2014 年臺灣國際科學展覽會
優勝作品專輯

作品編號　**090024**

參展科別　醫學與健康科學

作品名稱　**Development of Biomimetic Skins**

得獎獎項　一等獎

國　　家　**Singapore**

就讀學校　**Raffle Institution**

作者姓名　**P Kishore**

Abstract

The objective of the project is to develop a novel biomimetic membrane and/or a scaffold for the said membrane. The approach of the project is to use animal skin from the domesticated pig or fish as a scaffold material for the adherence and growth of human skin fibroblasts to create a biomimetic membrane that can be used in medical applications as an alternative to today's gold standards of Xenograft, Allograft and Autograft procedures. The biomimetic skin membrane can be used to treat victims of burns or scarring with a natural material that would be eliminated via natural bodily functions while eliminating the side effects and drawbacks such as scarring, secondary infections and tissue damage resulting from the current gold standard graft procedures on donor sites. Pig and fish skins were treated with ethanol and dehydrated followed by perfusion with Phosphate buffer solution and Cell culture media. Human skin fibroblasts (NF3 cells) were seeded on the animal skin scaffold. The human skin fibroblasts were then observed to determine their morphology and membrane formation properties. It was observed that the human skin fibroblasts were able to adhere to the non-human skin scaffolding and proliferate. More research is needed to determine their viability as a biomimetic membrane.

INTRODUCTION

Biomimetic skins are membranes that closely mimic the properties of natural membranes so that they can be used for a variety of purposes such as treatment of severe burns or skin replacement therapies. While many biomimetic membranes have been developed, the ideal membrane remains elusive. The demand for such biomimetic membranes has outpaced discovery and supply of such membranes. This project will aim to fill the gap. Many persons suffer debilitating skin diseases over the course of their lives and such illnesses may require skin grafts to recover. The skin is one of the most important organs in a human body. It is an amazingly durable, resilient and self-healing structure that exhibits extraordinary repair properties. The current gold standards for skin tissue grafting are the Allograft, Autograft and Xenograft processes. Many skin transplants performed today are skin Allografts which are graft skin tissues transplanted from one human donor to another human recipient. By grafting from one individual of the *homo sapien* species to another, risks arising from cross-species incompatibility can be reduced but require strong immune suppressants leaving recipients more vulnerable to opportunistic infections. Hence, an allograft may not be possible in the event that the patient is suffering from a debilitating, terminal or chronic illness. Skin Auto grafts are skin tissues transplanted from the patient's body to another region on his own body. Autografts pose no risk of immune rejection as skin segments are removed from discreet parts of a victim's body, using a dermatome, and transplanted to the region of skin injury. Allergic reaction and surgical risks are greatly reduced. However, no more than 50-60% of skin tissue can be removed and thus, this is not a viable method for treating large areas of damaged skin tissue without causing serious harm to the patients' health and wellbeing. The autograft procedure also scars the patient as the skin to be "donated" to himself has to be excised from his body that will leave scarring and pose an increased threat of secondary or opportunistic infections. Skin xenografting is least used and rarely successful. The immune reaction against xenografts is much more severe than in the case of auto or allografts ultimately causing rejection of the graft or even hyperacute rejection causing endothelial damage to the donor tissue, inflammation of tissues, thrombosis, and the formation of blood clots along with eventual necrosis of the donor tissue. Hence, current research is focused on the production of transgenic organs or tissues for the purposes of transplantation. This

too, is very challenging a demand as genetically modifying animals for the purpose of transplantation for example knocking out the gene that produces the enzyme that produces the receptor (Inciting a human immune response) does not mean that other causes for the rejection can be addressed. The human immune system is very proficient in identifying self from non-self and this in turn acts against such efforts.

MATERIALS AND METHOD

The growth of the biomimetic membrane can be categorized into 5 stages. The collection and interim storage of the cell scaffold material. The preparation and sterilization of the scaffold, initiation of the primary cell culture, initiation of secondary cell culture and the crafting of the membrane itself. Pig and fish skins were collected from a commercial source (i.e. Local market) and were stored at -80 ℃ immediately to prevent and degradation of the material. The scaffold material was sterilized to remove any contamination from various infectious agents such as bacteria, fungi and prototists due to human handling. The frozen pig/fish skin to eliminate moisture that is trapped in the cells and was subjected to ethanol exposure to eradicate contamination from infectious agents. This is achieved through gradual dehydration (using increasing ethanol concentrations (50%, 75% and 100%) of ethanol as it is a dehydrating agent. After complete dehydration, the scaffold will be rehydrated via perfusion of Phosphate buffer solution (PBS) at a neutral pH of 7 at (25 ℃) in preparation for the construction of the biomimetic membrane.NF3 from a commercial source are thawed and cultured in a complete growth medium in a cell culture flask. The cells are incubated in a CO_2 inhibitor for 24-48hrs and then checked for morphology, proliferation and viability.

