Application Note

Multilayer Bio-Films for Bone Tissue Regeneration

Nanofibrous polymers enriched with bioactive molecules have great potential to improve the durability of regenerated tissues. Here QCM instrument qCell T was successfully applied for real time characterization of a bioactive implant for bone tissue reconstruction, where basic fibroblast growth factor (FGF2) is incorporated into a 3-D nanofibrous scaffold via Layer-by-Layer (LbL) assembly.

Summary

Despite the enormous potential of tissue regeneration using synthetic yet biodegradable implants, the lack of durability of the regenerated tissue is a serious issue. Thus, bioactive implants, 3-D scaffolds which are functionalized with bioactive molecules essential for cellular growth and differentiation such as growth factors, are attractive additions and have proven better success in next generation of tissue engineering. Herein, we present a characterization of a bioactive implant for bone tissue reconstruction, where basic fibroblast growth factor (FGF2), an essential protein for bone tissue repair, is incorporated into a 3-D nanofibrous scaffold via Layer-by-Layer (LbL) assembly. The real time film formation by the LbL technique was monitored using a quartz crystal microbalance (QCM, qCell T by 3T Analytik GmbH, Germany) and the results indicate a stable film formation with a nominal thickness of 10 nm under set experimental conditions.

Background

The regeneration, re-growth or repair of cells and tissues, has long attracted biomedical interest due to the potential of replacing dysfunctional or damaged tissues with new ones. One of the promising strategies in modern tissue engineering involves localization of an optimal combination of regenerative cells (autologous, allogeneic, xenogeneic, genetically engineered, or stem cells) into a 3-D scaffold, which mimics a natural extracellular matrix [1,2]. These 3-D scaffolds are generally made up of cell-friendly nanofibrous polymers which are capable of spatially directing the complex multicellular processes of tissue formation and regeneration (see figure 1).
Despite the tremendous progress in tissue engineering, lack of durability of the repaired tissue still persists with 3-D scaffold-based approach. Thus, the scaffold surface is enriched with bioactive molecules such as growth-, angiogenic and differentiation factors to facilitate cell adhesion, proliferation, and differentiation.

Strategy

In this study, we surface functionalize a 3-D scaffold with an osteogenetic growth factor, basic fibroblast growth factor (FGF2), using the LbL approach. The process involves a sequence of simple alternate immersions of the 3-D scaffold into a solution of a polycation, and a solution of FGF2, with rinsing steps between adsorption steps. Successful LbL assembly formation, stability and physical properties of the film is characterized in real time using quartz crystal microbalance (QCM).

