Epithelial–Mesenchymal Interactions as a Working Concept for Oral Mucosa Regeneration

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Oral mucosa consists of two tissue layers, the superficial epithelium and the underlying lamina propria. Together, oral mucosa functions as a barrier against exogenous substances and pathogens. In development, interactions of stem/progenitor cells of the epithelium and mesenchyme are crucial to the morphogenesis of oral mucosa. Previous work in oral mucosa regeneration has yielded important clues for several meritorious proof-of-concept approaches. Tissue engineering offers a broad array of novel tools for oral mucosa regeneration with reduced donor site trauma and accelerated clinical translation. However, the developmental concept of epithelial–mesenchymal interactions (EMIs) is rarely considered in oral mucosa regeneration. EMIs in postnatal oral mucosa regeneration likely will not be a simple recapitulation of prenatal oral mucosa development. Biomaterial scaffolds play an indispensable role for oral mucosa regeneration and should provide a conducive environment for pivotal EMIs. Autocrine and paracrine factors, either exogenously delivered or innately produced, have rarely been and should be harnessed to promote oral mucosa regeneration. This review focuses on a working concept of epithelial and mesenchymal interactions in oral mucosa regeneration.

Introduction

ORAL MUCOSA PLAYS a crucial role as a barrier against exogenous substances and pathogens. Oral mucosa can be lost after tumor resection, trauma, or chronic diseases. Currently, oral mucosa loss frequently is treated with the patient's own (autologous) skin or mucosa grafts. However, current treatments have persistent drawbacks including donor site morbidity and limited donor site availability. Tissue engineering offers important tools for the regeneration of oral mucosa, potentially minimizing donor morbidity and overcoming issues of limited donor site availability. The present review proposes integrated biological and bioengineering approaches of oral mucosa regeneration with a focus on the adoption of epithelial–mesenchymal interactions (EMIs), which is pivotal for the development of multiple ectodermal and mesodermal/mesenchymal structures including oral mucosa. Thus, the present review covers an aspect of oral mucosa regeneration that previous meritorious reviews on oral mucosa biology and regeneration have rarely addressed.^{1–3} We recognize the paucity of experimental data in the area of oral mucosa regeneration that purposefully incorporates EMI and, therefore, have relied on some of the development studies and dermal regeneration studies for important clues along with our speculation of how EMI may facilitate oral mucosa regeneration.

Clinical Need for Oral Mucosa Tissue Engineering

Surgeons are frequently confronted with the shortage of oral mucosa for reconstruction of the oral cavity. Defects in oral and craniofacial tissues resulting from trauma or post-neoplastic ablation present a formidable challenge for reconstruction.^{2,4,5} There is also a need for a graft to repair congenital abnormalities such as cleft palate surgery⁵ or vestibuloplasty.⁶ Treatment of periodontal pathologies affecting oral mucosa often requires surgical reconstruction or even substitution of the excised tissues. Currently, oral mucosa defects are restored by skin and secondary epithelization, microvascular graft, mucosal grafts, or flaps.^{7,8} However, current surgical approaches have a number of deficiencies. Because of the limitations of oral mucosa grafts, surgeons have explored the use of skin grafts from radial forearm, lateral arm, anterolateral thigh flaps, or free-tissue transfer.^{4,9–12} Although autologous skin grafts are generally tolerated in the oral cavity, oral mucosa differs from the skin in many aspects. Oral mucosa is mostly nonkeratinized and moist with saliva at all times. The use of skin grafts frequently causes complications such as unwanted hair growth and excessive keratinization in the oropharyngeal recipient site.¹⁰ Skin harvesting also results in donor site morbidity.

