Japanese Dental Science Review 59 (2023) 365–374

Contents lists available at ScienceDirect

Japanese Dental Science Review

journal homepage: www.elsevier.com/locate/jdsr

Recent trends and perspectives in reconstruction and regeneration of intra/extra-oral wounds using tissue-engineered oral mucosa equivalents

Kenji Izumi [a,*,1], Witsanu Yortchan [a,b], Yuka Aizawa [a,c], Ryota Kobayashi [a,c], Emi Hoshikawa [a,d,2], Yiwei Ling [e], Ayako Suzuki [a]

[a] Division of Biomimetics, Faculty of Dentistry & Graduate School of Medical and Dental Sciences, Niigata University, Japan
[b] Department of Preventive Dentistry, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand
[c] Division of Oral and Maxillofacial Surgery, Faculty of Dentistry & Graduate School of Medical and Dental Sciences, Niigata University, Japan
[d] Division of Periodontology, Faculty of Dentistry & Graduate School of Medical and Dental Sciences, Niigata University, Japan
[e] Division of Bioinformatics, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

ARTICLE INFO

Keywords:
Oral mucosa
Tissue engineering
Clinical applications
Biomimetics
Scaffold

ABSTRACT

Many conditions, including cancer, trauma, and congenital anomalies, can damage the oral mucosa. Multiple cultures of oral mucosal cells have been used for biocompatibility tests and oral biology studies. In recent decades, the clinical translation of tissue-engineered products has progressed significantly in developing tangible therapies and inspiring advancements in medical science. However, the reconstruction of an intraoral mucosa defect remains a significant challenge. Despite the drawbacks of donor-site morbidity and limited tissue supply, the use of autologous oral mucosa remains the gold standard for oral mucosa reconstruction and repair. Tissue engineering offers a promising solution for repairing and reconstructing oral mucosa tissues. Cell- and scaffold-based tissue engineering approaches have been employed to treat various soft tissue defects, suggesting the potential clinical use of tissue-engineered oral mucosa (TEOMs). In this review, we first cover the recent trends in the reconstruction and regeneration of extra-/intra-oral wounds using TEOMs. Next, we describe the current status and challenges of TEOMs. Finally, future strategic approaches and potential technologies to support the advancement of TEOMs for clinical use are discussed.

1. Introduction

Significant tissue damage and loss of integrity in the oral mucosa caused by major trauma, congenital deformities, and pathological lesions that are unamenable for primary closure pose significant challenges to treatment. These critically sized oral mucosa defects cannot be left open owing to the risk of microbial infection, excessive fluid loss, pain, foreign material contamination, wound contracture, and scarring when healed by secondary intention [1]. Therefore, it is crucial to cover these defects with tissue or biomaterial grafts to prevent severe functional deficits, such as difficulties in chewing, swallowing, and speech, and to promote proper wound healing. Various reconstructive surgical methods have been employed for intraoral wounds and soft tissue defects, including split and full-thickness skin grafts, free oral mucosa grafts, local flaps, pedicled distant flaps, and microvascular flap transfers. While autologous grafts from other epithelial tissues remain the gold standard for critical-sized defects, this approach has drawbacks, such as donor-site morbidity, limited tissue availability, and poor integration. Therefore, alternative approaches are needed to replace or regenerate intraoral wounds and oral mucosa defects. Tissue engineering is a promising solution to address the limitations of autologous grafts, offering a way to compensate for the lack of autologous tissue and avoid the complications associated with graft harvesting. Thus, regenerative medicine and tissue engineering have contributed significantly to our understanding of tissue regeneration mechanisms and the development of reconstructive products for transplantation into tissue defects. These advances are of significant interest in oral and craniomaxillofacial surgery.

Previously, we reviewed the progress of tissue-engineered oral mucosa (TEOM), mostly at pre-clinical and clinical levels [2]. In this

* Corresponding author.
E-mail address: izumik@dent.niigata-u.ac.jp (K. Izumi).
[1] 0000-0002-6302-6828.
[2] 0000-0001-7008-7154

https://doi.org/10.1016/j.jdsr.2023.10.002
Received 25 July 2023; Received in revised form 25 September 2023; Accepted 19 October 2023

updated review, we focus on recent trends in reconstructing and regenerating extra- and intraoral wounds using tissue engineering of the autologous oral mucosa, emphasizing its clinical relevance instead of in vitro models for research evaluation. Furthermore, this review highlights the potential strategies of TEOMs, regardless of their components (acellular/cellular, natural/synthetic biomaterials, and monolayered/bilayered), and discusses perspectives and future directions to expedite clinical translation in regenerative dentistry.

2. Overview of the functions and structure of oral mucosa

The oral mucosa performs several clinical functions, including barrier protection, sensation, and secretion. The oral cavity is constantly exposed to mechanical, chemical, and biological challenges from daily activities and an intrinsically moist environment, and its primary function is to protect the underlying tissues from life-threatening trauma, infection, and dehydration. This barrier protection is achieved by multilayered oral epithelial cells, cell-cell junctions, and immune cells, which serve as both physical and immune barriers [3].

The oral mucosa comprises three layers. The surface is structured by a squamous stratified epithelial layer consisting of keratinocytes, with thickness and keratinization varying according to the region and functional requirements (i.e., masticatory and lining mucosa) [4]. Underneath the epithelium lies the lamina propria, a layer of connective tissue composed of blood vessels, nerves, fibroblasts, and macrophages. Type I collagen fibrils form a major component of the extracellular matrix (ECM). The undulating projections of the deeper layers of the epithelium were attached to the underlying papillary projections of the lamina propria. The nanostructure of the basement membrane lies underneath the epithelium and supports the connection of the lamina propria through hemidesmosomes. [5]. Some regions also have a submucosal layer containing adipose tissue, minor salivary glands, vasculature, lymphoid tissue, and muscles [6].

Restoring oral mucosa functions and structures can be achieved by enhancing regenerative abilities or developing biological tissue substitutes that compensate for the lost structures and functions. Tissue engineering has great potential for effectively facilitating reconstruction without the need for autologous intact tissue harvesting by generating a bioengineered solution. Options include using an oral mucosa epithelial cell sheet, off-the-shelf biomaterials such as acellular matrices, or a newly engineered equivalent scaffold seeded with cells consisting of the oral mucosa.

3. Updates of tissue-engineered oral mucosa (TEOM)

3.1. Clinical application for extraoral wound treatment

Based on recent literature reviews of TEOMs for human clinical use [7–9], along with our own research, a significant breakthrough and noteworthy development has been observed. Since 2015, our previous review was published, three autologous TEOMs have become available on the market worldwide.

3.1.1. Corneal epithelium

Two TEOMs have been commercialized in Japan as therapeutic options for limbal stem cell deficiency and severe ocular surface diseases. The first product, a cultured autologous oral mucosa epithelial cell sheet (COMECs) (Ocural®, J-TEC, Gamagori, Aichi, Japan), was developed using a cell sheet engineering (CSE) system. It was first used in a clinical trial by Nishida *et al.* [10] to treat bilateral limbal stem cell deficiency. One crucial step in the CSE system is to harvest intact cell sheets from the culture surface without disturbing the signaling proteins and molecules that promote cellular functions and biological processes. Among several systems designed to harvest cell sheets without using enzymes such as trypsin, autologous COMECs generated in temperature-responsive culture dishes were successfully transplanted into four patients for the first time in 2004. This groundbreaking study confirms the clinical applicability of COMECs transplantation in restoring visual acuity in patients with bilateral limbal stem cell deficiency. Since then, autologous COMECs have been used to reconstruct severe ocular surface diseases and have been evaluated by numerous institutes, demonstrating their safety, feasibility, and effectiveness in restoring visual function, as evidenced by their clinical success rates and long-term follow-up [11].

Another notable product is a bi-layered TEOM consisting of autologous oral mucosa keratinocytes and denuded human amniotic membrane (hAM) (Sakracy®, Hirosaki LI Inc., Hirosaki, Aomori, Japan). This product originated in a human clinical trial reported by Nakamura *et al.* in 2004 [12]. Clinical trials of ex vivo cultivated oral mucosal epithelial transplantation, known as COMET, have also been conducted worldwide [13] and have been applied for treating the scar phase of severe ocular surface disorders [14]. As a surgical waste with no ethical issues, hAM is a sufficient, cost-effective, readily available biomaterial that serves as a scaffold for TEOM. Its clinical usefulness stems from its biocompatibility, low immunogenicity, and various beneficial biological and mechanical properties, including anti-inflammatory and antifibrotic effects and transparency [15]. The safety and efficacy of COMET and its long-term follow-up for symblepharon release and fornix reconstruction in eyes with chronic cicatrization have also been demonstrated [16]. However, the behavior of oral keratinocytes in grafts and their long-term survival after transplantation remain unclear. Therefore, researchers must evaluate the presence of oral keratinocytes on the ocular surface to confirm the clinical outcomes of COMECs and COMET. Therefore, there is a need for a univocal oral keratinocyte marker to identify regenerated oral mucosa and understand clinical outcomes [7,17]. Recent case reports on COMECs transplantation concluded that progenitor/stem cells originating from autologous oral keratinocytes were successfully engrafted [18]. Although fabricating COMECs and COMET incurs high healthcare costs because they are regenerative medical products for orphan diseases, their advancements have significantly propelled the tissue engineering of the oral mucosa. As a result, oral keratinocytes have emerged as significant players in regenerative medicine, leading to the reconstruction and regeneration of extraoral epithelial tissue.

