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***Supporting Information**

Local Epidermal Growth Factor Delivery Using Nanopillared Chitosan-Gelatin Films for Melanogenesis and Wound Healing

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Cell viability on the C:G Films: Eight different test groups were analyzed to identify effects of nanotopography and EGF content on the cells. In addition to the EGF-doped films, EGF dropped films were also prepared via droping and drying the EGF solution onto the C:G films that contained the same EGF amount as the doped ones.. Briefly, NIH/3T3 (fibroblasts, ATCC® CRL-1658™), B16-F10 (melanocytes, ATCC® CRL-6475™) and coculture of them (NIH/3T3:B16-10 in 5:1 ratio) (10^4 cells/well) were seeded on the films in 96 well-plate at 37°C and 5% CO_2 humidity environment for 72 h incubation time. The cell lines were grown in 10% fetal bovine serum (FBS) -supplemented Dulbecco's modified Eagle's medium (DMEM) with a 1% antibiotic−antimitotic solution (Sigma Aldrich, A5955). Routinely, the cells were maintained in a humidified incubator and all cell mediums were altered every 72 h.

MTT assay was conducted according to the manufacturer's protocol (Thermo Scientific, M6494). To test viability, cells were rinsed with serum-free media and the media was replaced with fresh phenol red and serum-free DMEM media (Sigma Aldrich, D5796) supplemented with 10 µl of a 2.4 M 3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide salt (MTT) solution and the cells were incubated for 2 h at 37°C. To solve tetrazolium salt crystals, the media was then changed with 100 µl of dimethyl sulfoxide (DMSO) and the absorbance values of solutions were recorded at 520 nm. All experiments were performed in triplicates.

Quantification of Total Fibronectin Content of The Cocultured Cells on the C:G Films: All cell groups (10^4 cells/well) were incubated on the C:G films or TCP (Control) for 72 h in 96-well plates. Then the cells were washed with phosphate-buffered saline (PBS) for two times and then the plates were incubated with RIPA lysis and buffer extraction buffer (Thermo Scientific, 89901) on ice for 15 min. The solutions were transferred to microcentrifuge tubes and samples were centrifuged at 10K×g for 30 min. The supernatants were placed in new tubes and the manufacturer's ELISA protocol (BosterBio, EK0351) was followed to detect total fibronectin content in the supernatants following BCA analysis. All experiments were conducted in triplicates.

Quantification of Total Fibronectin Content of The Skin Biopsies: For protein extraction, 20-50 mg tissue was first frozen in liquid nitrogen and was transferred into the microcentrifuge tube where tissue was homogenized in the presence of protease inhibitor cocktail. After homogenization, the samples were centrifuged at 1K×g and collected supernatants were used to conduct BCA assay for protein estimation and ELISA for fibronectin quantification (BosterBio, EK0351) according to manufacturer's protocol. Total fibronectin content was expressed as µg/mg protein.

Evaluation of Tyrosinase Activity: The monocultured melanocytes and cocultured cells were seeded at a density of 10^4 cells/well on the C:G films and were incubated for 72 h in a 96-well plate. Afterwards, the films were rinsed with PBS, cells were trypsinized using trypsin/EDTA solution and were then centrifuged at 1K×g for 5 min. After centrifugation, the cell pellets were washed with PBS and were lysed using RIPA lysis extraction buffer on ice for 15 min. The samples were then centrifuged and the supernatant was transferred into a new microcentrifuge tube. Following micro-BCA analysis, total protein amount was normalized from sample to sample. For tyrosinase assay, 10 mM 10 µl 3,4-Dihydroxy-L-phenylalanine (Sigma Aldrich,

D9628) aqueous solution was added into 90 µl supernatants. The mixture was incubated for 1 h at room temperature in dark and absorbance values were read at 405 nm. For animal tissues, after protein extraction, BCA test was conducted and the aforementioned tyrosinase measurement protocol was followed. Tyrosinase activity was expressed as Units.mg^{-1} protein.

The tyrosinase activity of monocultured melanocytes and the cocultured cells was assayed by measuring hydroxylation of tyrosinase to dopa. The tyrosinase activity results of monocultured melanocytes and cocultured cells showed that the EGF-doped nano C:G films showed increased enzymatic activity compared to the TCP (Control) group (Fig. S1A and S1B).

