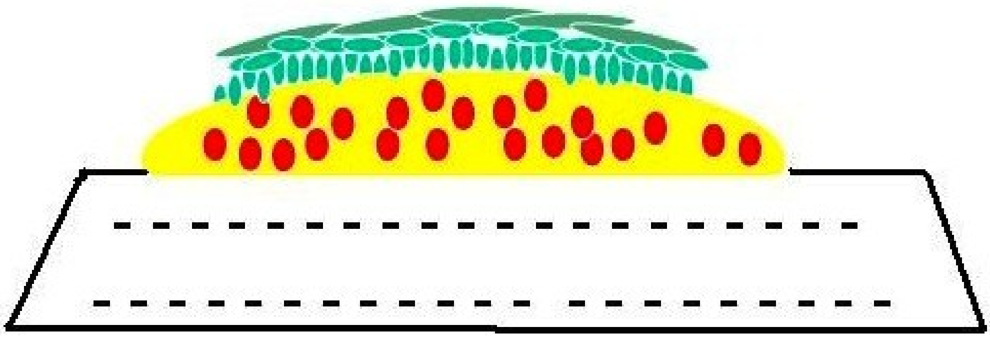
Healthcare industry BW

Gingiva grown in Petri dishes

We use teeth to break down food and gum disease is the major cause of tooth loss. Our teeth are firmly anchored in the jaw bone and surrounded and supported by the gum (gingiva), one of the five types of tissue that make up the human periodontium. A complex interplay of biomolecules maintains homoeostasis, i.e. the natural balance, in the gum tissue. A team of researchers led by Prof. Dr. Pascal Tomakidi from the Freiburg University Medical Centre is investigating the biomolecules that maintain this natural balance or restore it following injury.



Schematic representation of the organotypical interactive co-culture: metal grids (white area bordered by a black line) on which the collagen type I (yellow) cell culture matrix is placed. This cell culture matrix contains gingival connective tissue fibroblasts (red); gingival keratinocytes (also known as epithelial cells, green) are grown on the surface of the matrix. The interaction between the spatially separated cell types enables the keratinocytes to reconstitute a layered gingival epithelium in vitro. The cell culture medium is shown as an interrupted black line.
© Prof. Dr. Pascal Tomakidi

The researchers use a special in vitro test system to grow gingival epithelium in a culture dish. This in vitro tissue culture system also enables new biomaterials to be tested for their application in dental medicine – initially without any need for experimental animals.

Our teeth are anchored in the jaw bone and supported by what is generally referred to as periodontium. Five tissues make up the periodontium: the alveolar bone into which the teeth are anchored, the root cement on top of the tooth root, the periodontal ligament (a group of specialized connective tissue fibres that help the tooth withstand the compressive forces that occur during chewing) and the gum, which is also referred to as gingiva, which consists of gingival connective tissue and gingival epithelium. The gingival epithelium consists of several layers of epithelial cells which, depending on their differentiation stage, have a different morphology, and the lamina propria, which is located underneath the epithelial cells.

