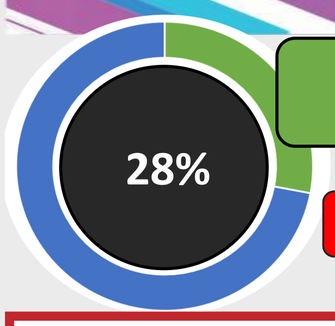
Human tissue-engineered arteries can mimic blood clotting: An alternative to current animal experimentation?

Jacob Ranjbar, Ying Yang & Alan G.S. Harper

Keele UNIVERSITY

British Heart Foundation

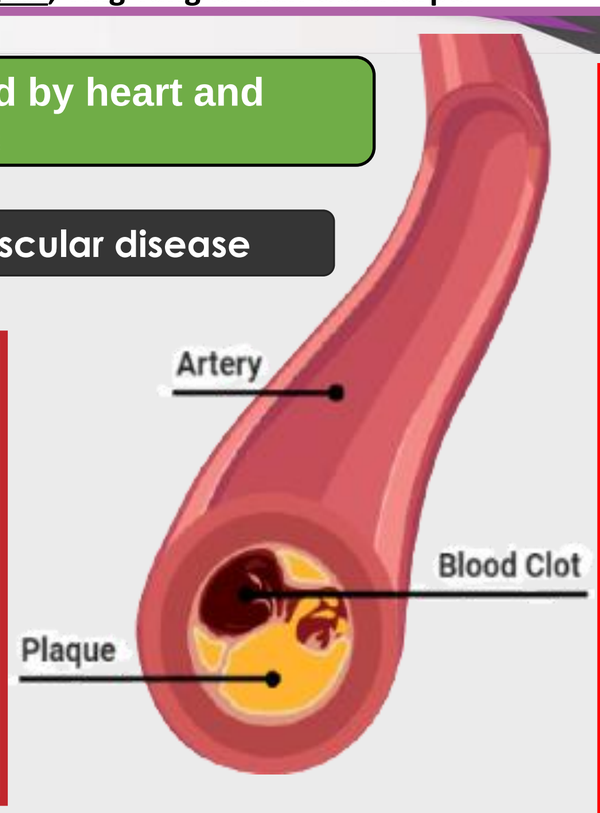
NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

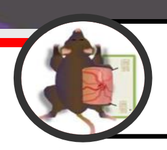


Proportion of all UK deaths caused by heart and circulatory diseases

1 In 10 people suffer from Cardiovascular disease

- Blood clots result from exposure of flowing blood to the damaged artery wall.
- The artery wall contains collagen that triggers uncontrolled activation of circulating platelets and tissue factor, which initiates blood clotting components.
- Activated platelets and blood clotting components create a stable clot that blocks the artery leading to heart attacks.
- Understanding how blood clots are formed is vital in…
  - **Identifying new ways to prevent heart attacks.**
  - **Preventing unwanted blood clotting.**





Animal Experimentation

The mechanisms involved in forming a blood clot is complex, and it is argued that the process can only accurately be captured in living animals. Mice are currently the preferred animal used to study blood clotting in cardiovascular disease research as they can be genetically modified to present the human disease.
Experimentation on mice has allowed researchers to analyse blood clot formation in real-time and has help us develop preventative drugs.

