Mini Review

Tissue engineering by transplantation of oral epithelial sheets cultivated on amniotic membrane for oral mucosal reconstruction

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Human amniotic membrane (AM), a thin intrauterine placental membrane is highly biocompatible, and possesses anti-inflammatory and anti-scarring properties. Using AM, we developed a novel method for cultivating oral mucosal epithelial cell sheets. We evaluated autologous transplantation of oral mucosal epithelial cells on AM in patients undergoing oral surgeries. Specimens of AM were obtained from women undergoing Caesarean section. Using oral mucosal biopsy specimens obtained from the patients, oral epithelial cells were cultivated on an AM carrier. The resultant sheet was transplanted on the oral mucosal defect. After 2-3 weeks of culture, the cultivated epithelial cells seemed well differentiated and showed stratification into 5-7 layers on AM. Immunohistochemistry demonstrated that the cultivated cells expressed highly specific mucosal epithelial cell markers and basement membrane proteins. After the surgical procedure, the reconstructed sites did not show infection, bleeding, rejection, or sheet detachment, and the sites achieved a new oral mucous membrane. The cultivated epithelial sheets maintained the properties of a mucous membrane and expressed basement membrane proteins. Autologous transplantation of cultivated oral epithelial sheets was performed, and the transplanted tissue showed adherence to the oral mucosal defect. A long-term follow-up of more than 12 months revealed absence of postoperative recurrence, and the postoperative courses were excellent. These findings showed that this novel epithelial sheet is a useful biomaterial for mucosal reconstruction.

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Introduction

Oral mucosal defects following intraoral surgery and traumatic injuries lead to open wounds. Autologous oral mucosa grafts have been used as a transplant material to reconstruct postoperative oral mucosal defects. These grafts are taken from a different part of the oral cavity (e.g., free gingival grafts, buccal mucosal grafts, palatal grafts); however, autologous mucousal grafts leave defects at the donor site, and it may be difficult to harvest enough oral mucosa for their reconstruction. The ideal reconstruction material would be an autologous tissue whose harvest would involve minimally invasive procedures at the donor site. Advancements of tissue-engineering studies have overcome these issues. Investigators have introduced cultured oral epithelial cell sheets for use as biomaterials in grafting[1,2]. A cultured, oral epithelial cell sheet transplant may be used for many types of membrane defects as an effective treatment method with wide application in surgical augmentation[3,4].

Amniotic membrane

Amniotic membrane (AM) is an easily available thin placental membrane that retains amniotic fluid, and consists of monostratal amniotic epithelium on the basement membrane with a certain parenchymal thickness. It is tough and lacks blood vessels, lymphatic system and nerves. Immunohistochemically, collagen III, collagen IV, and laminin are expressed in the basement membrane of AM; collagens are also expressed in the parenchyma[5,6]. Moreover, AM contains many growth factors[7], and exhibits anti-inflammatory[8] and anti-bacterial properties[9], and has been reported to reduce scarring. However, not much is known about its mechanism of action, and it can be expected to elicit a relatively mild immune response.

In the clinical use of AM as a biomaterial, several favorable advantages have been reported in the field of surgical procedures[10]. Davis first grafted human amniotic sac pieces into granulating wounds. AM has been used to prevent post-surgery abdominal adhesion and as a dressing for burn wounds[11,12], and it favorably influences wound protection and pain reduction. These beneficial influences have been reported in clinical case reports and excellent results were obtained in colpoplasty and ocular reconstruction[13]. With regard to the application of AM to the oral mucosa, Samadari et al. performed mandibular vestibuloplasty using fresh AM, and reported that the enhancement of wound cure was observed[14]. Furthermore, Nakamura et al. produced AM-cultured oral epithelial cell sheet, as the extracellular matrix, and obtained good clinical results using these sheets in severe intractable corneal disorders[15,16].

Therefore, the transplantation of AM can be considered advantageous, since rapid epithelialization occurs following its transplantation making the transplanted site very stable and avoiding postoperative infections, which are very important for the successful clinical application of this procedure.

