WET MOUNT MICROSCOPY OF HUMAN SKELETAL MUSCLE; UNVEILS THE ULTRA-STRUCTURAL PATTERN

Abstract

Wet mount microscopy employs water as a mounting medium for viewing the living micro-organisms of different kind. The refractive index of water not only enhances the image quality but also produces image in natural color with no or little artifacts. A qualitative observational study was done in which wet mount slide of the formalin fixed skeletal muscle from the human cadaver was prepared, and examined under light microscope. The images showed very fine striated sarcomeric pattern in myofibrils with quiet resemblance to the ultra-structure. The study was helpful in the better comprehension of histological structure of skeletal muscle and gave prevision for further melioration and application in the field of anatomy.

Keywords: Wet mount microscopy, skeletal muscle, Ultra structure, Striations, Sarcomere.

Introduction

Wet mount microscopy remained as a "Forgotten art" in the modern era of medicine. It primarily uses water as a mounting media in order to see living micro-organisms useful in diagnostic and clinical research.2 Being temporary in nature, it enticed few researchers for the advancement, despite of simple and minimal infrastructure requirements. The present study employs wet mount of formalin fixed skeletal muscle to describe its micro-architecture.

Microstructure of Skeletal muscle: The skeletal muscle consists of unbranched longitudinal myofibrils with peripherally located flattened nuclei, surrounded by endomysial connective tissue.4 The myofibrils show faint striations (sarcomeres) post tissue processing and H and E staining (Fig 1). The ultra structure reveals repeated pattern of sarcomere, the structural and functional unit of the skeletal muscle. Each sarcomere bounded within Z lines comprises of thick myosin (A band) and thin actin (I band) filaments.3, 4

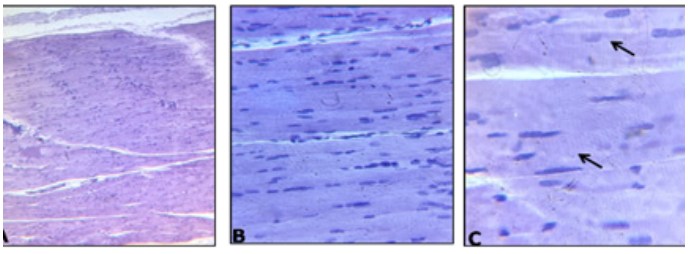

Figure 1: Human skeletal muscle as seen in light microscope (H and E Stain preparation A-10 X, B-40X, C- 100X Magnification respectively). The striations are faintly visible (marked with arrows) in C.

Objective

A qualitative observational study to develop a simple wet mount of the formalin fixed skeletal muscle from the human cadaver and its observation under different magnifications of light compound microscope. The study anticipates its further betterment and application in the modern science.

Materials and Methods

Formalin-fixed Deltoid muscle was procured from the human cadaver voluntarily donated to the Anatomy department of the ABC institute for teaching and research purpose. The specimen procured was dissected using dissection microscope (Fig-3C) into thin myofibrils with the help of tweezers and fine syringe needles. The sample was made to stick on the surface of microscope glass slide and made wet by pouring few drops of distilled water with the help of dropper (Fig 2).

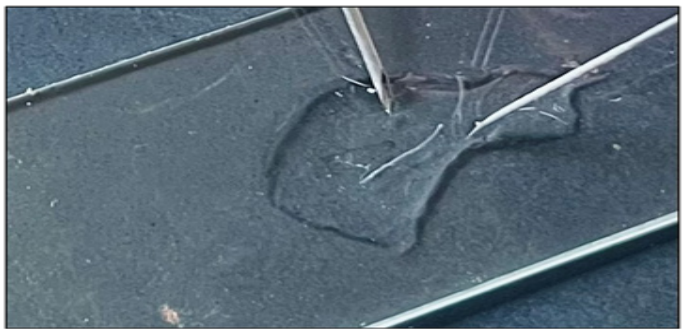

Figure 2: Wet mounting of skeletal myofibrils

A cover glass was placed over the wet mount preparation and is viewed under 10X (low power) and 40X (high power) of light (compound) microscope (Fig 3B).