Clinical uses for a tissue-engineered mucosa graft include not only intraoral defects,¹³ such as repair of acquired or

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congenital oral mucosal defects,¹⁴ but also the reconstruction of the cornea,¹⁵ eyelids, conjunctiva,¹⁶ esophagus,¹⁷ trachea,¹⁸ bladder,¹⁹ urethra,¹⁹ or vagina.²⁰ Other potential uses of tissue-engineered mucosa grafts are *in vitro* models to study the biology and pathology²¹ and as a vehicle for delivery and expression of transduced genes.²⁰ In reconstructed oral mucosa, the morphology and protein expression pattern such as that of keratins are similar to that in the native oral mucosa, but the epithelium is typically thinner.^{21–26} In general, rete ridge formation is limited and the basement membrane is not fully developed. Cultured oral mucosa equivalents have been applied in patients following resection of mucosal tumor with some levels of short-term clinical success.^{14,27} One of the goals of tissue engineering is to shorten *in vitro* cultivation time and apply tissue-engineered constructs *in vivo* at early time points in *in situ* regeneration approaches.²⁸

Stem/Progenitor Cells of Oral Epithelium

Oral mucosa consists of two tissue layers: the superficial epithelium and the underlying lamina propria. The epithelium consists of densely packed keratinocytes in four arbitrary layers: the basal, spinous, granular, and keratinized layers. The epidermis is a dynamic epithelium and undergoes constant renewal. Thus, it is not surprising that stem/progenitor cells are identified in adult epithelium, primarily in the basal layer.^{29–32} The basal layer harbors three cell subpopulations: stem/progenitor cells, transient amplifying cells, and post-mitotic differentiating cells.^{31,33} Transit-amplifying cells divide only three to five times before their daughter cells start to terminally differentiate.^{29,31,34} Stem/progenitor cells residing in the basal layer continue to migrate toward epithelium surface as they differentiate. Keratinocytes in oral mucosa exhibit regional diversity.^{30,35} In summary, we need to learn a great deal more about putative populations of oral mucosa stem/progenitor cells, including their stemness markers, self-renewal behavior, differentiation potential, and *in vivo* regenerative capacity. Tangible cell sources that can be used for oral mucosa regeneration are provided in Table 1.

Fibroblasts of Oral Submucosa

The dermal component plays important structural and physiological roles in oral mucosa. Lamina propria underlies and supports oral epithelium. In most regions of oral cavity, submucosa is a layer of loose fatty and/or glandular connective tissue that underlies the lamina propria. Lamina propria and submucosa determine the flexibility of the attachment of the oral mucosa. In the gingiva and hard palate, oral mucosa is directly attached to the periosteum of the underlying bone without submucosa, known as mucoperiosteum, which provides a firm, inelastic attachment.^{46,47} The lamina propria is composed of a highly woven network of type I collagen mixed with type III collagen, elastic fibers, glycosaminoglycans, proteoglycans, and glycoproteins. Located within this meshwork are capillaries, fibroblasts, and mesenchymal stem/progenitor cells. In addition to mesenchymal stem/progenitor cells, oral submucosa contains other cell populations including fibroblasts, blood vessel cells including endothelial cells, pericytes, and smooth muscle cells, peripheral nerve fibers and Schwann cells, and immune system cells including neutrophils, macrophages, and lymphocytes. The appendages of the oral mucosa are mainly small salivary glands and few sebaceous glands.

What we now know as mesenchymal stem cells were first identified as colony-forming unit fibroblast-like cells in the 1970s.^{48,49} Numerous reports have demonstrated that bone marrow, adipose tissue, tooth pulp, etc., contain a subset of cells that not only are capable of self-renewal for a number of passages, but also can differentiate into multiple end-stage cell lineages that resemble osteoblasts, adipocytes, chondrocytes, myoblasts, etc.^{43,50} Our understanding of mesenchymal stem cells (MSCs) has advanced tremendously because of their demonstrated and perceived therapeutic capacity.^{50–58} Why are MSCs perceived superior to autologous tissue grafts in the regeneration of human tissue and organs? Autologous tissue grafts often represent the current clinical gold standard for the reconstruction of defects resulting from trauma, chronic diseases, congenital anomalies, and tumor resection. However, autologous grafting is based on the concept that a diseased or damaged tissue must be

