

**Synopsis of Soft Tissue Engineering Methods in Reconstructive Oral and Maxillofacial Surgery**Sauerbier<sup>1</sup>, Günter Lauer<sup>2</sup>, N. Weyer<sup>1</sup>, J. Kuschnierz<sup>1</sup>, N. Liebehenschel<sup>1</sup>, R. Schön<sup>1</sup>, R. Schmelzeisen<sup>1</sup>, R. Gutwald<sup>1</sup><sup>1</sup> Department of Oral and Craniomaxillofacial Surgery, University Hospital Freiburg, Freiburg, Germany<sup>2</sup>Department of Oral and Craniomaxillofacial Surgery, University Hospital Carl-Gustav-Carus Dresden, Dresden, Germany

**Background and Introduction:** Operating on tumors in the oral cavity causes loss of tissue. Standard secondary surgical techniques on soft tissue are freeing of the tongue, lowering of the floor of the mouth and open vestibuloplasty. In routine interventions split skin grafts have been used for many years to cover extensive oral mucosal defects. The disposition to hyperkeratosis and growth of hair are the major disadvantages of this keratinized epithelium. Split skin grafts do neither differentiate into mucosa nor fulfil the demands of mechanical stress. Mucosal grafts do not show the disadvantages described above. In larger reconstructive procedures the limited availability of mucosa is a major concern. In reconstructive surgery the radial flap is a widely used microvascular, fasciocutaneous graft, too. To avoid the above mentioned disadvantages the radial flap with *in vitro* cultivated mucosa transplants has been prelaminated. The aim of this study was to test the feasibility of applying tissue engineered mucosa in the above mentioned surgical procedures.

**Methods:** Three to four weeks prior to the transplantation a 4 to 8 mm<sup>3</sup> biopsy of oral mucosa and a 40 ml venous blood sample were taken to cultivate autologous cell layers. These procedures were performed in local anaesthesia. The tissue was transferred into sterile isotonic salt solution and transported to the lab. After separating the serum from the blood by centrifugation the serum was added to the cell culture medium. The medium for culturing gingival keratinocytes consists of Dulbecco's modified Eagle's medium and nutrient mixture Ham's F-12 at a ratio 3:1 with 10% autologous patient serum and additives. Primary gingiva epithelial cultures were established according to the explant culture technique. The oral tissue was separated from connective tissues by microdissection, washed with 70% ethanol and rinsed three times with phosphate buffered saline (PBS). The biopsies were cut into small pieces (1 mm<sup>3</sup>), resuspended in 2 ml culture medium and seeded into a culture flask. After 14 to 21 days the cell layer of the primary culture was detached with trypsin for 20 min at a temperature of 37°C. The trypsinized keratinocytes were transferred onto membranes in a density of 2 x 10.000 cells per cm<sup>2</sup>. After a period of 3 to 4 weeks 2 to 3 layers of cells with an extension up to 15 cm<sup>2</sup> on top of the foil resulted. Then these constructs were transplanted. Biopsies were taken and examined immunohistochemically.

**Results:** Tissue engineered oral mucosa was applied successfully in all four surgical methods. Six months after transplantation a regular epithelial layering with a histological delimitation of the stratum, epithelial crest and a strong basal membrane appeared. According to the reception site the tissue engineered oral mucosa differentiated in several ways.

**Vestibuloplasty and Freeing of the Tongue:** .When the wound dressing was removed after seven days the surface still tended to bleed easily when being touched. After ten days it already seemed to be palely epithelialized. The wound generally stabilized within the next few days and healed after 20. There was a severe loss of vestibular dept within the first 6 months after the operation. Then the decrease ceased at this level. After freeing of the tongue there was one case of delayed healing, combined with severe scar formation. In the other cases healing proceeded without clinical complications.

**Prelaminating the Radial Flap:** After primary wound closure the donor site healed without complications like impairment of hand movement or finger force. At the recipient site no dehiscence between the borders of the flap and the resection was observed. All anastomosis functioned without failure. One case of delayed healing occurred due to an infection of the resection cavity. This was caused by a retention of saliva. Drainage of the right parotid duct was affected by the tumor resection. While the infection was drained it settled without endangering the flap. This incident resulted in a limited mouth opening. In the postoperative period the transplants showed a tendency to shrink. As table 3 shows the opening of the mouth and the movability of the tongue were good after 3 to 6 months. It was no longer possible to differentiate between the transplant and the local mucosa.

**Discussion and Conclusion:** The clinical results prove that cultured autologous tissue and tissue engineered grafts can be successfully applied in reconstructive oral and maxillofacial surgery. The application in clinical routine is often limited by the costs, which result from the quantity of preoperative work and laboratory preparations. These expenses could be decreased if the method was used for more indications, in other surgical disciplines and in a larger scale. To improve long term results it is necessary to achieve modifications in the field of cell cultivation and carrier materials as well as to obtain a better understanding of the physiological part of the healing process. To gain a more solid clinical evidence further controlled and randomized studies are needed, too.

**References:**

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