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Embroidered polymer–collagen hybrid scaffold variants for ligament tissue engineering

M. Hoyer [a,b], N. Drechsel [c], M. Meyer [c], C. Meier [a], C. Hinüber [d,e], A. Breier [d], J. Hahner [d,e], G. Heinrich [d,e], C. Rentsch [f], L.-A. Garbe [b], W. Ertel [a], G. Schulze-Tanzil [a,*,1], A. Lohan [a,1]

[a] Department of Orthopaedic, Trauma and Reconstructive Surgery, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Garystrasse 5, 14195 Berlin, Germany
[b] Department of Bioanalytics, Technische Universität Berlin Seestr. 13, 13353 Berlin, Germany
[c] Research Institute of Leather and Plastic Sheeting-FILK, Meißner Ring 1–5, 09599 Freiberg, Germany
[d] Department of Materials and Processing, Leibniz-Institut für Polymerforschung Dresden e.V., Hohe Strasse 6, 01069 Dresden, Germany
[e] Institute of Material Science, Technische Universität Dresden Helmholtzstraße 10, 01062 Dresden, Germany
[f] Department of Trauma and Reconstructive Surgery, University Hospital Carl Gustav Carus, Fetscherstrasse 74, 01307 Dresden, Germany

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ABSTRACT

Embroidery techniques and patterns used for scaffold production allow the adaption of biomechanical scaffold properties. The integration of collagen into embroidered polylactide-co-caprolactone [P(LA-CL)] and polydioxanone (PDS) scaffolds could stimulate neo-tissue formation by anterior cruciate ligament (ACL) cells. Therefore, the aim of this study was to test embroidered P(LA-CL) and PDS scaffolds as hybrid scaffolds in combination with collagen hydrogel, sponge or foam for ligament tissue engineering.
ACL cells were cultured on embroidered P(LA-CL) and PDS scaffolds without or with collagen supplementation. Cell adherence, vitality, morphology and ECM synthesis were analyzed. Irrespective of thread size, ACL cells seeded on P(LA-CL) scaffolds without collagen adhered and spread over the threads, whereas the cells formed clusters on PDS and larger areas remained cell-free. Using the collagen hydrogel, the scaffold colonization was limited by the gel instability. The collagen sponge layers integrated into the scaffolds were hardly penetrated by the cells. Collagen foams increased scaffold colonization in P(LA-CL) but did not facilitate direct cell-thread contacts in the PDS scaffolds. The results suggest embroidered P(LA-CL) scaffolds as a more promising basis for tissue engineering an ACL substitute than PDS due to superior cell attachment. Supplementation with a collagen foam presents a promising functionalization strategy.

1. Introduction

The anterior cruciate ligament (ACL) rupture is the most frequent ligament injury of the knee joint [1], but the ACL is not able to self-regenerate [2,3]. The ruptured tissue is usually surgically reconstructed to restore joint stability [4]. Ligament reconstruction based on autologous tendons such as *Musculus (M.) semitendinosus, M. gracilis* or patellar tendons is associated with donor site morbidity [5] and restricted by autograft availability. Rathbone et al. showed that many surgeons would prefer a tissue engineering based ACL substitute compared with actual standard therapies for the case that the construct meets the biomechanical requirements of the ACL [6].

A biomaterial suitable for ACL reconstruction should possess properties such as high biocompatibility, suitable biomechanics and durability. Both synthetic materials used in this study, a copolymer from 70% polylactide and 30% polycaprolactone (polylactide-co-caprolactone: P[LA-CL]) and polydioxanone (PDS) are in clinical use as biocompatible, slow degrading suture materials [7–10] and have a suitable flexibility needed for the embroidery process. Both polymers are aliphatic polyesters that are degradable by hydrolysis [11] and are therefore suitable for ligament tissue engineering [7,12–14]. Two dimensional substrates of P(LA-CL) manufactured in different ratios were already tested with lapine ACL cells and revealed a biocompatibility comparable with commercially available cell culture plastic [12,15]. PDS has been successfully implanted in large tendon defects in rabbits [16] and could therefore also be promising for the ACL tissue engineering.

