Improved tissue formation with PCL scaffold and reduced in-vitro cell expansion

More with less?

Improved tissue formation with PCL scaffold and reduced *in-vitro* cell expansion.

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Introduction

Tissue engineering of heart valves (TEHV) based on rapid degrading PGA-P4HB scaffolds is known to result in compaction (reduced width of scaffold) and retraction (shrinkage) of the leaflets (Fig 1) due to imbalance of cell traction forces and scaffold resistance. [1,2]. PCL represents a promising material as it might result in reduced compaction and retraction due to slower degradation of the scaffold material. Differences in tissue production and cell phenotype may occur due to the differences in degradation time of the scaffolds. Further, cell aging, due to in-vitro expansion, is also known to have an effect on tissue production and cell phenotype[3]. This study will focus on the effects of the use of PGA-P4HB and PCL scaffolds and cell aging on tissue formation, compaction and cell phenotype.

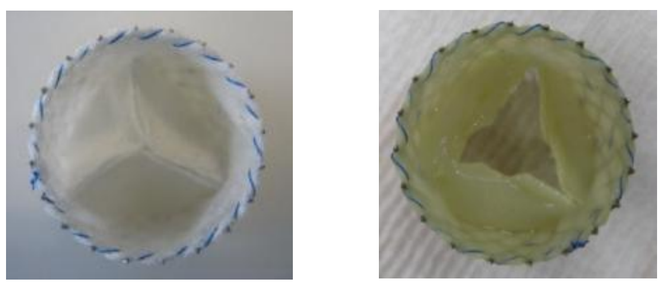

Figure 1. Heart valve based on PGA-P4HB scaffold before (left) and after (right) culture.

Material and methods

PGA-P4HB and PCL strips (n=6 per group) were seeded with p3, p5 or p7 human and ovine cells. Strips were cultured for four weeks and subsequently analyzed.

Results

Compaction and cell proliferation

Figure 2A shows a decrease in remaining width of PGA-P4HB strips compared to PCL strips. Control strips are seeded with fibrin only. No compaction is shown as 100%. Human cells demonstrate a higher proliferation rate in PCL scaffolds, while ovine cells proliferated more in PGA scaffolds (Fig 2B). Large variations in cell proliferation are present in the ovine PCL groups.

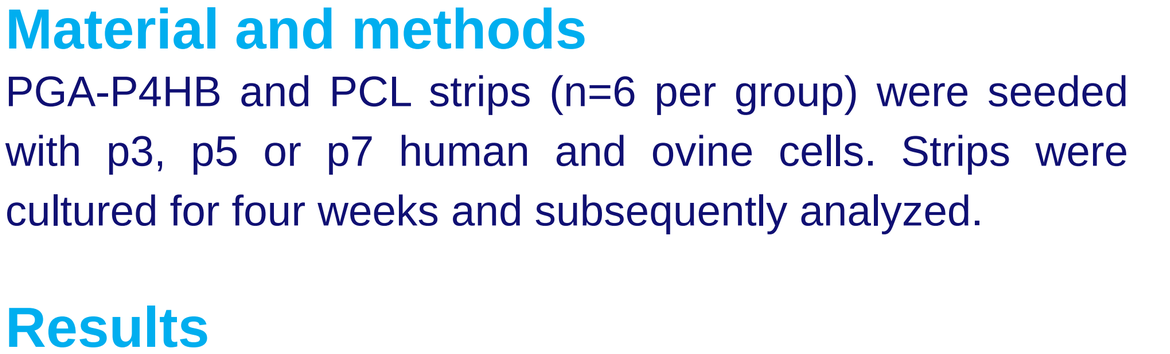

Figure 2. A. Remaining width of PGA-P4HB and PCL strips in percentage. B. Proliferation human and ovine cells on PGA-P4HB and PCL scaffolds.

Tissue formation per strip

Figure 3 shows the amount of tissue formed per strip after 4 weeks. Due to thickness differences, three times the amount of cells were seeded on PGA-P4HB scaffolds. In ovine strips, three times the amount of tissue is present in PGA-P4HB strips compared to PCL. In human only PGA-P4HB p3 shows three times more tissue compared to PCL.

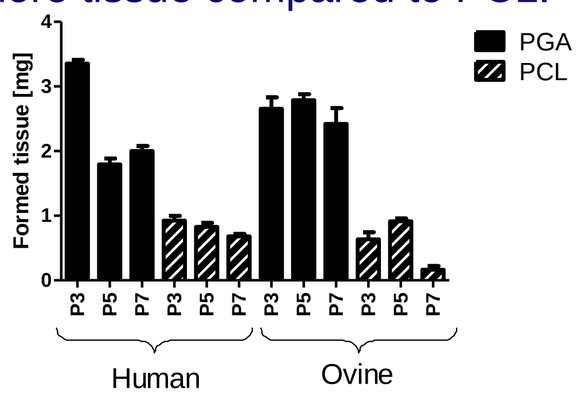

Figure 3. Tissue formation per strip present after 4 weeks in mg.

Extracellular matrix analyses

Collagen (Hyp per DNA) was increased in both human and ovine p3 tissues compared to p5 and p7, in PCL as well as PGA-P4HB scaffolds (Fig 4A). No collagen could be measured in ovine PCL p7 tissues.

GAG per DNA is decreased in human PGA p5 tissues compared to p3 and p7 (Fig 4B), while in PCL p5 and p7 are decreased compared to p3. In ovine tissues, p7 tissues show an decrease of GAG per DNA compared to p3 and p5.

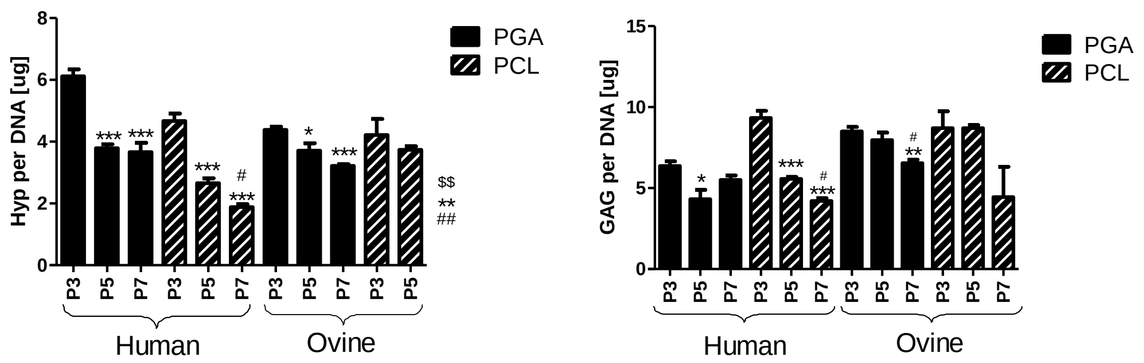

Figure 4. A. Hyp per DNA. B. GAG per DNA. * denotes a significant difference compared to p3.

# denotes a significant difference compared to p5.

Conclusion

Preliminary results indicate that PCL scaffolds may serve as alternative scaffold material for TEHV with reduced compaction. Cells from lower passages showed to improve tissue formation. In addition, reducing cell expansion will result in faster generation of TEHV and providing a more rapid treatment to patients. Histology analyses for cell phenotypes are ongoing. Ovine PCL strips should be cultured again to investigate if the experiment failed or p7 cells do not grow on PCL scaffolds.

1. Driessen-Mol *et al* , Ann. Biomed Eng (2005)
2. Flanagan *et al,* Tissue Eng Part A (2009)
3. Hoffman-Kim *et al*, Tissue engineering (2005)