Tissue engineering of composite grafts: Cocultivation of human oral keratinocytes and human osteoblast-like cells on laminin-coated polycarbonate membranes and equine collagen membranes under different culture conditions

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Abstract: In complex craniomaxillofacial defects, the simultaneous reconstruction of hard and soft tissue is often necessary. Until now, oral keratinocytes and osteoblast-like cells have not been cocultivated on the same carrier. For the first time, the cocultivation of human oral keratinocytes and human osteoblast-like cells has been investigated in this study. Different carriers (laminin-coated polycarbonate and equine collagen membranes) and various culture conditions were examined. Human oral keratinocytes and human osteoblast-like cells from five patients were isolated from tissue samples, seeded on the opposite sides of the carriers and cultivated for 1 and 2 weeks under static conditions in an incubator and in a perfusion chamber. Proliferation and morphology of the cells were analyzed by EZ4U-tests, light microscopy, and scanning electron microscopy. Cocultivation of both cell-types seeded on one carrier was possible. Quantitative and qualitative growth was significantly better on collagen membranes when compared with laminin-coated polycarbonate membranes independent of the culture conditions. Using perfusion culture in comparison to static culture, the increase of cell proliferation after 2 weeks of cultivation when compared with the proliferation after 1 week was significantly lower, independent of the carriers used. In conclusion, the contemporaneous cultivation of human oral keratinocytes and human osteoblast-like cells on the same carrier is possible, a prerequisite for planned in vivo studies. As carrier collagen is superior to laminin-coated polycarbonate membranes. Regarding the development over time, the increase of proliferation rate is lower in perfusion culture. Examinations of cellular differentiation over time under various culture conditions will be subject of further investigations. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res 93A: 704–715, 2010

Key words: cocultivation; oral keratinocytes; osteoblasts; biomaterials; tissue engineering

INTRODUCTION

Many studies reported the examination and optimization of tissue engineering procedures to replace single tissues. These methods gain in importance especially when other donor sites are rare or bearing the risk of severe donor site morbidity, transmission of disease, or problems in immunocompatibility.1,2

Grafts for, for example, head and neck reconstructions often require hard and soft tissue. Apart from the hard tissue component which is addressed by different approaches in many cases an intraoral lining by oral mucosa needs to be achieved.3–5 Lauer et al. (2001) introduced a radial forearm flap prelaminated with in vitro cultured oral keratinocytes.6

Besides the mucosa-like lining in the oral cavity, a primary wound closure could be achieved at the donor site. Schultz-Mosgau et al. (2004) transplanted preconfluent oral keratinocytes on the gracilis muscle of Wistar rats and achieved a uniform multilayered oral epithelium coverage.7

For the ideal transplant in the field of combined hard and soft tissue reconstruction by the means of tissue engineering, different cell types in coculture are necessary. Up to now, there are only few approaches of combined hard and soft tissue substitutes. Neovius and Kratz (2003) described the cocultivation of dermal keratinocytes and elastic chondrocytes for ear reconstruction.8 Many attempts were performed to create a dermal and an epidermal layer to treat full-thickness defects of the skin. For this purpose, dermal keratinocytes and with fibroblasts-seeded dermal equivalents were combined.9–11 To the best of our knowledge until now, oral keratinocytes and osteoblast-like cells have not been investigated on the same carrier even if they
are common cells in the head and neck region, whereas the combination of oral keratinocytes and fibroblasts has been already examined.12,13

The aim of this study was to investigate the cocultivation of human oral keratinocytes and human osteoblast-like cells on laminin-coated polycarbonate membranes and equine collagen membranes. Polycarbonate membranes and collagen are described to support the growth of human oral keratinocytes and human osteoblast-like cells.13–16 The laminin-coating of polycarbonate membranes seems to enhance the growth of human osteoblast-like cells and also the attachment of oral keratinocytes to culture dishes.17 Furthermore, we compared the influence of two different culture conditions (static culture in an incubator vs. perfusion culture system) on the growth of the cocultivated cells.

The perfusion culture system guarantees the continuous exchange of medium, constant removal of metabolic waste and has been used for the cultivation of a variety of cells and tissues.18–21 These circumstances have a positive influence on growth of osteoblast-like cells as well as on oral keratinocytes.18,22–24 Especially, less dedifferentiation of the cells as demonstrated by Strehl et al. (2005) for the long-term cultivation of cartilage, multilayered growth, and deposition of extracellular matrix of osteoblast-like cells were described.18,25,26 The latter is meant to be due to fluid-flow derived shear stress.27–29 The first successful in vitro cocultivation of human oral keratinocytes and human osteoblast-like cells on the same scaffold would be of main interest in the field of tissue engineering. It is a necessary prerequisite for planned in vivo studies and a further step to optimize craniomaxillofacial defect reconstructions.

