Tracing fast roads towards bone regeneration

Chapter 9

Impact

Scientific impact

Although bone has been studied since the birth of the tissue engineering (TE) field, scientists are still searching for the optimal strategy for its regeneration (**Figure 1A**). Bone is a complex tissue, in terms of mechanical properties, structure, and composition, and many molecular factors and cell types are involved in its formation and regeneration. For this reason, clinical problems, such as non-union fractures or craniofacial reconstructions, are also complex to approach, and not easy to resolve. Among the three components of the classical tissue engineering paradigm (cells, scaffolds and growth factors), scaffolds have been the main focus of study in the bone tissue engineering (BTE) field. Besides providing a bioactive environment for new tissue formation, scaffolds in bone regeneration have the additional significant role of providing mechanical support, mimicking the high strength of the native extracellular matrix. Aiming towards size and shape personalized structures with high mechanical properties, but at the same time porous for cell ingrowth, additive manufacturing (AM) has stood out within the last 15 years as one of the preferred biofabrication technologies for the fabrication of scaffolds for BTE applications. Over the years, large technological advancements have been made around AM to facilitate an efficient scaffold design, i.e. multiprinthead melt extrusion AM (ME-AM) platforms have been developed for the fabrication of multimaterial scaffolds, sophisticated light-based or bioprinting AM platforms have emerged, new printable polymer materials or bioinks have been designed. Yet, an AM scaffold with the optimal set of properties for efficient bone regeneration has not been fully attained, suggesting that further investigations and advancements are still required.

Within this thesis, courageous choices were made to generate large scientific and technological strides providing solutions to existing issues within the field. We believe that the diverse strategies presented, consisting on optimized or new composite materials, new surface modification techniques, and novel cell seeding methodologies, contribute to the better understanding of AM scaffolds and their use for bone regeneration applications and provides a stepping stone for future studies on the application of functional composites in BTE. We also consider that this research has a scientific impact beyond the BTE field and can inspire future research, in the biomedical, biomaterial and overall tissue engineering scientific community.

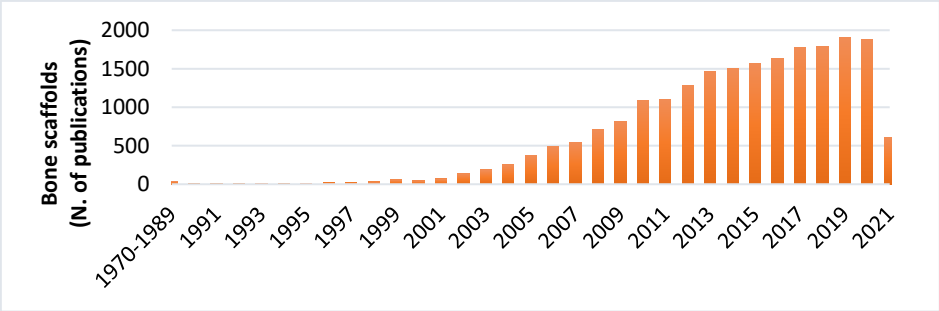

Figure 1. Scientific publications on new bone scaffolds since 1970. Pubmed search, April 2021.

Within the first chapters, we found extremely relevant to revisit the recurrent cell seeding efficiency problem on macroporous AM scaffolds. On one hand, in **Chapter 3** we proposed a simple, reproducible and universal cell seeding approach that could be easily implemented by other research groups working with 3D AM for different applications. We are certain of the high scientific impact of this method, since it helps minimizing the required cell stock for *in vitro* experimentation and maximize cell seeding efficiency on scaffolds fabricated with any existing or newly developed synthetic material. It encourages researchers to redirect their efforts towards investigating more relevant issues, once the basic cell seeding problem is tackled. In **Chapter 3,** we went one step further and, while addressing the cell seeding issue, we explored a portion of the possibilities that a hybrid platform consisting of a melt extrusion printhead and plasma jet could bring to the design of surface-functionalized scaffolds. Our results open the door to future research on the effect of different plasma treatments on cell behavior on 3D ME-AM scaffolds. Furthermore, since this technique allows for a single-step workflow of scaffold production and surface homogeneous treatment, we envision its implementation within the scaffold fabrication pipeline of many laboratories in the future, as well as its impact in a smoother translation of ME-AM scaffolds into the clinic.

