Ex Ovo chorion allantoic membrane as a pre-screening model for testing clinical biomaterials for bone tissue regeneration

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Background: Chorion allantoic membranes (CAMs) of the chicken embryos have been used as a model to study angiogenesis *in ovo* for nearly 20 years[1]. However, there are disadvantages associated with the traditional *in ovo* CAM assays which limit an easy access to the developing CAM.

We utilised the *ex ovo* method published before[2] and herein report the accuracy and feasibility of this method to examine and compare the angiogenic potential of clinically utilized scaffolds as well as scaffolds under development for bone tissue regeneration.

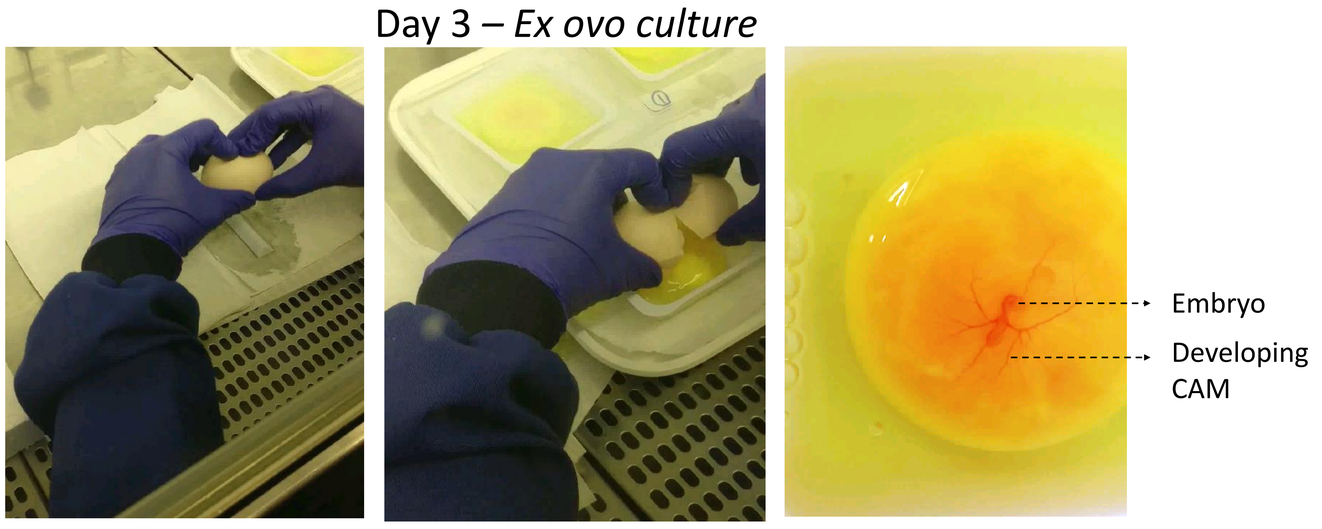
Aim: To establish a pre-screening method for assessing the suitability of biomaterials intended for clinical use

Method

Incubate eggs

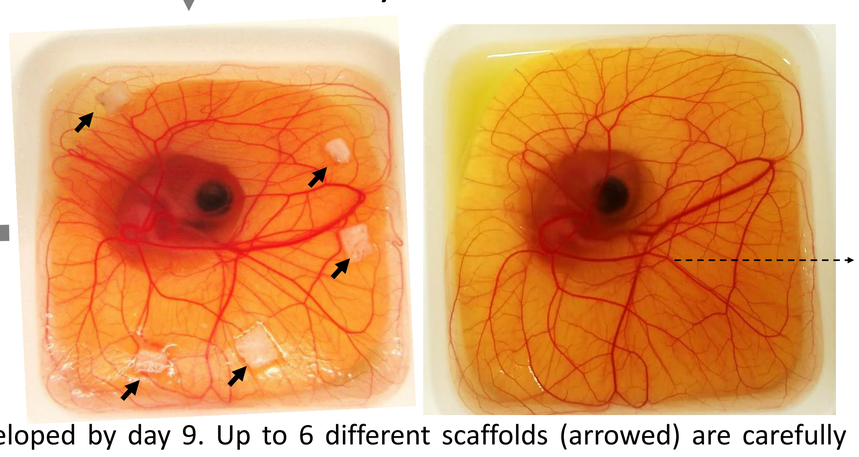
Fertile chicken eggs are incubated at 38°C and 35-45% humidity for 72 hours.

