Structural changes in skin and skeletal thigh muscle of the rat in experimental diabetes

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Background

Despite a large amount of data related to extracellular matrix protein (especially collagens) glycation with diabetes, little is known on how such a modification by glucose affects collagen structure mechanics and damage, cell-collagen fibrils interactions, and collagen turnover during this pathology development *[Snedeker JG, Gautieri A., 2014].*

The present study reports the application of scanning electron microscopy to determine structural changes in alloxan - induced diabetes in rat skin and skeletal muscles.

Experimental models of diabetes

The non-genetic form of experimental diabetes mellitus was modeled in outbred white rats by intramuscular injection of alloxan (100 mg/kg). The model of compensated diabetes mellitus was reproduced by intramuscular injection of alloxan (65 mg/kg). Two weeks after injection, glucose levels were 7-10 mmol/L in the compensated diabetes group and 27-30 mmol/L in the group with the model of uncompensated diabetes.

Sample preparation

Fixation of tissue samples was carried out in 10% neutral buffered formalin solution for 24 hours. Histologic wiring was conducted in a series of 95-96% ethyl alcohol solutions - 4 alcohol changes. After that, the tissue samples were soaked in 4 changes of paraffin, 1 hour in each paraffin, and then the biopsy specimens were cast into blocks using paraffin heated to 56-58℃. After the paraffin solidified (about 24 h), thin tissue slices were cut using a rotary microtome - about 4-5 µm thick, then the tissue slices were placed on slides and dried in the thermostat for 12 h at 37℃.

Before scanning electron microscopy (SEM), the slices were pre-deparaffinized in 3 changes of xylene for 10-15 minutes each and dehydrated in 3 changes of 95-96% ethyl alcohol solution for 10-15 minutes each, then dried for 1 hour in air. For morphological examination the slices were stained by standart histologic methods.

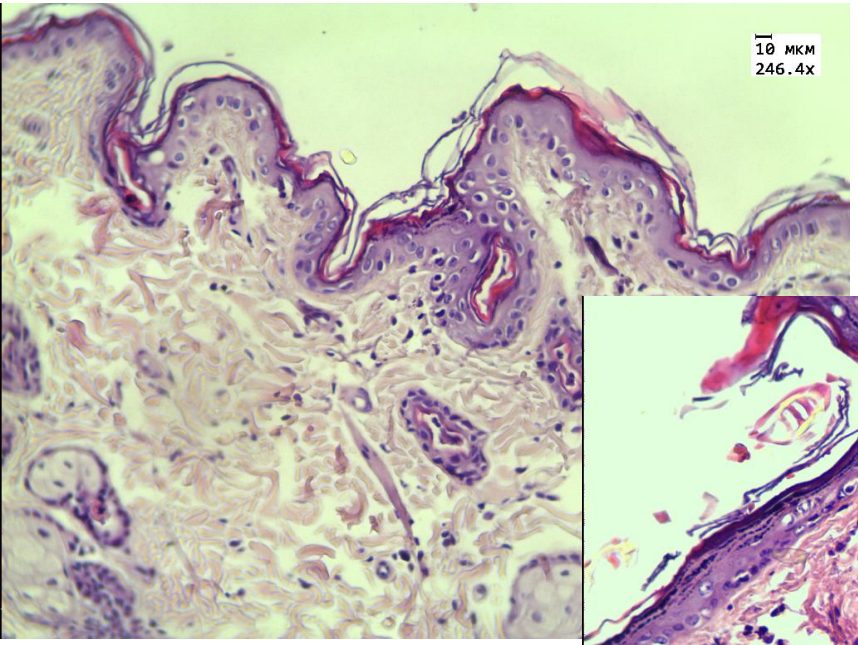
SEM: Sample preparation

After drying, the slices were fixed to the sample table using a vacuum double-sided carbon conductive adhesive tape. A thin conductive layer of metal is applied to them by spraying. Spraying takes place in a vacuum chamber. To do this, the chamber is pumped out to a pressure of less than 10-2 Pa. Then the working gas (argon) is let in until an equilibrium state is established inside the chamber. Plasma is then ignited in the chamber, which sprays a metal target. The ejected metal atoms are deposited on the surface of the sample. The thickness of the coating is controlled by the plasma current and the spraying time. The thickness of the coating is usually from 5 to 10 nm. This makes it possible to obtain high-magnification micrographs without self-visualization of the sprayed substance.

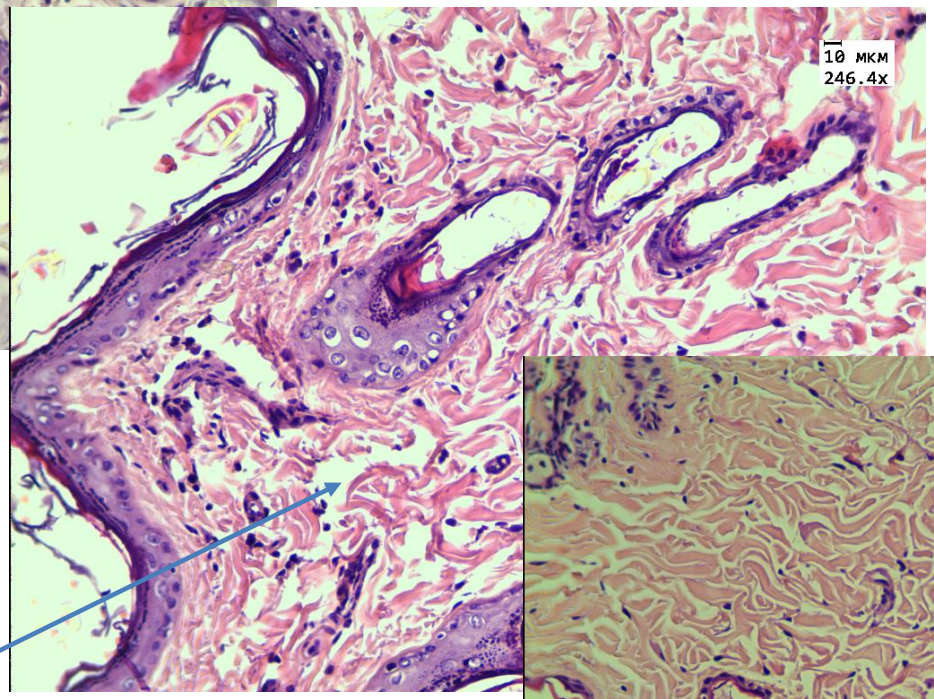
SEM was conducted using Mira II LMU microscope (TESCAN, Czech Republic) to determine structural changes in alloxan - induced diabetes in rat skin and skeletal muscles.