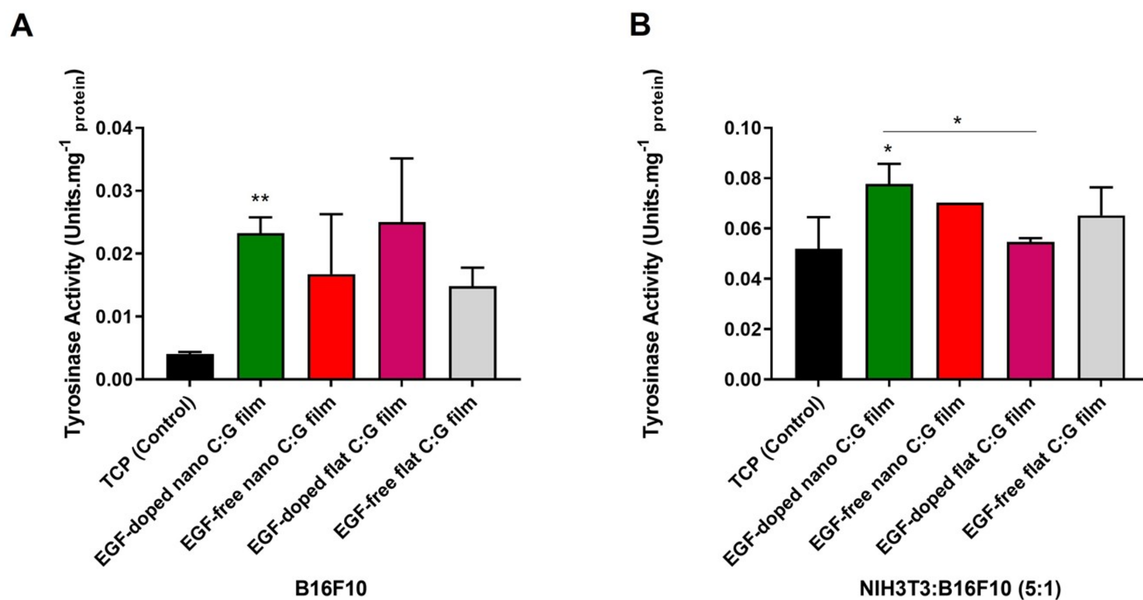
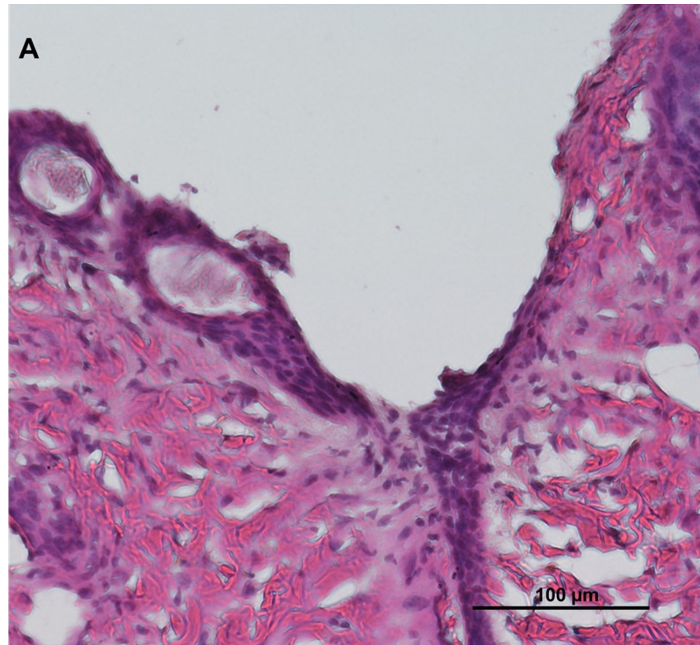


Figure S1. Tyrosinase enzyme activity measurements of B16-F10 and cocultured cells (NIH/3T3:B16-F10 (5:1)) grown on nano and flat C:G films. The tyrosinase activity of A) B16-F10 (melanocytes) and B) cocultured cells grown on nano and C:G films, respectively. TCP were used as control group. Data presented as mean ± SE, n=3/group. * $p<0.05$, ** $p<0.005$; TCP control vs test groups. * $p<0.05$; between respective test groups.



