Bone regeneration

Valorization addendum

The knowledge generated in this thesis has great potential to be utilized for both social and economic purposes. Any disease or health problem has a dramatic impact on the well-being of people, and bone diseases or conditions are no exception. Problems such as lower back pain and non-union are one of the leading causes of years lived with disability in many countries worldwide. One of the goals of this thesis was to generate knowledge that could eventually be applied to treat these patients and thereby improve their life quality.

As we are approaching the 2020s, the science of bone regeneration is facing a big challenge: the lack of novelty in the approaches taken and therapies available. Clinicians are stuck with a century-old method, the autologous bone graft. Or they may elect take a risk by using bone morphogenetic protein-2 (BMP-2) or unproven methods (e.g., mesenchymal stem cell therapies). And certainly many will be confused by what appears to be a wide selection of bone graft substitutes that are essentially the same (namely a combination of either allogenic bone, type I collagen and/or calcium phosphate). There is a great need for some breakthroughs in the science and clinical application of bone regeneration.

Throughout this thesis, we have explored several different approaches to look for a solution for the regeneration of bone. It is noteworthy that we used the cell line ATDC5 three times (in chapters 2, 3 and 4). There is a good reason for this. ATDC5 is a mouse cell line with the intrinsic potential to become hypertrophic chondrocytes. The collagen matrix of hypertrophic chondrocytes is rich in type X collagen, which is a natural template for bone formation in the body during growth and fracture healing.

Type X collagen is very rare in nature, and currently only available for research from sources such as placenta tissue, at very high price. It is why type X collagen, despite its relevance to bone formation, has not been used for bone regeneration. There is no clinical product on the market using type X collagen at the moment. Instead, type I collagen is used, isolated from sources such as bovine skin, which may not the best type of material for bone formation. Thus, there is a big opportunity for a product comprising type X collagen for bone regeneration.

In chapter 2, we did a high throughput screen to search for a drug that can stimulate the cell ATDC5 to produce more collagen matrix. We found and successfully validated a compound called tetradecylthioacetic acid (TTA). There are two possibilities to use the knowledge from this chapter. First, using TTA, we can readily optimize a culture protocol to stimulate ATDC5 cells to increase their collagen production, in particular type X collagen, which is discussed further below. Second, the method of using a collagen-binding fluorescent probe for high throughput screening can be adapted to screen on other cell types. For example, using it with skin progenitor cells could find a molecule that promotes the formation of a collagen matrix suitable for skin regeneration.

In chapter 3, we put our effort into upscaling the culture of ATDC5 cells *in vitro*. The most common method to culture these cells is using the surface of the plastic vessels (such as 300 cm^2 flasks), which is a 2D culture system. While these are convenient, ATDC5 thrive best in a 3D environment, where the cells are always in contact to each other. The work in chapter 3 described an upscaling culture method in which ATDC5 cells are grown as small aggregates of 1000 cells, formed in a mold containing thousands of micro-wells. We called these aggregates: micro-tissue-engineering cartilage (MiTEC). We demonstrated that MiTEC grew much better and produced collagen matrix in high quantity and quality. This method of micro-aggregate cell culturing is suitable to upscale to a much bigger scale using bioreactor vessels, and potentially producing large quantities of hypertrophic matrix biomass rich in type X collagen.

Next, in chapter 4, we did some basic research to prove the concept that surface topography of a material can be as potent as growth factors for directing cell fate. All materials implanted in the body are in contact with cells, and how cells respond to the materials influences the survival of the implants. In bone regeneration, surgeons have long realized that materials with rough surfaces are superior to those with smooth finishes for bone integration. But the mechanism is far more complicated than that, and we are still learning every day.

In order for this knowledge from chapter 4 to be applicable to life, more research needs to be done. However, as evidence builds for the mechanisms and reproducibility, the science of surface topography can be widely applied in almost all aspects of medicine. One can foresee its use in replacing or augmenting growth factor therapy and it can be used *in vitro* (e.g., in manufacturing of type X collagen matrix) or in the clinic (e.g., to modify surface topography of dental implants).

