SEM Characterization of Adipose Tissue Structure

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ABSTRACT

In this work the scanning electron microscopy (SEM) was used for the characterization of adipose tissue samples taken from volunteer obese patients. The adipose tissue was taken from 3 layers of adipose tissue (subcutaneous, preperitoneal and visceral). The obtained results provided information about structural composition of adipose tissue layers in human body, as well as main microstructural features. It was demonstrated for the first time to the best our knowledge that SEM is indispensable tools in order to investigate some specific morphological features of human adipose tissue, identifying surface structure of it. From the obtained results we concluded that such characterization of adipose tissue is an essential step for the possible prediction of appearance of symptoms of different diseases.

Keywords: adipose tissue, layers, surface, morphology, SEM

1 INTRODUCTION

Adipose tissue is generally considered as a storage depot for excess energy, which is stored as triglycerides. It was long considered as a passive organ, but the adipose tissue has been described as an endocrine organ recently with important physiological roles. For example, pericardial adipose tissue volumes were significantly increased and adrenal gland volume was slightly enlarged in patients with chronic major depressive disorder [1]. Obesity is increasingly recognized as a growing cause of cancer risk [2]. There is conflicting information about differences between the fatty acid composition of subcutaneous adipose tissue (SAT), preperitoneal adipose tissue (PAT) and visceral adipose tissue (VAT). Varying proportions of fatty acids from adipose tissue may be related to atherosclerosis, metabolic syndrome, type 2 diabetes mellitus, cardiovascular and other diseases and might exert a direct influence on serum lipids that may differ depending on the adipose tissue region [3-6]. During cancer development, loss of total adipose tissue occurs in most cancer patients [7]. However, loss of total adipose tissue did not reflect changes in SAT and VAT in the same direction or intensity. Intensity of SAT is more likely to be gained further way from death, whereas VAT loss remains constant throughout the disease progression.

The conventional methods for determining the composition of fats are gas and liquid chromatography [8, 9]. However, it is clear that new alternative analytical procedures for the analysis of adipose are very much desired. In this work the adipose samples were taken from obese patients and analysed using scanning electron microscopy (SEM). Also, the aim of the present study was to investigate the distribution of different morphological features in different layers of adipose tissue from obese patients.

2 EXPERIMENTAL

All subjects included in our study were recruited from patients at Vilnius University Hospitals' Department of General Surgery. During the laparoscopic gastric banding surgery three samples of adipose tissue was taken: subcutaneous (SAT), preperitoneal (PAT) and visceral (VAT). The study protocol was approved by the Lithuanian Ethics Committee, with the aim and design of the study explained to each subject, who in turn gave their informed consent. The adipose tissue samples (5 g from each region) were washed out in normal saline solution and frozen immediately. Adipose tissue samples were stored in -70 ºC temperature before the microscopical analysis was performed. For morphological characterization of the adipose tissue specimens scanning electron microscope (SEM) Hitachi SU-70 was used. Energy dispersive X-ray (EDX) elemental analysis was performed with a Hitachi Tabletop Microscope TM3000.

3 RESULTS AND DISCUSSION

The scanning electron micrographs of adipose tissue obtained from different layers of randomly selected obese, but without metabolic diseases patient are shown in Fig. 1 indicating the surface similarity and difference of specimens. The main morphological features of adipose tissue taken from adipose tissue layers SAT, PAT and VAT of individual patient are quite similar. The microstructure of the sample 1 obtained from adipose tissue layers SAT, PAT and VAT is characterized by a number of planar particles 1-1.5 μm in size.

