6. Evaluation of PLA membranes for wound healing

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6.1 Introduction

The incidence of burn injuries is fast reaching epidemic proportions in India and other developing nations (1). This changing socio-economic scenario has led to increased incidence of burn related injuries in industrial units and homes. Most of these patients who are in the rural and semi-urban areas have little access to proper burn care leading to high incidence of mortality and morbidity.

The gold standard for burn treatment in most hospitals presently is autologous skin grafting (2). Though it provides good results to most patients it has certain disadvantages. In patients with large areas of burn injuries it may be difficult to find enough harvesting sites for grafting. Biological coverings like amniotic membrane and cadaver skin though useful in burn patients have issues relating to proper storage and risk of contamination and infection. To circumvent these difficulties, artificial skin substitutes have been developed and have proven to be very effective for burn treatment especially in the western countries. Unfortunately, they are yet to be commonly available to patients in India and other developing countries, mainly due to their high cost. Artificial skin substitutes can have the cellular components of skin like keratinocytes or fibroblasts grown on scaffold. Theses can act as regeneration templates without any cells, thus classifying the skin substitutes as cellular or acellular. An example of a cellular skin substitute is ‘Apligraf’, which consists of viable allogenic neonatal fibroblasts grown in a bovine type I collagen gel matrix and combined with viable allogenic neonatal keratinocytes (3). ‘Apligraf’ has proven very effective for treatment of chronic ulcers (4). But it has not been adopted for widespread use in burns and large wounds due to its high cost, safety considerations and very short shelf life. In this regard, acellular artificial skin substitutes are more suited for wider use for burn wound cover due to their relatively lesser cost compared to their cellular counterparts, ease of handling and storage and the benefits for wound healing offered by the skin regeneration template. ‘Integra’ was one of the first acellular artificial skin substitutes to be developed and widely used in US with very good results in burn patients (5). An example of a biocompatible and biodegradable polymer based acellular skin substitute is ‘Suprathel’, which is a polylactic acid based copolymer membrane for burn treatment. These polymeric skin substitutes provide a
protective barrier for the open wound and decrease the chances of infections. These biodegradable membranes are semi-porous, thus reducing fluid loss, at the same time maintaining moist wound environment which is beneficial for wound healing.

In India and other developing countries, there is an urgent need for an artificial skin substitute that is affordable, effective and easy to use. It is proposed that our process of instantaneously making porous polymer membrane from PLA particles can be used as a method for making membranes which act as temporary cover and even as regeneration templates in burn injuries. The membrane has all the prerequisites to be developed as skin substitute. It is made from particles having porosity and made from biodegradable polymer. Large sized membranes depending upon the requirement can be fabricated easily. In this chapter, preliminary evaluation of PLA membranes as an artificial skin substitute for wound treatment was carried out in rat wound models. Collagen content was analyzed to assess the wound strength of the treated animal with membranes in comparison to animals treated with conventional wound dressings.

6.2 Materials and Methods

6.2.1 Evaluation of Polymer Membrane as Skin Substitute in Animal Wound Model

Animals were maintained according to the guidelines established by the Institute Animal Ethics Committee (IAEC) of the National Institute of Immunology, New Delhi. Preliminary evaluation of the polymeric membrane for wound healing was tested on Wistar rats. All surgical procedures were carried out under anesthesia. For the initial experiments, the polymeric membrane was evaluated for non-infected full skin thickness wounds (2×3 cm) on the dorsum of rats. The animals were divided into the treated and untreated groups (n=6) and in the treated group, the polymeric membrane immediately formed after the fusion process was directly transferred onto the wounds. The control rats were treated with conventional wound dressing of paraffin gauze and cotton bandage. Wound scoring according to the parameters given in Table 6.1 was carried out at different time points till the complete closure of the wounds in the animals (6).
6.2.2 Wound Collagen Assay