3.1.2. Urethral epithelium

The autologous tissue-engineered buccal mucosa (TEBM), as another TEOM, has been used for urethral reconstruction as a substitute for autologous buccal mucosa graft (MukoCell®, Urotiss Europe GmbH, Dortmund, Germany). Initially approved by Germany, it was authorized as an advanced medicinal drug product by the European Medicine Agency (EMA) and is now available on the market [19]. The prototype of MukoCell® was initially generated as an in vitro TEBM, where oral keratinocytes and fibroblasts were seeded together onto de-epidermized dermis [20]. Subsequently, clinical applications for the therapeutic use of TEBM and its clinical outcomes in five patients were reported, resulting in the survival of three patients with TEBMs in a 9-year follow-up [21,22]. Barbagli *et al.* presented the first case study of urethroplasty using the current form of MukoCell® for 12 patients. TEBM was fabricated by seeding epithelial cells obtained from the buccal mucosa on a biocompatible collagen scaffold and cultured for three weeks, indicating a protocol change with a different scaffold and without the use of oral fibroblasts [23]. Afterward, according to studies by Ram Liebig *et al.* and Karapanos *et al.*, a biodegradable membrane and a protein-containing biodegradable scaffold of animal origin was used as a scaffold, respectively [19,24]. Therefore, the exact composition of the scaffold used in MukoCell®, a substitute for autologous buccal mucosa graft, is unknown [22]. Schwab *et al.* concluded that the most suitable collagen-based biodegradable membrane to fabricate TEBM for genitourinary reconstruction was a bi-layered collagen matrix in an in vitro study [25]. Since then, TEBM has demonstrated acceptable clinical outcomes and success rates in various human clinical applications, including multi-centered studies [8,26–28]. In terms of the clinical setting as a regenerative medicinal product, this bi-layered graft

possesses remarkable features, including the unit size of its graft (2.8 × 3.8 cm) [24,29] fabricated by seeding non-split autologous buccal mucosa keratinocytes of passage 1 and durability that allows suturing to surrounding tissue [19,29]. These features are surgeon-friendly during implantation, making the development of MukoCell® admirable and encouraging for investigators and clinicians.

3.1.3. Other epithelia

According to Maurizi *et al.* [7], Ocural®, Sakracy®, and MukoCell® are successful examples of tissue-engineered epithelia that have bridged the gap between pre-clinical translational research and human clinical application of the commercialized product (availability of the final product). Moreover, another TEOM of autologous oral epithelial cell sheets has successfully treated ulcerative esophageal contracture, a common complication of endoscopic submucosal dissection (ESD) [11]. Since the pilot study of this novel therapy combining ESD with TEOM transplantation by Ohki *et al.* [30], several clinical applications of this surgical intervention have been completed and are ongoing, indicating that the grafts show preventive effects on scar formation and facilitate the re-epithelialization of ulceration after ESD [11]. Recently, they reported a novel system for air transportation of TEOM for esophageal repair in remote hospitals [31].

Despite being at the pre-clinical stage, promising in vivo studies of TEOMs for extraoral TEOM use have been conducted since 2015. Chen *et al.* generated TEOMs consisting of oral keratinocytes seeded on a decellularized amniotic membrane. When implanted into wounds in the uterus of rats, they showed that TEOM had great potential for preventing fibrosis and promoting regeneration of the endometrial epithelium [32]. Fukahori *et al.* reported the reconstruction of vocal fold mucosa in dogs by transplanting organotypically cultured TEOM fabricated by seeding autologous oral keratinocytes onto collagen gels in which oral fibroblasts were repopulated, leading to the successful restoration of vocal fold mucosa [33]. Additionally, Guzmán-Uribe *et al.* demonstrated the potential application of autologous bilayered TEOMs as a viable alternative source of tissue-engineered skin for treating diabetic ulcers in patients with diabetes mellitus [34].

The unique clinical characteristics of the oral mucosa have been attributed to the wide use of TEOMs for extraoral grafting, which include easy accessibility, minimal invasiveness, and no aesthetic problems when performing a small biopsy under simple local anesthesia. Moreover, the anatomical, histological, and physiological features of the oral mucosa, including high regenerative capacity, abundance of vascularization, absence of a cornified envelope, lack of hair, and easy handling due to its mechanical properties, are useful and favorable for manufacturing cell-based products. These encouraging properties have implications for other epithelial tissue reconstructions [6,34,35].

3.2. Clinical application for intraoral wound treatment

The regeneration of oral mucosa defects in patients is of utmost importance. Although there have been significant advances in the use of TEOMs for extraoral epithelial reconstruction, commercially available TEOMs for intraoral applications are still lacking. In addition, new human clinical applications and pilot studies on TEOMs for reconstructing intraoral mucosa defects are scarce [2,8].

3.2.1. Bi-layered TEOM

Our review of the literature after 2015 revealed only two pilot studies on the intraoral clinical applications of TEOMs. Sieira Gil *et al.* demonstrated unique techniques to replace the skin surface with full-thickness TEOMs using fibula flap grafts and fibula flap prelamination [36]. Their TEOMs, fabricated using autologous oral keratinocytes grown on a plasma-based scaffold with oral fibroblasts, were used to regenerate keratinized oral mucosal grafts for future placement of dental implants into jaw bones reconstructed by the fibula [36,37]. Moreover, Amemiya *et al.* reported five clinical cases of reconstruction of relatively small-sized oral mucosa defects with bi-layered TEOMs consisting of autologous oral keratinocytes and hAM, suggesting hAM as a useful biomaterial and a composite cultured with oral mucosal epithelial cells as a feasible graft [38].

Although TEOMs have been used in pre-clinical settings, Roh *et al.* developed a completely autologous TEOM using fibrin glue made from autologous blood samples as a scaffold [39,40]. TEOM, consisting of oral mucosa epithelial cells and oral fibroblasts, was transplanted into intraoral mucosa defects in rats, resulting in the rapidly healing oral mucosal and soft-tissue defects without scarring. Moreover, there was a pilot study of three cases of mandibular reconstruction with pre-fabricated and Integra®-prelaminated vascularized fibula flaps [41], which is a similar approach to the report of Sieira Gil *et al.* Integra® is a composite graft for dermal regenerative scaffold, with a porous layer made of cross-linked bovine type I collagen and chondroitin-6-sulfate glycosaminoglycan and an outer layer made of a thin silicone sheet. A few more case reports used Integra® for oral mucosa defects after tumor resection [42–44].

3.2.2. Epithelial cell sheet

Apart from bi-layered TEOMs, there have been no clinical studies to reconstruct full-thickness oral mucosa defects using autologous oral mucosa epithelial cell sheets owing to several drawbacks of clinical outcomes, such as difficult handling attributed to their fragility, blister formation by mechanical forces, and severe wound contraction [2].

3.2.3. Acellular dermal substitute and a new regenerative therapy

In terms of dermal substitutes for oral tissue regeneration, collagen is a major biomaterial with long-term results and many studies on its clinical applications. It is a natural polymer and a major component of the ECM of the lamina propria and is known for its low immunogenicity, high biocompatibility, and convenient extraction procedures [45]. Collagen-based biomaterials offer advantages in generating TEOMs, including both acellular and cellular constructs with incorporated oral fibroblasts, available in various forms such as hydrogels, sponges, fibrillar forms, or membranes [46]. Since 2015, several single-center case studies have demonstrated the efficacy of collagen matrix membranes in covering the oral mucosa, serving as effective substitutes for autologous grafts [46–48]. In addition to collagen matrices, human-made ECMs such as the human acellular dermal matrix (hADM) have been applied as graft materials for oral mucosa defects [49,50]. Furthermore, at the pre-clinical stage, hAM obtained by seeding oral keratinocytes shows great potential as a valuable and feasible biomaterial for oral mucosa reconstruction [51,52]. As an acellular matrix, there has been growing interest in hAM for use in soft and hard tissue reconstruction in the oral cavity owing to their biological and mechanical properties, despite the disadvantages of cost and preservation methods [6,53]. According to literature reviews by Paternoster and Vranckx and Allen *et al.* [8,9], dermal substitutes such as TEOM, collagen matrices, and hADM have been mainly applied for periodontal and peri-implant tissue regeneration to increase keratinized gingiva and soft tissue volume since 2015. Nonetheless, Li *et al.* developed a polymer-integrated amnion scaffold for cleft palate repair with great potential for wound healing in both soft and hard tissues [54].

Polyglycolic acid (PGA) sheets (Neoveil®, Gunze Ltd., Ayabe, Kyoto, Japan), a soft, non-woven fabric with its fixation using fibrin glue spray, have been utilized in surgical procedures to treat wounds, prevent bleeding, and reduce leaking during surgeries on the liver, pancreas, and lung due to their strong affixation to the wound [55–58]. Recently, the technique of MCFP (mucosal defects covered with fibrin glue and the polyglycolic acid sheet) has been used in oral and maxillofacial surgery to repair open wounds of the oral mucosa. After the first report in 2010 on reconstructing the tongue mucosa [59], Takeuchi *et al.* evaluated the clinical outcomes of MCFP for partial glossectomy, suggesting preventive effects on postoperative pain and wound contracture [60]. This technique has also been applied to cover the surface of the jawbones

with or without buccal fat pad grafts [61–63]. A comparison of the clinical outcomes between the groups receiving MCFP and split-thickness skin grafts revealed that MCFP reduced the time of the operation, bleeding during the operation, and days of hospitalization [64].

3.2.4. Hallmarks of wound healing in oral mucosa vs. skin

In general, it is well-recognized that oral mucosa wounds heal faster than skin wounds with minimal scarring, which is a specific feature of oral mucosa wound healing [6,35,65]. In a study on partial glossectomy wounds, PGA sheet grafts remained intact, and re-epithelization of the mucosal defect was successfully completed [60,66]. However, to the best of our knowledge, there have been no studies on the clinical application of this technique for skin defects, suggesting that MCFP could be a novel regenerative therapy for intraoral wound closure. This ability of oral mucosa wound healing by secondary intention could be attributed to various intrinsic and extrinsic factors that differ from skin wound healing, such as the unique environment of the oral cavity, inflammation, angiogenesis, keratinocyte proliferation, fibroblasts, the ECM, and molecular cues [67].

