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AN ULTRASTRUCTURAL STUDY OF THE SUPPORTING TISSUES OF SEA URCHIN (*LYTECHINUS VARIEGATUS*) TEETH

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ABSTRACT

The teeth of sea urchins are connected to the calcareous jaw plates, known as pyramids, by a ligament consisting of collagen fibers and microfibrils synthesized by fibroblasts in the aboral growth zone of the tooth. This ligament needs to be sufficiently stiff to hold the teeth firmly when the animal scrapes hard surfaces, but also needs to be flexible enough to allow the teeth to move outwards during growth. To understand the mechanisms that regulate the growth and stiffness of sea urchin teeth, we have examined the ultrastructural organization of the supporting structures of *Lytechinus variegatus* teeth. Electron microscopy showed that collagen fibrils were mechanically attached to the column-like structures in the tooth, to produce a firm, irreversible connection. The ligament-attaching region in the jaws was formed by cavities that ramificated in the deep portions. The collagen fibrils were not mechanically linked to the jaws. These findings suggest that the stiffness of the ligament is mediated by chemical bonding between the collagen fibrils and the jaw surface. The cavities present in the pyramids greatly increased the surface area and strengthened the area for the bonding of collagen fibrils.

Key words: Collagen, extracellular matrix, pyramid, sea urchin, teeth

INTRODUCTION

Sea urchins (phylum Echinodermata) are marine invertebrates with five elongated and slightly curved teeth that are part of the masticatory apparatus, the lantern of Aristotle. This apparatus has a five-fold radial symmetry and is located at the base of the lower oral hemisphere of the sea urchin's body, juxtaposed to the substratum. Calcareous jaw plates known as pyramids hold the teeth together. The pyramids are connected to each other by transverse muscle fibers [11]. Most sea urchin species feed by scraping algae from rock surfaces. The region involved in tooth formation has a high cell density and the teeth are continuously renewed [7]. To compensate for shortening of the teeth during chewing, new tooth material is constantly synthesized in the aboral soft growth zone, the plumula. During growth, the teeth slide slowly along the jaw at about 1-1.5 mm/week [6,9]. Each tooth is connected to the jaw by a ligament consisting of collagen fibers and microfibrils that are synthesized by fibroblasts in the aboral growth zone of the tooth. The tooth ligament contains highly sulfated acid proteoglycans that apparently connect to the collagen fibrils [2]. This ligament is sufficiently stiff to hold the teeth firmly when the animal scrapes hard surfaces, but is flexible or soft enough to allow the movement of the teeth as they grow. To understand more about the mechanisms that regulate the growth and stiffness of sea urchin teeth, we have examined the ultrastructural organization of the supporting structures of *Lytechinus variegatus* teeth.

This paper is dedicated to the memory of our colleague Prof. Gregorio Santiago Montes.

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MATERIAL AND METHODS

Transmission electron microscopy

The masticatory apparatus (lantern of Aristotle) of five adult sea urchins (*Lytechinus variegatus*) was removed intact from each animal. The lantern was divided into five equivalent subunits by cutting the muscle that separates these subunits. Each subunit, which consisted of a tooth encased in a calcareous plate (pyramid), was fixed in Karnovsky solution for 24 h at 4^{o}C. For transmission electron microscopy (TEM), three subunits were dehydrated in a graded series of acetone and embedded in araldite resin. Transversal sections 0.1 mm and 1 mm thick were obtained with a diamond disk at low speed. This procedure was necessary to avoid distortion of the soft tissues during decalcification, when the entire mineral skeleton or framework was removed. The 0.1 mm-thick sections were used in light microscopy, and the 1 mm slices were decalcified in 5% EDTA for 7 days. After decalcification, the slices were post-fixed in 1% osmium tetroxide and reembedded in araldite resin to obtain ultrathin sections. These sections were contrasted with uranyl acetate and lead citrate, and examined in a Zeiss EM-10 electron microscope.

