Introducing height to mechanobiology

Chapter 10

Valorization

Jip Zonderland

Should a single PhD thesis always have direct tangible benefits to society?

Complicated, fundamental biological research on its own cannot be directly valorized and translated to societal gains. This does of course not mean the research is not valuable to society in the long term. Fundamental research leading to the discovery of a protein in jellyfish that emits green light under UV light might have been hard to valorize when initially discovered in 1962. Years later, in 1994, this green fluorescent protein (GFP) was used to tag individual cells and proteins and revolutionized almost every field of biological research, resulting in the Nobel prize in 2008[1]. This is a classic among many examples, which question the need to immediately valorize fundamental research. The requirement for a direct societal and functional output of research seems productive, but, as argued for in this thesis, could actually prove to be counterproductive if this steers research away from much-needed fundamental research. A fundamental understanding of biological processes is required to design smart scaffolds for tissue engineering. From the build-up of such knowledge to results from practical use will exceed the length of a single PhD thesis, but could benefit society greatly in the long term.

Besides the fundamental research done in this thesis, chapter 8 describes the hydrocup, which could have direct practical applications. The hydrocup can be used to fix cell-laden or drug releasing hydrogels in place *in vivo* and the opportunities to commercialize these scaffolds are explored below.

Clinical relevance

Cytokines released by human mesenchymal stromal cells (hMSCs) have beneficial effects, including angiogenesis, immunomodulation, supporting tissue regeneration by local stem cells, and anti-scarring[2-4]. For this reason, MSCs are currently being widely used in clinical trials as 'secretion factories' in a wide variety of diseases[5-7]. The vast amount of ongoing clinical trials can be visualized by searching "mesenchymal stem cells" or "mesenchymal stromal cells" on clinicaltrials.gov, together resulting in over 1200 ongoing or completed clinical trials (January 2020). Many clinical trials inject MSCs, either intra-venously or directly in the tissue. However, these injected cells are difficult to hold in a specific place. On top of this, cells often die shortly after implantation by injection[8-10]. If hMSCs are to be used as secretion factories of biological factors to aid local regeneration of a specific tissue or immunomodulation, hMSCs should be maintained alive and in a specific location. For this purpose, we developed the hydrocup, a hollow electrospun (ESP) scaffold to deliver cells *in vivo*.

The hydrocup

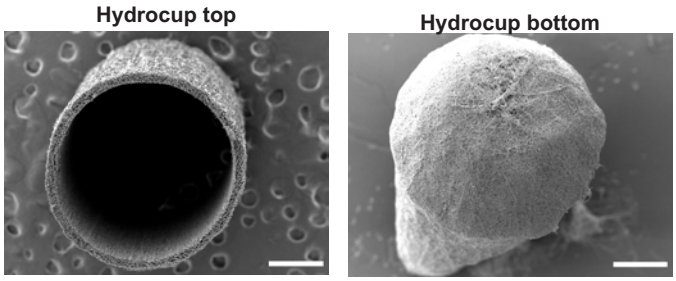

Figure 1. Scanning electron microscopy images of the hydrocup. The open end through which the hydrogel can be loaded into the hydrocup and the closed bottom that keeps the hydrogel in the hydrocup when being loaded are displayed. Scale bars are 500 µm.

The hydrocup was created by electrospinning a polymer solution on the open end of a rotating mandrel. This creates a hollow ESP scaffold with one open and one closed end (Figure 1). A hydrogel can be pipetted through the open end while the closed bottom end prevents the uncrosslinked hydrogel from leaking out. The hydrogel can then be crosslinked inside the cup and the top end is closed with sutures. The ESP scaffold wall is highly porous and, therefore, allows for cells to secrete factors out of the hydrocup, and for nutrients to diffuse in to maintain cells alive in the hydrocup. In chapter 8, we demonstrate that hMSCs release functional factors from the hydrocup and stay alive for at least 28 days. Also, 6 weeks after *in vivo* delivery, the hydrocup remained in place with the hydrogel inside. These proof of principle experiments show that the concept of the hydrocup for *in vivo* fixation of hydrogels works. However, functional *in vivo* experiments were not performed and should be the next step.

