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Wound repair in *Pocillopora*

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ABSTRACT

Corals routinely lose tissue due to causes ranging from predation to disease. Tissue healing and regeneration are fundamental to the normal functioning of corals, yet we know little about this process. We described the microscopic morphology of wound repair in *Pocillopora damicornis*. Tissue was removed by airbrushing fragments from three healthy colonies, and these were monitored daily at the gross and microscopic level for 40 days. Grossly, corals healed by Day 30, but repigmentation was not evident at the end of the study (40 d). On histology, from Day 8 onwards, tissues at the lesion site were microscopically indistinguishable from adjacent normal tissues with evidence of zooxanthellae in gastrodermis. Inflammation was not evident. *P. damicornis* manifested a unique mode of regeneration involving projections of cell-covered mesoglea from the surface body wall that anastomosed to form gastrovascular canals.

1. Introduction

Invertebrates experience injuries from many sources, and tissue loss is one of the lesions more frequently seen in scleractinian corals (Lindsay, 2010; Moses and Hallock, 2015). In corals, tissue loss has many potential origins such as competition and predation (Rodriguez-Villalobos et al., 2015; Moses and Hallock, 2015), anchor-induced trauma, or infectious disease (Work et al., 2012). Many coral diseases such as White Syndrome, White Plague, White Band, and Skeletal Eroding Band, are characterized by tissue loss (Ainsworth et al., 2007; Page and Willis, 2008; Work and Aeby, 2011; Work et al., 2012), that in some cases present multiples aetiologies that could even change over time (Work et al., 2012).

Regardless of cause, corals have abilities to recover from injuries (Bak and Steward-Van Es, 1980; Kramarsky-Winter and Loya, 2000; Soong and Chen, 2003). Healing and regeneration play a vital role for the individual allowing recovery of tissue integrity that permits normal ecological and biological functions (Sonnemann and Bement, 2011; Giangrande and Licciano, 2014; da Silveira and van't Hof, 1977). However many factors, such as high nutrients concentration, sedimentation, turbidity, high irradiances (Moses and Hallock, 2015; Sabine et al., 2015), health condition of the individual (Ruiz-Diaz et al., 2016), coral species or morphology (Hall, 1997), and even size (Bak and Steward-Van Es, 1980) and morphology of lesion (Meesters et al., 1997) can influence the capacity of corals to recover from injuries thereby affecting reproduction, colony growth and defenses abilities against pathogens (Moses and Hallock, 2015).

Whilst there is considerable information on wound repair in corals at the gross level, studies of repair processes at the tissue and cell level are uncommon. Wound repair is a process that includes cell migration, adhesion, and contraction involving complex signaling pathways that begin immediately after injury (Brockes and Kumar, 2008). Wound repair, has been studied at the cellular level in some Cnidaria including soft corals (da Silveira and van't Hof, 1977; Meszaros and Bigger, 1999) anemones (Singer, 1971; Patterson and Landolt, 1979; Young, 1974), and recently the scleractinian corals *Montipora capitata* (Work and Aeby, 2010) and *Porites cylindrica* (Palmer et al., 2011). These studies show that regeneration of tissue in cnidaria occurs by different mechanisms. For instance, in the perforate coral *M. capitata*, wound repair does not involve migration of mesogleal cells to the site of injury region (Work and Aeby, 2010) in contrast to *Porites* (Palmer et al., 2011), soft corals (Meszaros and Bigger, 1999), and anemones (DuBuc et al., 2014) that all manifest a prominent inflammatory response. Calicoblastic or epidermal pluripotentiality in *Montipora* may play a role in tissue regeneration (Work and Aeby, 2010) whereas in *P. cylindrica*, wound repair is exemplified by formation of a clot or plug of degranulating melanin-containing

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cells and the aggregation of cell debris. In *Porites*, inflammation is evidenced by the aggregation of granular amoebocytes at the wound edge (Palmer et al., 2011).

While data exists on wound repair (at the cellular level) for two perforate scleractinian corals, we have no information on this process in non-perforate corals such as *Pocillopora* spp. Given the vast difference in tissue organization between these two groups, it is likely that the process of wound repair might differ. Understanding wound repair in *Pocillopora* is important, because this genus is dominant in the Mexican Pacific (Reyes-Bonilla, 2003) and is affected by unexplained tissue loss (White Syndrome) that is widespread (Rodríguez-Villalobos et al., 2014; Rodriguez-Villalobos et al., 2015). *Pocillopora* are also particular in the Mexican tropical Pacific in that they are the first to colonize high-energy sites where conditions are not optimal for growth (Calderón-Aguilera et al., 2007) suggesting that, in addition to rapid growth and recruitment (Coles and Brown, 2007), Pocilloporidae may have efficient tissue repair mechanisms in face of injuries. Understanding the morphologic markers of wound repair in this species might also inform interpretation of histopathology investigations, just as understanding of wound repair in *Montipora* has facilitated interpretation of microscopic lesions in this genus in the Central and Western Pacific (Work et al., 2015, 2014). Therefore, the aim of this study was to describe the gross and microscopic pathology of wound repair in *Pocillopora damicornis*.