Iglesias-Bartolome et al. [68] reported that the human oral mucosa epithelium was primed for wound healing, resulting in a higher regenerative capacity than the skin. They revealed that SOX2 and PITX1 transcriptional regulators are vital in wound repair and tissue regeneration. Our recent gene expression data from human lip epithelial layers were consistent with their study, showing higher differential expression levels of molecular signatures in the oral mucosa > lip vermilion and those in the lip vermilion > the skin [69] [Database: GEO accession (https://www.ncbi.nlm.nih.gov/ (accessed on 26 Jan 2023), reference number GSE222604 and GSE222605)]. In addition to gene expression levels, compared with the skin, the unique environment of the oral mucosa bathed in saliva contributes to pro-wound healing mechanisms, such as antimicrobial peptides and local growth factors [6,35]; epidermal growth factor (EGF) is one of the key factors with positive biological effects on wound healing [67]. Our recent study on quality control and pharmacological manipulation of oral keratinocytes used in regenerative medicine revealed that EGF plays a crucial role in regulating oral keratinocyte cell motility and proliferative capacity, supporting the fact that the moist environment bathed in saliva is beneficial for re-epithelialization of the oral mucosa when healing by secondary intention [70]. Collectively, MCFP benefits from the intrinsic characteristics of oral mucosa wound healing, although it is incomparable to TEOMs owing to their different wound healing modalities.

4. Current status and tribulations of tissue-engineered oral mucosa

Although the concept of TEOMs is expected to cause a paradigm shift in therapeutic options to circumvent the disadvantages of autologous grafts, its clinical success is limited to minor and simple defects [38]. Therefore, tissue engineering principles must be followed to regenerate large-sized and more complexly structured oral mucosa. Furthermore, owing to the limitations of intraoral applications and the status quo of TEOM, oral mucosa tissue engineering remains rudimentary rather than burgeoning, and its clinical applications are far-fetched [8,71]. This is partly due to the lack of scientific information regarding the complex and compact structure of the oral cavity [6,72]. Therefore, it is necessary to recapitulate wound healing in the oral mucosa and emulate the microenvironment and cellular responses associated with autologous oral mucosa grafts. Other studies have developed unique composite TEOMs consisting of three different cell/tissue layers. These in vitro models include a three-dimensional (3-D) in vitro composite tissue mimicking the natural structure of the alveolar bone with overlying oral mucosa [73] and oral mucosal structures containing submucosal muscles [74]. Because the oral mucosa is highly vascularized, major hurdles encountered in tissue-engineered constructs involve a lack of vascular supply, especially in large-sized TEOMs [75]. In the skin, poor wound healing results from a lack of vascular networks and ischemia [76]. The amount of blood supply in the skin graft is the primary factor determining the quality of the transplanted grafts [77]. As described in a previous study, pre-vascularization approaches based on the production of microvessels inside tissue-engineered grafts before implantation provide a more immediate blood supply [78]; thus, the technology for fabricating TEOMs must consider methods of autologous skin grafting.

5. Potential strategies and future perspectives for the next generation of tissue-engineered oral mucosa

5.1. Pre-vascularization

For the clinical success of tissue-engineered constructs after implantation, it is essential to integrate them with host tissues to improve their lifespan. Achieving full adherence and integration of the TEOM in the open wound bed and surrounding oral mucosa requires early revascularization [79–81]. Pre-vascularization of the TEOMs is a critical and indispensable strategy for enhancing engraftment. To overcome the limitations of intraoral clinical translation, developing a new generation of TEOMs incorporating advanced technologies to induce vascularization is necessary. Emerging technologies have been developed for TEOMs [82–84]. Significant literature reviews have also reported the facilitation of craniofacial tissue regeneration [85–87]. Studies have shown that to prime TEOMs for rapid wound healing and tissue integration, other cell components, such as endothelial cells, endothelial progenitor cells, pericytes, and mesenchymal stem cells, which can induce vascular network structures and develop re-vascularization, need to be incorporated into the dermal matrix, such as gelatin, collagen, and fibrin, within the TEOMs [82–84]. However, angiogenic cell sources, which are primarily allogeneic, must be addressed. Although potential strategies to incorporate cellular components and vascular network structures are still in the exploratory stage, these novel oral mucosa equivalents are highly likely to lay the groundwork for the next generation of TEOMs, thereby increasing the clinical success rate of TEOM implantation. Re-vascularization and adequate blood supply are essential in any tissue engineering approach, as highlighted in many recent studies [2,8,88,89].

Electrospinning technology has also emerged as a pivotal tool for constructing novel scaffolds, including gingiva [90–92]. It enables various biomaterial customizations based on biodegradability, mechanical resistance, and compliance [93]. In terms of accelerating vascularization after grafting TEOMs, the specific approaches using leptin-encapsulated silk electrospun fiber and collagen electrospun scaffolds with optimized pore structure have shown potential utility for promoting pro-angiogenesis and oral mucosa regeneration [89,92].

5.2. Dermo-epidermal junction (DEJ): connective tissue papillae

The DEJ, which allows attachment between the oral mucosa epithelium and the underlying lamina propria in vivo, is a crucial aspect to consider when designing TEOMs that recapitulate and mimic the micro- and nanostructures [71,88,94,95] of the native oral mucosa. These structures include connective tissue papillae, an interdigitating complementary structure of the epithelial rete ridges, and a basement membrane between them. In the skin, this undulating microstructure, lacking in most nonhuman primates, provides enhanced mechanical strength and physical and physiological functions such as stem cell niches [96–98], which apply to the oral mucosa. A recent literature review on skin tissue engineering highlighted several emerging strategies for developing rete ridges, such as micromilling, electrospinning, and 3-D printing. These strategies have improved robust in vitro biomimetic research models for skin, ultimately enhancing clinical performances and outcomes of tissue-engineered skin substitutes [96,99]. Furthermore, acellular 3-D micropatterned dermal scaffolds, regardless

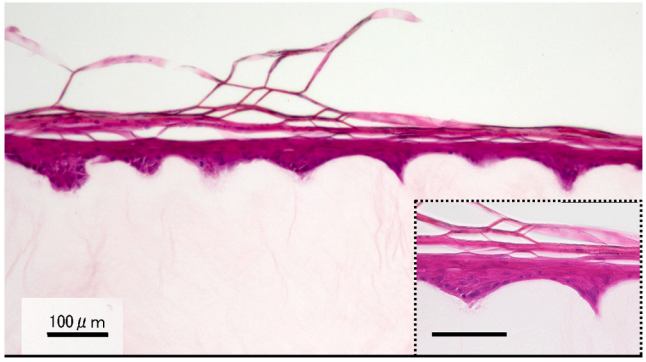


Fig. 1. Representative microscopic appearance of day 11 EVPOME generated on a micropatterned collagen scaffold. The day 11 EVPOME was fabricated as described previously [105]. A continuous, well-differentiated epithelial layer was formed with rete ridge-like structures corresponding to the scaffold micropattern. Original magnification, × 4 (scale bar =100 μm). An image with a higher magnification, boxed by a dotted line, is shown separately at the bottom right. The epithelium was well organized with distinct layers in which the basal cells were well aligned. Original magnification × 20 (Scale bar =100 μm).

of their shape and dimensions, have shown enhanced overall wound closure and wound healing outcomes [100,101], which may be attributed to the recent discovery of a wavy gap, leading to significantly faster wound healing [102].

Scaffold-based tissue engineering plays a key role in facilitating the clinical implementation of TEOMs. However, previous studies on TEOMs have not focused on DEJ restoration despite evidence showing that DEJ formation correlates with the mechanical stress associated with specific signaling molecules [103,104]. Recently, using soft lithography or stainless steel mold systems, we successfully developed a micropatterned collagen scaffold derived from the tilapia scale, mimicking the connective tissue papilla of the oral mucosa for manufacturing an ex vivo produced oral mucosa equivalent (EVPOME) [105,106]. This technology has enabled the development of a DEJ-like interdigitated structure in day 11 EVPOMEs (Fig. 1). Consequently, as a TEOM, EVPOME can withstand constant mechanical abrasion in the oral cavity, including tensile, shear, and repetitive stresses without undergoing plastic deformation or rupture after implantation. This advancement can potentially improve the clinical performance and biological functions of TEOMs.

5.3. Dermo-epidermal junction (DEJ): basement membrane

Similar to the undulating microstructure, the basement membrane, as a component of the DEJ, is crucial for TEOMs. The basement membrane is responsible for rigid epidermal–dermal adhesion and epithelial function and for regulating and maintaining biochemical signals between cells and their surrounding tissues [107]. Therefore, creating rete ridge–mimicking micropatterns and developing tissue-engineered basement membranes is essential to promote rapid and robust DEJ formation when fabricating TEOMs [108]. However, fabricating basement membrane mimics tailored to the scaffold is challenging because of their unique nanostructure and composition specific to the oral mucosa. Nonetheless, Jain *et al.* recently highlighted three major strategies for recapitulating the native basement membrane: porous polymeric membranes, hydrogels, and electrospun materials [107]. Recent reports have shown that basement membrane deposition can be enhanced by seeding keratinocytes into a micropatterned scaffold with fibroblasts [109,110]. The spatiotemporal properties of micropatterned scaffolds create a keratinocyte stem cell niche, making top-down scaffold-based tissue engineering approaches promising for basement membrane development [111]. Additionally, bottom-up tissue engineering, partial block fabrication, and assembly techniques for creating higher-order bioarchitectures with the continuous development of micro-nano technology have shown promise for tissue reconstruction [112,113]. Applying this technique to develop substrates that mimic the native structure and composition of basement membranes could be a favorable strategy for TEOMs [114]. In skin tissue engineering, Malara *et al.* developed a novel technique of cultured epithelial autografts in combination with lasered micro-patterned, electrospun collagen dermal templates and enhanced basement membrane deposition, which may also be applicable to TEOMs [110].

