STUDY ON INTERACTION OF SATELLITE CELLS WITH ELECTOSPUN COMPOSITE MATRIX

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Abstract – We report the fabrication of novel Fe_3O_4/TiO_2 hybrid nanofibers with the improved cellular response for potential tissue engineering applications. In this study, Fe_3O_4/TiO_2 hybrid nanofibers were prepared by facile sol-gel electrospinning. The physicochemical properties of the synthesized nanofibers were determined by FE-SEM, EDX and XRD pattern. To examine the *in vitro* cytotoxicity, satellite cells were treated with as-prepared Fe_3O_4/TiO_2 and the viability of cells was analyzed by Cell Counting Kit-8 assay at regular time intervals. The morphological features of unexposed satellite cells and exposed to Fe_3O_4/TiO_2 composite were examined with a phase contrast microscope. We observed that Fe_3O_4-TiO_2 composite nanofibers could support cell adhesion and growth. Results from this study therefore suggest that Fe_3O_4/TiO_2 composite scaffold can mimic the natural extracellular matrix well and provide possibilities for diverse applications in the field of tissue engineering.

Key Words – Fe_3O_4-TiO_2 hybrid, Biocompatibility, Scaffolds, Satellite cells, Electrospinning

I. INTRODUCTION

Modern trends in nanomaterials synthesis allocate preparation of hybrid nano-constructs with a variety of architectures such as nanowires, core shell, nanoflowers, nanofibers and so on through various synthetic routes. Electrospinning, an electrostatic fiber manufacture practice has substantiated concentrated notice and attention during recent years due to its flexibility and potential for applications in diverse fields. The noteworthy applications include tissue engineering, biosensors, filtration, wound dressings, drug delivery, enzyme immobilization and so on [1-4]. Moreover, the electrospun nanofibers are found to possess morphological resemblances such as high porosity and effective mechanical properties etc to the extracellular matrix (ECM) of natural tissue. Despite wide investigations of the interactions between different cells and nanostructured TiO_2 materials, the effect of electrospun Fe_2O_4/TiO_2 on satellite cell adhesion and growth has not been reported hitherto. Nevertheless; some studies have shown the interaction of iron oxide containing nanostructured material with cells. For instance, Kim et al reported the enhancement of neurite outgrowth in PC12 cells by iron oxide nanoparticles. They demonstrated that PC12 cells exposed to both polyethylene glycol coated iron oxide nanoparticles and nerve growth factor synergistically increased the efficiency of neurite outgrowth in a dose-dependent manner [5]. While Liu et al described low health hazard of plain iron oxide nanoparticles [6]. In the present study we investigated the potential effect of as-synthesized Fe_2O_4/TiO_2 composite nanfibers using satellite cells as model cell lines. On the basis of present results we can conclude that Fe_2O_4/TiO_2 nanofibers could be employed in guiding cell adhesion and spreading for a variety of advanced tissue engineering applications especially for muscle growth and regeneration.

II. MATERIALS AND METHODS

Synthesis and characterization of Fe_3O_4-TiO_2 hybrid nanofibers

PVAc solution (18 weight%) was prepared by dissolving PVAc in DMF under magnetic stirring for 8 h at room temperature. 5 g of TIP was taken in a separate bottle and a few drops of acetic acid were added to it till the solution turn out to be transparent. Appropriate amount (10 weight%) of the alcoholic solution of iron nitrate was dissolved into the TIP. Then 6 g of PVAc solution was added slowly into the aforementioned solution and stirred vigorously. The resulting composite solution was fed to a 10 ml syringe as the spinning head. A copper pin connected to a high voltage generator was inserted in the solution as a positive terminal whereas a ground iron drum served as counter electrode. The XRD pattern of synthesized composite nanofibers was recorded on a Rigaku/Max-3A X-ray diffractometer with CuK radiation (λ=1.540 Å) and the operating voltage and current were maintained at 30 kV and 40 mA, respectively. To examine the microstructure, the images were acquired at various magnifications using SEM, H-7650, Hitachi, Japan) .

