Cytokine induced angiogenesis in chick embryos

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Objective

This experiment seeks to determine the effect of the growth factor bFGF on blood vessel formation within the chorioallantoic membrane (CAM).

Introduction

Several growth factors are involved in the development of the blood system in both developing chick embryos and in the yolk sacs themselves (Gilbert, 2003). Basic fibroblast growth factor (bFGF, or FGF2) functions in vasculogenesis, the process during which blood vessels are created (Gilbert, 2003). It is this process which is responsible for the appearance of blood islands in the yolk sac (Gilbert, 2003). Prior to the actual appearance of these islands, mesoderm cells must first from cells called hemangioblasts, a specification brought about by bFGF (Gilbert, 2003). It is these cells that form the blood islands and give rise to either angioblasts or hematopoietic stem cells (Gilbert, 2003). These cells, then, respectively, form endothelial cells and blood cells, in time resulting in the formation of capillaries (Gilbert, 2003). Following vasculogenesis, the process of angiogenesis gives rise to a more complex vessel system derived from these established capillaries (Gilbert, 2003). Vasculogenesis and angiogenesis, in particular, are key within the chorioallantoic membrane (CAM) of the developing embryo. This membrane is located beneath the shell membrane removed from embryos in previous labs. It is here that calcium, needed for theformation of the skeleton of the chick embryo, is absorbed into the blood vessels in contact with the eggshell (Tuan and Lynch, 1983).

Materials

10 day old chick embryos
70 % ethanol
60 mm Petri dish
Whatmann 3MM Filter Paper 3 x 3 mm squares,
soaked in 3 mg/ml Hydrocortisone-acetate and air-dried.
fine forceps
fine scissors
Magic Scotch Tape
4 mL of either bFGF (1.5 μg/ml) or VEGF (0.5 μg/ml) in Dulbecco's Modified Eagles's Medium (DMEM) and 4 mL DMEM
Sterile Howard's Ringers with 50 μg/ml gentamycin (Invitrogen, GIBCO 3772)

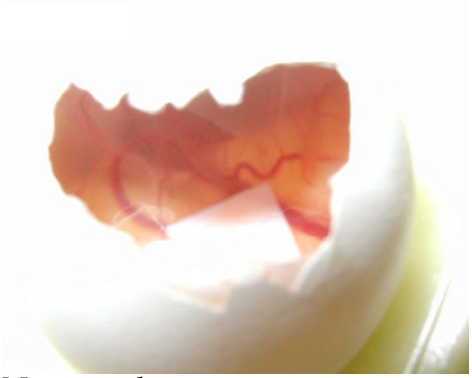
Procedure

1. Obtain 4 mL of either bFGF (1.5 μg/ml in DMEM) or VEGF (0.5 μg/ml in DMEM) and 4 mL of DMEM (as a control). Pour the solution into a petri dish that is labeled with the name of the compound you are working with. Soak four filter paper disks (3mm x 3mm) in the solution for 30 minutes.

2. While the filter paper is soaking, obtain four ten-day-old chick embryos and wash them in 70% ethanol. Label the eggs as either DMEM or bFGF using a pencil. Open the chick embryos at the blunt end of the egg as demonstrated in class. Make as small a hole as is practical.

3. Make a small nick in the shell membrane (the dry, white membrane that lines the air-space). Put two or three drops of Howard's Ringers in the hole and allow it to soak in to help separate the shell membrane from the CAM, which is immediately underneath. Peel back the shell membrane, being careful not to damage the CAM. The CAM is a transparent membrane containing blood vessels.

4. Drop the filter paper over the embryo in the area with the least number of visible blood vessels. Cover the hole in the shells with Scotch tape, and place in the incubator at 37 °C.



Next week

5. Remove the tape covering the holes and extract the filter paper using a pair of forceps. Examine the filter paper for the presence of blood vessels. Count all blood vessels on the filter paper and record the numbers within your lab notebook. Compare the number of blood vessels formed in response to bFGF with the DMEM controls.

References

http://www.millersville.edu/~jcebrathomas/cebra_thomas/DB_lab/Chick/KJohnson/Chick_blood.html

Gilbert, Scott. 2010. *Developmental Biology*, 9th ed. Sinauer Associates, Inc., MA.

Tuan, Rocky S., and Marion H. Lynch. 1983. Effect of experimentally induced calcium deficiency on the developmental expression of collagen types in chick embryonic skeleton. *Dev. Biol.* **100**: 374-386.