CULTIVATION OF FIBROBLASTS ON HYBRID SCAFFOLD FOR TISSUE ENGINEERING APPLICATIONS

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Abstract – We report the fabrication of novel hybrid micronanofiberous scaffold composed of virgin olive oil/copper oxide nanocrystals and poly(urethane) with improved cellular response for potential tissue engineering applications. In this study, hybrid nanofibers were prepared by facile sol-gel electrospinning. The physicochemical properties of the synthesized hybrid nanofibers were determined by scanning electron microscopy and electron probe microanalysis. To examine the *in vitro* cytotoxicity, NIH 3T3 fibroblasts were cultured on as-prepared hybrid scaffold and the viability of cells was analyzed by Cell Counting Kit-8 assay at regular time intervals. The morphological features of unexposed fibroblasts and cultured on composite scaffold were examined with SEM. We observed that the composite scaffold could support cell adhesion and growth. Results from this study therefore suggest that composite scaffold can mimic the natural extracellular matrix well and provide possibilities for diverse applications in the field of tissue engineering.

Key Words – Micronanofibers, Copper oxide, Polyurethane, Olive oil, Biocompatibility

I. INTRODUCTION

Recently the fabrication of nanofibers by electrospinning technique has attracted enormous interest as a method to craft tissue engineering appliances. Fibers with modifiable dimensions could be produced by electrospinng procedure; that is the diameter of electrospun fibers comparable to fibrils in extracellular matrix (ECM) [1] and these electrospun fibers has demonstrated huge potential as a substrate for cell growth. To this end we herein reported the synergistic outcome of copper oxide and virgin olive oil for enhanced wound healing application. The medicinal benefits of olive oil are undoubtedly huge and very much reported into the literature [2-7]. Besides, photoprotection to skin [8, 9], the olive oil has depicted direct antioxidant action on skin, mainly oleuropeine, [10], which acts as a free radical scavenger. Furthermore, in order to impart/or enhance the antimicrobial activity of wound dressing, it is necessary to incorporate efficient antibacterial agents into it. It is well-known that silver, copper and zinc are chemically stable and possess excellent antimicrobial activity with little possible toxic reactions. Besides, possessing the broad antimicrobial activity copper also plays a significant role in various physiological and metabolic processes such as stimulation of endothelial growth, angiogenesis and stabilization of extracellular skin proteins [11]. On the contrary, the unpleasant skin response due to copper is negligible [12]. Conclusively, considering the potential of PU to enhance epithelial growth[5] and aforementioned applications of olive oil and copper; the organic-inorganic hybrid micronanofiberous dressing was fabricated via facile electrospinning and we believe that these biodegradable micronanofibers aid in the quick healing as well as decrease the risk of wound infection.

II. MATERIALS AND METHODS

Synthesis of hybrid micronanofibers by electrospinning and characterization

Preparation of pure and hybrid micronanofibrous mats was carried out by electrospinning. 10 wt% PU polymeric beads were dissolved in DMF: THF (1:1 w/w) under magnetic stirring overnight at room temperature to get clear solution. The PU/olive blends were obtained by adding different weight% (5% and 10%) of oil. The respective blends were mixed at room temperature to get uniform olive/PU mixtures. Whereas the final composite mixture was prepared by mixing 5 wt% of virgin olive oil and 3 wt% of CuO nanocrystals in 10 wt% PU polymeric solution. The obtained composite mixture was stirred for an hour prior to electrospinning. The obtained pristine and composite sol-gels were transferred with the help of micropipette to a 10 ml plastic needle (Shinchang medical Co. Ltd., Republic of Korea). The micro-tip (internal diameter = 0.6 mm) was used as the spinning head. A voltage of 15 kV was applied to this solution and the distance between the syringe needle tip and collector was maintained at 10 cm during electrospinning. A copper pin inserted in the solution was used as positive terminal whereas a ground iron drum covered by a polyethylene sheet was used as counter electrode. To examine the microstructure, the images were acquired at various magnifications using SEM, H-7650, Hitachi, Japan). The distribution of elements was measured using electron probe microanalysis (EPMA).