Isolation of satellite cells from Korean Hanwoo cattle and in vitro cultivation

Satellite cells were isolated from 30-month old native Korean Hanwoo cattle according to the method of Dodson et al with suitable modifications [7]. The entire work involving the use of animals was approved by an Institutional Animal Care and Use Committee. Briefly, after the slaughter of Hanwoo cattle at a commercial abattoir, *the longissimus dorsi muscle* was excised from Hanwoo cattle immediately. The epimysium and most of the fat was trimmed off and discarded. Muscle strips were chopped in a sterilized meat chopper. 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. When the cells reached 80 % confluence, they were collected and re-suspended in phosphate-buffered saline (PBS) supplemented with 0.5 % BSA and 2 mM EDTA. Finally, cell suspensions (~10^7 cells in 500 µl PBS) were loaded into a magnetic cell sorting system (AutoMACS, Milteny Biotech, Germany) to isolate the satellite cells. The isolated cells were cultured in growth medium at 37 °C, 5 % CO_2 in air and near 95% relative humidity.

Cell viability test

The satellite cells were grown in 75 cm^2 culture flask (Bedford, MA, USA) to get the enough cells. The cell density of 1 × 10^6cells/well was seeded in a 96-well tissue culture plate and allowed to attach and grow in wells overnight before treatment. When satellite cells reached ~60% confluence, cells were treated with different concentrations (10 and 20 µg/ml) of Fe_3O_4/TiO_2 composite nanofibers for a specific time (3, 5, and 7 days) duration. Unexposed cells were set as the control. The CCK-8 assay was performed to check the cell viability in the presence of Fe_3O_4/TiO_2 composite nanofibers. In brief, media from the microplates after 3, 5, and 7 days of incubation time, was taken out and replaced with fresh media (160 µL), in which 10 µL of water-soluble tetrazolium-8 (CCK-8) solution in each well (100 µL medium) was added and incubated for 4 h at 37°C according to the manufacturer's instructions. At the end of the experiment, absorbance was measured at 450 nm for each well by a microplate spectrophotometer (model 680; Bio-Rad Laboratories, Hercules, CA).

III. RESULTS AND DISCUSSION

The XRD (Fig. 1) confirms the presence of iron oxide in the Fe_3O_4/TiO_2 composite nanofibers.

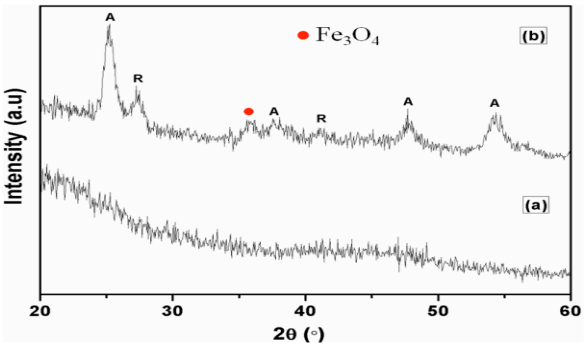

Figure 1. XRD pattern of Fe_3O_4/TiO_2 composite nanofibers (a) without calcination and (b) calcination at 500 °C (A represents anatase, R represents rutile phase)

Fig. 2a demonstrates the FE-SEM image of the material. The SEM image of the calcined sample at high resolution demonstrated distinctive nano-size particle building block morphology on the surface of composite nanofibers (Fig. 2c). The EDX is shown in Fig. 2d. It has been observed that

the sample contains Fe, Ti and O; no other element impurity is detected, indicating the final product is free of impurity.