Culture of oral epithelial cells on amniotic membrane

The acquisition of transplantable cultured structures requires a combination of stem cells, adequate extracellular matrix, and growth factors. Cultured oral epithelial cell sheets were developed for mucosal defects[3,4]; however, these epithelial sheets lack substrates and are fragile and difficult to handle for grafting[1]. The important element in oral grafting is the substrate that supports the oral epithelial cells. Selecting an ideal substrate with biocompatibility, stability, and mechanical properties is important for tissue engineering.

We used AM as a key substrate for the growth of oral epithelial cells, and performed basic studies of AM-cultivated oral epithelial cell sheets[17]. For oral epithelial cell culture, we modified the already reported procedure[17-19]. Briefly, oral mucosal biopsy specimens (approximately 3 mm in length) were incubated with dispase and treated with trypsin-ethylenediamine tetraacetic acid (EDTA) to separate the cells. Oral epithelial cells were then seeded onto AM, left to rest on cell culture plate inserts, and cocultured with MMC-treated 3T3 cells. The culture was submerged in culture medium for 1-2 weeks and then exposed to air for 1 week (Fig.1).

After 2-3 weeks, histomorphologically, they showed 5-7 stratification layers of measurable thickness. The basal cells of the cultivated epithelium were cuboidal and the superficial cells were flattened (Fig.2A). Immunohistochemically, it stained positive for antibodies specific for mucosal epithelium keratins 4/13 (Fig.2B,C), coinciding with our earlier findings[19]. Integrin alpha 6 and laminin alpha 5 chain were expressed in the basement membrane of the cultured oral epithelia (Fig.2D,E). Integrin alpha 6 was present in the basal layer of oral mucosa[20], and was a specific component of hemidesmosomes[21]. These findings indicated that cultivated oral epithelial sheets on AM maintained the properties of a mucous membrane, and reaction products for integrin alpha 6 and laminin alpha 5 chain were confined to the basement membrane of the oral mucosal epithelia in culture.

As integrin alpha 6 and laminin alpha 5 chain were expressed in the basal layer of cultured oral epithelial sheets, the basal epithelial cells adhered to AM with hemidesomosome attachments and produced the basement membrane. Clinically, an important

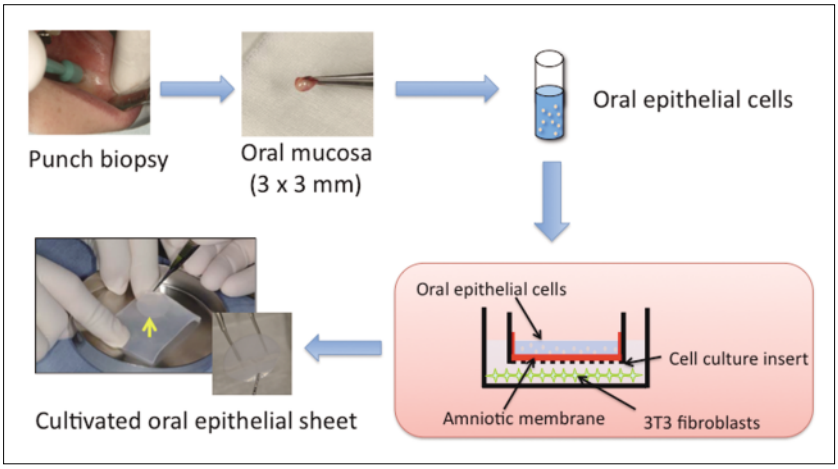

Fig.1 A schematic diagram indicating the concept of cultivation of oral mucosal epithelial cells

Table 1 Characteristics of subjects undergoing transplantation of AM-cultivated oral epithelial sheets

Case	Age	Sex	Diagnosis	Site	Follow up
1	36	M	Mucous cyst	Lower lip	18 months
2	42	M	Mucous cyst	Lower lip	12 months
3	75	M	Mucous cyst	Upper lip	12 months
4	45	F	Pleomorphic adenoma	Upper lip	12 months
5	74	F	Oral leukoplakia	Buccal mucosa	12 months