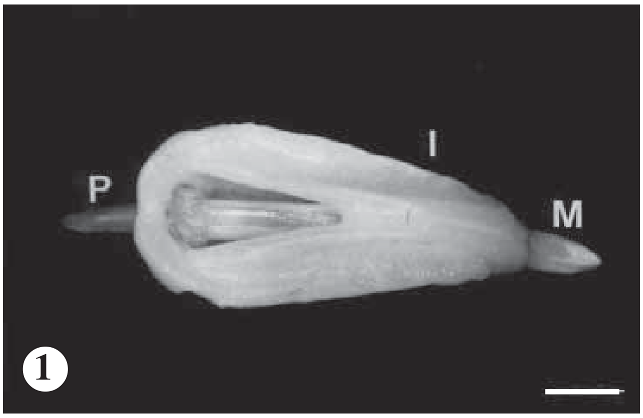
Scanning electron microscopy

The teeth of non-decalcified subunits were separated from the pyramids and immersed in 0.1% sodium lauryl sulfate (Sigma) for 10 min at room temperature in an ultrasonic cleaner in order to partially remove the organic material but still preserve the collagen fibers. The specimens were then mounted on metal stubs, coated with gold using a Balzers MED 010 sputter coater, and examined in a Zeiss DSM900 scanning microscope.

RESULTS

The sea urchin teeth consisted of three zones along their long axis: the mature zone, which emerges from the jaw and forms a chewing edge, the intermediate zone connected to the jaw, and the soft aboral growing zone or plumula where new tooth elements are formed (Fig. 1). The teeth were held by collagen fibers in two small regions (pyramids) of the mandible (Fig. 2). Scanning electron microscopy showed that the internal portion of the tooth, which faces the intermediate portion of the jaw, had a longitudinal groove (Fig. 3). In the intermediate zone this groove had column-like projections. Small cylinders of calcified material that resembled bridges linked the columns. These bridge-like structures appeared broken at the beginning of the mature zone, and gradually disappeared as the teeth erupted. Collagen fibrils formed bundles around the columns and bridges. The collagen fibrils embraced these structures (Fig. 4).

The pyramids had cavities that ramified into the deep portion to form galleries (Fig. 5). The collagen bundles appeared to penetrate these cavities and connected the tooth to the pyramid, as shown in figure 2. These cavities were only present in the intermediate zone, where the teeth were connected to the jaws.



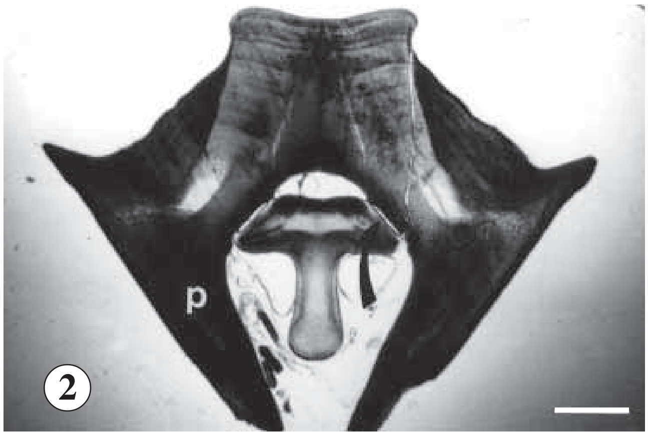
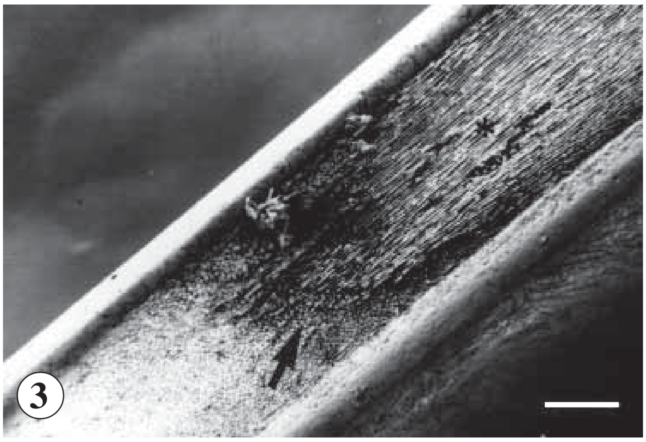


Figure 1. A pyramid, showing the mature (**M**), intermediate (**I**) and growth (**P**) zones. Bar = 2.8 mm.

Figure 2. Transversal section showing the intermediate portion of the pyramid (**p**) with the teeth. The arrow indicates the location of the ligament. Bar = 0.38 mm.



