From lab to patients…

How to pre-screen scaffolds intended for clinical application?

Ex Ovo chorion allantoic membrane (CAM) as a pre-screening model for testing biomaterials

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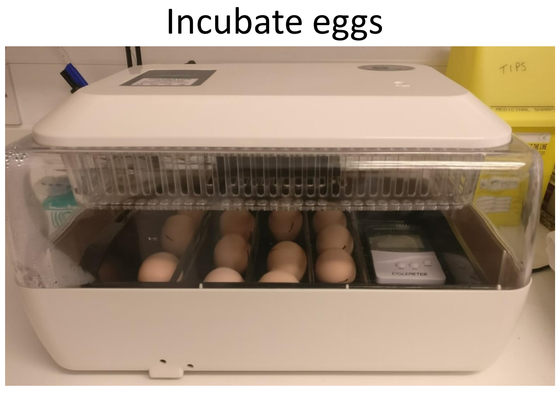
Background: Many biomaterials intended for clinical use either fail to make it to *in vivo* testing or fail as an implant due to a lack of the angiogenic capacity of these biomaterials[1].

We utilised the *ex ovo* method published previously[2] and report the accuracy and feasibility of this method for examining and comparing the angiogenic potential of clinically utilized scaffolds as well as scaffolds under development for different tissues such as skin, bone and breast.

Aim: To establish a pre-screening method for assessing the suitability of biomaterials intended for clinical use

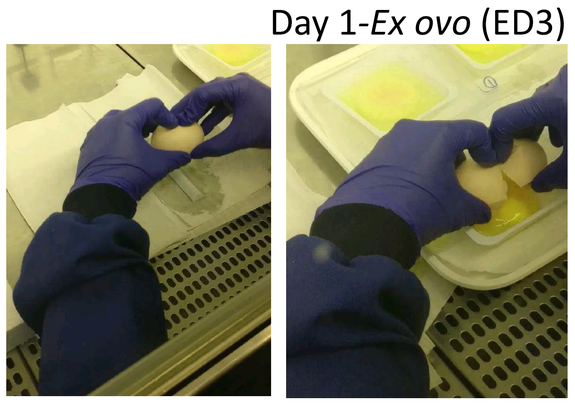
Method & Results

Incubate eggs



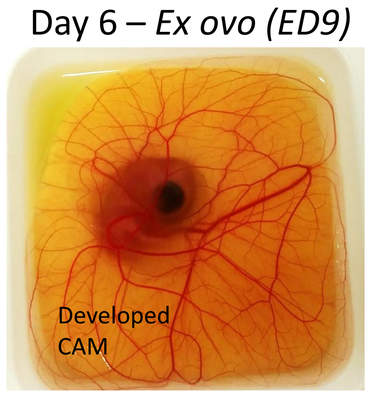
Fertile chicken eggs are incubated at 38°C and 35-45% humidity for 72 hours.

Day 1-*Ex ovo* (ED3)



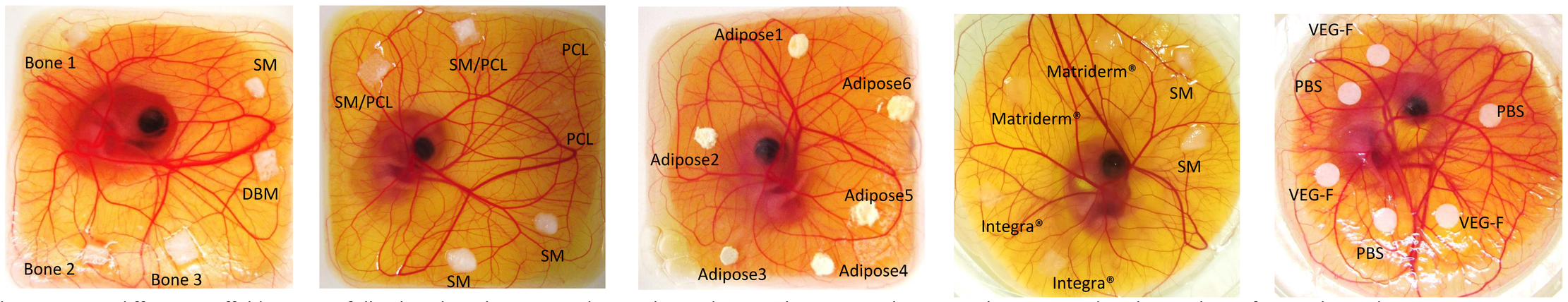
Under sterile conditions, the eggs are cracked and the contents are placed in a shell-less culture system. The embryos are grown in ~80% humidity, 37.5°C incubation temperature and 3% CO_2.

