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Potential of Bioprinted Films for Skin Tissue Engineering

WEI LONG NG

NTU Additive Manufacturing Centre, School of Mechanical & Aerospace Engineering, Nanyang Technological University, HW1-01-05, 2A Nanyang Link, Singapore 637372, Singapore
Singapore Institute of Manufacturing Technology, Agency for Science, Technology and Research, 71 Nanyang Drive, Singapore 638075, Singapore

WAI YEE YEONG

NTU Additive Manufacturing Centre, School of Mechanical & Aerospace Engineering, Nanyang Technological University, HW1-01-05, 2A Nanyang Link, Singapore 637372, Singapore

MAY WIN NAING

Singapore Institute of Manufacturing Technology, Agency for Science, Technology and Research, 71 Nanyang Drive, Singapore 638075, Singapore

ABSTRACT: Key roles of the healthy human skin are to prevent bacterial infection and excessive loss of water. However, such essential roles are compromised in patients with severe burns or chronic wounds. Synthetic materials such as petrolatum gauze backings and silicone membranes are commonly used in wound dressings to improve its mechanical properties and temporarily restore the role of the damaged skin. These synthetic materials are usually non-biodegradable and subsequent removal of such temporary wound dressings from the wound site is necessary. This necessitates the development of biodegradable films for skin tissue engineering. Although solvent casting is a simple fabrication technique for such films, properties such as mechanical strength and water transmission rate cannot be easily controlled via solvent casting technique. In this paper, a bioprinting approach will be explored for the fabrication of films for skin wound healing. The potential of such bioprinted films for skin tissue engineering is highlighted.

1. INTRODUCTION

The main goal of the tissue-engineered skin is to restore skin barrier function in patients with severe burns or chronic wounds (MacNeil et al. 2007). Synthetic materials such as petrolatum gauze backings (Carsin et al. 2000) and silicone membranes (Tausche et al. 2003, Hernon et al. 2006) in tissue-engineered skin substitutes improve the mechanical properties and prevent excessive moisture loss and bacterial infection. However, these materials are non-biodegradable and subsequent removal from the wound site is necessary. To overcome this limitation, biodegradable biomaterials are widely utilized for tissue engineering (Nair et al. 2007).

Chitosan has been widely utilized for skin tissue engineering due to its unique properties to induce hemostasis and accelerated tissue regeneration (Cho et al. 1999). The presence of chitosan expedited wound healing by inducing the migration of inflammatory cells and production of multiple cytokines (Mori et al. 1997, Peluso et al. 1994). Furthermore, chitosan and its biodegradation by-product do not pose any toxicity to the cells. At typical wound pH values, positively charged chitosan-based biomaterials facilitate interaction with negatively charged

molecules such as nucleic acids, proteins and anionic polysaccharides present at the wound site (Lloyd et al. 1998, Bhattarai et al. 2010). Notably, chitosan-based biomaterials exhibited antimicrobial properties, which is important for preventing bacterial infection (Tomihata et al. 1997). However, chitosan has poor mechanical integrity and degrades rapidly in the presence of lysozymes and acidic medium (Madhally et al. 1999). Gelatin is a partially denatured form of collagen and is composed of a unique sequence of amino acids. It is completely resorbable *in vivo* and does not show any forms of antigenicity. Markedly, gelatin improves the biological activity by forming polyelectrolyte complexes and facilitating cell adhesion and migration with its RGD sequences (Huang et al. 2005). Several chitosan/gelatin scaffolds have been reported for tissue engineering applications (Huang et al. 2005, Mao et al. 2003a, Mao et al. 2003b).

This paper hence focuses on the development of a biodegradable film that aims to improve mechanical properties of tissue-engineered skin and restore the skin barrier functions. Although solvent casting is a relatively simple technique of fabricating thin films, it is difficult to manipulate properties such as mechanical strength and moisture permeability. A novel bioprinted composite chitosan/gelatin film is fabricated and its potential for skin tissue engineering will be discussed.

2. MATERIALS AND METHODS

2.1. Materials

Gelatin (porcine skin, Type A) and chitosan (low molecular weight) were obtained from Sigma Aldrich (Singapore). 0.5M acetic acid, 0.25M sodium hydroxide (NaOH) and phosphate buffered saline (PBS) solution (pH 7.4 at 0.01M) were used.

2.2. Composite chitosan-gelatin bilayer film preparation

2.2.1. Solvent-casting approach

4ml of a 5% w/v gelatin solution was heated in an oven at 40°C and then poured into a tissue culture dish of diameter 5cm. The thermo-sensitive gelatin solution solidified upon cooling at room temperature (~15mins). 90mg of chitosan was dissolved in 4ml of 0.5M acetic acid to prepare a 2.25%w/v solution, followed by stirring with a magnetic bar for 20mins to obtain a homogeneous solution. The homogeneous chitosan solution was poured over the solidified gelatin solution and left to evaporate completely. Upon complete drying, the film was washed with 0.25M NaOH to remove residual acetic acid followed by repeated washing in distilled water. The average thickness of solvent-casted films was $0.203 \pm 0.022\text{mm}$.

Figure 1: Solvent-casted chitosan/gelatin bilayer film

2.2.2. Bioprinting approach

An extrusion-based Bioplotter was used in the printing process. The gelatin base layer and chitosan solution were prepared in a similar manner as described in the solvent-casting approach. The homogeneous chitosan solution was then poured into a 5ml printing cartridge and a 30mm X 20 mm multi-layered chitosan block was printed above the gelatin layer. Upon complete drying, the film was washed with 0.25M NaOH to remove residual acetic acid followed by repeated washing in distilled water. The average thickness of bioprinted films was $0.196 \pm 0.002\text{mm}$.

