Comparative Analysis of Mechanical Properties of Common Carotid Artery between Young and Old Specimen

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ABSTRACT

Objective: This study aimed to perform the carotid artery stretching experiments with young and elderly corpse for the study of carotid atherosclerosis pathogenesis and biomechanical basis.

Methods: 15 carotid arteries were randomly selected from young and elderly groups, respectively, for the longitudinal tensile test, and 1 specimens of each group was randomly selected for the morphological observation.

Results: The maximum longitudinal tensile stress, maximum strain, elastic limit strain of middle cerebral artery in young group were greater than those of elderly group ($P < 0.05$), while the elastic modulus of middle cerebral artery in young group was less than that of elderly group ($P < 0.05$).

SEM results showed that the muscle cells of middle cerebral artery in young group were in neat arrangement, with intact elastic membrane, while the muscle cells of elderly carotid artery showed active hyperplasia, some elastic membrane were ruptured, with disorganized arrangement.

Conclusions: The morphology and longitudinal tensile properties of carotid artery in elderly group of changed.

Keywords: Carotid artery, elderly, longitudinal stretching mechanical properties, youth

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INTRODUCTION

Carotid artery is the predilection site for carotid atherosclerosis, which is the most frequently involved in systemic atherosclerosis. Carotid atherosclerosis and stenosis are extremely easy to cause the cardiovascular and cerebrovascular diseases. Domestic and foreign scholars have performed on a lot of researches about the pathogenesis of atherosclerosis, stroke prevention, diagnosis and treatment (1-12). Homburg et al. (13) have carried out the correlation analysis on the relationship of CA and cerebral infarction, they believe that CA can be used as one of the most important risk indicator of cerebral infarction prediction; Simova et al. (14) have studied the relationship of media layer-thickness of carotid intima and CA and find that, the intima membrane thickness can be seen as the marker of atherosclerotic changes. Parmar et al. (15) have studied the relationship between stroke onset with the unstable carotid artery plaques or "vulnerable plaques" of ischemic stroke, and the results show that the complex carotid artery plaque was closely related to ischemic stroke. Sadekova et al. (16) have studied the effect of carotid artery calcification on cerebral arterial stiffness in mice by using $CaCl_2$ deposition and find that, the carotid artery calcification can induce the cerebral superoxide anion generation and increased neurodegenerative diseases, with decreased carotid artery elasticity, compliance and distensibility. The mouse model of $CaCl_2$ deposition in carotid artery conforms with the feature and specificity of arterial stiffness, and can be used to study the effects of arterial stiffness on the brain. Khorvash et al. (17) have comparatively analyzed the relationship between carotid artery intima-media thickness and type D personality in

40-60 years old persons, and measured the carotid artery intima-media thickness in placebo group and type D personality group using Doppler ultrasound. Results show that, the average carotid artery intima-media thickness between two groups is no significantly different (P = 0.19). However, there are 22.9% and 48.6% of individuals with abnormal intima-media thickness in placebo group and type D personality group, with significant difference between them (P = 0.001). Type D personality can increase the incidence of cardiovascular disease, especially for stroke and myocardial infarction due to hormonal imbalance, which lead to artery vasospasm and atherosclerosis. Therefore, it is necessary to conduct evaluation and treatment for patients with increased risk of atherosclerosis and myocardial infarction. The elderly metabolic syndrome (MS), such as hypertension, hyperglycemia, dyslipidemia and obesity, was the frequently-occurring and common diseases towards the elderly, and the independent risk factors of cardiovascular diseases. Von Hafe et al. (18) reported that the prevalence rate of elderly MS was 43.5%. The morbidity and mortality of elderly heart and cerebrovascular diseases were even higher. The main pathological basis of heart and cerebrovascular diseases were the atherosclerosis. In the recent years, the carotid had become a "window" that could reflect the systemic atherosclerotic lesions.

Jackson et al. (19) have copied rabbit carotid artery injury model and decreased the carotid artery tension with heteropleural transplantation method. Result show that, the reduced longitudinal tension can lead to vascular tissue reconstruction and arterial distortion. The size of the longitudinal tension has an important influence on the arterial injury transplantation.