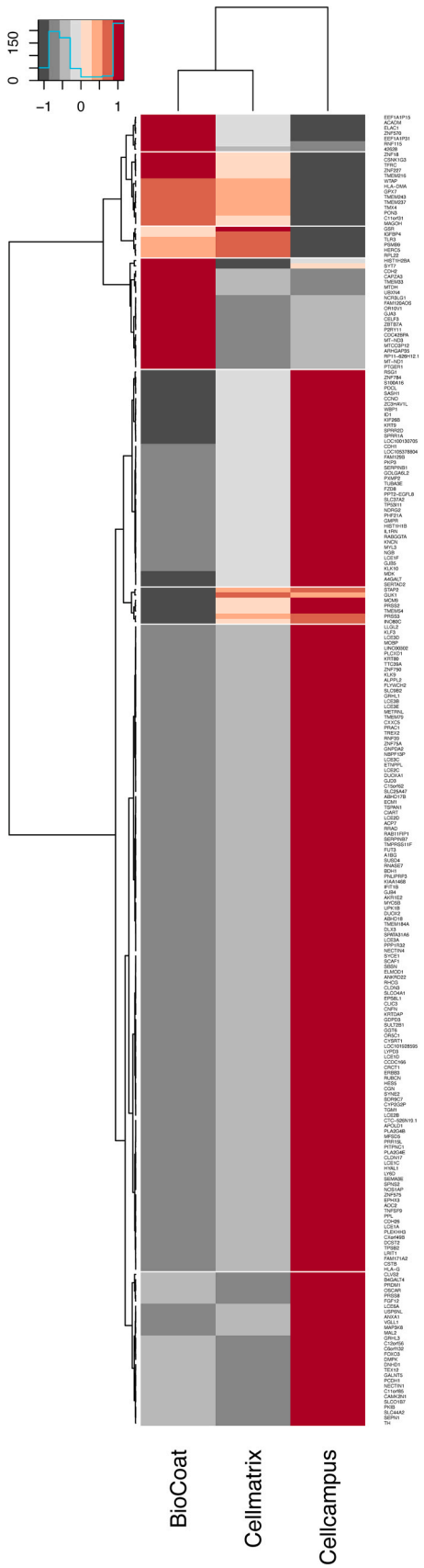
5.4. Mechanotransduction

Another insight into scaffold-based TEOMs is soft-tissue mechanotransduction during wound healing, which has been overlooked during the development of TEOMs [115,116]. Owing to the complex structure of the oral cavity, the biomechanical properties of the native oral mucosa differ from those of the oral cavity, such as attached gingiva, alveolar mucosa, hard palate, and buccal mucosa, owing to site-dependent functions and underlying tissue stiffness [117,118]. For example, the abrupt change in keratinization pattern at the mucogingival junction appears to be influenced by the oral mucosa's biomechanical properties and different functions [6,119]. The oral mucosa is constantly subjected to high mechanical stress, such as stretching and compression, to which oral keratinocytes can sense and respond. Therefore, oral keratinocytes interact with the viscoelastic properties of the ECM, thereby influencing their fate and function [108]. The proliferative capacity and migratory ability of cultured epidermal keratinocytes are regulated by matrix stiffness via distinct molecular mechanisms [120,121]. In our preliminary gene expression analysis of oral keratinocytes cultured in 'complete' EpiLife® containing 0.06 mM Ca^{2+} [70], we observed differential gene expression patterns dependent on the stiffness of the three collagen-based matrices/surfaces (Fig. 2; unpublished data). Notably, when cells were cultured on Cellcampus®, the expression level of the late keratinocyte differentiation genes such as PRSS3 and ZNF750 were upregulated compared to cells cultured on collagen I-coated tissue-culture polystyrene dish [122,123]. Our data suggest that the regional difference in the keratinization pattern of the oral mucosa is partly due to the underlying tissue stiffness resulting from the complex local anatomy of the oral cavity. Therefore, the biomechanical properties of site-dependent intraoral wound beds must be considered in future tissue engineering approaches because the oral mucosa is a mechanosensitive tissue [9].

Collagen-based biomaterials with biomimetic mechanical and topographic features are expected to transduce mechanical loading and enhance multi-tissue repair with matched stiffness. Although the mechanical and biological properties of collagen-based biomaterials remain a significant challenge in clinical settings, innovations based on nature-derived structures and the microenvironments of oral soft tissues could revolutionize the future of collagen-mediated therapeutics [45]. Kinikiglu *et al.* (2015) highlighted the importance of optimal porosity in scaffolds for highly vascularized lamina propria of the oral mucosa, allowing optimal perfusion of the interstitial fluid, imbibition, inosculation, and adequate fibroblast ingrowth during engraftment [124]. However, there is a trade-off between the porosity and mechanical properties of the collagen matrix. Recently, to gain keratinized tissue and increase the thickness of peri-implant soft tissues, grafting a bi-layered collagen matrix consisting of a smooth and compact layer and a porous and 3-D spongy layer has enhanced clinical outcomes [125,126]. The low-porosity collagen fibers on the surface layer enabled the bilayered collagen scaffold to be sutured to the wound margin, indicating its potential for fabricating TEOMs.

5.5. 3-D Bioprinting

The process of scaffold-based tissue engineering for the clinical



(caption on next column)

Fig. 2. Heatmap of differentially expressed genes (DEGs) of three groups in which oral keratinocytes were cultured on collagen-based matrices with different mechanical properties. Oral keratinocytes obtained from seven individuals were cultured for three days on three type I collagen-based matrices/surfaces with distinctly different mechanical properties in a complete EpiLife® culture medium containing Ca^{2+} after 0.06 mM, resulting. Three matrices/surfaces are BioCoat® type I collagen-coated culture dish made of polystyrene (Corning, New York, NY, USA), Cellcampus® (Taki Chemical Co. Ltd., Kakogawa, Hyogo, Japan), and Cellmatrix® Type I-A (Nitta Gelatin, Osaka, Japan), whose Young's modulus is approximately 3 GPa [Young's Modulus: Modulus of Elasticity Units & Formula (specialchem.com)], 1.78 MPa, and 5 kPa, respectively. RNA was extracted from oral keratinocytes, as previously described [63]. The expression values of cultured oral keratinocytes (N = 7) were used to analyze variance (ANOVA) among the three groups, followed by a paired post hoc test. Among 18912 genes, 247 DEGs were identified by applying a threshold of values of false discovery rate below 0.01. After scaling the mean expression values of individual genes among the three groups, a clustering analysis of 247 DEGs was performed.

translation of TEOMs is closely linked to efficient vascularization strategies incorporating angiogenic cells into the ECM. It involves the modification of micro- and nanostructures as well as the physicochemical properties of native oral mucosa. These technological advancements are expected to produce synergistic effects, leading to the development of biomimetic TEOMs for the next generation [127]. 3-D bioprinting technology is an additive manufacturing process and a promising tool for clinical research and future development. It enables tissue engineers to develop new treatments and medical devices by printing cells and biomaterials to form 3-D structures that support cell growth at a defect site. Using 3-D bioprinting and ECM-based bioinks to mimic the structure of native tissues provides a new direction for tissue regeneration and reconstruction [128]. Owing to the complex and compact structure of the oral cavity, these tools offer unique opportunities for fabricating and emulating the microstructures and microenvironments of oral soft tissues, thereby enabling personalized therapies [129]. In the initial stage of bioprinting, the regeneration of keratinized soft tissue with supporting bone can be considered for composite tissue defects in the oral cavity. However, despite the literature on the bioprinting of TEOMs, most studies remain in vitro and face obstacles hindering the clinical translation of 3-D_bioprinted TEOM implantation [129–132]. Collaborations across multidisciplinary fields are necessary to overcome these challenges.

5.6. *Delivery of bioactive molecules*

Apart from the function of scaffolds as a biomaterial for tissue-engineered construct, it serves as a carrier of various bioactive molecules to transfer onto the oral mucosa defects of the host tissue and promote tissue regeneration after intraoral grafting of TEOM [35,133]. Bioactive molecules, including growth factors as well as small endogenous vesicles such as exosomes and extracellular vesicles, could coordinate and promote cell proliferation, migration, adhesion, and differentiation [6,89,133]. Although direct injection of growth factor(s), such as EGF, transforming growth factor-β (TGF-β), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin growth factor-1 (IGF-1), is a simple way to deliver to improve oral mucosa wound healing, their use for regenerative applications is minimal due to short-acting time and uncontrollable concentration in vivo [35,134]. Similar to the previous report demonstrating the capability of the sustained release of bFGF using a collagen-gelatin sponge to accelerate the regeneration of palatal mucosa in the pre-clinical model, the scaffold of TEOMs can be a delivery vehicle of bioactive molecules [135]. A robust vascular network within the TEOM by promoting a pro-angiogenic milieu is required for successful engraftment and integration of TEOMs, which enables the delivery of oxygen and nutrition

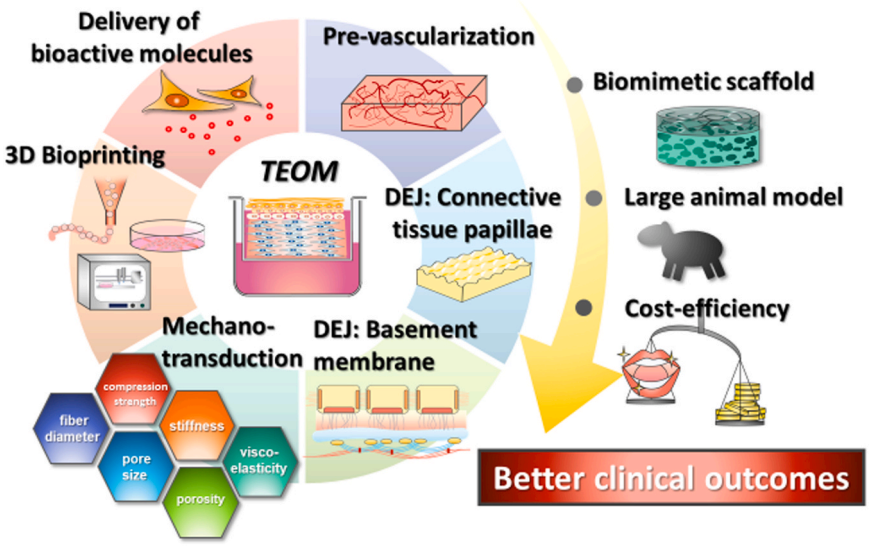


Fig. 3. Graphical representation of the potential vital strategies to be designed and incorporated to engineer scaffold-based TEOMs for intraoral grafting.

into the tissue constructs after grafting [89,136]. Therefore, the feasibility and rationale of applying growth factors to TEOM promote neovascularization, specifically angiogenic factors such as TGF-β, bFGF, PDGF, VEGF, HGF, and IGF-1. However, it is necessary to develop an appropriate delivery system to accurately mimic spatiotemporal and sequential release of multiple growth factors [134]. As previously mentioned, hAM serves as a scaffold for TEOM and a reservoir of multiple growth factors [6,35,137]. In addition to growth factors, recent studies revealed that exosomes and extracellular vesicles have great potential in regenerative dentistry [138,139]. The local administration of these vesicles alone or combined with transplantation of the tissue constructs could contribute to the future clinical applications of TEOMs.

