SciTech 2013 Summer Science Camp

הפקולטה להנדסה ביו-רפואית בטכניון
Department of Biomedical Engineering - Technion, Israel
מדע והנדסה לקידום הרפואה

Technion Israel Institute of Technology

True Blood (Vessel Creation)

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Introduction

The aim of tissue engineering is to create functional tissues and organs in the lab and to use them as grafts[1]. A major obstacle in tissue engineering is poor post implantation graft survival due to insufficient blood supply that can lead to the death of the tissue[4]. One solution is to populate the engineered tissue with endothelial cells (ECs) which can quickly form organized vessel networks cultured in the lab and integrate with the host blood vessels and form functional blood vessels in the animal[4].

In this project, ECs isolated from human umbilical vein (HUVEC) were seeded together with Human Neonatal Dermal Fibroblasts (HNDF) on biodegradable PLLA-PLGA scaffolds and left to proliferate for 4 days in vitro. Afterwards the scaffolds were implanted in the abdominal wall of nude mice and monitored for 3 weeks. Eventually, the animals were sacrificed, the scaffolds were extracted and stained for mouse CD31 and desmin.

Methods and Materials

Cell Culture: Human umbilical vein endothelial cells (HUVEC) were cultured in endothelial cell medium (LONZA) and Human Neonatal Dermal Fibroblasts (HNDF) were cultured in DMEM supplemented with 10% FBS, 1% nonessential amino acids and 0.2% b mercaptoethanol as described priouviusly[2,3] .

Scaffold Preparation: Porous sponges composed of 50% PLLA and 50% PLGA were fabricated (Fig 1). For seeding, the desired number of cells were pooled and resuspended in 5 µl of thrombin and 5 µl fibrinogen. This suspension was allowed to absorb into the scaffolds, after which they were incubated for 30 min at 37 °C. Culture medium was then added, the scaffolds were detached from the bottom, and incubated at 37 °C . The medium was changed every other day.

Implantation of Muscle Grafts: All surgical procedures were conducted according to protocols approved by the Institutional Animal Care and Use Committee. Female nude mice were anesthetized using a ketamine : xylazine cocktail. A small incision was made allowing access to the abdominal muscle and surrounding tissue, where a 3×2 mm full thickness defect segment was removed and an engineered graft was sutured in place. All mice were monitored closely for 1–2 h to ensure full recovery from the anesthesia[2].

Tissue Processing and Immunohistochemical Staining: , Immunohistochemical and immunofluorescent were performed both on whole mount scaffolds and on frozen cryostat sections. Grafts were fixed in 4% paraformaldehyde, followed by extensive washing in PBS and overnight blocking. Anti-mouse CD31 was injected into the tail vein of the mice before they were sacrificed . Anti mouse CD31 and anti mouse desmin were diluted in fresh blocking serum and incubated with samples overnight at 4 °C.

Imaging: All images were taken with a Leica TCS-LSI confocal microscope.

Fig 1. Synthetic PLLA/PLGA scaffold.

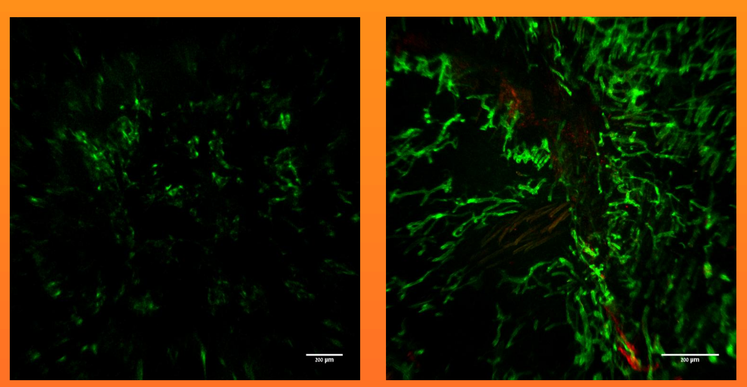

Fig 2. A whole mount staining for CD31 to detect EC after 8 days calture *in vitro*. **B** Section of an implant retrieved from the mouse 21 days post implantation. The section stained green for mouse CD31 to detect EC and red for Desmin to identify mouse smooth muscle cells (SMC). Scale bars 200 µm.

