Tissue and Cell 53 (2018) 68–75

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Differentiation of adipose derived stem cells to keratinocyte-like cells on an advanced collagen wound matrix

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ARTICLE INFO

Keywords:
Adipose derived stem cells
Keratinocytes
Co-culture

ABSTRACT

Despite advances in tissue engineering and regenerative medicine, skin regeneration and cutaneous wound healing remains a significant medical challenge. A bioengineered skin that stimulates the body's natural regeneration capability is needed to address the current lack of treatment options. To this end, a biocompatible collagen wound matrix was developed using an electrochemical deposition fabrication process. The advanced collagen wound matrix has relatively high tensile strength compared to normal collagen matrix made by the heat gelation process and open porosity, and serves as an excellent platform for cellular growth and differentiation. Human adipose derived stem cells (hADSCs) were cultured on this collagen matrix and a co-culture system with primary keratinocytes and keratinocyte conditioned media was developed for differentiation of the hADSCs to keratinocyte-like cells. After fifteen days, hADSCs in co-culture began to exhibit a "cobblestone-like" morphology, indicating preliminary signs of differentiation to a keratinocyte-like cell. Based on morphological analysis at day 30, the co-culture with keratinocyte conditioned media system shows promising preliminary evidence of hADSC differentiation to a keratinocyte-like cell on an electrochemically aligned collagen wound matrix.

1. Introduction

Wound healing is an intricate process that requires precise coordination of an extraordinary array of growth factors, signaling molecules and pathways, many tissue types, and multiple cell lineages (Bielefeld et al., 2013; Gurtner et al., 2008; Martin, 1997). Because of its complexity, skin wound healing represents one of the greatest challenges in medical care, and despite advances in tissue engineering and regenerative medicine, there is still a significant need for skin regeneration treatments. Currently, the clinical gold standard treatment in skin regeneration is the split-thickness autograft. However, in cases of extensive burns and skin wounds, adequate healthy skin donor sites may not be available. Additionally, many chronic wounds are complicated by a disease state, such as diabetes, that may contraindicate use of autologous skin.

To address this, collagen sheets for wound healing have been investigated for decades with promising results (Chattopadhyay and Raines, 2014). Collagen is the major component of the extracellular matrix of skin and confers little or no immune reaction upon implantation. Collagen also offers an excellent surface for cell adhesion and proliferation, and has been shown to be highly biocompatible (Chattopadhyay and Raines, 2014). The scaffold used here is created by the process of electrochemical deposition through isoelctric focusing and cathodic deposition, not traditional collagen gelation. In this method, a low voltage of direct current is applied to a collagen solution. A pH gradient is formed within the solution, and collagen molecules migrate and congregate along the isoelectric point, forming a uniform collagen sheet eventually on the cathode (Cheng et al., 2008; Uquillas and Akkus, 2012). This method produces a scaffold of densely-packed collagen molecules that has robust mechanical properties, high porosity, and a long shelf-life (Cheng et al., 2008).

With advances in tissue engineering and stem cell research, the addition of cells has been proposed for accelerated wound healing. Adipose derived stem cells (ADSCs) are mesenchymal stem cells that exhibit multi-lineage differentiation capacity and have been shown to play an important role in cutaneous wound healing (Cherubino et al.,

Abbreviations: hADSC, human adipose derived stem cells; CWM, collagen wound matrix

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https://doi.org/10.1016/j.tice.2018.06.001
Received 20 March 2018; Received in revised form 5 June 2018; Accepted 6 June 2018
Available online 11 June 2018
0040-8166/

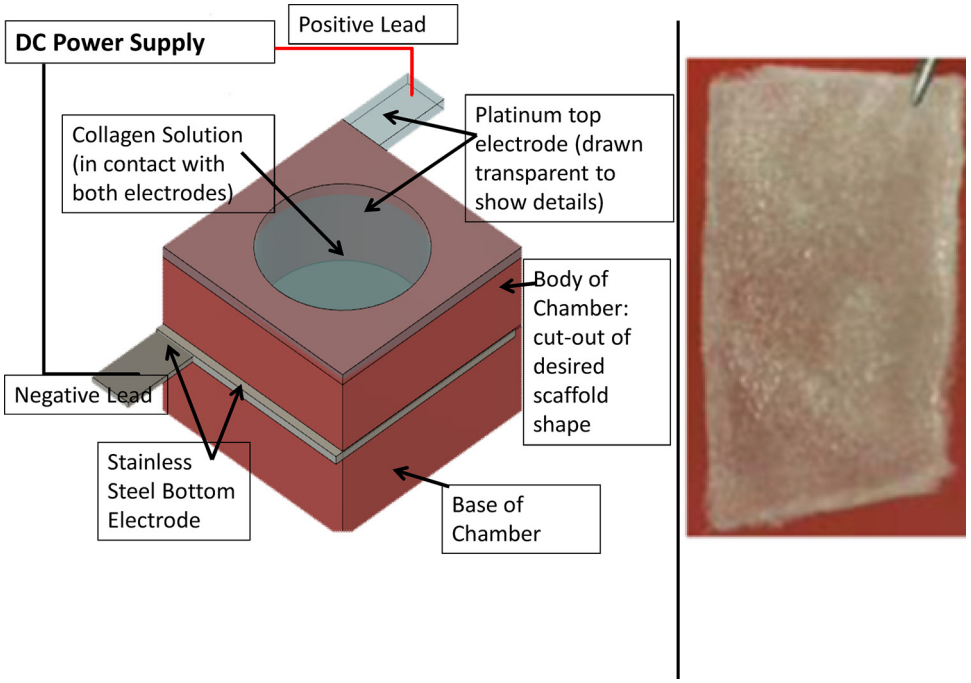

Fig. 1. Left: Electrochemical chamber set-up used for CWM fabrication. **Right:** Electrochemically aligned collagen wound matrix (CWM) after lyophilization.

