THE ROLE OF PORES IN ACELLULAR DERMAL MATRIX SUBSTITUTE

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SUMMARY. To promote the engraftment rate of autologous skin combined with acellular dermal matrix (ADM), ADM was punched to produce regular pores from 500 to 800 μm in diameter, separated by a distance of 3 to 5 mm. The porous ADM was then implanted beneath the flap and transplanted onto an open full-thickness defect wound combined with autografts about 0.2 mm thick in a rat model. The change in diameter of pores in ADM and the neovascularization of ADM matrix were evaluated, and the take rate of porous ADM combined with overlying autologous skin was compared with that of non-porous ADM. The results showed that when porous ADM was grafted onto the full-thickness skin excised wound, plasma penetrated from the wound bed to the surface of ADM through these pores, i.e. the pores punched on ADM were responsible for the imbibition function. Subdermal implantation of ADM indicated that one week post-operation the pores in ADM were still detectable, and some of them contained red blood cells. Two to three weeks after grafting the pores became smaller, partly because of newly synthesized collagen matrix deposition. In Sprague-Dawley rats the engraftment rate of autologous sheet skin graft placed over ADM with pores was 89.5%, which was significantly higher than ADM without pores (63.2%). It is concluded that porous ADM could serve as a good dermal substitute.

Introduction

Acellular dermal matrix (ADM) is easily prepared and stored, capable of large-scale production, and immediately available for grafting. It therefore has a potential for easy and relatively economic use to cover full-thickness skin defect wounds, serving as a dermal substitute.[1] However, autologous skin graft placed over ADM exhibits a low engraftment rate. The take rate of autologous ultra-thin split-thickness skin 0.3-0.5 mm thick placed over ADM simultaneously has been reported to be 60-70%, which limits the use of ADM in burn wounds.[2] In the present study, ADM was punched to make regular pores, which promote its imbibition and vascularization, resulting in a higher take rate in the overlying autografts.

Materials and methods

Preparation of porous ADM

Normal pig split-thickness skin 0.6 mm thick was washed and treated with Dispase II (GIBCO BRL Life Technologies, Inc., USA) followed by Triton X-100 (Sigma Chemical Co., St Louis, MO, USA) to completely remove epidermis and cellular components from the dermis.[3] Routine H & E section showed that tissue cells and epidermal appendages were absent. However, the basic dermal architecture of collagen bundles remained unaltered. ADM was then punched by carbon dioxide laser to make regular pores ranging from 500 to 800 μm in diameter, separated by a distance of 3-5 mm.

Subdermal implantation of porous ADM

Twenty-one male Sprague-Dawley (SD) rats (Animal Centre of Shanghai) weighing 200 ± 20 g were anaesthetized with pentobarbital (30 mg/kg) by intraperitoneal injection. The dorsal hair was shaved and the skin was scrubbed with 75% alcohol. A midline incision was made on the dorsum of the rats, and skin flaps were raised bilaterally. One-half of an ADM strip measuring 1×4 cm was punched to make pores as described above; the other half was not punched. ADM was then implanted beneath the flaps on the fascia of each rat. The wound was closed using nylon sutures. Three days, one week, and three weeks after implantation seven randomly chosen rats were killed, the gross appearance of implanted ADM was observed, and an excision biopsy of each implant was obtained, which was used for routine histological examination.

Composite transplantation of ADM combined with SD split-thickness skin

Forty-two male SD rats were divided equally into two groups, test and control. A split-thickness skin graft 0.2 mm thick and 3 by 3 cm in size was removed from the dorsum of the rats using a dermatome, and the remaining subcutaneous tissue in the donor site was then removed completely to produce a full-thickness skin defect wound. Skin defects in the test group (n = 21) were covered with porous ADM (0.6 mm thick and 3 by 3 cm in size) followed by autologous split-thickness skin, while in the control group non-porous ADM and autologous skin were used.

Two weeks after graft placement the wound was examined to determine the borders of the graft and areas of necrosis. The percentage of graft survival was determined using the paper template technique.[4] Percentage graft take was quantified as follows:

$$\frac{\text{(total area of graft)-(area of necrotic tissue)}}{\text{total area of skin graft}}$$

Wound contraction was evaluated by measuring wound area macroscopically one, two, and four weeks after grafting. The percentage contraction was quantified using the following formula:

$$\frac{\text{(original area of graft)-(graft area at the time point of observation)}}{\text{original area of graft}}$$

Statistical analysis

Data were expressed as the mean ± SEM. *P* values of less than 0.05 were considered significant. Non-paired t-tests were used to compare control versus test group in the engraftment rate data. Tukey's test was used to identify statistically significant differences between specific intergroup mean values.

