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Thicker Sections (300-500 nm) Observed in the Conventional TEM May Reveal Extended Structures Not Recognized in Ultrathin Sections

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Our Micro-Imaging facility focuses on the connective tissue field, with the majority of our effort dedicated to ultrastructural analysis of connective tissue matrices including bone, cartilage, eye, tendon and skin by transmission electron microscopy. Since the installation of our laboratory over 30 years ago, many of our investigations have included immuno-gold localization of the various collagens and associated molecules present within normal matrix. With these results in mind, we also evaluate the presence or absence of these components in disease states. Within the past decade, our focus is the evaluation of therapeutic intervention strategies designed to ameliorate the severe blistering disease Epidermolysis Bullosa. A form of this disease, Recessive Epidermolysis Bullosa, results if type VII collagen is missing or deficient. In normal individuals, type VII collagen forms anchoring fibrils, critical in dermal/epidermal adhesion. Life threatening, debilitating blisters result in the absence of anchoring fibrils. The strategies for introducing normal collagen VII into individuals deficient for the molecule vary widely, with several concurrent clinical trials ongoing. We evaluate the formation of anchoring fibrils in skin biopsies collected early and later in the treatment duration to determine the effectiveness of the therapy.

The dermal/epidermal junction of skin is a complex region, with many interconnected supra-molecular structures important to dermal/epidermal adhesion. Keratin composes intermediate filaments within the epithelial cells, which merge into dense cords on the epithelial side of hemidesmosomes, which punctuate the epithelial basal cell membrane. BP230 and plectin co-localize to the dense plaques of the hemidesomsomes, and on the basal side of the plaques, thin anchoring filaments composed of laminin 3-3-2 radiate through the lamina lucida of the dermal-epidermal junction to terminate at the lamina densa. Type VII containing anchoring fibrils anchor into the lamina densa, interacting with laminin 3-3-2, then radiate into the dermis, where they entrap collagen fibrils, acting much as Velcro to mediate dermal/epidermal adhesion. There are other molecules here too, but if any of these aforementioned components are missing, a form of blistering disease results.

For many years we explored the DEJ by observing ultrathin (80 nm) sections. More recently, however, we have been interested in the interaction of juxtaposed ultrastructural features and have come to appreciate how remarkable these structures appear in thicker sections (300-500nm). Our appreciation is not mediated by sophisticated hardware or 3-D rendering software. Our TEM is an FEI G20 200KV microscope, but we routinely operate it at 120KV. The microscope is equipped with a tilting stage; to collect an aligned tilt series of thick sections we operate the microscope at 200KV to penetrate the added apparent thickness of the section when tilted at 60 degrees relative to the beam. The resulting 3-D impression of a single aligned tilt series will often rival that gained by examining of many hundreds of ultrathin sections. The method has allowed us to image extended structures not previously described. The purpose of this report is to encourage others to collect and examine thick sections of their chosen tissue with the hope of added insight [3].

References:

[1] D Keene and S Tufa in “Methods in Cell Biology”, ed. R Mecham, (Academic Press) p. 1.
[2] J Gebauer et al., Matrix Biology **28** (2009).
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