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Research paper

Polyurethane membrane/knitted mesh-reinforced collagen–chitosan bilayer dermal substitute for the repair of full-thickness skin defects via a two-step procedure

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ABSTRACT

The advent of dermal substitutes provides a revolutionary strategy for the repair and reconstruction of deep skin defects. Dermal substitutes form a regenerative template that provides the porous structure and mechanical support necessary to guide cell migration, deposition of the extracellular matrix (ECM) and angiogenesis. Commercially available dermal substitutes, particularly collagen-based dermal scaffolds, are widely used in clinical practice. However, the poor mechanical properties of collagen-based dermal scaffolds compromise their biological effects, as well as the repair outcomes. Here, we describe a bilayer dermal substitute prepared by integrating a hybrid dermal scaffold with a polyurethane (PU) membrane to obtain a PU membrane/knitted mesh-reinforced collagen–chitosan bilayer dermal substitute (PU-PLGAm/CCS). The morphology of PU-PLGAm/CCS was investigated and, to characterize the effects of PU-PLGAm/CCS on tissue regeneration, dermal substitutes were transplanted to repair full-thickness skin wounds in Sprague-Dawley rats using a two-step surgical procedure. These results were then compared with those obtained using the PELNAC™ Artificial Dermis. In the weeks after the first operation, wound changes were analysed based on macroscopic observations, and tissue specimens were harvested for histology, immunohistochemistry,

Abbreviations: α-SMA, Alpha smooth muscular actin; CCS, Collagen–chitosan scaffolds; ECM, Extracellular matrix; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; H&E, hematoxylin and eosin; PELNAC, PELNAC™ Artificial Dermis; PLGA, Poly(L-lactide-*co*-glycolide); PLGAm/CCS, PLGA knitted mesh-reinforced collagen–chitosan hybrid scaffold; PU-PLGAm/CCS, PU membrane/PLGA knitted mesh-reinforced collagen–chitosan bilayer dermal substitute; PU, Polyurethane; PVP-I, Povidone iodine; RT-qPCR, Real-time quantitative polymerase chain reaction analysis; STSG, Split-thickness skin graft

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immunofluorescence real-time quantitative PCR, and Western blotting analysis. Following the second operation (i.e., transplantation of split-thickness skin grafts), the repair outcomes were investigated based on the mechanical strength and ECM expression. PU-PLGAm/CCS significantly inhibited wound contracture, promoted angiogenesis, and facilitated the ordered arrangement of neotissue, such that the repair outcomes were improved in the PU-PLGAm/CCS group compared with the PELNAC™ group. In conclusion, the favourable microstructure and structural stability of dermal substitutes facilitated tissue regeneration. PU-PLGAm/CCS achieved a balance between porous structure, biocompatibility and mechanical properties for dermal regeneration by integrating the advantages of biological and synthetic biomaterials, which demonstrates its potential for skin tissue engineering.

1. Introduction

Human skin, the soft outer covering of the body, can be easily damaged by acute injuries or chronic diseases (Schulz et al., 2000; Singer and Clark, 1999). Superficial skin injuries, such as epidermal loss, can heal via re-epithelialization, whereas severe skin defects, particularly extensive full-thickness skin defects, fail to repair spontaneously and typically require skin grafts or surgical interventions (Buchanan et al., 2014). To date, autologous split-thickness skin grafts (STSGs) remain the gold standard for the repair of full-thickness skin defects (Bottcher-Haberzeth et al., 2010; van der Veen et al., 2010); however, STSG interventions involving only a very thin dermis commonly lead to scar formation, tissue contracture and other long-term complications. Delivery of epidermal autografts, including cultured epidermal sheets (Rheinwald and Green, 1975) and epidermal suspension (Wood et al., 2012), is another common method used to cover extensive wounds from a small skin biopsy specimen; however, healed wounds treated by epidermal grafts typically exhibit long-term fragility due to the thin dermis. Therefore, dermal reconstruction or regeneration is pivotal to reduce scar formation and improve the quality of wound healing (Wang et al., 2012; Widgerow, 2012). All of these techniques have encouraged the initiation and development of dermal substitutes or equivalents (Rennekampff et al., 1996; Yannas, 1998).

In recent decades, numerous dermal substitutes have been developed to repair various skin defects, and have been applied in clinical practice (Banyard et al., 2015; Philandrianos et al., 2012). The available evidence indicates that the use of dermal substitutes in this setting results in high-quality healing with improved skin elasticity, and reduced scar contracture (Jiong et al., 2010; Sasidaran et al., 2008; Yim et al., 2010). The Integra® Dermal Regenerative Template, a bilayer dermal substitute developed by Yannas and Burke (Burke et al., 1981; Yannas et al., 1981), remains one of the best dermal substitutes that confers superior healing quality and aesthetic appearance; however, its collagen-based dermal matrix is commonly limited by their poor mechanical properties and its price is very high. PELNAC™ Artificial Dermis is a bilayer dermal substitute with an upper layer formed of silicone film and a lower layer formed of porcine-derived collagen sponge. This material is commercially available, and has been used clinically with good outcomes (Suzuki et al., 2000; Wosgrau et al., 2015). It is well-known that ideal dermal substitutes should exhibit excellent biocompatibility and biodegradability, good mechanical properties, and an appropriate microstructure to facilitate cellular infiltration, proliferation and differentiation (O'Brien et al., 2005; Zhong et al., 2010). Biological materials such as collagen have been widely used to prepare scaffolds with good biocompatibility, hydrophilicity and cell affinity (Zhong et al., 2010); however, collagen-based scaffolds tend to deform or contract in response to external physical loads or internal contractile forces, due to poor mechanical properties (Bell et al., 1979; Mao et al., 2009; Ng et al., 2004). Insufficient mechanical support typically leads to the closure of pores, which reduces the space and support for tissue ingrowth and functionalization (Wang et al., 2013). Artificial polymers typically exhibit superior mechanical properties compared to naturally derived biomaterials, and can also exhibit variable plasticity (Keane and Badylak, 2014). For example, poly(L-lactide-*co*-glycolide) (PLGA) is a type of polyester synthesized from lactic acid and glycolic acid; its biodegradability and mechanical properties depend on the ratio of lactic acid to glycolic acid. In addition to porous scaffolds, PLGA can also be fabricated into fabrics with highly ordered loop structures and controllable mechanical properties. Some studies have shown that PLGA knitted meshes exhibit internal connective space that promotes tissue ingrowth, and can act as a "skeleton" to reinforce collagen-based scaffolds (Chen et al., 2008; Wang et al., 2012).

Collagen–chitosan scaffolds (CCS) have been shown to exhibit suitable physical, chemical, and biological characteristics for dermal equivalents (Ma et al., 2003; Ma et al., 2007). To improve the mechanical stability of CCS, a warp-knitted mesh with PLGA yarns was developed and incorporated into CCS to obtain the PLGA knitted mesh-reinforced collagen–chitosan hybrid scaffold (PLGAm/CCS) (Wang et al., 2012). The results of this study indicate that PLGAm/CCS confers improved mechanical strength and a stable porous microstructure. When transplanted into wounds, PLGAm/CCS resisted wound contraction, and promoted the formation of neotissue (Wang et al., 2012). However, the in-vivo influence