Results

Physical property of porous ADM

Porous ADM with characteristics of softness and elasticity proved suitable for operations and suturing. After implantation onto the full-thickness skin defect, the porous ADM colour changed from milk-white to pink because of fluid, such as blood plasma, penetrating from the wound bed to the surface of ADM. The colour of nonporous ADM did not change.

Subdermal implantation of ADM in the SD rat model

- Macroscopic analyses of the grafts

Three days to one week after implanting, the surface of the part of ADM with pores, and especially that around the pores, presented a change of colour from milk-white to a nice pink, but not the other part without pores. Two weeks post-graft, the surface of both parts of ADM turned pink. No difference was observed macroscopically.

- Histological analyses

Three days after subdermal implantation, histological observation revealed host fibroblast infiltration into the collagen matrix, and limited neovascularization in the deep part of the implanted ADM. The pores in the ADM, without neocollagen deposition, still existed. One week post-graft, a number of fibroblasts infiltrated into the collagen matrix and lined along the collagen bundles. Neovascularization became more prominent, with some newly developed vessels extending to the uppermost surface of the ADM. In the meantime, the pores had become smaller because of the formation of new connective tissue. Some of the holes contained red blood cells, which suggested that new blood vessels had formed along the holes. Two weeks after grafting, the holes gradually disappeared and the well-organized collagen matrix structure in porous ADM resembled that of nonporous ADM.

- ADM and SD autologous skin implanted into open wounds

Two weeks after implanting, the autologous skin overlying the porous ADM appeared sufficiently integrated into the wound, without evident of necrosis, as judged by visual inspection. However, some parts of the autografts overlying the non-porous ADM gradually dried out with a brown crust, and the necrotic zones exhibited a clear border with surviving skin. The rate of autologous skin graft survival was 63.2 ± 7.8% in the control group and 89.5 ± 6.0% in the test group ($p < 0.05$ vs control group) (*Table I*).

Table I - Take rate of Sprague-Dawley autologous skin overlying ADM

Groups	Take rate (percentage)
Non-porous ADM	63.2 ± 7.8
Porous ADM	89.5 ± 6.0*

n = 14 for each group; data are given as mean ± SEM

* $p < 0.05$ vs non-porous ADM group, non-paired t-test.

The wound area in both groups decreased. Two and four weeks post-graft the contraction became more evident ($p < 0.01$ vs one week post-graft) (*Table II*). However, there were no significant differences between the test and the control groups at the same time point after grafting.

Table II - Contraction rate of Sprague-Dawley autologous skin overlying ADM

Groups	Time after implantation		
	1 wk	2 wk	4 wk
Non-porous ADM	5.4 ± 3.1	14.7 ± 2.9**	27.6 ± 3.3**
Porous ADM	6.3 ± 2.5	15.2 ± 3.7**	28.3 ± 3.9**

n = 14 in each group; data are given as mean ± SEM.

** $p < 0.01$ vs one-week group; Tukey's test.

Histological analysis of graft biopsies two and four weeks after implantation showed that porous ADM induced migration of normal fibroblasts and vessels into the material and served as a template for the formation of a "neodermis". The newly synthesized collagen matrix retained the well-organized anatomical structure of dermis, differing from the usual heavy and solidly packed collagen bundles seen in conventional scars.

Discussion and conclusion

ADM exhibited very low antigenicity, the capacity for vascularization, and stability as a dermal template. After implantation into full-thickness skin defect wounds, ADM acted as a dermal substitute, inducing infiltration of host fibroblasts, endothelial cells, and neovascularization, which

led to the formation of "neodermis" and improvement in the functional and cosmetic results of healed wounds.[5] However, the overlying autologous skin survival rate was low (less than 61.4%) when it was grafted with ADM simultaneously. The take rate reached more than 80% when autologous skin was grafted one or two weeks after ADM implantation. This would cause many clinical problems: first, two-stage therapy would prolong hospitalization; second, prolonged wound closure would increase the possibility of wound infection, resulting in poor survival of autologous skin grafts. It was therefore necessary to promote the take rate of overlying autologous skin grafted with ADM in a one-step procedure.

