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Culturing Chicken Skin from Embryos: Skin Culture Lab Protocol and Results

Based on Procedures Described on the Developmental Biology Web Site

http://www.swarthmore.edu/NatSci/sgilber1/DB_lab/DB_lab.html

Introduction

Within chick embryos, interactions between the epidermis and dermis of the skin result in the formation of preliminary feather buds, and later, specific feather types or cutaneous structures (Gilbert, 2003). The outer layer of skin, the epidermis, is formed from the ectoderm of the developing embryo; it covers the embryo following the development of the neural tube (Gilbert, 2003). After neurulation, the preliminary epidermis undergoes a series of cell divisions and differentiations to form the skin. The final product consists of the basal layer, or germinal epithelium, the spinous layer, the granular layer, and cells known as keratinocytes (Gilbert, 2003).

Altogether, the cells of the epidermis are connected and form an epithelium, or sheet of cells (Gilbert, 2003). Under the epidermis lies the dermis, which has its origins from the mesoderm and is a mesenchymal layer of “loosely” associated cells (Gilbert, 2003). The epidermis signals to the dermis through the production of sonic hedgehog and transforming growth factor-beta (SHH, TGF- β) proteins. Once the dermis receives these factors its cells aggregate, and in turn signal back to the epidermis, causing certain genes to be activated (Gilbert, 2003). As a result, different cutaneous structures are formed, based on the location of the dermis (Gilbert, 2003). Of particular note, such interactions between the epidermis and dermis layers lead to the initial development of feather buds. In the early stages, parts of the epidermis thicken and form epidermal palcodes, under which the dermal cells aggregate to form dermal papilla – a small

projection of tissue at the base of the feather (Bellairs et al., 1998). In time, feather buds appear as these structures elongate, forming the basis from which feathers will be formed (Bellairs et al., 1998)(figure 2C).

Understanding that the development of the skin can occur autonomously from the rest of the organism, specifically in culture, has proven vital to the development of techniques to treat burn injuries, as well as factored into the creation of artificial skin such as Apligraf (OI, 2003; Scott, 2000). Apligraf, composed of a collagen matrix, fibroblast cells (which contribute to the dermis), and keratinocytes, has been used to treat both diabetic and venous ulcers. (OI, 2003; Cebra-Thomas, 2004). If the complexities of skin formation can be understood, strides could be made in the development of skin grafts, allowing for greater availability to patients. In addition, greater understanding of the chemical signals between the dermal and epidermal layers could be used to design treatments for burn patients whose skin layers have been damaged, helping the patient's own skin regenerate rather than depending on whole skin grafts .

This experiment seeks to demonstrate that the skin of eight-day-old chick embryos, if removed, can continue to develop to the feather bud stage in culture(figure 2C). We expect that, as in past experiments, this development will be successful to the point of late feather bud development, but will arrest before any actual feather development takes place (as it would in-ovo) (Cebra-Thomas, 2004).

Procedure

1. Obtain a six-well plate. In each well, pipette 1.8 ml of Hank's Balanced Salt Solution 1X.

Rest Transwell 3452 culture inserts (24mm diameter) over the wells containing the salt solution.

2. Wash eight eight-day-old chick eggs with 70% ethanol. Open the chick embryos as demonstrated in class (at the blunt end of the shell, where the air sac is located), using forceps. Record the stages of the embryos, making sure to keep track of which embryo is at which stage as they are removed from their shells.
3. Empty the embryos into separate petri dishes and remove them from their yolks. Then place the isolated chick embryos, using a plastic spoon, into smaller petri dishes (60mm) containing Howard Ringers's Solution (see DB Lab CD; Cebra-Thomas, 2001).
4. Cut away the anterior portion (i.e. remove the head) of the embryo using either scissors or forceps. Make a shallow incision along the trunk of the embryo and peel off as large a portion of skin as possible. Place this skin segment onto an insert covering one of the wells filled with Hank's solution. Make sure the "inside " portion of the skin is touching the disk (DB Lab CD; Cebra-Thomas, 2001).
5. Place well plates with skin cultures into an incubator, set at 5% CO₂ and 37° C. Observe development of skin cultures periodically over a week's time, noting the appearance and development of feather buds. Record observations and photograph skin cultures at every observation point to determine the extent to which the skin develops.
6. Limbs from the embryos may also be placed in analogous culture conditions (same media) with the caveat of using non-collagen culture inserts so that the limbs won't stick too closely to the insert. These too may be observed periodically to determine if there would be any further development of the limbs, or feather buds on the limbs.