Fabrication of a Decellularized Human Amniotic Membrane (DAM) Nanoparticle Coating for Small-Vessel ePTFE Grafts

Wesley Glime, BA[1], Bo Wang, PhD[2],
[1]Medical School, Medical College of Wisconsin; [2]Department of Biomedical Engineering, Medical College of Wisconsin, Milwaukee WI

Background

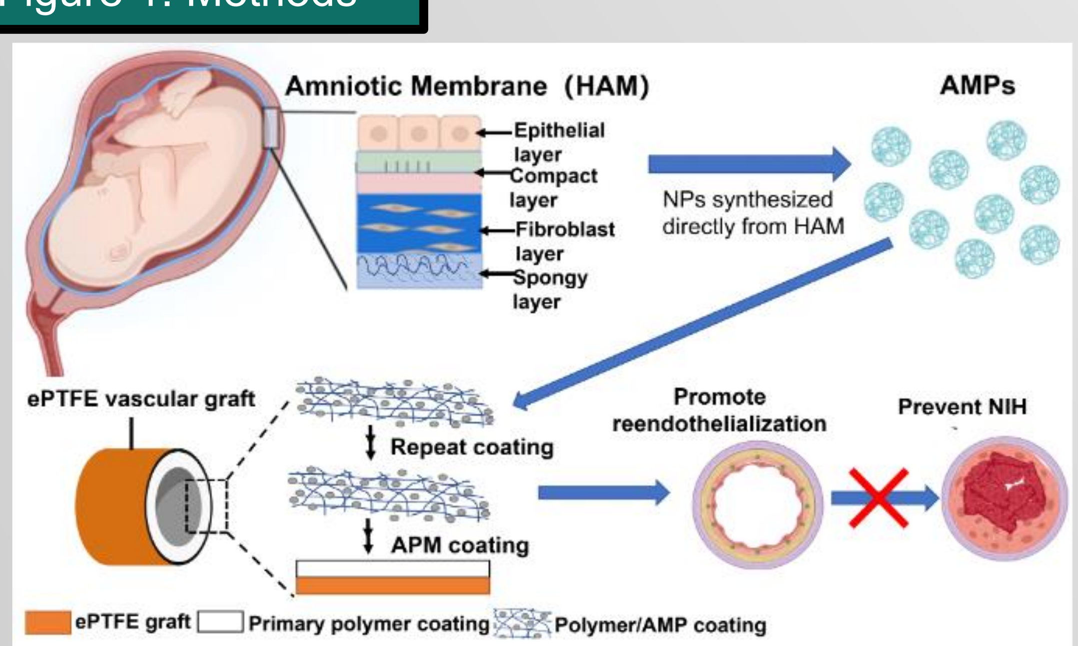
- Cardiovascular disease (CVD) is the leading cause of death in the United States, and grafting is an important surgical intervention.
- Autologous (harvested from the patient) grafts are the gold standard, but they are not always feasible for every patient. Synthetic grafts have been developed but have poor long-term patency, especially in vessels less than 6 mm in diameter.
- Wang et al. 2023 showed a genipin-crosslinked DAM graft that displayed prolonged stability, superior biocompatibility, and improved patency over a synthetic ePTFE graft. Yet, the size and mechanical properties are limited by the amnion membrane[1].
- Other research has shown the ability to use polymers to coat ePTFE grafts with a variety of materials to promote reendothelialization or reduce thrombogenicity, but its clinical application has been limited[2,3,4].

Hypothesis

- The goal is to engineer a DAM nanoparticle coating for small-vessel ePTFE grafts that will improve long-term patency and allow for tunable size and mechanical properties.
- We hypothesize that a DAM-coated ePTFE graft will increase reendothelialization, reduce thrombogenicity, and maintain the mechanical properties of a native vessel.

