*Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 20 (December Suppl.) : 2018 : S43-S47*

***ISSN-0972-3005***

# ELECTROSPUN FIBERS AS A WOUND DRESSING MATERIAL USING COMBINATION OF CELLULOSE ACETATE/COLLAGEN SEEDING STEM CELL

**PURWATI[1,2]*, BAGUS SATRIO NURWITO[3] AND HENDITA NUR MAULIDA[1]**

[1]Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia
[2]Adjunct Associate Professor, Department of Biotechnology, Asia University, Taichung, Taiwan
[3]Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

(Received 25 September, 2018; accepted 15 November, 2018)

***Key words:*** *Wound dressing, Electrospun fibers, Electrospinning, Cellulose acetate, Collagen, Stem cell*

**Abstract–** Wound healing is a complex tissue regeneration process that the body undergoes as a response to wound openings or missing cellular structures as a result of various types of traumatic injury. Because of the ability of materials to induce a high immune response or limited donor tissues, the skin repair and regeneration methods using allografts and autografts cannot be widely used. Many researches have shifted into tissue engineering approaches using scaffolds. To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements include biocompatibility, biodegradability, and mechanical properties. Our study aimed to fabricate composite cellulose acetate-collagen (CA/Collagen) scaffolds by electrospinning and determine the appropriate compositions of CA:Collagen for obtaining skin substitutes as wound dressing through investigating the morphological of stem cell seeded on electrospun CA/Collagen membranes. High proliferation of mesenchymal stem cells on electrospun CA/collagen 75:25 (wt.%) confirmed the capability of CA/collagen 75:25 nanofibers as a tissue-engineered scaffold, while the electrospun CA/collagen 75:25 can be a potential low-adherent wound dressing.

## INTRODUCTION

Wound healing is a complex tissue regeneration process that the body undergoes as a response to wound openings or missing cellular structures as a result of various types of traumatic injury (Mulugeta *et al.,* 2018). In adult humans, optimal wound healing involves: (1) rapid hemostasis; (2) appropriate inflammation; (3) mesenchymal cell differentiation, proliferation, and migration to the wound site; (4) suitable angiogenesis; (5) prompt re-epithelialization (re-growth of epithelial tissue over the wound surface); and (6) proper synthesis, cross-linking, and alignment of collagen to provide strength to the healing tissue (Gosain *et al.,* 2004; Mathieu *et al.,* 2006). To facilitate effective wound healing, a wound site is typically covered with a sterile dressing material to avoid infection and to promote the healing process.

In the last decade, several skin repair and regeneration from xenografts, allografts and autografts have been used for wound healing, such as human amnion or chorion membrane. However, because of the ability of materials or antigen to induce a high immune (antigenicity) response or limited donor tissues, the skin repair and regeneration methods mentioned above cannot be widely used (Boyce, 2011; Schulz *et al.,* 2005). Many researches have shifted into tissue engineering approaches. This technique is an interdisciplinary field of study that emerges by applying the principles of biology, chemistry and engineering science to tissue regeneration (Hoerstrup *et al.,* 2004). The approach used in tissue engineering is more to the use of biomaterials, cells or combination of both, and suitable biochemical and physico-chemical factors to restore, maintain and improve biological functions.

The most important thing in skin tissue engineering is the construction of scaffolds. Scaffolds are materials that have been engineered to cause desirable cellular interactions to contribute to the formation of new functional tissues, that serves in infiltration and physical support to guide cell

***Corresponding author's email**: purwatisumorejo@gmail.com

differentiation and proliferation into targeted functional tissues (Mertsching *et al.,* 2009). To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. Ideal scaffolds for skin tissue engineering applications must have good biocompatibility, suitable microstructure such as average pore size of 63-150 ìm and porosity values above 90%, biodegradation can be controlled and suitable mechanical properties (O'Brien *et al.,* 2005; Newman *et al.,* 2013). Many different biomaterials have been investigated and being already employed as skin tissue engineering. Examples of these materials are cellulose acetate and collagen.