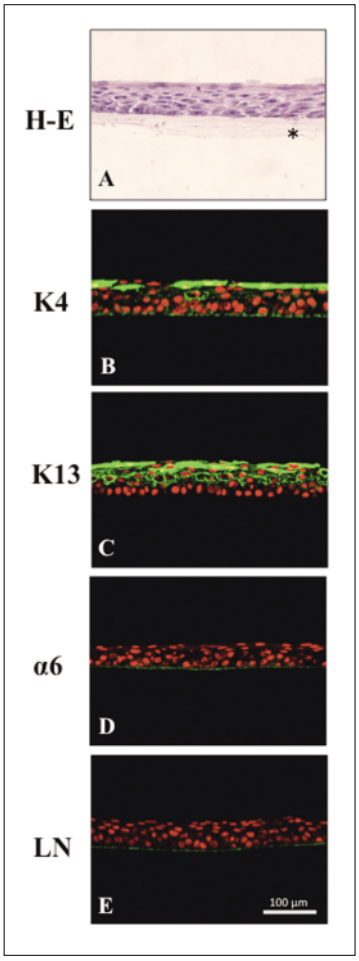

Fig.2
(A): Oral epithelial cells cultured on AM showed 5-7 stratified layers with measurable thickness. The asterisk marks amniotic membrane. (B-E): The cultured oral epithelia sheet stains immunohis-tochemically for keratins 4/13, integrin alpha 6 and laminin alpha 5 chain (green). The nuclei are positive for propidium iodide staining (red).

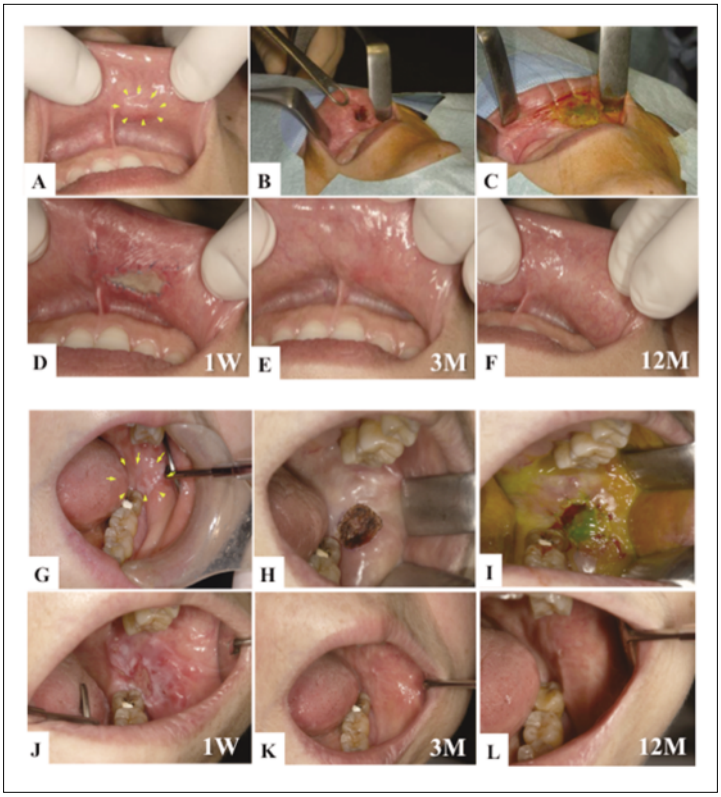

Fig.3 Transplantation to patients of autologous oral mucosal epithelial cells cultivated on AM (A-F: patient 4, G-L: patient 5)

(A): A lesion was detected below the left mucosa of the upper lip. (B): The lesion was excised. (C): The cultured cell sheet was applied. (D): The threads were removed. (E): 3 months after surgery. (F): 12 months after surgery. (G): A lesion was detected at the left buccal mucosa. (H): The lesion was excised. (I): The cultured cell sheet was applied. (J): The threads were removed. (K): 3 month after surgery. (L): 12 months after surgery.

key point for success in carrying out cultivation of oral epithelial sheets is understanding how the basal cells are attached to the underlying AM, and these findings encouraged us to carry out the transplantation of cultivated oral epithelial cells on AM.