Potential applications

MSCs as secretory factories and drug releasing hydrogels are being used as potential treatment for a wide variety of diseases[2-4, 11]. The hydrocups can in principle be used for any application where cells or drug releasing hydrogels needs to be delivered and stay in a specific location. Applications of hMSCs for systemic release of immunomodulatory factors through the whole body, for example, are therefore not suited to benefit from hydrocups. Other applications where MSCs are now being delivered systemically to repair a specific tissue, such as the intervertebral disc[12], or myocardium[13], could benefit from a more local release of MSC-secreted factors. Applications where MSCs are delivered locally and are expected to stay in place by direct injection could also benefit from the hydrocup, such as heart[14], kidney[15] or radiation burns[16]. Another benefit of the hydrocups as opposed to systemic or local injection of MSCs is that the hydrocup could be removed. Unless transplanted cells die, the cells are impossible to remove from the local tissue and will therefore keep affecting the

tissue, even after regeneration would be complete. This could not be desired in tissues where MSCs normally don't reside in large numbers. To prevent potential complications, an MSC-laden hydrocup could be implanted and removed after sufficient regeneration. As briefly described, there is a wide range of potential applications. For each application, however, the efficacy of the treatment remains to be tested.

Competition

While hydrogels can be directly implanted, to our knowledge there is currently no scaffold used to fix hydrogels in place *in vivo*. Scaffolds have been developed for *in vivo* delivery of cell aggregates ([17-20] to name a few examples), but not specifically for hydrogels. Even though these cell aggregate-holding-scaffolds could theoretically also hold hydrogels, the wells are much smaller than the 1.8 mm inner-diameter and up to multiple cm long hydrocups that could be fully filled with hydrogels. Because of the large hollow interior, the hydrocup could deliver large quantities of hydrogel and cells. The lack of direct competition is a great benefit for business opportunities, but also means that the benefits of such a system still need to be proven.

Further improvements

Depending on the application of the hydrocup, further improvements could be made to the hydrocup. As described in chapter 8, the hMSCs are able to escape to the outside of the hydrocup. In chapter 6, we found that the ESP scaffold polymer solution used to create the cups indeed allows for cell migration through the scaffolds. By decreasing the ESP fiber diameter from 3 µm to 1 µm or less, the escape of MSCs could be prevent. In addition, this could prevent host cells from infiltrating the hydrocups and interacting with the MSCs.

For each application, the MSC secretome should be analyzed and the hydrogel properties should be optimized. Depending on the application, other improvements could be made to the hydrocup, such as increasing mechanical properties to protect the hydrogel, or increase the interior volume to increase total gel volume.

The hydrocup is currently made from 300PEOT55PBT45, which is not approved for clinical use. To ease the direct application of the hydrocup, it could be produced from clinically approved polymers, such as polycaprolactone (already available in medical grade).

The production process is currently quite labor intensive. An experienced user can produce 10-15 hydrocups per hour. For commercialization, this process should be fully automated. The electrospinning jet has to be positioned in a precise location, so that it hits the open end of the rotating mandrel at the right spot. However, each time the electrospinning jet started to produce a new cup, the position of the jet changes. This makes full automation difficult and required a manual process by an experienced user. Automation processes could make use of a camera focused on the electrospinning jet to calculate the position to create the hydrocup. Such an automated system could increase both scalability and reproducibility.

Another labor-intensive part of the hydrocup process is the closing of the open end of the hydrocup after loading the hydrogel. This is currently done by closing a suture tightly around each hydrocup. To improve scalability, this should also be automated.

Conclusion

The hydrocup is the first scaffold developed for *in vivo* delivery and fixation of cell-laden or drug-releasing hydrogels. It has a wide range of potential applications and the proof-of-principle experiments in chapter 8 show promising initial results. Functional *in vivo* tests should now be performed, for which the hydrocup and contained hydrogel could be modified for the specific purpose. In parallel, the production process should be automated to allow scalability. With promising *in vivo* results and a scalable production process, the hydrocups could be used to treat a wide variety of diseases and be a viable commercial opportunity.

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