인공피부 제조시 기저막 재건의 효과

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Reconstruction of basement membrane in the artificial skin

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Abstract

We attempted to reconstruct basement membrane (BM) in between the epidermal compartment and dermal compartment in the artificial skin preparation and examine its effect on the skin architecture as well as on the epidermal differentiation. Laminin, one of the component of BM, stimulate the migration of the basal cells but type IV collagen which is a major component of the mechanical network of BM did not stimulate epidermal migration. However laminin in the presence of type IV collagen at a 1:1 molar ratio did not stimulate epidermal migration but provide nice demarcation between epidermis and dermis. This mixture of laminin and type IV collagen enhanced epidermal differentiation in the artificial skin based on the morphological observation as well as biochemical criteria. The epidermal acquirement of migratory ability on the laminin-rich substrate suggest that this type of unbalance in the expression of the components of BM may prevail in the area of healing tissue and the invasive transition of the tumor. The result in this study provide the technical improvement in the artificial skin preparation and further application of this technique for the reconstruction of other bio-artificial organ.

Introduction

Epidermis protects our body from a variety of noxious stimuli such as sun light, chemicals, dehydration, and microrganisms. Severe epideraml loss is often observed in burn patients and other accidents, under the situation where intensive care must be taken to prevent the body-fluid loss as well as to provide suitable microenvironment for better wound-healing. In this aspect, the artificial skin which has been developed for the experimental model and now is available at commercial level in the USA and Japan has long been suggested for the clinical application for the burn patients. Recently their application was attempted in several hospitals in other countries and successful results in terms of wound healing effects and less scar formation came out.

The artificial skin development may be roughly categorized in three types: a simple epidermal cell sheet, single fibroblast cell sheet on the type I collagen, or fully stratified epidermis on the dermal matrix. The former two may be better suited for the transient emergency application as a biodressing. The third one is the most developed artificial skin which can be considered as a skin equivalent in the aspect of biochemical characteristic and morphological property. Therefore this type

of artificial skin which can be regenerated from the patient's own epidermal cells and dermal fibroblasts may be more useful for the scheduled application such as plastic surgery and for in vitro skin model.

The technique for the skin equivalent at this stage also has to be modified for the complete resemblance to in vivo skin.
In an attempt to reconstruct basement membrane situated in between epidermis and dermis in manufacturing the artificial skin, we reconstructed basement membrane by the mixtures of laminin and type IV collagen existing at different molar ratio and examined their effects on epidermal architecture and differentiation. The effect of the exogenously supplied laminin, fibronectin, and type IV collagen on the expression in the endogenous ones was examined. The possible clinical application of this result was discussed.

Materials and Methods

Preparation of epidermal cells and the raft culture method:

The epidermal cells were prepared from the new born foreskin (Cha Hospital) by serial trypsinization as described previously (Fuchs's Cell culture manual). The raft culture method which generates a stratified artificial skin was basically followed as described with a few modifications(Choi and Fuchs, 1990). Artificial dermis was reconstructed by mixing neutralized-porcine type I collagen with J2 fibroblast at a concentration of $3X10^5$ cells/ml. After gel formation of the artificial dermis, the components of basement membrane were applied as thin film and allowed to be semi-dryed overnight. Epidermal cells in the exponential phase were plated on the collagen matrix embedded with fibroblasts, cultured at submerged state for 7 days, and at air-liquid interface for 2 weeks.

Morphological examination of the raft culture and immunocytochemical staining:

The raft culture may be treated as tissue for all the other applications. The raft section was fixed in Carnoy's solution composed of ethanol, chloroform, and acetic acid in a ratio of 60; 30;10 for 30 min at 4 ^{o}C and washed with 60 % ethanol extensively. The paraffin block was made in general method and 5 mM sections were used for the immunocytochemical staining and hematoxylin/eosin staining for the morphological observation. The paraffin section after deparafinization was allowed to bind with primary antibodies and subsequently detected with secondary antibodies. These complexes were developed by the immunogold sliver enhancement methods as described in the manufacturer' specification (Amersham, USA).

