Guardians of the oral cavity
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CHAPTER 1

GENERAL INTRODUCTION
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The mouth

The oral cavity consists of the teeth, palate, tongue and oral mucosa. The oral mucosa is a mucous membrane that lines the inside of the oral cavity and consist of an epithelial and a connective tissue layer. There are 3 types of oral mucosa, the masticatory, the specialized and the lining mucosa. The masticatory mucosa covers the gingiva and hard palate and its epithelial layer is heavily keratinized to endure the forces that are placed on it during chewing. The specialized mucosa covers the tongue and is similar to the masticatory mucosa, with additional papillae and taste buds. The lining mucosa covers the other areas of the oral cavity and is highly flexible and not keratinized.

The main functions of mucosa in general are lining and protecting, as they form the largest and most important interface between the human body and its environment. Interaction with the environment is of vital importance for the uptake of oxygen and nutrients and for the excretion of waste products, but it also makes the body susceptible to damage and infection. The oral cavity is challenged by antigenic and toxic stimuli in food and in the air, as well as (pathogenic) bacteria and viruses. Food, heat, cold, mechanical forces and acidic food components all put a substantial amount of stress on the soft tissue in the mouth. Since the mouth is the entrance to the gastrointestinal and the respiratory systems, the mucosal tissues form a first line of defense against pathogens.

Saliva

Saliva covers the surfaces of the oral cavity and creates a micro-environment around the teeth and other structures in the mouth (e.g. tongue, mucosa). It is produced by the major salivary glands (parotid, submandibular, and sublingual glands) and many minor salivary glands (labial, buccal, lingual, and palatal glands). The fluid in the oral cavity is also called mixed saliva because it contains a mixture of contributions of the individual glands, which varies over time.

Saliva protects the oral cavity in many ways: it keeps the mouth moist, enables speech and taste, supports digestion, dilutes noxious materials, cleanses, and acts like a buffer as it contains bicarbonate and phosphate ions to control the pH of the oral cavity. Saliva is also a lubricant since specialized glycoproteins, namely mucins, present in saliva make it mucous and protect the lining mucosa by forming a physical barrier against toxins and other stresses. Additionally, saliva contains proteins with antibacterial properties such as lysozyme and lactoferrin, histatins and peroxidase, and others such as proline-rich proteins, salivary agglutinin, Immunoglobulin A antibodies and mucins that bind to bacteria, causing them to clump and impeding their attachment to oral surfaces, thereby making it easier to remove them. In the absence of saliva, for example when the salivary glands are dysfunctional due to radiotherapy in the head-neck area, the oral mucosa become highly vulnerable to infection and inflammation and the teeth become sensitive to chemical and mechanical wear. The key function of saliva, besides supporting the first step of food digestion, is keeping the teeth and the soft tissues in good condition.
Tissue integrity

Saliva contains a multitude of proteins and peptides that are involved in the protection of the oral cavity, thereby adding to the barrier function of the epithelium. The oral cavity is a part of the body that is heavily colonized and is an important point of entry for toxins and harmful microorganisms, therefore tissue integrity and an intact epithelial barrier are crucial to prevent penetration of such agents. Tissue integrity is maintained via cellular adhesion, which comprises both cell-substrate and cell-cell adhesion. There is a functional relation between cell attachment to the extracellular matrix and cell-cell adhesion via common molecules and connected signaling pathways. The expression adhesive crosstalk is commonly used to describe this relation. Besides maintaining tissue integrity, cell adhesion is also required for many cellular functions including cell communication and differentiation, as loss of adherence often leads to apoptosis.

The epithelial barrier depends on cell-cell adhesion through structures called desmosomes, adherens junctions and tight junctions that form connections between neighboring cells. The formation of adherens junctions is controlled by cadherins: calcium-dependent cell adhesion proteins. E-cadherins on adjacent cells interact with each other on the outside of the cell membrane through homophillic interaction, while they are also associated with the actin cytoskeleton on the inside of the cell membrane. E-cadherin is also involved in epithelial-to-mesenchymal transition (EMT) and is activated in the reversed process called mesenchymal-to-epithelial transition (MET)—the transformation of mesenchymal cells into epithelial cells. MET and EMT are important for the maintenance of tissue morphology, cell differentiation, cell polarity and barrier function. The barrier function of the epithelia in the oral cavity is of paramount importance to protect against stresses and assaults.

Histatins

Histatins (Hsts) are histidine-rich peptides present in the saliva of humans and higher primates. Hsts are derived from 2 genes, HTN1 and HTN3, encoding histatin 1 and 3, respectively. All other variants (Hst2, Hst4-12) originate from these two parent gene products. Hsts are secreted in human saliva by the parotid and submandibular glands. Hst1, 3 and 5 comprise about 85% of the total histatin concentration in human saliva.

A wide range of functions has been attributed to Hsts in general, with Hst5 being the subject in the majority of studies because of its ability to combat Candida infections. Hsts are implicated in processes such as inhibition of enamel demineralization, detoxification of noxious food constituents, and killing of bacteria and yeast. Hsts bind tannins, polyphenolic compounds from specific foods that can interfere with nutrient absorption. More recently, several studies reported that Hst1 and 2 enhance wound healing, as they promote wound closure in vitro.
SCOPE OF THE THESIS

Hst1 is the most abundant of the histatin peptides and has been attributed many functions, including a stimulating role in wound healing. But what is its role under healthy conditions when there is no wound present? Our hypothesis is that Hst1 helps to protect the oral cavity via maintaining the epithelial barrier and improving tissue integrity. In order to answer the question how the oral mucosa is affected by Hst1 we examined the effects of Hst1 in different cell types and model systems.

In chapter 2 we studied the effects of Hst1 with respect to cellular adhesion with a focus on the barrier function of epithelial cells. The effect of Hst1 on the epithelial barrier function was explored in detail in chapter 3. In chapter 4 we propose an application for Hst1 in dental implantology. Chapter 5 describes a protocol for the scratch or wound healing assay, including critical guidelines to tackle the problems associated with this assay. The protocol was used in chapter 3 to monitor the outgrowth of tumor cell spheroids. Finally, in chapter 6, we discuss the physiological functions of Hst1 with a focus on a possible role in pathologies.
REFERENCES