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Wound healing after excision of mantle tissue from the Akoya pearl oyster, *Pinctada fucata*

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Abstract

Pearl oysters are usually sacrificed to donate mantle tissue for pearl production. However, if oysters are anaesthetized, they are able to survive mantle excision and regenerate this tissue. Mantle excision causes a large wound and severs the pallial artery that necessitates rapid wound repair to avoid death by bleeding. This study was undertaken to assess the wound healing process in the mantle of the Akoya pearl oyster, *Pinctada fucata*, following mantle excision. Forty-seven *P. fucata* were relaxed with 2.5 mL L^{-1} propylene phenoxetol before mantle tissue was excised. Oysters were relaxed and sacrificed 1, 3, 6, 12, 25, 36, 48, 66, 80 and 105 h after excision to assess mantle healing using histological techniques. Muscular contraction that effectively reduced the size of the wound was observed within 1 h after mantle excision. Accumulation of haemocytes and connective tissue occurred 3–6 h after excision and wound plugging was achieved within 6 h of excision. Proliferation of epithelial cells to cover the wound site was observed within the first 25 h after mantle excision and growth of connective tissue and formation of the pallial artery were observed within 105 h after mantle excision.

Keywords: Pearl oyster; *Pinctada*; Mantle tissue; Wound healing

1. Introduction

Mantle tissue in bivalves has a wide array of functions including nutrient storage, direction of feeding currents and sensorial capacity (Morton, 1983a,b; Zandee et al., 1980). Another major function of the mantle is biomineralization in which specialized secretory cells produce the shell (Simkiss and Wilbur, 1989). Biomineralization is also used as a defence mechanism by which foreign particles or parasites coming into contact with the bivalve body, are 'insulated' in shell material (Lowenstam and Weiner, 1989). This defence mechanism frequently produces natural pearls. In species with a nacreous lining to their shells, such as pearl oysters (family Pteriidae), this process provides the basis for cultured pearl production where a piece of mantle tissue from a donor oyster is grafted with a round nucleus into the gonad of a recipient pearl oyster. The grafted mantle tissue proliferates to form a layer of secretory tissue that deposits layers of nacre onto the nucleus. This process results in the formation of a cultured pearl after approximately 2 years (Norton et al., 2000).

Mantle donors are usually sacrificed for cultured pearl production (Kafuku and Ikenoue, 1983); however, we recently reported that mantle tissue can be excised from pearl oysters while keeping them alive (Acosta-Salmón et al., 2004). Oysters are able to survive the removal of a large piece of mantle tissue and are capable of complete regeneration of this part of the mantle and all its internal structures (Acosta-Salmón et al., 2004; Acosta-Salmón and Southgate, 2005). Excision of mantle tissue from pearl oysters causes a large wound and severs the pallial artery; the wound must be quickly repaired to avoid death by bleeding.

The wound healing process in bivalves involves rapid accumulation of haemocytes shortly after wounding. This prevents loss of haemolymph by forming a mechanical barrier or plug which seals the wound and produces an 'extra-cellular matrix' or basement membrane which is then used to regenerate epithelial cells (Ruddell, 1971; Suzuki et al., 1991; Suzuki, 1992). Hodgson (1982) reported that wounds resulting from

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amputation of the siphonal tissue of the Tellinid bivalve, *Scrobicularia plana*, healed quickly. Connective tissue began accumulating around the site of the lesion within three hours and epidermal cells fully covered the site of the wound 72 h after amputation (Hodgson, 1982).

There is little information available on wound healing in pearl oysters and the majority relates to wounds to the gonad produced after the grafting procedure for pearl production (Suzuki et al., 1991; Suzuki, 1992; Awaji and Suzuki, 1995). Furthermore, there are few studies related to the healing of mantle tissue in bivalve molluscs (e.g., Ruddell, 1971) and, other than the study of Hodgson (1982), there are no other detailed reports describing progressive wound healing in bivalves. This paper provides a detailed description of the healing response of the Akoya pearl oyster, *Pinctada fucata*, following excision of mantle tissue.

2. Material and methods

The *P. fucata* (Gould) used in this study were hatchery propagated (Southgate and Beer, 1997) and maintained on a surface long-line (Gervis and Sims, 1992) at Orpheus Island, Great Barrier Reef (north Queensland), Australia (18°35′S, 146°29′E).