Day 3 – *Ex ovo culture*

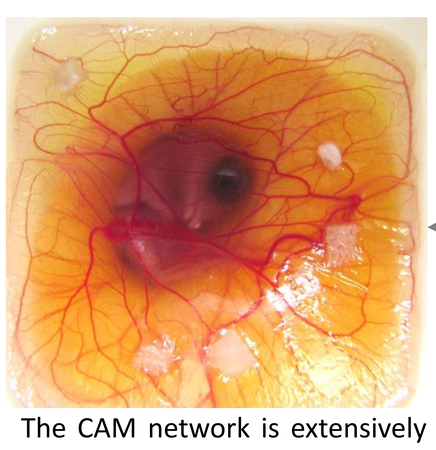


Under sterile conditions, the eggs are cracked open using a triangular magnetic stirrer and the contents are placed in a sterile plastic weighing boat. The viability of the eggs is assessed by looking for a beating heart. The embryos are grown in the shell-less culture system with ~80% humidity ,37.5°C incubation temperature and 3% CO_2.

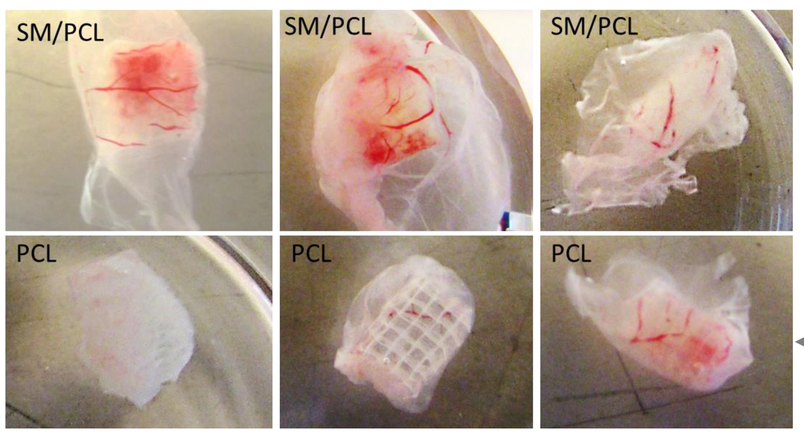
Day 9 – *Ex ovo*



Day 12 – *Ex ovo*



The CAM network is extensively developed by day 9. Up to 6 different scaffolds (arrowed) are carefully placed on the CAM under sterile conditions. The *ex ovo* cultures are then returned to the incubator for another 3 days.



At day 12, the embryos are sacrificed by decapitation and the scaffolds with the surrounding CAM are carefully harvested. The scaffolds are washed in PBS and fixed in 4% PFA and imaged using the GXM-XTL3T101 stereo microscope. SM: Smart Matrix® (fibrin/alginate scaffold); PCL: Poly-caprolactone.