Day 6 – *Ex ovo (ED9)*



The CAM network is extensively developed by day 9.

Day 1 –ED9



At day 9, up to 6 different scaffolds are carefully placed on the CAM under sterile conditions. The *ex ovo* cultures are then returned to the incubator for another 3 days.

Key: Bone 1, Bone 2 and Bone 3 are scaffolds under development for bone; SM: Smart Matrix® (fibrin/alginate scaffold); PCL: Poly-caprolactone, SM/PCL: Smart Matrix®/ Poly-caprolactone; DBM: Demineralised bone matrix; Adipose 1,2,3,4,5,6 are scaffolds under development for breast tissue regeneration; Integra® and Matriderm® are commercially available scaffolds for dermal regeneration; VEG-F: Vascular endothelial growth factor soaked filter disks (positive control), PBS: phosphate buffered saline soaked filter disk (negative control).

Microscopy and Image analysis

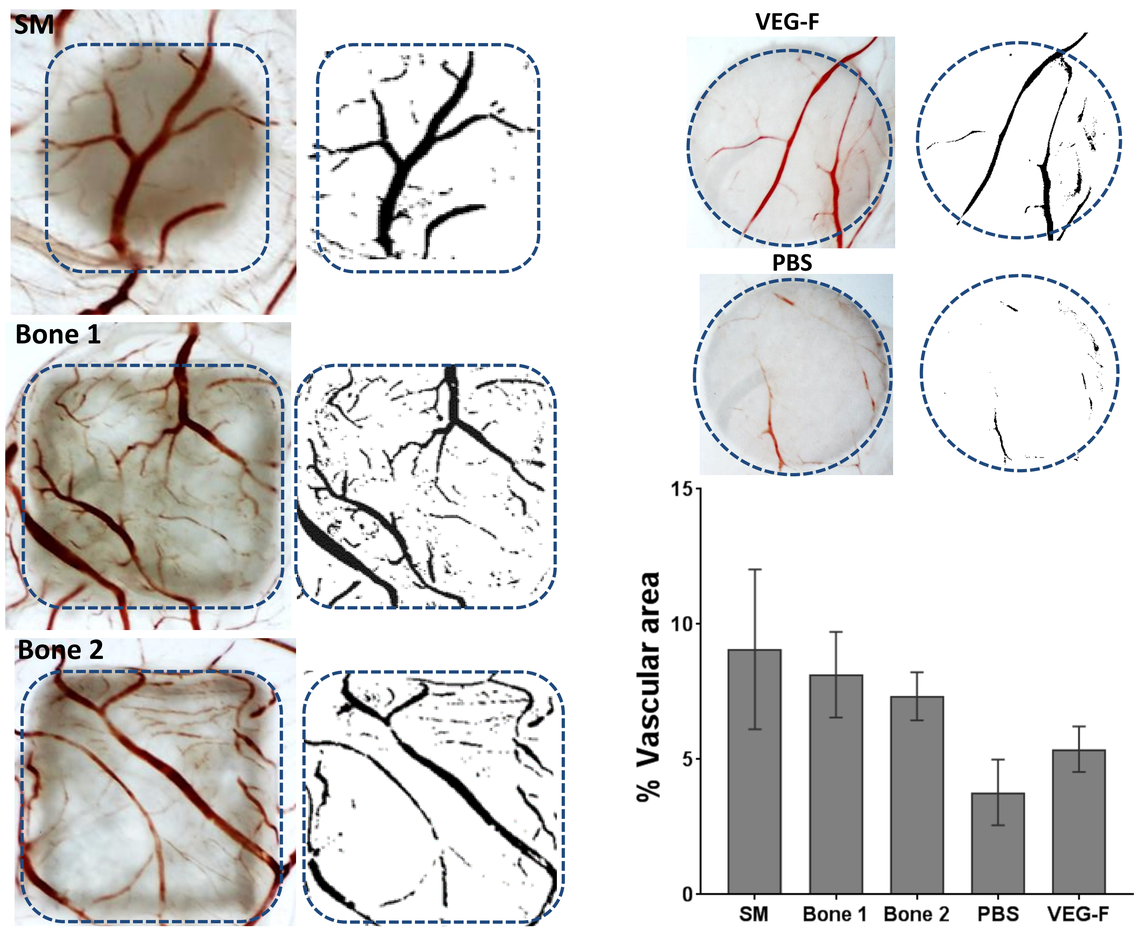


Figure 1. Representative coloured stereo microscope images are shown of different scaffolds placed on the same CAM. Using Image J software, binary images were created of each sample to calculate percentage vascular area that was normalised to the size of the scaffolds. As shown in the graph, SM showed the highest percentage vascular area compared to other scaffolds. PBS (-ve control) showed the lowest percentage of vascular area. Data are presented as means±SEM of n=3 samples. The data were not statistically significant.

