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# The use of a biostatic fascia lata thigh allograft as a scaffold for autologous human culture of fibroblasts – An in vitro study

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SUMMARY

The method for covering gingival recession defects and augmenting keratinized gingiva involves the use of autogenuous connective tissue grafts obtained from palatal mucosa in combination with various techniques of flap repositioning or tunnel techniques. In the case of multiple gingival recession defects the amount of connective tissue available for grafting is insufficient. Therefore, the use of substitutes is necessary. The most widely used material in recent years has been the acellular dermal matrix allograft. The disadvantage of its application lies in the absence of cells and blood vessels, which increases incorporation time. Primary cultured human autologic fibroblasts are commonly used to optimize the healing process. The aim of this study was to examine the in vitro biocompatibility of human fascia lata allograft as a new scaffold for primary cultured human autologic fibroblasts. For that, a fibroblast culture obtained from a fragment of gingival tissue taken from the hard palate mucosa of a subject was used. After 14 days the colony cells were inoculated on a fragment of human fascia lata allograft. After a further 7 days of incubation the material was frozen, cut and prepared for histochemical examination. After two weeks of incubation, and 7 days after inoculation on a fragment of fascia lata allograft numerous accumulations of the cultured fibroblast were found that had a typical structure and produced collagen fibres. A human fascia lata allograft can be used as a scaffold for primary cultured human autologic fibroblasts. Further studies should confirm the clinical efficacy of this solution.



## 1. Introduction

Patients with mucogingival problems represent a growing percentage of the adult human population. Most often these problems take the form of gingival recessions and a narrow zone of keratinized gingiva or a total lack thereof. The treatment is carried out mainly in a surgical manner. The methods used to cover a gingival recessions as well as augment and expand keratinized gingiva make use of utilize coronal and lateral flaps, tunnel techniques alone or in combination with autologous transplantation (free gingival graft – FGG and connective tissue graft – CTG) or allogeneic, Guided Tissue Regeneration (GTR), growth factors (autologous or recombinant), biologically active proteins (amelogenins), primary culture of fibroblasts and mixed techniques (Cardaropoli et al., 2012; McGuire and Scheyer, 2010; Schlee and Esposito, 2011; Zuhr et al., 2014).

Nowadays, in plastic mucogingival surgery, tissue substitutes are sought, especially in cases of multiple gingival recessions. The group of autogenous tissue substitutes includes natural matrices – acellular and cellular dermal grafts, collagen matrices, simple (gel, foam, netting) or complex ones, fibrin, gelatine, synthetic, hybrid (semisynthetic) matrices (Moharamzadeh et al., 2007; Schlee and Esposito, 2011). Xenogeneic and allogeneic materials are used most frequently. Xenogeneic materials include the following devices: Mucograft® (Cardaropoli et al., 2012; Nevins et al., 2011; Sanz et al., 2009), Mucoderm® (Rothamel et al., 2014) and DynaMatrix® (Nevins et al., 2010). On the other hand, only allogeneic material used in soft tissue augmentation until now has been the human acellular dermal matrix allograft (Alloderm) (Aichelmann-Reidy et al., 2001; Allegrini et al., 2008; Harris, 1998; Henderson et al., 2001; Tal, 1999). However, the drawback of using this matrix is its dry form – prior to its usage it must be hydrated to improve its thickness (to about 2 mm) – its technical and clinical processing is difficult. Alloderm requires, inter alia, a specific cutting technique prior to implantation in the recipient site and often large flat mobilization, and significant tension to the flap increases the risk of a

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postoperative relapse. The human fascia lata allograft offers a new promising alternative in the surgical treatment of mucogingival defects (*FascioDerm®*).

The tissue has been used in various areas of medicine since the 1970s (Coulam et al., 1973; Davidorf et al., 1974; Hinton et al., 1992; Ionescu et al., 1972). In maxillo-facial surgery Smolka et al. used autogenous thigh fascia lata to reconstruct the palatal aponeurosis in secondary radical intravelar veloplasty (Smolka et al., 2008). However, this procedure required a second surgical site. Sezer et al. used femoral fascia lata allograft in oral vestibuloplasty (Sezer et al., 2004). They evaluated the surgical site after it had healed histologically and found complete allograft resorption had occurred, replaced by the host tissue. A characteristic feature of tissues harvested for bacteriostatic grafts (bone, cartilage, fascia, dura mater) is that they contain small numbers of cells and a large amount of extracellular matrix whose main component is collagen. The tissues are cadaveric and thus require sterilization. Radiation sterilization is a widely used method (Dufrane et al., 2002). Human fascia lata femoral allograft has low immunogenicity and is well tolerated by the host tissue (Defrere and Franckart, 1994). Generally, however, the absence of cells and blood vessels in allogeneic materials hinders and prolongs the incorporation of the graft at the recipient site. To optimize the healing process for allografts, keratinocytes, fibroblasts and growth factors are used, and the allograft itself acts as a scaffold (Novaes et al., 2007).

The aim of this study was to evaluate the possibility of using hydrated biostatic fascia lata thigh allograft as a scaffold for primary cultured autologic human fibroblasts harvested from the hard palate mucosa.