Hystological examination: skin

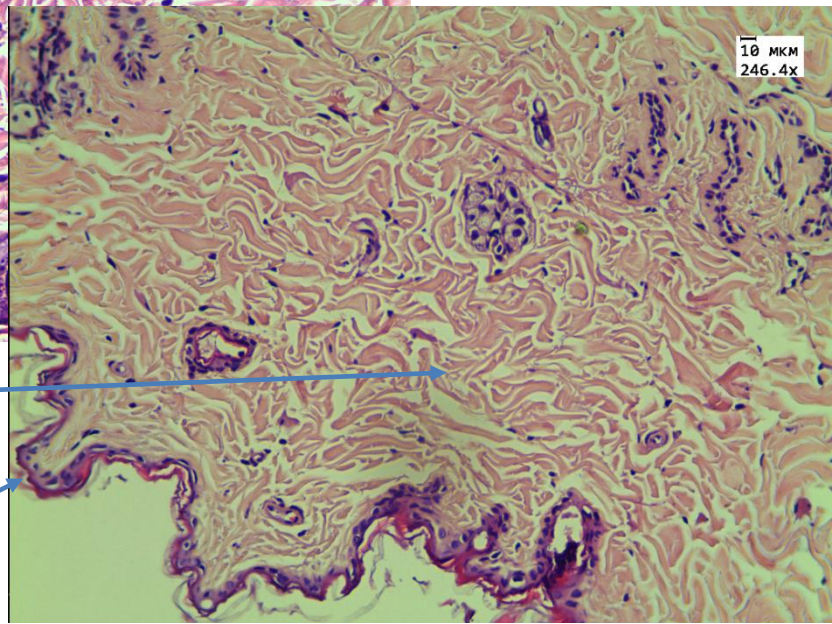
Control group



Diabetes II type



Diabetes I type

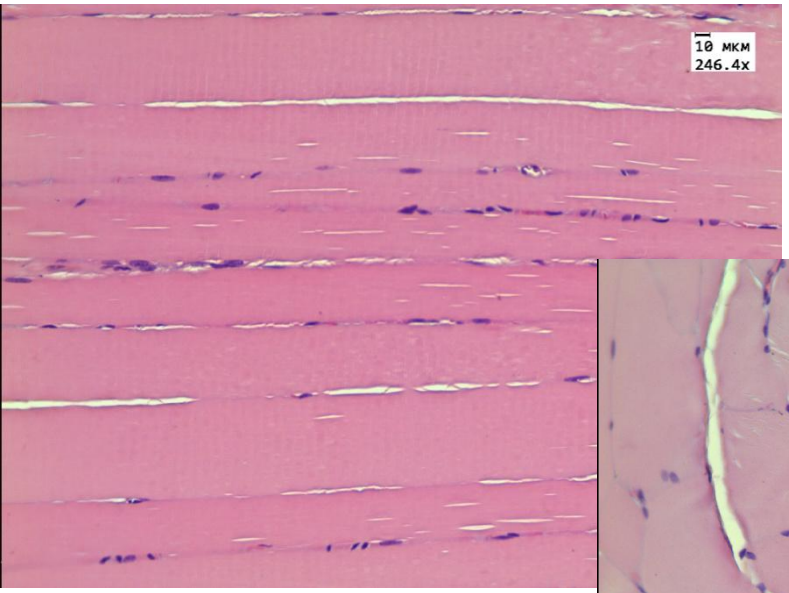


Collagen unraveling, dermal edema

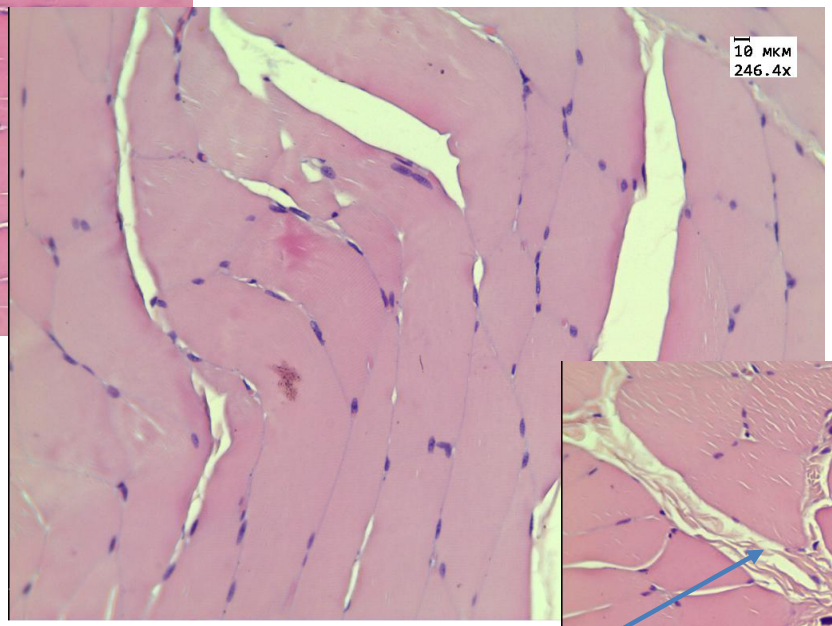
Epidermis thinning

Hystological examination: muscle

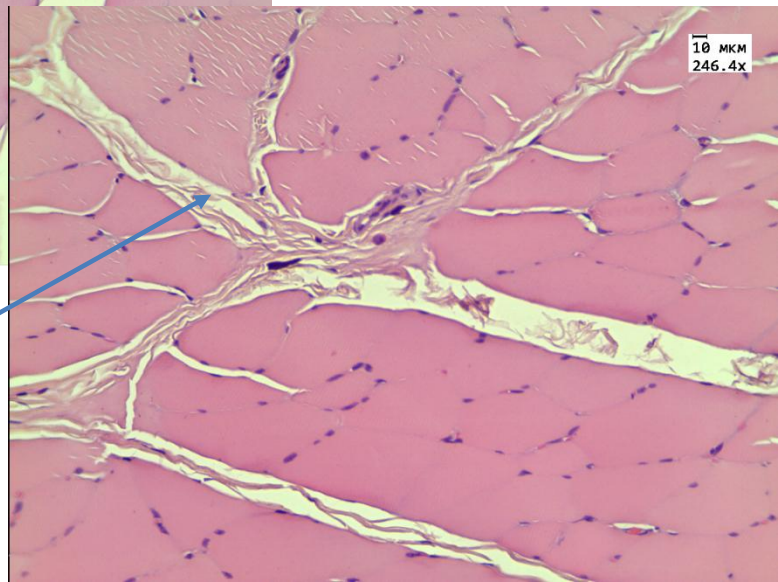
Control group



Compensated diabetes

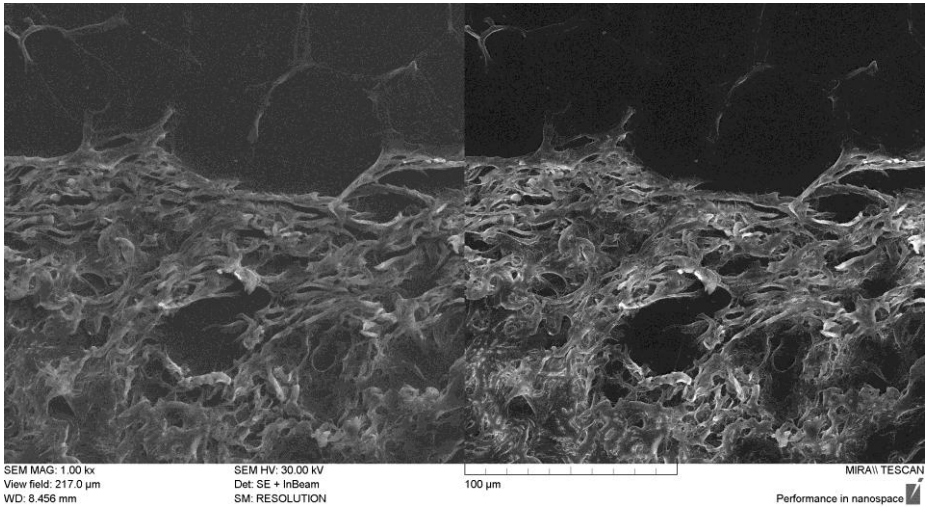


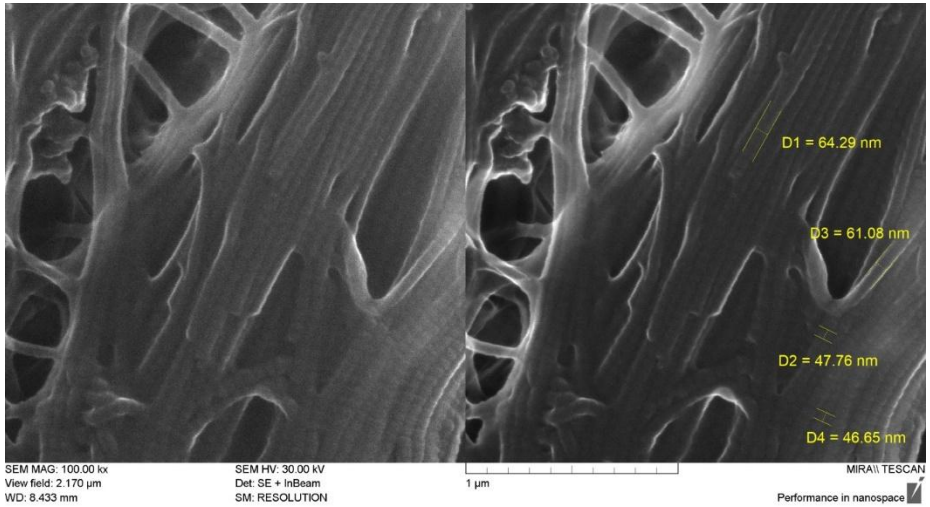
Diabetes



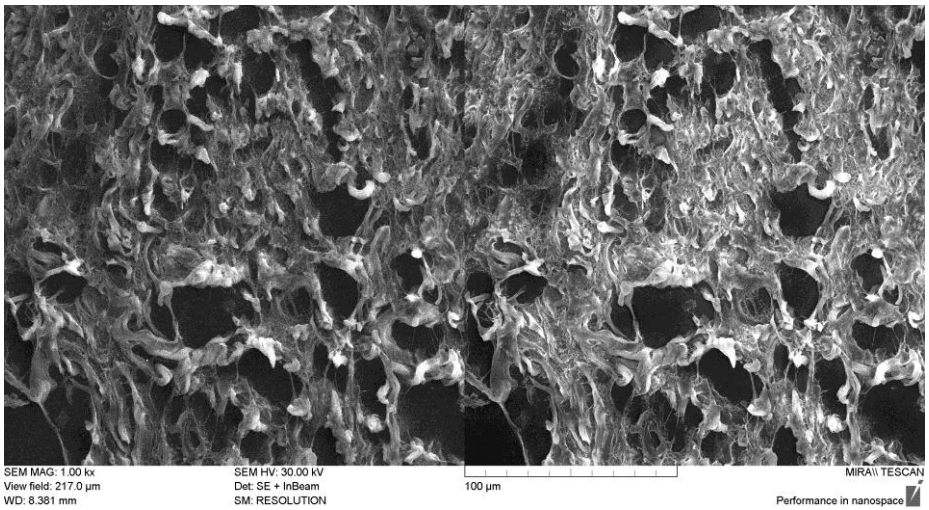
areas of dystrophically altered muscle with swelling of fibers

