

# Novel Application of Artificial Dermis Plus Autologous Vital Epithelial Cells: Improved Wound Epithelialization

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The purpose of this study was to evaluate artificial dermis with the simultaneous addition of autologous epithelial cells for oral lesion defect reconstruction. Surgical wounds reconstructed with artificial dermis plus scraped epithelial cells were evaluated in 5 patients with oral benign lesions or squamous cell carcinoma. Clinical follow-up indices included scar formation and tissue surface texture observation. The neomucosal layers were analyzed histologically to establish the degree of epithelialization. Clinical observation showed that the oral mucosal texture was smoother in artificial dermis with added epithelial cells at 4 weeks postoperation compared with artificial dermis alone. The wound contraction and scar formation processes were slow. Viable epithelial cells with flat rete ridges remained in the artificial dermis, and a neoepithelial layer was present in the histological findings. We showed that healthy granulation tissue and neoepithelial formation in artificial dermis with epithelial cells was beneficial for the repair of oral defects. Scraping oral epithelial cells and applying them to artificial dermis assisted in the early preparation of composite grafts and minimized requirement for donor sites. This technique may improve the treatment of patients with oral benign tumors and early-stage squamous cell carcinoma. [*J Chin Med Assoc* 2010;73(2):108–112]

**Key Words:** artificial dermis, epithelialization, scar contraction, squamous cell carcinoma, tissue engineering

## Introduction

Neoplastic diseases of the oral cavity frequently result in significant defects and cause reconstruction challenges. Split-thickness skin grafts (STSGs) and full-thickness skin grafts (FTSGs) are routinely used to cover oral mucosal defects.<sup>1</sup> However, reconstruction with STSGs may cause donor site morbidity, pain, infection and hypertrophic scar formation. In addition, tissue shortage may complicate scar contraction in the wound healing process.<sup>2</sup> This makes clinical follow-up of cancer recurrence more difficult because of mouth opening limitations.<sup>3</sup>

The degree of contraction and scarring is directly related to wound healing time. It has been shown that the risk of contraction and scarring is markedly decreased if wounds heal within 3 weeks. An open wound has been found to contract significantly faster than a wound treated with a skin graft.

Recently, commercially available artificial dermis has been used in clinical practice. Two major advantages of using artificial dermis have been proposed. One advantage is that it avoids additional donor site morbidity and the other is that it accelerates wound healing, thereby reducing postoperative contraction.<sup>4</sup> One type of artificial dermis is Terudermis. It is composed of a collagen sponge with a silicone layer. The collagen sponge is dermis-like tissue made from bovine type I atelocollagen.<sup>5</sup> It has been reported that artificial dermis is effective in promoting hemostasis, relieving pain, inducing granulation, and preventing contraction.<sup>6–8</sup> No infection or allergic reaction has been reported in patients who received reconstruction with artificial dermis during the postoperative follow-up period.<sup>9</sup> However, because of wound size and wound base condition, epithelialization of the surgical wound is uncertain. Lack of angiogenesis has been found in artificial dermis reconstruction alone.



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E-mail: a2888@vghtc.gov.tw • Received: April 2, 2009 • Accepted: December 1, 2009

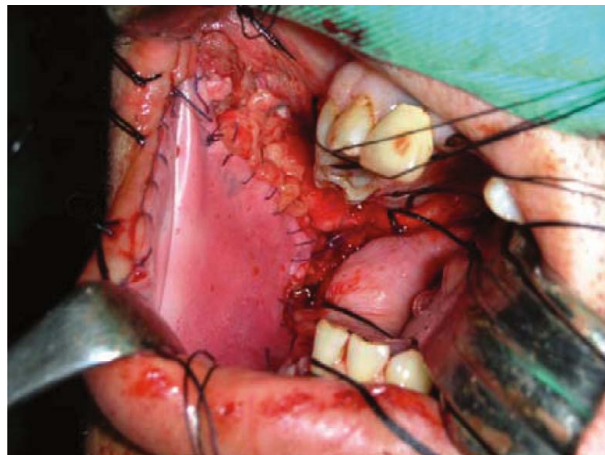
Soejima et al combined cultured cells and fibroblasts to artificial dermis and found acceleration of wound healing and angiogenesis.<sup>10</sup>

There are 2 major differentiation patterns of the oral epithelium: non-cornified epithelium of the lining mucosa and cornified epithelium of the masticatory mucosa. Cornified epithelium includes granular and horny layers, which lack non-cornified epithelium.<sup>11</sup> Loss of epithelial layer formation may play a role in postoperative wound contraction. The degree of wound contraction may also be influenced by recipient location, surgical defect size, and duration of artificial dermis in surgical defects.

The purpose of this study was to evaluate the clinical performance and histological findings of artificial dermis with the addition of epithelial cells to the wound healing process.