It has been reported that split-thickness skin grafts have survived because of the diffusion of nutrients through the graft for two days. This is followed by the establishment of capillary vascular flow in the graft for two to five days. If nutrients are not diffused through the graft during this critical time, graft survival is compromised. ADM alone possessed few natural pores - resulting from the movement of hair, ducts, and glands - which were unable to provide sufficient nutrients by diffusion of plasma in the wound bed for the graft for two to three days post-graft. At the same time, ADM did not contain a network of capillaries, and it vascularized slowly. We observed that the majority of ADM did not have enough new blood vessels until two weeks after subdermal implanting in SD rats. Consequently, poor nutrition, resulting from insufficient diffusion of plasma and slow neovascularization after ADM implantation, has been proposed as the most likely reason for the poor survival of overlying autografts.

In our present study, ADM was punched regularly to improve the diffusion of nutrients. After implantation onto full-thickness skin defect, the porous ADM colour changed from milk-white to pink. This was caused by fluids, such as blood plasma, penetrating from the wound bed to the surface of ADM. To investigate the effect of pores in ADM on the take rate of overlying autografts, highly porous ADM and non-porous ADM combined with autologous skin were implanted onto full-thickness skin defect wounds in an SD rat model. One week after grafting, the autograft, placed over non-porous ADM, gradually dried and sloughed; the engraftment rate of autograft placed over porous ADM was 89.5%, which was significantly higher than that of non-porous ADM.

Depending on the reason for the diffusion, the pores in ADM may be responsible for the penetrating function as well as the cellular and vascular population, which largely depends on the size of pores. When the pore sizes were too large, it took longer for newly synthesized connective tissue to fill the pores, and the collagen bundles became irregularly arranged, which led to a meshed pattern in the healed wound. In addition, the larger the pore sizes, the weaker the penetrating function, for physical reasons. Pore sizes smaller than those of normal dermis were found to retard cellular invasion and to promote a thick fibrous capsule surrounding the implanted artificial material, and vascularization of the implant did not occur.[6] The pore structure of the artificial material is thus very important. Some studies have found that sponge collagen membrane with a pore size of about 50 μm and a pore volume fraction of more than 95% could support the diffusion of nutrients and the invasion of host cells. The pore size in polyglycolic acid or polyglactin mesh containing cultured human fibroblasts, a living dermal substitute, is 120 x 250 μm or 280 μm x 400 μm.[7] In this study, the diameter of pores in ADM was 500-800 μm, separated by a distance of 3-5 mm. Macroscopic and histological examination of the subdermal implantation of porous ADM in the SD rat model indicated that this pore structure improved the imbibition of ADM and the invasion of host cells and blood vessels of the collagen matrix.

In conclusion, highly porous ADM could be useful for the survival of overlying autologous skin.

RÉSUMÉ. Pour promouvoir le taux de greffage de la peau autologue associée à une matrice dermique acellulaire (MDA), la MDA a été perforée pour créer des pores réguliers de diamètre de 500 à 800 μm, séparés d'une distance de 3 à 5 mm. La MDA poreuse a été ensuite implantée sous le lambeau et transplantée sur une lésion ouverte à toute épaisseur associée à des autogreffes d'épaisseur d'environ 0,2 mm dans un modèle de rat. Le changement de diamètre des pores de la MDA et la néovascularisation de la matrice de MDA ont été évalués, et le taux de prise de la MDA poreuse associée à la peau autologue sur-jacente a été confronté avec celui de la MDA non-poreuse. Les résultats ont démontré que lorsque la MDA poreuse a été greffée sur la lésion cutanée excisée à toute épaisseur, le plasma pénétrait du site de la lésion jusqu'à la surface de la MDA à travers ces pores, c'est-à-dire les pores créés sur la MDA étaient responsables de la fonction d'imbibition. L'implantation subdermique de la MDA indiquait que les pores dans la MDA étaient encore détectables une semaine après l'opération, et quelques-un d'entre eux contenaient des globules rouges. Deux ou trois semaines après la greffe les pores sont devenus plus petits, partiellement à cause de la déposition de la matrice de collagène fraîchement synthétisée. Dans les rats Sprague-Dawley le taux de prise de la greffe de la peau autologue appliquée sur la MDA perforée était 89,5%, ce qui était significativement plus élevé que la MDA non perforée (63,2%). Les Auteurs concluent que la MDA poreuse pourrait servir comme un bon substitut dermique.

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