Polysaccharides are natural biopolymers available from plant sources and they show excellent biocompatibility (Miao *et al.,* 2011). Cellulose, the most abundant renewable polysaccharide in the form of woven cotton gauze, has been utilized for many years as wound dressings (Elham *et al.,* 2014). While collagen is known as the most promising materials in tissue engineering application because of its biocompatibility and biodegradability. Scaffold from collagen has a homeostatic effect, antigenicity and can increase cell growth and cell adhesion (O'Brien *et al.,* 2005).

Nanofibrous membranes due to their porous structure, high surface area and structural similarity to the native extracellular matrix (ECM) can serve as an excellent functional skin substitute for deep wounds. Among the different methods of nanofiber fabrication, electrospinning is a simple, cost-effective and versatile technique for generating nanofibers of polymers as wound dressings (Elham *et al.,* 2014; Zahedi *et al.,* 2010). In recent study, cellulose acetate (CA) was combined with polyurethane to fabricate a composite scaffold as wound dressing by electrospinning (Liu *et al.,* 2012). However, very few reports are available on the application of electrospun CA composite scaffolds with natural polymers such as collagen for tissue regeneration (Powell *et al.,* 2008).

Our study aimed to fabricate composite CA/Collagen scaffolds by electrospinning and determine the appropriate compositions of CA:Collagen for obtaining skin substitutes as a wound dressing through investigating the morphological of stem cell seeded on electrospun CA/Collagen membranes. Stem cells are unique types of cells that are undifferentiated. So the main focus of creating these constructs is to be able to safely deliver these stem cells, and create a structure that is physically and mechanically stable so that these stem cells can differentiate.

## MATERIALS AND METHODS

### Materials

Cellulose acetate (CA; white powder; Mr ~29,000; acetyl groups ~40%), acetone and formic acid were all purchased from Sigma-Aldrich, collagen type 1 from snapper scales was purchased from the National Nuclear Energy Agency of Indonesia. Mesenchymal stem cell was derived from rat tissues. Dulbecco Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and penicillin streptomycin solution were purchased from Gibco.

### Electrospinning

The CA/Collagen solutions were prepared in three different weight ratios, including 95:5, 85:15 and 75:25 (wt.%). To achieve beadless fibers, CA were dissolved in aceton with the concentration of 10% (w/v). After stirring for 1 h, each solution was loaded into a 5-mL syringe attached to a 23G blunted stainless steel needle at a flow rate of 0.01 mL/h. A high voltage of 14 kV was applied to the tip of the needle. The fibers were placed on a flat aluminum foil-wrapped collector kept at a distance of 10 cm from the needle tip. Nanofibrous membranes were dried in a room temperature for at least 24 h to ensure the solvent residuals vaporized completely.

### Cell Culture

Mesenchymal stem cells (MSCs) were derived from rat tissue using aspiration and separation on Histopaque-1.077 (Sigma). Harvested cells were cultured in Dulbecco's Modified Eagles Medium containing 1.0 g/L glucose. MSCs characterization were performed by analyzing the expression of 90+, CD34- and CD105+ by using DAB immunostaining and FACS (BD). Confluent stem cells were seeded on the scaffolds placed in a 24-well plate and tissue culture at a density of 5000 cells per well.

### Membrane Morphology

The morphology of CA/collagen seeded stem cell on electrospun scaffolds was observed by scanning electron microscopy (SEM). After 7 days of cell seeding, samples were washed with PBS and fixed with 3% glutaraldehyde. After being washed with

distilled water, scaffolds were dehydrated through ethanol solutions. Subsequently, the samples were treated with hexamethyldisilazane and air-dried in a fume hood. Completely dried specimens were sputtercoated with gold. The SEM images were analyzed with image analysis software to determine average fiber diameter.