Scaffolds were perfused with growth medium and cell culture was added to the top of the scaffold in an even fashion. The structure is then incubated in a CO_2 incubator for 24-48 hrs. This process is repeated thrice to ensure consistent results. The cells will then be observed to check their adhesion to the scaffold, their proliferation and viability through the use of optical, florescence and scanning electron microscopy.

Repeat all steps. Before the reperfusion of the skin scaffold with phosphate buffer solution, place the skin tissue samples in a vacuum oven and ensure complete dehydration and removal of residual ethanol. Repeat all steps but coat the scaffolds with biocompatible polymer such as polycaprolactone.

RESULTS AND DISCUSSION

The treatment of the animal skin scaffold material with ethanol and dehydrating the skin scaffold material was done to ensure complete sterilization of the skin scaffold, a photomicrograph of the skin scaffolds were obtained using a optical microscope. This was done in order to ensure that contaminants like bacteria, fungi, protists and other infectious agents were not present on the sterilized skin scaffolding material.

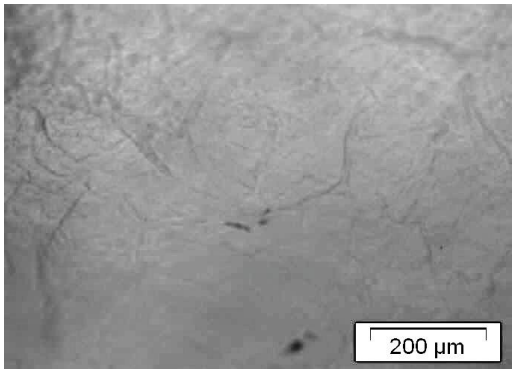
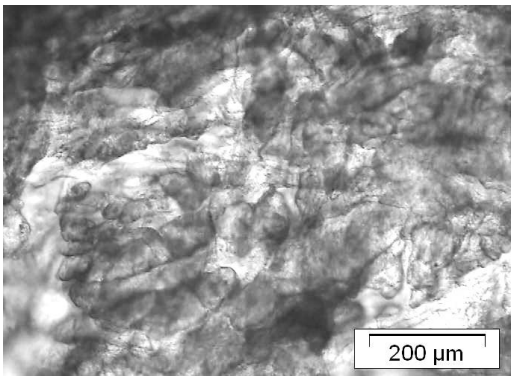
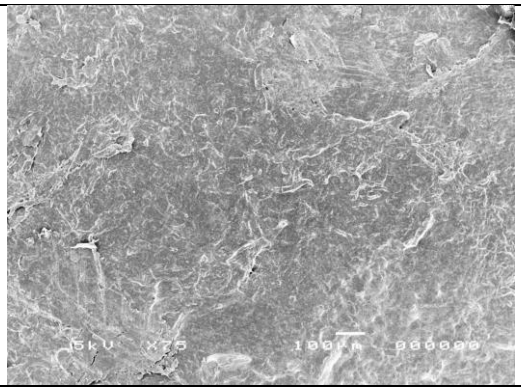
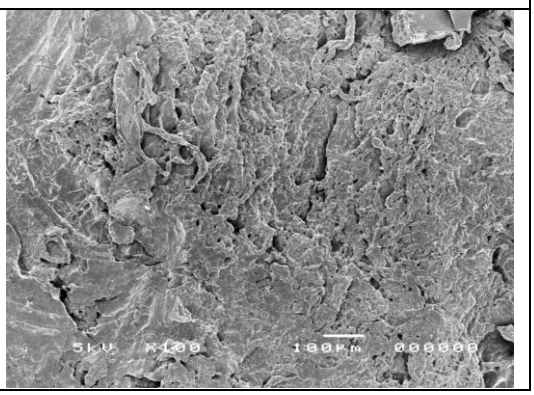
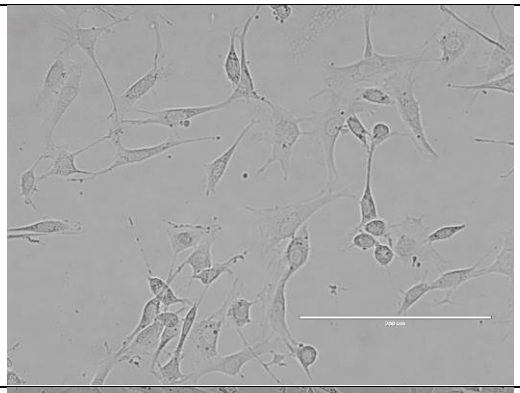
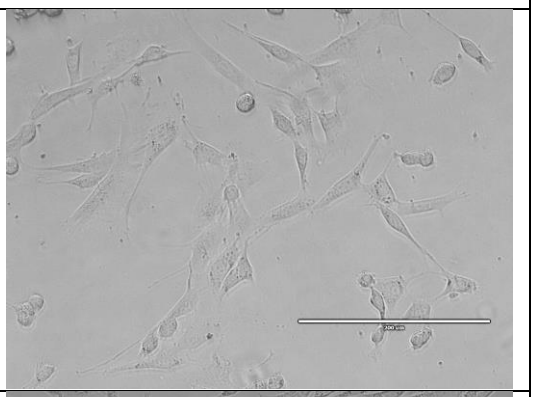
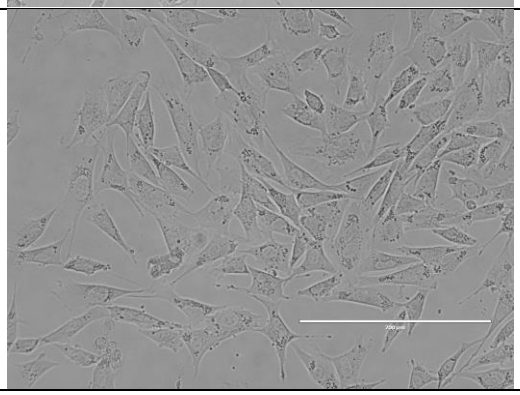
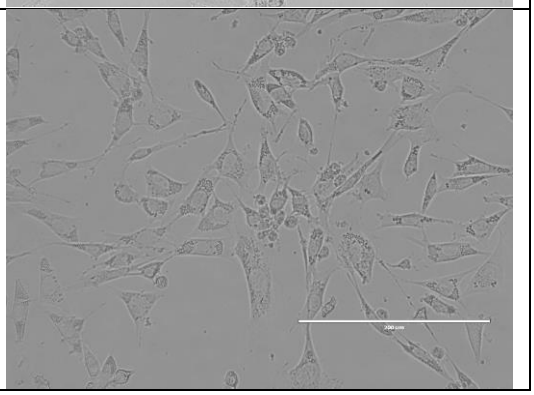
	FISH	PIG
A		
B		