Haematoxylin & Eosin Stain

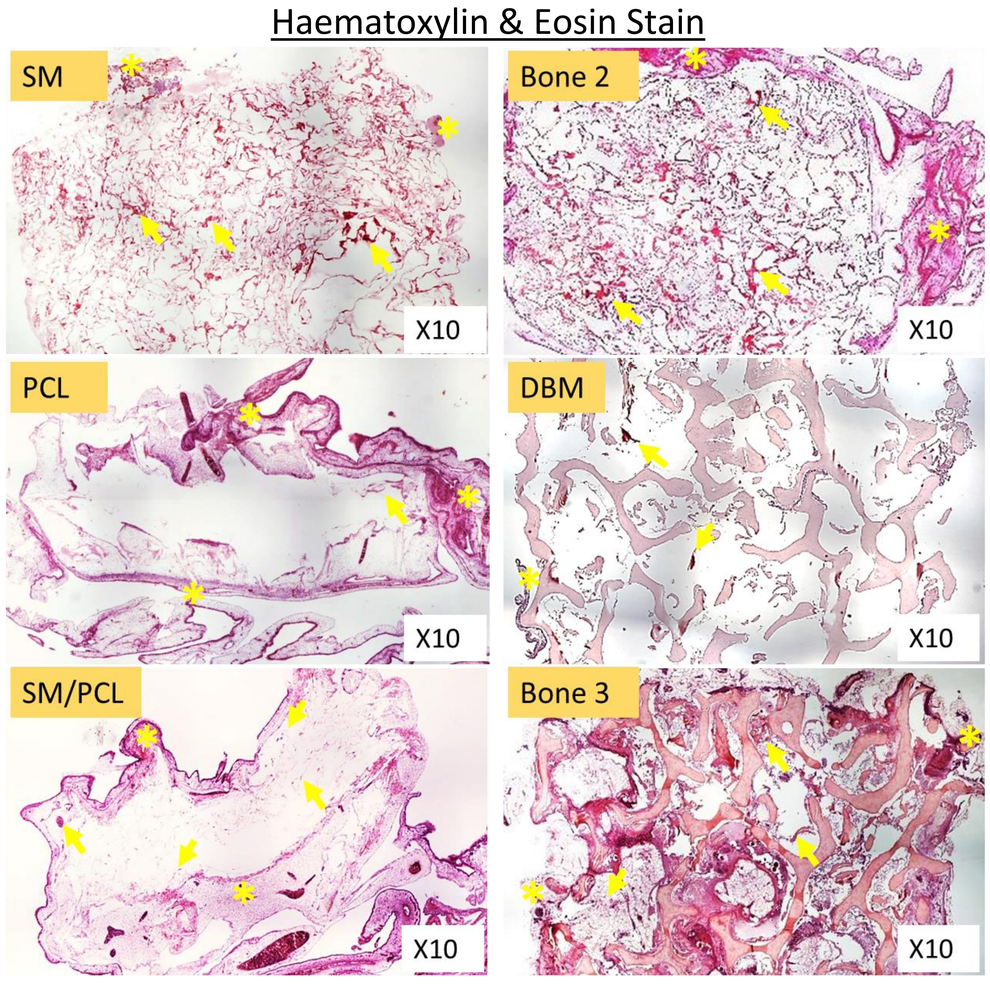


Figure 2. Histological analysis corroborated the image analysis, with more number of blood vessels seen in scaffolds that appeared more angiogenic (SM, SM/PCL & Bone 3) compared to scaffolds that appeared less angiogenic (PCL & DBM). Yellow asterisks denote the surrounding CAM and the yellow arrows point at the blood vessels seen within the scaffolds.

Conclusion

- This *ex ovo* method is an effective way of assessing the angiogenic potential of scaffolds compared to Matrigel assays or ELISAs which are expensive and complex and are far from mimicking the *in vivo* situation.
- This method could potentially be applied routinely as a pre-screening assay to validate scaffolds prior to *in vivo* animal studies.

References:

[1] O'Brien, F.J., (2011). Materials Today, 14(3)

[2] D.S. Dohle, S.D. Pasa, S Gustmann, et al., (2009) *J Vis Exp*. (33).

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