A physicochemical approach to design bioactive scaffolds for tissue engineering

Summary

In this thesis, a number of novel strategies to design and fabricate instructive scaffolds for the regeneration of skeletal tissues are presented. Chapter 1 describes recent advanced in technologies for manufacturing nanofibrous scaffolds with a structure resembling the native ECM. Strategies for improving cell-scaffold interaction were also illustrated before discussing nanofibrous scaffolds for tissue engineering and regenerative medicine applications. From the review of literature proposed in chapter 1, it appears that traditional nanofibrous scaffolds do not display instructive properties, despite their physical resemblance to the native ECM. Thus, there is a great need for fabricating bioactive fibrillar scaffolds, which are capable to instruct cellular functions like adhesion, proliferation, and differentiation. Such bioactive scaffolds could be constructed via tailoring their physical properties (e.g. surface topography, mechanical properties, and structural features), and chemistry.

Chapter 2 shows functional electrospun fibers were fabricated from a new class of poly(butylene succinate)-based (PBS-based) polyesters containing either butylene thiodiglycolate (BTDG) or butylene diglycolate (BDG) sequences. Controllable properties including mechanical strength, wettability, and hydrolysis, could be achieved for these scaffolds through manipulating the molecular architecture and introducing an etheroatom along the polyesters backbone. Polyesters containing BDG sequences were more hydrophilic compared to those containing BTDG units. The higher hydrophilicity of polyesters led to a higher degradation rate. The polymer chemistry of the scaffolds also showed to have an important effect on cell-scaffold interactions. Here, the pure PBS scaffolds were beneficial to stimulate early hMSCs osteogenic differentiation. With the introduction of ether units (BDG) along its polymer backbone, the scaffolds showed enhanced mineralization properties. Having the presence of BTDG functional sequences in the polymer chains, scaffolds supported hMSCs chondrogenisis, while inhibiting their osteogenic differentiation. Tailoring the chemistry composition of nanofibrous scaffold could, therefore, serve as an appealing strategy for deigning bioactive scaffolds in the regenerative medicine field.

Apart from the chemistry point of view, the structure and texture are also essential for functional scaffolds. In chapter 3, yttrium stabilized zirconia (YSZ) ceramic nanofibrous

scaffolds were fabricated via electrospinning a mixture of its precursors and polyvinylpyrrolidone (PVP), followed by annealing of the obtained green fibers. These ceramic nanofirbous scaffolds behaved like a paper, having high flexibility and good fatigue behavior at macroscale, which is not common seen in traditional ceramic mats. Yet, the scaffolds retained their stiffness at the microscale. The surface roughness and grain size of YSZ nanofibers were dependent on the annealing processing parameter. Cell biology results demonstrated that the osteogenic differentiation and mineralization of seeded hMSCs was supported by the nanofibrous structure of YSZ scaffolds, in contrast to the known bioinert behavior of bulk YSZ. In addition, a better mineralization of hMSCs was observed on the scaffolds with a higher surface roughness. Altogether, these flexible ceramic scaffolds presented highly potential application for bone regeneration.

As a logical consequence of the findings of chapter 3 related to the effect of surface topography on mineralization, the influence of surface roughness on cell behaviors were further investigated. Whereas the influence of surface roughness and specific surface topographies on hMSCs activity is known in a number of two-dimensional substrates, limited knowledge is present when translating these studies to 3D scaffolds. In chapter 4, the surface nanoroughness of individual fibers in PEOT/PBT electrsopun scaffolds was varied via adjusting the relative humidity in the spinning chamber during electrospinning. The obtained scaffolds had the same polymer chemistry, similar porosity and pore size, and comparable fiber dimensions, yet a different surface roughness on individual fibers. The scaffolds having a rougher surface roughness of 71 nm had a higher protein adsorption than those having a surface roughness of 14 nm. To investigate the influence of the surface roughness of individual fibers on cellular activities, hMSCs were seeded in scaffolds and cultured in osteogenic and chondrogenic differentiation media for 21 days. The higher surface roughness (Ra=71.0 nm) promoted the expression of some osteogenic genes including osteopontin , bone morphogenetic protein 2 , and runt-related transcription factor 2 , while the lower surface roughness (Ra=14.3 nm) showed to better up-regulate other osteogenic genes, such as bone sialoprotein, collagen type I alpha 1, and osteocalcin. The lower surface roughness (Ra=14.3 nm) was more beneficial for chondrogenic genes expression at day 7. In summary, this research proposes a promising approach to fabricate electrospun scaffold with tailored topographical cues for skeletal differentiation of hMSCs.

The fibrous structure of native tissue has a complex and tissue dependent organization. For example, wavy patterns are observed in many connective tissues, while the collagen

fiber orientation in cartilage, tendons, or ligaments is anisotropic. Thus, traditional electrospinning, which normally produce non-woven meshes, could not fairly meet all the biomimicry requirement for fabricating scaffolds in regenerative medicine. As such, new electrospinning techniques were described in chapter 5 and chapter 6. Chapter 5 offers a simple and effective technique to fabricate wavy or crimped fibrous scaffolds. A wide range of crimped or wavy patterns could be achieved via tuning the processing conditions, including the orientation of the deposited fibers, additional sacrificial polymer layers, and the kind of thermally-shrinkable films. To the best of our knowledge, this research for the first time demonstrated the capability to generate multiscale crimped patterns spanning from fibers to fiber meshes through controlling the thickness of fiber layers. Cellular infiltration is one of the main concerns for electrospun scaffolds regarding their application in regenerative medicine. Here, hMSCs showed a more even cellular penetration in wavy electrospun fiber meshes compared to the non-wavy fiber meshes. Given the obtained wavy patterns mimicking the configuration observed in connective tissues, a mink lung epithelial cell line harboring a TGF-*β* reporter construct was used to examine the effect of such topographical cues on TGF-*β* signaling expression, which is a key signaling pathway involved in the development of several connective tissues. Compared to the non-wavy scaffolds, the wavy scaffolds promoted the expression of TGF-*β* signaling.

Although solution electrospinning technique has been widely used for varied application, its inherent working principle of exploiting jet instability causes also the difficulty to deposit fibers in an accurate manner. This may hinder the further application of electrospinning where more hierarchical organization of nanofibers is needed. As such, chapter 6 shows a new technique for solution electrospinning with the capability to make electrospinning jet follow a stable path from the tip of the spinneret needle to the collector plate. Complex patterns and 3D multiscale structures with controllable porosity could be easily produced using this technique in contrast to traditional electrospinning. Using this new technique, a scaffold that mimics articular cartilage with its distinct multi-zone morphology was fabricated. To estimate their potential application in cartilage tissue engineering, hMSCs was seeded in scaffolds in chondrogenic differentiation medium for 21 days. SEM analysis, histological and immunostaining results demonstrated that the scaffold directed tissue organization and fibril matrix orientation in a zone-dependent way. Moreover, gene expression results revealed that chondrogenic markers including Sox9 and aggrecan were significant upregulated in the scaffolds compared to traditional

electropsun mesh. Overall, the research here provided a simple way to fabricate customized fibrous scaffolds for regenerative medicine, especially those for which anisotropy is of importance.

In chapters 7, themes are summarized and discussed in relation to the results presented in this thesis and a few possible ideas for future work. Finally, chapter 8 describe the valorization potential of the research presented in this thesis.