Interactions between host and oral commensal microorganisms are key events in health and disease status

Mahmoud Rouabhia PhD

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The oral cavity has sometimes been described as a mirror that reflects a person's health. Systemic diseases such as diabetes or vitamin deficiency may be seen as alterations in the oral mucosa. A variety of external factors cause changes in the oral mucosa, thus altering mucosal structure and function, and promoting oral pathologies (most frequently bacterial, fungal and viral infections). Little is known, however, about immune surveillance mechanisms that involve the oral mucosa.

There is no direct contact between specific immune cells in the basal epithelium and microorganisms in the upper layers of the oral mucosa. The author's hypothesis is that the protective immunity is conveyed through epithelial cells. The present brief review assesses the oral mucosa's role as the main defence in the interactions between the host and the oral microbial community. A unique model was used to investigate these interactions as the cause of oral disease and to develop new treatments that exploit our knowledge of the host-microorganism relationship.

Key Words: *Epithelial cells; Fibroblasts; Oral mucosa; Oral pathologies; Tissue engineering*

Relations entre l'hôte et les micro-organismes commensaux dans la bouche : différences clés entre la santé et les maladies

On a déjà dit que la bouche était le miroir de la santé d'une personne. Les maladies générales comme le diabète ou les carences vitaminiques peuvent se percevoir par des modifications de la muqueuse buccale. Différents facteurs externes peuvent agir sur la muqueuse buccale et, par le fait même, en altérer la structure et le fonctionnement, d'où création d'un milieu propice aux affections buccales, le plus souvent sous forme d'infections virales, fongiques ou bactériennes. Pourtant, on connaît peu de choses sur les mécanismes de surveillance immunitaire de la muqueuse buccale.

Il n'y a pas de contact direct entre les cellules immunitaires se trouvant dans les couches basales de l'épithélium et les micro-organismes se logeant dans les couches supérieures de la muqueuse buccale. L'auteur croit que la protection immunitaire passe par les cellules épithéliales. Le présent survol examine le rôle de la muqueuse buccale comme principal moyen de défense dans les interactions entre l'hôte et la faune microbienne buccale. Un modèle unique a été utilisé pour permettre l'étude des interactions à l'origine des affections buccales et la mise au point de nouveaux traitements qui tiendraient compte des relations entre l'hôte et les micro-organismes.

Faculté de médecine dentaire et Groupe de recherche en écologie buccale, Pavillon de médecine dentaire, Université Laval, Sainte-Foy, Québec
Correspondence and reprints: Dr Mahmoud Rouabhia, Faculté de médecine dentaire, Bureau 1728, Pavillon de médecine dentaire, Université Laval, Sainte-Foy, Québec G1K 7P4. Telephone 418-656-2131 ext 16321, fax 418-656-2861, e-mail mahmoud.rouabhia@FMD.ulaval.ca
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THE ORAL CAVITY ENVIRONMENT

More than six billion people inhabit this planet. This number is the same as the number of new microbial cells that are produced in 1h or 2h in each of our mouths (1). This complex oral microbiota contains more than 500 bacterial species (2), as well as many species of viruses and yeasts (Table 1). Multiple ecological niches maintain at least five major bacterial ecosystems (3-5). They are: bacteria on the tongue; bacteria on the buccal mucosa; tooth-adherent bacteria that are coronal to the gingival margin (supragingival plaque); bacteria that are apical to the gingival margin (subgingival plaque), and bacteria in the saliva.

Studies of a wide range of distinct ecosystems have shown that the vast majority of microorganisms exist often in nature as sessile communities called biofilms. These communities develop structures that are morphologically and physiologically different from free-living bacteria. The biofilm plaque that accumulates on tooth surfaces includes over 30 categories of microorganisms from 500 bacteria, yeasts, etc (6,7). Despite this complexity, plaque formation follows a distinct sequence. It begins with colonization by a group of Gram-positive organisms, mainly streptococci, followed by further colonization by a succession of species and culminating in the arrival of Gram-negative anaerobic bacteria such as *Porphyromonas gingivalis*, a predominant pathogen in severe adult periodontitis (8). Biofilms provide microorganisms with protected niches where they are safe from antimicrobial materials and can become a source of persistent infection.