## RESULTS

The morphology of electrospun nanofibers was investigated by SEM and Figure 1 shows the SEM micrographs of electrospun scaffolds. The interaction between mesenchymal stem cells and electrospun nanofibrous scaffolds were evaluated after 7 days of cell culture and the results are shown in Figure 2. The cells were found to attach on all the electrospun nanofibers, but the compositional variations of the scaffolds caused some differences in the extent of proliferation such that the scaffold containing the highest collagen content showed the stretching of the cells across the nanofibrous substrate.

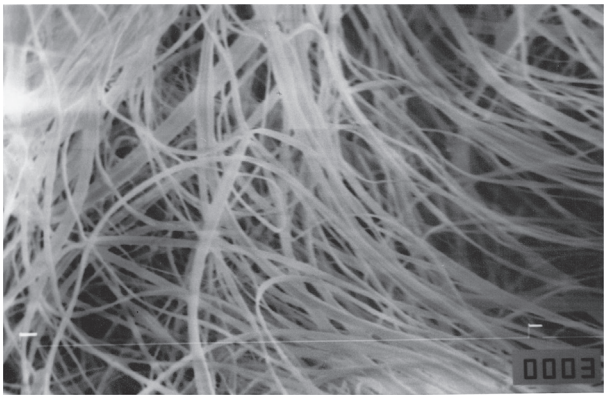


**Fig. 1.** Morphology of electropun CA/Collagen (75:25 wt.%) using scanning electron microscopy (SEM)

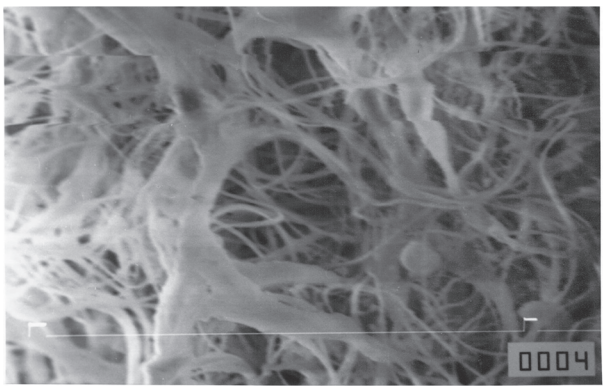


**Fig. 2.** Morphology of electropun CA/Collagen (75:25 wt.%) after 7 days of cell culture using scanning electron microscopy (SEM)

## DISCUSSION

Wound healing is a dynamic process consisting of four continuous, overlapping, and precisely programmed phases. The events of each phase must happen in a precise and regulated manner. Interruptions, aberrancies, or prolongation in the process can lead to delayed wound healing or a non-healing chronic wound (Guo *et al.*, 2010). Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure. Non-healing wounds affect about 3 to 6 million people in the United States, with total cost estimated at more than $3 billion per year (Gou *et al.*, 2010; Mathieu *et al.*, 2006; Menke *et al.*, 2007).

Trauma at the epidermis layer could be healed through re-epithelialization without any skin grafting and wound dressing is recommended for such applications. Healing process of deep dermal injuries owing to the lack of remaining cell sources at the site for regeneration takes a long time and the process remains complicated (Chong *et al.*, 2007). Tissue engineering such as wound dressing is a promising method to provide functional alternatives to allografts and autografts for skin regeneration, without restrictions involving donor site limitations, disease transmission or risk of immunological rejection (Venugopul *et al.*, 2006).

Dressing selection should be based on its ability to 1) provide or maintain moist environment, 2) enhance epidermal migration, 3) promote angiogenesis and connective tissue synthesis, 4) allow gas exchange between wounded tissue and environment, 5) maintain appropriate tissue temperature to improve the blood flow to the wound bed and enhances epidermal migration, 6) provide protection against bacterial infection, 7) should be non-adherent to the wound and easy to remove after healing, 8) must provide debridement action to enhance leucocytes migration and support the accumulation of enzyme and 9) must be sterile, non-toxic and non-allergic (Selvaraj *et al.*, 2015).