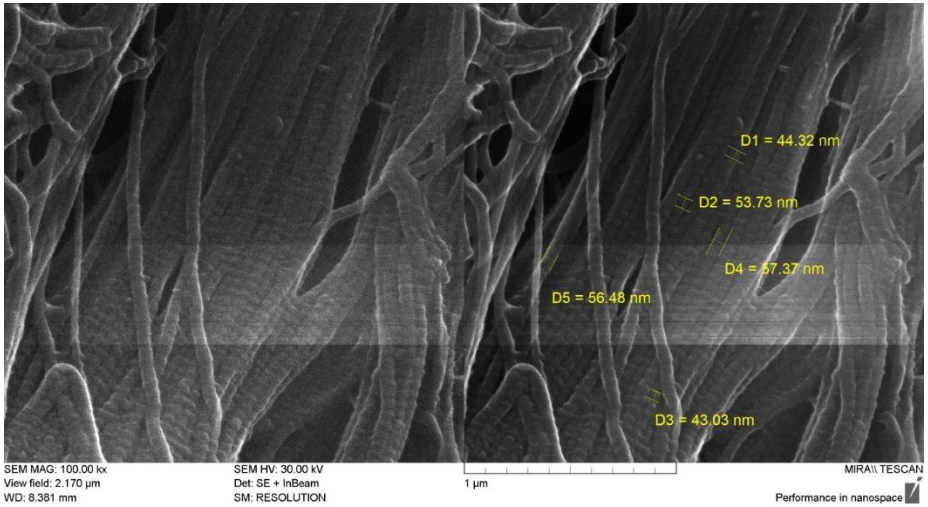
Skin – control group



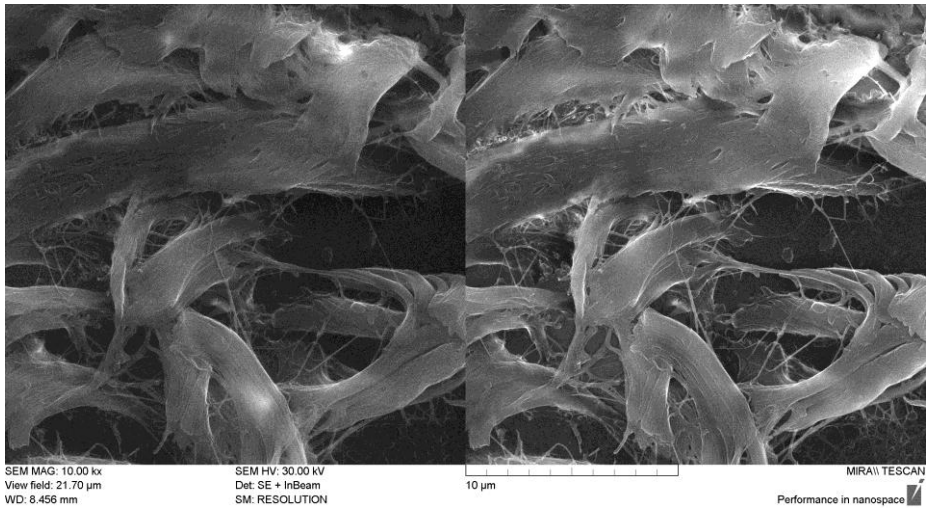


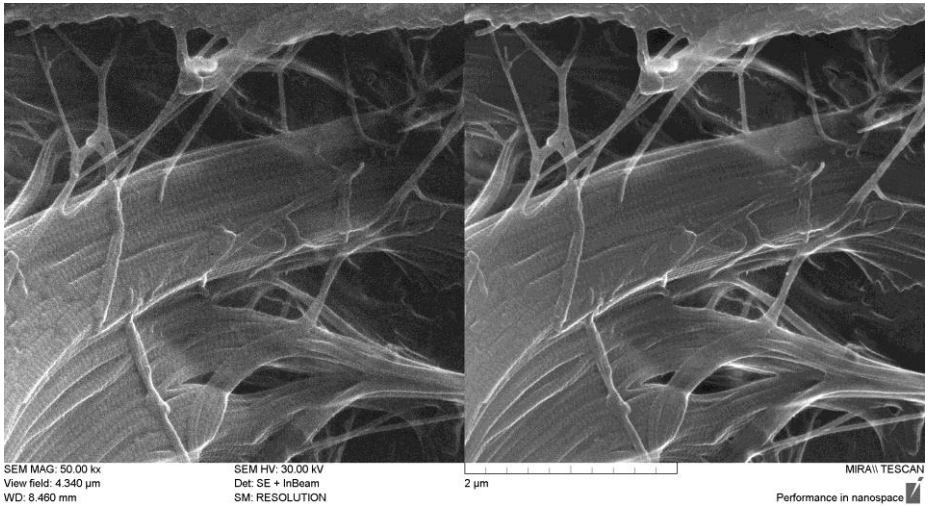
Skin – compensated diabetes



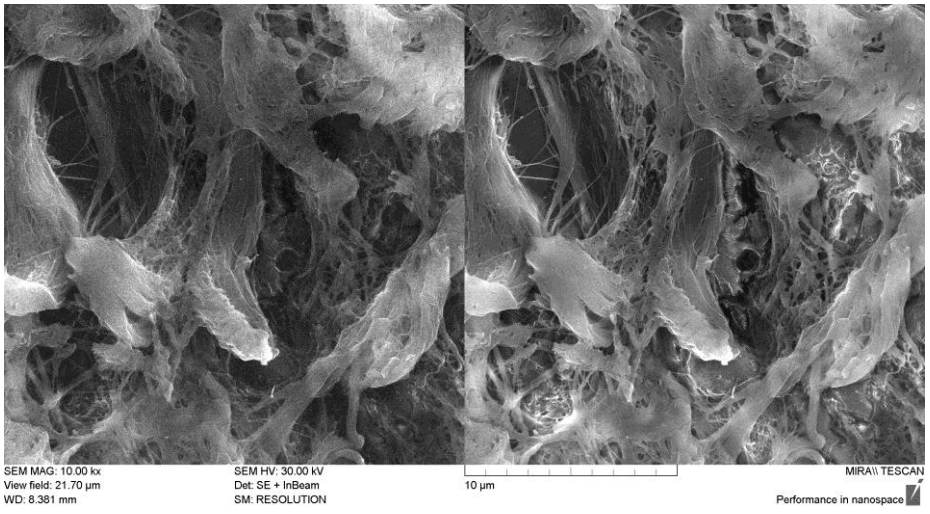


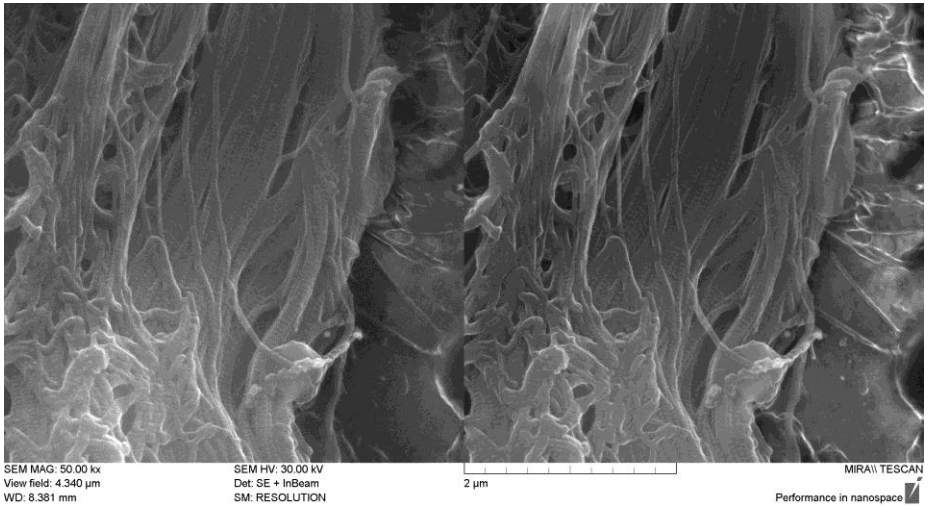
Skin – control group



