GENE EXPRESSION OF KOREAN HANWOO SATELLITE CELLS ON AG/TIO$_2$ NANOMATRIX: A MOLECULAR STUDY

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Abstract –Herein we report synthesis of novel silver decorated titanium dioxide nanofibers via e-spinning technique with improved cellular response for possible muscle tissue engineering applications. For a model application in muscle tissue engineering, satellite cells of native Korean Hanwoo were cultured on synthesized hybrid nanofibers. Standard microarray technique has been employed to screen potential effects of synthesized nanofibers on genome after culture of cells. Microarray analyses of gene expression response indicated clear changes in gene expression in a range of proliferation and apoptosis. Our results provide insights into molecular mechanisms of response of muscle cells to Ag/TiO$_2$ hybrid matrix. These results open-up new ways to develop and analyze gene expression biomarkers for satellite cell proliferation and finally muscle tissue engineering.

Key Words – Microarray, Satellite cells; muscles

I. INTRODUCTION

Nanocrystalline silver has verified to be the most effective antimicrobial agent[1] as silver and its compounds have powerful antimicrobial activity[2] and have depicted extensive biocidal spectra for miscellaneous groups of microbes [3-5].Similarly the influence of TiO_2 nanotubes on cellular response has been investigated using a variety of cell types including osteoblasts[6], fibroblasts[7], chondrocytes[8], endothelial cells[9], muscle cells[9], epidermal keratinocytes[7] and mesenchymal stem cell (MSC)[10,11]. The present study was aimed to evaluate the potential of silver decorated TiO_2 nanofibers as tissue engineering scaffolds. Herein; microarray technique was utilized to identify the expression of specific genes after the interaction of muscle satellite cells with synthesized hybrid matrix. On the basis of present results we can wrap up the conclusion as Ag/TiO_2 hybrid nanofibers could be employed in guiding cell adhesion and spreading for muscle growth and regeneration.

II. MATERIALS AND METHODS

Satellite cells were isolated from *longissimus dorsi* muscle of native Korean Hanwoo. The entire work involving use of animals was approved by an Institutional Animal Care and Use Committee. Aseptically epimysium and fat were trimmed off and discarded. Muscle strips were chopped in a sterilized meat chopper and after enzymatic digestion with pronase (1 mg/ml) at 37 °C for 60 minutes; single cells were separated from the tissue fragments by repeated centrifugation. The primary muscle cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco® life technologies, Grand Island, NY, USA) supplemented with 15% fetal bovine serum (FBS; GIBCO), 100 IU/ml penicillin, and 100 µg/ml streptomycin in a humidified incubator at 37°C with 5 % CO_2. The satellite cells were grown in 75 cm^2 culture flask (Bedford, MA, USA) to get the enough cell count. The hybridization images were analyzed by Agilent DNA microarray Scanner (Agilent Technology, USA) and data quantification was performed using Agilent Feature Extraction software 10.7 (Agilent Technology, USA). The average fluorescence intensity of each spot was calculated and local background was subtracted. All data normalization and selection (fold-change) were performed using GeneSpringGX 7.3.1 (Agilent Technology, USA). Reliable genes were filtered by flag following Agilent manual. The average of normalized ratio was calculated by dividing average of control normalized signal intensity by average of test normalized signal intensity. Functional categorization of gene families over represented was performed using program from NIAID web site (http://david.abcc.ncifcrf.gov/summary.jsp).

III. RESULTS AND DISCUSSION

Fig. 1 shows XRD of Ag/TiO_2 hybrid nanofibers. Fig.2 demonstrates FESEM images after calcinations at 500 °C (Fig. 2a, b, c). Fig. 2d shows EDX of Ag/TiO_2 hybrid nanofibers. The elemental composition of nanofibers was further confirmed by EPMA (Fig. 3).

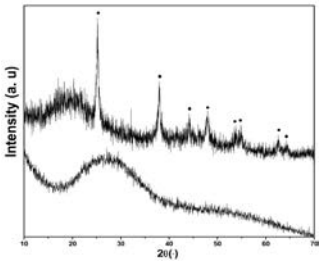

Figure 1. XRD pattern

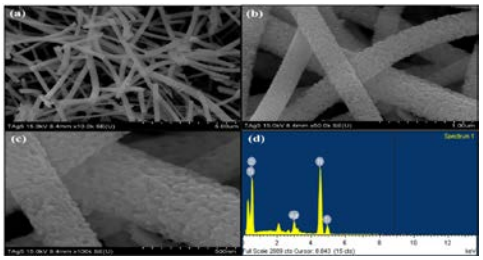

Figure 2. FESEM and EDX images

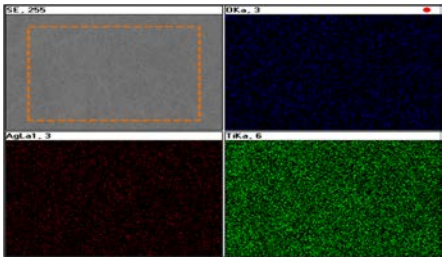

Figure 3. EPMA images

Fig.4 (a, b) shows microscopic images of unexposed and exposed satellite cells respectively. To acquire insight into biological effects of synthesized hybrid nanofibers; microarray analysis was used to obtain functional information about whole genomic expression profile in satellite cells in particular significant genes involved in proliferation and apoptosis. In present study functional information could be attained for 32,965 genes. We compared gene expression profiles of satellite cells grown on hybrid matrix with control cells. Out of 32,965 genes, a total of 9654 genes were up-regulated, whereas 12,325 genes were down-regulated after cultivation on nanofibers. Furthermore, in the scatter plot (Fig.5) position of each gene on the plot is determined by its expression level (cells cultured on hybrid matrix-*x*-axis and control cells-*y*-axis). Genes with identical or similar expression are depicted by a black line, whereas the gene clusters around the red line depicts the ≥2 fold up-regulation. The green solid line demonstrates the ≤ 2 fold down-regulation. The red spots at top of red line indicate ≥ 3 fold up-regulation; whereas the blue spots at the bottom of green line illustrate ≤ 3 fold down-regulation respectively.

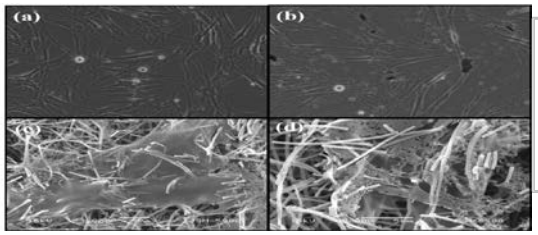

Figure 4.BIOSEM of unexposed and exposed satellite cells

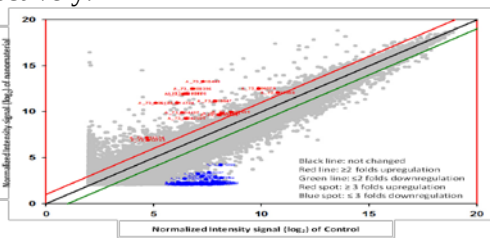

Figure 5. Microarray assay of satellite cells

IV. CONCLUSION

In summary, we isolated satellite cells from adult Hanwoo muscle and the isolated muscle precursor cells were for the first time cultivated on Ag/TiO_2 hybrid nanofibers under *in vitro* conditions. The morphology of Ag/TiO_2 nanofibers was characterized by SEM and TEM whereas the crystallinity was analyzed by X-ray diffraction pattern. The present study provides constructive information regarding the muscle tissue engineering.

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