## 2. Material and methods

### 2.1. Preparation of scaffold

The allogeneic grafts used in the present study were collected from deceased donors in accordance with Polish law (Act on the Retrieval and Transplantation of Cells, Tissues and Organs – Dz. U. 2005. no. 169 pos. 1411) and EU Directive 2004/23/WE dated 23.03.2004 setting the standards of quality, safety, commissioning, grafting, testing, processing, preservation, storage and distribution of human tissues and cells.

A tissue fragment is collected from the fascia thigh of a donor and is then frozen at a temperature below −20 °C for up to 6 months. During this time the donor's serum is tested for the presence of infectious diseases: HIV, hepatitis B and C, syphilis, and other diseases of viral and bacterial origin and in the event of a negative result (qualification of the donor) the tissue is thawed in a refrigerator at about 8 °C. After thawing, it is rinsed in a saline solution (0.9% NaCl), twice in 2× 500 ml of water over a period of 15 min × 2. All manipulations are performed in sterile conditions. The rinsed tissue is mechanically cleared of the underlying tissue using a scalpel until a uniform collagen structure is achieved so as to obtain a 2 mm thick membrane. The membrane obtained in this way is cut along the collagen fibres and then digested in 50 ml of 70% ethyl alcohol for 48 h. Subsequently, it is washed again with a saline solution two times (2× 50 ml) to wash out the residual alcohol. After rinsing, the membrane is packaged in a foil container, the sterilization indicator included, and transferred for sterilization with a 35 kGy radiation dose for 5 min.

### 2.2. Gingival fibroblasts culture

During the surgery, a 140 mg fragment of mucosa was collected from the patient's hard palate. The collected tissue was immediately placed in a sterile transport tube containing Ham's F12 (PAA) nutrient with 1 mg/ml Penicillin/Streptomycin (PAA) and 2 μg/ml (PAA) Amphotericin. The samples were transported at a temperature of +2° to +8°.

Keratinocytes and fibroblasts were isolated from the collected tissue. The tissue was washed overnight in a dispase solution (1 mg/ml) (Worthington). The next day an epithelial layer was separated. The epithelium was digested for 10 min in 0.05% trypsin (PAA). The cells were washed and seeded in T25 culture bottles – the culture was not continued due to lack of a culture nutrient (Lauer et al., 2006). After the epithelial layer had been separated the connective tissue was cut into 3 mm × 3 mm pieces and digested in 0.1% collagenase type II (Worthington) in Ham's F12 nutrient (PAA) for 4 h. The cells were inoculated and grown in DMEM/Ham 'SF12 nutrient (PAA) with 1 mg/ml Penicillin/Streptomycin (PAA) and 2 μg/ml Amphotericin (PAA) and 10% serum, in an incubator (SANYO MCO 18AIC) at +37 °C, 90% humidity and 5.5% $CO_2$. The nutrient was prepared immediately prior to use and changed after every 48 h. The culture was grown for 14 days. All activities related to the pre-treatment of tissue and further cultivation were conducted in aseptic conditions and while observing the principles of asepsis under laminar air-flow (KOJAR GL – 130) in specially prepared cell culture rooms. The cells were trypsinized when they reached 80% confluence.

### 2.3. Fibroblast culture within scaffold

The nutrient was removed from the culture, washed twice with PBS (PAA) deprived of calcium and magnesium ions, after which trypsin was added so that it just covered the layer of cells present. They were then incubated for 3 min in the incubator (KOJAR GL – 130), with the peeling process monitored using an inverted microscope. The trypsin was neutralized by adding double (relative to the trypsin) the volume of the culture nutrient DMEM/Ham'sF12 (PAA) with 1 mg/ml Penicillin/Streptomycin (PAA) and 2 μg/ml Amphotericin (PAA) and 10% serum, centrifuged for 5 min at 100 × *g* (Eppendorf Centrifuge 5702). The supernatant was removed.

Human serum was used during the culturing process. The serum contained the nutrients necessary for cell growth. The blood was collected in Serum Beads Clot Activator (BD) tubes using the appropriate needle and holder. The blood was collected in a closed system. The serum was prepared in the following way – blood was subjected to centrifugation in an Eppendorf Centrifuge 5702 for 10 min at 800 × *g*. The serum was then retrieved in form of the supernatant using a pipette. The RBC mass was then removed.

After sterilization, the allogeneic graft was removed from the foil packaging in a sterile room, and in laminar air-flow conditions the autologous cells were applied by pipette to the surface of the graft. The allogeneic membrane graft with autologous cells obtained in this way was placed in a culture bottle, in a culture medium, and stored in a $CO_2$ incubator for a period of 7 days. The culture medium was renewed every 72 h. Once the culture was completed, the material was frozen at a temperature of −30 °C so that it could be cut with a microtome.

## 3. Histological assessment

The evaluated tissue was obtained from frozen slivers. The preparations were stained using the immunohistochemical technique. A mouse anti-vimentin antibody was used as a primary antibody. These antibodies primarily mark cells of mesenchymal origin in normal tissue, inter alia, fibroblasts. A 1:200 dilution was used. The reaction was visualized with the DAKO EnVision™ G|2 System/AP detection system. This system is employed to detect antigens present in low concentrations. The system does not contain biotin, thus the non-specific staining resulting from