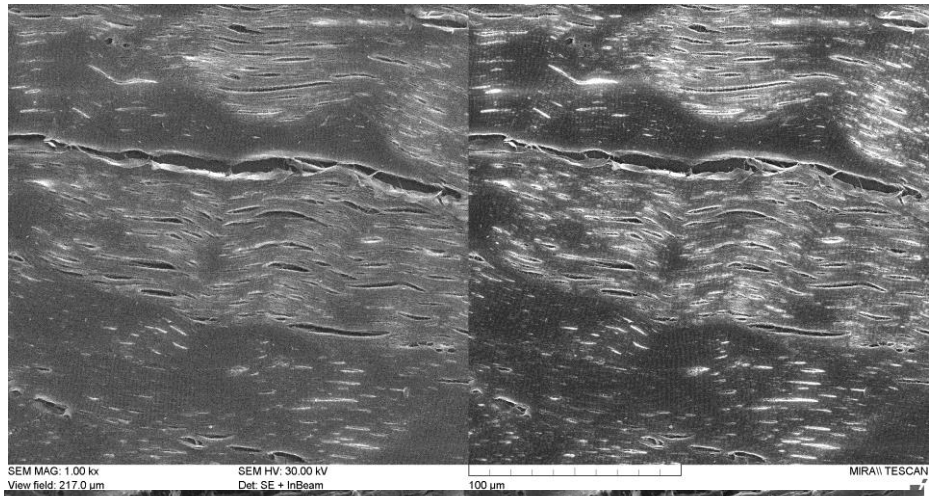


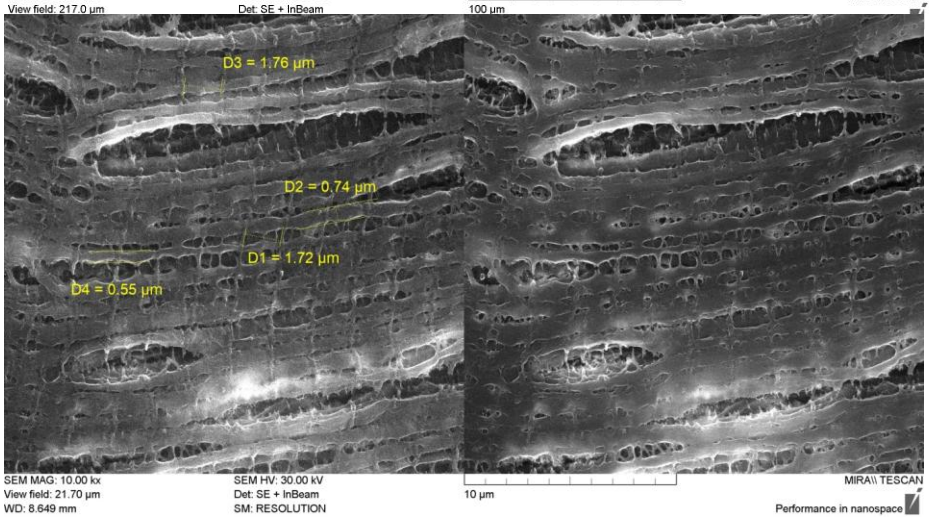
Skin – compensated diabetes



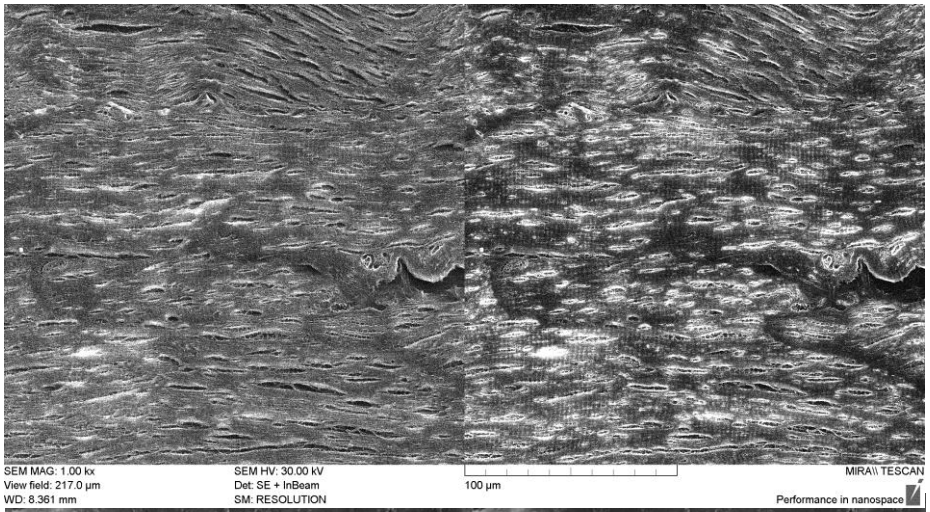


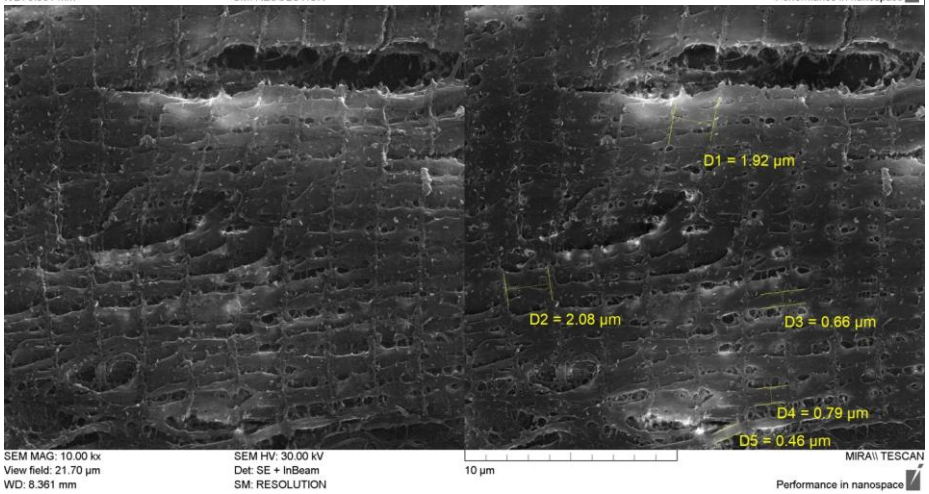
Muscle – control group



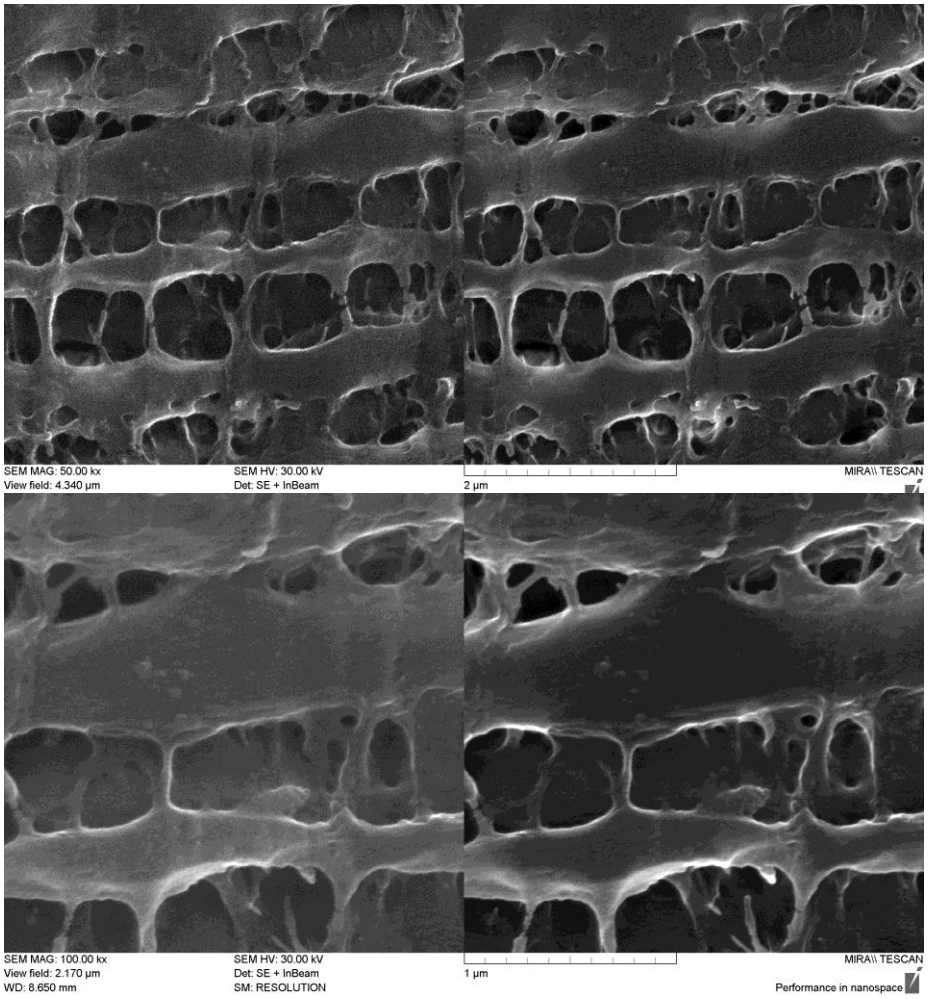


Muscle – compensated diabetes