The oral cavity is also the gateway for a wide array of antigenic challenges (9). These are represented by the substantial bacterial colonization that exists in the oral cavity. Many species of the complex oral microbiota maintain a symbiotic relationship with the host (10-12). To maintain homeostasis within the oral cavity, the host has two distinct but interrelated immune response systems: the salivary immune system (13-16) and the serum immune system (17,18). The changing balance of conditions in the mouth influences the stability and integrity of the oral mucosal tissues. The balance may be disturbed by either an increased local stress or a decreased innate immunity.

TABLE 1
Some species of microorganisms that constitute the oral microbial community

Virus	Bacteria species	Yeast
Cytomegaloviruses	*Porphyromonas*	*Candida*
Herpes simplex	*Prevotella*	*Rhodotorula*
Hepatitis	*Fusobacterium*	*Saccharomyces species*
Epstein-Barr virus	*Eubacterium*	
	Propionibacterium	
	Veillonella	
	Peptostreptococcus	
	Actinobacillus	
	Actinomyces	
	Neisseria	
	Streptococcus	
	Staphylococcus	

STRUCTURE AND FUNCTION OF THE ORAL MUCOSA

The mucosa of the oral cavity is composed of wet epithelium and underlying connective tissue. While modified by frictional forces, the oral mucosa, either lining or masticatory tissue, is composed of epithelium, which contains mainly keratinocytes, and a subjacent connective tissue (lamina propria), which contains mainly fibroblasts within an extracellular matrix. The epithelium and lamina propria are linked by an acellular region – the basement membrane. The major function of the oral mucosa is to protect the deeper tissues of the oral cavity (19,20). It also acts as a sensory organ (21) and serves as a site for some glandular activities. The normal activities of seizing, biting and chewing food expose the soft oral tissues to mechanical forces and surface abrasions. Both epithelium and the connective tissues of the mucosa adapt to this trauma and to the microorganisms that normally reside within the oral cavity and would cause infection if they were to access deeper tissues (22-24). These organisms produce many potentially toxic substances such as lipopolysaccharides and proteases.

The epithelium of the oral mucosa is the major barrier between the organism and the environment. Stratified, surface, squamous epithelium has been long considered to be a physical barrier to microbes, and recent studies have found that keratinocytes function as fixed or immobile immunocytes (25-27). Keratinocytes are capable of secreting a variety of pro-inflammatory cytokines (28-30), including interleukin-1b and the recently described interleukin-18 (31). These cytokines play a critical role in the development of protective immunity against intracellular pathogens (32,23). Contact with microorganisms also leads epithelial cells to produce a variety of antimicrobial proteins, including defensins (33,34). These cationic antimicrobial peptides are produced by neutrophils, macrophages and epithelial cells, and are important to the innate defence of a wide range of species (35).

Thus, the oral mucosa serves an immunological and biochemical function, rather than serving strictly as a physical barrier for the external environment. The mucosa remains vulnerable to environmental insults, including microbial infections. The mucosa's integrity and function depend on the stability of the immediate environment.

INTERACTION BETWEEN THE HOST AND THE ORAL MICROBIAL COMMUNITY

The host monitors and responds constantly to the colonizing organisms of the oral cavity (Figure 1). This includes nonspecific and specific mucosal immunity to maintain health and limit infection (36,37).

Control of infection by the host is managed through a highly efficient, innate host defence system that continual-

ly monitors the status of bacterial colonization and prevents bacterial intrusion into local tissues (38,39). Monocytes and macrophages in the mononuclear phagocyte system are a first line of defence against pathogenic microbes. Viral and bacterial infections activate multiple transcriptional systems and post-translational events in these cells, resulting in cytokine production. The stage of cellular differentiation of monocytes and macrophages may enhance the cell's capability to produce cytokines in response to bacterial and viral infections (40,41). On the other hand, bacteria have evolved mechanisms to ensure their survival and reproduction by monitoring their environment and evading or modifying the host as needed (37,39). Bacteria have adapted to the ecological niche that is provided by both the tooth surface and the gingival epithelium, as well as to the surrounding environmental conditions of the oral cavity; thus, a dynamic equilibrium usually exists between the oral microbial community (free microorganisms and dental plaque bacteria) and the host (42).

