計畫名稱：燒燙傷傷口暫時性敷料之細胞毒性及生物商應性分析

計畫主持人：林峰輝
執行單位：台大醫學工程研究所
聯絡處：台北市仁愛路一段一號
電話：23912641　　**傳真：**23940049

Abstract

A novel method of preparation of easily stripped off temporary wound dressing material is disclosed. A tri-layer membrane system for artificial skin is prepared in this study. In this process, the N-isopropyl acrylamide monomer is successfully grafted on the non-woven fabric by copolymerization. It is initiated by plasma to activate the surface of the non-woven cloth. N-isopropyl acrylamide is then grafted onto the surface of the non-woven cloth by -ray irradiation. The last layer but the most important of a bovine gelatin with glycosaminoglycans (chondroitin-6-sulfate) is grafted by UV light, which serves as a matrix for the infiltration of fibroblasts, macrophages, lymphocytes, and capillaries derived from the wound bed.

The goal in this work is to provide such a nonantigenic membrane closely resembling dermis in its anatomic structure and chemical composition, which would act as a scaffolding inducing the synthesis of a new dermis. The following describes the construction and animal testing of this artificial skin in extensively damage. In the experiment, the specimen are divided into 4 groups: (1) controlled group without dressing material, (2) non-woven fabric, (3) non-woven fabric grafted with NIPAAm, and (4) non-woven fabric grafted with NIPAAm, bovine gelatin, and glycosaminoglycans from bottom to top in sequence.

After operated for 6 weeks, both controlled group and non-woven fabric group stayed in the proliferative phase where no epidermis or dermis structure has been traced in the section. 6 weeks of postoperation, the third group has been healed completely in the maturation phase. The wound site has been totally recovery with normal dermis and epidermis structure around but the dressing material still stayed on the wound site. In the group of the non-woven fabric grafted with NIPAAm, geltain, and glycosaminoglycans, it has been recovery to the final stage of maturation phase. The wound site has been totally recovery at the 4th week of postoperation. The dressing material of the group fall off automatically from the wound site without any damage to the skin after recovery. We believe the dressing material will have a great potential in medical application in the near future

1. Introduction

Artificial skin is urgently needed to maintain the body temperature of severely burned patient. Actual permanent replacement of skin by biomaterials is still a great clinical challenge. The treatment of extensively burned patients is a difficult clinical problem not only because of the extent of the physiologic abnormalities caused by the burn itself, but also because of the small area of normal skin available to provide replacement of the large area of skin destruction, which must take place if the patient is to survive the injury. There have been substantial improvements in the physiologic management of burn shock, infection, and metabolism which have considerably improved acute burn management. However, physiological replacement of destroyed skin has not kept pace with the improvement in systemic management so that the burn illness is greatly complicated by the persistence of a large, open wound. If this wound is not promptly closed, malnutrition and bacterial invasion set the stage for extensive

complications and a high mortality rate [1-3].

To reduce the extent and duration of open wound, measures such as the use of allograft in moderate and large injuries, and temporary transplantation and immunosuppression for massive injuries, as well as technical innovations, such as autograft meshing, are used and have improved prognosis following an extensive burn [4]. Unfortunately, the full benefits of prompt excision of necrotic burned tissue and immediate wound closure have not been realized because there is no immediately available and physiologically acceptable replacement for extensive areas of destroyed skin [4,5].

We try to prepare a tri-layer membrane system for artificial skin. The first layer is a dermal replacement layer which is made of a 3-dimentional porous cross-linked bovine gelatin with -ray irradiation. The second layer is a so called connection-layer that is poly-N-isopropylacrylamide (PNIPAAm) polymer. The third layer is a bovine gelatin with glycosaminoglycans (chondroitin-6-sulfate) that is manufactured with a controlled porosity and defined degradation rate. The temporary epidermal substitute layer is made of synthetic non-woven cloth and functions to control moisture loss from the wound. The gelatin dermal replacement layer serves as a matrix for the infiltration of fibroblasts, macrophages, lymphocytes, and capillaries derived from the wound bed.

A major drawback in a conventional dressing materials, mainly composed of gauze, is the adhering between the fibers of the gauze materials and the tissue. When fibers of the gauze is enclosed by the newly grown tissue as the wound is cured, the separation between the dressing material and the tissue become very difficult [6, 7]. If separation is not correctly performed, a secondary damage to the wound will be experienced and the recovery is also prolonged. The PNIPAAm connection layer have a good adhesion with the tissue when the wound is in the moisture condition. It will automatically and easily apart from the tissue once wound closed and in dry condition. Epidermal substitute of the tri-layer membrane will serve as protective film to prevent wound from infection, fluid loss, and bacteria invasion as well.

The goal in this work is to provide a nonantigenic membrane closely resembling dermis in its anatomic structure and chemical composition, which would act as a biodegradable scaffolding inducing the synthesis of a “neodermis”. The following describes the construction and animal testing of this artificial skin in extensively damage.

2. Materials and Experiments

2-1 Materials Preparation

A widely used non-woven fabric of polythylene is used as the substrate and this substrate can be pretreated or surface activated in a plasma chamber. The substrate will be immersed in a solution of N-isopropyl acrylamide monomer (NIPAAm). An additive (vitamin B2) have to be added into the solution to prevent free radical from oxidation in the air. The substrate will then exposed to irradiation by -ray which NIPAAm can be grafted onto the surface of the substrate by copolymerization. The radiation dose of the -ray is preferably in the range of 10-100 kGy and irradiated for about 10-20 hours. If UV light is used as the radiation source, the duration is preferably 10-90 mins [8].