Selection of material compositions is required. The dermal matrix consists of collagen (mainly type I and III) and elastic fibers surrounded by the ECM made up of proteoglycans. Therefore, a nanofibrous architecture composed of a combination of polysaccharide-protein might be an ideal choice for treatment of skin injuries. Various researchers have

develop scaffolds of collagen/chitosan, silk fibroin/chitosan, or collagen/hyaluronic acid by electrospinning for skin tissue engineering. Therefore, we considered the development of electrospun skin substitutes using cellulose acetate or CA (a linear organic polysaccharide) and collagen (Elham *et al.,* 2014).

Fibers obtained from electrospinning are considered as ideal dressing materials for non-healing wounds since the method is versatile and can deliver various biological agents long-term to local tissues at the wound site (Mulugeta *et al.,* 2018). Electrospun nanofibrous membranes serve as a biomimetic fibrous structure of the native dermis, provide good support for wound healing and increase the rate of epithelialization and dermal organization (Elham *et al.,* 2014). In this study, the obtained nanofibrous membranes from scanning electron microscopy had an average fiber diameter and pore size that might not only provide sufficient space for efficiently cell housing and exchanging of nutrient and metabolic waste between the membrane and environment but also engage in presenting excess exudate absorbability and oxygen permeability.

The epithelium of the skin has a remarkable ability of self-renewal over the lifetime and also produces daughter cells that differentiate into one or multiple lineages. Although epidermal stem cells in the basal layer, as an endogenous source of stem cells, can regenerate skin, but these cells are not sufficient to provide perfect repair after deep and extensive skin damage. Thus, exogenous supply of stem cells in traumatic conditions may be one of the novel therapeutic strategies to achieve perfect skin repair (Suman *et al.,* 2017). In this study, the stem cells were found to attach on all the electrospun nanofibers.

Once a wound occurs, mesenchymal stem cells (MSCs) mobilize to the wound site, where they manage cell proliferation and migration during the inflammation phase of cicatrization. MSCs influence the wound´s ability to progress beyond the inflammatory phase and not regress to a chronic wound state. The mechanism of action of these cells is that they directly attenuate inflammatory response so that they decrease secretion of the proinflammatory cytokines while increasing the production of antiinflammatory cytokines. These anti-inflammatory properties make them particularly beneficial to chronic wounds by advancing the wound past a chronic inflammatory state into the next stage of healing. Furthermore, MSCs secreted several growth factors so these cells promote dermal fibroblast proliferation, angiogenesis and collagen deposition (Suman *et al.,* 2017; Menendez *et al.,* 2014).

This study approves the feasibility of electrospun CA/Collagen scaffolds seeded stem cells for skin treatment especially for wound dressing and shows the importance of compositional designing to provide appropriate features of the target application. Further research is needed to find out how the scaffold mechanism in wound dressing able to deliver stems cells to the injured area. It is also needed another study to determine the mechanical properties of the scaffold and its biodegradability.

## ACKNOWLEDGEMENTS

We would like to acknowledge the support of all laboratory technicians in Stem Cell Research and Development Centre, Airlangga University; and Cell and Tissue Bank, Dr. Soetomo General Hospital, Surabaya, Indonesia.

### Potential Conflict of Interest

The authors declare that there is no conflict of interest.

## REFERENCES

Boyce, S.T. 2011. Design principles for composition and performance of cultured skin substitutes. *Burns.* 27 : 523–533.

Chong, E.J., Phan, T.T. and Lim, I.J. 2007. Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. *Acta Biomater.* 3 (3) : 321–330.

Elham Vatankhah, Molamma, P. Prabhakaran, Guorui Jin, Laleh Ghasemi Mobarakeh and Seeram Ramakrishna, 2014. Development of nanofibrous cellulose acetate/gelatin skin substitutes for variety wound treatment applications. *Journal of Biomaterials Applications.* 28 (6) : 909–921.

Gosain, A. and Di Pietro, L.A. 2004. Aging and wound healing. *World J Surg.* 28 (3) : 321-326.