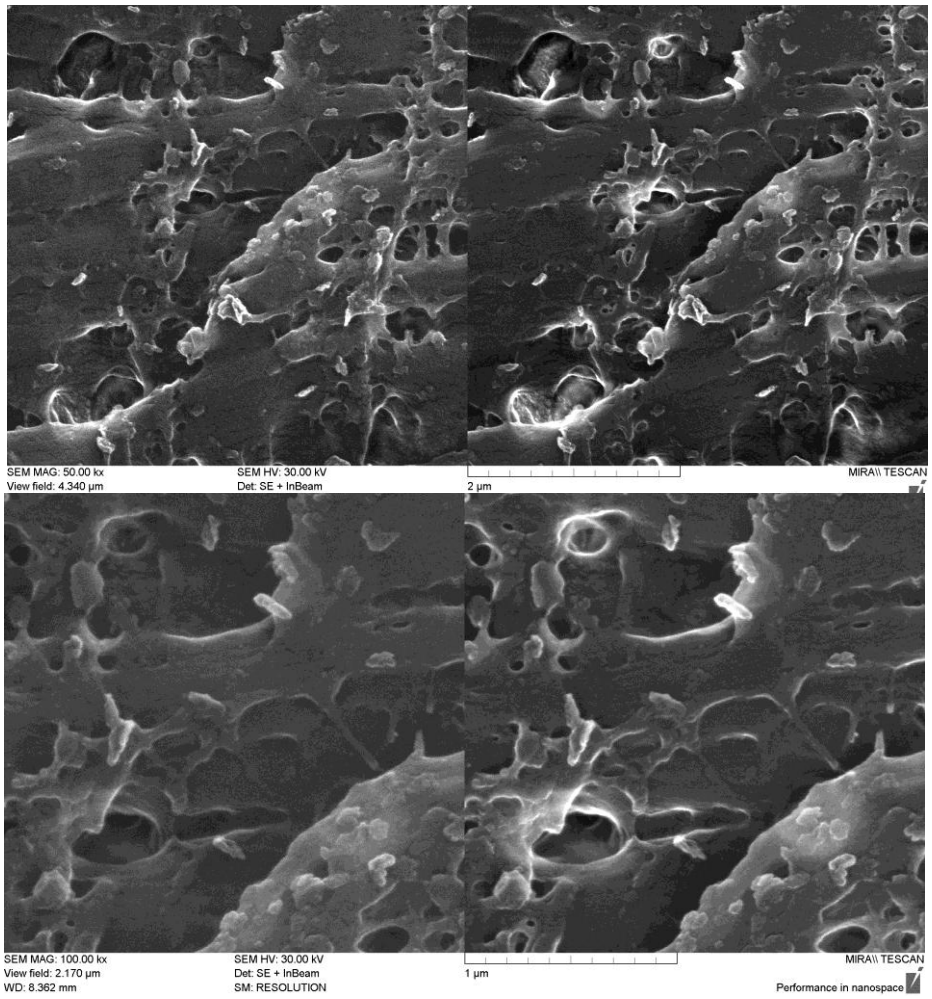




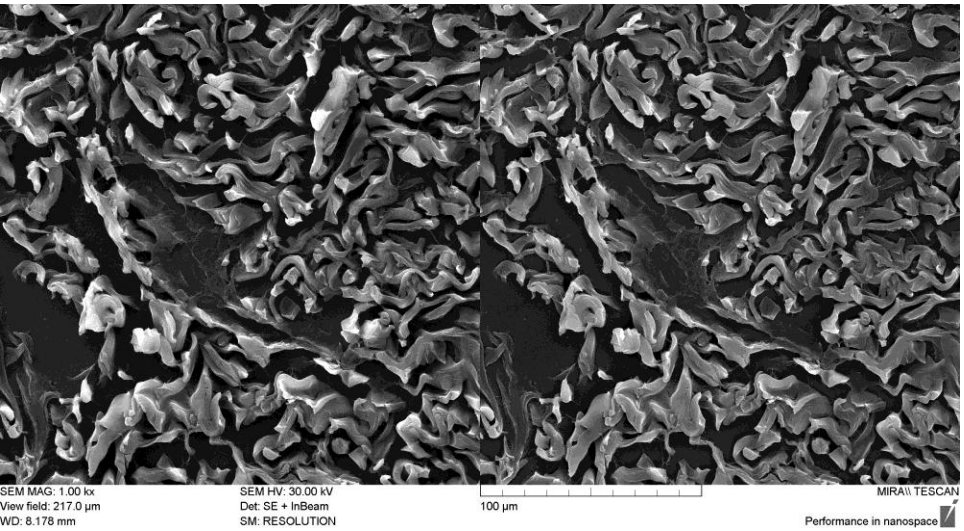
Muscle – control group

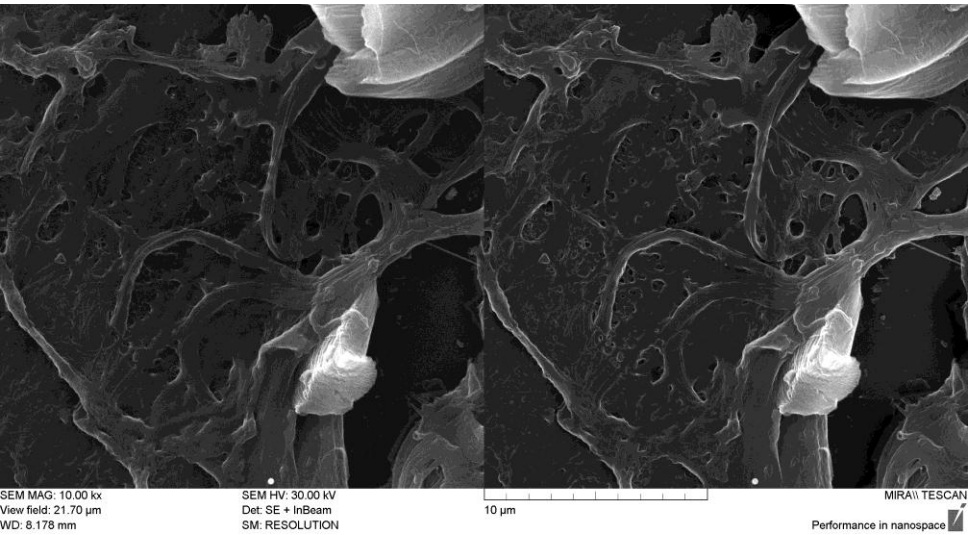


Muscle – compensated diabetes

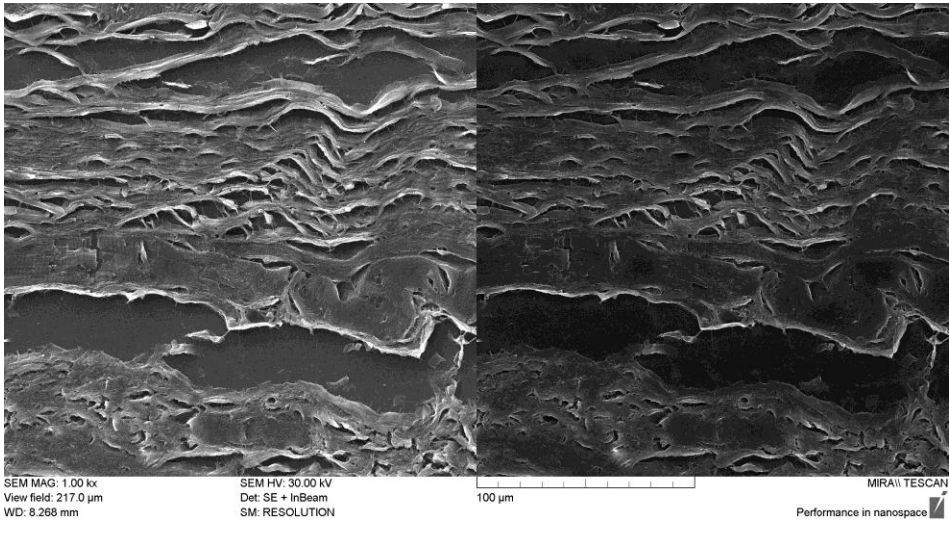


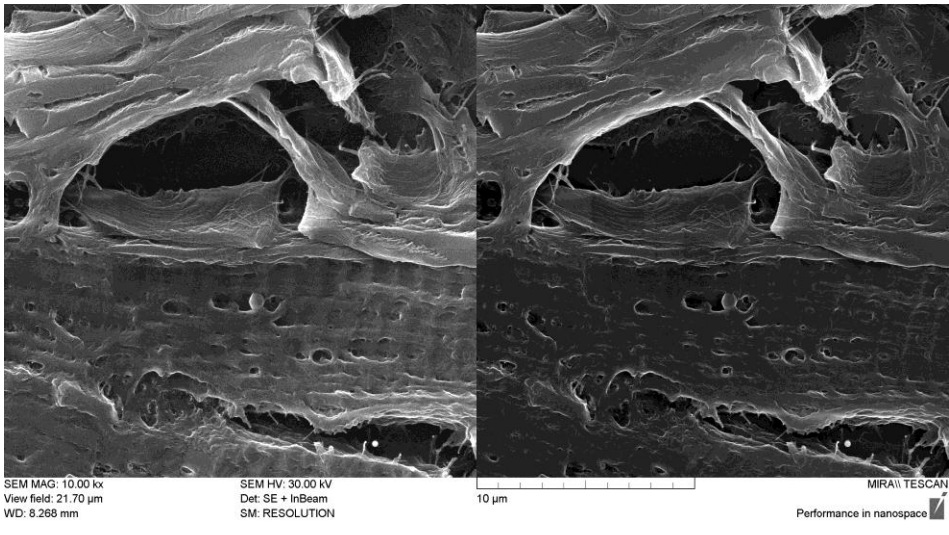
Skin – control group



