실리콘으로 개질된 Hydroxyapatite particulate 나노섬유에 관한 연구 : 표면개질을 위한 접근

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Hydroxyapatite Particulate Nanofiber Modified Silicon : An Approach for Surface Modification

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1. Introduction

It was since early 1970s, electron probe microanalysis, reported the presence of silicon (~0.5 wt %) *in-vivo* within the active calcification sites [1, 2]. This study suggested that Si is associated with calcium in an early stage of calcification. Now a day it has been widely explored in the development of implantable biomedical devices such as: neural prostheses, controlled drug delivery system, chemical/biological sensors etc [3, 4]. Any device for long term *in-vivo* application has to fulfill rigorous biocompatibility and biostability requirements [5]. Cell-substratum interaction is central to many biological phenomena. Study and findings of this interaction is crucial to the understanding of many fundamental biological questions and to the design of medical devices. Although,the design and engineering of active implantable medical devices have reached an advanced state of development, important clinical parameters are still limited by the host's reactions against the implant as a foreign body resulting inflammatory response. According to the US National Center for Health, more than 20 million people in US have at least one medical implant and the latest statistics shows nearly 20% of the hip replacement surgeries performed in 2001 was to "revise" the original implants. Central to tackling this issue, considerable research interest has focused on investigating implant-prosthesis failure and on developing biomaterials with extended life time. With the findings of Carlise, presence of silicon in calcification sites, in young mice and rats, silicon is placed at the material of interest along with hydroxyapatite (HA) for bone tissue engineering [6-8]. This research reports the surface modification of Si with HA particulate nanofiber by using a simple electrospinning method.

2. Materials and Methods

Homogeneous aqueous slurry of calcium glycerol phosphate (CGP) and calcium acetate (CaAc) with Ca/P ratio 1.67 was treated with 9 wt% PVA (aq)solution in the ratio of 3:7 by wt. to get a clear sol-gel and electrospun by applying 15 kV at an electrode distance of 15 cm. The fibers were collected on Si wafer (Si wafer was previously washed with distilled water/acetone and dried) and subjected to calcination at various temperatures. Different physiochemical characterization technique

(FT-IR, FE-SEM, XRD, AFM, ICP) were employed to characterized the materials.

A human osteoblast cell line CCRL-11372 was cultivated on a six-well cell culture plate with and without Si-HA matrix in Dulbecco's modified Eagle's medium supplemented with 2% penicillin/Streptomycin and 10% fetal bovine serum. Cells were cultivated in 5% CO_2 at 37°C in 95% relative atmospheric humidity for 3 days. In a typical experiment, prior to culture Si-HA composite matrix was cut in dimension 15mm×11mm×1mm and was sterilized on both side in UV light for 30 min. the cytotoxicity of the materials was evaluated by lactate dehydrogenase (LDH) test.

3. Results and discussion

Figure 1 shows the interesting finding of crystallographic reflection at $2\Theta=32.9°$, the characteristic (300) plane of HA, usually ceramics exhibits number of reflections according to its structural alignment. Also, presence of matrix, types of precursors, and instrumental conditions influence the structure integrity. This crystallographic purity shown by exhibiting single reflection towards (300) plane, with simultaneous increase in intensity along with calcinations temperature in electrospinning derived matrix shows the key role of fibrous structure of nanofiber mats, which has never been observed in conventional pure HA. However, the numbers of XRD reflection were observed (Figure 1, inset) when simply dipping Si into sol-gel precursor instead of electrospinning. Possibly, with the steady increase in calcinations temperature and steady evaporation of polymer the intra and inter fibril minerals fused resulting inter-connected particulate nanofiber as shown in FE-SEM images (Figure 2). Also this phenomenon is favored by complexation between Ca^{2+} and acetate ions in solid state reaction because in wet chemistry the rate of precipitation reduces efficiently due to the complexation and hence decreases the solution supersaturation and their adsorption on the surface of the initially formed crystal nuclei, thereby, blocking the growth active centers [9]. Due to the high crystalline and chemical purity, elemental ratio (Ca/P=1.66) mimicking to that of natural apatite, in the sample calcined at 600°C, as indicated by intense X-ray diffraction (XRD) and inductive coupled plasma (ICP) analysis, it was chosen as material of interest for further experiment.

Surface chemistry and surface topography has an important influence on the performance of bone implants. Microscale surface topography, e.g. surface porous beads, can improve implant fixation mainly through mechanical interlocking. This surface architecture can profoundly affect the behaviors of cells. Cell commonly shows different shapes when cultured on substrates as with different roughness; there is abundant evidence in rough Ti surface. These studies have shown that titanium implant surfaces may modulate phenotypic expression and metabolism of osteoblast cells [10, 11]. Anselme et. al. [10] have shown confluent layer of human osteoblast on Ti alloy surface. Also, have reported the responsiveness of osteoblasts to systemic hormones at the implant surface is also influenced by surface roughness. Cells on rough surface show increased production of osteocalcin and matrix produced by these cells is collagen base and forms a suspension bridge like structure, which is very difficult to remove.

