Development of cellular substitutes for oral mucosa

Principal investigator : R. Ophof, Msc, DDS
R.Ophof@dent.kun.nl
Co-investigators : Prof. Dr. A.M. Kuijpers-Jagtman, PhD, DDS
J.W. Von den Hoff, PhD
J.C. Maltha, PhD
M.P.A.C. Helmich, BSc
R.E.M. van Rheden, BSc
Institution : Department of Orthodontics and Oral Biology,
University of Nijmegen, The Netherlands

Relevance

The incidence of a cleft lip and palate is about 0.2% of the live births. The multidisciplinary treatment of these children starts soon after birth and extends until the end of growth (17-20 years old). In a complete palatal cleft at least three primary and several secondary surgical corrections take place during this period. Additional treatment takes place in relation to hearing and speech problems and impaired psychosocial development. A long-lasting facial orthopedic and orthodontic treatment is usually also required. The project is aimed at the prevention of maxillary growth disturbances in cleft palate patients after surgical closure of the cleft. This will substantially reduce the need for extensive facial orthopedic and surgical treatments in these patients. In the end the patient will have the benefit of a reduced treatment burden and the community will have the benefit of reduced health care costs. In addition to the treatment of cleft palate patients, the mucosal substitutes can also be used for reconstructions after the resection of intra-oral tumors and for vestibuloplasty in edentulous patients.

Experimental design

This research project can be divided into three phases: A. The construction and characterization of the mucosal substitutes *in vitro*. B. The evaluation of the mucosal substitutes *in vivo* in the dog model. C. The effects of mucosal substitutes on long term maxillary growth in a simulated Von Langenbeck procedure in dogs. These three phases are worked out below.

Phase A:

The principal investigator will conduct the tissue culture of canine keratinocytes and fibroblasts, and of the composite cultures consisting of dermal substrates (skin-derived and collagen based) and canine cells (supervised by Dr. J.W. Von den Hoff). For the histological procedures the principal investigator will be assisted by the technicians of the laboratory for Oral Biology (supervised by Dr. J.C. Maltha). The immunohistochemistry for the analysis of the differentiation state of the cultured composites will be conducted.

Phase B:

The composites selected after the characterization in phase A will be cultured by the principal investigator for transplantation on the palate of beagle dogs in order to select the most suitable material. After the *in vivo* experiment the principal investigator will prepare the palatal tissue for histology and immunohistochemistry. The histological work will be organized as in phase A.

Phase C:

Based on the results from phase A and B, the most suitable composite material will be selected for application in a simulated Von Langenbeck procedure in beagle dogs. The principal investigator will culture this composite material in sufficient quantities for six beagle dogs. The maxillary growth will be analyzed with dental casts (supervised by Prof. A.M. Kuijpers-Jagtman). The principal investigator will measure contraction of the palatal wounds through the displacement of tattoo marks adjacent to the wounds. The displacement will be measured on intra-oral pictures using image analysis software (supervised by Dr. J.W. Von den Hoff). After the *in vivo* experiment the palatal tissue will be processed for (immuno)histology and as described in phase A.

Results

In our first phase, we concluded that that the oral mucosal equivalent composed of oral canine keratinocytes cultured on skin-derived substrates (de-epidermised dog skin or Alloderm®) showed histological and immunohistochemical characteristics close to normal oral mucosa. Therefore, we are planning now an *in vivo* study to transplant a constructed mucosal substitute on the palate of a beagle dog to evaluate the tissue response to the substrates as described in phase B.