## Case Reports

Five patients with a diagnosis of oral benign lesions or squamous cell carcinoma, stages I–II, were included in this study. The defects were reconstructed with artificial dermis and addition of epithelial cells. Artificial dermis (Terudermis; Terumo Corp., Tokyo, Japan) was applied to the intraoral defect. Autologous oral epithelial cells, which were scraped from the non-lesion oral mucosa were sprayed onto the Terudermis (Figure 1). The artificial dermis was then attached to the oral cavity defect in a continuous fashion with iodoform packing (Figure 2). The packing and silicone layer of the artificial dermis remained in place for 10–14 days and were then removed (Figure 3). The wounds were assessed for granulation tissue formation



**Figure 2.** The Terudermis was placed onto the oral cavity defect, fastened in a continuous fashion and iodoform tie-over packing was applied.



**Figure 3.** The silicone layer of the Terudermis was removed 1 week postoperatively.



**Figure 1.** (A) Oral epithelial cells were scraped and meshed, and then (B) sprayed onto the Terudermis.

at 2 weeks postoperation. Contractions were assessed during outpatient clinical examinations at 4 weeks postoperation. The biocompatibility of artificial dermis with the added epithelial cells and epithelial-layer formation were observed in histological examinations.

At clinical follow-up, we found that most of the artificial dermis remained in the defect at 1 week postoperation. However, because of oral masticatory movement and speech, the silicone layer of the artificial dermis was no longer in place at 2 weeks postoperation. At that time, the wounds were completely filled with granulation tissue (Figure 4). Healthy granulation tissue formation was present below the Terudermis after removal of the silicone layer. At 2 months postoperation, the defect surface was soft and smooth (Figure 5). However, scar formation and decreased mouth opening were noted in some patients, especially when the defect was near the buccal mucosa posterior portion or the anterior pillar region (Figure 6).

In the histological findings, fibroblasts proliferated to the collagen layer at 1 week postoperation and epithelial cells remained vital (Figure 7A). A neoepithelial layer was present at the 2-month postoperative follow-up (Figure 7B). However, the neomucosa was thinner than normal oral mucosa and lacked a rete ridge. Compared with defects reconstructed with artificial skin alone, the Terudermis was infiltrated with neutrophils at 1 week postoperation (Figure 8).

## Discussion

In the wound healing process, there are 2 types of contractions: primary contractions and secondary contractions. Primary contraction is caused by the shrinkage of the harvested grafts, such as STSGs or FTSGs. The percentage of elastic fibers within the dermis influences the contraction. FTSGs have more primary contraction than STSGs because they retain more elastic fiber. A secondary contraction, or wound contraction, is of greater clinical concern. The fibroblasts/myofibroblasts from the donor site or the recipient site play a role in wound contraction. The more myofibroblasts are present, the lesser the wound contraction. Myofibroblasts within the granulation tissue in the wound healing process contract and pull on collagen fibrils.<sup>12</sup> A cell contraction results in a reduction of the granulation tissue and draws in the margins of the wound. The behavior of fibroblasts or myofibroblasts may alternate with the scar contraction.<sup>2,13</sup> The artificial dermis may have a positive influence in stimulating healthy granulation tissue formation. However, the longer the silicone layer of artificial dermis



**Figure 4.** Granulation tissue in the reconstructed defect.

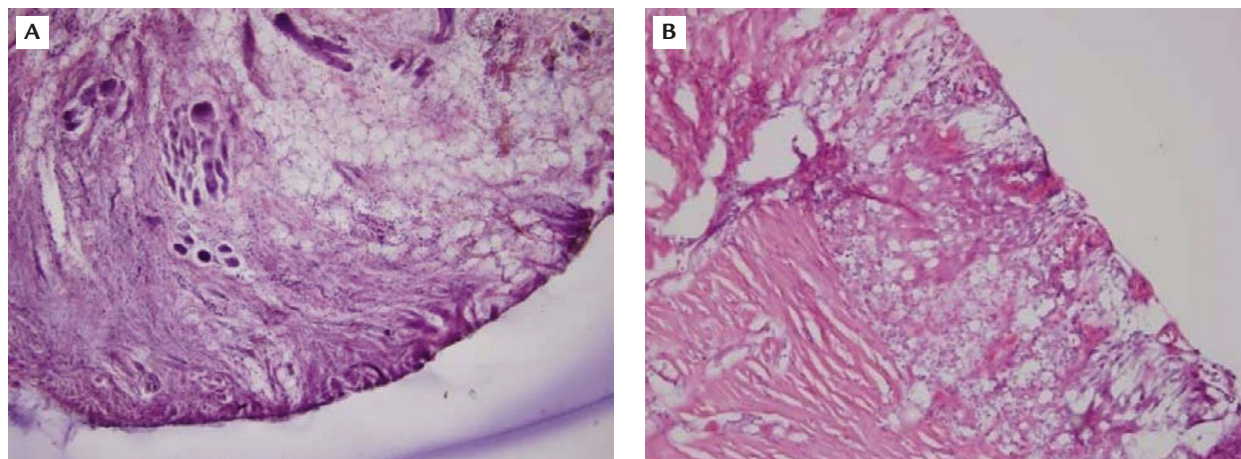


**Figure 5.** Smooth oral mucosa, which was reconstructed using artificial dermis with the addition of epithelial cells 2 months postoperation.

