Fantastic prints and where to find them

Chapter 7
IMPACT

7.1 SCIENTIFIC IMPACT

Due to the constant subjection to loading and the related injury risk, bone was one of the first tissues to be studied in the context of Tissue Engineering (TE). However, despite the large amount of available research and the undeniable scientific progress along the years, tissue engineers are still searching for a highly reproducible and upscalable solution to promote and speed up bone regeneration *in vivo*. The current lack of a golden standard is due to the complex interplay among bone properties such as composition, structure, and mechanics. In the classical paradigm of TE, scaffolds have been used to mimic these features to provide a temporary tissue replacement in terms of mechanical support, porosity and surface properties, able as much as possible to carry out the same biological functions while the surrounding native bone regenerates. Additive Manufacturing (AM) is a class of techniques that allow the production of constructs with personalized size and shape, and that give a certain control on the construct's mechanical properties by adjusting the disposition pattern and the thermal management. Being first born for industrial and technical applications, AM has been employed for TE purposes because it offers that degree of control over the fabricated object required in the context of personalized medicine. More than in other tissues, the mechanical properties of bone are highly linked to its structure, as in the case of cancellous regions where the tissue is organized to dissipate stresses and propagate the residuals towards the stiffer cortical tissue. Therefore, it is not surprising how widely AM techniques have been adopted for the regeneration of such mechanically-complex tissues. However, despite the large technological advancements since the adoption of AM in TE, production is still far from being optimal and standardized, a fact that is highly hindering the employment of such class of technique on a larger scale.

Within this thesis, innovative scientific knowledge was generated and novel strategies based on it were proposed, with the goal of providing solutions to current issues in melt-extrusion AM (ME-AM) for bone tissue engineering. We believe that the information presented, consisting of a numerical model of the scaffold fabrication process, new methods to induce material bioactivity or to introduce roughness and microporosity with no chemical processing, and on an insight of thermal history effects, contributes to a better understanding of the scaffold manufacturing process and to the optimization thereof, providing a steppingstone for future studies on the upscaling of ME-AM for bone tissue engineering. We also trust that this research will have a scientific impact beyond tissue engineering for bone regeneration and can be the foundation of future research in the biomedical, biomaterial and overall regenerative medicine fields.

Within the first chapters, we found necessary to provide a deep physical description of the scaffold manufacturing process via ME. On one side, in **Chapter 3** we proposed a simple numerical model based on fluid-dynamics that could be easily used by other researchers to estimate the final morphology of the manufactured scaffolds, for different applications. We believe in the great scientific impact of this model, since it helps minimizing costly material and time waste for material processing screening and optimization, and allows to maximize throughput and scaffold manufacturing rate, which currently are still at research level. By having a support tool to tackle the initial optimal fabrication issue, researchers not only are free to explore with higher flexibility different scaffold designs or materials, but can also dedicate greater efforts to evaluate the final biological response. In **Chapter 3**, we went one step further and, while modelling the impact of processing conditions on scaffold morphology, we also implemented tools to predict the compatibility of a specific couple material/printer and to estimate in advance the scaffold mechanical response. The completeness of our model lays the foundation for future additions by describing further phenomena involved in the scaffold manufacturing process, such as crystallinity and molecular dynamics. Furthermore, since established and optimized protocols are a prerequisite for upscaling of the scaffold fabrication process, we envision the implementation of our model into a software embedded in ME-AM printers in the scaffold production pipeline, allowing the manufacturing of standardized constructs and thus a faster translation to the clinic.

In subsequent chapters, the scaffold manufacturing process via ME-AM was optimized to enhance scaffold performance in biological environments, but also further studied to investigate its intrinsic impact on material properties and consequently on cell response. Complex methodologies from several scientific fields were employed, from material science and rheology to processing engineering and biology, to design, develop and optimize strategies to overcome the current biological limitations of ME-AM for TE applications, such as the lack of bioactivity and biomimicry, and gain further insights on the impact of processing on cell activity. In particular, **Chapter 4** showed that plasticizers, a typically industrial solution to improve the processability of a material, can be applied in ME-AM for TE applications to widen the palette of selectable materials, thus allowing to turn the attention to those high molar mass thermoplastic polymers with favorable mechanical properties that cannot be usually processed with research-scale equipment. In addition to being (necessarily) biocompatible, they can be accurately chosen to carry out a double function by additionally having a bioactive effect, such as inducing osteogenic differentiation in human mesenchymal stromal cells (hMSCs). This allowed to overcome the inherent bioinertness of thermoplastic polymers for ME-AM for TE. However, non-physiological workarounds to achieve cell attachment were necessary, thus raising questions related to manufacturing scaffolds with more biomimicking surfaces. For this reason, we believe that our work in **Chapter 5** on inducing roughness on the otherwise not-biologically-alike smooth surfaces of melt-extruded scaffolds constitutes a solid contribution to the field of scaffold fabrication for TE. In fact, the presented scaffold manufacturing strategy allowed to achieve a higher cell seeding efficiency without any media modification or scaffold handling during cell attachment, by creating a more physiological surface morphology. Moreover, it had the further effect of introducing microporosity within the filaments structuring the scaffolds, providing them with a more physiological ductile behavior under loading, thus further improving scaffolds biomimicry. Finally, we believe that the study presented in **Chapter 6** provided, for the first time, meaningful knowledge on the inherent effects of typical ME-AM thermal stresses on those properties of semicrystalline thermoplastics that are known to influence cell activity, namely surface stiffness and surface roughness. In a field where researchers use chemical, morphological and mechanical cues to drive cell fate, we trust that the results of our investigation can generate great awareness about the importance of proper thermal history management, so that to avoid any undesired properties modification and consequent cell response. In addition, we are confident that the provided knowledge serves as a basis for future research to manufacture scaffolds with controlled gradients in surface properties, so that to fabricate suitable implants for those interface regions such as the osteochondral tissue, which currently are not properly mimicked.

