Structure and function of the human periodontium
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Citation for published version (APA):

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Chapter 9

Summary and general discussion
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The idea to apply 3D histochemistry to gingiva was born when a publication by Chung et al. (2013) appeared in Nature on CLARITY. The publication described the preparation and clearing of intact mouse brain for immunohistochemical analysis of neuronal networks in 3D. Clearing of intact mouse brain was based on electrophoretic tissue clearing by perfusion with solvents to remove lipids of cell membranes to render brains transparent. Furthermore, acrylamide monomers were infused for the generation of a polyacrylamide network in the brain that is devoid of cell membranes to provide it with an artificial skeleton. As we had ample experience with polyacrylamide scaffolds to image metabolism in individual cells by metabolic mapping (De Schepper et al., 1985; van Noorden et al., 1982, 1984), we decided to apply CLARITY to study blood vessels in gingiva in 3D. However, after 5 months of experimenting with various modifications of CLARITY (e.g. active and passive CLARITY), we realized that CLARITY was not the proper approach for 3D histochemistry of gingiva which is an extracellular matrix (ECM)-rich tissue. Right then, Renier et al. (2014) published a novel clearing method called iDISCO which was an improvement of 3DISCO that had been developed by Becker et al., 2012. iDISCO appeared to be a simple and fast clearing method that could be performed without the use of expensive equipment. Parallel to iDISCO, we decided to apply another technique called BABB. We applied a modification of BABB that was published by Dodt et al., 2007 and was a modification of the original BABB method that had been developed by Spalteholz (1911).

As described in Chapter 2 of this thesis (Azaripour et al., 2016), we learned that there are roughly 2 approaches to clear tissue for 3D microscopy. The first approach is to dissolve and remove all cellular lipid bilayers in a tissue. When a tissue consists mainly of cells without ECM such as brain or an embryo, this approach is sufficient to render the opaque tissue clear as is the principle of CLARITY. However, ECM is unaffected by clearing methods such as CLARITY. ECM can be considered as a dense protein network outside cells, for example, in basal laminas that form a border between adjacent tissues, and connective tissues such as stroma but also bone and cartilage. In fact, Spalteholz’s research in the early 20th century described correctly that the refractive indexes (RIs) of tissues and embedding media need to be similar in order to be able to image tissues without diffraction of light (Spalteholz 1911). Tissues containing ECM have a rather high RI whereas aqueous embedding media have significantly lower RIs. His solution was to replace water in tissue by hydrophobic media with
a RI that matches the RI of tissues containing ECM. BABB is such a medium that was
developed by Spalteholz (1911) and modified by Dodt et al. (2007).

Three steps are needed to achieve 3D images of an opaque tissue (Azaripour et al. 2016): first,
clearing of the opaque tissue to render it transparent for microscopy, second, fluorescence
labeling of compounds in the tissues that are the subject of the study and third, 3D imaging
including 3D image processing.

For 3D histochemistry of gingiva, the first step was achieved successfully using iDISCO and
BABB. After using various fluorescence dyes and microscopes, 3D images of blood vessels in
human gingiva were obtained for the first time after clearing gingiva with BABB, staining
with fluorescent dyes and imaging of gingiva immersed in DBE using light-sheet microscopy
and 3D image processing with Imaris software (Chapter 3). Future steps will be studies of
the effects of smoking and diseases such as diabetes on the 3D structure of blood vessel
networks in gingiva to establish whether the gingiva suffers in a similar way as the retina
from diabetes leading to hemorrhages due to the formation of leaky blood vessels induced by
VEGF. In that case, anti-VEGF treatment would be an option to limit damage to gingiva.

In severe periodontitis cases, additional use of antibiotics to the standard therapy of scaling
and root planing (SRP) is necessary, but the use of antibiotics may lead to resistance.
Furthermore, systemic treatment with antibiotics may cause unwanted side effects such as
allergies or gastrointestinal complaints. Photodynamic therapy (PDT) seems to be an
attractive bactericidal alternative to support and improve periodontal therapy with minor side
effects. The increasing numbers of clinical studies on the efficiency of PDT as adjunct therapy
of chronic periodontitis was the subject of a systematic review and meta-analysis that is
presented in Chapter 4. Pooled overall effects of 26 randomized clinical trials (RCTs)
demonstrate significant benefits of PDT adjunct to SRP after 3 and 6 months. However, the
effect after 6 months is limited from a clinical perspective. In most RCTs (24 out of 26
RCTs), only a single PDT treatment was performed. Therefore, we suggest to perform further
RCTs to investigate whether 2-4 PDT treatments pro year after non-surgical treatment during
the maintenance phase of periodontitis patients improves the clinical outcome on a long-term
basis.
After the meta-analysis of the clinical benefits of PDT, we applied an in-vitro study to investigate the effects of PDT in vitro on human gingival fibroblasts and osteoblasts to determine possible side effects under conditions that reflect the clinical situation in the periodontium of patients as much as possible (Chapter 5). Cells were analyzed for viability and migration capacity. Our findings indicate that PDT as is used in periodontitis patients has no significant negative side effects on gingival fibroblasts and osteoblasts in vitro, suggesting that PDT can be safely used for antimicrobial treatment in periodontal diseases.