6. Other relevant topics of tissue-engineered oral mucosa

6.1. Large animal model

Non-clinical implantation studies are crucial for assessing the safety of tissue-engineered cell-based constructs for clinical translation, primarily stem cell therapy. These constructs require complex macroscopic and microscopic pathological evaluations owing to their physical presence and unique tissue responses [140]. However, transitioning from small-animal research, which often includes immunodeficient animals, to advanced pre-clinical studies in large animals presents a challenge in testing the safety and efficacy of products. Large animal models offer the advantage of simulating tissue engineering and regenerative medicine principles, from harvesting tissue from a patient to the implantation of 3-D autologous tissue substitutes. However, harvesting tissue samples and grafting TEOMs is challenging in small animals because of the size of the oral cavity. The Food and Drug Administration and EMA require a large animal model to evaluate cell-based devices, including TEOMs, prior to initiating human clinical studies because novel therapies, particularly cell-based TEOMs, have made substantial progress and have the potential to translate into better healing outcomes for a variety of intraoral mucosa defects [141]. Selecting the most appropriate animal model requires an in-depth knowledge of specific species and breeds to ascertain the adequacy of the model and outcome measures that closely mirror clinical situations [142]. A major challenge in the clinical translation of TEOMs is the lack of relevant animal models for intraoral use, which necessitates the development of TEOMs obtained from oral mucosa cells in the animal [6,143]. Currently, studies on pre-clinical large animal models, such as pigs and dogs, in which intraoral mucosa defects are created and treated with bilayered TEOMs are limited [35]. The location of experimental oral mucosa defects reconstructed using TEOMs must be carefully considered, given the complexity of the oral cavity, which varies depending on the type of oral mucosa [6,35]. Considering the advantages and disadvantages of anatomical and physiological issues in pigs and dogs [144,145], establishing well-validated pre-clinical models of TEOMs is essential to understanding their efficacy in healing mucosa defects and accelerating their clinical translation [146].

6.2. Cost-efficiency of TEOMs

Besides scientific issues, cost efficiency and performance are major concerns in tissue engineering products and human clinical trials. Extensive research and development towards the clinical commercial utilization of tissue engineering applications involving time-consuming cell culturing leads to high costs [71]. Additionally, regulatory approval, preservation, storage, distribution, shelf life, safety, and handling can further impact the financial solvency of these custom-engineered products, including TEOMs, for intraoral grafting [89]. Insufficiently powered studies have hindered the widespread implementation of tissue engineering in clinics funded by medical care authorities [26]. Considering the lack of clinical evidence of TEOMs that reconstruct small-sized and delicate/complicated tissue defects, the economic viability of pursuing TEOMs for intraoral clinical applications compared to the success of skin substitutes has become a question. This literature review shows that TEOMs still remain underdeveloped in clinical settings [2,124]. Our ultimate goal is to restore, regenerate, and enhance the oral functions impaired by oral mucosa defects. To successfully translate TEOMs from the bench to the bedside and replace autologous grafts, the focus should be on confirming their efficacy [143,147]. Therefore, investigators must be aware of translational research and how their work affects the future TEOM market, potential adoption, and the scalability of established companies [26]. Encouraging examples of clinical and industrial success, such as MukoCell® for urologic patients, demonstrate the feasibility of TEOMs in environments subjected to mechanical stress; however, the information on scaffolds used in the product is not disclosed.

7. Discussion

Extensive tissue engineering research has been conducted over the past three decades. Although extraoral grafting of TEOMs used in ophthalmology, urology, and esophageal applications has been

successful and is one of the front-runners in current epithelial regenerative medicine, TEOMs as intraoral grafts have not yet reached the level of clinical trials or applications. Although the scientific community has addressed this subject and witnessed broad collaboration among research organizations worldwide, there is a pressing need to build more robust cross-disciplinary collaborations and funding possibilities [89]. The success rate of tissue-engineered templates is higher when designed to mimic the niche or microenvironment of the target tissues [71]. Therefore, the biomaterials utilized for TEOMs should adapt to the dynamic environment of the oral mucosa. Because the niche of the target cells changes with time during intraoral wound remodeling, it is important to focus on developing tissue-engineered biomaterials and scaffolds that can mimic micro- or nano-architectural technique features and modify themselves according to the native environment [71]. Scaffolds play a pivotal role in the clinical translation of TEOMs, especially in promoting angiogenesis after implantation, because they interact directly with intraoral open wounds. The mechanical strength and biophysical properties, including viscoelastic modulus, porosity, and surface characteristics, should match the complex and compact oral mucosa. Researchers and clinicians should identify the intended oral sites in future studies and tailor the materials accordingly. Remarkable progress in biomaterials will contribute to the regeneration of native oral mucosa and will benefit future research into functional restoration and clinical translation [6].

An overview of the potential key strategies to be designed and incorporated into the scaffold to manufacture the next generation of TEOMs discussed in this review is illustrated in Fig. 3. This promising construct enables better clinical outcomes of intraoral grafting for future clinical applications of TEOMs.

8. Conclusions

In conclusion, tissue engineering for the reconstruction and regeneration of intraoral mucosa defects requires integrating biochemical and biomaterial engineering aspects with cell transplantation to generate better-quality biomimetic scaffolds, pre-vascularize 3-D tissue structures, and engineer composite interfaces depending on the type of oral mucosa.

Declaration of Competing Interest

None.

Acknowledgments

We are grateful to Prof. Shujiro Okuda, Division of Bioinformatics, Niigata University Graduate School of Medical and Dental Sciences, for technical support. This research was supported by JSPS KAKENHI grants (number 20H03870 to K.I.). We would like to thank Editage (www.editage.jp) for English language editing.