TABLE 1. CELL SOURCES FOR ORAL MUCOSA REGENERATION

<i>Epithelial stem/progenitor cells</i>	<i>Dermal/mesenchymal stem/progenitor cells</i>
<ol style="list-style-type: none"> 1. Oral mucosa or other epithelial cells including keratinocytes³⁶ 2. Epithelial cells from skin biopsy³⁷ 3. Bone marrow stem cells via mesenchymal–epithelial transition³⁸ 4. Adipose stem cells that differentiate into keratinocytes³⁹ 5. Other stem cells including induced pluripotent stem cells, embryonic stem cells, and umbilical cord blood stem cells⁴⁰ 	<ol style="list-style-type: none"> 1. Foreskin fibroblasts⁴¹ 2. Dermal fibroblasts from biopsy samples⁴² 3. Bone marrow MSC-derived fibroblasts⁴³ 4. Fibroblasts from oral mucosa biopsy samples²⁰ 5. Oral mucosa fibroblasts from soft tissue removed during tooth extraction or alveolar surgeries⁴⁴ 6. Epithelial stem/progenitor cells via epithelial–mesenchymal transition 7. Adipose stem cells that differentiate into mesenchymal and fibroblast lineages 8. Other stem cells including induced pluripotent stem cells, embryonic stem cells, and umbilical cord blood stem cells⁴⁵

MSC, mesenchymal stem cell.

replaced by like tissue that is healthy. Thus, the key drawback of autologous grafting is donor site trauma and morbidity. For example, healthy cartilage must be surgically isolated to repair arthritic cartilage. A patient who receives an autologous soft tissue graft for facial bone reconstruction may be hospitalized for extended stay because of donor site trauma and morbidity, beyond the normal morbidity of the facial surgery. Also, spare healthy tissue is scarce in patients who suffer from loss of oral mucosa. In contrast, MSC-based therapeutic approaches may circumvent the key deficiencies associated with autologous grafting. First, MSCs may act as progenitors of replacement cells and/or signaling cells that interact with immune-competent cells and vascular endothelial cells.^{59,60} Second, MSCs can differentiate into multiple cell lineages, thus providing the possibility that a common cell source can heal many tissues, as opposed to the principle of current surgical practice to heal a defect by healthy tissue. Despite common practice of MSC differentiation into osteoblasts, chondrocytes, and adipocytes,^{59,60} little is known whether MSCs differentiate into fibroblasts until our recent report.⁴³

One of the key players of submucosa is fibroblasts. Fibroblasts are ubiquitous cells and constitute the stroma of virtually all tissues. Fibroblasts play irreplaceable roles in homeostasis of oral mucosa and are responsible for the synthesis of collagen fibers. Upon injury, fibroblast contraction of granular tissue is a process of normal wound healing.^{61,62} Thus, collagen synthesis is an important component of oral mucosa development and regeneration. Importantly, scar formation is minimal in oral mucosa following wounding. To date, it is unclear why scar tissue formation is minimal in oral mucosa, similar to fetal wound healing. In pathological wound healing, the activation of fibroblasts by acquiring α -smooth muscle actin phenotype and excessive contractility are among the factors responsible for fibrosis or aberrant scarring,^{63–65} including keloids and hypertrophic scars to which there is currently no satisfactory therapy.^{64,66} We recently demonstrated that fibroblasts derive from bone marrow MSCs.⁴³ Clonal analysis showed that single clonal progenies of bone marrow MSCs differentiate into common mesenchymal lineage cells such as osteoblasts, chondrocytes, adipocytes, and most importantly, fibroblasts.⁴³ The mesenchymal origin of fibroblasts provides possibilities of a new cell source for oral mucosa regeneration. For example, in a patient with trauma in oral mucosa, it is now possible to derive fibroblasts from bone marrow MSCs, which can be readily

isolated and expand from a clinically accepted bone marrow aspiration procedure. Otherwise, additional oral mucosa trauma will need to be induced for oral mucosa regeneration.

