A Proof of Concept for Biomaterial Testing: *Ex Ovo* Chorioallantoic Membrane Assay for Pre-Screening Biomaterials intended for Clinical Application

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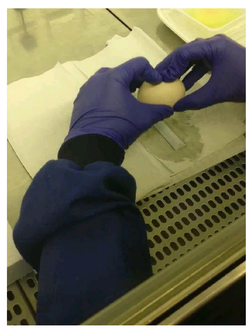
Background: Chorioallantoic membrane (CAM) of chicken embryos have been used as a model to study angiogenesis *in ovo* for nearly 20 years[1] Whether or not it could be used as proof of concept for biomaterial testing is currently unknown. We used the *ex ovo* CAM assay to substantiate whether structure or composition of a biomaterial could influence its angiogenic properties and therefore, determine the feasibility and accuracy of this method for biomaterial testing.

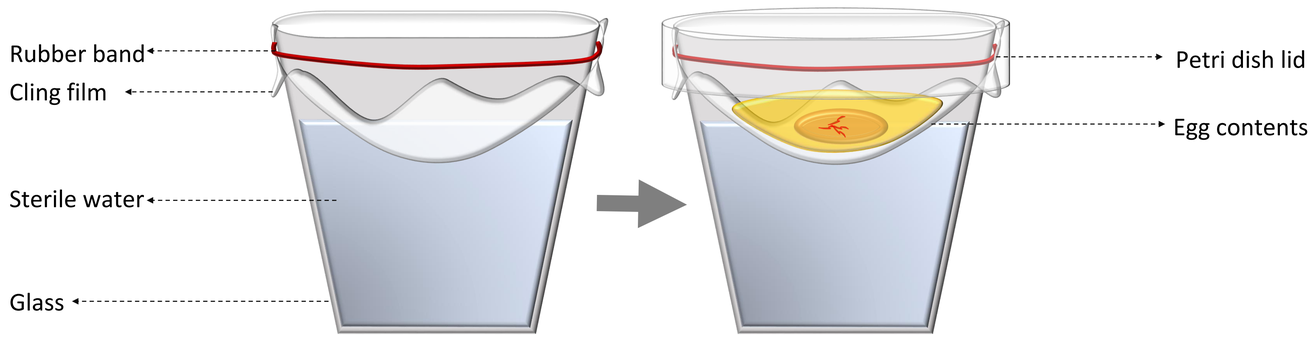
Aim: To assess the feasibility of CAM assays for examining how porosity and composition of biomaterials affects their angiogenic potential

Methods & Results

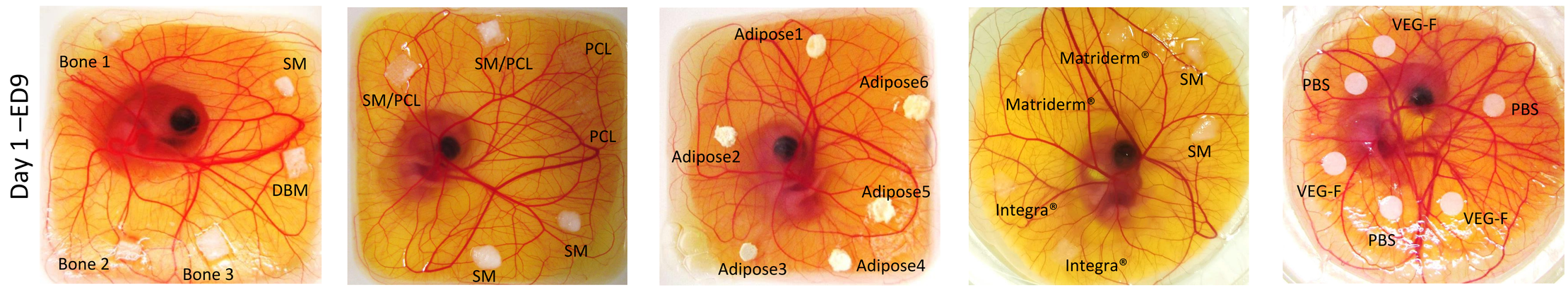
Incubate eggs

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





At day 3, 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.