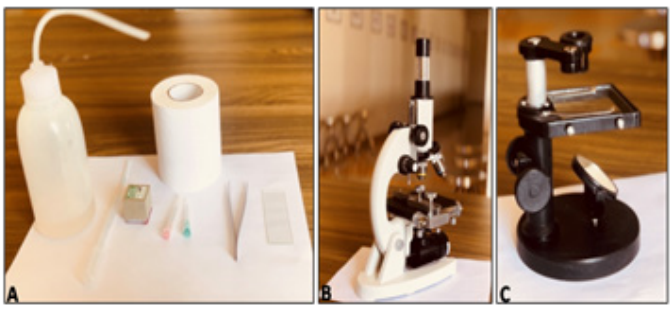

Figure 3: A- Materials used during preparation of wet mount microscope slide. B- Monocular light microscope (Compound) C- Dissection microscope

Results

The wet mount slide of skeletal muscle under low magnification (10X) revealed a fine longitudinal structure of myofibril with striations (Fig 4A). A single myofibril too depicted similar striation pattern (Fig 4B)

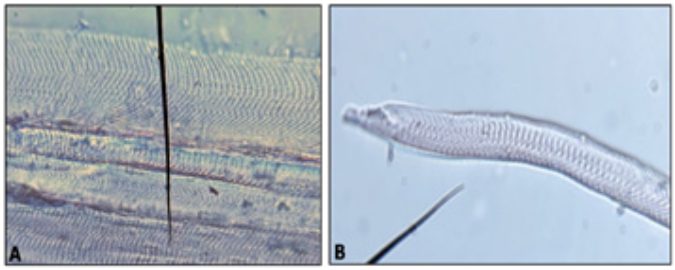

Figure 4: Wet mount microscopic slide of skeletal myofibrils (10X Magnification). A- Bundle of myofibrils with clearly visible pattern of striations (black pointer) B- Single myofibril with striations.

Under higher magnification (40X), similar repeated sracomeric pattern was seen with vivid internal architecture depicting Z lines as well as A and I bands (Fig 5 and 6)

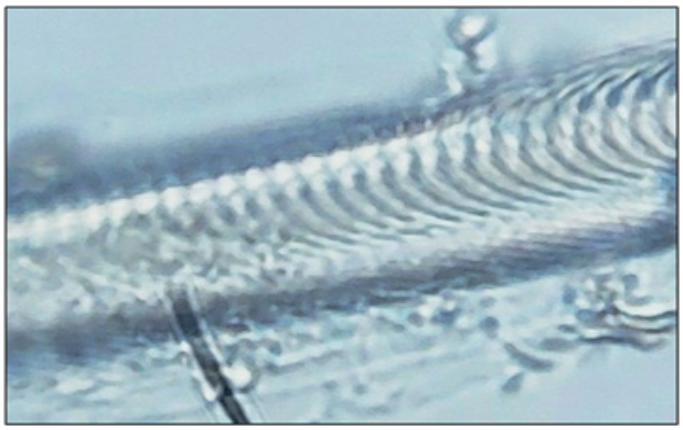

Figure. 5: Myofibril (single) of skeletal muscle seen in wet mount preparation (40X Magnification).

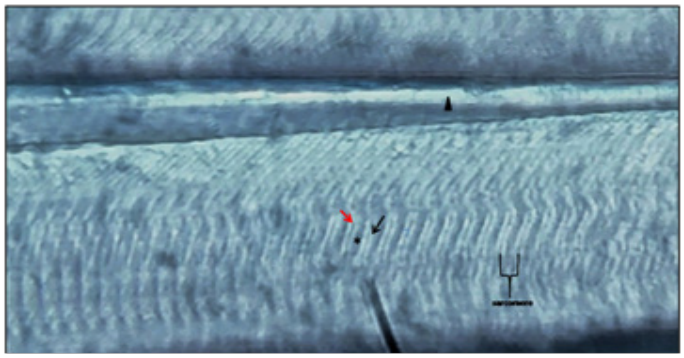

Figure 6: Myofibrils of skeletal muscle seen in wet mount preparation (40X Magnification). Striations (Sarcomeres) are clearly visible with dark A-Band (*), Z-Line (red arrow), light I-Band (black arrow). The connective tissue is also seen between myofibrils (black solid arrow).

Discussion

The present study acknowledges vivid and distinct skeletal muscle striation pattern which is two of a kind as ultra structural srcomeric pattern. The clearly visible thin and dark encircling Z lines10. of Krause bilaterally (Fig 6) forms the boundary of the sarcomere with an intervening space occupied by one dense and dark (A band- thick myosfilaments) and equally bisected light (I bands- thin action filaments).5, 9 Myofibril bundle as well as single myofibril at low magnification (Fig 4B) showed well defined striations than the H an E histology. Wet mount microscopy delivers no or minimum artifacts with a natural color of the tissues.1 The same is significantly consistent with the present study. The endomysial connective tissue appears white between two myofibrils (Fig 6) as viewable under electron microscopy.8 The circularly arranged striations clearly visible (Fig 5) conform double hexagonal configuration7 and the "sliding filament" theory of muscle contraction.6 The study provided new insight and will prompt future researchers for the development and utilization of the wet mount technique in anatomy teaching and research.

Conclusion

Wet mount microscopy of human cadaveric tissue can contribute significantly in the comprehension of its histological structure.

Refernces

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Desh Bhagat Dental College and Hospital
Desh Bhagat University,
Mandi Gobindgarh