MORPHOLOGICAL CHANGES IN SKELETAL MUSCLE ENDOMYSIAL AND PERIMYSIAL COLLAGEN NETWORKS SUBJECTED TO STRAIN

Allison R. Gillies, Nozomu Inoue and Richard L. Lieber

University of California San Diego, La Jolla, CA, USA
Rush University Medical Center, Chicago, IL, USA
VA San Diego Healthcare System, San Diego, CA, USA
email: rlieber@ucsd.edu web: http://muscle.ucsd.edu

INTRODUCTION

The skeletal muscle extracellular matrix (ECM) plays an important role in muscle fiber force transmission [1], however its structure-function relationships are not clearly understood. Some of this uncertainty is due to the lack of studies specifically describing endomysial and perimysial structures and their response to strain. The reorientation of endomysial collagen in highly digested tissue has been described [2], but ECM structures and their changes with strain have not been described in undigested tissue, which was the purpose of this study.

METHODS

All procedures were performed in accordance with the NIH Guide for the Use and Care of Laboratory Animals. The 5th toe of the extensor digitorum longus (EDL) muscle was dissected from wildtype mice for scanning electron microscopy (SEM) analysis. Muscles were formalin-fixed at resting length or longitudinally stretched 30% past resting length. Muscles were then dehydrated in graded ethanol, frozen and lyophilized. Muscles were sputter coated with iridium and viewed with a FEI XL30 SEM.

Endomysial collagen fibril diameter was measured by a custom software program (resting length: n=2 muscles; stretched: n=3 muscles). Each void space of the collagen network was identified by a particle analysis technique. Surfaces of the collagen fibrils were then registered using an edge tracking algorithm. Collagen fibril diameters were determined by the least distance algorithm [3]. Perimysial collagen diameter was measured manually with ImageJ software (resting length: n=2; stretched: n=3). Collagen diameter distributions from resting length and longitudinally stretched muscles were compared by Mann-Whitney nonparametric tests with p<0.05 considered significant.

RESULTS AND DISCUSSION

Surprisingly, SEM observations revealed structures specific either to skeletal muscle endomysium or perimysium. During processing, patches of ECM were separated from muscle fibers (Fig. 1, P). Based on morphology, it appeared that these patches were likely endomysium that pulled away from the muscle fiber surface (Fig. 1, F). Endomysial collagen networks were typically composed of thin fibrils in a mesh-like structure.

In contrast to the endomysium, perimysial collagen was organized as thick cables of collagen and had no apparent preferential direction in muscle at resting length. However, perimysial collagen cables were preferentially aligned with the axis of strain in stretched muscles (Fig. 2A). These collagen cables were composed of many collagen fibrils bundled together (see close-up in Fig. 2B).
shift toward smaller diameters at stretched length, however stretched perimysial collagen diameter was not different from resting length. These results suggest that endomysial and perimysial structures may be distinct and that thin fibrils of the endomysial collagen mesh may be more sensitive to strain than thicker perimysial collagen cables. The different morphologies of endomysial and perimysial collagen may be a result of different functional requirements at each level of ECM organization. Further studies are required to define specific biochemical and biomechanical differences between these important load-bearing ECM components.

REFERENCES


ACKNOWLEDGEMENTS

The authors acknowledge the Department of Veterans Affairs, NIH grant R24HD050837, and NSF for a Graduate Research Fellowship (ARG).