Cell culture

DMEM medium (containing 10% fetal calf serum and 1% penicillin and streptomycin solution) was used for the cultivation of cells and the cultured cells were maintained at 37 ^{o}C with 5% CO_2 and 95% air. To avoid contamination, UV was used for the sterilization of pristine and hybrid micronanofibers. Typically for cell seeding; the synthesized scaffolds were soaked in the growth medium for a few hours to aid in the appropriate attachment of cells to nanomatrix. Subsequently fibroblasts were seeded (60% confluent; cell count-1×10^5 cells/ml) in 96 well microplates and allowed to grow for 3 days. However the growth medium was changed at regular time intervals. The cells without the treatment of micronanofibers were treated as a control.

Cell viability test

Cell viability was measured by MTT [3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay. MTT reagent was prepared strictly following the manufacturer's instructions and stored in the dark at refrigeration temperature until used. Briefly, following the incubation of cells with pristine and hybrid wound dressings for 1, 2 and 3 days of incubation time, MTT reagent (40µl) was added to each well which contains growth medium media (160µL) and was afterward incubated for 4 h at 37°C. Absorbance at 490 nm was detected using an ELISA plate reader (Biotek, Winooski, VT).

III. RESULTS AND DISCUSSION

Figure 1 shows the SEM micrographs of the plain PU, virgin olive oil blended PU and olive oil/CuO-PU hybrid micronanofibers.

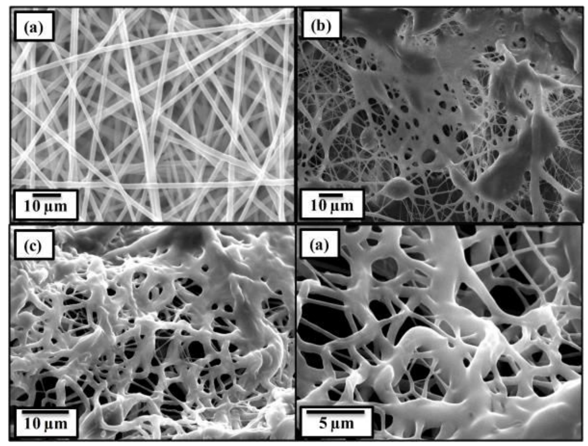

Figure 1. SEM images of pristine PU (a) Olive oil (10 wt%)/PU blend micronanofibers (b) Olive oil (5 wt%)/CuO-PU micronanofibers (c, d) at different magnifications.

Electrospinning of pristine PU solutions yielded nanofibers with 250–550 nm (±10 nm) diameter demonstrated in Figure 2a. Undoped PU nanofibers were bread free with even surfaces (Fig. 2a). Conversely, the nanofibers obtained from olive oil doping (5%) in PU were comparatively beaded (Fig. 2b). Furthermore, it was also observed that the merging of olive oil and CuO naocrystals in PU yielded micronanofibers which ranged from 0.15 µm – 1.5 µm in diameter with fused morphology (at certain positions) and enhanced mechanical strength (Figure 2c, d).

Figure 2 represents the chemical composition of hybrid micronanofibers. The successful blending of CuO nanocrystals and olive oil with PU fibers was confirmed by the EPMA which demonstrated the consistent dispersion of C, O and Cu elements in hybrid mat.

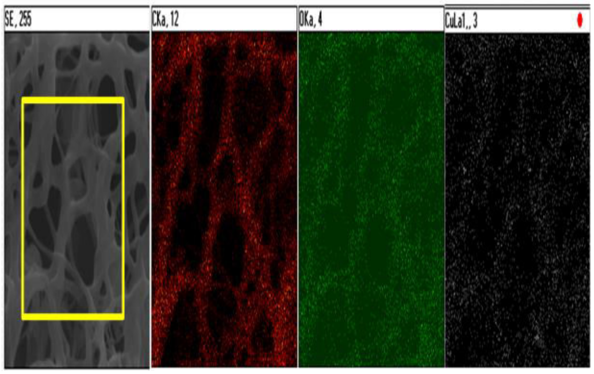

Figure 2. EPMA of hybrid micronanofibers.

The cytobiocompatibility of as-spun organic-inorganic hybrid micronanofibers was investigated against fibroblasts. Figure 3 shows the MTT assay results after specific incubation time as earlier referred in this manuscript. No toxic effects were seen in the fibroblasts lying on hybrid micronanofibers and untreated control. The cells increased exponentially during the culture period. The cells were active throughout the incubation time.

