Advanced Nanofibrous Scaffolds to Influence Endothelial Cell Activity

Summary

The use of tissue-engineered scaffolds has found widespread applications in the field of regenerative medicine for the treatment of injured tissues. In my thesis, I focus on the fabrication of scaffolds for vascularized tissue regeneration. **Chapter 1** describes recent literature review on advanced nanofibrous scaffolds, which are widely used in tissue engineering. Especially, electrospun scaffolds for promoting vascular tissue regeneration has been introduced in more details. According to the review we described in **chapter 1**, many requirements should be considered for designing angiogenic scaffolds. First, an ideal scaffold should be biomimetic to the native targeted tissue. Second, the scaffolds should deliver angiogenic factors. Therefore, in **chapter 2-6** we investigated a few different design options to create biomimetic nanofibrous scaffolds and immobilize angiogenic factors on them.

Chapter 2 describes the investigation of polycaprolactone (PCL) aligned fibers and co-culture with human umbilical endothelial cells (HUVECs) on the osteogenic differentiation of mesenchymal stromal cells (hMSCs). The main results demonstrated that aligned structure strongly influenced the morphology and orientation of cells, yet without interfering with the osteogenic differentiation of hMSCs. Moreover, co-culture with endothelial cells showed a positive influence to the osteogenesis of hMSCs.

Chapter 3 presents a simple and effective method to fabricate honeycomb nanofibrous meshes. This self-assembly method could produce honeycomb nanofibrous meshes with controllable diameter by adjusting electrospinning processing parameters. Gradients honeycomb meshes ranging from 800 μm to 300 μm were successfully fabricated. Structural gradients can be found mainly in interface tissues. The concept of gradient scaffolds has been applied to mimic complex gradients found in native interface tissues, such as bone-cartilage interfaces. Gradient honeycomb scaffolds may provide structural

cues to guide cells to migrate or differentiate, which may be beneficial for interface tissue regeneration.

In **chapter 4**, the influence of a honeycomb pattern on endothelial cell morphogenesis is discussed. Honeycomb nanofibrous scaffolds proved to promote cell proliferation and regulate HUVECs morphogenesis into capillary-like structures. HUVECs generated stronger cohesion and cell-cell junctions when cultured on honeycomb scaffolds. Therefore, this scaffold is promising for those tissue engineering applications demanding the formation of capillary networks.

Hydrogen sulfide (H_2S), a unique gasotransmitter, has been considered as a signaling molecule to modulate angiogenesis. In **chapter 5**, we demonstrated a method for bonding NTAs (N-thiocarboxyanhydrides, an H_2S donor) on fibrous scaffolds by azide-alkyne click conjugation. These experiments showed a new strategy to fabricate H_2S releasing fibrous scaffolds by conjugating NTAs, as other strategies providing H_2S in culture focused on traditional H_2S donors (e.g. sulfide salts, NaSH and Na_2S), which are hard to control and often cause burst release. The use of NTAs as H_2S donors could result in controlled and sustained release. The NTA functionalized scaffolds supported better cell proliferation and formed more rapidly a confluent endothelial monolayer than non-functionalized scaffolds. A chicken chorioallantoic membrane (CAM) assay indicated a significant increase in vascular growth on NTA scaffolds *in vivo*. The NTA-functionalized scaffolds could, therefore, offer a biochemical route towards promoting angiogenesis for vascularized tissue regeneration.

Vascular endothelial growth factor (VEGF) has been widely reported to stimulate endothelial cell proliferation and tube formation. VEGF-mimetic peptides have the ability to activate VEGF receptors, therefore possessing similar bioactivity of VEGF. In **chapter 6**, we prepared VEGF peptide functionalized fibrous scaffolds by thiol-ene click chemistry. *In vitro* studies proved that the VEGF peptide functionalized fibrous scaffolds significantly

maintained higher HUVECs survival compared with non-functionalized scaffolds in starving conditions. HUVECs cultured on both VEGF peptide functionalized scaffolds and unfunctionalized scaffolds activated VEGFR1 and VEGFR2 phosphorylation. Moreover, spatial control of the fibrous scaffolds functionalization is another advantage of using the photochemically promoted radical thiol−ene conjugation. Photopatterning experiments showed the potential of using photomasks to spatially control the presentation of the VEGF-mimetic peptide used in these studies.

A general discussion of our results and future perspectives are introduced in **chapter 7**. The valorization potential along with future applications of this research is described in **chapter 8**.