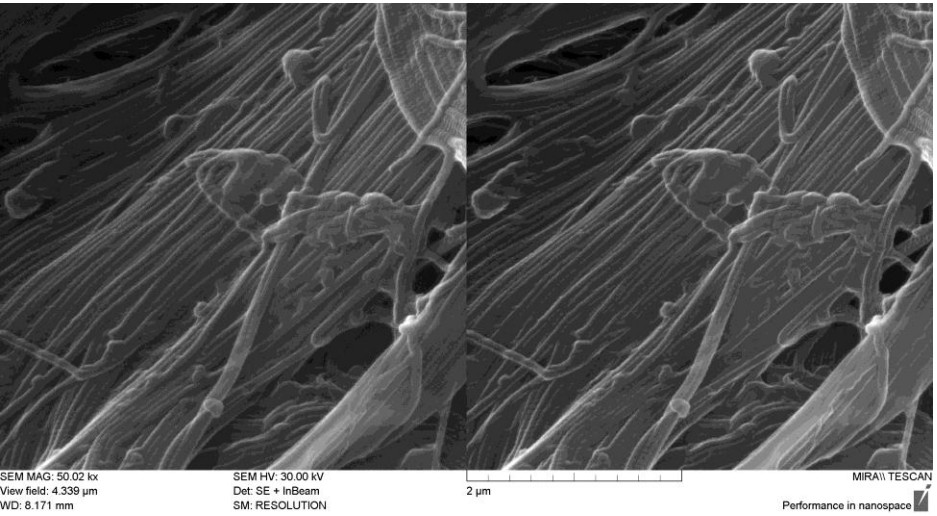


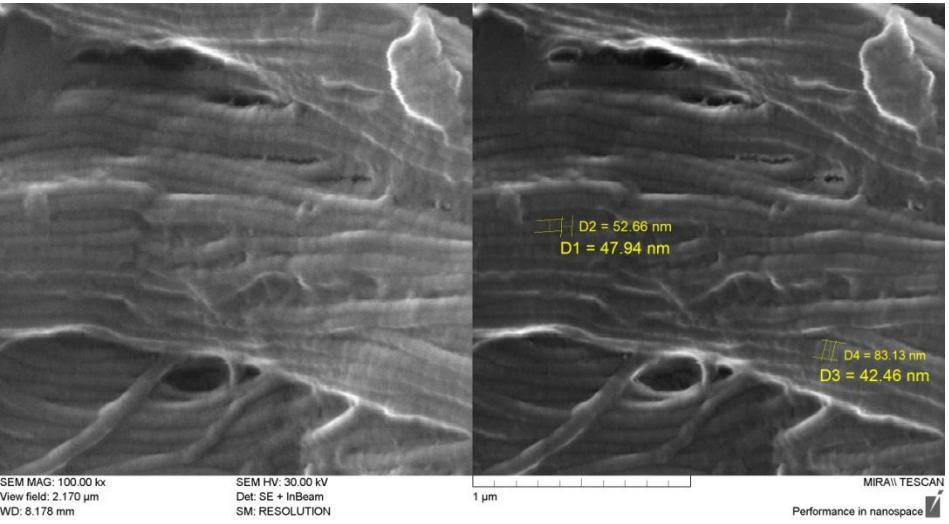
Skin – diabetes



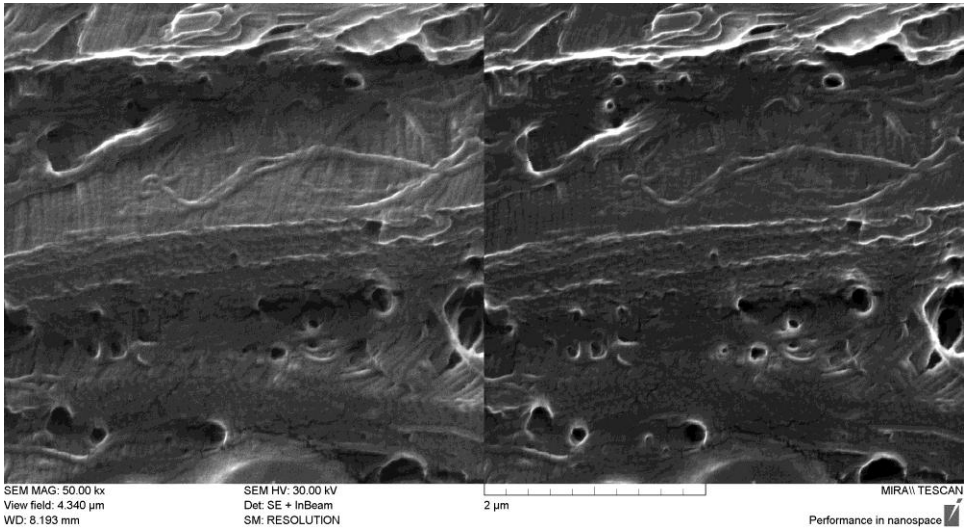


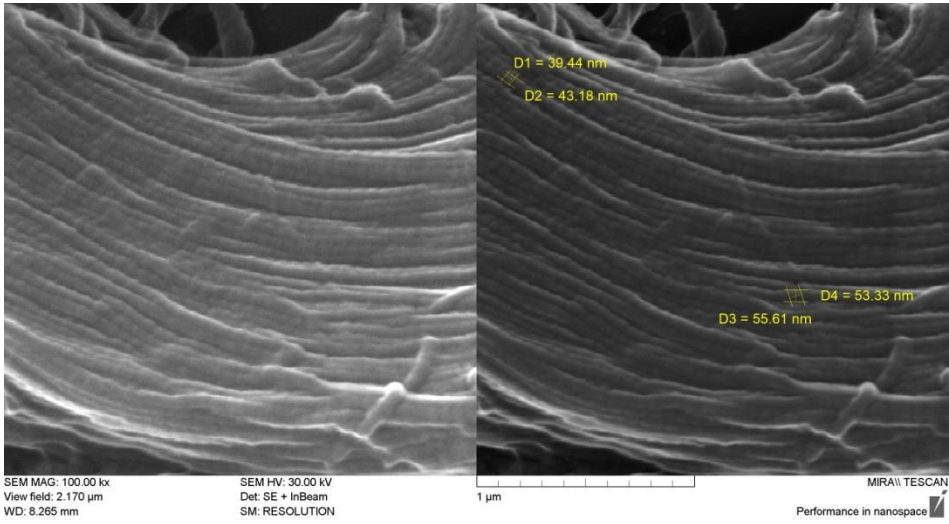
Skin – control group



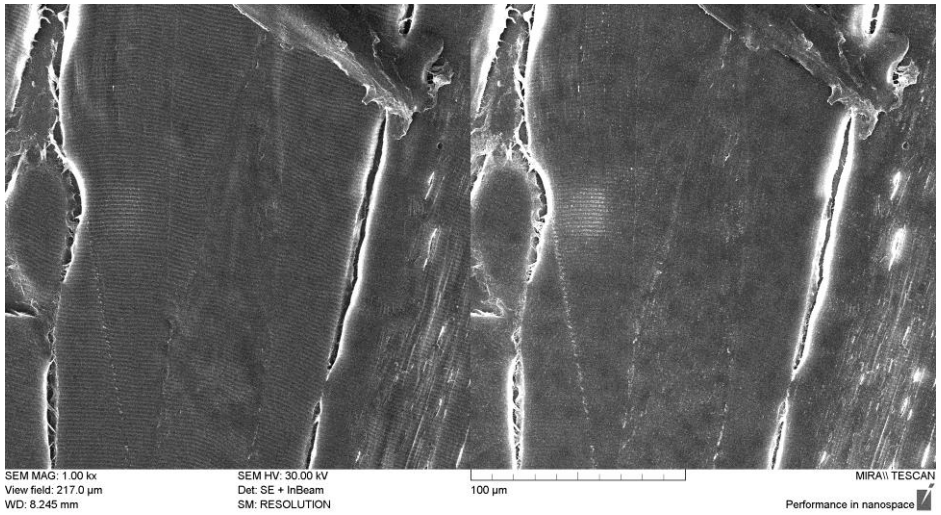


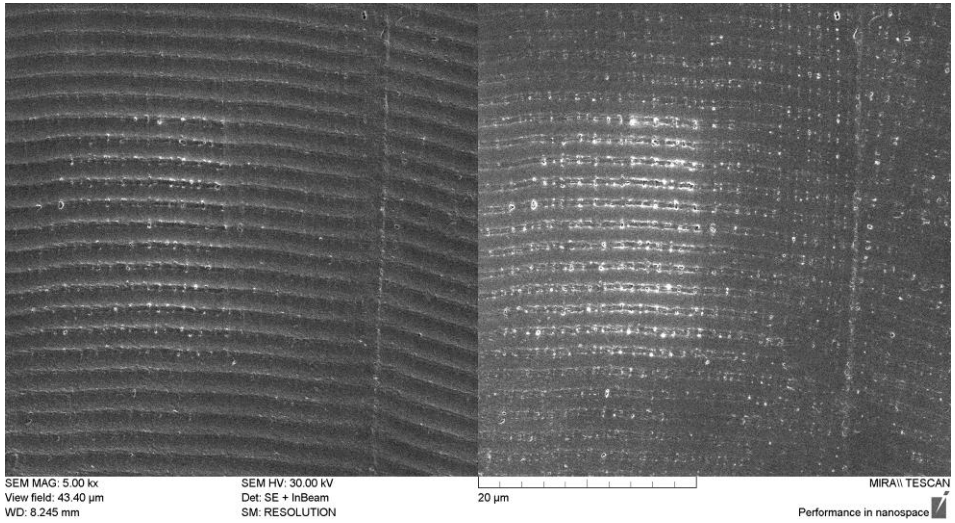
Skin – diabetes I type



