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.

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 control the formation of these structures. Experiments were performed to understand

**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.

**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:**