Scientific concerns

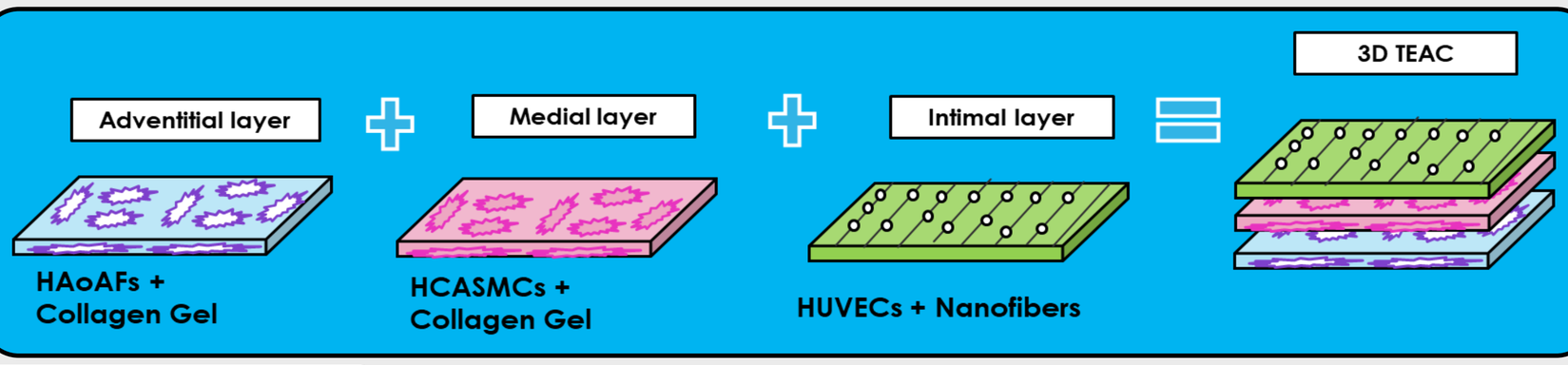
- **Anaesthetics used to put the animals to sleep has shown to impact how blood clots.**
- **Results from mice may not be directly comparable to humans.**
- **There is variation in results between research groups.**

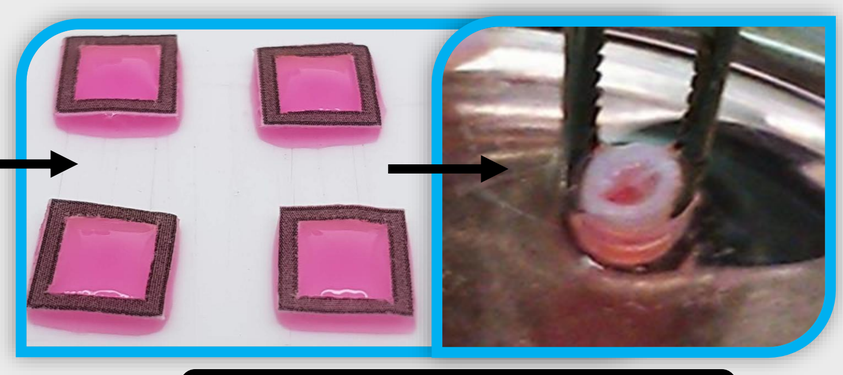
Concerns for animal welfare

- **Surgical exposure of veins/arteries is necessary for experiment.**
- **Mouse is put down at the end of the experiments.**

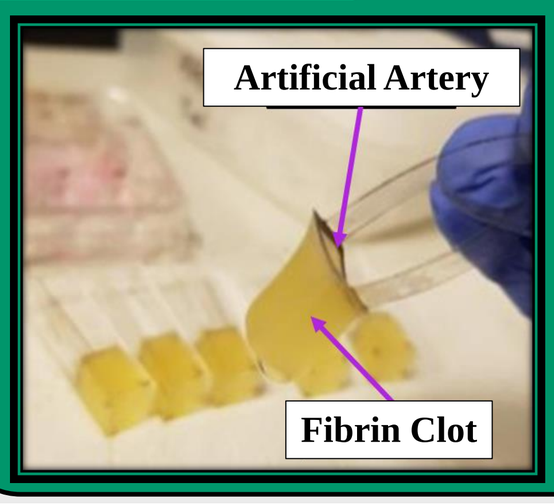
Project Aim

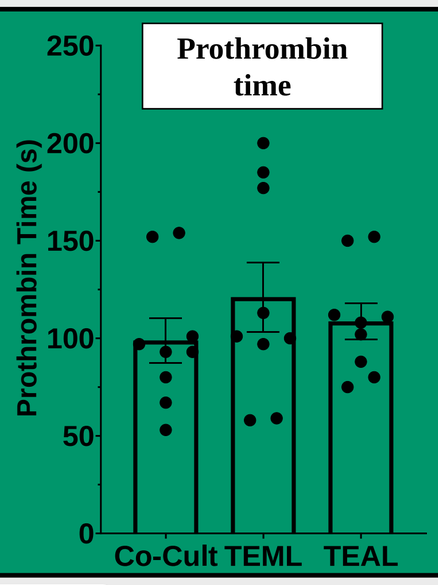
This project aims to create a tissue-engineered human artery (TEAC) and a 3D printed flow chamber. Together they can be used as a viable replacement to mouse models used in cardiovascular disease research.