Embroidery techniques allow the design of appropriate biomechanical properties of the scaffolds. Adaption of the embroidery patterns can

Abbreviations: ACL, anterior cruciate ligament; DAPI, 4',6-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; DMMB, dimethyl methylene blue; ECM, extracellular matrix; EtBr, ethidium bromide; FCS, fetal calf serum; FDA, fluorescein diacetate; HE, hematoxylin and eosin staining; HMDS, hexamethyldisilazane; P(LA-CL), polylactide-co-caprolactone; PBS, phosphate buffered saline; PDS, polydioxanone; PFA, paraformaldehyde; PVA, polyvinyl alcohol; SEM, scanning electron microscopy; RT, room temperature; sGAG, sulfated glycosaminoglycan; TBS, TRIS buffered saline.

* Corresponding author at: Dep. of Orthopaedic, Trauma and Reconstructive Surgery, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, FEM, Garystrasse 5, 14195 Berlin, Germany. Tel.: +49 30 450 552385; fax: +49 30 450 552985.
E-mail address: gundula.schulze-tanzil@charite.de (G. Schulze-Tanzil).
[1] Joined senior authorship.

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modulate the shape, pore sizes, stiffness and elasticity of the scaffolds [17]. Here mechanical requirements of the ACL are mimicked by the zig-zag embroidery pattern along the longitudinal axis so that the meshes can be extended during tension. In spite of the favorable properties of embroideries, to our knowledge this is the first study using embroidered hybrid scaffolds for ligament tissue engineering with primary ligament cells. Since the biomechanics of native ligaments depend on the parallel aligning of the collagen fibril bundles along the longitudinal axis of the tissue [12] and a wave-like extracellular matrix (ECM) texture in relaxed ligaments due to the presence of elastic fibers, a unidirectional zig-zag embroidery pattern of the scaffolds was selected for the present study. Also wrapping of the collagen fiber bundles by the ligament cells observed in ligaments and tendons could be mimicked by the ACL cells growing along the longitudinal threads [12].

With the aim to create a preformed tissue for the ACL reconstruction, the adherence of cells to the synthetic polymers P(LA-CL) and PDS has to be improved using functionalization techniques [18]. The embroidered structures were combined with collagen materials manufactured according to different preparation techniques. Collagen is the main component of connective tissue. The protein chains are twisted into triple helices, the latter in tissues being structured in microfibrils, fibers and fiber bundles [19]. Weak immunogenic reactions are observed, both humoral and the cell mediated, depending on the donor and the recipient species and reactions against antigens located in the triple helical as well as the telopetidal region [20]. For the clinical use triple helical collagen molecules may be assessed as immunocompatible [9,21] and the use of collagen may improve the biocompatibility of other implant materials. Furthermore, collagen is compatible with synthetic polymers [9] and the combination of both will result in hybrid-implants [22], which are more stable than solely collagen-based scaffolds [12,23]. It is assumed that the combination of synthetic polymers in hybrid scaffolds with the biopolymer collagen, which is the main ECM component in ligaments, could be a suitable strategy to improve cell distribution and growth within the scaffold [22], without affecting polymer chemistry and thereby stability or biomechanics.

Therefore, this study was executed to analyze whether P(LA-CL) or PDS is a suitable material to prepare embroidered scaffolds to be colonized by lapine ACL cells. Furthermore, strategies were developed to improve cell retention within the textile scaffolds using different collagen supplementations. The pore size of the embroidered structures shows a bottom limit, because with too low numbers of meshes the structure gets stiffer than desired to mimic ACL properties. To enhance biocompatibility of the synthetic material and to achieve a supporting biocompatible structure between the meshes the embroidered structures can be combined with collagen sponges, foams and hydrogel.

In this study a commercially available chicken collagen gel was used as reference certified for establishing Threedimensional (3D) cultures for tissue engineering purposes, while bovine soluble collagen, bovine non soluble collagen (dispersion) and sponge from bovine collagen were prepared on our own according to medical device legislation. The bovine collagens were not treated with proteases. Therefore this collagen still contained telopeptides whereas the chicken collagen was described as atelomeric collagen by the supplier.