In subsequent chapters, several optimized or novel composite materials were investigated and complex methodologies from several scientific fields, transiting from material engineering, chemistry, and biology were used to analyze their suitability for BTE applications and their impact in the field. In particular, **Chapter 4** showed that blending high concentrations of nanohydroxyapatite within a polymer, as a common focus of the community trying to mimic the bone composition, does not necessarily result in a highly osteoinductive material. Thus, here we raised important questions to the scientific community related to the appropriate calcium phosphate material and their incorporation

method in ME-AM composite scaffolds for the stimulation of bone regeneration. On the other hand, the incorporation of graphene based materials into ME-AM scaffolds is not yet a well-stablished line of research in the field. For this reason, we believe that our study in **Chapter 5** about the effect of rGO density and concentration on composite scaffolds production and cell-material interaction constitutes a stepping stone for scientists entering this field in the future. Finally, we believe that the approach presented in **Chapter 6** to fabricate antibiotic-releasing AM scaffolds could open the door to future research on the use of other relevant inorganic nanofillers, antibiotics and biodegradable polymers to further optimize the system towards clinical applications.

Social impact

Bone is one of the most commonly injured organs in our body. Bone fractures can result from trauma, such as car accidents, or sport injuries, as well as from osteoporosis, a disease that weakens the bones and increases the risk of fragility fractures. Inappropriate initial treatments and co-morbidities, such as diabetes, genetic factors and poor life style, can increase the risk of delayed bone healing or non-unions. Resection of bone tumors, infections or prosthetic revisions in long bones can also result in non-unions. Around 4 million fractures are estimated to occur in Europe annually, from which approximately half a million require hospitalization and bone repair intervention. [1] Non-unions, which occur in 5-10% of the cases, are the most difficult to treat and have a high financial impact, with a total cost of € 8,000 to € 91,000 per case. [2-3] In European healthcare systems, around 82.8–93.3% of this cost has been estimated to derive from indirect costs, such as productivity losses, due to long healing times leading to patients being unable to resume daily life activities and work within a short time. [4] The remaining expenditures derive from care and intervention costs.

Current available clinical solutions for bone repair are based on natural and synthetic bone grafts. The natural grafts are divided into autografts and allografts, the latter ones including demineralized bone matrices (DBMs). The synthetic materials are based on ceramic, collagen-ceramic composites, polymers and bone morphogenetic proteins. In 2020, the global bone graft market size, based on allografts and synthetic substitutes, was estimated at € 2.36 billion (Grand View Research, market analysis report 2021, ID: GVR-1-68038-154-2). Due to the aging population and the increased incidence of osteoporosis and bone fractures, this market is expected to grow at an annual rate of 5.8% to reach € 3.7 billion

by 2028. Although the market is currently dominated by allograft materials (~ 60% share in 2020, mainly DBMs), the synthetic segment is anticipated to show significant growth over the years, due to the investment in product development, arise of new technologies and acceptance growth among patients. Bone grafts and substitutes are mostly used for spinal fusion, dental, and joint cemented prosthesis applications, while only 5% of the products are dedicated to long bone repair. This is partly because current marketed solutions, such as DBMs, or resorbable calcium phosphate cements have been shown to be inefficient in managing non-union scenarios in long bones, mainly due to their poor mechanical properties, unable to withstand the load required in these locations. Similar limitations are offered by collagen sponges infused with bone morphogenetic proteins (BMPs), which additionally have shown to induce undesired uncontrolled ectopic bone formation in clinical trials.