MATERIALS AND METHODS

Isolation and cultivation of cells

After informed consent (Ethic commission of the Medical Faculty of Freiburg University, Germany) samples of bone and oral mucosa were harvested from five patients during oral and maxillofacial interventions and placed into sterile 0.9% NaCl solution.

For the cultivation of osteoblast-like cells, samples of bone were minced into pieces of 1-2 mm diameter and placed into culture flasks (25 cm², Greiner, Frickenhausen, Germany) using Opti-MEM1 + Glutamax (Opti Minimal Essential Medium, Gibco Invitrogen Life Technologies, Paisley, UK) supplemented with 10% foetal calf serum (FCS, PAA Laboratories, Linz, Austria), 2% 1M N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES, Roth, Karlsruhe, Germany), 1000 mg/L CaCl₂ (Roth, Karlsruhe, Germany), and 1% penicillin/streptomycin solution (PAA Laboratories, Linz, Austria). The explants were incubated at 37°C in a humidified atmosphere with 5% CO₂ (Heraeus Instruments, Osterode, Germany). After reaching confluence cells were trypsinized (0.5% trypsin in PBS, PAA Laboratories, Linz, Austria) and transferred into 75 cm³ culture flasks (Greiner, Frickenhausen, Germany). RPMI 1640 (Gibco Laboratories Life Technologies, NY) containing 10% FCS, 1% penicillin/streptomycin solution, and 5% HEPES was used as medium for further cultivation. Cells were used for experiments after second passage.

For the cultivation of oral keratinocytes, samples of mucosa were minced into pieces of 1-2 mm diameter and placed into culture flasks (75 cm³, Greiner, Frickenhausen, Germany). The used medium consisted of 750 mL Dulbecco’s Modified Eagle Medium (DMEM) with 1 g/L Glucose (Bio Whittaker, Cambrex Bio Science Verviers, Verviers, Belgium), 250 mL Nutrient Mixture Ham’s F-12 with Glutamine (Gibco Invitrogen Life Technologies, Paisley, UK), 10% FCS, 2% HEPES, and 1% penicillin/streptomycin solution supplemented with 293 g/L L-glutamine (PAA Laboratories, Linz, Austria), 10 µg/L epidermal growth factor (Sigma, Roedermark, Germany), 5 mg/L insulin (Sigma, Roedermark, Germany), 5 mg/L human transferrin (Sigma, Roedermark, Germany), 1.36 µg/L triiodothyronine (Sigma, Roedermark, Germany), 0.2 mg/L hydrocortisone (Sigma, Roedermark, Germany), 0.0085 mg/L cholaera-toxine (Sigma, Roedermark, Germany), 0.024 g/L adenine (Sigma, Roedermark, Germany), and 10.5 mg/L spermine (Sigma, Roedermark, Germany). The explants were incubated at 37°C in humidified atmosphere with 5% CO₂ (Heraeus Instruments, Osterode, Germany). Cells were used for experiments after the first passage.

Cells were trypsinized and seeded on different carriers. Cells seeded in culture dishes served as controls and for cell characterization analysis.

Carriers

Polycarbonate membranes (diameter 13 mm) with a pore size of 0.4 µm (Millipore, Minucells, and Minutissue, Bad Abbach, Germany) and equine collagen membranes (Tissue Foil E®, Baxter, Resorba, Nuremberg, Germany) were used. The polycarbonate membranes were coated with laminin (Sigma-Aldrich, Deisenhofen, Germany). The coating was performed with a concentration of 1-2 µg/cm² on both sides.