The periodontal maintenance phase is as important as the periodontal therapy itself and plays a decisive role whether the long-term outcome is successful or not (Axelsson et al., 1991). Therefore, patients with periodontitis need to be enrolled in a periodontal treatment protocol. The removal of dental plaque and food debris is necessary to maintain oral health and plays an important role in the prevention of caries and periodontal diseases. Therefore, special toothpastes and mouthrinses can support the oral hygiene of periodontal patients during the maintenance phase. During the past decades, alternative medical treatment concepts have received attention especially among Western cultures. These concepts led to the development and marketing of oral health products with natural ingredients that are now promoted as alternatives for the established oral hygiene products. Recently, dentifrices with miswak extracts have become commercially available (Gupta et al., 2012). The chewing stick or miswak (meswak or siwak) which originates from trees of the family Salvadoracea (Salvadora persica) is the first known tooth-cleaning tool in history, and its use dates back as early as 3500 BC (Gurudath et al., 2012). In Chapter 6, the effectiveness of a Miswak extract-containing toothpaste was evaluated. Sixty-six patients were recruited and enrolled. After 3 weeks of brushing, the use of the Miswak extract-containing toothpastes caused a significant reduction in gingival inflammation and amount of plaque and it was it was concluded that it can be safely used for domestic oral hygiene in patients with gingivitis.

Additional chemical plaque control as part of domestic oral hygiene has always been playing an important role in the treatment of gingival inflammation (Löe and Schiott, 1970; Paraskevas and van der Weijden, 2006). Chlorhexidine digluconate (CHX) is currently considered to be the most effective antiseptic mouthrinse due to its high substantivity and strong anti-bacterial effects. However, CHX has side effects such as reversible dysgeusia, black hairy tongue and tooth discolorations (Van Strydonck et al., 2012; Eley, 1999; Gent et al., 2002; Gurgan et al., 2006) and is therefore not suitable for daily use in long-term
periodontal maintenance. As a consequence, the search for effective alternatives without side effects continues. In Chapter 7, the effects of aluminium triformate (ATF), an aluminium salt as active component in a commercially-available mouthrinse as an adjunct to mechanical oral hygiene in the maintenance phase of periodontal patients was evaluated. ATF is freely available without prescription at the pharmacy to be used for a period of maximally one week. Longer periods of application have to be under surveillance of a clinician. We have performed a short-term study as a pilot for a larger clinical trial for two reasons: 1° we wanted to test the compliance of patients who participate in the study and 2° we wanted to monitor side effects. Forty patients were enrolled and have completed this study. After a period of 7 days, a significant reduction in gingival bleeding was observed. On the basis of the patients’ evaluation reports, it was concluded that ATF mouthrinse seems to be safe and not causing major side effects. A long-term trial with a larger number of participants is needed to confirm these findings.

More than ever, esthetics play an important role in our society. Social competence of a person is at least partly evaluated by his or her physical appearance and presence. Also in dentistry, esthetics in personal appearances play an important role. White, healthy and perfectly ordered teeth are a sign of vitality, sympathy, assertiveness and erotic attraction. Orthodontic treatment is performed not only on the basis of medical indications, but also on the basis of the desire to obtain perfect rows of teeth and is not only requested by adolescent patients but also by adult patients. However, fixed orthodontic appliances (FOA) can promote accumulation of bacterial plaque. FOA limit good oral hygiene, which can lead to destructive periodontal processes (Chapter 8; Azaripour et al. 2015). In the majority of patients, particularly during childhood and adolescence, FOA are the treatment of choice. Because of esthetics, this treatment is not very popular for adult orthodontics. Therefore, other orthodontic techniques have been developed to increase esthetics and simplify oral hygiene procedures. An alternative for FOA is Invisalign® which has been available since 1999 and offers not only the advantage of improved esthetics but also the convenience of removal during food and beverage consumption, as well as oral care. The aim of our study was to compare periodontal health status and patients’ satisfaction during orthodontic treatment of patients with FOA and Invisalign® (Chapter 8). Thorough examination of 100 patients showed that a better periodontal health and greater patient satisfaction was achieved during orthodontic treatment with Invisalign®.
In conclusion, the research project that is described in the present thesis has been a joint effort of clinicians and cell biologists (science meets the clinician) to push the limits of our knowledge of the structure and function of the human periodontium in health and diseases. On the basis of my experiences in the past years in this research project, I have learned how fruitful such a cooperation is between the clinic and research institutes. I am determined to continue my career as a clinician with one foot in a life sciences research institute. It was an exciting experience.

References


Löe H, Schiott CR (1970). The effect of mouthrinses and topical application of chlorhexidine...
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References