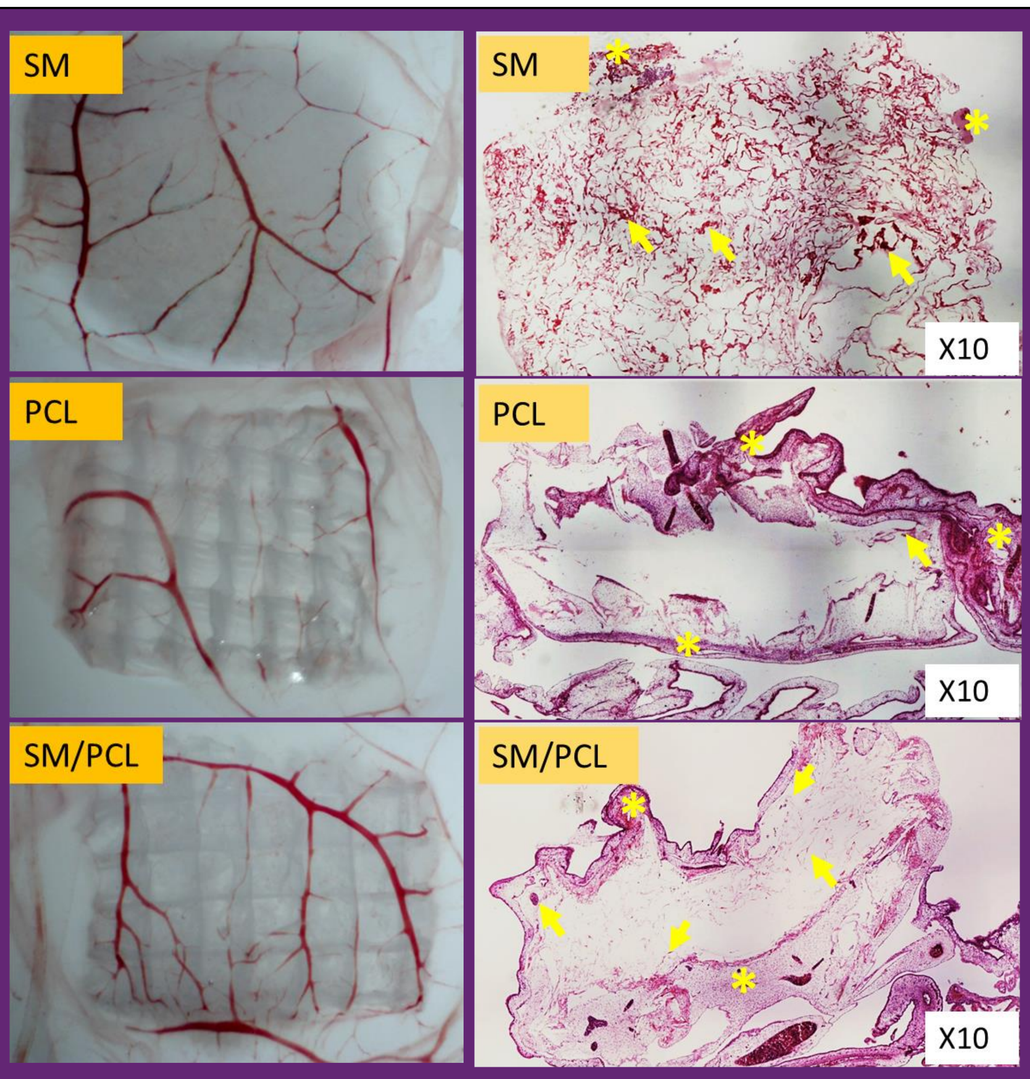


Figure 1. Representative images (left panel) are shown of the macroscopic appearance of different scaffolds placed on the same CAM. Histological images (right panel) confirmed the differences in the blood vessel infiltration seen in different scaffolds. Yellow asterisks display the surrounding CAM and the yellow arrows point at the blood vessels seen within the scaffolds.

Results & Discussion

- At day 12, numerous allantoic vessels were seen to develop radially towards the more angiogenic scaffold in a spoke-wheel pattern compared to the less angiogenic scaffolds.
- Histological analysis corroborated with macroscopic analysis where more number of blood vessels were seen in scaffolds that appeared more angiogenic compared to scaffolds that appeared less angiogenic. An example is shown in figure 1.

Conclusion

- This *ex ovo* method is a safe and inexpensive 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.
- The method described herein allows for a direct comparison of different scaffolds on the same CAM.
- This method could potentially be applied routinely as a pre-screening assay to validate scaffolds for bone tissue engineering prior to *in vivo* animal studies.

References:

[1] P Schlatter, M.F. König, L.M. Karlsson, P.H. Burri, (1997) *Microvasc Res*. 54(1):65-73.

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