Guo, S. and Di Pietro, L.A. 2010. Factors Affecting Wound Healing. *Dent Res.* 89 (3) : 219-229.

Hoerstrup, S. and Joseph, P. Vacanti, 2004. Tissue Engineering of a Trileaflet Heart Valve—Early *In vitro* Experiences with a Combined Polymer. *Mary Ann Liebert inc.*

Liu, X., Lin, T. and Gao, Y. 2012. Antimicrobial electrospun nanofibers of cellulose acetate and polyester urethane composite for wound dressing. *J Biomed Mater Res*

*Part B.* 100B (6): 1556–1565.

Mathieu, D., Linke, J.C. and Wattel, F. 2006. Non-healing wounds. In: *Handbook On Hyperbaric Medicine*, Mathieu DE, editor. Netherlands: *Springer*, pp. 401-427.

Menendez Yolanda, Alvarez-Viejo Maria, Ferrero-Gutierrez Amaia, Perez-Basterrechea Marcos, Perez Lopez Silvia, Escudero Dolores and Otero-Hernandez Jesús, 2014. Adult Stem Cell Therapy in Chronic Wound Healing. *J Stem Cell Res Ther.* 4 : 162.

Menke, N.B., Ward, K.R., Witten, T.M., Bonchev, D.G. and Diegelmann, RF. 2007. Impaired wound healing. *Clin Dermatol.* 25 (1) : 19-25.

Mertsching, H., Schanz, J., Steger, V., Schandar, M., Schenk, M., Hansmann, J., Dally, I., Friedel, G. and Walles, T. 2009. Generation and transplantation of an autologous vascularized bioartificial human tissue. *Transplantation.* 88 : 203-10.

Miao, J.J., Pangule, R.C. and Paskaleva, E.E. 2011. Lysostaphinfunctionalized cellulose fibers with antistaphylococcal activity for wound healing applications. *Biomaterials.* 32 (36) : 9557–9567.

Mulugeta Gizaw, Jeffrey Thompson, Addison Faglie, Shih-Yu Lee, Pierre Neuenschwander and Shih-Feng Chou, 2018. Electrospun Fibers as a Dressing Material for Drug and Biological Agent Delivery in Wound Healing Applications. *MDPI Bioengineering*: 5 : 9.

Newman Peter, Minett Andrew Ellis-Behnke, R. and Zreiqat Hala, 2013. Carbon nanotubes: their potential and pitfalls for bone tissue regeneration and engineering. *Nanomedicine: Nanotechnology, Biology and Medicine*. 9 (8) : 1139–1158.

O'Brien, F.J., Harley, B.A., Yannas, I.V. and Gibson, L. 2005. The effect of pore size on cell adhesion in collagen-GAG scaffolds. *Biomaterials.* 26 : 433–441.

Powell, H.M. and Boyce, S.T. 2008. Fiber density of electrospun gelatin scaffolds regulates morphogenesis of dermal-epidermal skin substitutes. *J Biomed Mater Res Part A*; 84A (4) : 1078–1086.

Schulz, J.T., Tompkins, R.G. and Burke, JF. 2005. Artificial skin. *Annu Rev Med.* 51: 231–244.

Selvaraj Dhivya, Viswanadha Vijaya Padma and Elango Santhini, 2015. Wound dressings – a review. *Biomedicine.* 5 (4) : 22.

Suman Kanji and Hiranmoy Das, 2017. Advances of Stem Cell Therapeutics in Cutaneous Wound Healing and Regeneration. *Mediators Inflamm.* 5217967.

Venugopal, J.R., Zhang, Y.Z. and Ramakrishna, S. 2006. In vitro culture of human dermal fibroblasts on electrospun polycaprolactone collagen nanofibrous membrane. *Artif Organs*. 30 (6): 440–446.

Zahedi, P., Rezaeian, I. and Ranaei-Siadat, S.O. 2010. A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. *Polym Advan Technol*. 21 (2) : 77–95.