References

[1] Izumi K, Feinberg SE. Skin and oral mucosal substitutes. Oral Maxillofac Surg Clin North Am 2002;14(1):61–71.
[2] Izumi K, Kato H, Feinberg SE. Chapter 53 - Tissue engineered oral mucosa. In: Vishwakarma A, Sharpe P, Shi S, Ramalingam M, editors. Stem cell biology and tissue engineering in dental sciences. Boston: Academic Press; 2015. p. 721–31.
[3] Wang SS, Tang YL, Pang X, Zheng M, Tang YJ, Liang XH. The maintenance of an oral epithelial barrier. Life Sci 2019;227:129–36.
[4] Groeger S, Meyle J. Oral mucosal epithelial cells. Front Immunol 2019;10:208.
[5] Brizuela M, Winters R. Histology, Oral Mucosa. 2023 May. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023.
[6] Ha P, Liu TP, Li C, Zheng Z. Novel strategies for orofacial soft tissue regeneration. Adv Wound Care (N Rochelle) 2023;12(6):339–60.
[7] Maurizi E, Adamo D, Magrelli FM, Galaverni G, Attico E, Merra A, et al. Regenerative medicine of epithelia: lessons from the past and future goals. Front Bioeng Biotechnol 2021;9:652214.
[8] Paternoster JL, Vranckx JJ. State of the art of clinical applications of tissue engineering in 2021. Tissue Eng Part B Rev 2022;28(3):592–612.
[9] Allen BN, Wang Q, Filali Y, Worthington KS, Kacmarynski DSF. Full-thickness oral mucoperiosteal defects: challenges and opportunities. Tissue Eng Part B Rev 2022;28(4):813–29.
[10] Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E, et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. New Engl J Med 2004;351(12):1187–96.
[11] Hu D, Li X, Li J, Tong P, Li Z, Lin G, et al. The pre-clinical and clinical progress of cell sheet engineering in regenerative medicine. Stem Cell Res Ther 2023;14(1):112.
[12] Nakamura T, Inatomi T, Sotozono C, Amemiya T, Kanamura N, Kinoshita S. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. Br J Ophthalmol 2004;88(10):1280–4.
[13] Cabral JV, Jackson CJ, Utheim TP, Jirsova K. Ex vivo cultivated oral mucosal epithelial cell transplantation for limbal stem cell deficiency: a review. Stem Cell Res Ther 2020;11(1):301.
[14] Nakamura T, Takeda K, Inatomi T, Sotozono C, Kinoshita S. Long-term results of autologous cultivated oral mucosal epithelial transplantation in the scar phase of severe ocular surface disorders. Br J Ophthalmol 2011;95(7):942–6.
[15] Walkden A. Amniotic membrane transplantation in ophthalmology: an updated perspective. Clin Ophthalmol 2020;14:2057–72.
[16] Komai S, Inatomi T, Nakamura T, Ueta M, Horiguchi G, Teramukai S, et al. Long-term outcome of cultivated oral mucosal epithelial transplantation for fornix reconstruction in chronic cicatrising diseases. Br J Ophthalmol 2022;106(10):1355–62.
[17] Attico E, Galaverni G, Pellegrini G. Clinical studies of COMET for total LSCD: a review of the methods and molecular markers for follow-up characterizations. Curr Ophthalmol Rep 2021;9(1):25–37.
[18] Toshida H, Kasahara T, Kiriyama M, Iwasaki Y, Sugita J, Ichikawa K, et al. Early clinical outcomes of the first commercialized human autologous ex vivo cultivated oral mucosal epithelial cell transplantation for limbal stem cell deficiency: two case reports and literature review. Int J Mol Sci 2023;24(10):8926.
[19] Ram-Liebig G, Barbagli G, Heidenreich A, Fahlenkamp D, Romano G, Rebmann U, et al. Results of use of tissue-engineered autologous oral mucosa graft for urethral reconstruction: a multicenter, prospective, observational trial. EBioMedicine 2017;23:185–92.
[20] Bhargava S, Chapple CR, Bullock AJ, Layton C, MacNeil S. Tissue-engineered buccal mucosa for substitution urethroplasty. BJU Int 2004;93(6):807–11.
[21] Bhargava S, Patterson JM, Inman RD, MacNeil S, Chapple CR. Tissue-engineered buccal mucosa urethroplasty-clinical outcomes. Eur Urol 2008;53(6):1263–9.
[22] Osman NI, Hillary C, Bullock AJ, MacNeil S, Chapple CR. Tissue engineered buccal mucosa for urethroplasty: progress and future directions. Adv Drug Deliv Rev 2015;82–83:69–76.
[23] Barbagli G, Ram Liebig G, Fahlenkamp D, Lazzeri M. 2 new bulbar urethroplasty using tissue-engineered oral mucosal graft: a preliminary clinical report. J Urol 2013;189(4):e1.
[24] Karapanos L, Akbarov I, Zugor V, Kokx R, Hagemeier A, Heidenreich A. Safety and mid-term surgical results of anterior urethroplasty with the tissue-engineered oral mucosa graft MukoCell®: a single-center experience. Int J Urol 2021;28(9):936–42.
[25] Schwab R, Heller M, Pfeifer C, Unger RE, Walenta S, Nezi-Cahn S, et al. Full-thickness tissue engineered oral mucosa for genitourinary reconstruction: a comparison of different collagen-based biodegradable membranes. J Biomed Mater Res B Appl Biomater 2021;109(4):572–83.
[26] Ławkowska K, Rosenbaum C, Petrasz P, Kluth L, Koper K, Drewa T, et al. Trauma and reconstructive urology working party of the European association of urology young academic urologists. Tissue engineering in reconstructive urology-The current status and critical insights to set future directions-critical review. Front Bioeng Biotechnol 2023;10:1040987.
[27] Larsson HM. Is tissue engineering of patient-specific oral mucosa grafts the future of urethral reconstruction? EBioMedicine 2017;24:30–1.
[28] Horiguchi A. Editorial comment to safety and mid-term surgical results of anterior urethroplasty with the tissue-engineered oral mucosa graft MukoCell®: a single-center experience. Int J Urol 2021;28(9):943.
[29] Barbagli G, Akbarov I, Heidenreich A, Zugor V, Olianas R, Aragona M, et al. Anterior urethroplasty using a new tissue engineered oral mucosa graft: surgical techniques and outcomes. J Urol 2018;200(2):448–56.
[30] Ohki T, Yamato M, Ota M, Takagi R, Murakami D, Kondo M, et al. Prevention of esophageal stricture after endoscopic submucosal dissection using tissue-engineered cell sheets. Gastroenterology 2012;143(3):582–588.e2.
[31] Yamaguchi N, Isomoto H, Kobayashi S, Kanai N, Kanetaka K, Sakai Y, et al. Oral epithelial cell sheets engraftment for esophageal strictures after endoscopic submucosal dissection of squamous cell carcinoma and airplane transportation. Sci Rep 2017;7(1):17460.
[32] Chen X, Sun J, Li X, Mao L, Cui L, Bai W. Transplantation of oral mucosal epithelial cells seeded on decellularized and lyophilized amniotic membrane for the regeneration of injured endometrium. Stem Cell Res Ther 2019;10(1):107.
[33] Fukahori M, Chitose S, Sato K, Sueyoshi S, Kurita T, Umeno H, et al. Regeneration of vocal fold mucosa using tissue-engineered structures with oral mucosal cells. PLoS One 2016;11(1):e0146151.
[34] Guzmán-Uribe D, Alvarado-Estrada KN, Pierdant-Pérez M, Torres-Álvarez B, Sánchez-Aguilar JM, Rosales-Ibáñez R. Oral mucosa: an alternative epidermic cell source to develop autologous dermal-β substitutes from diabetic subjects. J Appl Oral Sci 2017;25(2):186–95.
[35] Toma AI, Fuller JM, Willett NJ, Goudy SL. Oral wound healing models and emerging regenerative therapies. Transl Res 2021;236:17–34.