The wound collagen assays of the rat’s healed skin were performed using sircol collagen assay (7). It contains the reagent Sirius red which reacts specifically with the side chain groups of the basic amino acid groups of collagen. In brief, after an average healing period of 15 days the rats were sacrificed. The healed skin were excised and thoroughly washed with PBS. The skin was sliced into small bits; equal amounts of skin (70 mg) from both groups were boiled in 7 ml of phosphate buffer for 80°C for 50 minutes to extract the cross-linked insoluble collagen from the healed skin of the wounds. The supernatant was filtered using 0.45 micron filters and 100 μl of the solution was added to 1 ml of the Sircol dye. After shaking for 30 minutes, the tubes were centrifuged for 5 minutes for 12,000 rpm. Unbound dye solution was drained and 1ml of alkali reagent was added to release the unbound dye. The absorbances of the solutions were measured at 540 nm using spectrophotometer and the collagen content was calculated using a standard curve.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Weighing factors</th>
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<tbody>
<tr>
<td>Haematoma</td>
<td>-1</td>
</tr>
<tr>
<td>Exudate</td>
<td>-2</td>
</tr>
<tr>
<td>Membrane</td>
<td>-3</td>
</tr>
<tr>
<td>Pus</td>
<td>-4</td>
</tr>
<tr>
<td>Odour</td>
<td>-2</td>
</tr>
<tr>
<td>Perifocal reddening</td>
<td>-2</td>
</tr>
<tr>
<td>Granulation tissue impairment</td>
<td>-4</td>
</tr>
<tr>
<td>Granulation tissue</td>
<td>4</td>
</tr>
<tr>
<td>Wound contraction</td>
<td>3</td>
</tr>
<tr>
<td>Epithelialisation</td>
<td>4</td>
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Table 6.1: Wound scoring chart of the animals
6.3 Results

Polylactide membrane formulated by the fusion process was evaluated as artificial skin substitute for wound healing in experimental animal models. As described in chapter 3, PLA membrane of different sizes could be fabricated using petri dishes of varied sizes. For wound healing experiments, membranes of approximately 2 x 2 cm size were fabricated and used (Fig 6.1). The membranes were directly transferred onto the non infected full thickness wounds of Wistar rats to evaluate whether the polylactide membranes were biocompatible and did not delay wound healing (Fig 6.2 A,B). The freshly prepared PLA membrane readily stuck to the wound bed (Fig 6.2 A). As the wound starts healing, the PLA membrane starts degrading and giving way to the regenerating skin (Fig 6.2B). Since the rats are highly mobile, the polymer membrane was held in place by absorbable sutures. The polymeric membrane adheres well to the wound and starts slowly degrading. The control rats were treated with traditional wound dressing of paraffin gauze and cotton bandage. Preliminary evaluation of the polymeric membrane showed that they were biocompatible and did not delay the healing process. The polymeric membrane composed of the fused polylactide particles adhered to the wound bed and did not cause adverse reactions or wound infection during the entire closure of the clean wound as evaluated by wound scoring of both the sets of animals (Fig.6.3 A-D). In case of rats treated with conventional dressing material of paraffin and cotton gauze, the dressing had to be changed frequently. Change of dressings can be painful and also damage the sensitive regenerating skin of the wound, causing raw areas to appear (Fig 6.3 A). In case of PLA membrane treated rats, the membrane was able to stably cover the wound till its complete closure (Fig 6.3 B). The wounds in both groups of rats healed completely in about 21 days and this proves that the PLA membrane is biocompatible in the wound environment and does not interfere with the healing process (Fig C, D). This is a major advantage of biodegradable wound coverings, since it is not necessary to change the dressings frequently, thus causing minimal damage to the regenerating skin. The main functions of the PLA membrane as a skin substitute will be to provide barrier function to decreases the chances of wound infections and to decrease the fluid loss from the wound. Cross linked collagen content in healed skin of the animals treated with polylactide membrane was found to be higher than that of untreated animals (Fig 6.4). It has been shown that increase in the cross-linked
collagen content in the healing skin leads to increased wound strength (8). This showed that the wound strength of rats treated with the PDLLA membrane were higher than that of rats treated with conventional wound dressing.