The CAM network is extensively developed by day 9. At day 9, up to 6 different biomaterials are placed on the developing CAM. At day 12 the developing embryo is sacrificed by cryopreservation and fixation in 4% paraformaldehyde. The biomaterials are harvested and examined for vascular infiltration using imaging techniques.

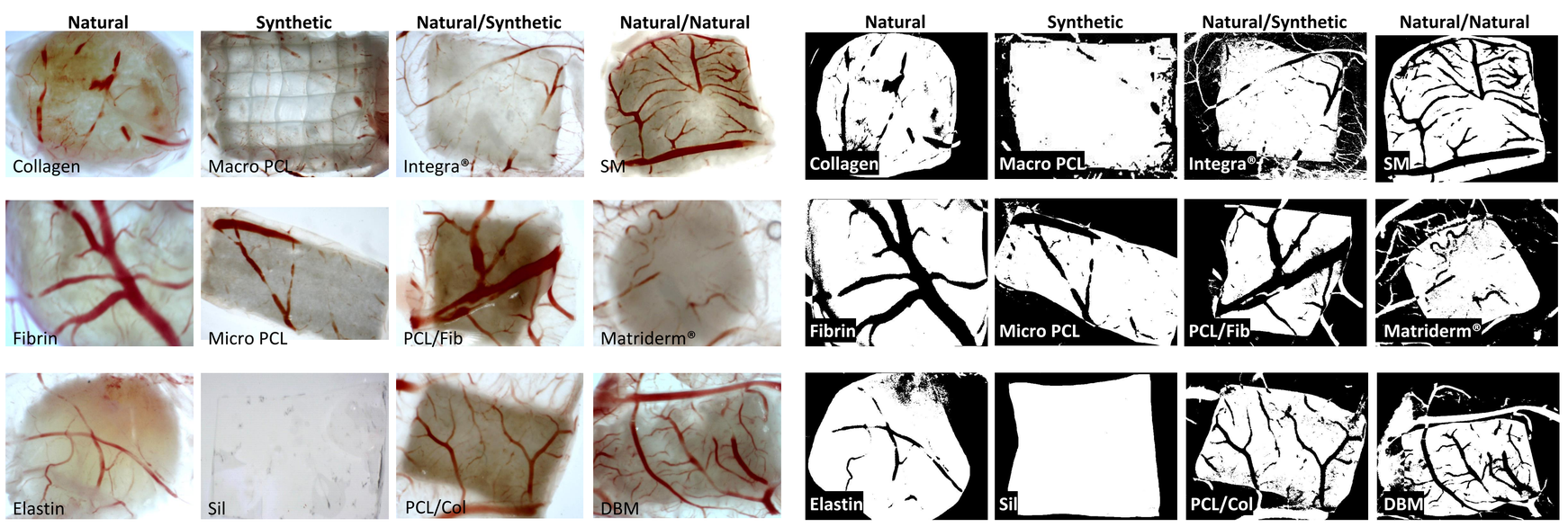


Figure 1. Representative coloured stereo microscope images are shown of different biomaterials. Using Image J software, binary images were created of each sample to calculate percentage vascular area that was normalised to the size of the scaffolds. As shown in the graph, comparative differences were observed in the total percentage vascular area of different combinations of biomaterials used. Data are presented as means ± SEM of n=3 samples.