Autologous oral epithelial transplant for oral mucosal reconstruction

Using AM, we developed a novel material for cultivating mucosal epithelial cell sheets. The protocol for this experiment comprises a preparatory stage in the development of clinical applications for mucosal reconstruction of oral mucosal defect after oral surgery[22]. We started clinical application after approval from the Human Studies Committee of Kyoto Prefectural University of Medicine (RBMR-R-19).

We investigated autologous transplantation of oral mucosal epithelial cells on AM in patients undergoing oral surgeries. The study included five patients with no history of any disorders and who underwent autologous cultured oral epithelial transplantation for oral surgical procedure. All patients were followed up for more than 12 months after transplantation (Table 1).

Oral mucosal biopsy specimens were taken from each patient under local anesthesia, 2-3 weeks before surgical procedure for developing cultivated oral epithelial sheet. A safety margin of approximately 2 mm was established around the lesion, and blunt detachment of the lesion was performed after dissection of the mucosa using a carbon dioxide gas laser under local anesthesia (Fig.3A,B,G,H). The AM-cultivated oral epithelial cell sheet was applied to the oral mucosa defect region after oral surgery, and sutured together with AM (Fig.3C,I). At postoperative day seven, the sutures were removed.

Clinically, the cultivated oral epithelial cell sheets on AM had sufficient strength to be handled. In all cases, we observed the transplanted sheets adhered to the mucosal defect at one week (Fig.3D,J). The transplanted sites did not show infection, bleeding, or sheet detachment. At three months, the mucosal defects epithelialized well and seemed to have replaced the oral epithelial sheets (Fig.3E,K). In the long-term follow-up of more than 12 months, neither contracture nor recurrence was detected, and the postoperative course was excellent (Fig.3F,L).

Conclusion

The advantage of AM-cultivated oral epithelial sheet transplants is that following transplantation, rapid epithelialization makes the transplanted site very stable and avoids postoperative infections. In the long-term follow-up of more than 12 months, no postoperative recurrence was detected, and the postoperative courses were excellent. To the best of our knowledge, no previous report has documented the use of transplanted epithelial sheets on AM in intraoral grafting. In view of these finding, AM-cultivated oral epithelial sheet on AM is an ideal mucosal graft for oral mucosal reconstruction.

This was a primary clinical study that evaluated a limited oral mucosal defect. In future studies, we will attempt to study the usefulness of AM-cultivated oral epithelial sheets on extensive size, depth, and regions of lesions by increasing the number of cases with variety of those lesions.

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References

1) Moharamzadeh K, Brook IM, Van Noort R, Scutt AM, Thornhill MH: Tissue-engineered oral mucosa: a review of the scientific literature. J Dent Res, 86: 115-124, 2007.
2) Feinberg SE, Aghaloo TL, Cunningham LL Jr: Role of tissue engineering in oral and maxillofacial reconstruction: finding of the 2005 AAOMS research summit. J Oral Maxillofac Surg, 63: 1418-1425, 2005.
3) Hata K, Kagami H, Ueda M, Torii S, Matsuyama M: The characteristics of cultured mucosal cell sheet as a material for grafting; comparison with cultured epidermal cell sheet. Ann Plast Surg, 34: 530-538, 1995.
4) Sumi Y, Hata KI, Sawaki Y, Mizuno H, Ueda M: Clinical application of cultured oral epithelium for palatal wounds after palatoplasty: a preliminary report. Oral Dis, 5: 307-312, 1999.
5) Malak TM, Ockleford CD, Bell SC, Dalgleish R, Bright N, Macvicar J: Confocal immunofluorescence localization of collagen types I, III, IV, V and VI and their ultrastructural organization in term human fetal membranes. Placenta, 14: 385-406, 1993.
6) Nanaev AK, Rukosuev VS, Shirinsky VP, Milovanov AP, Domogatsky SP, Duance VC, Bradbury FM, Yarrow P, Gardiner L, d'Lacey C, Ockleford CD: Confocal and conventional immunofluorescent and immunogold electron microscopic localization of collagen types III and IV in human placenta. Placenta, 12: 573-595, 1991.
7) Koizumi NJ, Inatomi TJ, Sotozono CJ, Fullwood NJ, Quantock AJ, Kinoshita S: Growth factor mRNA and protein in preserved human amniotic membrane. Curr Eye Res,

