Co-cultivation of oral keratinocytes and osteoblast-like cells on different carriers

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Statement of Purpose: The present investigation is focused on the co-cultivation of human oral keratinocytes and human osteoblast-like cells cultured on different carriers and under various culture conditions. The different carriers were laminin-coated polycarbonate membranes and equine collagen membranes. The compared conditions were static culture in an incubator and a perfusion culture system. To our knowledge there are no descriptions of co-culture of human oral keratinocytes and human osteoblast like cells on one carrier in the literature so far.

Methods: Human oral keratinocytes and human osteoblast-like cells were isolated from tissue samples and after reaching an adequate number of cells plated on the opposite sides of laminin-coated polycarbonate membranes with a pore size of 0.4 µm (Millipore, Minucells and Minutissue, Bad Abbach, Germany) and equine collagene membranes (Tissue Foil E®, Baxter, Resorba, Nuremberg, Germany). The tissue samples derived from 5 different patients undergoing oral and maxillofacial surgery. The seeded membranes were cultured for one and two weeks under static conditions in the incubator and in a perfusion chamber (Minucells and Minutissue, Bad Abbach, Germany) with a medium flow of 3 ml/h. Proliferation and morphology of the cells were analysed by cell proliferation analysis (EZ4U, Easy for you, Biomedica, Vienna, Austria), light microscopy and scanning and transmission electron microscopy. Results of cell proliferation analysis were analysed by repeated measures ANOVA.

Results: It could be shown that co-cultivation of the both cell-types seeded on one carrier was possible. Quantitative and qualitative growth was significantly better on collagen membranes compared to laminin-coated polycarbonate membranes independent of the culture conditions. Cell proliferation analysis indicates a significant difference between the two membranes ($p<0,001$). The increase of proliferation after two weeks of cultivation compared to the proliferation after one week was markedly less using perfusion culture in comparison to static culture in the incubator. This phenomenon was detectable using collagen carriers as well as using laminin-coated polycarbonate membranes in the same extension. The average of the measured absorbance in cell proliferation test EZ4U after one and two weeks is shown in Fig. 1 depending on the carrier and culture condition used.

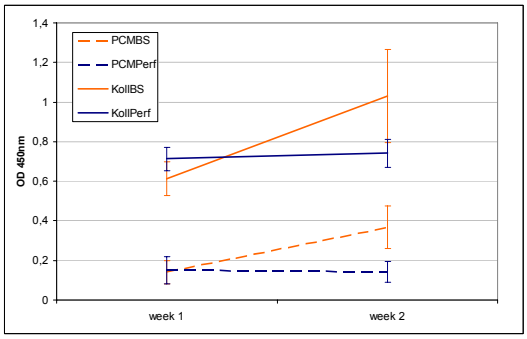

Figure 1. Cell proliferation analysis after one and two weeks. The conditions are polycarbonate-membrane under static conditions (PCMBS) and in perfusion culture (PCMPerf) and collagen membrane under static conditions (KollBS) and in perfusion culture (KollPerf). It is shown the average of the measured absorbance (OD 450nm) Error bars indicate 95% confidence interval.

Conclusions: The contemporaneous cultivation of human oral keratinocytes and human osteoblast-like cells on one carrier is possible. As carrier for co-cultivation of human oral keratinocytes and human osteoblast-like cells collagen is superior to laminin-coated polycarbonate-membranes. Regarding the development over time the increase in proliferation seems to be less in perfusion culture than in static culture. The development of cellular differentiation over time regarding the different culture conditions will be subject of further investigation.