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Toward reconstruction of the subcutaneous fat layer with the use of adipose-derived stromal cell–seeded collagen matrices

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Abstract

Background aims. Complex injuries of the upper and lower extremities often result in scarring and subsequent adhesion formation, which may cause severe pain and distinctly reduce range of motion. In revision surgery, replacement of the missing subcutaneous tissue is desirable to prevent new adhesions, to cushion scarred tendons and nerves and to regain tissue elasticity. Therefore, the objective of this study was the *in vitro* evaluation of cell-seeded collagen matrices to serve as the basis for the reconstruction of the subcutaneous adipose tissue layer. *Methods.* Five commercially available acellular dermal collagen matrices were seeded with human adipose-derived stromal cells (hASC). Size and shape stability of cell-matrix constructs were assessed and cell adhesion onto the matrix surface was evaluated histologically. Adipogenic differentiation of hASC on matrices was evaluated by means of histological staining, triglyceride quantification, and quantitative real-time polymerase chain reaction gene expression analysis. *Results.* The collagen matrix Permacol was the only cell-seeded material that exhibited excellent size and shape stability. For Permacol and Strattice, successful seeding with continuous cell layers on top of the matrices was observed. For both matrices, histological staining, triglyceride quantification and messenger RNA expression of adipogenic transcription factors indicated substantial adipogenic differentiation of hASC after long-term induction as well as after short-term induction of only 4 days. *Conclusions.* Of all matrices investigated, only Permacol exhibited adequate handling stability and the development of a thin adipose tissue layer on top of the matrix. Thus, this matrix appears promising to be used in the development of a subcutaneous cushioning layer after complex injuries involving large scar formation.

Key Words: *adipogenesis, adipose-derived stromal cells, collagen matrix, scar revision surgery, subcutaneous fat layer*

Introduction

Complex injuries of the upper and lower extremities, in many cases, lead to excessive scarring. Because of severe damage to the subcutaneous fat layer, a common sequela is adhesion formation to mobile structures such as tendons, nerves and blood vessels resulting in restricted motion and disabling pain [1,2]. Thus, in revision surgery of such injuries, adequate reconstruction of the subcutaneous fat layer would be desirable not only to add volume and prevent new adhesions but also to cushion scarred tendons, nerves and blood vessels and, thus, regain tissue elasticity. Current strategies for severe full-thickness wounds are autologous free tissue transfer, composite tissue flaps or artificial replacements [3,4]. The drawbacks of these methods are donor site morbidity, complex and cost-intensive surgical procedures as well as the rejection and shrinking of autologous and artificial materials [5,6].

From a surgical point of view, to date, there is no adequate matrix or tissue available for the reconstruction of the subcutaneous fat layer, especially in atrophic and marginally perfused scar tissue, in which microsurgical tissue transfer may not be an ideal option. An attractive treatment to overcome the current limitations would be the insertion of a laminar matrix seeded with mesenchymal stromal cells that give rise to a thin fat layer on top of the matrix. Naturally derived decellularized collagen materials have shown great promise in a range of clinical applications, mainly in abdominal wall and breast reconstruction and in wound repair. They are characterized by a superior biocompatibility and have been shown to have regenerative potential in terms of

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revascularization, recellularization and providing tissue support in these applications [7–9]. With regard to tissue engineering approaches, different types of cells have been proven to adhere and proliferate on these scaffolds [10–12]. A readily available autologous source for mesenchymal stromal cells is adipose tissue. Adipose-derived stromal cells (ASC), which easily can be obtained from tissue harvested by means of liposuction procedure, are immature precursor cells and have been shown to be more resistant to mechanical damage and ischemic conditions than are mature adipocytes. They also proliferate rapidly and differentiate into mature adipocytes both *in vitro* and *in vivo* [13,14]. The combination of these cells with a scaffold matrix as cell carrier, as compared with cell application alone, allows for a better dosing, control of location as well as a better determination of cell survival, extracellular matrix generation and host integration [15].

Therefore, with the use of ASC and collagen matrices, the overall goal of this study was to develop a tissue-engineered basis for the generation of a thin adipose tissue layer for the reconstruction of the subcutaneous tissue, which in future applications can deliver functional as well as aesthetic results. Specifically, 5 different commercially available collagen matrices were evaluated *in vitro*. Handling stability of the matrices in cell seeding and culture was assessed. Furthermore, cell adherence to the matrices and adipogenic differentiation of the seeded ASC assessed by triglyceride synthesis and adipogenic gene expression were investigated.

Methods

Collagen matrices

Five commercially available acellular dermal collagen matrices were investigated in this study. Three matrices are of porcine origin: Strattice was obtained from LifeCell Corp (Branchburg, NJ, USA) and Permacol and OptiMaix were kindly provided by Covidien (Norwalk, CT, USA) and Matricel (Herzogenrath, Germany), respectively. Two matrices are of bovine origin: Hypro-Sorb was obtained from Hypro Otrokovice (Otrokovice, Czech Republic) and Collagen Cell Carrier was kindly provided by Viscofan BioEngineering (Weinheim, Germany).

Isolation and expansion of human ASC

Human ASC were isolated from subcutaneous adipose tissue obtained in liposuction procedures of 3 healthy female donors. Patients were between 35 and 55 years of age and had a body mass index ranging from 25 to 30. The study was approved by the ethics committee of the University of Wuerzburg, Germany, and written informed consent was obtained from all patients.

In brief, the fat tissue was digested with 0.1% collagenase NB4 (Serva Electrophoresis, Heidelberg, Germany) for 2 h at 37°C on an orbital shaker. The resulting suspension was filtered through 100-µm mesh and centrifuged at 300*g* for 10 min. Floating fat was aspirated and pelleted cells were washed with phosphate-buffered saline and again filtered through a 100-µm mesh. The cells were resuspended in basal medium containing Dulbecco's modified Eagle's medium/F12 medium 1:1 (Invitrogen, Karlsruhe, Germany) supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (both from Invitrogen), seeded in tissue culture flasks and cultured in a humidified atmosphere at 37°C and 5% CO_2. ASC were expanded under these conditions and used for this study in passage 3.

Culture and adipogenic differentiation on collagen matrices

All collagen matrices were cut with the use of a biopsy punch to obtain discs with a diameter of 5 mm. Matrices were pre-wetted in pre-adipocyte growth medium (consisting of PBM-2 (Lonza, Walkersville, MD, USA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin) and subsequently seeded on top with a cell number of 300,000 hASC per disc in pre-adipocyte growth medium, and size and shape stability of the matrices were assessed during 14 days of culture.

To investigate adipogenesis of hASC, cells were seeded on collagen matrices in pre-adipocyte growth medium. After 24 h of cultivation, cell-matrix constructs were either incubated in adipogenic differentiation medium (growth medium supplemented with 1.7 µmol/L insulin [kindly provided by Sanofi-Aventis, Frankfurt, Germany], 1 µmol/L dexamethasone, 200 µmol/L indomethacin [both from Sigma-Aldrich, Steinheim, Germany] and 500 µmol/L 3-isobutyl-1-methylxanthine [Serva, Heidelberg, Germany]) or in growth medium for not-induced control. Constructs were cultured for up to 14 days in 24-well plates, and culture medium was exchanged every 2 to 3 days.

In a further experiment, to assess feasibility of a short-term induction, Permacol matrix was seeded with 300,000 cells/disc and incubated for 24 h in growth medium as described above. Subsequently, cells were differentiated according to different induction protocols. A positive control group received differentiation medium throughout the entire culture (14 days). For short-term induction, cell-seeded constructs were incubated in differentiation medium only for 2 or 4 days