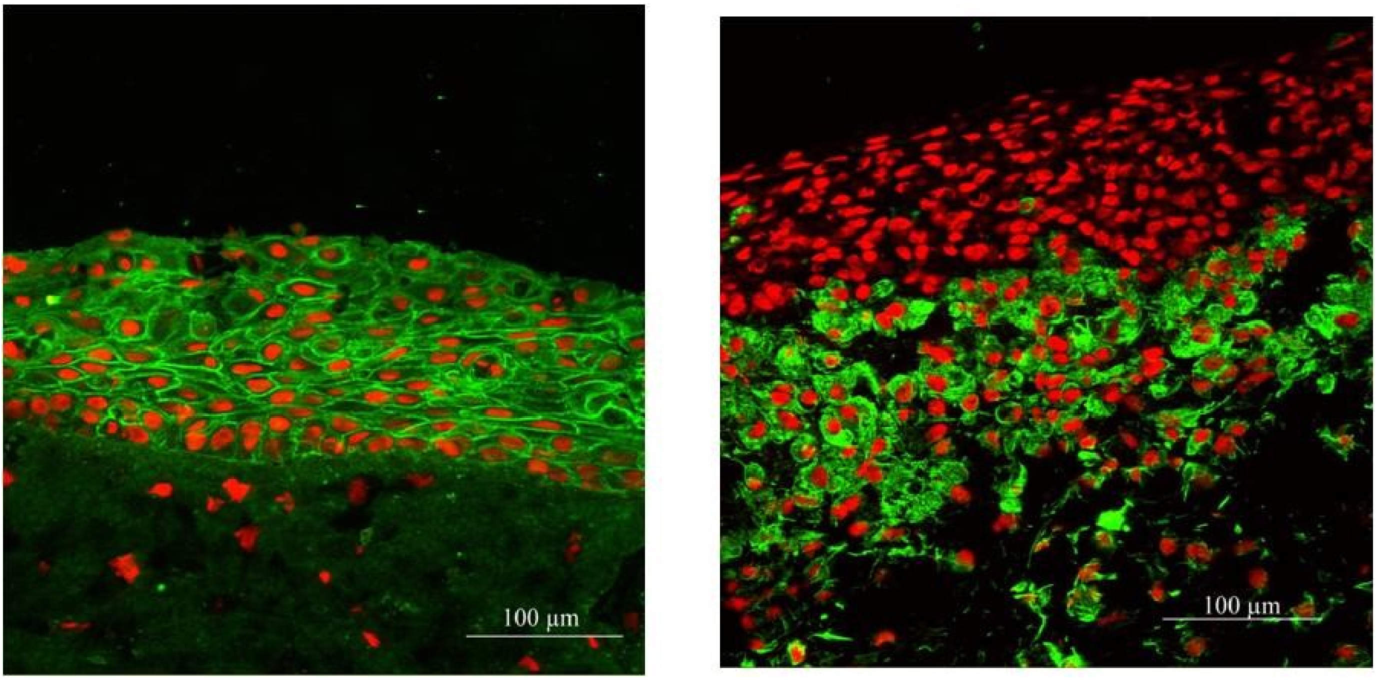
The structure of the gingiva is similar to that of human skin where epithelial cells also form the outer layer. The epithelial and connective tissue cells produce molecules that are involved in the creation of the epithelial basement membrane. This layer consists of interlinked proteins and carbohydrates; epithelial cell adhesion, which is mediated by proteins known as integrins, prevents the gum from breaking up upon mechanical strain.

“The gingiva is an extremely dynamic tissue; epithelial cells divide, mature and disappear relatively quickly – an endless cycle of creation and degeneration,” said Prof. Dr. Pascal Tomakidi, head of the Department of Oral Biotechnology at the University Clinic and Dental Hospital at the University of Freiburg. “The homoeostasis of this complex organization of epithelial cells and connective tissue is mediated by a dynamic interaction of different molecules. One of our major interests is to characterize this molecular interaction.”

Cell cultures that closely resemble the structure of natural tissue

The biologist Dr. Pascal Tomakidi spent many years at the German Cancer Research Center (DKFZ) in Heidelberg working on the investigation of skin tumour diseases, which is why he is extremely knowledgeable about the different states of layered epithelial tissues. He is also aware that it is difficult to investigate the cellular and molecular processes in the gingiva in culture dishes and that the tissue cultures have to mimic the natural tissue as closely as possible. This is why Tomakidi and his team have developed a test system in which epithelial cells grow alongside connective tissue cells. These organotypical co-cultures even enable the Freiburg researchers to get the cells to form a relatively correct basement membrane.

“Such co-cultures form different layers of epithelial cells just like real gingival tissue,” said Tomakidi. “In addition, they produce almost the same biomarkers.” These biomarkers are special molecules that are a prerequisite for a well-functioning gum, for example the proteins of the basement membrane or the keratins that form the cytoskeleton of the epithelial cells. Integrins can also serve as biomarkers; they not only anchor the epithelial cells in the basement membrane, but also serve as sensors for extracellular biomechanical stimuli. In response to pressure, tension and shear forces, the integrins initiate an intracellular signalling cascade that triggers reactions that can switch on genes, suppress gene function, lead to cell division, differentiation, apoptosis or to the synthesis of specific molecules.