Cell seeding

50 µL osteoblast-like cells (2 x 10⁶ cells/mL) were seeded onto the membranes fixed in carrier-devices (Minusheet, Minucells and Minutissue, Bad Abbach, Germany) and incubated for 24 h at 37°C/5% CO₂ atmosphere, followed by seeding of oral keratinocytes in the same way on the opposite side of the membrane. After further incubation for 24 h at 37°C/5% CO₂ atmosphere half of the carriers were placed into the perfusion chamber (Minucells and Minutissue, Bad Abbach, Germany; Fig. 1). The medium is transported by a peristaltic pump (Ismatec, Glattbrugg-Zurigo, Switzerland) with a flow rate of 3 mL/h.
from the bottle with fresh medium to the culture container which is placed on a heating plate (37°C). The waste medium is collected in another bottle (Fig. 1). The other carriers were incubated at 37°C/5% CO2 atmosphere under static conditions. Additionally, for cell characterization plates were seeded with 1 × 10^5 cells and incubated for 1 week at 37°C in 5% CO2 atmosphere. In static culture, RPMI 1640 containing 10% FCS, 1% penicillin/streptomycin solution, and 2% HEPES was used. For perfusion culture experiments, 4% HEPES instead of 2% was used to avoid an increase of pH outside the incubator. After 7 and 14 days, samples were examined by cell proliferation analysis, light microscopy, and scanning electron microscopy.

Cell proliferation analysis

The cell proliferation was analyzed by the nonradioactive assay EZ4U (Easy for you, Biomedica, Wien, Austria). This method is based on the finding that living cells are capable to reduce slightly yellow colored tetrazolium salts into intense red colored formazan derivatives by an intracellular reduction system, mostly located in the mitochondria. These formazan derivatives are excreted into the culture medium and the absorbance can be measured with a microplate reader. The amount of colored formazan derivatives correlates with the amount of living cells in the sample. The proliferation assay was carried out according to the manufacturers’ instructions.

Scanning electron microscopy

The cell colonization analysis was assessed by scanning electron microscopy. For scanning electron microscopy, the samples were fixed in 8% formaldehyde for 2 days followed by dehydration in graded alcohol (30, 50, 70, 90, 90, each one time and two times in 99.8%). After critical point drying (CPD 030, BAL-TEC, Wallruf, Germany), according to standard procedure using liquid carbon dioxide, the samples were sputtered (SCD 050, BAL-TEC, Wallruf, Germany) with goldpalladium (Provoc, Oestrich-Winkel, Germany). The samples were examined via Zeiss Leica 32 scanning electron microscope (Zeiss, Kochern, Germany) at 10 KV. Images were digitalized.

Light microscopy

For light microscopy, samples were fixed in 8% formaldehyde. Over an amounting alcohol gradient, the segments were dehydrated and embedded in 2-hydroxyethyl-methacrylate (Technovit 8100, Haereus-Kulzer, Wehrheim, Germany). By microtome (Leica, Nussloch, Deutschland), thin sections of ~2.5 μm were manufactured and stained with Nucleus Fast Red 0.3%/5% Al2(SO4)3 in Aqua dest. (C14H8NNaO7S, Fluc Chemie GmbH, Buchs, Switzerland). The sections were examined by light microscopy (Axiioskop, Zeiss, Oberkochen, Germany) with 10X, 20X, 50X, and 100X magnification. Images were digitalized.

Cell characterization

For cell characterization, cells were seeded in 24-well plates and examined after a cultivation period of 7 days. Osteoblast-like cells were examined for alkaline phosphatase and osteocalcin, whereas oral keratinocytes for cyto-keratine 13 and 19.

Assay for osteocalcin

Osteocalcin is exclusively synthesized by osteoblasts and is believed to prevent premature mineralization of newly formed, but yet disorganized bone matrix. For the quantification of osteocalcin in the cell culture, supernatant of human osteoblast-like cells the METRA osteocalcin EIA Kit (Quidel Corporation, San Diego, CA) was performed according to the manufacturer’s instructions. The absorbance was measured at 405 nm.

Alkaline phosphatase assay

For staining of osteoblast-like cells an alkaline phosphatase assay kit (Sigma, Roedermark, Germany) was used. The culture dishes were air dried, fixed in citrate-aceton-formaldehyde solution for 30 s, and rinsed gently with Aqua dest. Cells were incubated with alkaline phosphatase staining solution for 15 min protected from direct light and washed with Aqua dest. The citrate-aceton-formaldehyde solution as well as the alkaline phosphatase staining solution were prepared according to the manufacturer’s instructions. The culture dishes were counterstained with neutralred for 5 min and rinsed with Aqua dest. Positive staining for alkaline phosphatase (red-violet) was identified by light microscopy and evaluated by morphometry (analySIS, Soft Imaging System GmbH, Münster, Germany).
Detection of cytokeratines 13 and 19