In chapter 5, we attempted to devise a reporter cell line for the gene BMP-2. There has not been a cell line that can faithfully produce a fluorescent signal concomitant to the change of BMP-2 expression. Such a reporter cell line has tremendous use for scientists, who need to understand the role of BMP-2, a universal body growth factor, in organogenesis and the natural healing response. A BMP-2 reporter cell line could also be used for essentially any kind of high throughput screening such as drug screening or material screening, because of its convenient reporting properties. However, generating such a cell line is a difficult task, and we have complete perhaps 50% of the job. What is left to be done is the optimization of the genome editing tools, such as CRISPR, to efficiently induce a double-strand break at the desired location.

Now to elaborate on the potential of chapters 2–4 and the use of ATDC5 cells to produce collagenous matrix rich in type X collagen (hypertrophic cartilage matrix).

Type X collagen has a potential to revolutionize bone regeneration, but just getting enough type X collagen for a single experiment is already a challenge. Take one clinical example, treating severe degenerative intervertebral disc diseases with spinal fusion. The aim of this procedure is to stimulate bone fusion between two or more vertebrae in the affected region. Currently, recombinant human BMP-2 delivered in a type I collagen sponge, is recommended to use for this procedure by the US FDA. To guarantee a successful outcome, a very large dose of BMP-2 is often used by surgeons, which results in not only adverse effects but also an

expensive therapy. The fact is that surgeons depend solely on BMP-2 (or BMP-7) to regenerate bone, while the type I collagen sponge is simply a carrier.

But type I collagen alone is often used as a negative control in animal models of bone regeneration. Why is it that a material with no intrinsic bone forming capacity is used so often? Only because of its availability. Bovine or porcine type I collagen can be easily harvested from skin. If type X collagen could be available in only one tenth of the quantity of type I collagen, it would have a very good chance of competing in the market.

We all know that Maastricht produced the first million-dollar burger, made from bovine muscle cultured in the lab, and several companies followed to bring the cost down. If cells can be cultured for food at this high cost, there is absolute no reason why cells cannot be cultured for medical needs at similar cost. Type X collagen matrix has a great potential for bone regeneration, which has been shown in a rich body of literature, but steps need to be taken to bring a product into a clinical trial.

The work in this thesis has consistently suggested the use of the ATDC5 cells in producing such a collagen matrix. We have shown the feasibility to grow MiTEC in the lab, and have shown the ability to produce 1CC of decellularized matrix from four 12-well plates. The next step is to produce 100CC of matrix, using similar plates. For reference, a single interbody spinal fusion case needs 2-10CC of collagen matrix. This amount would be sufficient to bring into an animal trial.

Next, engineers need to be involved to up-scale the culture of MiTEC in larger vessels. MiTEC can be cultured in suspension, thus eliminating the need for surfaces. Engineers should be able to make bioreactors in the size of 10, 50, 100 and up to 1000 L, with sensors to control temperature, pH, and chemical levels. Every batch of ATDC5 culture can take up to 14 days to produce.

ATDC5 matrix can also be used directly as a decellularized matrix, as demonstrated in chapter 3, or can be used as a raw material for the isolation type X collagen. The benefit of further purifying the matrix is to ensure a medical grade product can be obtained through stringent manufacturing processes. Medical grade type X collagen can be applied in all bone site injuries (where type I collagen is currently used), from fracture healing, spinal fusion to filling of dental socket. Without a doubt, a type X collagen sponge will offer another interesting approach for future bone regeneration approaches.

Overall, this thesis has tapped into several approaches that can be useful for the research and development of products for bone healing and regeneration. Of which, the knowledge from chapter 2, 3 and 4 can be most applicable for the development of a type X collagen matrix from cultured ATDC5 cells.