Journal of Plastic, Reconstructive & Aesthetic Surgery (2017) xx, 1–11

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

Implant for autologous soft tissue reconstruction using an adipose-derived stem cell-colonized alginate scaffold

Tobias Hirsch [a], Christine Laemmle [a], Bjoern Behr [a], Marcus Lehnhardt [a], Frank Jacobsen [a], Dirk Hoefer [b,*], Maximilian Kueckelhaus [a]

[a] *Department of Plastic Surgery, BG University Hospital Bergmannsheil, Ruhr University Bochum, Bochum, Germany*
[b] *Department of Hygiene, Environment and Medicine, Hohenstein Institutes, Boennigheim, Germany*

Received 31 January 2017; accepted 6 August 2017

KEYWORDS
Adipose-derived stem cells;
ASCs;
Scaffold;
Implant;
Alginate

Summary *Background:* Adipose-derived stem cells represent an interesting option for soft tissue replacement as they are easy to procure and can generate their own blood supply through the production of angiogenic factors. We seeded adipose-derived stem cells on a bioresorbable, biocompatible polymer alginate scaffold to generate autologous soft tissue constructs for repair.
Materials and methods: We built and optimized an alginate scaffold and tested its biocompatibility using the MTT assay and its hydration capacity. We then isolated, characterized, and differentiated murine, porcine, and human adipose-derived stem cells. We characterized their angiogenic potential *in vitro* by VEGF ELISA and HUVEC tube formation assay in traditional cell culture substrate and in the actual three-dimensional scaffold. We assessed the angiogenic potential of adipose-derived stem cell-colonized scaffolds *in ovo* by chorion allantois membrane angiogenesis assay.
Results: Adipose-derived stem cells differentiated into adipocytes within the alginate scaffolds and demonstrated angiogenic activity. VEGF secretion by adipose-derived stem cells decreased significantly over the 21-day course of adipocyte differentiation in traditional cell culture substrate, but not in scaffolds. Adipose-derived stem cells differentiated for 21 days in scaffolds led to the longest HUVEC tube formation. Scaffolds colonized with adipose-derived stem cells resulted in significantly improved vascularization *in ovo*.
Conclusions: We demonstrate the feasibility of implant production based on adipose-derived stem cell-colonized alginate scaffolds. The implants demonstrate biocompatibility and

* Corresponding author. Department of Hygiene, Environment and Medicine, Hohenstein Institutes, Schlosssteige 1, 74357 Boennigheim, Germany.
E-mail address: d.hoefer@hohenstein.de (D. Hoefer).

http://dx.doi.org/10.1016/j.bjps.2017.08.009

Please cite this article in press as: Hirsch T, et al., Implant for autologous soft tissue reconstruction using an adipose-derived stem cell-colonized alginate scaffold, Journal of Plastic, Reconstructive & Aesthetic Surgery (2017), http://dx.doi.org/10.1016/j.bjps.2017.08.009

promote angiogenesis *in vitro* and *in ovo*. Therefore, they provide a combination of essential properties for an implant intended for soft tissue replacement.

Introduction

The management of acute and chronic soft tissue defects represents a complex challenge for the plastic surgeon, especially in context of high prevalence of diseases such as diabetes mellitus, chronic venous insufficiency, and peripheral artery occlusive disease or after radiation therapy. These soft tissue defects potentially led to prolonged hospitalization with protracted individual and economic burden.[1–4] Allogenic materials for soft tissue reconstruction introduce the problem of insufficient vascularization, leading to consecutive implant loss. Transfer of large volumes of autologous adipose tissue results in central necrosis and consecutive volume loss. In consequence, there is a high demand for alternatives to conventional defect coverage.[5,6] The ideal material for soft tissue replacement should not only replace the defect volume but also retain it by promoting its successful integration with the surrounding vascular system. Tissue engineering deals with the construction of tissue-like biologic materials that can fulfill these requirements.[7,8]