In a first step thin films of P(LA-CL) and PDS, prepared by spin coating were further covered with fibrillated collagen to analyze the benefit of collagen in combination with P(LA-CL) and PDS. In a second step embroidered scaffolds from the synthetic polymers were combined with a collagen hydrogel for a homogeneous cell distribution, or the scaffolds were directly embroidered on sheets (plies) of a collagen sponge that should absorb the cell suspension and help to over span the embroidery pores. Finally, the scaffolds could also be impregnated using collagen foam that should increase the cell adherence and also initially scale down the scaffolds pore diameter.

2. Materials and methods

2.1. Preparation of P(LA-CL) and PDS embroidered scaffolds and thin films

2.1.1. Embroidered P(LA-CL) and PDS scaffolds

Textile scaffolds were embroidered on a JCZ 0209-550 embroidery machine (ZSK, Germany) from surgical suture threads of P(LA-CL) (Gunze Ltd., Japan) and PDS (Samyang Biopharmaceuticals Corp., Korea) (thread diameters: 125 μm corresponds to the USP 6-0 [6-0] and 85 μm to the USP 7-0 [7-0], provided by the company Catgut, Germany). Threads were processed on plies of water soluble polyvinyl alcohol (PVA [Veline 581 white, Freudenberg Einlagestoffe, Germany]) as described elsewhere [24]. The base material was washed out after the embroidery process in several rinsing steps with aqua dest. All scaffolds were designed in a zig-zag stitch embroidery pattern with a stitch length of 1.5 mm and a stitch angle of 30° (Fig. 1). The height of the scaffolds was obtained by embroidering either three layers (6-0) or seven layers (7-0) one upon the other. All scaffolds were sterilized using ethylene oxide as proposed by Ray et al. [14].

Tensile testing of used monofilaments was executed following DIN EN 13895:2003 using a Zwick/Roell UPM 2.5 tensile testing machine (parameters: 15 mm clamping length, 50 mm/min testing velocity, rubberized mechanical clamps, 0.2 N minor load). Embroidered scaffolds were initially tested for proper biomechanics compared to native ACL references and were classified as suitable for ACL reconstruction in the rabbit (results not published yet).

2.1.2. Spin coating

P[LA-CL] and PDS films were produced by spin coating (RC5; Suess Microtec, Germany). The suture materials P(LA-CL) and PDS were dissolved in chloroform (>99%, Merck, Germany) and 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma-Aldrich, Germany), respectively. Coverslips (d = 15 mm) were freshly cleaned with ethanol (J.T. Baker, Netherlands) for 30 min in ultrasonic bath and thereafter, to achieve adherent coating, the surface was modified with tridecafluoro-1,1,2,2-tetrahyrdoctyl (trichlorosilane 97%, AB 111 444, ABCR, Germany) in gaseous phase, a procedure called silanization. Then, the surface was covered with 0.5% polymer solution. Spin coating was conducted at 3000 rpm for 30 s. The thickness of the polymer thin films was determined by ellipsometry (VASE 44 M; Woolam, USA); the contact angle was measured by means of dynamic contact angle measurement (OCA 30, dataphysics, Germany).

2.1.3. Bovine soluble collagen

Soluble collagen was extracted from calf skin, which was obtained from a local abattoir. The extraction method was described in detail by [25]. In short, skin was shaved, cut and intensively washed and immersed into 0.1 M acetic acid (Carl Roth, Germany) under intermittently stirring for three days without using enzymes. This acidic solved collagen was then precipitated by adding solid sodium chloride (Carl Roth) up to a total concentration of 5%. The precipitate was centrifuged, dialyzed against deionized water and freeze-dried. This dried collagen was then re-dissolved in 0.01 M hydrochloric acid (Carl Roth) at 4 °C (2 mg/ml). For coating 50 μl TRIS-buffer (50 mM Tris [Sigma-Aldrich], 150 mM NaCl [Carl Roth], pH 9) was placed at the surface of the thin film. 50 μl collagen solution was injected into the buffer; the final pH of this mixture was 8.9. Fibrillation started between some seconds and some minutes and a thin layer of collagen fibrils adsorbed at the thin film surface. The samples were allowed to incubate at 4 °C for 24 h – these conditions were found to be appropriate to generate fibrils – before being rinsed with deionized water. Finally, the thin films covered with this fibrillated collagen were dried at room temperature (RT).