Methods

Figure 1. Methods

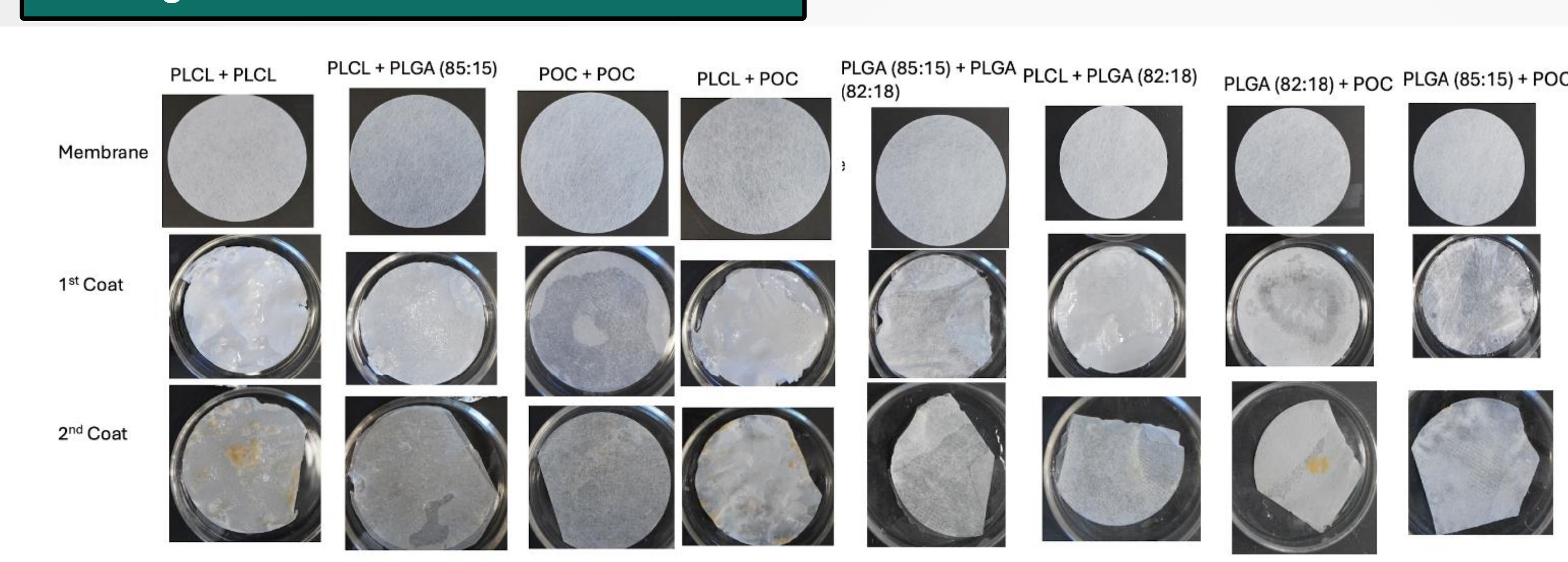


Methods

- Human amnion membranes obtained from the MCW Tissue Bank are decellularized and turned into ICG-labeled nanoparticles (AMPs). – Figure 1
- ePTFE membranes or grafts are coated with one of 3 polymers (PLGA, PLCL, or POC) as a primary polymer coating. Then additional layers of polymer with AMPs are added. – Figure 1
- The efficacy of the AMP-polymer coating is tested through SEM, fluorescence imaging, degradation, cell culture, mechanical testing, and in vivo rat models.