Cytokeratines 13 and 19 were examined immunhistochemically using the avidine-biotine-method. The culture dishes were washed in PBS for 5 min, fixed with 70% ethanol for 1 h, washed in PBS for 5 min, allowed to air-dry, and washed again in PBS. After an incubation with 0.3% H2O2 in methanol for 10 min, unspecific immune reactions were blocked with 10% bovine serum albumin (Merck, Darmstadt, Germany) for 10 min, before the murine antibody against human Cytokeratines 13 or 19 (Sigma, Roedermark, Germany) was added and incubated over night at 4°C. The detection of the attached antibody was performed using the ABC-Kit (Vectastain, Burlingame, CA) according to the manufacturer’s instructions. Staining reaction was achieved by DAB-Chromogen (4 mg 3,30-diaminobenzidintetrahydrochloride (C12H14N4⁺/4HCl, DAB, Sigma, Roedermark, Germany) in 10 mL 0.05M Tris-(hydroxylmethyl)aminomethane (C4H11NO3, Trisbuffer, Roth, Karlsruhe, Germany; pH 7.2–7.4 with 17 lL 30% H2O2), added for 15–25 min. Washing with PBS and counterstaining with hemalaun (Mayers Hämalaunlösung, Merck, Darmstadt, Germany) for 2 min followed. Afterwards the samples were washed with Aqua dest. and air-dried. Positive staining (brown cytoplasm and blue nuclei) was identified by light microscopy.

Statistics

The results of the proliferation test were analyzed by repeated measures ANOVA (SAS 8.2 PROC GLM) after converting the data according to log(0.3 + x) − log(0.3) to optimize the skewness of the distribution. Furthermore, student's t-tests were performed.

RESULTS

Cell proliferation and vitality

The vitality and proliferation capacity of the cells from five different patients seeded onto different membranes and cultured under static and perfusion culture conditions were studied by the EZ4U – EASY FOR YOU test. The average of the measured absorbance (OD450nm) after 1 and 2 weeks of cultivation is shown in Table I and Figure 2 in dependence on the two different membranes and the culture conditions.

Cells seeded on collagen membranes showed significantly higher proliferation rates when compared with cells seeded on laminin-coated polycarbonate membranes (p < 0.001). An overall difference between culture conditions is not detectable (p = 0.4).

On both investigated materials, a significant influence of culture conditions on the increase of cell viability between week 1 and week 2 could be observed (p < 0.05). Under perfusion culture conditions, cell vitality on polycarbonate and collagen membranes was stable between 1 and 2 weeks of cultivation (p = 0.482, p = 0.448, respectively), whereas under static conditions a significant increase could be detected (p < 0.001, p = 0.001, respectively).

Light microscopy

For light microscopy, cultivated membranes were stained with nucleus fast red. Nuclei appeared red, whereas cytoplasm and the collagen membrane were slight rose. The polycarbonate membrane is not stained. The different cell types cannot be distinguished. Cells on both sides and multilayered cell growth were visible more frequently on collagen membranes.
membranes when compared with polycarbonate membranes (Fig. 3). Morphological differences between static and perfusion culture as well as between cultivation periods for 1 week and 2 weeks were not detectable by light microscopy (data not shown).

Scanning electron microscopy

The surface of the membranes with respect to cell morphology and growth pattern was examined by scanning electron microscopy. Although osteoblast-like cells appeared as longish cells arranged like a draught of fishes in confluent layers [Fig. 4(A)], oral keratinocytes appeared more compact, polygonal, and flat and showed a cobble stone pattern when confluent [Fig. 4(B)].

Cell growth was detectable on both sides of polycarbonate and collagen membranes after cultivation under static conditions as well as in perfusion culture (Fig. 5–8).

On polycarbonate membranes isolated keratinocytes [Fig. 5(A)] and osteoblasts [Fig. 5(B)] or small conglomerates of cells were visible on both sides after 1 week of cultivation in the incubator [Fig. 5(A,B)]. After 2 weeks, more conglomerates of oral keratinocytes were present [Fig. 5(C)], whereas the number of osteoblast-like cells did not increase [Fig. 5(D)].

The results after a cultivation period for 1 week in perfusion chamber were similar when compared with the cultivation under static conditions: isolated cells and some conglomerates on both sides of the membranes were visible [Fig. 6(A,B)]. Only isolated, partly detached keratinocytes could be observed after a cultivation period of 2 weeks in perfusion culture [Fig. 6(C)], whereas the number of osteoblast-like cells increased [Fig. 6(D)].

