Melanogenesis Potential of EGF-Loaded Nano-Pillared Chitosan-Gelatin Films

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Statement of Purpose: Epidermal growth factor (EGF) has critical roles in regulation of fibroblastic activity, stimulation of keratinocytes and melanogenesis pathway. However, stability of EGF can be compromised because of physical and chemical properties of wound and this condition leads to permanent tissue defects like hypopigmentation and infection [1]. Therefore, there is a growing demand for EGF doped chitosan and gelatin scaffolds in the skin tissue engineering applications due to their biocompatible nature and optimal mechanical characteristics. The present study involves the fabrication, characterization, and potential in-vitro/in-vivo wound healing application of EGF-doped nano-pillared chitosan-gelatin films prepared using non-lithographic anodic alumina molds (AAMs) technology (Figure 1).

Methods: The films are fabricated using reusable nano-porous AAMs synthesized by the two-step anodization technique in oxalic acid solution. The stability of the chitosan-gelatin films was increased by adding 0.3 v/v % poly (ethylene glycol)diglycidylether and was tested in (DMEM) media. The AAMs and films were characterized using SEM, AFM, NMR, swelling and surface energy analysis. To test the proliferation potential of EGF-doped films in wound healing we used individual and co-culture system of fibroblasts and melanocytes in 5:1 ratio to mimic wound conditions. Additionally, EGFR, fibronectin, tyrosine activity and melanin release studies were conducted for 3 day incubation time. Expression levels of tyrosinase, TRP1 and TRP2 genes was followed for the melanocytes in addition to Western Blot analysis of tyrosine protein.C57BL/6 mouse model was chosen to follow wound closure and melanin release studies. Same protein and gene related analyses were conducted for EGF-doped and free- nano pillar surface compared to saline control group. (*p<0.05, one way ANOVA, n=3, for all test)

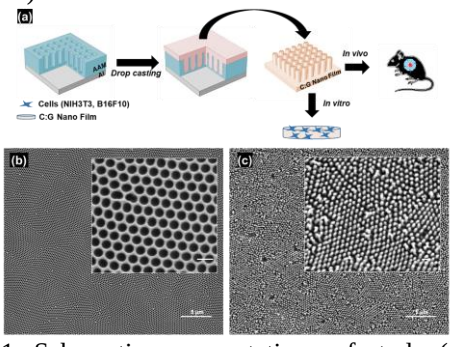

Figure 1. Schematic representation of study (a). SEM images of AAMs (b) and nano- films (c).

Histology analyses of skin biopsy samples were conducted via hematoxylin & eosin and Masson`s trichrome staining.

Results: The flat films showed 100 % release of EGF at course of 5 days at 37°C. Because of the increased surface area the release (%) /mm2 was decreased for the nano-films. To test the potential of EGF-doped films in wound healing we used individual and co-culture system of fibroblasts and melanocytes in 5:1 ratio to mimic wound conditions. The cell viability results of melanocytes and fibroblasts showed increase in viability by 1.2 fold after 3 days of incubation as compared to flat films. EGF doped nano- films was showed prominence results compared to EGF free nano- films and flat counterparts. For example, total secreted melanin amount of melanocytes approximately 2.5 folds higher compared to EGF doped flat films (Figure 2a). Same analysis were completed for coculture system and optical microscope analysis of the EGF doped nano- and flat- films are shown that proliferation of the fibroblast and melanocytes (red cells) is clearly observed at EGF doped nano- films (Figure 2b-c).

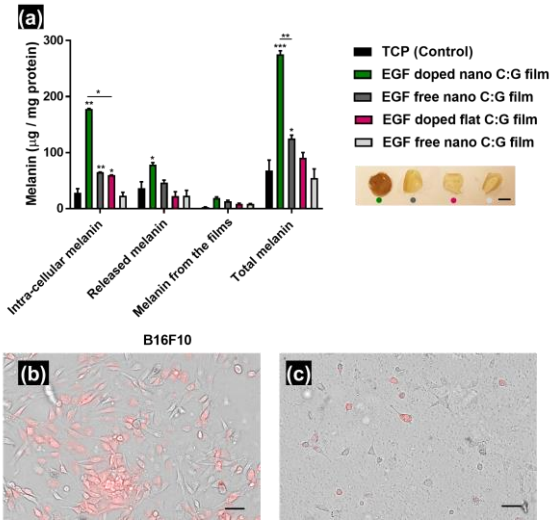

Figure 2. Melanin contents in presence of TCP control and EGF doped or free nanostructured and flat C:G films of B16F10 cells (a) (* p<0.05, ** p<0.005, *** p<0.0005). Inset scale bar: 3 mm (a). Optical images of NIH3T3 + B16F10 cells in co-culture (5:1) on EGF doped nano (b) and flat (c) films. Red signal came from B16F10. Scale bar: 50 µm (b,c)

In addition to in vitro analysis, EGF doped films were applied to excisional wound area and wound closure analyses were followed. At the end of the day 7, the

wounds on which were applied EGF doped nano- films was completely closed compared to saline and EGF free group (Figure 3). The same genomic and proteomic assays were repeated for skin biopsy samples. Fibronectin released results does not show significant difference between groups, while after 5 days melanin secretion significantly increased EGF doped nano- films compared to other groups ($p<0.05$). On the other hand, PCR analysis and histology staining results showed that EGF existence positively affect wound closure and melanin secretion.

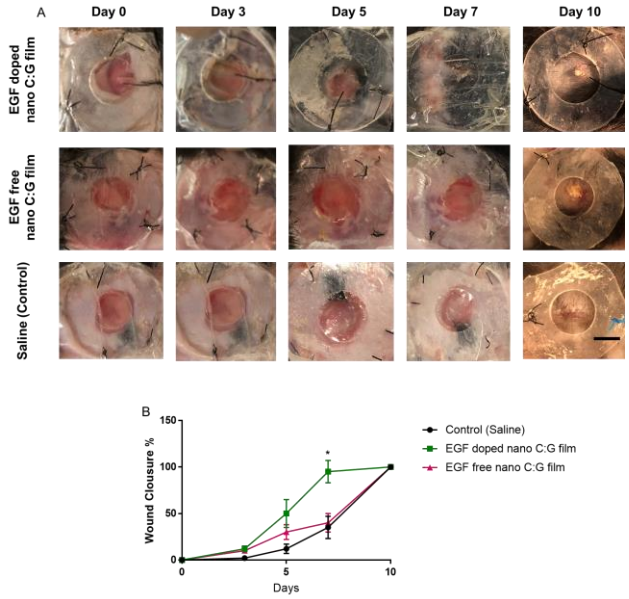

Figure 3. Images of time dependent wound closure studies (a). The graphic of wound closure (%) versus time (b).($n=3$, $p<0.05$).

Conclusions:

The results indicates that EGF doped nano films have a great potential for wound healing and melanin secretion related studies.

References: [1] D. R. Bijukumar, A. Segu, J. C. M. Souza, X. Li, M. Barba, L. G. Mercuri, J. J. Jacobs, M. T. Mathew, Nanomedicine: Nanotechnology, Biology, and Medicine 2018, 14, 95