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Ultrastructure of basement membranes in monkey and shark teeth at an early stage of development

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Abstract The basement membrane, which separates the inner enamel epithelium from the dental papilla in the early stages of tooth development, is known to play a significant role in odontogenesis. In this review article, this basement membrane was described in detail based on our recent findings with the use of high-resolution electron microscopy. Tooth germs of a monkey (Macaca fuscata) and a shark (Cephaloscyllium umbretale) were processed for thin-section observations. During the early stage of development, the basement membrane of the inner enamel (dental) epithelium was composed of a lamina lucida, lamina densa, and much wider lamina fibroreticularis. At higher magnification, the lamina densa in both species was made up of a fine network of cords, which are generally the main constituents of the basement membranes. In the monkey tooth, the lamina fibroreticularis was rich in fibrils, which were now characterized as basotubules, 10-nm-wide microfibrill-like structures. The space between the basotubules was filled with a cord network that extended from the lamina densa. Dental papilla cell processes were inserted into the lamina fibroreticularis, and their surface was closely associated with numerous parallel basotubules via 1.5- to 3-nm-wide filaments. In the shark tooth during its early stage of development, the basotubules were absent in the lamina fibroreticularis and only narrow extensions, 60–90nm wide and 1–2μm long, of the cord network of the lamina densa were present. The dental papilla cells were immobilized by means of the binding of their processes to the extensions. These results indicate that basement membranes in both monkey and shark teeth at early stage of development are specialized for functions as anchoring and firm binding, which are essential for the successful differentiation of the odontoblasts.

Key words Basement membranes · Inner enamel epithelium · Odontogenesis · Monkey · Shark · Ultrastructure

Introduction

The tooth is the hardest organ in the body and is organized in a highly elaborate manner. Developmentally, the mammalian tooth is formed by an epithelial-mesenchymal interaction,1–3 which also occurs in other organs. In the early stages of development, the inner enamel epithelium and the dental papilla are separated by a basement membrane during differentiation which plays a significant role in odontogenesis.4–7 The differentiation of odontoblasts was shown to occur only when the mesenchymal cells (dental papilla cells) are in direct contact with the dental basement membrane.8 This basement membrane has been known to be associated, as its connective tissue side (dental papilla), with a characteristic layer containing numerous fibrils,9–11 which becomes most conspicuous beneath the preameloblasts during odontoblast differentiation. The detailed nature of this layer, i.e., its ultrastructure, composition, and role, is not yet well understood. In our study, this fibrillar layer was ultrastructurally and immunohistochemically characterized, and it was found to be a highly specialized lamina fibroreticularis of the basement membrane of the inner enamel epithelium.12

On the other hand, characterization of the tooth basement membrane in nonmammalian species is still to be determined.13–15 For further understanding of the fundamental mechanisms of the formation of the mammalian tooth, comparative studies on the basement membrane will provide significant information.

In this short review article, we summarize recent findings from high-resolution studies of the ultrastructure of the basement membranes in both monkey and shark teeth at an early stage of development.
Materials and methods

The animals used in this study were Japanese Macaques (*Macaca fuscata*) provided by the Primate Research Institute of Kyoto University, Kyoto, Japan, and a Blotchey swell shark (*Cephaloscyllium umbratile*) freshly caught off the coast of Suruga, Shizuoka Prefecture, Japan.

Ultrastructure

Monkeys. The heads and neck regions of 1- to 3-year-old monkeys were perfused, under anesthesia, with a fixative containing 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, through the carotid arteries for 60 min. The isolated upper and lower jaws were further fixed by immersing them in the same fresh fixative for 24 h at 4°C. Tooth germs were isolated and washed with buffer. Both the demineralized with EDTA and non-demineralized tissues were postfixed with 1% osmium tetroxide for 1.5 h, dehydrated in ethanol and embedded in an epoxy resin. Thin sections were prepared for high-resolution observation using an H-7100 electron microscope (Hitachi, Tokyo, Japan).