Under normal, 'healthy' conditions, the host receives the appropriate inflammatory stimulus from these commensal bacteria to maintain an effective but nondestructive inflammatory barrier against potential pathogens (43,44). It remains unclear how commensal oral microorganisms become pathogenic in the face of an intact immune surveillance system. The host's physical contact with the oral microbial community takes place through the oral mucosa, especially the epithelial cells (45). As it changes from a commensal form to a pathogenic form, a microbial community may induce significant changes in tissue structure, with a breakdown of oral homeostasis.

Studies have investigated this interaction by using three models – animals, cell lines that were grown in monolayers, and people. Animal models contribute substantially to the understanding of the biological basis of the development of human oral pathology that follows microorganism infection (46,47). Mouse models have helped to define cellular and molecular targets in the initiation of oral pathology, biochemical pathways involved in promotion of acute and chronic inflammation, and intracellular mechanisms of pathogenesis (48,49). Studies of cell lines that are grown in monolayers (50) are hampered by the absence of epithelial and lamina propria cell-cell interactions and matrix environments, which play an important role in intra- and intercellular communications (51,52). Finally, studies of people are limited by the small number of participants and the ethical problems that are associated with harvesting tissues when clinical intervention is not required; thus, an appropriate in vitro model to study the interaction between the host and the oral microbial community is needed to elucidate more fully oral pathology.

ALTERNATIVE MODEL FOR HOST-MICROORGANISM INTERACTION IN THE ORAL CAVITY

The past decade has seen remarkable advances in tissue engineering technology to create organoids, in vitro, from cells and cellular scaffolding. Tissue-engineered organoids such as skin (53) and cartilage (54), with comparatively simple architectures, are currently in the clinical stage of development (55).

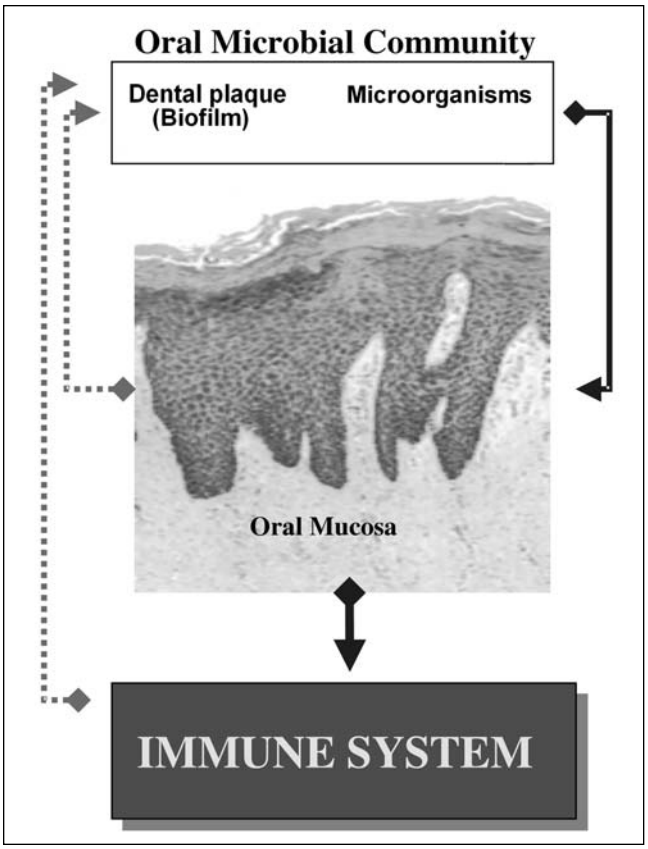

Figure 1) *Suggested schema that shows the possible interactions between the host and the oral microbial community, and the first contact between oral mucosa and oral microorganisms. This first contact can be initiated with free microorganisms of dental plaque (biofilm). There may be a systemic immune activation following this contact*