Similar evidencein cell proliferation is found in silicon substituted HA. As shown in Figure 3, the cultured cells spread with inter cell-communication and become dense thereby covering the entire matrix, a confluent layer. This cell proliferation and its "jump in"from one fibril to another is a kind of interlocking. As demonstrated in scheme 1, cells adapt to the fibril shape. This

scheme is also favored by the insets in Figure 5 (the proliferation of cells clearly shows the elongated cell structures which are fused with surrounding cells). Additionally, the maintained structural integrity at this stage was clearly observed in high resolution FE-SEM images (Figure 4). No apparent change in fiber arrangement instead cell penetration was observed.

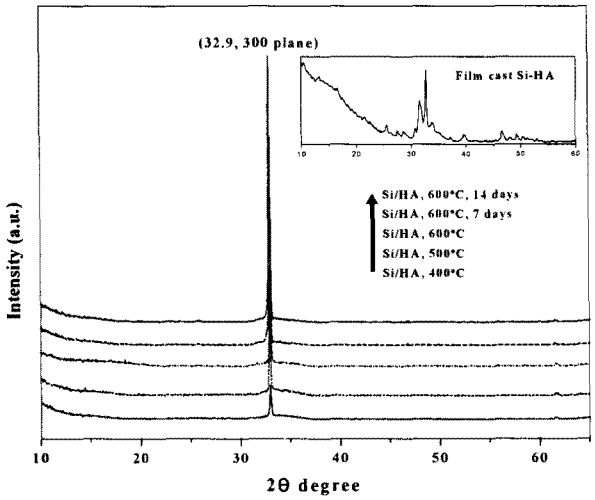

Figure 1. X-ray diffraction patterns of Si-HA matrix. Inset represents the pattern obtained from S-HA matrix prepared by simple film casting and cancined at 600 °C.

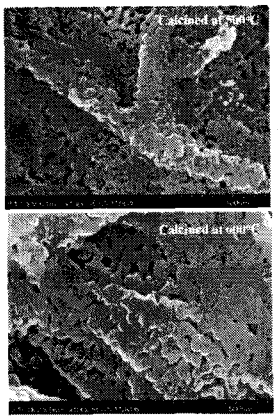

Figure 2. FE-SEM images of Si-HA marix

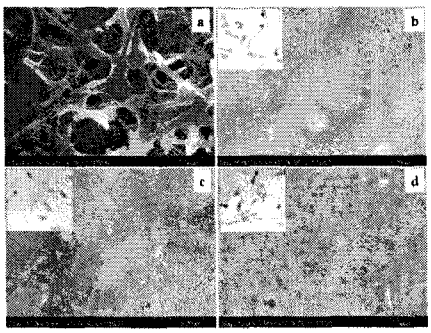

Figure 3. SEM images of the human osteoblast cell cultured in-vitro: (a) Si-HA control (b) 1 day (c) 2 days and (d) 3 days. Inset shows the morphology of cells cultured in wells plate

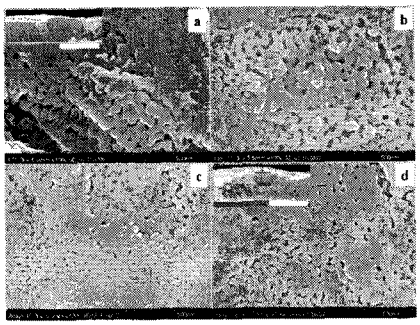

Figure 4. High resolution of FE-SEM images of the human osteoblast cell cultured in-vitro: (a) Si-HA control (b) 1day (c) 2 days and (d) 3 days. Inset shows the cross section image of respective samples (scale bar 250 nm).

4. Conclusions

In conclusion, this study highlighted the surface modification of Si wafer via electrospinning resulting hierarchal structure when utilize along with sol-gel technique, with out any chemical etching. Thus obtain composite matrix is found to have a superior topographical structure which

promotes the human osteoblast proliferation. This finding will add a new dimension in the surface modification of implant materials to create an appropriate environment for growth and differentiation of cell.

5. Acknowledgement

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6. References

[1] E. M. Carlisle, Science, 1970,167, 279 - 280.
[2] E. M. Carlisle, Science, 1972,178, 619 - 621.
[3] D. V. McAllister, M. G. Allen, M. R. Prausnitz, Annu. Rev. Biomed. Eng. 2000, 2, 289 - 313.
[4] J. T. Santini Jr, M. J. Cima, R. Langer, Nature, 1999, 397, 335 - 338.
[5] J. M. Anderson, J. J. Langone, J. Control Release,1999, 57, 107 - 113.
[6] A. E. Porter, C. M. Botelho, M. A. Lopes, J. D. Santos, S. M. Best, W. Bonfield, J. Biomed. Mater. Res. A, 2004, 15/69, 670 - 679.
[7] A.E. Porter, N. Patel, J. N. Skepper, S. M. Best, W. Bonfield, Biomaterials 2004, 25, 3303 - 3314.
[8] A. E. Porter, S. M. Best, W. Bonfield, J. Biomed. Mater. Res., 2003, 68, 133 - 141.
[9] N. Spanos, A . Patis, D. Kanellopoulou, N. Andritsos, P. G. .Koutsoukos, Cryst. Growth Des., 2007, 7, 25-29.
[10] K. Anselme, P. Linez, M. D. Bigerelle, A. Le Maguer, P. Hardouin, H.F. Hildebrand, A. Iost, J. M. Leroy,Biomaterials, 2000, 21, 1567-1577.
[11] J. Y. Martin, D. D. Dean, D. L. Cochran, Clin. Oral. Impl. Res., 1996, 7, 27-37.