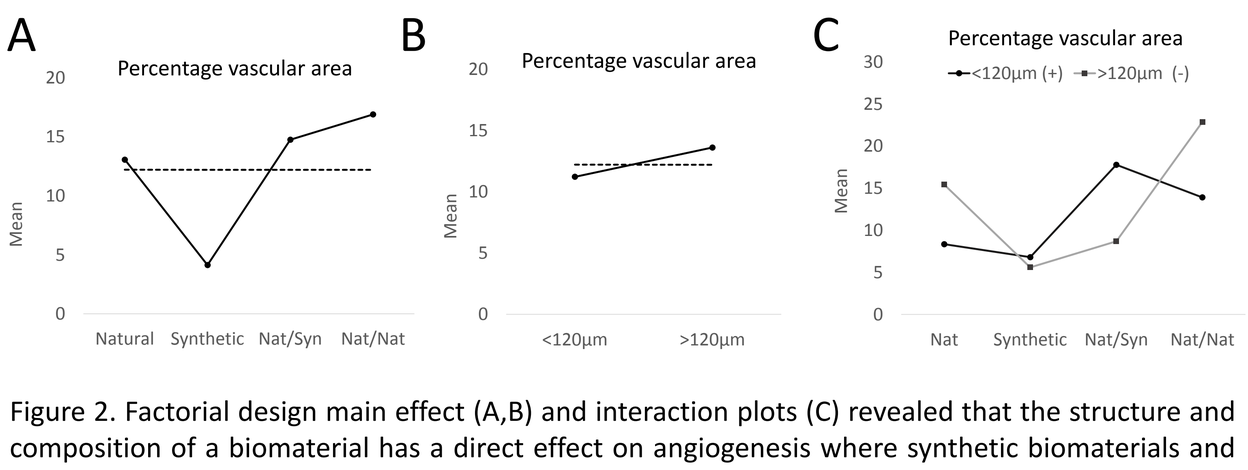


Figure 2. Factorial design main effect (A,B) and interaction plots (C) revealed that the structure and composition of a biomaterial has a direct effect on angiogenesis where synthetic biomaterials and biomaterials with pores less than 120 µm are less angiogenic than either of the combinations. The interaction plots revealed a strong interaction between pore size and composition where higher pore size and a combination of Nat/Nat polymer shows the highest percentage vascular area.

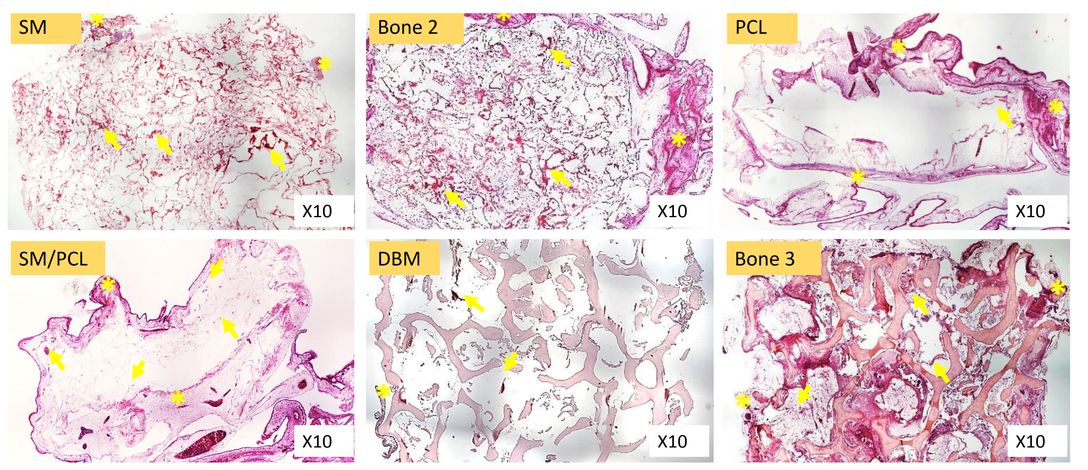


Figure 3. Histological analysis corroborated the image analysis, with more number of blood vessels seen in scaffolds that appeared more angiogenic (SM, SM/PCL & Bone 3) compared to scaffolds that appeared less angiogenic (PCL & DBM). Yellow asterisks denote the surrounding CAM and the yellow arrows point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

Conclusion

- The data presented suggests that a biomaterials' structure as well as composition has a direct affect on its angiogenic capacity and that this *ex ovo* method is an effective way of assessing a biomaterials angiogenic potential.
- This method could potentially be applied routinely as a pre-screening assay to validate scaffolds prior to *in vivo* animal studies.

References:

1. Schlatter P,et al. Microvasc Res. 1997;54(1):65-73.

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