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Collagen Ultrastructure and Skin Mechanics in DDR1 KO Mice

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The mechanical properties of skin are due in large part to the extracellular matrix (ECM) whose main structural component is collagen I. Not only is collagen I an infrastructural portion of the matrix, but it also interacts with and is affected by local cells. It has been previously shown that collagen binding proteins such as discoidin domain receptors (DDR1 and DDR2) influence collagen fibrillogenesis and structure *in vitro* [1, 2]. Therefore, the goal of this study was to determine if the structure of collagen *in vivo* is altered by lack of DDR1 and if these alterations impact the mechanical properties of the underlying tissue as well as the ability for cells to interact with the collagen.

Skin was isolated from DDR1 knock-out (KO) mice and their age and gender matched wild type (WT) littermates. Transmission electron microscopy (TEM) was used to identify the collagen ultrastructure and fibril diameters which were measured in both the loose and dense areas of connective tissue. Dynamic tensile testing was performed on the skin to determine the failure and relaxation properties of the skin. Relaxation data was obtained by placing the skin under a constant strain while simultaneously measuring the force exerted by the skin. Skin extracted from both genotypes of mouse was decellularized to leave only the collagen. This was then homogenized and allowed to repolymerize into gels. These gels were seeded with fibroblasts which were allowed to grow within the collagen matrix. Contraction of these collagen gels was used to evaluate the effect of cell traction forces on collagen from DDR1 KO vs. WT mice.

Consistent with our earlier in-vitro studies [1, 2], collagen fibers residing in both the loose and dense connective tissue areas of the skin of DDR1 KO mice were larger in diameter with a different distribution as compared to their WT counterparts. This can be seen in the TEM images and histogram of figure 1. While no significant differences in the failure properties of skin was observed in the two genotypes, figure 2 shows that the relaxation rate for the DDR1 KO skin was faster than WT. Expression of DDR1 in cells also resulted in enhanced contraction of collagen gels.

These studies provide insight into how collagen binding proteins like DDR1 can impact collagen ultrastructure. Our results indicate that the expression of DDR1 *in vivo* is necessary for the normal modulation of collagen fibrils. The skin from DDR1 KO appears to be less resistant to applied loads compared to the wild type mice. From this work, it can be elucidated that changes in collagen structure and organization in turn modify mechanical properties of the tissue and the underlying cell-matrix interactions. The increased relaxation rate in the skin of KO mice serves to illustrate this alteration in tissue level mechanics, while the reduced contraction of collagen gels exhibits the principle at a cellular

level. Expression of proteins such as DDR1 would be important in analyzing tissue and extra cellular matrix remodeling in health and disease.

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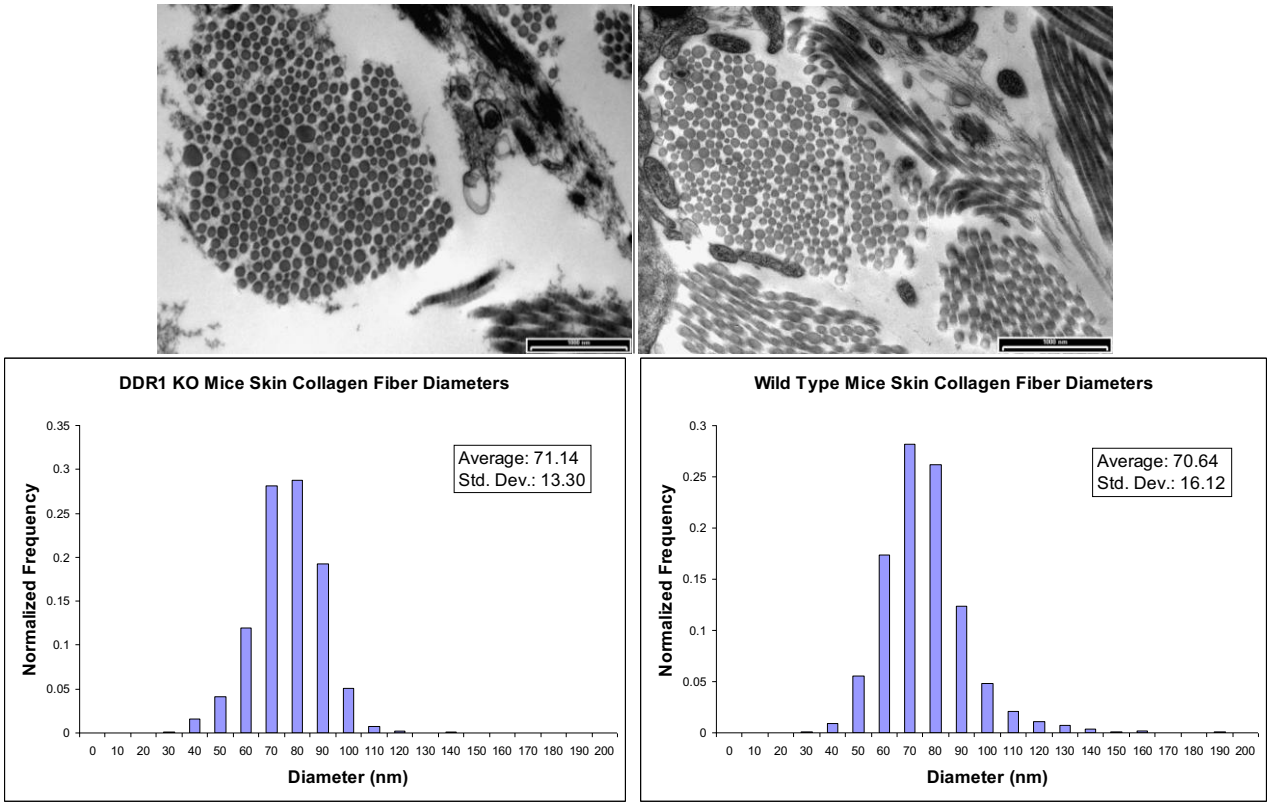

Figure 1. Transmission electron microscopy (TEM) image of collagen I cross sections in loose connective tissue of skin from a two month old (left) DDR1 KO and (right) WT female mouse. Scale bars are 1000nm. Below are the respective histograms of the fibers from each mouse.

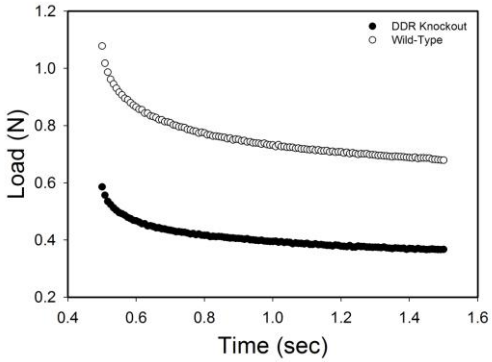

Figure 2. Dynamic tensile testing of skin. Skin was strained to 10% gauge length and held for 60 seconds while load was continuously recorded. DDR1 KO and WT mice had relaxation rates of 0.3178 and 0.2941 N/s respectively.