Figure 2: (A) Image of a printed single-layered chitosan solution under a inverted microscope (B) Bioprinted chitosan solution before drying (C) Bioprinted chitosan solution after drying

2.3. Tensile mechanical test

Figure 3: Tensile mechanical testing of composite chitosan/gelatin bilayer film.

The tensile mechanical property was tested by a Universal Tester (Instron 5566, Singapore) with 50N load cell. The sample size of each group was 4. The composite films were tensile tested along the printed orientation and the load was applied until ultimate fracture of the specimen. Sand papers were used to prevent slipping of specimens during tensile testing. The grip separation was set at 10mm and a testing speed of 5 mm/min was used. The ultimate tensile strength was determined as the maximum point of the force–strain curve.

2.4. Water vapour transmission rate

The moisture permeability of the film was determined by measuring the water vapour transmission rate (WVTR). Specimens from each group plus control set-up were used in this experiment. A digital pipette was used to add 4ml of distilled water into each 10cc plastic bottle with an opening

diameter of 16.6mm. The weight of each bottle with 4ml of distilled water was recorded. The composite films from each group were placed over the bottle openings while the control set-up was left uncovered. The weight loss in each sample was recorded after a period of 24 hours.

2.5. Water uptake ability

A completely dried composite film was accurately weighed and recorded as W_{dry} , followed by immersing the specimen in distilled water for 24 h. Excessive water was removed by blotting the film with a piece of filter paper; the wet sample was weighed and recorded as W_{wet} . The water uptake rate was calculated according to the formula:

$$\text{Water uptake rate (\%)} = [(W_{\text{wet}} - W_{\text{dry}}) / W_{\text{dry}}] \times 100\% \quad (1)$$

2.6. Degradation profile

The degradation behavior of the composite film was investigated by immersing the specimen in a petri dish containing 10 mL of phosphate buffered saline (PBS) solution at pH 7.4 and 37°C. The change in weight and thickness of the films were recorded.

3. RESULTS AND DISCUSSION

3.1. Tensile strength

Table 1: Comparison of ultimate tensile strength

Samples	Ultimate Tensile Strength (N)
Solvent-casted	2.92 ± 0.03
Bioprinted	4.19 ± 0.10

Notably, the bioprinted film has a higher ultimate tensile strength than the solvent-casted film. The observed phenomenon can be attributed to the alignment of the polymer chains within the polymeric films. It is very likely that polymer chains in the solvent-casted films are randomly orientated, whereas the polymer chains in the bioprinted films are more highly aligned. As illustrated in figure 4, the bioprinting process enabled the formation of highly-aligned chitosan lines. Furthermore, the high surface tension of solvent molecules at the liquid-air interface caused the edges of closely-packed chitosan lines to eventually merge into a single compact layer. The bioprinting process was repeated in a layer by layer manner to obtain a multi-layered chitosan construct with orderly alignment. The accumulative effects of such alignments in the bioprinted films resulted in higher ultimate tensile strength.

Figure 4: Image of highly-aligned chitosan lines during the bioprinting process

3.2. Water vapour transmission rate (WVTR)

Table 2: Comparison of water vapour transmission rate

Samples	WVTR (g/m ² h)
Solvent-casted	20.46
Bioprinted	12.92
Control	26.94

It is important to note that both temperature and humidity of surrounding environment can influence the WVTR. The solvent-casted films demonstrated higher WVTR than the bioprinted films. This can be attributed to the orderly alignments of polymer chains within the bioprinted film which hindered the transmission of water vapour. The water permeability of both films circumvents the accumulation of wound exudates and retains a moist wound environment to expedite wound healing. According to a study, different body parts have different water vapour permeation (10-50g/m²h) (Uhlir et al. 2007). Different line widths and spacings within each chitosan layer could be explored to obtain the desired WVTR for different body parts.

3.3. Water uptake ability

Table 3: Comparison of water uptake ability

Samples	Water Uptake Ability
Solvent-casted	270 - 277%
Bioprinted	241 - 245%

It was observed that the bioprinted films have lower uptake rates than the solvent-casted films. This is probably due to the densely packed polymer chains inside the bioprinted films which limited their ability to absorb more water. Both types of films have very high water uptake rate, which make them suitable for wounds with large amounts of exudates. It is of paramount importance to select suitable biomaterials with appropriate water uptake ability (Jones et al. 2006). Maceration of skin would occur when a low absorptive film was placed over a heavily exuding wound. Conversely, the use of a highly absorptive film on a dehydrated wound would hinder tissue regeneration.

3.4. Degradation profile

A huge decrease in weight for both films (~50% decrease) was observed after 1 day due to the disintegration of gelatin base at physiological temperature (36.9°C). After this stage, the remaining chitosan layer presented a gradual weight and thickness loss over the next few days. The remaining chitosan layer still remained intact in both groups after a week.

4. CONCLUSION

In summary, a novel bioprinted film is experimentally demonstrated to be effective as a carrier template for skin tissue engineering. Bioprinting technique could be used to manipulate the ultimate tensile strength, moisture permeability and water uptake ability of the film. Varying

printing parameters and designs could be explored to fabricate customized films with specific requirements and this huge potential has yet to be fully uncovered.

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