Clotting Ability





Prothrombin time captures the time taken for a fibrin clot to form in platelet poor plasma when exposed to the artificial artery layers.

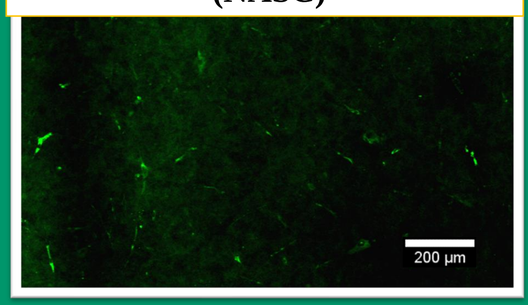
All layers showed significant clotting ability compared to controls.

- **TEML** = Tissue engineered Medial Layer
- **TEAL** = Tissue engineered Adventitial Layer
- **Co-Cult** = Medial + Adventitial layer
- **Control** = Collagen gel with no cells

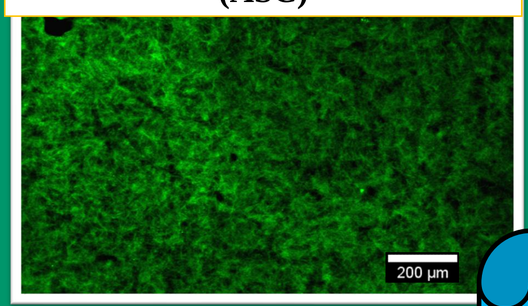
Collagen production

We have shown that we can increase collagen production within our artificial artery by adding ascorbic acid to the medial layer. This has shown to significantly improve the clotting ability and reduce the time and associated costs with producing the artificial artery.

No ascorbic acid supplementation (NASC)



Ascorbic acid supplemented (ASC)