7.2 SOCIAL IMPACT

Bone is one of the most exposed tissues to injuries because of its constant engagement and loading during daily life, being it involved in the support and motion functions of the body. Bone can undergo further stresses in the case of sport activities or because of the onset of osteoporosis, a disease mostly related to ageing that causes weakening of the tissue. Whereas loads exceed the bone bearing capabilities, fractures may occur. Although these are usually properly treated, prolonged healing or non-unions may take place in the case of patients suffering from diabetes, obesity, genetic conditions or osteoporosis too. In addition, removal of bone tumors can leave large defects in bone tissue, which may be beyond the bone's own self-healing ability. It is estimated that the risk of developing a non-union reaches up to 5% worldwide, with higher peaks in country with less advanced medical care [1]. The highest location incidence is given by the tibial bone with up to 15% of the occurring fractures, followed by the femur with 11%. These percentages further increase when focusing on elderlies, which are becoming a more important segment of the population in developed countries, due to higher life standards and better medical care. Besides involving a long medical recovery path, non-unions are often accompanied by pain and can cause functional and psychosocial disability [2], [3]. The costs associated with non-unions can reach up to 100k € depending on the case, most of which often derive from collateral issues, such as inability to start working soon.

A current widespread clinical practice for bone repair is based on natural grafts, which are tissue portions harvested from a donor and implanted into the defect to be treated. Donors can be either the patient himself, another person entirely or a cadaver. In the first two cases, grafts come from specific healthy areas where the harvesting procedure will not affect the functionality of the area itself. Instead, synthetic grafts make use of ceramic materials, collagen-ceramic composites and polymers as source materials to create bone substitutes. As these materials are indeed synthetic, the use of such grafts does not require a donor, which is the reason why this approach has been recently gaining great interest, resulting in predictions of strong market growth over the coming years. However, the manufacturing of synthetic implants with adequate mechanical properties, in particular for long bones repair, still represents an issue and an open quest.

Implants fabricated by AM could be of great benefit for the treatment of bone defects, with particular focus on non-unions in long bones. The inherent adavnatge of this technology is the morphological freedom, which allows to manufacture scaffolds that perfectly fit a specific defect simply by using from CT or MRI images of the site anatomy as models. In addition, mechanical properties can be tailored by optimizing the manufacting pattern and, thus, pore size and distribution. Porosity, together with the specific chemistry of the material of choice, allows to tune another key property for defect treatment such as resorption rate. Whereas scaffolds are degraded by the body at a pace matching the formation of new bone, implant stability is maintained all along defect recovery and revision surgeries can be avoided. Despite the high degree of freedom and tunability offered by AM, it seems that the application of additively-manufactured scaffold in the clinics is still quite limited and that most of the work is still being done at research level. Mainly emerging small companies have started exploring the sector by commercializing products fabricated via AM. For example, *Xilloc* has been manufacturing custom-made craniomaxilofacial implants in titanium or polyetheretherketone (PEEK) for over 10 years within Maastricht University Medical Centre (MUMC) and has recently started providing their solutions to surgeons and patients outside MUMC. However, their two main materials of choice are non-resorbable, which means that a perfect match of timed mechanical properties cannot be achieved. *Osteopore International* is the only company to our knowledge that has managed to enter the market with polymeric scaffolds fabricated via ME-AM for bone defects. Recently, a poly(caprolactone) (PCL) scaffold from *Osteopore* was successfully implanted to restore a tibial non-union at MUMC, in a surgery that drew the media's attention [4]. The uncountable explorative and optimization studies available in literature on ME-AM of 3D polymeric scaffolds for bone regeneration provide a large pool of data to draw from to start evaluating the most feasible solution in the clinical activity. We believe that the research presented in this thesis will have an impact in guiding the future research on such products, as we propose strategies to optimize their manufacturing and enhancing their biofunctionality, with the goals of improving the surgical outcome, of shortening healing times and reducing the burden for patients and society. The combination of the knowledge presented in the single chapters of this thesis into a single product can be seen as a reasonable next step to research and translate into clinic. Such scaffold would: i) be manufactured following an optimal procedure, which would take care of the morphological accuracy as well as of the mechanical properties, ii) induce greater cell attachment and have more ductile behavior under compression, iii) have osteogenic effect, iv) present a gradient in stiffness to properly mimic the osteochondral region. Of course, as previously mentioned, further optimization would be required for each of the strategies and solutions presented along this thesis, before obtaining a "ready-to-use" scaffold. Yet, we believe we traced the road towards more morphologically and mechanically optimal, cheaper, more biomimicking and bioactive products for bone non-union treatment compared to the current state-of-the-art.

References

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