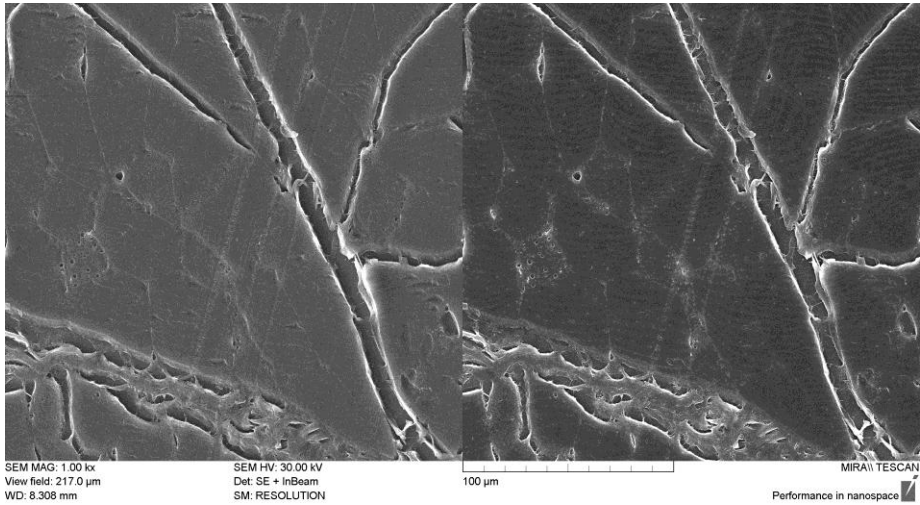


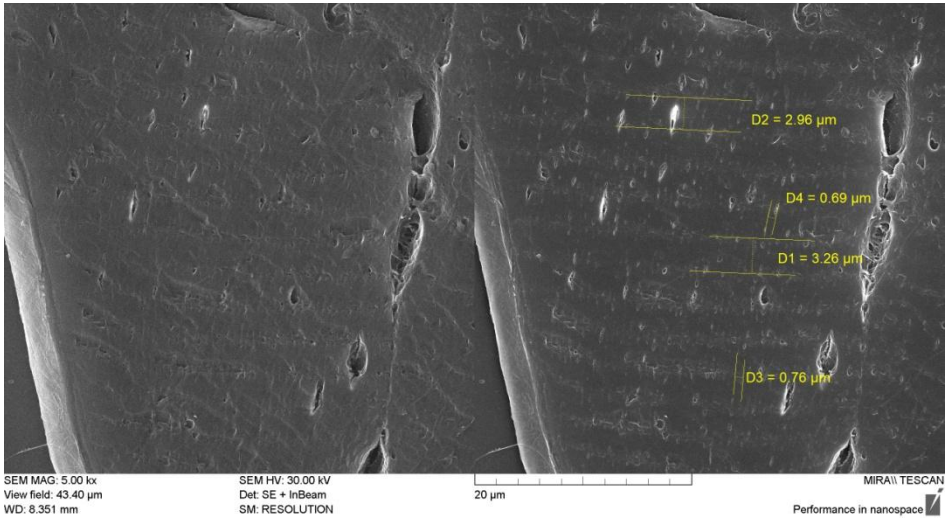
Muscle – control group



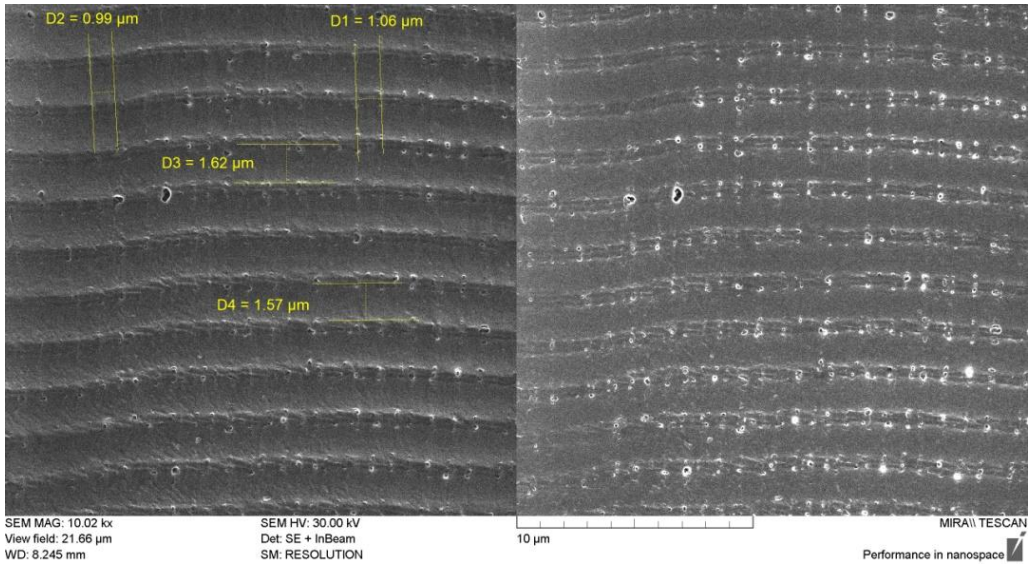


Muscle – diabetes

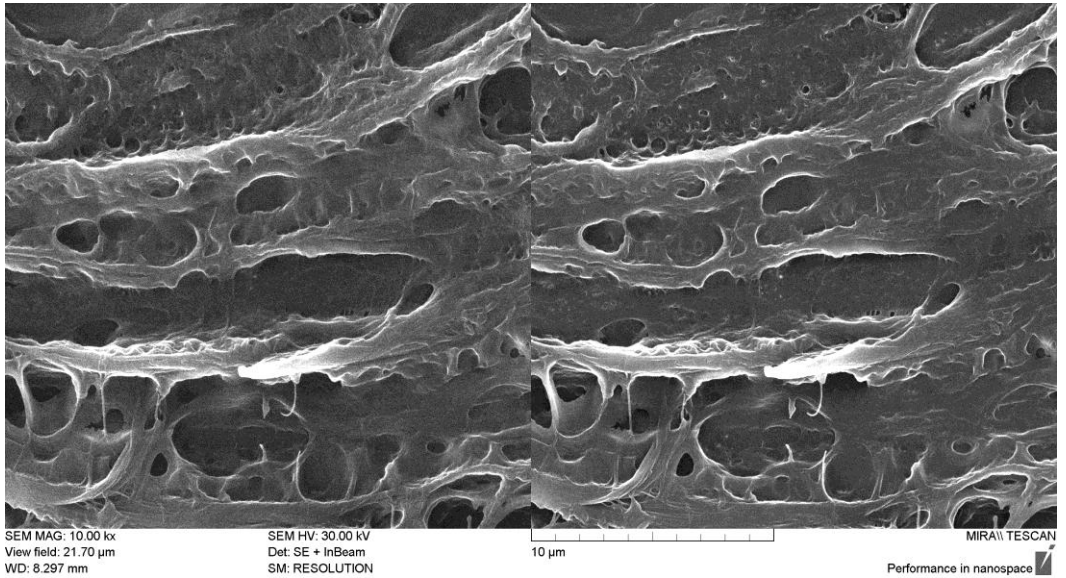




Muscle – control group



Muscle – diabetes



Conclusions

The decrease in the integrity of collagen molecules was found in muscle in both types of modeled diabetes.

The severity of structural changes in skin and muscle depended on glucose levels in experimental diabetes model.

Thank you for attention !

Acknowledgments

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