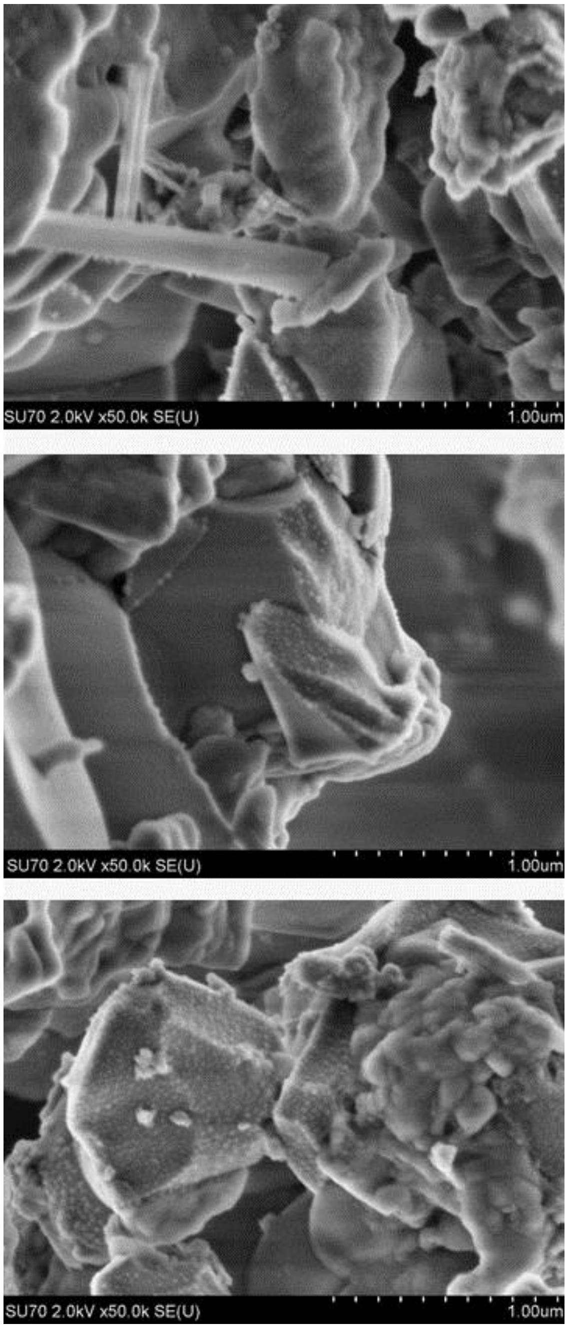

Figure 1: SEM micrographs of adipose tissue obtained from subcutaneous (top), preperitoneal (middle) and visceral (bottom) layers of obese healthy patients.

The adipose material shows rather open structure and a large surface area with no microscopic evidence for the existence of pores or interparticle voids. However, some rod- and/or stick-like individual particles 0.5-1.0 μm in size also could be seen in the SAT layer. Very small (50-100 nm) spherical particles are located on the surface of planar particles of adipose tissue in VAT layer. However, the microstructure of adipose tissue taken from preperitoneal (PAT) layer does not show any specific features.

Fig. 2 shows the representative SEM micrographs of PAT layer of adipose tissue samples taken from obese patients having different metabolic diseases. No progressive changes in morphology of PAT samples in comparison with sample from healthy patients were observed.

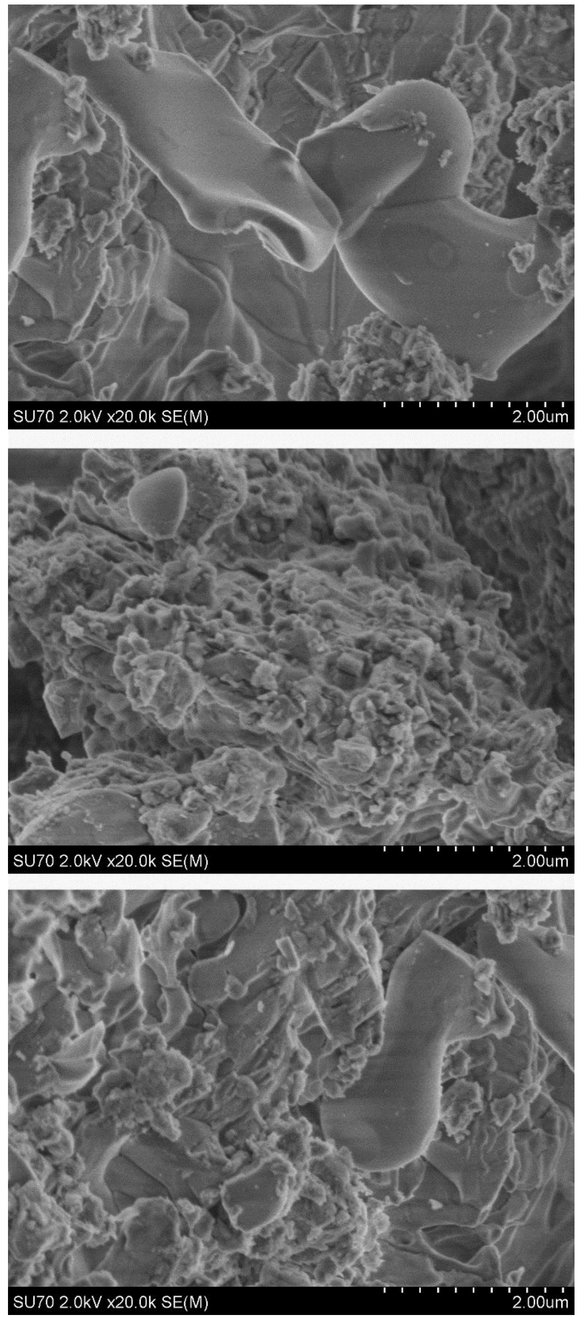

Figure 2: SEM micrographs of adipose tissue obtained from preperitoneal layers of obese patients with different etabolic diseases.