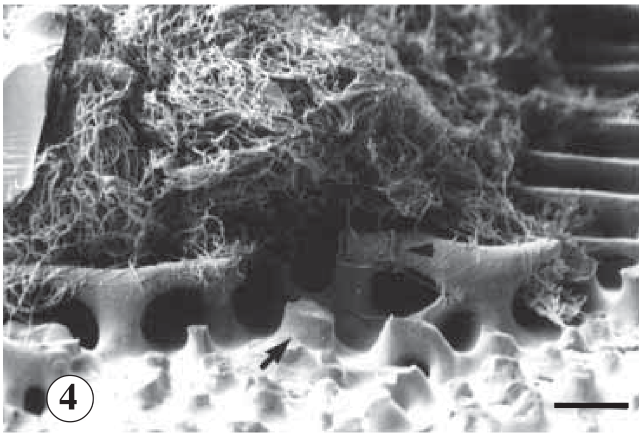


Figure 3. Scanning electron micrograph showing the internal part of a tooth. Note that the bridge-like structures (*****) are broken at the beginning of the mature portion (**arrow**). Bar = 77 µm.

Figure 4. Scanning electron micrograph showing collagen fibrils (**arrowhead**) involving bridge-like structures in the intermediate portion of a tooth. Note that these structures appear to be broken at the beginning of the mature portion (**arrow**). Bar = 10 µm.