Shark. The tooth-bearing jaws of a shark were dissected out under anesthesia with MS222 (Sigma, St. Louis, MO, USA), and were cut into small pieces. The pieces were fixed by placing them in a fixative containing 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium phosphate buffer for 24 h at 4°C. Some specimens were demineralized with ethylenediamine tetraacetic acid (EDTA). Both the demineralized and mineralized tissues were postfixed with 1% osmium tetroxide for 1.5 h. They were further processed as described above for observation of the thin sections.

Tooth germs at the cap and bell stages before enamel (enameloid) matrix secretion were selected for this study.

Immunostaining

The previously described preembedding immunoperoxidase method was used for immunostaining of the basement membrane and related structure of the inner enamel epithelium in the monkey tooth for laminin, type IV collagen, heparan sulfate proteoglycan (HSPG), and fibronectin.

Results and discussion

Basement membrane of inner enamel epithelium in monkey tooth

In tooth germs during their early stages of development (bell stage of tooth development), the basement membrane of the inner enamel epithelium demarcating the dental papilla was closely associated with a unique fibrillar layer (Fig. 1). The fibrillar layer was composed of numerous fibrillar structures arranged parallel to one another and approximately perpendicular to the basement membrane. More detailed ultrastructural studies showed that the basement membrane of the inner enamel epithelium was composed of a lamina densa and lamina lucida with widths of approximately 25 and 50 nm, respectively. The fibrillar layer immediately next to the lamina densa of the basement membrane was composed of fibrils approximately 15 nm width and up to 2 µm long (Fig. 2a). The processes of the dental papilla cells were elongated toward the inner enamel epithelium and inserted into the fibrillar layer. During the more advanced stage of development, the layer developed most prominently at the base of the differentiating ameloblasts and, on the opposite side, it was closely associated with the early mantle predentin. Numerous collagen fibrils in the surface layer of the predentin were localized within the fibrillar layer at this stage of development and were arranged parallel to the fibrils (Fig. 2b). At the onset of mineralization of the predentin, the entire fibrillar layer as well as the basement membrane was completely removed by the differentiating ameloblasts and replaced by dense assemblies of parallel collagen fibrils.

The sections for immunoperoxidase staining of the epithelial-mesenchymal border of the tooth germs for laminin, type IV collagen, and HSPG (Fig. 3a,b) showed that not only the basement membrane but also the fibrillar layer of the inner enamel epithelium was positively stained. Under high-resolution electron microscopy, the lamina densa of the inner enamel epithelium was found to be composed of a fine network of irregular anastomosing strands. The strands were identified as “cords,” which are the major component of all the basement membranes examined so far. Based on detailed immunohistochemical studies, it is indicated that the cord is composed of a core of type IV collagen filaments and a sheath containing and integrating the rest of the basement membrane components such as laminin and HSPG. An individual fibril within the layer was a rodlike structure with a thickness ranging from 8 to 15 nm, and they extended through the basement membrane and reached the cell surface. The fibril was made up of a surface layer and a core. The surface layer was composed of irregular assemblies of “double tracks,” a 4-5 nm-wide ribbon-like structure, which was positively immunostained for HSPG. The core of the fibril sectioned longitudinally through its center showed a set of three parallel lines in which the middle one appeared to be a string of successive dots. A transverse section of the core was seen as a 7- to 10-nm-wide pentagonal frame with an electron-lucent lumen containing a central dark dot. These structures have the same characteristic features as the core of the microfibrils.

The spaces between the fibrils were filled with a fine network structure resembling the cord network in the lamina densa of the basement membrane. The network structure was positively immunostained for laminin and HSPG (Fig. 3b). Occasional “stripped” strands, appearing as 1.5-nm-wide filaments after losing their sheath material, were positively immunostained for type IV collagen. These observations indicate that this interfibrillar structure is the