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Development of novel prevascularized 3D microtissues for scientific and clinical application

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Each year, thousands of people die waiting for an organ transplant, and millions suffer from tissue injuries or degeneration requiring interventions such as donor grafts, animal tissues or artificial prosthetics. Lab engineered tissues have the potential to restore function without requirement for donor tissue, artificial materials, or immunosuppression. However, current clinically used engineered tissues are thin and avascular (e.g. skin and cartilage), Thicker tissues require vascularization to avoid graft failure from necrosis due to lack of blood perfusion. In the human body cells are found not more than 200µm from the nearest capillary, which reflects the diffusion limit of oxygen. Tissues whose thickness is greater than this diffusion limit require a dedicated system for mass transfer. In the past great effort has been put into vascularizing tissues and delivering microvasculatures. However, many attempts lack in vivo data or feasibility for scientific or clinical application.

In this thesis, an innovative high throughput and highly scalable method of vascularizing a tissue and delivering a microvasculature is presented. Focus is directed on applicability as a research instrument as well as a therapeutics platform technology.

Microtissues are small functional 3D tissue units that can be assembled to build a larger tissue. Microtissues of varying size were produced containing vessel-forming endothelial cells surrounded by densely packed mesenchymal stem cells (MSC). To form microtissues, cell suspensions of human umbilical vein endothelial cells (HUVEC) and MSCs were placed in microwells of an alginate hydrogel. Because the alginate environment is non-adhesive, the cells formed strong attachments to each other and thus “self-assembled” into a spherical microtissue. Within these microtissues, the HUVECs and MSCs also self-segregated into two

layers, a process termed “self-sorting”. Microtissues were cultivated in a vasculogenic medium, to induce vessel formation. The self-assembly, self-sorting as well as in vitro vascular formation were investigated by means of time-lapse microscopy. To demonstrate the flexibility of this platform, microtissues of varying size and HUVEC to MSC ratios were formed. In vitro fusion of several microtissues to one macrotissue was tested. Vascularized and non-vascularized microtissues were placed in a newly developed mouse window chamber set up to allow for in vivo microscopy. The formation and maturation of the resulting vasculature was observed.

Microtissues were stable over the entire in vitro cultivation period of up to 11 days and had developed extensive vessel-like-structures prior to implantation. They were capable of fusing in vitro to form one large tissue as well as one connected vascular network. Window chamber assays proved that after implantation in vivo the microtissues and their respective vessel-like-structures became connected to each other forming a vascular network. Between day 4 and day 7 this vasculature became connected to the host, blood-filled, and thus functional. Microtissues without preformed vasculatures showed no signs of vascularization, supporting the need for a preformed vasculature within tissue constructs in order to achieve rapid vascularization.

For a future application, the vessel forming ability of this technology can be used standalone or in conjunction with differentiated stem cells to form specific types of vascularized tissues.

To summarize, this dissertation has shown a novel high-throughput, highly scalable platform technology for fabricating an injectable vasculature as well as injectable vascularized microtissues for future scientific and clinical application.