The SEM micrographs of these samples show that adipose tissue of preperitoneal layer is composed of irregularly shaped 200 nm–2 μm in size particles which are closely connected to each other forming hard agglomerates. The existence of a continuous network of particles is evident. Some parts of the samples are composed of bigger particles, some contain significantly smaller particles. However the microstructure of the adipose tissue PAT laeyrs obtained from different patients is quite similar. The morphology of most PAT samples was consisting of similar particulate matter. Only very seldom some additional small nanoscaled separate particles could be seen on the surface of adipose tissue of these specimens. These initial SEM observations let us to conclude that preperitoneal layer perhaps could be considered as a passive organ, i. e. is not associated with metabolic changes in human body.

The SEM micrographs of subcutaneous adipose tissue layer taken from obese patients having different metabolic diseases are presented in Fig. 3.

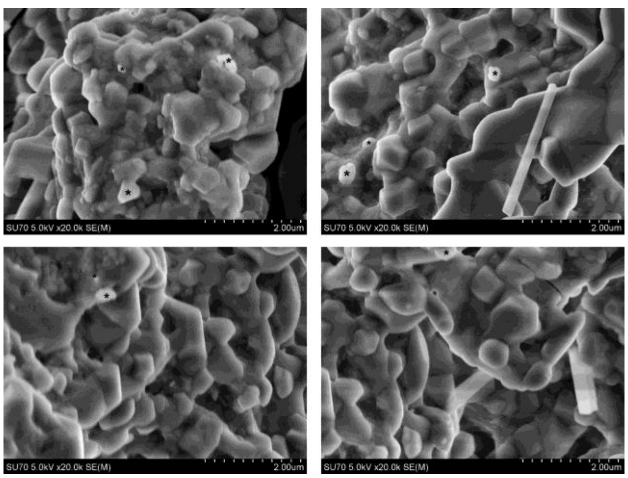

Figure 3: SEM micrographs of adipose tissue obtained from subcutaneous layers of obese patients with different metabolic diseases. The bright spots are marked *.

These SEM images are slightly different in comparison with ones taken from SAT layers of people without metabolic diseases. It can be seen from Fig. 3 that the adipose tissue samples are composed of cubes, prisms and spherically shaped granules particles less than 0.5 μm in size. These different geometric shapes are connected by solid matrix. Moreover, pores and voids can also be seen, which result probably from the different natural condition of adipose tissue. The interesting morphological feature is rarely observed bright spots in all SEM micrographs of SAT adipose tissue picked up from different patients. These bright spot probably are associated with metabolic changes in body of obese patients. However, only few samples contain rod- and/or stick-like individual particles 0.5-2.0 μm in size which were seen in the SEM images of SAT layers of healthy volunteers.

The SEM micrographs of visceral adipose tissue revealed individual surface morphology of adipose tissue samples taken from different patients. Fig. 4 shows the representative SEM micrographs of VAT layer of adipose tissue samples taken from obese patients having different metabolic diseases. Interestingly, the individuality of morphology of different VAT adipose tissue samples is strongly expressed. One part of the samples are composed of large particles necked to each other. These single cloudy particles are usually covered with randomly distributed differently shaped nanoparticles. The second part of SEM micrographs could be attributed to the adipose tissue samples showing the network of multishaped nanoparticles (50-100 nm) distributed on the surface of hard agglomerates of plate-like microparticles (0.3-0.5 μm). The surface of third part of the samples instead of continuous network is composed of the separate spherical clusters ~1.0-1.5 μm in size. In some cases, these spherical clusters are prolonged in one direction and form the dimeric or oligomeric fragments.