2. Materials and methods

Three colonies of apparently healthy *P. damicornis* were collected from Pichilingue Bay, La Paz, BCS, Mexico and immediately transported to the laboratory. There, they were fragmented into ca. 3 cm pieces and placed into a single water tank (300 L) with flow through filtered (100 μm) seawater under ambient temperature and natural light. After fragmentation, a 1 cm wide section of tissue was removed at the base of the fragment using a double action airbrush (Paasche Air Brush®). All fragments (180 in total) were maintained in the same tank during the experiment. Tanks were cleaned by vacuuming the walls three to four times per week in order to prevent algal overgrowth. Two replicate fragments per colony were sampled following tissue removal on Day 0, and the process repeated daily until day 40. All fragments were photographed, fixed with 10% formalin seawater solution, and decalcified in HCl (10%) buffered with 0.7 g/L EDTA, 0.14 g/L sodium tartrate, 0.008 g/L potassium sodium tetrahydrate. Samples were then embedded in paraffin, sectioned (5 μm thick), and tissues were stained with Harris's hematoxylin and eosin (Humason, 1967). Two tissue sections (slides) per colony per day were analysed under microscope. For microscopic interpretation, tissues were divided into surface body wall (SBW) comprising the coenenchyme and polyp and the basal body wall (BBW) including mesenterial filaments. All experiments were done at the Laboratorio de Histología, Universidad Autónoma de Baja California Sur (UABCS).

3. Results

3.1. Gross pathology

At Day 0, the edge of the lesion appeared irregular with bare white skeleton bereft of tissues or small clumps of mucus. The lesion remained essentially unchanged until Day 8 when a smooth and white margin a few millimeters wide became evident progressing from the edge of pigmented tissues. This white tissue continued to overgrow bare skeleton at the base of the fragment as new polyps developed. Extended tentacles of the polyps were seen at day 30 onwards. Re-pigmentation of regenerating tissues was not evident during the 40 days of experimentation (Fig. 1).

3.2. Microscopic pathology

At Day 0, mesoglea of the BBW was exposed and bereft of cells and polyps (Fig. 2A and B). At day 2, bare mesoglea at the edge of the lesion began to be colonized by clusters of cuboidal cells mixed with mucocytes with formation of gastrovascular canals (Fig. 2C and D). Near the edge of the lesion, projections of mesoglea covered by cuboidal cells originated from SBW (Fig. 2C) and eventually

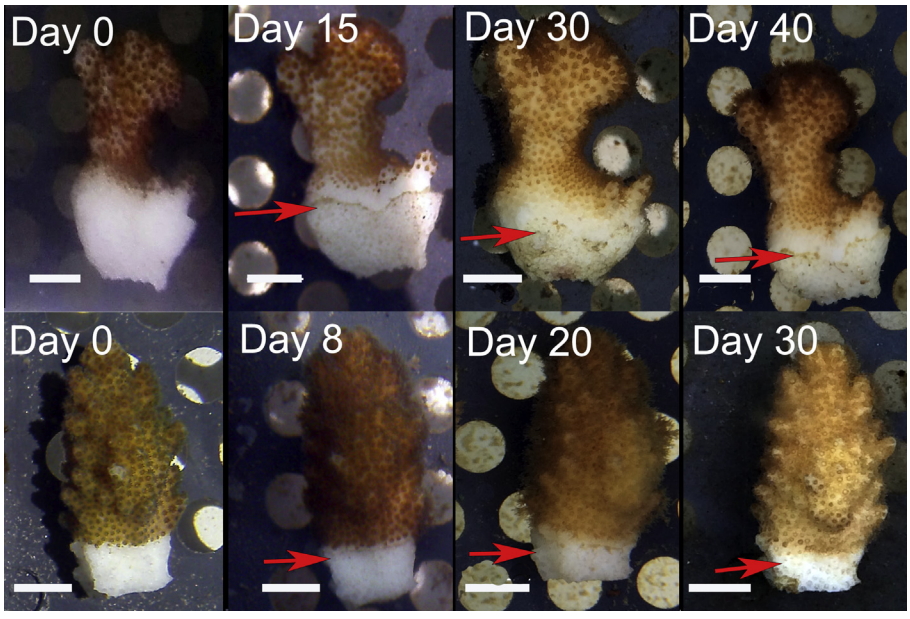

Fig. 1. Fragments of *Pocillopora* sp. with experimentally induced trauma showing progression of healing over time (red arrow). Scale bar = ca. 1 cm. Arrows at days 8–40 point to leading edge of apigmented regenerating tissues overgrowing bare skeleton. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)