HISTOLOGICAL AND BIOMECHANICAL EVALUATION OF BRAIDED TISSUE ENGINEERED ARTERIAL GRAFTS IN A MOUSE MODEL

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abstract

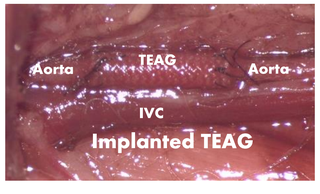
Tissue Engineered Arterial Grafts (TEAGs) are designed from biodegradable materials capable of forming a neoartery of autologous tissue that can grow and remodel within the body. Textile braiding techniques have the potential to aid in the creation of compliant TEAGs with tunable mechanical properties. In this work, TEAGs were fabricated from braided polyglycolic acid fibers with or without polyglycerol sebacate coating. Female C57BL/6 (WT) and Beige mutation (Bg) mice were implanted with TEAGs as infrarenal aortic interposition grafts and followed with ultrasound to complete scaffold degradation at three months. Neoarteries were then explanted and subjected to biaxial mechanical testing and histological analysis. While WT mice demonstrated a high rate of surgical thrombosis (5/10), Bg mice demonstrated no incidence of surgical thrombosis (0/10). One WT mouse and two Bg mice died from blood loss following surgery. Notably, all patent neovessels dilated at approximately one-month post-implantation. Following this dilation, three mice were found to rupture and die from the resulting hematoma, one WT mouse and two Bg mice, all with uncoated implants. On explant, 2/3 WT neoarteries were patent, compared to 6/6 patent neoarteries in Bg mice. Mechanical testing demonstrated neoarteries were much more compliant in both the axial and circumferential directions than unimplanted scaffolds, although neoarteries still remained stiffer than native aortas. Explants demonstrated robust collagen and elastin staining within the neoarteries, as well as numerous smooth muscle cells and limited macrophages. Von Kossa and Alizaeran Red staining demonstrated freedom from calcification in all samples. These findings support the use of braiding as a fabrication technique for TEAGs, as well as the use of a Bg mouse model to study TEAG evolution. Future studies will aim to integrate braiding techniques and mathematical modeling to develop TEAGs with optimal neoartery development.

background

There is a vital need for the creation of clinically acceptable prosthetic small-diameter arterial grafts due to widespread insufficient autograft availability.[1] The use of synthetic grafts has been limited by high rates of early occlusion secondary to thrombosis.[2] An ideal vascular implant would be (1) biocompatible, (2) non-thrombogenic, (3) compliant, (4) fatigue resistant, (5) flexible yet robust, (6) readily available, and (7) easy to manufacture.[3] These factors are dependent not only on the material used in the implants, but also depend heavily on the manufacturing methods used to fabricate them. Braiding technology benefits from a long history of textile manufacturing practices, as well as removing the need for a seam as in some other textile methods, and allowing for better radial expansion of the resulting tubes.[3] This work investigates the use of a braided Tissue Engineered Arterial Grafts (TEAG) as an aortic interposition graft in a mouse model to evaluate the mechanical and histological nature of the developing neoartery.

methods

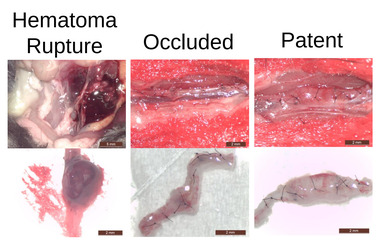
Braided scaffolds (provided by Secant Group, LLC) were constructed from Poly(Glycolic Acid) (PGA) fibers (16um diameter) with and without a Poly(Glycerol Sebacate) (PGS) coating with an internal diameter of 0.66mm. Scaffolds were implanted as infra-renal aortic interposition grafts in 8-12 week old female C57BL/6 (WT) and Beige mutation (Bg) mice. After 12 weeks, neoarteries were explanted for biaxial mechanical testing[4], followed by formalin fixation and paraffin embedding for histology evaluation. Histology images were quantified using FIJI image analysis software.



results

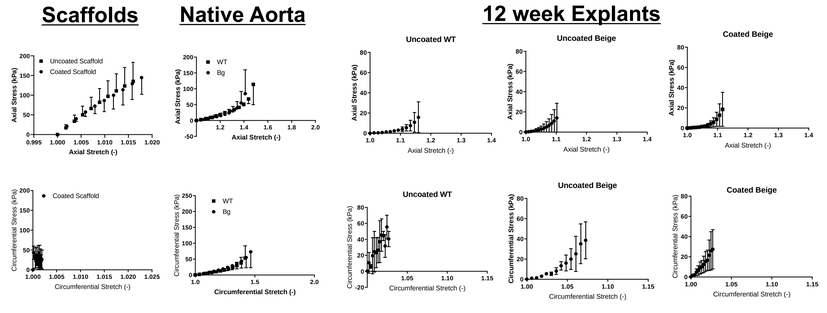
Surgical Outcomes

		Total Implanted	Early Death <1day: Thrombosis	Early Death <1day: Bleeding	Mortality >1 Day	3 Month Explant Outcomes: Occluded	3 Month Explant Outcomes: Patent	Explant Diameter (mm)
Uncoated	WT	5	1	1	1 (34d)	0	2	1.138 ± 0.173
	Beige	5	0	1	2 (28d, 46d)	0	2	0.823 ± 0.118
Coated	WT	5	4	0	0	1	0	N/A ± N/A
	Beige	5	0	1	0	0	4	0.918 ± 0.118



