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Introduction

Tissue engineering has advanced a lot over the last years. One of the challenges we're facing now is creating thicker constructs. The thickness of a tissue engineered construct is now limited to the diffusion of nutrients to the cells in the center. In order to create thicker constructs in which cell viability throughout the construct is maintained vascularization in vitro is necessary [1].
It was observed that endothelial cells (EC) seeded on top of myofibroblast (MF) seeded PGA/P4HB scaffolds make tube like structures. Experiments were performed to understand and control the formation of these structures.

Material and methods

Twelve rectangular PGA/P4HB scaffolds (20x7x1 mm) were glued into a wells culture using 20% polyurethane solved in THF (figure 1). The scaffolds were seeded with human vena saphena MFs using fibrin as a cell carrier. After 3 days of culture human vena saphena ECs were seeded on top of the construct.

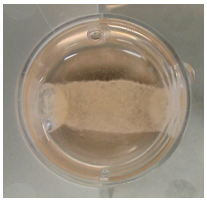

Figure 1 *PGA/P4HB scaffold glued in wells plate*

The constructs were cultured for 22 days in EGM-2 medium supplemented with 20% FBS and growth factors. At day 5, 8, 11, 15 and 21 two constructs were cut loose and stained with Cell Tracker Orange, staining the cytoplasma of viable cells and a UEA-1 lectin staining which is a specific for ECs. Different z-stacks of the EC side of the constructs were made, using a confocal laser scanning microscope (CLSM). These z-stacks were analyzed for the presence of an EC monolayer and EC tube like structures.

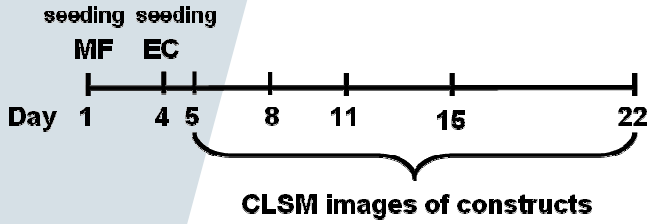

Figure 2 *Culturing protocol*

Results

After seeding, the EC monolayers started to grow, resulting in 80-100% confluent layers at day 21. At day 11 the first signs of the tube like structures appeared. On day 15 and 21 tube like structures were seen in most of the samples. They are up to 500 μm long and can be found up to 80 μm below the surface of the construct. Figure 3 shows a typical example of tube like structures lying under layers of ECs and MFs.

The EC tube like structures were detected at different positions: (1) under an EC and a MF layer, (2) under a MF layer only and (3) on top of a MF layer. Some tube like structures seem to be connected to the overlying EC monolayer (figure 4), indicating that they originate from that layer.

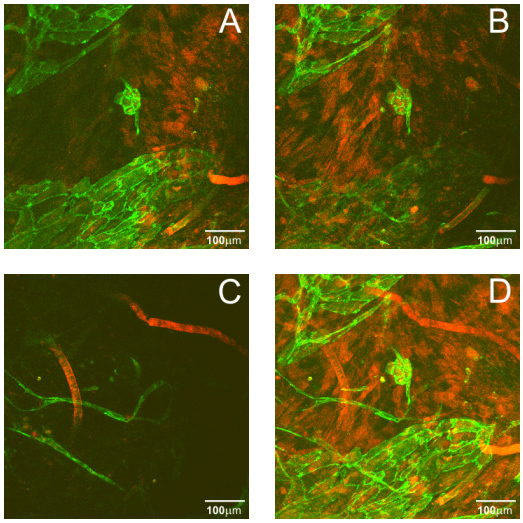

Figure 3 *A: CLSM image at 0μm with pieces of the EC monolayer (green). B: CLSM image at a depth of -23μm with the MF layer (red). C: CLSM image at a depth of -80μm with EC tube like structures (green) and fibers of PGA (red). D: Projection of all slices in z direction.*

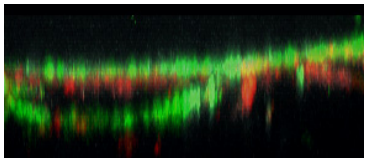

Figure 4 *Side projection of part of the stack, showing a EC tube like structure (green) that it is connected with the top monolayer of ECs (green).*

Discussion

The EC tube like structures were found in most of the constructs after 15 days. It is hypothesized that the MFs in the center of the constructs are experiencing a low oxygen concentration and are producing substances which trigger the ECs to form the tube like structures.

Future research

Histological slides will be made of the constructs in which the ECs will be stained with a different endothelial specific immunological staining, in order to visualize the tube like structures in another way. In order to verify if the MFs in the center of the constructs are hypoxic, a hypoxy probe will be used in coming experiments.

References:

[1] Levenberg S, et al.: *Nat Biotechnology, 2005, 23: 879-884*