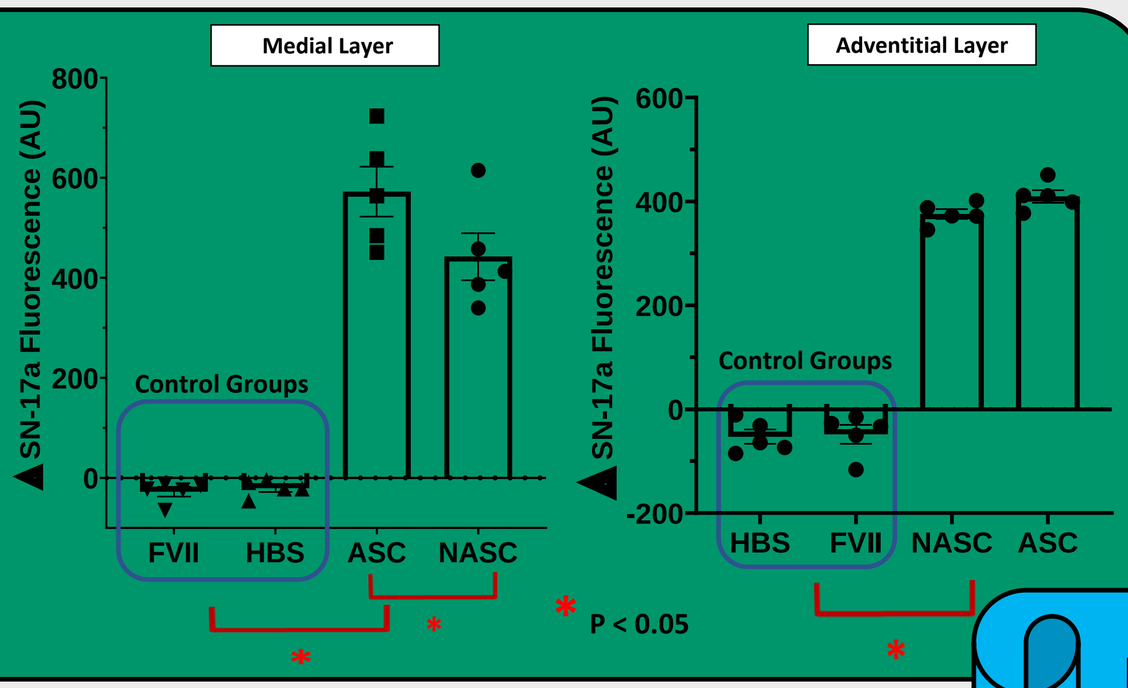
Tissue Factor Production

Tissue factor is a vital component in the production of a stable blood clot. It is found in both the medial and adventitial layers of the human artery.

Our artificial artery must contain tissue factor.

Using a specially designed experiment, we identified tissue factor through the dye SN-17a, which fluoresces when tissue factor is present.

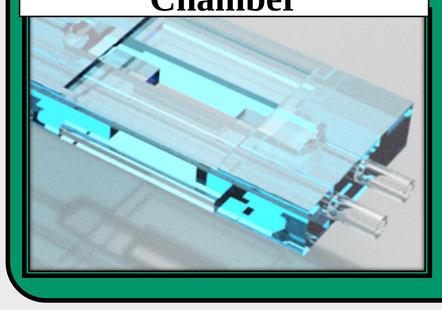
Both the medial and adventitial layers of our artificial artery contained tissue factor. Furthermore, the use of ascorbic acid significantly increased tissue factor production within the medial layer.



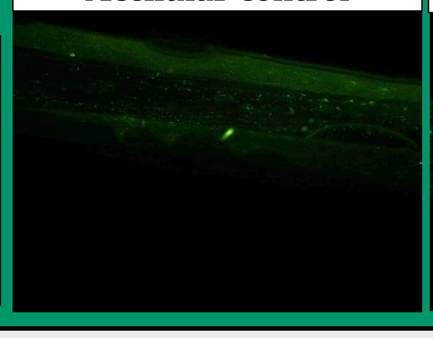
Activation of platelets under flow

A 3D printed chamber was used to house the medial layer of the artificial artery. Platelets were then perfused through the chamber and over the medial layer under flow conditions seen in humans. Activated platelets can be seen as green dots below. Ascorbic supplementation of medial layer increased platelet activation.

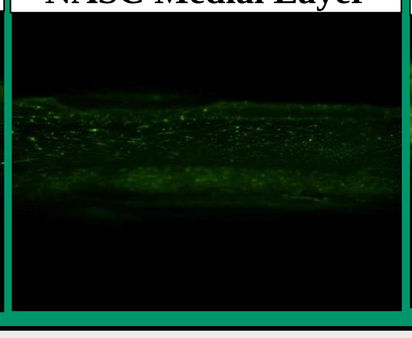
3D Printed Flow Chamber



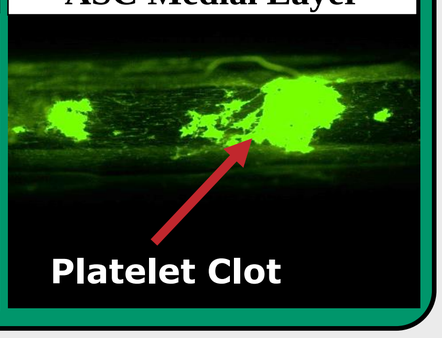
Acellular control



NASC Medial Layer



ASC Medial Layer



Lab-Grown Artery Check list

- [ ] Is it comparably relevant to humans?
- [ ] Will it mimic physiological conditions?
- [ ] Will it clot fresh blood upon injury?
- [ ] Will it show similar shape to mouse/human clots?
- [ ] Can it be recreated and used with ease?
- [ ] Is it a viable method to replace mice in clot research?

References

British Heart foundation—Heart and Circulatory Disease Statistics (2019), F. Musa (2017), Ipsos MORI—public attitudes to animal research (2016).