Figure 3. Light microscopy of collagen membrane (A) and laminin-coated polycarbonate membrane (B) seeded with human oral keratinocytes and human osteoblast-like cells after a cultivation period of 1 week in perfusion culture. Cells on one side (I) and a multilayer of cells on the other side (II) of the collagen membrane (Coll) are visible. On polycarbonate membrane (PCM) numerous cells (III) on one side are detectable. Magnification ×20. Nucleus fast red staining. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 4. Scanning electron micrographs of osteoblast-like cells on polycarbonate membrane (A) and of oral keratinocytes on collagen membrane (B) after 1 week under static conditions. Magnification ×1000.
On collagen membranes confluent and multilayered cell growth was detected more often when compared with polycarbonate membranes (Figs. 7 and 8). On most of the collagen membranes confluent, partly multilayered growth was visible after a cultivation period of 1 week in the incubator [Fig. 7(A,B)]. After a cultivation period for 2 weeks, the cell density of keratinocytes and osteoblasts increased [Fig. 7(C,D)].

Confluent growth of osteoblast-like cells was visible on collagen membranes after a cultivation period of 1 week in the perfusion chamber [Fig. 8(B)]. Oral keratinocytes were not as spread as after cultivation under static conditions [Fig. 8(A)]. After a cultivation period of 2 weeks in perfusion chamber on all collagen membranes, confluent and more dense growth of osteoblast-like cells [Fig. 8(D)] was visible, whereas only conglomerates or even singular cells with the aspect of keratinocytes [Fig. 8(C)] on the other side of the membranes were detectable.

**Cell characterization**

**Osteocalcin determination and alkaline phosphatase assay**

The characterization of osteoblast-like cells was determined by the amount of osteocalcin and the relative amount of alkaline phosphatase.

The amount of osteocalcin in the supernatant varied between 9.2 and 14.5 ng/mL.

The alkaline staining of these cells typically resulted intensively positive (about 70%; Fig. 9).

**Cytokeratine 13 and 19**

The characterization of oral keratinocytes was determined by the presence of expressing cytokeratine 13 and 19 (Fig. 10). Immunocytochemistry of the fixed cells showed the presence of cytokeratine
DISCUSSION

Human oral keratinocytes and human osteoblast-like cells are common in tissues involved in head and neck defects. For the later combined reconstruction of hard and soft tissues, the cocultivation of these cells was investigated for the first time. As carriers, laminin-coated polycarbonate membranes and equine collagen membranes were used. The cultivation was performed under static conditions and in perfusion chamber. In this study, we could show that it is possible to cocultivate human oral keratinocytes and human osteoblast-like cells on the two different sides of one biomaterial. According to previous findings of our laboratory, the cells do not support each other but the proliferation seems to be less than expected when compared with the growth of the cultivation of each cell type alone. Up to now, no examination exists concerning the combination of human oral keratinocytes and human osteoblast-like cells.

Cocultivation of different cells was mainly investigated studying skin models, consisting of dermal and epidermal layers to treat full-thickness skin defects. Rheinwald and Green (1975) described that the growth of dermal keratinocytes is supported by cocultivation with fibroblasts. According to this for the cultivation of keratinocytes, a feeder layer of mostly lethally irradiated fibroblasts was used and the effects of cocultivation of these cells even together with adipocytes were investigated. In a rat skin model, Sugihara et al. (2001) found that the proliferation and differentiation of keratinocytes was supported by adipocytes but cocultivated fibroblasts were inhibited. Also for the cultivation of oral keratinocytes, the cocultivation with fibroblasts was used. Additionally, effects of interactions

Figure 6. Scanning electron microscopy of a polycarbonate membrane seeded with human oral keratinocytes and human osteoblast-like cells after 1 week (A, B) and after 2 weeks (C, D) in perfusion culture. Typical appearance of oral keratinocytes (A, C) and osteoblast-like cells (B, D). Magnification ×1000.
between both cell types were studied. Even if oral and dermal keratinocytes show many similarities, the former seem to be more difficult to cultivate. For example, human oral keratinocytes stop proliferating much earlier under the same culture conditions when compared with dermal keratinocytes. Therefore, it is not possible to transfer results obtained with dermal keratinocytes to oral keratinocytes. This is also be true for studying the interaction with other cell types.