The surface modified non-woven fabric will be immersed into a gelatin solution with 16.7% in concentration and then exposed to UV light for 90 mins. Finally, the treated non-woven fabric will soak in the glycosaminoglycans solution for 2 hours to form the dressing material [8-9].

In the experiment, the specimen are divided into 4 groups: (1) controlled group without dressing material, (2) non-woven fabric, (3) non-woven fabric grafted with NIPAAm, and (4) non-woven fabric grafted with NIPAAm, bovine gelatin, and glycosaminoglycans from bottom to top in sequence.

2-2 Animal Experiment

From a professional stock breeder 20 mature New Zealand male rabbits in average weight of 3.0 0.5 kg were obtained. After at least 2 weeks in quarantine and examination by a veterinary surgeon they were operated upon. All surgical procedures were performed under sterile circumstances and followed by 3 days of preoperative antibiotic protection with ampicillin 20% (Alfasan, 0.1mL/2.5kg). The guideline of the ethical committee of National Taiwan University for the care and use of laboratory animals were observed [10]. Rabbits were anesthetized with subcutaneous medetetomidine (Dormitor) 0.3 mg/kg, ketamine (Ketalar) 20 mg/kg, and diazepam (Diapam) 0.15 mg/kg. A circle defect with 5 cm in diameter was created at the central area of dorsum along the dorsimesad. Around the defect of each rabbit was shaved and scrubbed before operation. Through a short incision, the epidermis, dermis and superficial fascia were monoblocally excised from the dorsal area of the experimental animal. The prepared dressing materials was then covered onto the created hole and fixed with 4-0 nylon. The rabbits were scarified after 2, 4, and 6 weeks of operation. There were 3 rabbits for each group in every experimental period.

2-3 Histology

The entire specimen with surrounding tissue were collected and fixed in 4% buffered formaline solution. Specimens were processed through serial alcohol solution, toluene and full-faced section embedding in paraffin. The procedure used is the celloidin-paraffin double-embedding method which will take 96 hours on an automatic specimen processor. Then impregnate the specimen with paraffin wax in the usual manner and embed. Transverse sections about 5 m in thickness were obtained using a microtome. Sections were stained with Hematoxyline & Eosin (HE) for optical microscopic examination.

3. Results and Discussion

Results of Histological Evaluation

	2 weeks	4 weeks	6 weeks
A: Bandage	***Inflammatory Phase:*** A blood clot forms in the wound and loosely unites the wound edges, epithelial cells begin migrating across the wound, vasodilation and increased permeability of blood vessels deliver nfeutrophils and monocytes that phagocytize microbes, and mesenchymal cells develop into fibroblasts.	***Migratory phase*** Sporadic and loose fibrous tissue filled on the wound sites.	***Proliferative phase*** No epidermis or dermis structure has been traced in the section.
B: Unwoven Cloth	***Migratory phase:*** The clot becomes a scab and epithelial cells migrate beneath the scab to bridge the wound, fibroblasts migrate along fibrin threads	***Proliferative phase*** No epidermis or dermis structure has been traced in the section.	***Proliferative phase*** No epidermis or dermis structure has been traced in the section.

	and begin synthesizing scar tissue, and damaged blood vessels begin to regrow. During this phase, tissue filling the wound is so called granulation tissue.		
C: Unwoven Cloth + NIPPAN	Upper part of the wound site has been in the ***final phase (maturation phase)*** that the scab sloughs off once the epidermis is restored to normal thickness, collagenous fibers become more organized, fiberblasts begin to dissapear, and blood vessels are restored to normal. In the lower part of the wound site is still in ***proliferation phase***.	***Maturation Phase*** The wound site has been totally recovery with normal dermis and epidermis structure around.	***Maturation Phase*** The wound site has been totally recovery with normal dermis and epidermis structure around.
D: Unwoven Cloth + NIPPAN + Gelatin	***Proliferative phase***: An extensive growth of epithelial cells beneath the scab, the deposition of collagenous fibers in random patterns by fiberblasts, and the continued growth of blood vessels.	***Proliferative phase*** The dermal paplliae, hair follicle and sudoriferous (sweat) gland have been developed.	***Maturation Phase*** The wound site has been totally recovery with normal dermis and epidermis structure around.
E: Unwoven Cloth + Gelatin	***Proliferative phase*** No epidermis or dermis structure has been traced in the section.	***Proliferative phase*** The dermal paplliae and sudoriferous (sweat) gland have been developed. Hair follicle was not appeared in the section.	***Proliferative phase*** The dermal paplliae, hair follicle and sudoriferous (sweat) gland have been developed. The wound site becomes narrow because of the slicing place on the periphery of the wound site.

5. Conclusion

The tri-layer wound dressing has been successfully in the treatment of extensive skin injury. The histological changes in and around the dressing on the wound bed were over a period that extended from 7 days to 42 days. The process of wound healing and repair was divided into 4 phases that were recognized by certain histological features. Occasionally, eosinophiles or giant cells were observed. Their appearance did not correlate with clinical problems. Such cellular infiltrates appear to be entirely healing and repair under sucha circumstances. No immune reaction that correlated with rejection of the dressing was observed. The dressing will fall off automatically without any damage once the wound site healed completely.

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7. References

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