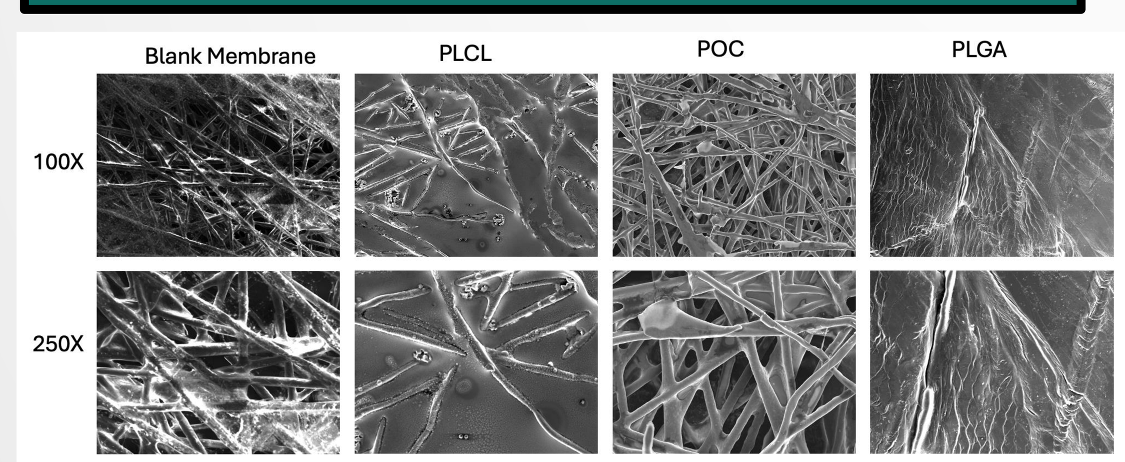
Results

Figure 2. Coated Membranes



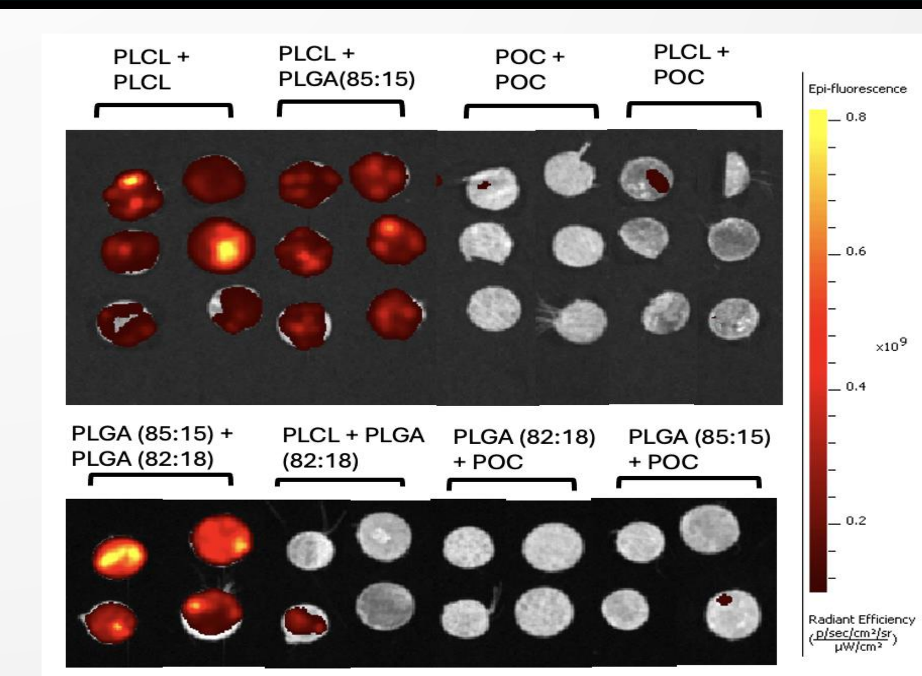
- 8 polymer combinations (1st layer + 2nd layer w/ AMPs): PLCL+PLCL, PLCL+PLGA(85:15), POC+POC, PLCL+POC, PLGA(85:15)+PLGA(82:18), PLCL+PLGA(82:18), PLGA(82:18)+POC, and PLGA(85:15)+POC

Figure 3. SEM of 1st Layer Coating



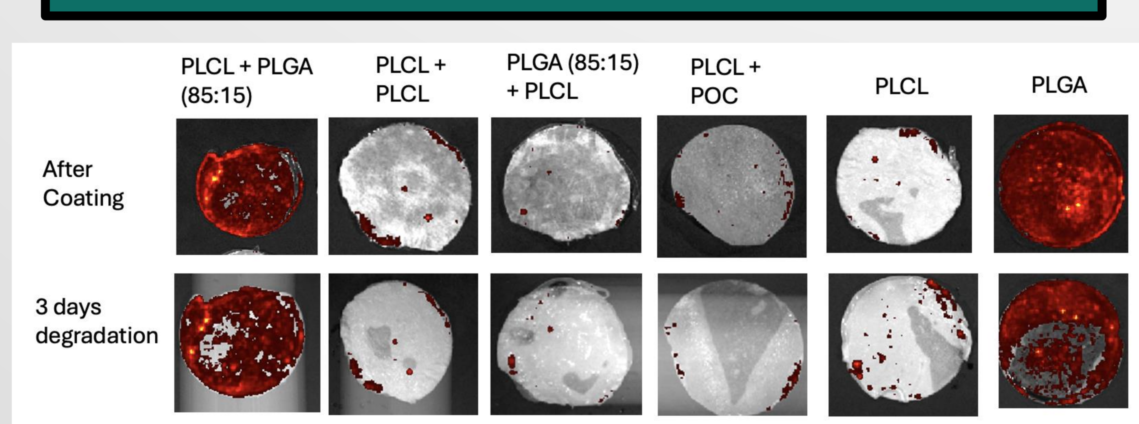
- Under SEM, PLCL integrates between ePTFE fibers, providing a thick, even coating
- POC coats the fibers but doesn't fill the gaps
- PLGA provides an even, crystalline coating