[36] Sieira Gil R, Pagés CM, Díez EG, Llames S, Fuertes AF, Vilagran JL. Tissue-engineered oral mucosa grafts for intraoral lining reconstruction of the maxilla and mandible with a fibula flap. J Oral Maxillofac Surg 2015;73(1). 195.e1-16.
[37] Llames S, Recuero I, Romance A, García E, Peña I, Del Valle AF, et al. Tissue-engineered oral mucosa for mucosal reconstruction in a pediatric patient with hemifacial microsomia and ankyloglossia. Cleft Palate Craniofac J 2014;51(2):246–51.
[38] Amemiya T, Nakamura T, Yamamoto T, Kinoshita S, Kanamura N. Autologous transplantation of oral mucosal epithelial cell sheets cultured on an amniotic membrane substrate for intraoral mucosal defects. PLoS One 2015;10(4):e0125391.
[39] Roh JL, Jang H, Lee J, Kim EH, Shin D. Promotion of oral surgical wound healing using autologous mucosal cell sheets. Oral Oncol 2017;69:84–91.
[40] Roh JL, Lee J, Jang H, Kim EH, Shin D. Use of oral mucosal cell sheets for accelerated oral surgical wound healing. Head Neck 2018;40(2):394–401.
[41] Vassiliou LV, Sinha D, Dawood A, Kalavrezos N. Vascularized free fibula flap oral rehabilitation using tissue engineered mucosa: Report of 3 cases. J Craniomaxillofac Surg 2022;50(6):485–92.
[42] Rúa González L, de Villalaín Álvarez L, Novoa Gómez A, de Vicente Rodríguez JC, Peña González I. Use of Integra in oral reconstruction: a case series. Oral Surg Oral Med Oral Pathol Oral Radio 2018;125(3):e72–5.
[43] Deganello A, Bosio P, Giannini L, Parolini F, Berretti G, Sordi A, et al. Matrix for mucosal regeneration in transoral glossectomy for squamous cell carcinoma: objective and subjective functional evaluation. Curr Oncol 2023;30(2):1354–62.
[44] Beech A, Farrier J. Use of the integra skin regeneration system in an intraoral mandibular defect in osteoradionecrosis. Int J Oral Maxillofac Surg 2016;45(9):1159–61.
[45] Zhang B, He Y, Liu J, Shang J, Chen C, Wang T, et al. Advancing collagen-based biomaterials for oral and craniofacial tissue regeneration. Collagen Leather 2023;5(1):14.
[46] Zhu J, Li Z, Zou Y, Lu G, Ronca A, D'Amora U, et al. Advanced application of collagen-based biomaterials in tissue repair and restoration. J Leather Sci Eng 2022;4.
[47] Ahmad F, Lata J. Collagen matrix membrane as a biological dressing in defects of the oral mucosa. Natl J Maxillofac Surg 2021;12(2):199–205.
[48] Preidl RHM, Reichert S, Coronel TV, Kesting M, Wehrhan F, Schmitt CM. Free gingival graft and collagen matrix re-vascularization in an enoral open wound situation. J Oral Maxillofac Surg 2021;79(5):1027–37.
[49] Tognetti L, Pianigiani E, Ierardi F, Lorenzini G, Casella D, Liso FG, et al. The use of human acellular dermal matrices in advanced wound healing and surgical procedures: state of the art. Dermatol Ther 2021;34(4):e14987.
[50] Calì Cassi L, Massei A. The use of acellular dermal matrix in the closure of oronasal fistulae after cleft palate repair. Plast Reconstr Surg Glob Open 2015;3(3):e341.
[51] Qi F, Yoshida T, Koike T, Aizawa H, Shimane T, Li Y, et al. Construction and characterization of human oral mucosa equivalent using hyper-dry amniotic membrane as a matrix. Arch Oral Biol 2016;65:26–34.
[52] Arrizabalaga JH, Nollert MU. Human amniotic membrane: a versatile scaffold for tissue engineering. ACS Biomater Sci Eng 2018;4(7):2226–36.
[53] Odet S, Louvrier A, Meyer C, Nicolas FJ, Hofman N, Chatelain B, et al. Surgical application of human amniotic membrane and amnion-chorion membrane in the oral cavity and efficacy evaluation: corollary with ophthalmological and wound healing experiences. Front Bioeng Biotechnol 2021;9:685128.
[54] Li W, Fu Y, Jiang B, Lo AY, Ameer GA, Barnett C, et al. Polymer-integrated amnion scaffold significantly improves cleft palate repair. Acta Biomater 2019;92:104–14.
[55] Maniwa T, Kaneda H, Saito Y. Management of a complicated pulmonary fistula caused by lung cancer using a fibrin glue-soaked polyglycolic acid sheet covered with an intercostal muscle flap. Interact Cardiovasc Thorac Surg 2009;8(6):697–8.
[56] Gika M, Kawamura M, Izumi Y, Kobayashi K. The short-term efficacy of fibrin glue combined with absorptive sheet material in visceral pleural defect repair. Interact Cardiovasc Thorac Surg 2007;6(1):12–5.
[57] Kobayashi S, Nagano H, Marubashi S, Wada H, Eguchi H, Tanemura M, et al. Experience with the use of fibrin sealant plus polyglycolic acid felt at the cut surface of the liver in laparoscopic hepatectomy. Surg Endosc 2011;25(11):3590–6.
[58] Hayashibe A, Sakamoto K, Shinbo M, Makimoto S, Nakamoto T. New method for prevention of bile leakage after hepatic resection. J Surg Oncol 2006;94(1):57–60.
[59] Sato Y, Watanabe S, Murakami K, Yokoe H, Hada S, Nakajima J. Clinical experience using a combination of PGA sheet and spraying of fibrin glue to cover partial resection of tongue carcinoma. Hosp Dent 2010;22(2):203–6.
[60] Takeuchi J, Suzuki H, Murata M, Kakei Y, Ri S, Umeda M, et al. Clinical evaluation of application of polyglycolic acid sheet and fibrin glue spray for partial glossectomy. J Oral Maxillofac Surg 2013;71(2):e126–31.
[61] Rokutanda S, Yanamoto S, Yamada S, Naruse T, Inokuchi S, Umeda M. Application of polyglycolic acid sheets and fibrin glue spray to bone surfaces during oral surgery: a case series. J Oral Maxillofac Surg 2015;73(5). 1017.e1-6.
[62] Shibata A, Kawahara K, Oguri T, Ohira M, Oriyama T, Niwa A. An approach to marginal mandibulectomy and reconstruction for lower gingival carcinoma: a case report. Oral Maxillofac Surg Cases 2020;6(3):100172.
[63] Udayakumar SIV, Kwon D, Kwon TG, Paeng JY. Secondary healing property using Neoveil®, a polyglycolic acid bioabsorbable sheet on the oral mucosal defects. J Stomatol Oral Maxillofac Surg 2023;124(1):101259.
[64] Mochizuki Y, Tomioka H, Tushima F, Shimamoto H, Hirai H, Oikawa Y, et al. Clinical evaluation of coverage of open wounds: polyglycolic acid sheet with fibrin glue spray vs split thickness skin. Ann Maxillofac Surg 2016;6(2):228–34.
[65] Glim JE, van Egmond M, Niessen FB, Everts V, Beelen RH. Detrimental dermal wound healing: what can we learn from the oral mucosa? Wound Repair Regen 2013;21(5):648–60.
[66] Yonezawa H, Yanamoto S, Kawasaki G, Umeda M. Polyglycolic acid sheets with fibrin glue (MCFP technique) for resection of oral mucosa. Br J Oral Maxillofac Surg 2012;50(3):e43–4.
[67] Pereira D, Sequeira I. A scarless healing tale: comparing homeostasis and wound healing of oral mucosa with skin and oesophagus. Front Cell Dev Biol 2021;9:682143.
[68] Iglesias-Bartolome R, Uchiyama A, Molinolo AA, Abusleme L, Brooks SR, et al. Transcriptional signature primes human oral mucosa for rapid wound healing. Sci Transl Med 2018;10(451):eaap8798.
[69] Kobayashi E, Ling Y, Kobayashi R, Hoshikawa E, Itai E, Sakata O, et al. Development of a lip vermilion epithelium reconstruction model using keratinocytes from skin and oral mucosa. Histochem Cell Biol 2023;160(4):349–59.
[70] Kobayashi R, Hoshikawa E, Saito T, Suebsamarn O, Naito E, Suzuki A, et al. The EGF/EGFR axis and its downstream signaling pathways regulate the motility and proliferation of cultured oral keratinocytes. FEBS Open Bio 2023;13(8):1469–84.
[71] Singh PN, Byram PK, Das L, Chakravorty N. Natural polymer-based thin film strategies for skin regeneration in lieu of regenerative dentistry. Tissue Eng Part C Methods 2023;29(6):242–56.
[72] Emara A, Shah R. Recent update on craniofacial tissue engineering. J Tissue Eng 2021;12. 20417314211003735.
[73] Almela T, Brook IM, Moharamzadeh K. Development of three-dimensional tissue engineered bone-oral mucosal composite models. J Mater Sci Mater Med 2016;27(4):65.
[74] Yamane S, Higa K, Umezawa T, Serikawa M, Shimazaki J, Abe S. Engineered three-dimensional rabbit oral epithelial-mesenchymal-muscular hybrid sheets. Int J Oral Sci 2016;8(3):145–54.
[75] Kato H, Marcelo CL, Washington JB, Bingham EL, Feinberg SE. Fabrication of large size ex vivo-produced oral mucosal equivalents for clinical application. Tissue Eng Part C Methods 2015;21(9):872–80.
[76] Turissini JD, Elmarsafi T, Evans KK, Kim PJ. Major risk factors contributing to split thickness skin graft failure. Georget Med Rev 2019;3(1):1–11.
[77] Hosseini M, Brown J, Shafiee A. Strategies to induce blood vessel ingrowth into skin grafts and tissue-engineered substitutes. Tissue Eng Part C Methods 2022;28(3):113–26.
[78] Laschke MW, Menger MD. Vascularization in tissue engineering: angiogenesis versus inosculation. Eur Surg Res 2012;48(2):85–92.
[79] Tremblay PL, Hudon V, Berthod F, Germain L, Auger FA. Inosculation of tissue-engineered capillaries with the host's vasculature in a reconstructed skin transplanted on mice. Am J Transpl 2005;5(5):1002–10.
[80] Baltazar T, Merola J, Catarino C, Xie CB, Kirkiles-Smith NC, Lee V, et al. Three dimensional bioprinting of a vascularized and perfusable skin graft using human keratinocytes, fibroblasts, pericytes, and endothelial cells. Tissue Eng Part A 2020;26(5–6):227–38.
[81] Xiong S, Wang J, Zhu W, Yang K, Ding G, Li X, et al. Onlay repair technique for the management of ureteral strictures: a comprehensive review. Biomed Res Int 2020;2020:6178286.
[82] Heller M, Frerick-Ochs EV, Bauer HK, Schiegnitz E, Flesch D, Brieger J, et al. Tissue engineered pre-vascularized buccal mucosa equivalents utilizing a primary triculture of epithelial cells, endothelial cells and fibroblasts. Biomaterials 2016;77:207–15.
[83] Nishiyama K, Akagi T, Iwai S, Akashi M. Construction of vascularized oral mucosa equivalents using a layer-by-layer cell coating technology. Tissue Eng Part C Methods 2019;25(5):262–75.
[84] Blanco-Elices C, Chato-Astrain J, Oyonarte S, Bermejo-Casares F, España-López A, Fernández-Valadés R, et al. Generation of a novel model of bioengineered human oral mucosa with increased vascularization potential. J Periodontal Res 2021;56(6):1116–31.
[85] Smirani R, Rémy M, Devillard R, Naveau A. Engineered prevascularization for oral tissue grafting: a systematic review. Tissue Eng Part B Rev 2020;26(4):383–98.
[86] Xu X, Liao L, Tian W. Strategies of prevascularization in tissue engineering and regeneration of craniofacial tissues. Tissue Eng Part B Rev 2022;28(2):464–75.
[87] Liang J, Zhao J, Chen Y, Li B, Li Y, Lu F, et al. New insights and advanced strategies for in vitro construction of vascularized tissue engineering. Available online 16 August Tissue Eng Part B Rev 2023. https://doi.org/10.1089/ten.TEB.2023.0044.
[88] Masson-Meyers DS, Bertassoni LE, Tayebi L. Oral mucosa equivalents, prevascularization approaches, and potential applications. Connect Tissue Res 2022;63(5):514–29.
[89] Anand M, Bhagania M, Kaur K. Tissue engineering in plastic and reconstructive surgery: fostering advances in the 21st century via an understanding of the present state of the art and future possibilities. Arch Aesthetic Plast Surg 2023;29(2):64–75.
[90] Qian C, Xin T, Xiao W, Zhu H, Zhang Q, Liu L, et al. Vascularized silk electrospun fiber for promoting oral mucosa regeneration. NPG Asia Mater 2020;12(1):1–16.
[91] Webb BCW, Glogauer M, Santerre JP. The structure and function of next-generation gingival graft substitutes-a perspective on multilayer electrospun constructs with consideration of vascularization. Int J Mol Sci 2022;23(9):5256.