To examine the toxic effects of Fe_3O_4/TiO_2 composite nanofibers, satellite cells were incubated with different concentrations (10, 20 µg/ml) of as-synthesized nanofibers and viability was determined at 3, 5 and 7 days after treatment respectively. Fig. 3 (a, b) shows the microscopic images of unexposed and exposed satellite cells respectively. From the images we can observe 100% cell growth in case of control cells. Meanwhile, the satellite cells cultured with the hybrid nanofibers showed a similar trend as that of control cells. Fig. 3 (c, d) depicts the results of CCK-8 assay for aforesaid incubation time. It was also observed that growth proceeds in an exponential manner with respect to incubation days. Overall, the satellite cells showed nearly confluent growth even in the presence of as-spun Fe_3O_4/TiO_2 composite nanofibers during culture period. These results further clarify that prepared Fe_3O_4/TiO_2 composite nanofibers are non-toxic to the cells, therefore, suggests them to be used in biomedical applications especially muscle revival.

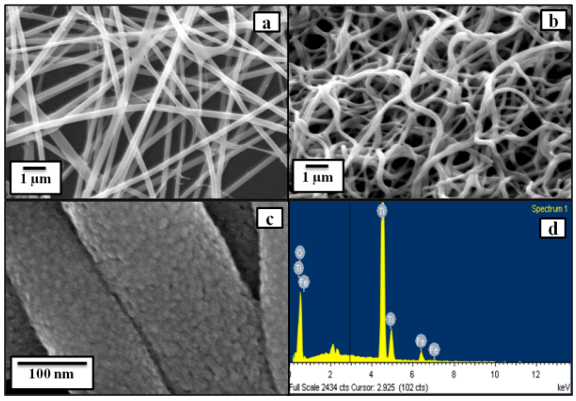

Figure 2. FE-SEM micrographs of the Fe_3O_4/TiO_2 composite nanofibers without calcinations (a) calcinations at 500 ^oC (b) and at high magnification of the calcined sample (c) and EDX spectra (d) of Fe_3O_4/TiO_2 composite nanofibers

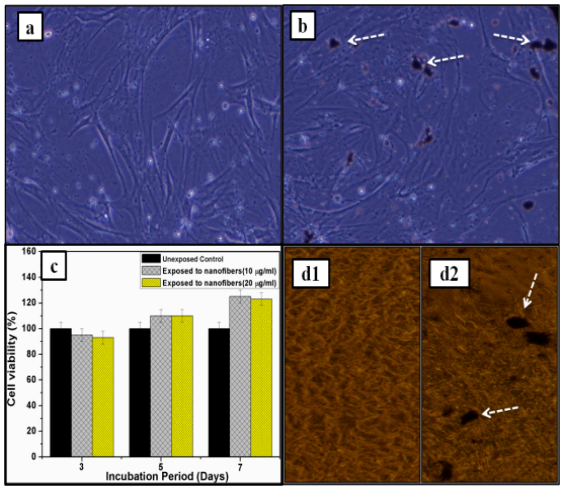

Figure 3. Representative phase contrast images of satellite cells (a) unexposed control (b) exposed to Fe_3O_4/TiO_2 composite nanofibers (Magnification 40×), (c) CCK-8 assay results, the viability of control cells was set at 100%, and viability relative to the control was expressed. The experiments were conducted at least in triplicate, (d1) control cells (d2) exposed cells after (CCK-8) solution treatment.

IV. CONCLUSION

In summary, we isolated the satellite cells from adult Hanwoo muscle and these muscle cells are able to proliferate and differentiate into myotubes under *in vitro* conditions. Moreover, we explored the electrospinning process for the fabrication of Fe_3O_4/TiO_2 composite nanofibers. Novel Fe_3O_4/TiO_2 composite nanofibers with increased surface area were prepared via cost effective electrospinning method. From the *in vitro* test results, it was concluded that Fe_3O_4/TiO_2 composite nanofibers showed a beneficial effect on the adhesion and propagation of satellite cells and could guide the spreading behavior of muscle cells. Thus, the current work demonstrates that the as-synthesized Fe_3O_4/TiO_2 composite nanofibers represent a promising biomaterial to be exploited for various tissue engineering applications especially muscle regeneration.

ACKNOWLEDGEMENTS

This work was supported by research funds of Chonbuk National University in 2012. This work has been partly supported by a grant from Next-Generation BioGreen 21 Program (no. PJ008191 and PJ008196) Rural Development Administration, Republic of Korea.

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