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The Comparison of the Bioactivity of Fibroin/Titanium Dioxide Nanoparticles and Fibroin/Fluoridated Titanium Dioxide Nanoparticles Scaffolds for Bone Tissue Engineering

Narges Johari[1] HamidReza MadaahHosseini[2]

Ali Samadikuchaksaraei[3]

1- Introduction

One of the most important human health issues is the defecting of an organ or tissue. The tissue engineering scaffold provides a means for the delivery of cells and/or growth factors to the site of damage and an appropriate template for new tissue formation throughout the construct. Its function is different from traditional bone fillers, in that the goal of tissue engineering is to entirely replace the implanted scaffold with new bone tissue. While for bone fillers, integration with existing bone and equivalent mechanical strength are fundamental design criteria, the tissue engineering scaffold should ideally possess a three-dimensional structure that endures only as long as necessary for replacement after new bone growth. A scaffold for bone tissue engineering should thus approximate the mechanical function of the callus in natural bone repair, providing a dynamically durable–degradable three-dimensional structure, which over time can be replaced by cell-derived tissue function. Also, bone is a heterogeneous composite material with constituents including hydroxyapatite mineral ($Ca_{10}(PO_4)_6(OH)_2$), a mixed organic component (type I collagen, lipids and non-collagenous proteins) and water. During scaffold manufacture it would therefore seem logical to include a combination of materials to create a composite scaffold, potentially allowing greater scaffold bioactivity and structural biomimicry to be achieved. Scaffold bioactivity is also increased by incorporating materials that possess the ability to interact with or bind to living tissues. Increased scaffold bioactivity can in turn improve bone cell ingrowth, stable anchoring of scaffolds to host bone tissue, stimulation of immature host cells to develop into osteogenic cells, and increase vascularization.

In current study, two groups of nanocomposite scaffolds have been prepared with silk fibroin/titanium dioxide nanoparticles (SF/N-TiO_2) and silk fibroin/fluoridated titanium dioxide nanoparticles (SF/N-TiO_2-F) and their bioactivity have been evaluated by immersing in simulated body fluid (SBF) up to 28 days.

2- Experimental study

Silk fibroin (SF) was extracted from *Bombyx mori* silk cocoons (Iran Silkworm Research Center, Guilan, Iran). Accordingly, silk cocoons were boiled in 0.02 M sodium carbonate solution for 1 h to remove sericin. Then, they were absolutely rinsed with consecutive immersion in cold and hot distilled water to complete degumming treatment. Degummed silk fibers were dried overnight at room temperature and dissolved in 9.3 M lithium bromide solution at 50–60 °C for 4 h. Afterward, the solution was dialyzed for 3 days using deionized water. To prepare the SF/TiO_2 solution, the SF solution was added to the dispersed TiO_2 in DI water. The obtained mixture was frozen at −20 °C for 4 h and at −80 °C for 12 h; eventually the frozen mixtures were freeze-dried overnight. The prepared SF/N-TiO_2 nanocomposite scaffolds were immersed in methanol for 1 h to treat the structure of SF. To prepare the SF/N-TiO_2-F nanocomposite scaffolds, TiO_2 nanoparticles were incubated in 2.0 vol% HF for 120 s. Thereafter, N-TiO_2-F nanoparticles were uniformly dispersed in deionized water. The SF/N-TiO_2-F nanocomposite scaffolds were prepared similar to the SF/TiO2 nanocomposite scaffolds.

The phase structure of SF/N-TiO_2 and SF/N-TiO_2-F nanocomposite was analyzed using a STEO-D 64295 diffractometer (XRD, STEO, Germany) at 40 kV and 40 mA. The functional groups of SF/N-TiO_2 and SF/N-TiO_2-F nanocomposite were studied using a Fourier Transform Infrared Spectroscopy (FTIR, MB series, ABB Bornem, Canada) in an IR spectrum range within the range of 400–4000 cm^{-1}.

In vitro bioactivity of SF/N-TiO_2 and SF/N-TiO_2-F nanocomposite was evaluated by immersing the composite in SBF with pH of 7.4 at 37 °C. The standard SBF solution was prepared according to Kokubo's protocol. The apatite formation on the surface of SF/N-TiO_2 and SF/N-TiO_2-F nanocomposite was observed by SEM and analyzed using an energy-dispersive X-ray spectroscopy (EDX) device (TESCAN, Czech Republic) and X-ray diffractometer.

3- Conclusion

In this study, silk fibroin (SF) was extracted from Bombyx mori cocoons and SF/N-TiO_2 and SF/N-TiO_2-F nanocomposites were synthesized. The effects of SF, TiO_2 nanopowders and flour ions on bioactivity of SF/N-TiO_2 and SF/N-TiO_2-F nanocomposites were investigated. It was found that after immersing the composite in SBF, some agglomerates of white sphere-like particles were formed on the surface of SF/N-TiO_2 and SF/N-TiO_2-F nanocomposites (Fig. 1).