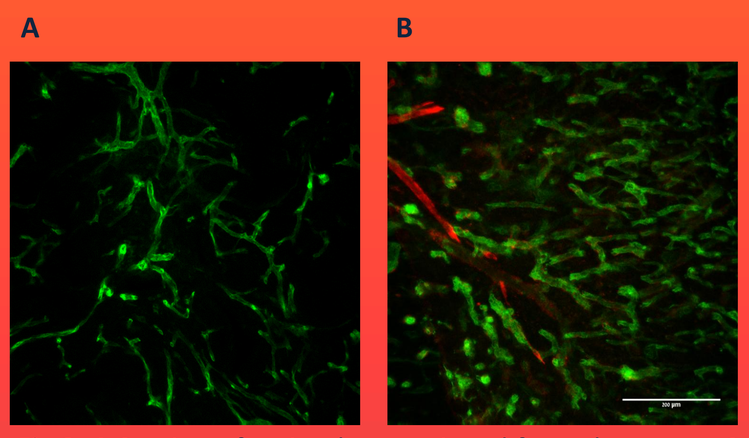

Fig 3. A Section of an implant retrieved from the mouse 21 days post implantation. The section stained green for CD31 to detect mouse EC. **B** native mouse tissue stained green for CD31 to detect mouse EC and red for Desmin to identify SMC. Scale bar 200 µm.

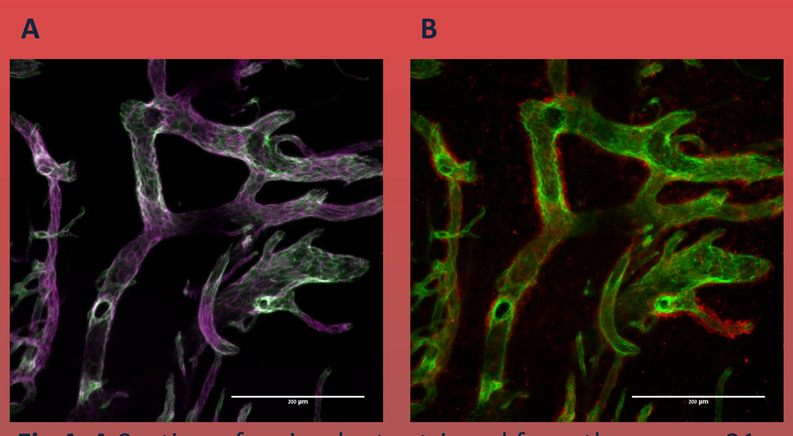

Fig 4. A Section of an implant retrieved from the mouse 21 days post implantation. The mouse was injected with Alexa Flour 647 anti mouse CD31 to detect functional vessels. The section was stained green for CD31 to detect mouse EC. White indicates co localization of green and magenta CD31 stains. **B** section stained green for CD31 to detect EC and red for Desmin to detect SMC. Scale bars 200 µm.

Results

After being grown *in vitro* for 4 days and *in vivo* for 21 days, the scaffolds were stained with different antibodies: DAPI stained the nuclei, Desmin stained the smooth muscle cells, and CD31 stained mouse endothelial cells. Fig 3 shows that the scaffold is filled with mouse blood vessels. CD31 that was injected into the tail vein of the mice before they were sacrificed shows which vessels are functional (Fig 4.)

Conclusion

Our results confirmed that the polymeric PLLA/PLGA scaffold is viable *in vivo*, as demonstrated previously[2,4] . we also saw that the construct is capable of inducing angiogenesis *in vivo.* The co localization shown in figure 4A confirmed that the new blood vessels, formed in the scaffold, are viable and functional.

Bibliography

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Acknowledgements and Special Thanks

We would like to thank Luba Shargarodsky for making this project possible and for her great help throughout the whole project, and Professor Shulamit Levenberg for hosting us in her lab.