Figure 1 A1 and A2 show no contamination of the bare fish and pig skin scaffolds respectively after PBS and cell culture media treatment. We can observe only the pseudo-stratified squamous cell layers of the fish and pig skins. The morphological observations indicate that the skin scaffold material was sterilized with the ethanol treatment and dehydration processes. But the human skin fibroblasts were unable to adhere to the scaffold. Figure 1 B1 and B2 are the SEM images of polymer coated pig and fish scaffolds respectively. Figure 2 A1 and A2 show the control study consisting of only the human skin fibroblast cells seeded on cell culture plates. It was performed to reaffirm the morphology and viability of the human skin fibroblasts used in the study. Figure 2 B1 and B2 show NF3 proliferation on cell culture plates with bare fish and pig skin scaffolds respectively. We observed that NF3 cells adhered to the base of the cell

culture plate and have proliferated exhibiting a normal cellular morphology and viability.

	1	2
A		
B		

Toxic substances, if present in the scaffolds, would diffuse into the cell culture media and destroy the cells ensuring zero or poor growth of human skin fibroblasts in the cell culture plate. Figure 2 B1 and B2 clearly show that the NF3 cells were able to proliferate and thrive in the cell culture plate with the scaffolds indicating that there was no toxic effect. However, insufficient time for the NF3 cells to adhere to the scaffolds before adding culture media or the scaffold assembly had floating in its well during incubation may have caused them to move to the base of the plate.

Future extension/current work

NF3 cells had poor cell attachment on bare skin. Further suggests that an adhering agent such as gelatin or PEG will facilitate the adherence of the NF3 cells to scaffold. Biocompatible adhering agent will merely facilitate the adherence of the cells to the scaffold leading to the formation of the biomimetic membrane.

評語

To develop a novel biomimetic membrane and scaffold for the said membrane. Human skin fibroblasts (NF3) were seeded on the animal skin scaffold. The preliminary data indicated the human skin fibroblast were able to adhere to the non-human skin scaffold. However, the human test was prohibited, can't compare its advantage over commercial available skin.