The cultivation of bone was widely investigated in the field of head and neck reconstructions and dental implantology. To the best of our knowledge up to now, the combination of oral keratinocytes and osteoblast-like cells to create hard and soft tissue replacements was not described and interactions of osteoblasts and keratinocytes were not examined. Spector et al. (2002) investigated the cocultivation of rat osteoblasts and immature dural cells, which leads to higher proliferation and differentiation of the osteoblasts when compared with osteoblasts cultured alone due to paracrine factors. Maurin et al. (2000) described the inhibition of the proliferation of human bone-derived osteoblasts by cocultivated human adipocytes. For human bone-marrow-derived osteoblast-like cells and human adipocytes, this effect could not be found.

In this study, collagen membranes showed significant better results relating to cell proliferation when compared with laminin-coated polycarbonate membranes although both materials were previously described as suitable carriers for the cultivation of human oral keratinocytes and human osteoblast-like cells. One reason for the better cell proliferation rate could be that the attachment of cells on collagen as a typical protein of extracellular matrix is facilitated when compared with the attachment on polycarbonate membranes. Schleicher et al. (2005) described a better attachment of osteoblasts to collagen in comparison to polyglycic acid. Also Webster et al. (2002) explained the better adhesion of osteoblasts to yttrium-pretreated hydroxylapatite (HA)
with binding of calcium and following binding of collagen to this material. In our study, the laminin-coating does not support the cells in the same way as collagen membranes do. This is in contrast to other studies which reported that laminin-coating of polycarbonate membranes enhances the growth of osteoblast-like cells and supports the attachment of oral keratinocytes to culture dishes. Another aspect could be the avoidance keratinocyte-osteoblast cell contact because of the thicker collagen membrane. A direct cell contact of the different cell types could lead to a growth inhibition.

In this in vitro study, not only different carriers but also different culture conditions (static culture in an incubator and perfusion culture) were compared. Overall after 1 week of cultivation, we could not find any significant differences between the two culture conditions. In contrast, if the cell proliferation after 1 week and after 2 weeks for static culture and perfusion culture were compared, we could find sig-

Figure 8. Scanning electron microscopy of collagen membrane seeded with human oral keratinocytes and human osteoblast-like cells after 1 week (A, B) and after 2 weeks (C, D) in perfusion culture. Typical appearance of oral keratinocytes (A, C) and osteoblast-like cells (B, D). Magnification ×1000.

Figure 9. Detection of alkaline phosphatase (blue), alkaline phosphatase negative cells are stained red. Magnification ×10. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
significant differences in proliferation over the culture period depending of the culture conditions. After a cultivation period of 2 weeks under static culture conditions, the proliferation of the cells was significantly higher when compared with a cultivation period of 1 week. In the perfusion culture, there were negligible differences in proliferation rates after 1 and 2 weeks of cultivation. These results seem to be in contrast to the fact that developing renal microvasculature can be maintained under perfusion culture. There are investigations to maintain gastric epithelium under perfusion culture which would be dissolved under static conditions. Additionally, for the long term cultivation of auricular cartilage and human gingiva perfusion culture is shown to be appropriate. The tissues keep their typical appearance and biochemical qualities. One aspect for the differentiation of osteoblast-like cells is fluid flow mediated shear stress. This aspect supports our findings because differentiated cells do not proliferate to the same extent than dedifferentiated cells do. After 2 weeks of perfusion culture, less keratinocytes were visible by scanning electron microscopy when compared with a cultivation period of 1 week, whereas osteoblasts were even increased in cell number. This could be due to the facilitated detachment of cells in perfusion culture caused by the flow rate when compared with the cultivation under static conditions, especially when oral keratinocytes have reached their maximum life span. The life span of oral keratinocytes is much shorter than the life span of dermal keratinocytes when cultured under the same conditions. Detached cells could be also a reason for lower results in the proliferation test. Additionally, in perfusion culture osteoblast-like cells proliferate more when compared with oral keratinocytes. This could lead to a growth inhibition of oral keratinocytes. In experiments of our laboratory, it could be demonstrated that osteoblast-like cells and oral keratinocytes grow better if they are separated by a barrier. In conclusion, for the first time the cocultivation of human oral keratinocytes and human osteoblast-like cells is feasible. Collagen membranes are more advantageous than polycarbonate membranes even if the latter are coated with laminin. Furthermore, under perfusion culture conditions the cell proliferation does not increase over time. This could be attributed to differentiation of the cells or the loss of cells after reaching their maximum life span or because of the inhibition of oral keratinocytes by the more expanding osteoblast-like cells. The examination of cellular differentiation under perfusion culture conditions in comparison to static culture will be subject of further investigations. Additionally, these in vitro results are necessary to provide a basis for planned in vivo studies (animal model) to reconstruct craniomaxillofacial defects.

References