[92] Sakulpaptong W, Clairmonte IA, Blackstone BN, Leblebicioglu B, Powell HM. 3D engineered human gingiva fabricated with electrospun collagen scaffolds provides a platform for in vitro analysis of gingival seal to abutment materials. PLoS One 2022;17(2):e0263083.
[93] Adamowicz J, Kuffel B, Van Breda SV, Pokrwczynska M, Drewa T. Reconstructive urology and tissue engineering: Converging developmental paths. J Tissue Eng Regen Med 2019;13(3):522–33.
[94] Wang F, Cai X, Shen Y, Meng L. Cell-scaffold interactions in tissue engineering for oral and craniofacial reconstruction. Bioact Mater 2022;23:16–44.
[95] Shah G, Costello BJ. Soft tissue regeneration incorporating 3-dimensional biomimetic scaffolds. Oral Maxillofac Surg Clin North Am 2017;29(1):9–18.
[96] Shen Z, Sun L, Liu Z, Li M, Cao Y, Han L, et al. Rete ridges: morphogenesis, function, regulation, and reconstruction. Acta Biomater 2023;155:19–34.
[97] Lawlor KT, Kaur P. Dermal contributions to human interfollicular epidermal architecture and self-renewal. Int J Mol Sci 2015;16(12):28098–107.
[98] Wong VW, Levi B, Rajadas J, Longaker MT, Gurtner GC. Stem cell niches for skin regeneration. Int J Biomater 2012;2012:926059.
[99] Aleemardani M, Trikić MZ, Green NH, Claeyssens F. The importance of mimicking dermal-epidermal junction for skin tissue engineering: a review. Bioengineering 2021;8(11):148.
[100] Magin CM, Neale DB, Drinker MC, Willenberg BJ, Reddy ST, La Perle KM, et al. Evaluation of a bilayered, micropatterned hydrogel dressing for full-thickness wound healing. Exp Biol Med 2016;241(9):986–95.
[101] Shen Z, Cao Y, Li M, Yan Y, Cheng R, Zhao Y, et al. Construction of tissue-engineered skin with rete ridges using co-network hydrogels of gelatin methacrylated and poly(ethylene glycol) diacrylate. Mater Sci Eng C Mater Biol Appl 2021;129:112360.
[102] Xu H, Huo Y, Zhou Q, Wang LA, Cai P, Doss B, et al. Geometry-mediated bridging drives nonadhesive stripe wound healing. Proc Natl Acad Sci USA 2023;120(18):e2221040120.
[103] Chen H, Luo T, He S, Sa G. Regulatory mechanism of oral mucosal rete peg formation. J Mol Histol 2021;52(5):859–68.
[104] Wu T, Xiong X, Zhang W, Zou H, Xie H, He S. Morphogenesis of rete ridges in human oral mucosa: a pioneering morphological and immunohistochemical study. Cells Tissues Organs 2013;197(3):239–48.
[105] Suebsamarn O, Kamimura Y, Suzuki A, Kodama Y, Mizuno R, Osawa Y, et al. In-process monitoring of a tissue-engineered oral mucosa fabricated on a micropatterned collagen scaffold: use of optical coherence tomography for quality control. Heliyon 2022;8(11):e11468.
[106] Suzuki A, Kodama Y, Miwa K, Kishimoto K, Hoshikawa E, Haga K, et al. Manufacturing micropatterned collagen scaffolds with chemical-crosslinking for development of biomimetic tissue-engineered oral mucosa. Sci Rep 2020;10(1):22192.
[107] Jain P, Rauer SB, Möller M, Singh S. Mimicking the natural basement membrane for advanced tissue engineering. Biomacromolecules 2022;23(8):3081–103.
[108] Roig-Rosello E, Rousselle P. The human epidermal basement membrane: a shaped and cell instructive platform that aging slowly alters. Biomolecules 2020;10(12):1607.
[109] Blackstone BN, Malara MM, Baumann ME, McFarland KL, Supp DM, Powell HM. Fractional CO_2 laser micropatterning of cell-seeded electrospun collagen scaffolds enables rete ridge formation in 3D engineered skin. Acta Biomater 2020;102:287–97.
[110] Malara MM, Blackstone BN, Baumann ME, Bailey JK, Supp DM, Powell HM. Cultured epithelial autograft combined with micropatterned dermal template forms rete ridges in vivo. Tissue Eng Part A 2020;26(21–22):1138–46.
[111] Nadine S, Chung A, Diltemiz SE, Yasuda B, Lee C, Hosseini V, et al. Advances in microfabrication technologies in tissue engineering and regenerative medicine. Artif Organs 2022;46(7):E211–43.
[112] Tiruvannamalai-Annamalai R, Armant DR, Matthew HW. A glycosaminoglycan based, modular tissue scaffold system for rapid assembly of perfusable, high cell density, engineered tissues. PLoS One 2014;9(1):e84287.
[113] Wang X, Wang Z, Zhai W, Wang F, Ge Z, Yu H, et al. Engineering biological tissues from the bottom-up: recent advances and future prospects. Micromachines 2021;13(1):75.
[114] Palchesko RN, Funderburgh JL, Feinberg AW. Engineered basement membranes for regenerating the corneal endothelium. Adv Health Mater 2016;5(22):2942–50.
[115] Wong VW, Longaker MT, Gurtner GC. Soft tissue mechanotransduction in wound healing and fibrosis. Semin Cell Dev Biol 2012;23(9):981–6.
[116] Jansen KA, Atherton P, Ballestrem C. Mechanotransduction at the cell-matrix interface. Semin Cell Dev Biol 2017;71:75–83.
[117] Chen J, Ahmad R, Li W, Swain M, Li Q. Biomechanics of oral mucosa. J R Soc Interface 2015;12(109):20150325.
[118] Choi JJE, Zwirner J, Ramani RS, Ma S, Hussaini HM, Waddell JN, et al. Mechanical properties of human oral mucosa tissues are site dependent: a combined biomechanical, histological and ultrastructural approach. Clin Exp Dent Res 2020;6(6):602–11.
[119] Vielmuth F. Anatomy of the oral mucosa. In: Schmidt E, editor. Diseases of the Oral Mucosa: Study Guide and Review. Cham: Springer International Publishing; 2021. p. 5–19.
[120] Kenny FN, Drymoussi Z, Delaine-Smith R, Kao AP, Laly AC, Knight MM, et al. Tissue stiffening promotes keratinocyte proliferation through activation of epidermal growth factor signaling. J Cell Sci 2018;131(10):jcs215780.
[121] Zarkoob H, Bodduluri S, Ponnaluri SV, Selby JC, Sander EA. Substrate stiffness affects human keratinocyte colony formation. Cell Mol Bioeng 2015;8(1):32–50.
[122] Cohen I, Birnbaum RY, Leibson K, Taube R, Sivan S, Birk OS. ZNF750 is expressed in differentiated keratinocytes and regulates epidermal late differentiation genes. PLoS One 2012;7(8):e42628.
[123] Miyai M, Matsumoto Y, Yamanishi H, Yamamoto-Tanaka M, Tsuboi R, Hibino T. Keratinocyte-specific mesotrypsin contributes to the desquamation process via kallikrein activation and LEKTI degradation. J Investig Dermatol 2014;134(6):1665–74.
[124] Kinikoglu B, Damour O, Hasirci V. Tissue engineering of oral mucosa: a shared concept with skin. J Artif Organs 2015;18(1):8–19.
[125] Bevilacqua L, Pipinato G, Perinetti G, Costantinides F, Rizzo R, Maglione M. The use of a xenogenic collagen matrix (Mucograft®) in the treatment of the implant site: a literature review. Front Oral Maxillofac Med 2020;2:23.
[126] De Santis D, Gelpi F, Castellani R, Palumbo C, Ferretti M, Zanotti G, et al. Bi-layered collagen nano-structured membrane prototype collagen matrix CM-10826 for oral soft tissue regeneration: an in vivo ultrastructural study on 13 patients. J Biol Regul Homeost Agents 2019;33(1 Suppl 1):29–41.
[127] Frueh FS, Menger MD, Lindenblatt N, Giovanoli P, Laschke MW. Current and emerging vascularization strategies in skin tissue engineering. Crit Rev Biotechnol 2017;37(5):613–25.
[128] Wang H, Yu H, Zhou X, Zhang J, Zhou H, Hao H, et al. An overview of extracellular matrix-based bioinks for 3D bioprinting. Front Bioeng Biotechnol 2022;10:905438.
[129] Nesic D, Durual S, Marger L, Mekki M, Sailer I, Scherrer SS. Could 3D printing be the future for oral soft tissue regeneration? Bioprinting 2020;20:e00100.
[130] Tabatabaei F, Moharamzadeh K, Tayebi L. Fibroblast encapsulation in gelatin methacryloyl (GelMA) versus collagen hydrogel as substrates for oral mucosa tissue engineering. J Oral Biol Craniofac Res 2020;10(4):573–7.
[131] Koopaie M, Nassar DHMA, Shokrolahi M. Three-dimensional bioprinting of mucoadhesive scaffolds for the treatment of oral mucosal lesions; an in vitro study. 3D Print Med 2022;8(1):30.
[132] Nesic D, Schaefer BM, Sun Y, Saulacic N, Sailer I. 3D printing approach in dentistry: the future for personalized oral soft tissue regeneration. J Clin Med 2020;9(7):2238.
[133] Cao L, Su H, Si M, Xu J, Chang X, Lv J, et al. Tissue engineering in stomatology: a review of potential approaches for oral disease treatments. Front Bioeng Biotechnol 2021;9:662418.
[134] Amirsadeghi A, Jafari A, Eggermont LJ, Hashemi SS, Bencherif SA, Khorram M. Vascularization strategies for skin tissue engineering. Biomater Sci 2020;8(15):4073–94.
[135] Ayvazyan A, Morimoto N, Kanda N, Takemoto S, Kawai K, Sakamoto Y, et al. Collagen-gelatin scaffold impregnated with bFGF accelerates palatal wound healing of palatal mucosa in dogs. J Surg Res 2011;171(2):e247–57.
[136] Vranckx JJ, Hondt MD. Tissue engineering and surgery: from translational studies to human trials. Innov Surg Sci 2017;2(4):189–202.
[137] Mohan R, Bajaj A, Gundappa M. Human amnion membrane: potential applications in oral and periodontal field. J Int Soc Prev Community Dent 2017;7(1):15–21.
[138] Alqurashi H, Ortega Asencio I, Lambert DW. The emerging potential of extracellular vesicles in cell-free tissue engineering and regenerative medicine. Tissue Eng Part B Rev 2021;27(5):530–8.
[139] Sjöqvist S, Ishikawa T, Shimura D, Kasai Y, Imafuku A, Bou-Ghannam S, et al. Exosomes derived from clinical-grade oral mucosal epithelial cell sheets promote wound healing. J Extra Vesicles 2019;8(1):1565264.
[140] O'Brien MT, Schuh JCL, Wancket LM, Cramer SD, Funk KA, Jackson ND, et al. Scientific and regulatory policy committee points to consider for medical device implant site evaluation in non-clinical studies. Toxicol Pathol 2022;50(4):512–30.
[141] Kinikoglu B, Hemar J, Hasirci V, Breton P, Damour O. Feasibility of a porcine oral mucosa equivalent: a pre-clinical study. Artif Cells Blood Substit Immobil Biotechnol 2012;40(4):271–4.
[142] Ribitsch I, Baptista PM, Lange-Consiglio A, Melotti L, Patruno M, Jenner F, et al. Large animal models in regenerative medicine and tissue engineering: to do or not to do. Front Bioeng Biotechnol 2020;8:972.
[143] Izumi K, Song J, Feinberg SE. Development of a tissue-engineered human oral mucosa: from the bench to the bed side. Cells Tissues Organs 2004;176(1–3):134–52.
[144] Štembírek J, Kyllar M, Putnová I, Stehlík L, Buchtová M. The pig as an experimental model for clinical craniofacial research. Lab Anim 2012;46(4):269–79.
[145] Foster JR, Mowat V, Singh BP, Ingram–Ross JL, Bradley D. Chapter 19 - animal models in toxicologic research: dog. In: Haschek WM, Rousseaux CG, Wallig MA, Bolon B, editors. Haschek and Rousseaux's Handbook of Toxicologic Pathology. (Fourth edition).,. Academic Press; 2022. p. 721–50.
[146] Mosaddad SA, Hussain A, Tebyaniyan H. Exploring the use of animal models in craniofacial regenerative medicine: a narrative review. Available online 16 August Tissue Eng Part B Rev 2023. https://doi.org/10.1089/ten.TEB.2023.0038.
[147] Kim RY, Fasi AC, Feinberg SE. Soft tissue engineering in craniomaxillofacial surgery. Ann Maxillofac Surg 2014;4(1):4–8.