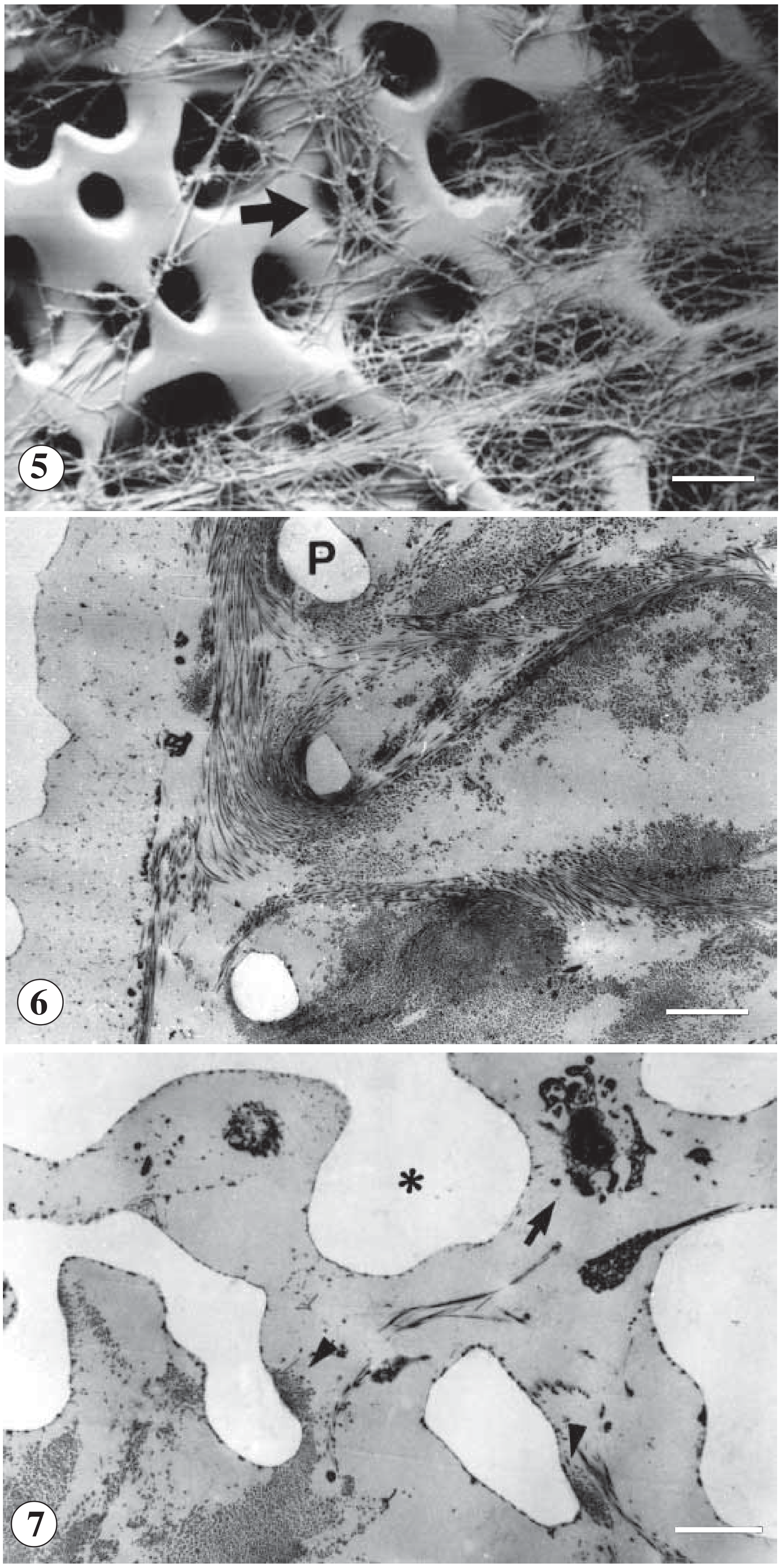


Figure 5. Scanning electron micrograph showing the cavities in the pyramid. Note the extensive net of collagen fibrils (**arrow**) around these structures. Bar = 7.4 µm. **Figure 6.** Transmission electron micrograph showing collagen fibrils around the bridge-like structures (**P**) in the intermediate portion of the tooth. Bar = 3.4 µm. **Figure 7.** Transmission electron micrograph showing collagen fibrils in the pyramid cavities. Note that the collagen fibrils are in contact with the surface of the cavities (**arrowheads**), and that there are cells within these cavities (**arrow**). The clear areas correspond to decalcified calcareous plates (*). Bar = 3.4 µm.

In transmission electron microscopy thick collagen fibers were observed around the columns (Fig. 6), and extended towards the deep cavities to form an extensive net inside the calcareous plates. The collagen fibrils were in close contact with the surface of the cavities (Fig. 7).

DISCUSSION

The teeth of sea urchins are used to scrape rocks. This scraping wears down the teeth, which must grow continuously to replace the worn-down surfaces. The teeth are connected to the jaws by a collagenous ligament that must be sufficiently stiff to hold the teeth firmly when the animal scrapes hard surfaces, but flexible enough to allow the teeth to move outwards during growth [4]. Continuous tooth growth also occurs in other animals, and the physiology of tooth growth has been studied best in mammals [5]. As in mammals, sea urchin teeth are connected to the jaws by a collagenous ligament. The movement of the teeth during growth in mammals is facilitated by a very high collagen turnover. However, the growth of sea urchin teeth is unlikely to be supported by a mechanism similar to that of mammalian teeth, since mammals have higher metabolic rates than other animals, including marine invertebrates such as sea urchins. The eruption of sea urchin teeth is likely to be regulated by a mechanism that does not require the rapid synthesis and degradation of the collagenous ligament. This assumption is supported by the absence of fibroblasts or other cell types among the collagen fibrils in the ligament of sea urchin teeth.

Based on these morphological observations of the sea urchin jaw, teeth and collagenous ligament, we have formulated a hypothesis to explain the "stiff/soft" dilemma of the sea urchin collagenous ligament. The long collagen fibrils of the ligament are linked to columnar structures in the teeth but are not mechanically attached to the cavities in the pyramids. The collagen fibrils appear to simply touch the surface of the jaws. Attachment of the collagen fibrils to the pyramid jaws involves an interaction between these fibrils and a cementing substance in the pyramid cavities. These cavities greatly increase the surface area in this region and strengthen the attachment of collagen fibrils. A possible candidate for this cementing substance could be the proteoglycan molecules present in the sea urchin collagenous ligament [10]. Proteoglycans can be degraded without affecting collagen [8]. As shown by Birenheide [1], papain loosens the ligament of sea urchin teeth, indicating that proteoglycans are responsible for this ligament stiffness. The stiffness of the dermal connective tissue in echinoderms is controlled by neuropeptides known as holokinins, which are homologous to bradykinin [3]. Such neuronal control allows rapid changes in the stiffness of the connective tissue, and triggers the degradation of putative adhesive substances in the pyramid cavities.

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