Here, AM scaffolds could bring many benefits for non-union defects. Such scaffolds can be fabricated to fit the specific patient complex defect using CT scan images of the patient's anatomy. This avoids the need of manipulation or modification of the scaffold intraoperatively, reducing the surgery time and ensuring good implant fixation within the defect. Moreover, AM scaffolds can be fabricated with specific structural parameters and mechanical properties from polymeric biodegradable materials or composites, to allow new bone formation and avoid a revision surgery. Despite their advantages, the implementation of biodegradable AM scaffolds in the clinics is still slow, as it is a rather new field and new medical devices require long regulatory paths, which subsequently leads to the current absence of companies marketing such products. Accordingly, many key players in the business of bone grafts, such as *DePuy Synthes, Medtronic, Wright Medical, AlloSource, Stryker, Johnson and Johnson and Zimmer Biomet*, only offer products based on DBM, ceramics or BMPs, and so far do not possess 3D AM scaffolds within their product portfolio. On the other hand, emerging small companies, such as *Mimetis* and *Cerhum*, have entered the sector by jumping in the 3D printing wave, yet developing AM ceramic scaffolds based on tricalcium phosphate (TCP) and hydroxyapatite (HA), whose performance in load bearing scenarios is arguable and still to be proven. Up to now, only one company (*Osteopore International*) has been able to commercialize 3D ME-AM polymeric scaffolds, for craniomaxilofacial defects, and currently is performing first-in-human trials for their future application in long bone non-unions. *Osteopore*'s poly(caprolactone) (PCL) scaffold was recently implanted in a patient with a non-union tibial defect at Maastricht University Hospital. The success of this clinical case, and many

others to follow, will certainly open in the future the clinics' door to other novel ME-AM scaffolds. We believe that the research presented within this thesis will have a direct impact in shaping the future of such novel products, as we propose several strategies to improving their functionality, which could further accelerate bone healing times and reduce the socioeconomic burden associated with non-unions. Joining the success of the aforementioned basic polymeric ME-AM scaffolds with the extensive road of ceramics in the clinics, the clinical success of the PEOT/PBT-nHA scaffolds presented in **Chapter 5** can be seen as a natural next step to come. Such scaffolds would combine i) the bioactivity and osteoconductivity provided by the ceramic component, ii) the mechanical support brought by the polymer, the reinforcing ceramic, and the scaffold structure itself, and iii) the concept of personalized medicine. Overall, a blend of properties that current commercial products lack. Although not directly investigated within this thesis, the further addition to these scaffolds of the antibiotic loaded lamellar fillers presented in **Chapter 7**, would certainly further boost their functionality and transform them into a very unique product. As mentioned in previous chapters, further optimization would be required before obtaining a "ready to use" scaffold, but we believe that we are on a good track, which will surely guide us to implement a novel, more reliable, probably cheaper and, importantly, functional therapy for bone non-union regeneration, compared to current gold standards.

References

1. Hernlund, E.; Svedbom, A.; Ivergård, M.; Compston, J.; Cooper, C.; Stenmark, J.; McCloskey, E. V.; Jönsson, B.; Kanis, J. A., Osteoporosis in the European Union: Medical Management, Epidemiology and Economic Burden. A Report Prepared in Collaboration with the International Osteoporosis Foundation (Iof) and the European Federation of Pharmaceutical Industry Associations (Efpia). *Arch Osteoporos* **2013,** *8* (1-2), 136.

2. Nandra, R.; Grover, L.; Porter, K., Fracture Non-Union Epidemiology and Treatment. *Trauma* **2016,** *18* (1), 3-11.

3. Mills, L. A.; Aitken, S. A.; Simpson, A., The Risk of Non-Union Per Fracture: Current Myths and Revised Figures from a Population of over 4 Million Adults. *Acta orthopaedica* **2017,** *88* (4), 434-439.

4. Hak, D. J.; Fitzpatrick, D.; Bishop, J. A.; Marsh, J. L.; Tilp, S.; Schnettler, R.; Simpson, H.; Alt, V., Delayed Union and Nonunions: Epidemiology, Clinical Issues, and Financial Aspects. *Injury* **20**