Using tissue engineering technology, the author's lab engineered human oral mucosa by isolating epithelial cells and fibroblasts from oral mucosa biopsies (56). Sequential seeding of fibroblasts into collagen, followed by epithelial cell seeding, forms a complex multilayered tissue that exhibits an orderly sequence of cell proliferation and differentiation (Figure 2). These multicellular properties differentiate engineered oral mucosa from traditional monolayer culture systems. As reported previously (52,53), fibroblasts play a critical role in directing epithelial differentiation. Indeed, epithelial cultures without fibroblast interaction show a reduced expression of regional epithelial differentiation markers. The cells that formed this model proliferated and remained viable and well organized on a multilayered tissue for several weeks. Monolayer cultures stopped growing shortly after confluence. This demonstrates that interaction between epithelial cells and fibroblasts is critical for tissue organization in a three-dimensional model.

Engineered oral mucosa is a powerful tool for developing future technologies in dental research. It is a stable, repro-

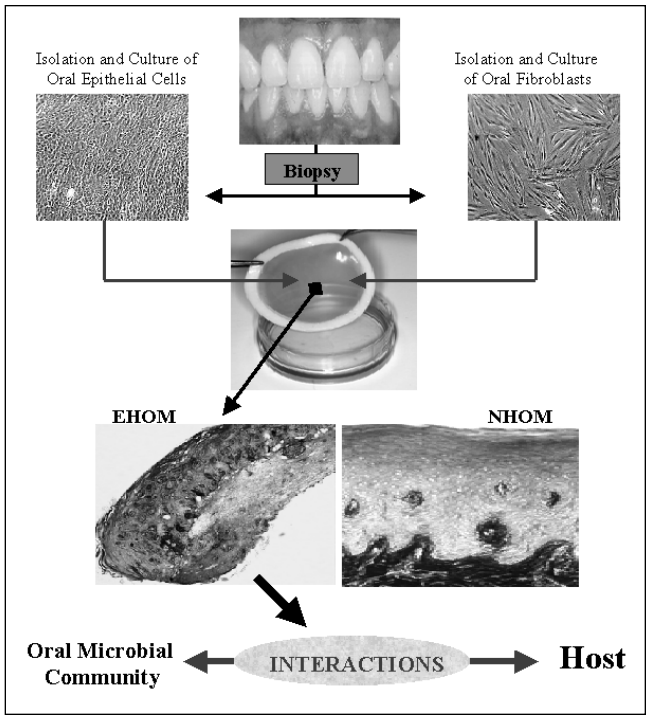

Figure 2) *Engineered oral mucosa tissues and their possible use in investigating the interaction between the host and the oral microbial community. This engineered tissue was produced with normal human oral epithelial cells and fibroblasts. EHOM Engineered human oral mucosa; NHOM Normal human oral mucosa*

ducible source of oral mucosa tissue for comparative studies, with the advantage of versatility. It allows the design of step-by step studies that incorporate each type of immune and nonimmune cell in the oral mucosa. This model is a potentially useful tool for the study of periodontal tissue responsiveness to different stimuli and pathological situations in the oral cavity. It should be a powerful tool for modeling the interaction between the host and the oral microbial community.

CONCLUSIONS

The host monitors and responds constantly to bacterial colonization in the oral cavity. Oral organisms engage the host in an intricate cellular and molecular dialogue, the outcome of which usually serves to constrain the bacteria in a commensal state. Studies of people with impaired innate host response demonstrate that a normal, innate inflammatory response is necessary for periodontal health. The examination of innate host responses in clinically healthy people has revealed a low-level, 'inflammatory surveillance' state, in which the host maintains an effective barrier against bacterial infection. Normal oral microflora may not only form a series of nonpathogenic commensal communities, but may also participate in establishing this protective state, thus functioning as symbiotic partners with the host. Mechanisms of bacterial recognition that should help to explain how members of the microbiota maintain an effective host-defense barrier are emerging. Also, studies of the host activation potential of periodontal pathogens suggest that dysfunctional host responses may contribute to pathogenesis. Additional studies to examine the bacterial and host dynamic, through appropriate models such as engineered human oral mucosa in vitro; and through clinically normal and diseased hosts in vivo, will better describe the creation of different microbial communities and their interaction with the host.

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