Biomaterial Needs for Oral Mucosa Regeneration

Scaffolds for mucosa reconstruction include synthetic materials, natural derivatives such as acellular dermis, extracellular matrix (ECM) protein-based scaffolds, and hybrid scaffolds of both natural and synthetic matrices (Table 2). Historically, a common technique for fabricating epidermal sheets from a minimal skin biopsy has been described.⁶⁷ This approach has been widely adopted in skin and oral mucosa cell culture studies. Initially, irradiated mouse 3T3 fibroblast feeder cells were incorporated. However, the use of bovine serum-based culture and murine feeder layers is problematic for clinical applications because of potential pathogen transmission. Bovine serum may also elicit xenoinmunization against bovine antigens that may escape irradiation or the mitomycin C treatment.⁶⁸ Oral epithelial cell culture is similar to skin keratinocytes but has several important differences. Oral epithelial cells appear to proliferate at a higher rate than skin keratinocytes.⁶⁹ Cell morphology and keratin expression of cultured oral epithelial cells seem to depend on the site of origin in the oral cavity. During the past 20 years, epidermal sheets have been applied for the reconstruction of oral mucosal defects.^{70–72} The cultured keratinocyte grafts enhance healing process and promote reepithelialization. However, epidermal sheets frequently lack a dermal component and are difficult to handle. Epidermal sheets are also susceptible to wound contraction and scarring in full-thickness oral mucosa defects.

Synthetic materials that have been used as a dermal substrate include polymers such as poly-(L)-lactic acid and polyglycolic acid in the form of films, foams, and sprays (Table 2). The advantages of synthetic materials are their reproducible mechanical and physical properties and unlimited availability.³ Synthetic materials can be made turnable as a function of *in vivo* oral mucosa regeneration needs, although this area has rarely been exploited. Specifically, degradation rate of synthetic materials can be controlled to synchronize with the rate of oral mucosa regeneration. However, synthetic materials may elicit a foreign body response and may facilitate infections leading to the formation of dense scars and fibrosis.

TABLE 2. SCAFFOLD OPTIONS FOR ORAL MUCOSA REGENERATION

Materials	Pros	Cons	Representatives
Synthetic materials	Reproducible; turnable structural and mechanical properties; unlimited availability	Cyto- and biocompatibility	Poly-(L)-lactic acid, polyglycolic acid
Acellular dermis	Preservation of basement membrane	Suboptimal cell ingrowth	Deepidermized dermis, AlloDerm
ECM protein-based scaffolds	Biocompatibility	Suboptimal mechanical and physical properties	Collagen, fibrin, hyaluronic acid
Hybrid scaffolds	Improved biodegradable and biocompatible properties	Complicated production process	Combined natural and synthetic matrices

ECM, extracellular matrix.

ECM protein-based scaffolds are derived from human or animal tissues (Table 2). Collagen is a natural substrate and is generally biocompatible and nontoxic. Purified collagen has been used in the form of gels, sponges,⁷³ meshes, and membranes.⁷⁴ However, collagen has a tendency to shrink and lose volume upon cell seeding or *in vivo* applications. In addition, dermal substitutes made of collagen are not stable and can undergo rapid degradation. Collagen scaffolds implanted as skin and oral substitutes in animal experiment are biocompatible and promote wound healing.^{73,75} Human fibrin has been used as a dermal substitute to construct different tissue substitutes and has the advantages of low cost, availability, and cytocompatibility. In contrast to collagen gels, fibrin gels are less likely contracted by fibroblasts.⁷⁶ Fibrin sealant promotes skin and mucosal wound healing *in vivo*.^{77,78} However, the initial mechanical properties of fibrin are not sufficient for abrasive and microbial environment of the oral cavity because of a lack of barrier function. Hyaluronic acid, another natural polymer, has been used in mucosa regeneration. Hyaluronic acid is one of the macromolecules of the ECM with the advantage of structural conservation among species. Hyaluronic acid is hypoallergenic, is water soluble, and has homeostatic properties.^{79,80} Hyaluronic acid spongy matrix combined with collagen gel is biodegradable and acceptable for wound bed *in vivo*.⁸¹