Figure 4. Fluorescence of each Polymer Combination with AMPS



- After incubation and 2 days of washing with PBS, fluorescence imaging reveals that POC does not hold the AMPs as well as PLCL and PLGA
- The density of AMPs is greatest for the PLCL+PLCL, PLCL+PLGA(85:15), and PLGA(85:15)+PLGA(82:18) combinations

Figure 5. Fluorescence Degradation of Polymer Combination with AMPs



- Coating with POC does not provide a very durable attachment of AMPs
- Coating with PLGA provides the most durable attachment of AMPs

Discussion

- PLGA is most promising for its ability to hold the AMPs and evenly coat the ePTFE membrane, but its hydrophobicity and crystallinity may prevent reendothelialization and alter the mechanical properties of the graft.
- PLCL also shows some promise for adhering AMPs to the ePTFE while maintaining the mechanical properties of the graft and providing a more hydrophilic surface for reendothelialization.
- POC holds the AMPs at first, but is degraded quickly and likely would not withstand the shear stress of a native vessel.
- Further testing will investigate several polymer combinations with PLGA and PLCL including coating of the graft with just a single layer of either polymer.

Future Work

- Determine whether the coated ePTFE can support reendothelialization through cell culture, histology, and SEM
- Quantitative measurement of the degradation rate and loss of AMPs
- Measure mechanical properties such as contact angle, elasticity, and compliance
- Test with actual ePTFE graft
- Test in in vivo rat model

Acknowledgements

This research was funded by the Medical College of Wisconsin Department of Biomedical Engineering. A special thank you to the principal investigator, Dr. Bo Wang, for their guidance on this study.

References

1. Wang et al. 2023 - Wang B, Wang X, Kenneth A, Drena A, Pacheco A, Kalvin L, Ibrahim ES, Rossi PJ, Thatcher K, Lincoln J. Developing small-diameter vascular grafts with human amniotic membrane: long-term evaluation of transplantation outcomes in a small animal model. Biofabrication. 2023 Jan 30;15(2). doi: 10.1088/1758-5090/acb1da. PMID: 36626826.
2.Kim et al. 2013 - Kim H, Park S, Kim D, Park J. New Coating Method for Sustained Drug Release: Surface Modification of ePTFE Grafts by inner coating PLGA. Bulletin of the Korean Chemical Society. 2014 May. 35. 1333-1336. doi: 10.5012/bkcs.2014.35.5.1333.
3.Kim et al. 2019 - Kim D, Chung JJ, Jung Y, Kim SH. The effect of Substance P/Heparin conjugated PLCL polymer coating of bioinert ePTFE vascular grafts on the recruitment of both ECs and SMCs for accelerated regeneration. Sci Rep. 2019 Nov 19;9(1):17083. doi: 10.1038/s41598-019-53514-6. PMID: 31745143; PMCID: PMC6863833.
4.Yu et al. 2021 - Yu L, Newton E, Gillis D, Sun K, Cooley B, Keith A, Sheiko S, Tsihlis N, Kibbe M. Coating Small-Diameter ePTFE Vascular Grafts with Tunable Poly(diol-co-citrate-co-ascorbate) Elastomers to Reduce Neointimal Hyperplasia. Biomaterials Science. 2021 Aug. Doi: 10.1039/D1BM00101A.