RNAi functionalized collagen-chitosan/silicone membrane bilayer dermal equivalent for full-thickness skin regeneration with inhibited scarring

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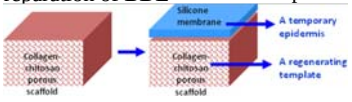
Introduction

Various polymer-based dermal equivalents have been developed in recent years to treat skin losses, however it remains a challenge to fulfill the criteria for “regenerated skin” as the original uninjured skin. For an ideal “regenerated skin”, scarring should be inhibited because it causes loss of dermal functions, disfiguration, itching, and local ulceration. Essentially, the excessive and disordered accumulation of extracellular matrix such as collagen directly leads to the formation of scars. Recently, it has been proved the over-expression of transforming growth factor β1 (TGF-β1) activates the excessive synthesis of granulation tissue constituents by fibroblasts and eventually induces scar formation[1]. It is a potential approach to inhibit scarring by targeted suppression of TGF-β1 gene by the recent emerging biomolecular cue of RNA interference (RNAi). For efficient siRNA delivery, apart from particulate vehicles which can overcome extracellular and intracellular barriers, the incorporation of vehicle/siRNA complexes with 3-D scaffolds provides possibilities of combining RNAi with tissue engineering and regenerative medicine. In our previous studies[2], a porous scaffold composed of collagen and chitosan was fabricated by freeze-drying method and subsequently combined with a silicone membrane to obtain a bi-lay dermal equivalent (BDE). The BDE has shown very positive effects in the repair of full-thickness skin defects. Encouraged by all of the above backgrounds and results, in the present study, trimetylchitosan/siRNA complexes that targets TGF-β1 are loaded into the BDE aiming at fabricating a “RNAi-BDE” for skin regeneration with inhibited scarring.

Materials Methods

1. Preparation of BDE



2.Prepation of TMC/siRNA loaded BDE

3.Implantation of RNAi-BDE in pig model

Conclusions

- TMC can compact siRNA to form nano-sized complexes
- RNAi-BDE is successfully fabricated by loading TMC/siRNA via physical adsorption and serves as reservoir for siRNA release
- In vitro, RNAi-BDE suppressed TGF-β1 and collagen type I mRNA expression in fibroblasts
- In vivo, RNAi-BDE down-regulates TGF-β1 expression ,inhibits collagen over accumulation, and suppresses scarring

References

[1] Rhett JM et al. Trends in Biotechnology 2008;26(4):173-180
[2] Ma L et al. J Mater Sci Mater Med 2007;18(11):2185-2191

Acknowledgements

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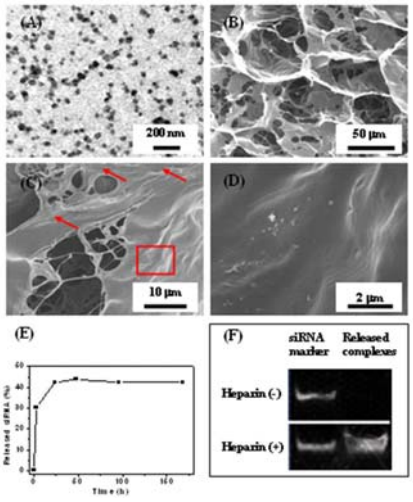
In vitro results：

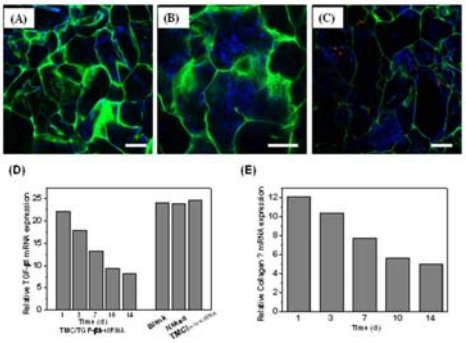


➢Fig.1(A), TMC/siRNA complexes are mainly spherical with a diameter under 100 nm under TEM

➢Fig.1(B), collagen-chitosan scaffold shows connective porous structure

➢Fig. 1(C)and (D),TMC/siRNA complexes are loaded into scaffold and locate on pore walls, (D) is amplification of rectangle area of (C)

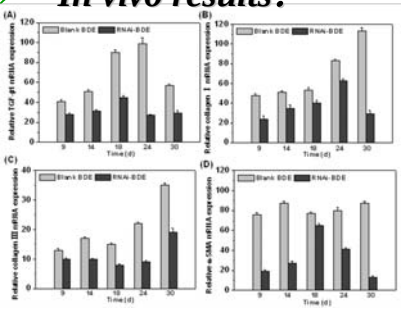
➢Fig.1(E), siRNAs profile confirms that the scaffold acts as siRNA reservoir; (F) siRNAs release from the scaffold with a form of complex and intact structure



➢Uptake of siRNA (red) by fibroblasts (DAPI labeled nucleus) seeded in scaffold (FITC labeled collagen) without siRNA(Fig.2A),with naked siRNA(Fig.2B) and complexes(Fig.2C).Scal bar indicates 100μm

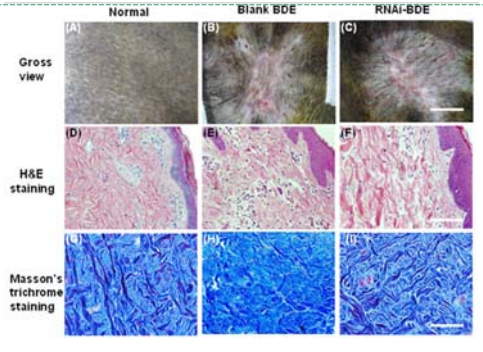
➢Downregulation of TGF-β1(Fig.2D) and collagen type I (Fig.2E) at mRNA level in fibroblasts seeded within scaffold

In vivo results：



➢Variation of mRNA levels with time post implantation of blank BDE and RNAi-BDE, for TGF-β1(Fig.3A),collagen type I (Fig.3B),),collagen type III (Fig.3C) and α-SMA (Fig.3D)

➢RNAi-BDE suppresses TGF-β1 expression, extracellular matrix deposition and myofibroblasts differentiation



➢73 days post implantation, compared with blank BDE, RNAi-BDE can result in flatter wound appearance with less irregular contour(Fig.4A-C) , collagen bundles with more ordered distribution(Fig.4D-I), and overall a repaired skin structure with inhibited scarring, which is more similar to that of normal skin