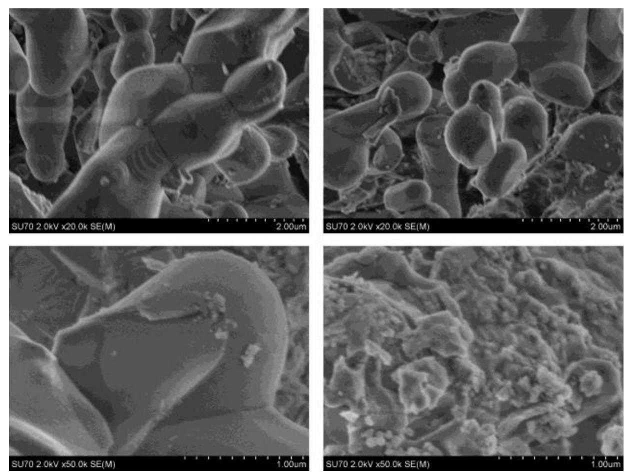

Figure 4: SEM micrographs of adipose tissue obtained from visceral layers of obese patients with different metabolic diseases.

Thus, careful morphological observations revealed that the surface of visceral adipose tissue could serve very important biomedical information.

It is known, that certain amount of different metals accumulated in the blood plasma or other human internal organs could be as signal of the seriousness and depth of the disease [10-13]. Therefore, we have evaluated chemical composition of the adipose tissue samples by energy dispersive X-ray spectroscopy. It was determined, that the concentration of sodium and potassium in adipose layers is much higher in comparison with other elements. The concentration of magnesium is higher than calcium almost in all samples. Moreover, Mn and Ni were not detected in all analysed samples of adipose tissue. The EDX analysis results obtained for the determination of selected transition elements in PAT, SAT and VAT layers of adipose tissue specimens showed, that the concentrations of Fe and Zn do not vary significantly in the adipose tissue samples from different patients. However, the amount of iron was highest in the PAT layers, and the VAT layers generally contained slightly smaller amount of Fe in comparison with SAT layers. On the other hand, the random distribution of zinc in different layers of adipose tissue was determined. Evidently, some adipose tissue samples contain significantly higher amount of copper. Interestingly, the higher amount of copper is prevailing in VAT layer. Also, rather large amount of Cu was determined also in some samples of SAT layer. Chromium was found only in the adipose of few patients. The relative standard deviation (RSD) values obtained for the determination of metals in the adipose from the obese patients (3.4%-7.1%) indicate a high degree of homogeneity, which could be expected for adipose samples. No doubt, the elemental analysis results obtained show various distributions of different metals in adipose tissue of patients with different metabolical state. We may assume, however, that the results of distribution of copper and chromium in adipose tissue layers in obese patients are promising for further medical observation. The change of these metals concentrations in adipose tissue might be the sign or the possible reason of appearance of symptoms of different diseases. Finally, the initial observations show such a tendency that the higher concentration of metals prevail in SAT and VAT layers. However, the distribution of metal levels in adipose tissue layers is rather chaotic and important information could be probably obtained after more intensive study.

4 CONCLUSIONS

It was demonstrated for the first time to the best our knowledge the application of scanning electron microscopy (SEM) and metal content determination by energy dispersive X-ray spectroscopy (EDX) for the characterization of adipose tissue from obese patients. The SEM micrographs of samples from the preperitoneal layer of adipose tissue showed that adipose tissue of PAT is composed of irregularly shaped 200 nm–2 μm in size particles which are closely connected to each other forming hard agglomerates. The microstructure of the adipose tissue PAT layers' obtained from different patients is quite similar. The SEM micrographs of subcutaneous adipose tissue layer taken from obese patients having different metabolic diseases were slightly different in comparison with ones taken from SAT layers of people without metabolic diseases. The adipose tissue samples were composed of cubes, prisms and spherically shaped granules particles less than 0.5 μm in size. The interesting morphological feature was rarely observed bright spots in all SEM micrographs of SAT adipose tissue picked up from different patients. The SEM micrographs of visceral adipose tissue revealed individual surface morphology of adipose tissue samples taken from different patients. These initial SEM observations let us to conclude that preperitoneal layer perhaps could be considered as a passive organ, i. e. is not associated with metabolic changes in human body. The bright spot seen in the SEM images of SAT layers probably are associated with metabolic changes in body of obese patients. Moreover, careful morphological observations revealed that the surface of visceral adipose tissue could serve very important biomedical information. The elemental analysis results obtained showed various distributions of different metals in adipose tissue of patients with different metabolical state. However, the results of distribution of copper and chromium in adipose tissue layers in obese patients are promising for further medical observation. Obviously, further investigation is needed to prove the impact of chemical composition, structural and morphological features to adipose tissue activity and their relationship with disease stage.

5 ACKNOWLEDGEMENTS

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