[1] Assistant professor, Department of Materials Science and Engineering, Golpayegan University of Technology, Golpayegan, Iran.
[2] Professor, Department of Materials Science and Engineering, Sharif University of Technology, Tehran, Iran.
[3] Corresponding author, Professor, Department of Medical Biotechnology, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran.
Email: Johari_narges@yahoo.com

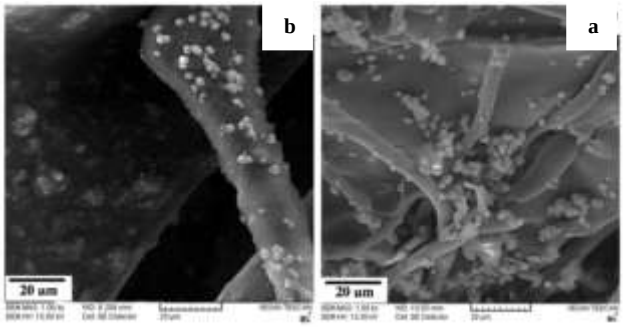

Figure 1 SEM images of a) SF/N-TiO_2-F and b) SF/N-TiO_2 nanocomposites immersed in SBF for 28 days.

Fig. 2 and 3 show the changes of calcium ions and phosphorous ions of SBF solution which SF/N-TiO_2 and SF/N-TiO_2-F nanocomposite scaffolds were immersed in.

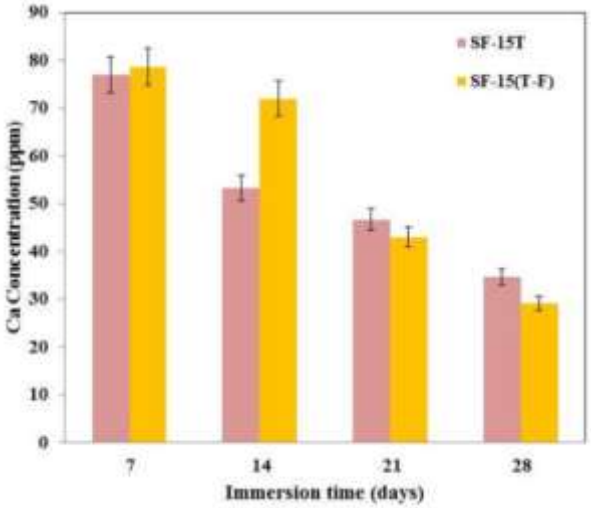

Figure 2. The changes of Ca concentration of SBF which SF/N-TiO_2-F and SF/N-TiO_2 nanocomposites immersed in.

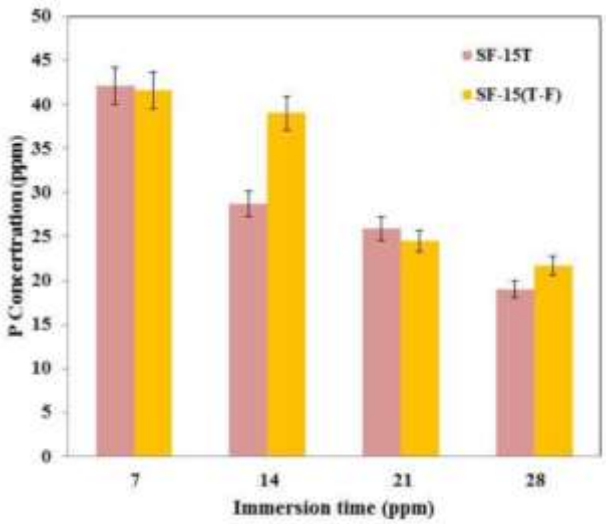

Figure 3. The changes of P concentration of SBF which SF/N-TiO_2-F and SF/N-TiO_2 nanocomposites immersed in.

The decrease of Ca and P concentrations reveals that these ions have been consumed and changed into apatite and deposit on the surface of the prepared scaffolds.
Fig. 4 shows the EDS spectrum of white sphere-like particles precipitated on the surface of SF/N-TiO_2-F nanocomposites which reveal the presence of Ca and P ions in white sphere-like particles.

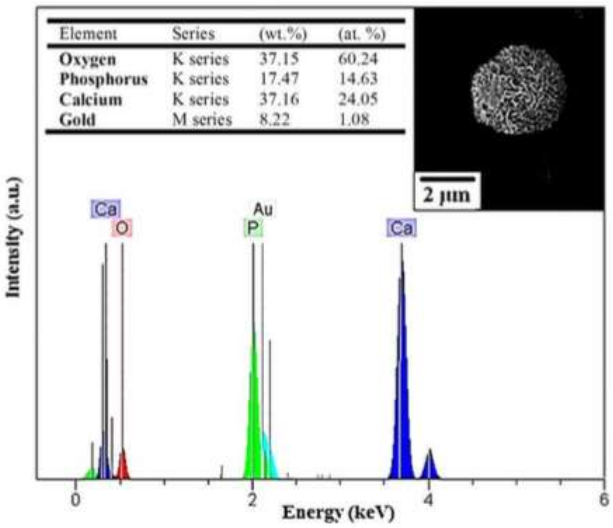

Figure 4. EDS spectrum of SF/N-TiO_2-F nanocomposites.

Bioactivity evaluation showed that these white sphere-like particles are bonelike apatite. The presence of flour ions in SF/N-TiO_2-F nanocomposite enhanced the bioactivity of SF.