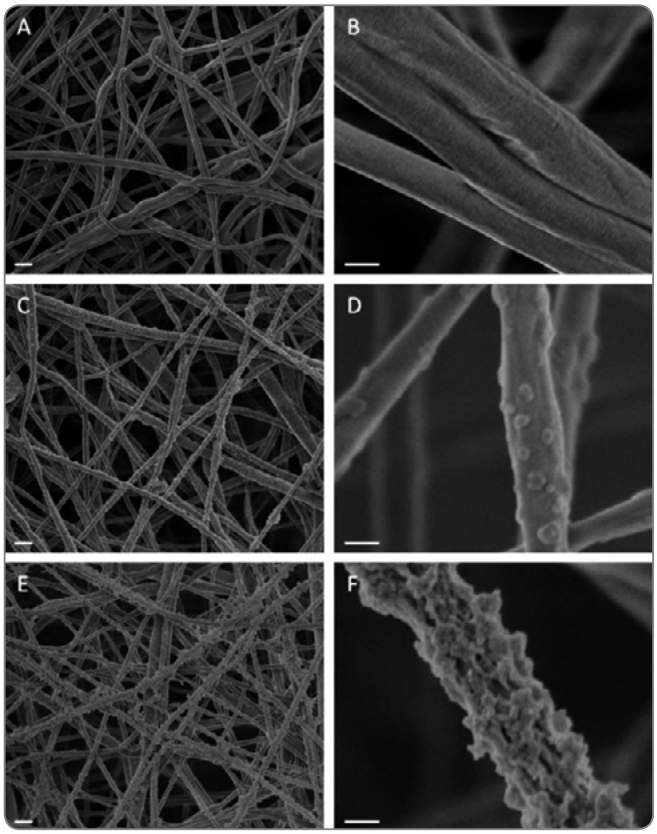

Figure 1. SEM visualization at different magnifications of the electrospun PCL nanofibers A,B) before LbL treatment, C,D) after the deposition of (PLL/BMP-2)3 spheroid nanoarchitectures,and E,F) after the deposition of (CHI/BMP-2)3 fish scale-like nanoarchitectures. Scale bars: A,C,E) 1mm and B,D,F) 100 nm [2].

Method

The LbL assembly of poly-L-lysine (pLys, a polycation), poly-L-glutamic acid (pGlu) and FGF2 with a film archi-

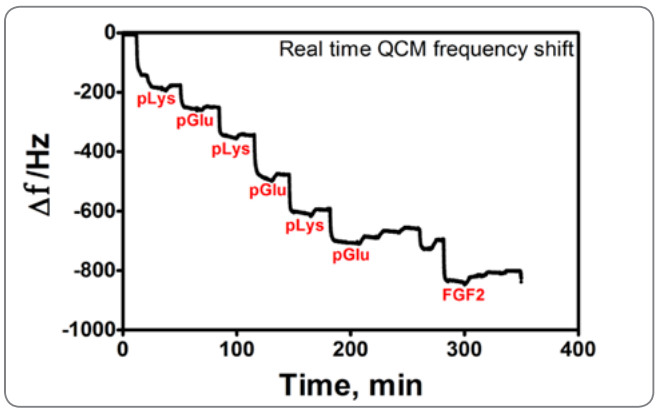

Figure 2. Real time QCM frequency shift (Δf) upon formation of the film $(pLys/pGlu)_3$/FGF2 on a 3-mercaptopropionic acid coated on a 10 MHz QCM resonator at 25 °C.

tecture of $(pLys/pGlu)_3$/FGF2 was constructed on 10 MHz QCM resonators (provided by 3T Analytik GmbH), which are already functionalized with a negatively charged monolayer of 0.5 mM 3-mercaptopropionic acid in ethanol overnight. The film construction process involved brief, subsequent flow periods of polyelectrolyte solutions over the sensor surface accompanied by saline buffer (pH 7.4) washings after each layer, with the QCM frequency change being monitored in real time (see figure 2).

Conclusion

The QCM represents an advanced and very useful technique to investigate the properties of a layer-by-layer assembled biofilm. Progressive reduction in resonance frequency upon introduction of polyelectrolytes and FGF2 was successfully monitored by QCM instrument qCell T. The result indicates stable LbL film formation under our experimental conditions. The nominal thickness of the film is 10 nm.

Reference

1. Porous scaffold design for tissue engineering. Hollister, S. J., 2005, Nature Materials, 4, pp.518-526.
2. Osteogenetic Properties of Electrospun Nanofibrous PCL Scaffolds Equipped with Chitosan-based Nanoreservoirs of Growth Factors. Ferrand, A. et. al., 2014, Macromol. Biosci., 14, pp. 45-55.
3. Biomolecular Films: Design, Function, and Applications. Rusling, J. F., 2003, Marcel Dekker: New York.

Acknowledgement

The results were carried out by **Dhanuka P. Wasalathanthri** and **Prof. Dr. Liisa Kuhn** in **Prof. James F. Rusling**'s lab at the **University of Connecticut Health Center** in Storrs, CT, USA

3T analytik GmbH & Co.KG
Gartenstraße 100
D-78532 Tuttlingen

Tel.: +49 (0)7467-947-66-0
Fax: +49 (0)7467-947-66-29
E-Mail: info@3t-analytik.de
www.3t-analytik.com