Engineering micro for repairing macro

Impact paragraph

Currently, in the USA and Europe alone, each year, over half a million patients require a bone repair surgery for which a bone graft (substitute) is needed.[1, 2] Examples of this clinical need include spinal fusion, and treatment of bone defects caused by a trauma or tumor removal. The use of a patient's own tissue removed from elsewhere in the body (autologous bone) is the most effective treatment for challenging bone defects. Nevertheless, important disadvantages are associated with this treatment, including limited availability and complications related to harvesting of bone tissue such as long-term pain. Bone tissue obtained from other sources, like deceased humans or animals carry the risk of triggering an immune reaction and disease transmission. Synthetic bone graft substitutes, i.e., materials that can be synthesized without the involvement of a natural source, can solve the supply problem, and are not associated with an immune reaction. In particular, calcium phosphate (CaP) ceramics have been used in orthopedic, dental and craniomaxillofacial applications for a long time, owing to their similarity to the mineral found in bones. Therefore, CaPs are biocompatible, meaning that they are well accepted by the body without adverse reactions. While CaP bone graft substitutes can be produced in large quantities, and are relatively inexpensive, their bone regenerative performance varies and they are in general considered inferior to natural bone grafts. The bioactivity of CaPs, in terms of their ability to regenerate challenging bone defects is very much dependent on their properties: exact chemical composition (phase), as well as structural and mechanical properties. These properties are, however, not independent; for example, changing the chemical composition can cause a change in the microstructure, that in turn may affect the mechanical properties. In order to improve the performance of CaP bone graft substitutes, it is important to understand the role of individual properties in the biological response to a biomaterial, and for this, we need tools that are helpful in decoupling the normally intertwined properties.

This is the challenge we tried to address in this thesis: finding the ways to decouple the role of individual or combinations of material properties in how cells or tissues respond to a material, and using this knowledge to develop improved biomaterials. We first performed a literature study to collect information about the strategies that were already developed by other researchers in the past, analyzed the pros and cons of each of them, and developed ideas for our own research. Second, we applied microfabrication technologies to transfer the microstructure of a CaP bone graft substitute into polymer and polymer/ceramic composite materials, to decouple the effects chemical composition and microstructure on the differentiation of bone marrow-derived mesenchymal stromal cells towards the bone lineage. The results

showed that both chemistry and microstructure, and specially the combination of both, drive the cells towards osteogenic differentiation. The impact of this study was twofold: the technique we used can be applied to other biomaterials, and the knowledge generated can inform the design of new bone graft substitutes.

In the second study, we pursued a different approach, giving emphasis to an as comprehensive as possible characterization of biomaterials with known performance in the body. For this, we used six CaPs to obtain close to a hundred numerical parameter describing each of them, including the chemical composition, degradation behavior, and microstructure. We then employed bioinformatics to correlate this dataset with another set of data describing the transcriptomic profile of osteoblasts cultured on these six ceramics. The results of the correlation allowed us to make suggestions about the role of the individual material properties on the activation of different genes. While this study had a proof-of-concept character, it is impactful as it offers tools to better understand the property-function relationships of other CaP bone graft substitutes, and even completely different biomaterials, intended for the regeneration of other parts of the body. Furthermore, given that transcriptomics analyses can already be performed in a high-throughput way, and that some material characterization techniques are also high-throughput,[3] there is room to develop (automated) massive screening of biomaterials and their interactions with biological systems.

While the knowledge of how individual properties of CaPs influence cell and tissue behaviour is essential for the design of highly functional synthetic bone graft substitutes, developing synthesis and production methods that provide close control over these properties is also required. To contribute to the efforts in this direction, in our research, we used microfluidics, i.e. the technology for precise manipulation of very small amounts of fluids using microfabricated devices (known as chips) with micrometer-size features. We fabricated a microfluidic device capable of producing transparent droplets with a diameter in the range of tens to hundreds of micrometers, which served as miniature reactors for production of CaP microparticles with well-defined properties, dependent on the precursors that were introduced into the droplets. Cell culture experiments showed that the materials produced using this method were biocompatible and stimulated the osteogenic differentiation. Although we focused on the production of CaPs, droplet microfluidics is a highly versatile technique to rapidly produce libraries of different types of materials, which then can be screened to identify promising candidates at the early stages of development.

Finally, we also used microfluidics to create a bone-on-a-chip model. In conventional *in vitro* experiments, cells are cultured on flat, stiff cell culture plates,

thus in an environment that is far from the physiological microenvironment of a cell. This is often a reason why the findings from the *in vitro* experiments are not comparable or predictive for the *in vivo* situation. To bridge this gap, and potentially reduce the need for animal experiments, we combined the techniques applied in the rest of the thesis to create a microenvironment resembling that of bone in terms of microstructure, chemistry, and flow of nutrients and studied the behavior of human mesenchymal stromal cells inside this microenvironment. The potential value of such a model has been demonstrated before, in another bone-on-a-chip device that was used to study the effects of a radiation countermeasure drug used during bone cancer treatment, showing different results from those obtained with conventional *in vitro* cell culture methods.[4] Therefore, biomimetic organ-on-chip models, including those of bone can have an important scientific, as well as clinical and societal impact, as the advantages of miniaturization (experiments cost reduction) and parallelization (increase of screening throughput) may accelerate and personalize the therapy development, making it affordable even for rare diseases.[5, 6]

In summary, this thesis contributed to the insights into the mechanisms of bioactivity of CaPs and the role of individual material properties in this mechanism, which can be used as input for developing new, improved synthetic bone graft substitutes. Moreover, the thesis delivered some practical tools to produce CaP-based materials in a controlled manner.

In the context of an ageing population, the number of surgical procedures to treat bone defects is expected to continue increasing, leading to an expected annual growth of 5-8% of a market that is currently around 3 billion US dollars.[7, 8] This places an enormous burden on the healthcare systems, once again stressing the importance of effective and affordable bone graft substitutes such as CaP-based ones.

References

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