Natural derivatives include deepidermized dermis and AlloDerm (Table 2). AlloDerm is a cadaveric dermal graft that is processed for deepithelialization and decellularization to produce a completely acellular dermal matrix. AlloDerm is believed to act as a scaffold for the migration of host fibroblasts and retains its basement membrane complex to facilitate attachment of surface epithelium. However, sub-optimal cell ingrowth and migration have been observed using deepidermized dermis as a scaffold for skin and mucosal regeneration.²¹ Natural derivatives are also used for intraoral resurfacing in gingival augmentation procedures, cleft palate repair,⁸² or reconstructions after tumor resection.⁸³

Composite substrates are composed of two or more polymers, naturally derived and/or synthetic, are designed with a goal to combine the advantages of biodegradable and biocompatible properties of native and synthetic polymers (Table 2). From a development standpoint, scaffold materials for oral mucosa regeneration should accommodate separate layers of epithelial cells and underlying mesenchymal cells with a basement membrane in between. However, little is known on the interactions of epithelial cells and mesenchymal cells in the repair of full-thickness oral mucosa defects *in vivo*.⁸¹ An additional challenge is numerous autocrine and paracrine cues that regulate the EMI process. In oral mucosa regeneration, multiple reiterations of *in vitro* and *in vivo* experiments are likely needed to identify effective strategies to take advantage of putative EMI in the adult wound healing environment.

Summary and Future Directions

Oral mucosa provides a barrier between the underlying tissue and the external environment. Reconstructions of the oral cavity after tumor resection, congenital anomalies, and trauma pose an acute and unmet clinical need on oral mucosa regeneration. Ideally, an engineered oral mucosa

equivalent should approximate the properties of the original tissue during functional recovery. Future directions for oral mucosa regeneration are perceived as follows:

1. Further incorporation of EMIs in oral mucosa regeneration is needed. EMIs are understudied and rarely utilized concept in the regeneration of oral mucosa. Given that EMI is indispensable in native development of oral mucosa, there are reasons to believe that manipulation of EMI will enhance oral mucosa regeneration.
2. Composite biomaterials are those that accommodate separate cell behavior and functions. In oral mucosa regeneration, composite biomaterials are likely of increasing relevance and may promote EMI. However, oral mucosa regeneration needs to be realized primarily in adult human patients. Therefore, EMI in native development may need to be modified to maximize the outcome of oral mucosa regeneration.
3. Although cell transplantation is likely obligatory, additional studies should pay attention of roles of host endogenous cells in regeneration of oral mucosa. In a conducive environment, host endogenous cells are capable of being recruited to reside in an anatomical compartment. It is important to delineate the relative contribution between transplanted cells and host cells. Recently, we showed that transforming growth factor β 3-induced cell homing was sufficient to regenerate an entire synovial joint surface with cartilage and subchondral bone.²⁸ If a single protein, in this case, transforming growth factor β 3, can regenerate complex tissues of cartilage and bone, there is a reason to believe that endogenous cell homing may play important roles in oral mucosa regeneration that has not been recognized earlier.

In summary, previous work in the field of oral mucosa regeneration has yielded important clues for proof of concept. Increasingly integrated biology and biomaterial/engineering approaches will further advance the field of oral mucosa regeneration. EMIs between oral epithelium and submucosal connective tissue are projected to be a pinnacle for successful regeneration of scalable oral mucosa for ultimate clinical applications.

Acknowledgments

The authors thank Michael Diggs and Kening Hua for administrative assistance. This work was supported by grants from the National Natural Science Foundation of China (No. 30970740 to L.C.) and the China Hubei Provincial Health Department (No. JX4B10 to L.C.). This work was supported by NIH grants RC2DE020767 and R01EB006261 (to J.J.M.).

Disclosure Statement

No competing financial interests exist.

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Received: August 19, 2010

Accepted: November 3, 2010

Online Publication Date: December 27, 2010