20: 173-177, 2000.
8) Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F: Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. Cornea, 19: 348-352, 2000.
9) Talmi YP, Sigler L, Inge E, Finkelstein Y, Zohar Y: Antibacterial properties of human amniotic membranes. Placenta, 12: 285-288, 1991.
10) Trelford JD, Trelford-Sauder M: The amnion in surgery, past and present. Am J Obstet Gynecol, 134: 833-845, 1979.
11) Colocho G, Graham WP 3rd, Greene AE, Matheson DW, Lynch D: Human amniotic membrane as a physiologic wound dressing. Arch Surg, 109: 370-373, 1974.
12) Waikakul S, Chumniprasas K, Setasubun S, Vajaradul Y: Application of freeze-dried amniotic membrane: a control trial at the donor site of split-thickness skin grafting. Bull Hosp Jt Dis Orthop Inst, 50: 27-34, 1990.
13) Tseng SC, Prabhasawat P, Barton K, Gray T, Meller D: Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. Arch Ophthalmol, 116: 431-441, 1998.
14) Samandari MH, Yaghmaei M, Ejlali M, Moshref M, Saffar AS: Use of amnion as a graft material in vestibuloplasty: A preliminary report. Oral Surg Oral Med Pathol Oral Radiol Endod, 97: 574-578, 2004.
15) 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, 88: 1280-1284, 2004.
16) Nakamura T, Kinoshita S: Cultivated oral mucosal epithelial transplantation for ocular surface reconstruction. Inflammation and Regeneration, 24: 43-46, 2004.
17) Nakamura T, Endo K, Cooper LJ, Fullwood NJ, Tanifuji N, Tsuzuki M, Koizumi N, Inatomi T, Sano Y, Kinoshita S: The successful culture and autologous transplantation of rabbit oral mucosal epithelial cells on amniotic membrane. Invest Ophthalmol Vis Sci, 44: 106-116, 2003.
18) Amemiya T, Yamamoto T, Oseko F, Nakamura T, Kinoshita S, Kanamura N: Development of rabbit oral mucosa epithelium cells and periodontal ligament cells sheet using the amniotic membrane, J Jpn Regenerative Dent, 1: 25-35, 2003.
19) Amemiya T, Nakamura T, Oseko F, Yamamoto T, Fukushima A, Nakanishi A, Kinoshita S, Kanamura N: Human Oral epithelial and periodontal ligament cells sheets cultured on human amniotic membrane for oral reconstruction. J Oral Tissue Engin, 1: 89-96, 2004.
20) Maragou P, Bazopoulou-Kyrkanidou E, Panotopoulou E, Kakarantza-Angelopoulou E, Sklavounou-Andrikopoulou A, Kotaridis S: Alteration of integrin expression in oral squamous cell carcinomas. Oral Dis, 5: 20-26, 1991.
21) Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK: Alpha 6 beta 4 integrin heterodimer is a component of hemidesmosomes. Proc Natl Acad Sci USA, 87: 8970-8974, 1990.
22) Yamamoto T, Amemiya T, Nakanishi A, Nakamura T, Kinoshita S, Kanamura N: Usefulness for a cultured human oral epithelial cell sheet on human amniotic membrane following removal of minor salivary gland tumor surgery. J Oral Tissue Engin, 5: 54-58, 2007.