The aim of this study was to engineer an implant using a three-dimensional biocompatible alginate scaffold colonized with angiogenic adipose-derived stem cells and test its potential for soft tissue replacement both *in vitro* and *in ovo*. The biopolymer alginate is derived from algae or produced by bacteria.[9] Since its discovery in 1881, it has been widely applied in the food and pharmaceutical industry.[10] Hydrogels built from alginate are hydrophilic, biocompatible, and non-immunogenic.[11] Its physicochemical properties such as viscosity and stiffness are easily controllable and therefore make it an attractive candidate for tissue engineering.[9]

Materials and methods

Establishing a three-dimensional alginate scaffold for stem cell colonization

An aqueous solution of sodium alginate powder (3%) in double-distilled water (ddH_2O) was homogenized and subsequently autoclaved. Then 15 grams (g) of the solution was mixed with 2 milliliters (ml) of a 0.5 molar (M) calcium carbonate ($CaCO_3$) suspension using a magnetic stirrer, and 1 ml of a 1 M glucono-δ-lakton solution was added. The mixture was stood under ambient temperature, pressure, and saturation until complete gelation was accomplished (Supplemental Figure 1). Then the supernatant was discarded, and the cured hydrogel was lyophilized at 0.05 millibar (mbar) and −55 °C for 24 ± 2 h to produce the protoscaffold in cylindrical shape.

Cytotoxicity of protoscaffolds

L929 (murine fibroblast cell line) cells were seeded in a 96-well microtiter plate (Omnilab, Bremen, Germany). After 72 h, the Dulbecco's Modified Eagle Media (DMEM) used for incubation was removed. New DMEM, which had been incubated for 24 h at 37 °C with a protoscaffold (scaffold prior to washing cycle) ($n = 8$), was added at different dilutions with regular DMEM (33%, 22%, 14.8%, and 9.9%). Cells were cultured for an additional 72 h as described above, and 0.5 milligram (mg)/ml 3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) (Sigma, Taufkirchen, Germany) was added. After 4 h, 0.01 M hydrochloric acid (HCl) containing 10% sodium dodecyl sulfate was added to stop the reaction. Cells were lysed with dimethyl sulfoxide, and absorbance at 562 nanometers (nm) was measured using an enzyme-linked immunosorbent assay (ELISA) plate reader (BioTek Instruments, Bad Friedrichshall, Germany).

Cell culture

All cells were cultured at 37 °C, with 5% carbon dioxide (CO_2), and 90% humidified atmosphere. Different cell types were procured as shown in Table 1.

The adipogenic differentiation media consisted of DMEM + 10% FCS HyClone, 2% L-glutamine, 1% insulin, 0.4% indomethacin, 0.2% 3-isobutyl-1-methylxanthin, and 0.1% dexamethasone.

Procurement of ASCs

Following approval from the local research ethics committee, the human ASCs were isolated from a single female donor from subcutaneous adipose tissue as part of an esthetic plastic surgery. Prior to the surgery, the patient signed a written informed consent, conforming to the World Medical Association Declaration of Helsinki (June 1964) and subsequent amendments. Murine cells were isolated at the University of Stuttgart after approval by German authorities. The porcine ASCs were procured from subcutaneous fat tissue from a local slaughterhouse.

Isolation of ASCs and cell culture were performed according to the description by Yang et al.[12] with some modifications. The subcutaneous tissue was washed extensively with sterile PBS containing 1000 U/ml penicillin and 1000 μg/ml streptomycin to remove contaminating blood cells. The specimen was then cut carefully. Connective tissue and blood vessels were removed, and the tissue was cut into 1 mm^3 pieces. The extracellular matrix was digested with 0.1% collagenase type I

Please cite this article in press as: Hirsch T, et al., Implant for autologous soft tissue reconstruction using an adipose-derived stem cell-colonized alginate scaffold, Journal of Plastic, Reconstructive & Aesthetic Surgery (2017), http://dx.doi.org/10.1016/j.bjps.2017.08.009