This experiment was conducted in November 2004 (austral summer). Forty-seven 3-year-old oysters were anaesthetised with 2.5 mL L^{-1} propylene phenoxetol (Norton et al., 1996; Acosta-Salmón et al., 2005) and sections of mantle tissue were removed from the left mantle lobe (Fig. 1). After mantle excision, oysters were placed into a seawater aquarium system (20 °C) and between three and five oysters were randomly removed from this population 1, 3, 6, 12, 25, 36, 48, 68, 80 and 105 h after mantle excision to assess mantle healing. No food was provided during this period. Oysters used for this purpose were relaxed as described above and sacrificed. Samples of mantle tissue were sectioned for standard histological preparation. All samples were preserved in aqueous Bouin's solution for 24 h, rinsed in 70% ethanol, dehydrated, embedded in paraffin, sectioned to 5 μm and stained with MSB trichrome (Bradbury and Gordon, 1977; Hodgson, 1982; Culling et al., 1985). Microphotographs were obtained using an Olympus BH-12 microscope equipped with an Olympus DP12 digital camera.

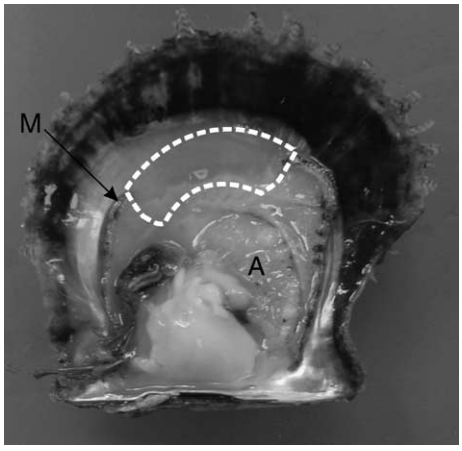

Fig. 1. *Pinctada fucata* with one shell valve removed showing adductor muscle (A) and mantle edge (M). The area outlined by the dotted line indicates the section of mantle tissue excised.

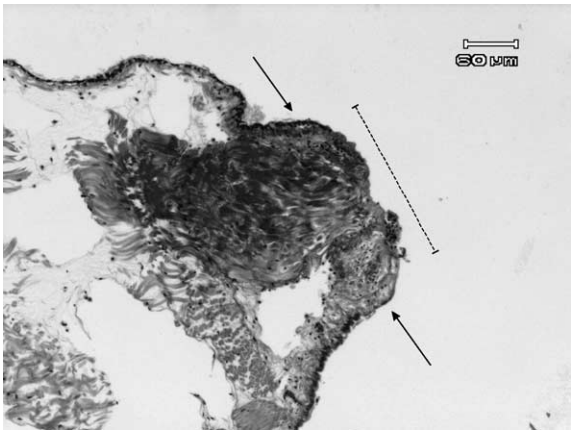

Fig. 2. Histological view of the mantle tissue of *Pinctada fucata* one hour after excision showing the reduction of the wound site (arrows). The dotted line indicates the wound size.

3. Results

No mortality of *P. fucata* was observed after mantle excision. The first visible response to mantle excision was seen

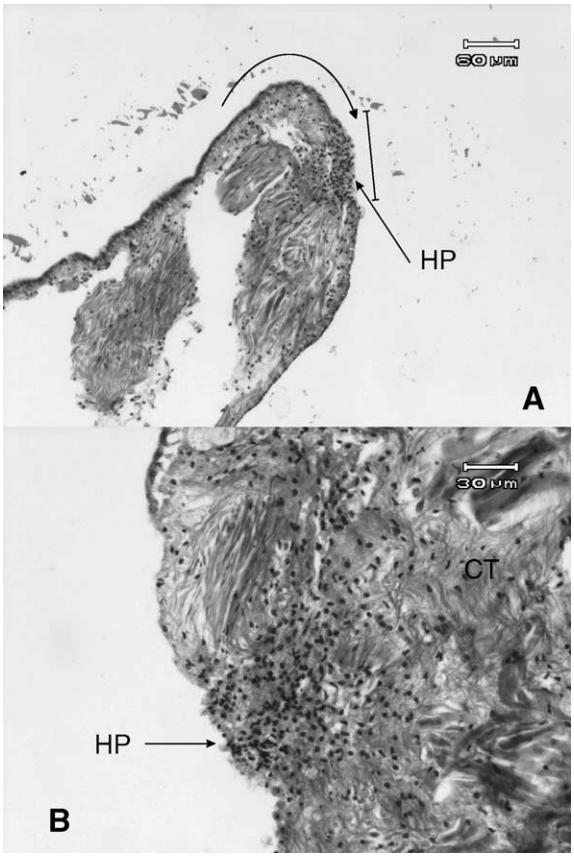

Fig. 3. Sections of the mantle tissue of *Pinctada fucata* six hours after excision showing A: muscular contraction at the wound site (curved arrow) and haemocyte plug (HP), and B: detail of HP and connective tissue (CT).