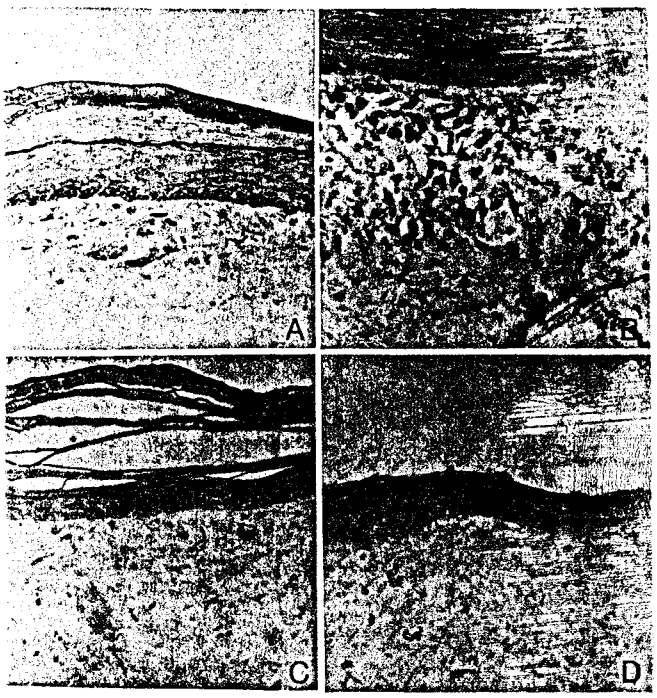
Results and Discussion

Laminin stimulates migration of epidermal basal cells:

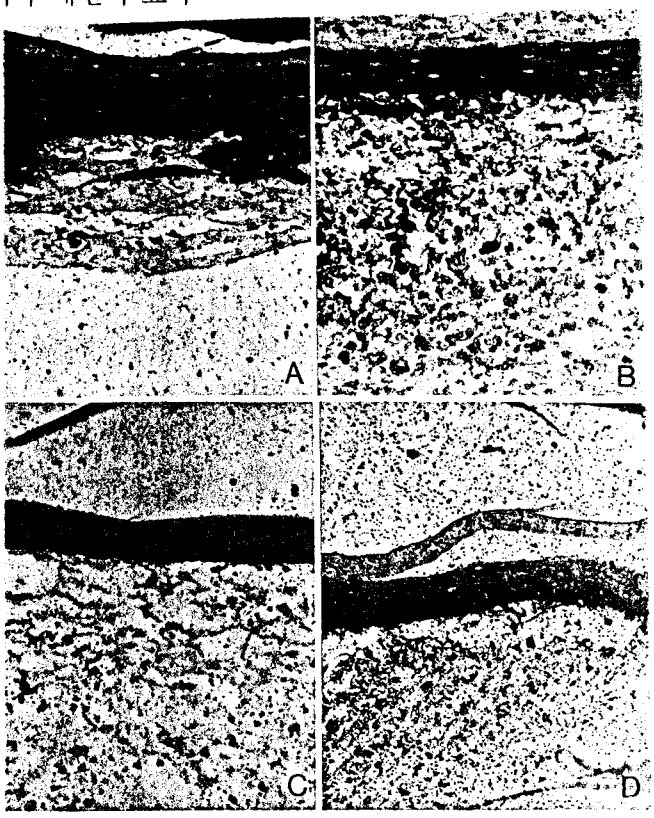
When the raft culture was performed in the presence of laminin substrate in between epidermal compartment and dermal compartment, the basal cells were actively penetrating into the dermal matrix and some cells migrated out of the derma matrix. However in the raft culture with type IV collagen insertion between epidermal and dermal boundary, any basal cell penetrating into dermal compartment was not observed and clear demarcation between two compartments existed. Therefore type IV collagen mesh may provide enough structural barrier for the basal cell's transpassing.

Type IV collagen/laminin at 1:1 ratio established nice demarcation between epidermal and dermal compartments:

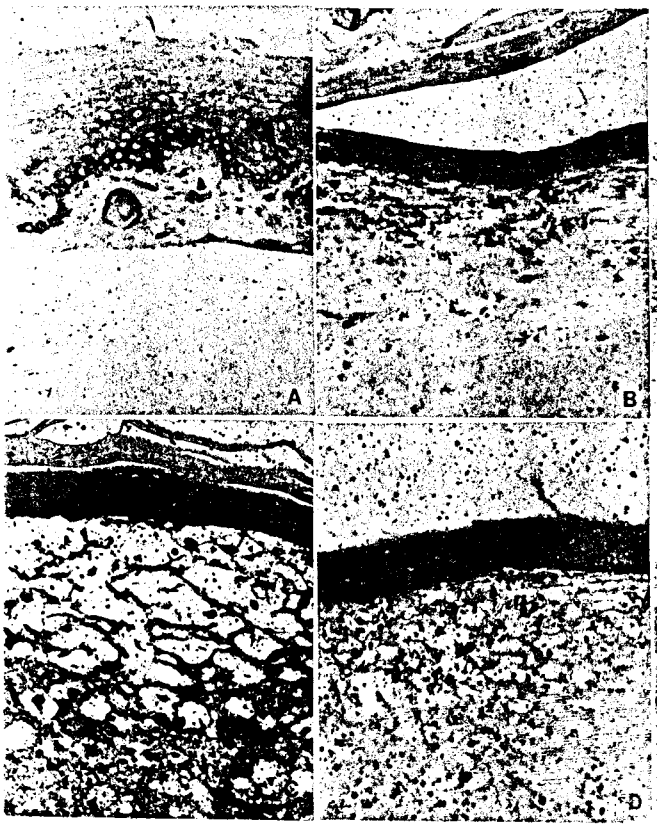
In the basement membrane of the in vivo skin, laminin and type IV collagen are two major components. Tenascin, fibronectin, and heparin sulfate proteoglycan are coexisting as minor components. To explore the suitable molar ratio for the epidermal basement membrane reconstruction, we carried out the raft culture in the presence of both laminin and type IV collagen in a 1:1 ratio which is probably close to the in vivo ratio. The supplement of type IV collagen to the laminin , which probably allows the association of



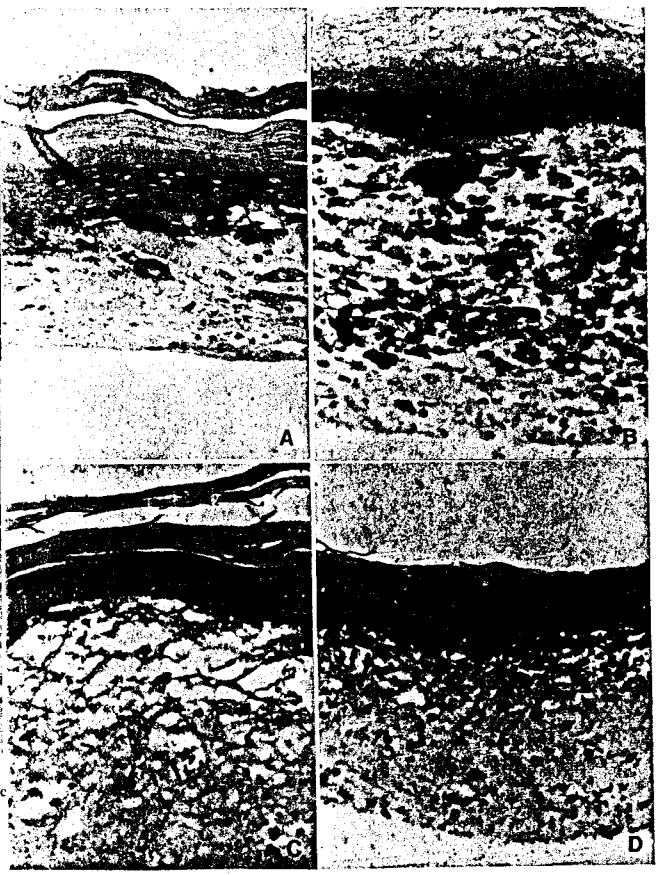
Effect of laminin, type IV collagen, and laminin + type IV collagen on the morphology of the artificial skin.
A) control B) laminin C) type IV collagen D) laminin + type IV collagen



Immunocytochemical staining of the artificial skin regenerated in the presence of basement membrane components with the antibodies against human fibronectin receptor ($\alpha 5\beta 1$).
control B) laminin C) type IV collagen D) laminin + type IV collagen



Immunocytochemical staining of the artificial skin regenerated in the presence of basement membrane components with the antibodies against human laminin.
A) control B) laminin C) type IV collagen D) laminin + type IV collagen



Immunocytochemical staining of the artificial skin regenerated in the presence of basement membrane components with the antibodies against human type IV collagen.
A) control B) laminin C) type IV collagen D) laminin + type IV collagen

laminin to the type IV collagen matrix, strongly inhibited epidermal-migrating potential of laminin. In this study we can not answer the question what is a major mechanism in the laminin-induced migration of epidermal cells.

Exogenous supplement of ECM inhibited its endogenous expression in the raft culture:

The immunocytochemical staining with specific antisera to human laminin showed very little expression of laminin in the raft culture with supplemt of mouse laminin but high level of human laminin expression without its supplement. This expression patterns were similarly true for type IV collagen. These results suggest that cells somehow sense the lack of certain ECM component for the basement membrane formation and induce the synthesis of the missing component However molecular event underlying this phenomena remains to be elucidated in the future study.

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