Surface Treatment to Promote Endothelialization on TCP

By: Lukas Hruby, Tranquillo Group, UROP Summer 2022

Abstract

This project analyzed the use of new surface coatings on tissue culture plastic to determine whether the proposed coating material would act as an effective alternative to endothelialization on TCP. The main method involved seeding cells onto 18 total TCP well plates, all with varying concentrations. Nine of the plates had fibronectin applied first, followed by the application of Poly-L-Lysine, with the other nine reversing the order. Based on the results, the plates that had Poly-L-lysine applied first had better overall cell count, and subsequently adhesion, than the plates with fibronectin first. Further experimentation must be done to determine whether the combination of substrates is overall more effective than solely using one substrate to complete endothelialization. This project outlines the importance of forming a monolayer of endothelial cells and is the first step to eventually synthesizing a completely biological transplant organ..

Introduction

Over a million cardiovascular related surgeries are performed every year, complicating these people's lives and reducing their quality of living.

The most common heart related surgery that is performed is coronary artery bypass surgery

A fully biological tissue engineered vascular bypass graft that fully integrates with the patient and can reduce or eliminate the need for follow up surgeries and long term treatment is becoming more and more relevant with the number of heart related disease cases increasing every year.

These grafts are the solution to improving and even saving the lives of millions of people worldwide that suffer from coronary artery heart disease and begin with determining the most effective way to produce a complete monolayer of cells, which would act as the foundation for these autonomous grafts.

The research outlined in this presentation studied what conditions, specifically the surface coating utilized, would enhance endothelialization the greatest.

Results

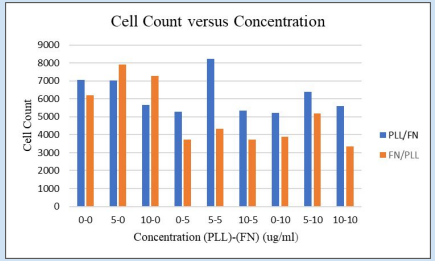

Figure 1: Data collected for a total of 18 wells with the displayed concentrations respective to coatings(PLL and FN)

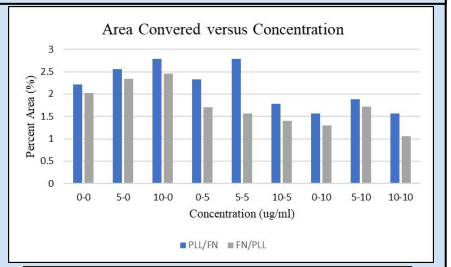

Figure 2: Percent area of well covered with endothelial nuclei for sample(18 total).

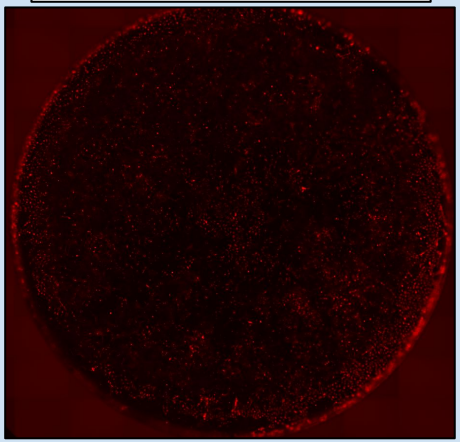

Figure 3: Von willebrand factor expressing the presence of endothelial cells and the subsequent monolayer formed for the PLL/FN mixture at 5 ug/ml each Less gaps in the monolayer were observed than the FLL/PLL counterpart.

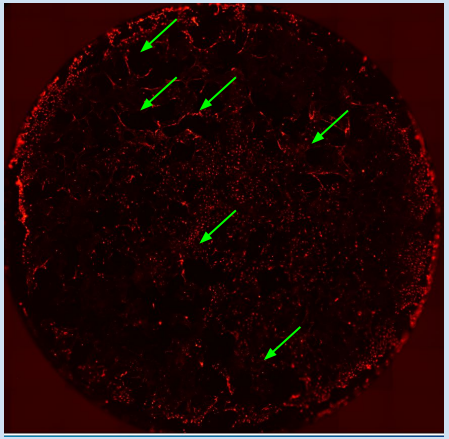

Figure 4: Same concentration (5 ug/ml each) for the reversed order (FN/PLL) of Von willebrand factor. Gaps in the endothelium are larger and more frequent, aligning with hypothesis compared to figure 3.

Discussion

The collected data supported the initial hypothesis that applying PLL first, followed by fibronectin, to the TCP surface would be more effective than the reverse order due to the cationic PLL polymer exhibiting a greater affinity to the anionic TCP surface than fibronectin. Further analysis concluded that fibronectin is a better coating to directly attach to the seeded cells, further supporting the hypothesized coating order.

This is mostly due to the biological interactions that the extracellular matrix has with the endothelial cells and are overall stronger than the electrostatic interactions that the PLL has at the cell interface.

The PLL and fibronectin mixture did not greatly surpass the samples containing only one of the coatings in overall cell count meaning that further experimentation should be done to determine whether the use of a mixture is viable or ultimately redundant.

Due to fluorescent imaging being conducted over a weeks span, it is possible that apoptosis factored into the cell counts, reducing the overall count for several of the samples and interfering with results.

Conclusion

The collected data supports the initial hypothesis that applying Poly-L-lysine first, followed by fibronectin is more effective in promoting endothelialization. Furthermore, the total area covered by endothelial cells was greater for all sample solutions, as depicted by figure(2). Figure(3) expresses a more uniform monolayer compared to figure(4) which expresses more gaps in the endothelium. Having a completely uniform monolayer is necessary for the construction of these biological transplant organs and is the most vital part in the synthesis process. A further extension of this work will be to conduct multiple rounds of trials, focusing on the effectiveness of the PLL/FN mixtures against mono-coating samples to determine their feasibility for future use. Once a satisfactory monolayer has been achieved, the adhesive properties of the coating mixture will need to be tested to mimic the in-vivo conditions that the endothelium experiences. Preliminary testing will involve rinsing the monolayer followed by imaging to determine the relative strength of the proposed coatings materials. The data analyzed is favorable to the integration of PLL into current methods, however; whether that difference is considerably large will need to be determined through further experimentation.

Acknowledgement

A special thanks to the University Of Minnesota UROP Program as well as the Tranquillo Lab Group for supporting this project