Towards the creation of an atlas of scaffold patterns

Summary

Every tissue in the human body is composed of a unique mixture of cells and extracellular matrix (ECM) components. A mixture, which is specifically arranged and is often related to the function of the tissue. The ultimate goal of tissue engineering is to replace damaged or diseases tissues with engineered biological analogues that can restore their original functionality until the tissue would be again fully recovered. Researchers are trying to imitate or recreate a tissue through a process defined as biomimicry or “the imitation of life and nature”. The term biomimicry itself is still rather vague as it is broadly used in tissue engineering and regenerative medicine, since it does not specify what exactly is being mimicked. Therefore, **Chapter 2** is focused on what biomimicry encompasses within the scope of regenerative medicine. This chapter first describes that biomimicry can be used in three different forms: mechanical biomimicry, morphological biomimicry and biological biomimicry. Using current biofabrication techniques, this chapter explores how each form of biomimicry is achieved within regenerative medicine. One of these commonly used biofabrication techniques is fused deposition modeling (FDM). This technique is an extrusion-based technique that allows a material to be extruded through a nozzle in a molten form that solidifies directly after extrusion to form a filament. With FDM, any 3D design is possible to recreate, but only limited infill patterns can be generated with current software. This thesis aimed at investigating alternative patterns that can be fabricated through extrusion-based techniques, taking FDM as a testbed. These patterns were used to create scaffolds with enhanced biomimicry, which were further explored for different tissue engineering applications, namely vascular and skeletal regeneration. A methodology to generate patterns was established to create a foundation of patterns to investigate in **Chapter 3**. By expanding the available patterns to use, this chapter makes unconventional patterns more accessible and easier to understand. These patterns are based on various mathematical curves. The results revealed that the differences in Young’s

modulus between the patterns can have a factor of 100 using the same material. In addition, the study found that the mechanical behavior of the scaffolds was significantly changed when a different loading regime was applied. One of the patterns that was further investigated in **Chapter 4** is based on the hypotrochoidal curve. This curve mimics the organization of collagen type II bundles in articular cartilage. Here, we investigated the mechanical properties of the scaffolds designed with the hypotrochoidal pattern compared to a classical woodpile 0-90 scaffold design. We studied what was the influence of the scaffold architecture on collagen type II deposition when cultured in static and dynamic conditions. The research showed that the hypotrochoidal pattern had improved mechanical properties over the conventional 0-90 woodpile structure. In addition, it revealed an improved collagen type II deposition and reduced type X collagen when cultured in dynamic conditions, indicating that the hypotrochoidal pattern could be better used for cartilage regeneration.

An additional application investigated in this thesis was vascular regeneration. In spite of many efforts, scaffolds used for vascular regeneration still do not correspond to the vessel's biomechanics or its structural design. The reason for this is that there is a limitation in creating hollow tubular structures using traditional FDM platforms. This is caused by the lack of support during fabrication and the possibility to crash into previously deposited filaments. **Chapter 5** introduces a solution to this by replacing the flat collecting surface of the traditional FDM system and introducing a cylindrical 4^{th} axis, which is controllable. A variety of new patterns for tubular scaffolds could be created using this system for the regeneration of hollow tubular tissues such as blood vessels. This chapter showcases the technology and compares two different patterns, which cannot be created using a traditional FDM platform. The results showed that small variations within the patterns can be made to change the mechanical behavior. These pattern changes can be used to mimic the mechanical properties for a targeted tissue. Tissues such as large

diameter arteries have unique mechanical properties. The stress-strain curve of a carotid artery can be described with a sigmoidal shape when tensile forces are applied. **Chapter 6** is focused on using a corrugated pattern to mimic the stress-strain curve of large arteries. The corrugations within the pattern allowed the scaffold to extend during tensile testing before the stress increased mimicking the mechanical behavior of a large artery. This behavior could be controlled by varying the corrugation amount. It was found that the stress-strain curves and mechanical properties of the corrugated patterns were similar to those of carotid arteries. The downside of the patterns that were used is that the gaps between the fibers is too big to fully populate with cells and to maintain a barrier function. To solve this limitation, a technique that is further explored in **Chapter 7** is called melt spinning. With melt spinning a thin fiber is being drawn around a mandrel to create highly aligned fibers in the circumferential direction. The resulting fibers mimic the orientation of the tunica media of an artery. This study used melt spinning in combination with the four axis FDM described in **Chapter 5** to create a scaffold that had mechanical biomimicry from the large supporting fibers, but also morphological biomimicry from the melt spun fibers. The results showed that the process of melt spinning was reliable enough, hence could be described with equations predicting the fiber outcome. The process could be controlled to such an extent that gaps just small enough for cells to pass through could be created facilitating cells growth while still maintaining a barrier function when seeded with cells. The co-culture with endothelial and smooth muscle cells revealed that the endothelial cells had a cobblestone morphology forming a barrier in the luminal side of the scaffold and the smooth muscle cells populated directly underneath between the melt spun fibers.

In **Chapter 8**, all the above results are discussed and placed into a state-of-the-art context, in addition to providing future perspectives. In **Chapter 9**, we conclude by reviewing the scientific and societal impact of the research presented in this thesis.