Fluorescence-optical representation of in-vitro gingival epithelium and co-cultured gingival fibroblasts: (A) The epithelial compartment fluoresces green, the cell nuclei of the in vitro gingival epithelium equivalent and the gingival fibroblasts located in the collagen cell culture matrix fluoresce red. (B) Green fluorescing gingival fibroblasts in the collagen cell culture matrix and red fluorescing fibroblast nuclei and gingival keratinocytes that have led to a multilayered epithelium on the surface of the cell culture matrix after 14 days of culture.

The in vitro co-cultures can be used for different applications. The researchers can use them to study the molecular mechanisms related to the maintenance of the natural tissue homoeostasis. Which proteins and signalling molecules ensure that the tissue remains in a healthy/normal state? Over the last few years, the Freiburg researchers have used molecular biology methods to investigate the signalling processes and have succeeded in identifying some signalling components.

The Freiburg researchers are also interested in molecular signal processing, for example when biomechanical forces act on the cells of periodontal, inflamed or injured gum tissue. Such investigations have the potential to contribute to the regeneration of injured gum tissue, for example, using biochemical and/or biomechanical strategies involving bioactive matrix molecules, cell-supporting growth factors or biomaterials with cell-specific biomechanical elasticity characteristics. “We can for example test whether and where the relevant biomarkers are expressed in the epithelium after a period of regeneration,” said Tomakidi. “This helps us to find out whether the tissue has reconstituted correctly and is fulfilling all important functions.”

Restoring tissue defects with biomaterials

Co-cultures can for example be used in biomaterials research, a field of research that has only gained in importance over the last few years. When a tissue defect occurs in the human periodontium, it can be repaired if suitable biomaterial is available. However, the biomaterial needs to be compatible with the tissue of the periodontium and needs to be able to grow effectively on it. “In cooperation with materials researchers, we are investigating the effects that biochemical/biomechanical compositions of novel biomaterials, made from polymers for example, have on the cells of epithelia and/or connective tissue,” said Tomakidi who, together with his team, will need to answer questions related to mechanical characteristics such as the elasticity of candidate materials that are mechanically linked with the natural tissue. On the other hand, the researchers are investigating the cell and tissue compatibility of the material components used. Do undesired metabolic degradation products occur when a material directly or indirectly interacts with the cells of target tissues? Do the materials compromise the homoeostasis of the gum tissue?

Such in vitro investigations are related to “interfacial problems”. “These questions can be addressed and partially answered in vitro using our cell systems,” said Tomakidi. “The complexity of our cell systems is closer to the natural gum tissue than standard test systems. In contrast to the standard test systems that mainly involve rodent cells, we are able to extract all cell types directly from the human target tissue and culture them. The interactive co-culturing of different cells, which effectively mimics the physiological cell-cell interactions, enables the development of in vivo relevant statements on putative material effects in terms of cell and tissue compatibility.”

From basic research to clinical and therapeutic benefit

The more Tomakidi’s team of researchers learn about the biochemical and biomechanical conditions of the periodontium at the cellular and molecular level, the more likely that it will one day be possible to use the basic research results for clinical and therapeutic applications. Amongst other things, what modern orthodontics will be able to achieve in the future will very much depend on the type of basic research that is carried out. “The challenges we are facing can only be solved by taking an interdisciplinary approach,” said Tomakidi. “Materials researchers, biomechanics, clinicians, biologists, physicists, chemists and biotechnologists need to join forces and work together. I am sure that the Freiburg University Medical Centre with its diverse academic research and teaching activities provides us with the necessary conditions for tackling periodontal challenges.”

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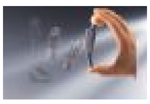

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