**Fig 6.1.** PLA membrane formed by fusing PDLLA-CTAB particles in presence of ethanol in sterile petri dish to be used to cover the wounds in animal wound models.
Fig 6.2A Wound treated with polymeric membrane, Day 1

Fig 6.2 B Polymeric membrane on the wound, Day 6
Fig 6.3 Evaluation of polylactide polymeric membrane formed by fusion process for wound healing. (A) 2×3 cm uninfected open wound, day 5. (B) Open wound treated with the polymer membrane, day 5. (C, D) Rats after 21 days of treatment. (C) Rat with open wound. (D) Rat treated with polymeric membrane
Fig 6.4: Collagen assay of the healed rat skins. Skin of the scaffold treated rats and rats treated with conventional wound dressings (control) were used for collagen assay.
6.4 Discussion

Artificial skin substitutes need to comply with three major requirements before they can enter clinics, namely that they should be safe for the patient, be clinically effective and be convenient in handling and application (9). Our major focus is on polymer based acellular skin substitutes since they are cost effective, user friendly and possesses a long shelf life. The membrane should have certain properties like non-toxicity and cause minimal immunological reaction and it should prevent fluid loss from wound surface and protect the wound from infection (10). ‘Integra’, one of the first acellular regeneration templates to be introduced in the wound market has now been widely adopted for the treatment of full thickness burns (11). It consists of a porous dermal component made of bovine type I collagen and shark chondroitin-6-sulphate glycosaminoglycan which is bonded to a silicone layer. The matrix becomes populated with fibroblasts in the wound and contributes towards neo dermis formation, at the same time the scaffold degrades in a controlled way. The silicone layer protects the wound from fluid loss and bacterial infection. ‘Suprathel’ is a polylactide based composite membrane for wound treatment (12). Suprathel rapidly adhered to the wound bed, reduced pain, protected against infections and promoted wound healing.

In the present study, polylactide membrane formulated by fusion of PDLLA-CTAB particles was evaluated as an artificial skin substitute for treatment of wounds in rats. The scaffold had proved to be suitable for the growth of animal cells and did not cause any toxicity to the growing cells. The ethanol treatment during the fabrication process ensured the sterility of the membranes and the removal of the surfactant molecules. Preliminary evaluation in rats showed better performance of the polylactide membrane on wound healing over traditional wound dressings with higher cross linked collagen content of the polylactide membrane treated wounds. PLGA based scaffolds to be used for skin tissue engineering have shown successful attachment and growth of fibroblasts (13). The polylactide membrane in addition to providing wound cover and protection against infections, may be acting as a regeneration template for more organized growth of fibroblasts in the wound bed, resulting in higher cross-linked collagen content. Also, it is not necessary to change the polylactide membrane dressing frequently, since they are
biodegradable and biocompatible, while frequent changes of traditional wound dressings are required causing injury to the underlying healing skin. The porous nature of the polymeric membrane maintained a moist environment needed for proper wound healing, at the same time prevented excessive loss of fluids. Since infections of open wound with microbes and subsequent delay in healing are major problems faced in burn cases (14, 15), it would be advantageous if the scaffold also releases drugs in a controlled manner to combat wound infections. Presently work is being carried out to encapsulate broad spectrum antibiotics that combat wound infections in the PDLLA-CTAB scaffolds, so that the efficiency of the membrane to combat wound infections is increased by the controlled release of the drugs to the wound bed.

6.5 Conclusion

Polymer membrane designed using PLA particles acted as passive membrane for wound dressing. It is proposed that using surfactant coated polylactide particles encapsulated with antibiotics and growth factors can be made and conveniently stored till needed. When a burn case arrives, the particles can be spread out into desired sizes and wetted with ethanol to immediately form the polymeric membranes to be used to cover the wounds. This step also removes the surfactant molecules and effectively sterilizes the membrane. Polylactide is a biocompatible and biodegradable polymer and polylactide based composite membrane for burn treatment is already approved for human use. Preliminary evaluation in animal wound models showed that the membranes did not interfere with the normal healing process, at the same time the treated animals showed increased cross linked collagen indicative of increased wound strength. It would be advantageous for the patient if the porous polylactide membrane composed of the fused particles act both as a temporary wound cover as well as provide a template for skin regeneration.
6.6 References


