

## Synopsis

Tissue repair and regeneration is a natural healing process seen in human body in response to injuries and infections. The regenerative capacity of most tissues decelerates with age and its associated pathological conditions. In particular, bone pathology leads to serious challenges for an individual's physical activity and survival. Large bone defects in most cases become irreversible. Surgical and therapeutic interventions are common clinical practices to promote the natural healing process. In particular, substitutes such as auto-grafts and allografts, and permanent implants are the conventional approaches. Common risks associated with aforementioned strategies involve multiple surgeries, immune rejection, graft failure due to non-integration and subsequent side effects. The growing demand for improved and lasting solutions to bone pathologies necessitates engineering of bone-like tissue substitutes. Bone tissue engineering aims to design bone-like three dimensional (3D) scaffolds that closely mimic the native architecture of bone tissues and accelerate bone regeneration and integration with the host tissue. Among many types of scaffolds that are available, nanofibrous scaffolds are particularly promising as they closely mimic the fibrous architecture of the extracellular matrix of native bone tissue. Furthermore, composite nanofibrous scaffolds prepared from blending synthetic biodegradable polymers and ceramic nanoparticles are being widely investigated as an alternative to the use of labile and expensive growth factors. This motivated us to prepare composite nanofibrous scaffolds that offer low cost and effective therapeutic benefits for bone tissue engineering.

**Chapter 1** outlines the detailed literature review on the basic principles involved in preparing nanofibrous scaffolds through electrospinning technique and their importance in bone tissue engineering. Furthermore, recent advances in the technique to generate more complex 3D scaffolds with variety of nanofibrous architecture and material compositions for efficient tissue generation are described. In this chapter, we discuss the progress in improving the equipment design to overcome the limitations associated with electrospinning as a technique to prepare scaffolds that can be used clinically as bone graft substitutes.

**Chapter 2** focusses on finding the mechanism through which the nanofibrous scaffold architecture induces osteogenic differentiation of hMSCs. The role of cytoskeletal organization of hMSCs grown on two-dimensional flat films (2D Films) and 3D nanofibrous scaffolds (3D NFs) was studied. The 3D NFs were observed to induce osteogenic

differentiation and mineralization even in the absence of soluble osteoinductive growth factors when compared to 2D Films. It was observed that both actin filaments and microtubules on 3D NFs regulate cell and nuclear shape, which influences the heterochromatin arrangement thereby inducing the differentiated phenotype through enhanced expression of osteogenic markers and subsequent mineralization.

**Chapters 3, 4 and 5** focus on the preparation and osteogenic potential of bioactive polymer/ceramic composite nanofibrous scaffolds. **Chapter 3** describes the fabrication of strontium carbonate nanoparticle encapsulated PCL scaffolds. The composite scaffolds released significant amount of strontium ions that induced higher proliferation rate, osteogenic differentiation and enhanced mineralization of hMSCs when compared to neat PCL scaffolds.

**Chapter 4** explains a facile sol-gel method to fabricate silicated nanofibers by electrospinning *in situ* silica gelled PCL solution. This novel *in situ* sol-gelation method overcomes the limitation associated with silica particle agglomeration in polymer solution during the mixing process that limits the electrospinning process. Citric and acetic acids were used as acid catalysts in the sol-gelation process, where citric acid was found to promote rapid synthesis of near uniform silica nanoparticles when compared to acetic acid. The presence of citric acid in the silicated scaffolds increased the surface water wettability that triggered significant amount of silicon ion release. The released ions stimulated angiogenic activity of human vascular endothelial cells (HUVECs) and osteogenic differentiation of hMSCs *in vitro*.

**Chapter 5** describes the preparation of composite nanofibrous PCL scaffolds encapsulating *in situ* synthesised multi-ceramic particles using electrospinning. This method allows for rapid and simultaneous synthesis of particles, having silica and calcium composition (calcium oxide or calcium hydrogen phosphate) that mimics the conventional bioactive glass composition, within the polymer solution. The degradation of polymer during fabrication at high pH was prevented by using morpholine as an alternative to pH catalysts. Further, the composite scaffolds released both calcium and silicon ions with or without phosphate ions. These multicomponent composite scaffolds enhanced angiogenic activity of HUVECs as well as hMSC osteogenesis.

In conclusion, the present thesis work focusses on providing insight into the role of nanofibrous scaffolds in stimulating osteogenic activity and for further improving the osteogenic activity by engineering low cost and yet potent multi-biofunctional nanofibrous scaffolds for bone tissue regeneration.