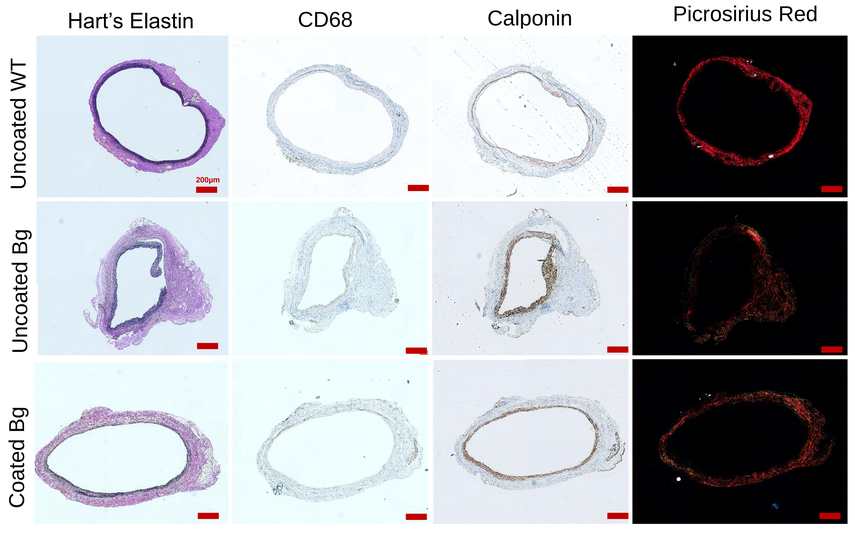
WT mice demonstrated a high rate of surgical thrombosis, which was not seen in the Bg mice. Three mice, all in the uncoated groups, experienced a hematoma and rupture between the 1st and 2nd month. Patent grafts were noticeably dilated to around 1mm diameter at explant (confirmed by histology). Dilation was noted on ultrasound between the 1 and 2 month time point. Sutures were added to neoarteries prior to explantation as markers for mechanical testing.

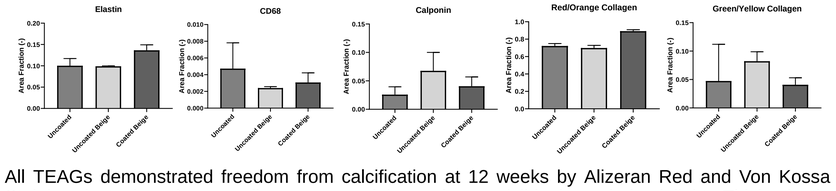
Mechanical Testing



Uncoated scaffolds were not water-tight and unable to hold pressure for circumferential testing. Scaffolds were significantly stiffer than native vessels by mechanical testing, and PGS coated did not appear to affect the axial stiffness. Explanted neoarteries were significantly more compliant than implanted scaffolds, but did not reach the compliance of the native artery by 12 weeks. Noticeable biological variation was detected between samples with the same implanted TEAG.

Histological Analysis





All TEAGs demonstrated freedom from calcification at 12 weeks by Alizeran Red and Von Kossa Staining (not shown), as well as no remaining polymer visible by polarized light. Thick elastin staining, robust smooth muscle staining, and minimal macrophage staining were seen in the neoarteries. Picrosirius Red staining demonstrated large amounts of collagen deposition.

discussion

Beige mice were noticeably free from early thrombosis seen in the wild type mice. PGS coating appears to play a role in preventing hematoma and rupture at the TEAGs. All explanted TEAGs were free from calcifications at 12 weeks. Mechanical testing demonstrated substantial remodeling of the scaffold into a more compliant neovessel, although all neoarteries were still much less compliant than native aorta at 12 weeks. Longer time course studies will be required to evaluate the long term outcomes of the TEAG's neoarteries. Histology demonstrated large amounts of elastin and collagen deposition, as well as little inflammatory staining. Future work will evaluate the time course of neoartery development at both earlier and later time points.

These findings support the use of Beige mice as an animal model for TEAG implantation. Future work will evaluate the effect of braiding parameters on neotissue formation. This work will also be used to establish a mathematical model for the development of neotissue in TEAGs in an effort to design a TEAG with optimal neotissue development.

acknowledgements

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references

1.Tara S, Rocco KA, Hibino N, et al. Vessel bioengineering. Circ J 2014;78:12-9.
2.Malm CJ, Risberg B, Bodin A, et al. Small calibre biosynthetic bacterial cellulose blood vessels: 13-months patency in a sheep model. Scand Cardiovasc J 2012;46:57-62.
3.Singh C, Wong CS, Wang X. Medical Textiles as Vascular Implants and Their Success to Mimic Natural Arteries. J Funct Biomater 2015;6:500-25.
4. Ferruzzi, J., M.R. Bersi, and J.D. Humphrey, Biomechanical phenotyping of central arteries in health and disease: advantages of and methods for murine models. Ann Biomed Eng, 2013. 41(7): p. 1311-30