2011; Gimble et al., 2007; Kim et al., 2009; McLaughlin and Marra, 2013). ADSCs have been shown to improve wound healing through secretion of growth factors and cytokines, stimulating the body's natural wound healing response and cellular cascades (Cherubino et al., 2011; Bertozzi et al., 2017). ADSCs are easily harvested with minimally invasive techniques, have high donor yields, and are well suited to tissue culture (Kern et al., 2006). Keratinocytes, the major cellular component of the skin's epidermis, also play a vital role in wound healing. Keratinocytes are crucial for wound closure, barrier repair, and have been shown to facilitate cross-talk between the many cell types involved in wound healing (Pastar et al., 2008, 2014). Thus, the incorporation of keratinocytes in a wound healing treatment may facilitate quicker wound closure and more complete skin regeneration. To this end, we propose a method for hADSC differentiation to a keratinocyte-like cell on our novel advanced collagen wound matrix.

The objective of the current study is to investigate the seeding of hADSCs onto our advanced collagen wound matrix and the differentiation of seeded hADSCs into keratinocyte-like cells. Instead of using primary human keratinocytes cell lines, hADSC differentiation to keratinocytes has several advantages. First, harvesting hADSCs from a patient with extensive skin wounds may be more practical than keratinocyte harvest. Second, the use of autologous hADSCs instead of donor keratinocytes eliminates the risk of transplant rejection by the immune system due to donor mismatch. If autologous cell use is impractical, donor hADSCs may also be used. Stem cells are largely regarded to be immune privileged, perhaps differentiated keratinocytes, in the presence of stem cells, will show lower host rejection than transplant of primary adult cells. Finally, differentiation of hADSCs into keratinocytes-like cells also allows for more customization of the final cell type to be implanted. Theoretically, custom methods for cell differentiation could eventually be tailored to result in keratinocytes at different stages of differentiation and migration, which could be correlated to the optimal keratinocyte cell and cell markers for wound healing (Pastar et al., 2014).

At first glance, hADSC differentiation to keratinocytes may seem improbable due their different embryonic germ layer origin (hADSCs are mesenchymal, keratinocytes are derived from the ectoderm) (Liu, 2007). Despite the mismatch, several groups have shown that ADSCs have shown the capability to differentiate to keratinocyte like cells in culture (Chavez-Munoz et al., 2013; Kasap et al., 2017; Shokrgozar et al., 2012). This study, however, uses a novel collagen wound matrix as a substrate for hADSCs and the differentiation of hADSCs under mono-culture and co-culture was compared. After fifteen days, hADSCs in co-culture began to exhibit the polygonal cobblestone-like morphology typical of keratinocytes, indicating preliminary signs of differentiation to a keratinocyte-like cell. Through morphological analysis at day 30, hADSCs seeded on the CWM in the co-culture with keratinocyte conditioned media system shows promising preliminary evidence of hADSC differentiation to a keratinocyte-like cell on the CWM.

2. Materials and methods

2.1. Collagen wound matrix fabrication

To fabricate the CWM, a novel electrochemical process was used that produces a planarly aligned, densely packed, porous collagen scaffold that closely mimics the structure of the collagen components of the extracellular matrix (ECM) of human skin, and has shown high cellular viability (Cheng et al., 2008, 2016). Highly purified bovine corium pepsin-soluble collagen, atelo collagen, in 0.01 N HCl (predominantly type I collagen, with approximately 5% Type III) (Collagen Solutions, LLC, San Jose, CA) was dialyzed *versus* e-pure water at 5 °C to facilitate removal of Cl^- ions. The dialyzed collagen was placed in a circular electrochemical chamber with a diameter of 3 cm between two electrodes, with the top electrode being the anode. First, 3.3 mL of collagen was added to the chamber, water was added on top of the collagen to completely fill the chamber, and 2.5 V was applied for one hour. After one hour, the anode was removed and excess water was poured out of the chamber. The CWM that had formed on the cathode remained. An additional 3.3 mL of collagen was added on top of the remaining collagen, empty space in the chamber again filled with water, and the top anode was replaced. 4 V was applied for one additional hour. Next, the CWM that formed on the cathode was collected and incubated in 1X phosphate buffered saline (PBS) at 37 °C for at least four hours. After incubation, the CWM was collected and submerged in 5 mL 0.1% *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC-HCl) in e-pure water at room temperature overnight to crosslink the collagen fibers. Following cross-linking, the CWM was