Publishable summary – final report

PreVascIn

Engineering a vascularized and innervated tissue using a building block approach

1. Description of the project objectives

The project objectives for the entire project are as follows:

- To design hydrogel formulations with mechanical properties, which guide differentiation towards osteogenic, myogenic, endothelial and neuronal cells.
- To design growth factors localization methods that enhance cellular differentiation in cell laden hydrogels.
- To develop cell laden hydrogel building blocks that are prone to self-assemble into a controlled three dimensional configuration.
- To combine the different building blocks using a self-assembly approach to engineer vascularized and innervated bone and skeletal muscle tissue.

When successful, this will result in a new tissue engineering paradigm where complex tissues containing multiple supportive structures can be acquired using a building block approach.

2. Description of the work performed and the main results

Within this project, a composite hydrogel system has been developed consisting of a mixture of gelatin methacryloyl (GelMA), which is a chemical modification of gelatin, and poly(ethylene glycol) dimethacrylate (PEGDMA). By changing the PEGDMA content of the hydrogel while keeping the GelMA content constant, the compressive modulus of the hydrogel can be tuned in the range of < 1 kPa to > 200 kPa. The composite hydrogel system has been well characterized, including mechanical properties, porosity, degradation and swelling behavior.

The effect of the composition, and thus mechanical properties, of the composite hydrogel system on the differentiation of human mesenchymal stromal cells (hMSC) has been investigated. Cell viability, cell proliferation, and cell spreading have been determined in the different hydrogel compositions for up to one week. Even though myogenic and endothelial cell differentiation could not be detected in any of the hydrogel formulations, the neural and osteogenic differentiation of hMSC in the different hydrogel compositions has been well characterized, both in basic and osteogenic differentiation medium. This showed that gels with a compressive modulus of approximately 20 kPa were optimal for osteogenic differentiation, while neural differentiation was optimal in gels with a compressive modulus of approximately 2 kPa.

An aptamer based methodology to pattern growth factors inside hydrogels in space and time has been developed. Experiments have shown that acrydite functionalized aptamers are chemically bound to the hydrogel matrix upon photopolymerization, and that these aptamers subsequently specifically bind to growth factors. The inclusion of growth factor binding aptamers did not have an adverse effect on the proliferation and survival of hMSC. Specific conjugation of the growth factor VEGF to the aptamers within the hydrogel environment resulted in the specific organization of endothelial cells.

Protocols have been developed to prepare individual tissue building blocks and to combine different building blocks into a tissue construct. Using different photomasks, differently shaped building blocks were prepared. Different building blocks were successfully combined using a lock-and key methodology. Using fluorescent beads to track different hydrogel compositions, up to 3 different fractions could be combined.

Using this tissue building block approach, tissue constructs containing regions with different hydrogel compositions were prepared. hMSC seeded in the building blocks and cultured for 1 week remained viable and differentiated based on their specific mechanical environment, with osteogenic differentiation in the stiff regions and neural differentiation in the soft regions, as can be seen in figure 1. Even though a tissue that contains both neural and vascular structures could not be obtained due to the lack of endothelial differentiation of hMSC in the hydrogels, this does prove the concept that a tissue containing different structures can be prepared from a single cell source using controlled mechanical environments and a building block approach.

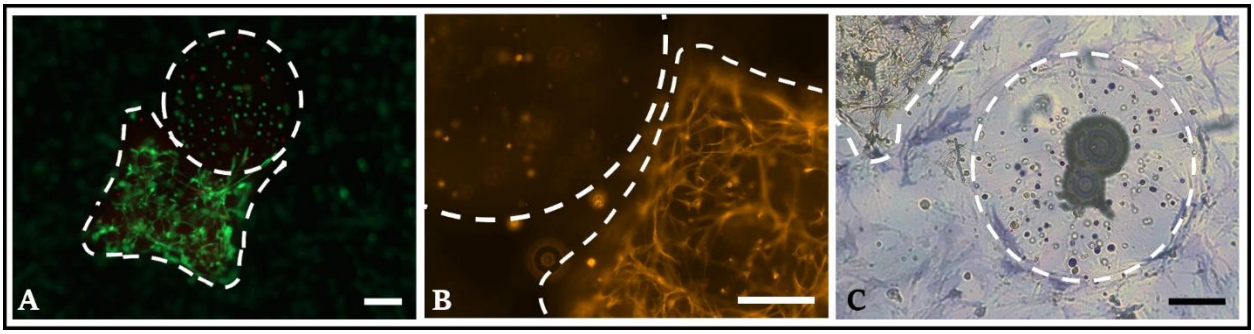

Figure 1 Multi-structural tissue engineering using building blocks
A stiff (circle; ≈ 20 kPa) and a soft (square; ≈ 2 kPa) tissue building block containing hMSC were combined using subsequent photomasks and illumination steps. The combined tissue was cultured in basic medium for 7 days. **Panel A** shows a life-dead staining showing that the cells remain viable in both environments. **Panel B** shows the expression of the neural marker β3-tubulin and extensive cellular organization in the soft environment, but not in the stiff environment. **Panel C** shows the expression of the osteogenic marker ALP and a rounded cell morphology in the stiff environment, but not in the soft environment. Scale bar = 200 µm. White lines indicate the approximate shapes of the separate environments.

3. Expected final results and impact

Even though both the combination of bone tissue with neural structures and bone tissue with endothelial structures has been investigated in this project, the combination of a base tissue with both a vascular and a neural network still remains to be investigated due to the lack of endothelial differentiation of hMSC in the developed hydrogels. Apart from that, the localization of growth factors using specific aptamers patterned in the tissue constructs needs to be further optimized and employed. Dr. Jeroen Rouwkema will continue working on this.

PreVascIn addresses a highly relevant bottleneck in the field of tissue engineering. Prevascularized and pre-innervated engineered tissues are expected to perform superior in a clinical setting due to a better integration and therefore a better functioning in the patient. Apart from that, the technologies for growth factor localization and tissue building blocks can be easily adapted for other tissue engineering applications where a high level of control over tissue development is needed. As such, the tools developed in this project have a significant impact on the field of tissue engineering and could aid in the promotion of the clinical application of engineered tissues.