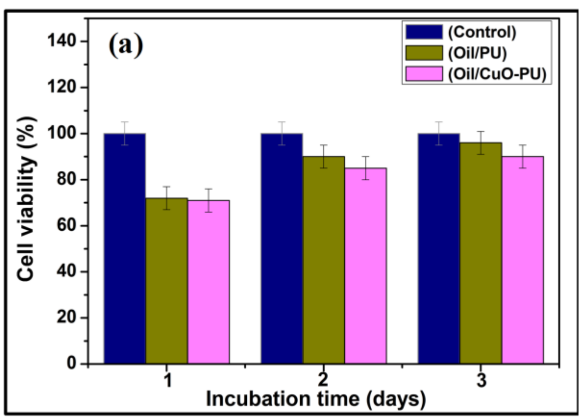

Figure 3. MTT assay results (a), the viability of control cells were set 100%, and viability relative to the control was expressed. The experiments were conducted at least in triplicate,

IV. CONCLUSION

In summary, our study clearly suggests the role of virgin olive oil, copper oxide in wound management and usefulness of the electrospinning process for the fabrication of organic-inorganic composite wound dressing. The as-spun composite dressing possesses multifunctionality such as disallow the access of infectious microbes to wounds; maintains a hydrated environment; help in deodorizing the wounds; prevents the apoptosis of wound repair cells and so forth. Nevertheless; before these wound dressings will be applied to humans; detailed curative effects of full thickness skin wounds in *in vivo* model will be investigated. Thus, the current work demonstrates that the as-synthesized composite scaffold represent a promising biomaterial to be exploited for various tissue engineering applications.

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REFERENCES

1. Friess, W. (1998). Collagen–biomaterial for drug delivery. European Journal of Pharmaceutics and Biopharmaceutics, 45(2), 113-136.
2. de Backer, G., Bagnara, S., Crepaldi, G., Fernandez-Cruz, A., Godtfredsen, J., Jacotot, B. & Varela, G. (1997). International consensus statement on olive oil and the Mediterranean diet: implications for health in Europe. European Journal of Cancer Prevention, 6, 418-421.
3. Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., Würtele, G., Spiegelhalder, B., & Bartsch, H. (2000). Olive-oil consumption and health: the possible role of antioxidants. The lancet oncology, 1(2), 107-112.
4. Ozyilkan, O., Colak, D., Akcali, Z., & Basturk, B. (2005). Olive: Fruit of peace against cancer. Asian Pacific Journal of Cancer Prevention, 6(1), 77-82.
5. Shah, M., Adams-Huet, B., & Garg, A. (2007). Effect of high-carbohydrate or high-cis-monounsaturated fat diets on blood pressure: a meta-analysis of intervention trials. The American journal of clinical nutrition, 85(5), 1251-1256.

6. Strychar, I., Ishac, A., Rivard, M., Lussier-Cacan, S., Beauregard, H., Aris-Jilwan, N., & Yale, J. F. (2003). Impact of a high-monounsaturated-fat diet on lipid profile in subjects with type 1 diabetes. Journal of the American Dietetic Association, 103(4), 467-474.
7. Visioli, F., & Galli, C. (1998). The effect of minor constituents of olive oil on cardiovascular disease: new findings. Nutrition reviews, 56(5), 142-147.
8. Kelly, G. S. (1999). Squalene and its potential clinical uses. Alternative medicine review: a journal of clinical therapeutic, 4(1), 29.
9. Thiele, J. J., Dreher, F., & Packer, L. (2002). Antioxidant defense systems in skin 1. Cutaneous and Ocular Toxicology, 21(1-2), 119-160.
10. Ancora, C., Roma, C., & Vettor, M. (2004). Evaluation of cosmetic efficacy of oleoeuropein. In Symposium on the New Frontiers of Dermo-cosmetology: Efficacy, Stability and Safety. Rome, Italy.
11. Sen, C. K., Khanna, S., Venojarvi, M., Trikha, P., Ellison, E. C., Hunt, T. K., & Roy, S. (2002). Copper-induced vascular endothelial growth factor expression and wound healing. American Journal of Physiology-Heart and Circulatory Physiology, 282(5), H1821-H1827.
12. Gorter, R. W., Butorac, M., & Cobian, E. P. (2004). Examination of the cutaneous absorption of copper after the use of copper-containing ointments.American journal of therapeutics, 11(6), 453-458.