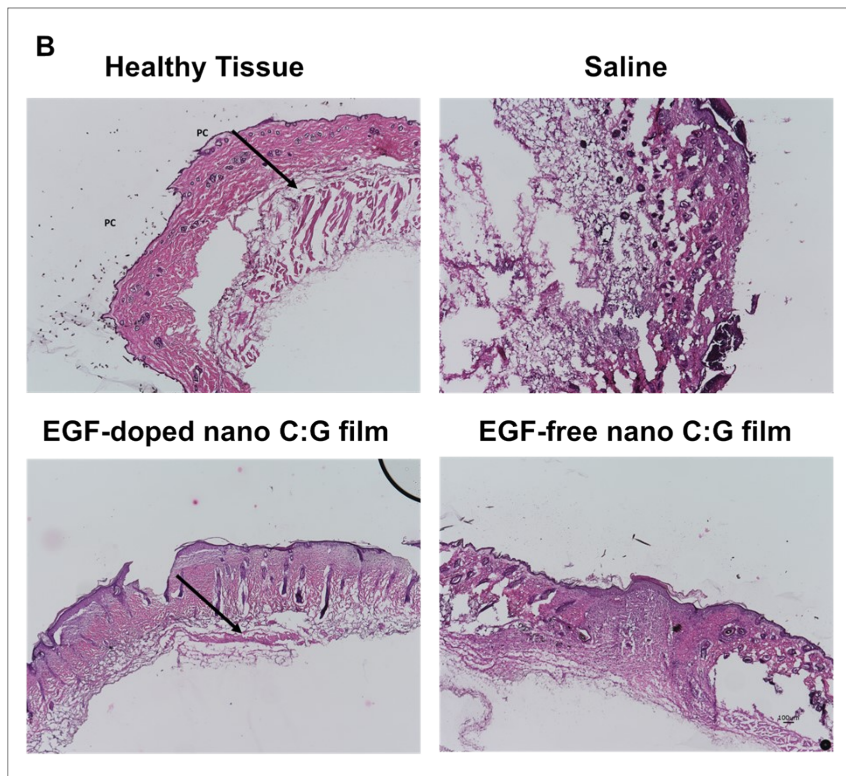


Figure S2. A) Magnified version of the EGF-doped nano C:G films-applied and H&E-stained tissue B) Histological analysis of the tissues using H&E staining to show the edge of the panniculus carnosus (PC) muscle with black arrow. The black arrows indicate border of PC formations. Scale bar represents 100 µm.

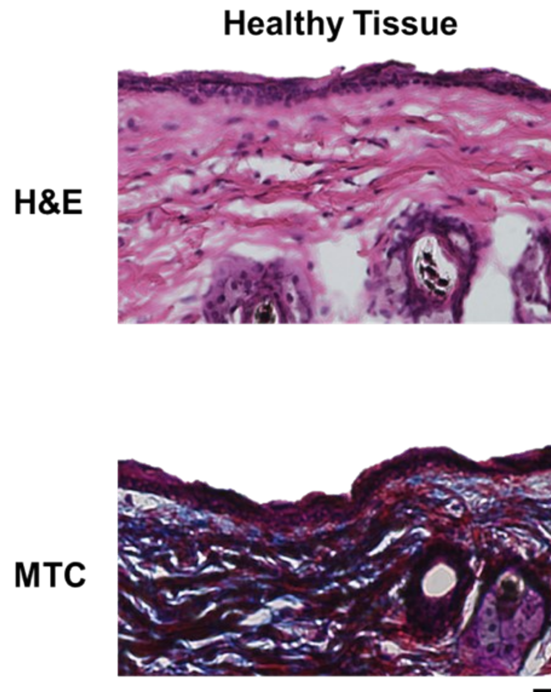


Figure S3. Histological analysis of healthy tissue using H&E and MTC staining. Scale bar represents 100 µm.

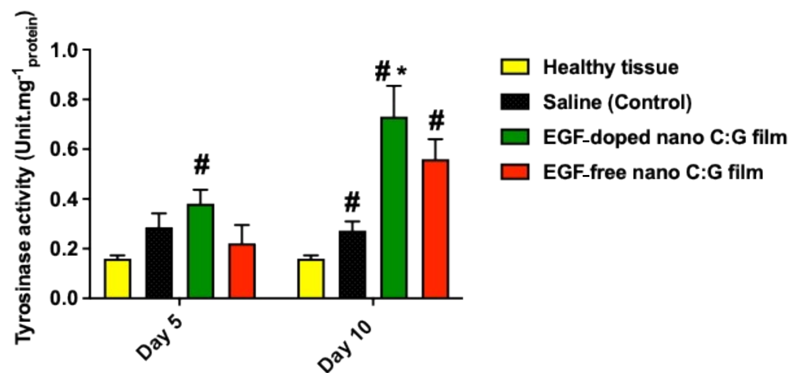


Figure S4. The tyrosinase activity of wounds after 5 and 10 days of treatment with Saline (Control), EGF-doped nano C:G films and EGF-free nano C:G films. Data presented as mean ± SE, n=3/group. * $p<0.05$; Saline (Control) group vs test groups. # ($p<0.05$); wound treatment groups vs. healthy tissue.

Table S1. Profile controlled EGF release at pH 7.5 from EGF-doped nano C:G films and EGF-free nano C:G films.

Korsmeyer-Peppas Equation

$$Ct/C\infty = kt^n$$

$Ct/C\infty$ = fraction of drug release at time
k = rate constant
n = release exponent