffolds, using a microsyringe (Gilmont). At least six measurements were performed on each surface (n = 5). The errors in contact angle were ±1°.

2.4. Pore size and porosity

The volumes of the scaffolds (cylinders of 0.5 cm × 0.1 cm) were (V_s) were calculated from their geometry. The mean size of the pores was measured from the SEM images of the scaffolds with the software ImageJ (NIH). 20 measurements were acquired per image. The overall porosity of the scaffolds was calculated as described elsewhere, through an ethanol infiltration method [31]. Values are expressed as means ± standard deviation (n = 3).

2.5. Swelling and in vitro degradation

The swelling properties (PBS uptake) of the scaffolds were determined as previously described [31]. Briefly, scaffolds were weighted after lyophilization (dry), and after incubation in PBS at 37 °C, at different time points. The uptake ratio was defined as%