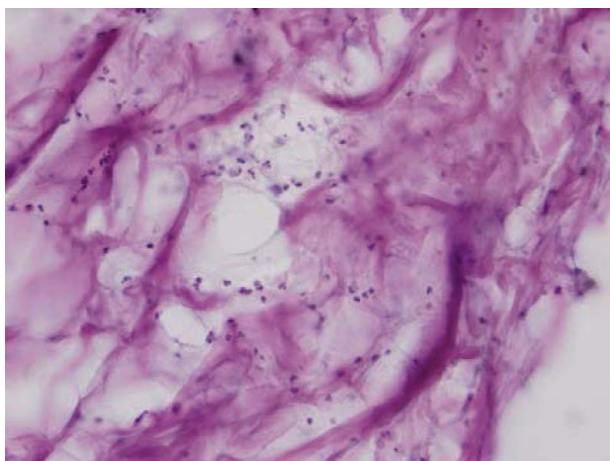


**Figure 6.** Scar formation of the oral defect, which was reconstructed using artificial dermis with the addition of epithelial cells 3 months postoperatively.





**Figure 7.** (A) The defect was reconstructed with artificial dermis and epithelial cells. Epithelial cells and fibroblasts infiltrated into the Terudermis 1 week postoperation. (B) Artificial dermis and epithelial cells 4 months postoperation. Note the neoepithelial layer formation (100 $\times$ ).



**Figure 8.** The defect was reconstructed with artificial dermis alone and the Terudermis was infiltrated with neutrophils 1 week postoperation (400 $\times$ ).

remains, the more likely it is that there will be an inflammatory reaction, which may delay wound healing and cause scar contraction, as noted by Reid et al.<sup>3</sup>

In a study by Zhang et al, in which superficial defects of oral mucosa were covered with artificial dermis, the contraction rate was  $3.7 \pm 1.1\%$  at week 4, and no scarring appeared.<sup>14</sup> However, in patients with squamous cell carcinoma, because of the tumor size and safety margin concern, the deep margin often extends to the muscular layer in the tongue and even to the buccal fat pad in the buccal mucosa. The influence of contractions is more significant with a decrease in mouth opening and limitation of tongue movement.

Reid et al reported that wounds treated with STSG contracted more than those with artificial dermal substitute until day 21.<sup>3</sup> By using Terudermis, we found

that more granulation tissue was present when the silicone layer of artificial dermis was removed at 2 weeks postoperation, and delayed contraction was noted at 4 weeks postoperation. The wound condition remained granulated for a longer time when artificial dermis was used for reconstruction, and patients had more time for mouth opening practice.

Even though artificial dermis can support the migration of peripheral cells, Wei et al showed that the non-vital dermal matrix is not capable of directing cytodifferentiation of the covering epithelium.<sup>15</sup> It is important that the microenvironment in the recipient site influences epithelial differentiation. In a study by Izumi et al, keratinocytes from the oral cavity were harvested and seeded onto a cadaveric dermis (AlloDerm; LifeCell Corp., Branchburg, NJ, USA).<sup>16</sup> The results showed that a well-stratified, parakeratinized epithelial layer was present, similar to native oral keratinized mucosa. In our study, we added epithelial cells onto the Terudermis and applied them to the wound bed. Epidermis function was restored earlier, and smooth mucosa was present at 4 weeks postoperation. In the histological findings, neutrophils migrated to the artificial dermis at 1 week postoperation in artificial dermis reconstruction alone and an epithelium layer was found. In artificial dermis with autologous epithelial cells, stratified epithelial cells were present above the connective tissue at 2 months postoperation. Compared to the natural oral mucosa, there were no rete ridges and the epithelium was in a loose condition.

In addition to seeded keratinocytes, the differentiation of oral epithelial cells is also influenced by the remaining fibroblasts/myofibroblasts in the connective tissue.<sup>11,17</sup> Shephard et al speculated that keratinocytes

may induce myofibroblast differentiation.<sup>18</sup> Some interaction occurred between the seeded keratinocytes and fibroblasts on the wound bed. There is evidence that keratinocytes stimulate fibroblasts to secrete more factors, and fibroblasts may also stimulate keratinocyte proliferation.<sup>19</sup> The use of artificial skin can provide scaffolding for the remaining fibroblasts/myofibroblasts to proliferate in the wound healing process.<sup>19-22</sup>

Using tissue engineering concepts to reconstruct the oral mucosa is a promising treatment option.<sup>23</sup> Establishment of epidermal function can help smooth mucosa formation and promote interaction with the underlying connective tissue. Early wound healing and epithelialization reduces contraction and scar formation.

In conclusion, we achieved good clinical results by using artificial dermis to repair defects in the oral mucosa. This stimulated healthy granulation tissue formation and reduced wound contraction. Artificial dermis with epithelial cells may accelerate epithelial formation and help smooth mucosal texture faster than using artificial dermis alone.

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