There are rare studies of longitudinal tensile with the fresh cadaver carotid artery of Chinese people and fresh elderly carotid artery. It is deduced that the elderly carotid artery will change due to the changes of mechanical properties of CA tissue morphological changes. There are some differences between the mechanical properties of young and elderly carotid artery, which must verify through the tests. In view of this, the author performed the longitudinal tensile test on normal fresh carotid artery and elderly carotid artery of people cadaver, to comparatively analyze the tensile mechanical properties of fresh and elderly carotid artery of Chinese people cadaver, discuss the pathogenesis of atherosclerosis and cerebral infarction from the biomechanical perspectives.

SUBJECTS AND METHODS

Subjects

The young carotid arteries were obtained from 10 fresh normal adult male corpses, aged 22-25 years old, and the senile carotid arteries were from 10 elderly male bodies, aged 65-70 years old, who had the history of atherosclerosis and high blood pressure before the death. The bodies were provided by College of Basic Medical Science, Jilin University. The body was dissected within 24 hr of death: the body was lied on the back, with a wooden pillow padded under the rear part of neck, so that the head could be lied back as far as possible. The jugular vein incision was made from the middle chin, along the midline and to the sternum, along the upper end of incision and the lower jaw, the incision was expanded to the bilateral mastoid bones, along the lower end of incision and the lateral side of clavicle, the incision was expanded to the acromion. Then along the anterior neck midline, close to the surface of platysma muscle, the skin was dissected and turned back to the front edge of trapezius muscle, thus the platysma muscle was revealed. The platysma muscle was then cut along the edge of clavicle, followed by flipped up close to the deep side until the basis mandibulae, the cervical plexus branches of sternocleidomastoid surface could then be recognized; found the anterior jugular vein from the superficial fascia on the bilateral sides of anterior neck midline, and found the initial segment of external jugular vein from the posterior mandibular angle, thus the external jugular vein could be seen obliquely pass through the sternocleidomastoid surface and penetrate the deep fascia, then removed all the superficial fascia. Cleaned and observed the cuff fascia, and it could be seen that it wrapped the sternocleidomastoid and trapezius, and linked up to the hyoid body; then cut the cuff fascia along the upper edges of sternum and clavicle, flipped up, and stripped the cuff fascia on the surface of sternocleidomastoid. The heads of sternum and clavicle of sternocleidomastoid were cut off, separated and turned upwards this muscle to observe the deep carotid sheath, under which surface the hypoglossal nerve loop could be seen, with deep lateral cervical lymph nodes surrounded, and divided into two groups (up and down) from the scapular hyoid muscle. The carotid sheath was then vertically incised to reveal the common carotid artery, according to experimental needs, 10 left common carotid arteries and 10 right common carotid arteries were obtained. The specimens were placed in the physiological saline for the future use. And the use of specimens was approved by the ethics committee of Medicine Department of Jilin University.

Preparation of the carotid artery specimens

20 young and elderly carotid artery specimens, respectively, were stored for one day, then removed the carotid artery. The S-5 sterile plastic handle scalpel, Lianheyikang, Huai'an, Jiangsu Province, was used to cut and obtain 20 15 mm-long samples in the proximal site of the elderly common carotid artery group, as well as 20 samples from the same site of the youth group for the tensile test. And in both elderly and young groups, a 5 mm length sample from the bifurcation of the common carotid artery was used for the tissue morphological observation.

Tensile test method

The laboratory equipment was Css4410 automatic-controlling electronic universal testing machine (Changchun, Jilin, China), Changchun Institute of Testing Machine, with ambient temperature box. The reading microscope, the 3rd Optical Instrument Factory (Changchun, Jilin, China), was used to measure the lengths and diameters of specimens in each group. The common carotid artery sample was 15 mm in length and 1.19-1.21 mm in thickness, with the inner diameter as 6.5-6.8 mm outer diameter as 8.89-9.12 mm. Firstly, each sample was performed pre-emptive adjustment according to the reference method (20-22). The experiment simulated the normal body temperature at (36.5 ± 1.0) °C conditions, the carotid artery specimens of young and elderly groups were clamped in the test machine, respectively, then applied the elongation load at the speed of 0.5 mm/min, at the same time, continuously leached some solution to the sample to maintain the humidity of the sample during the test. After the experiment, the computer would automatically output the stress, strain, elastic modulus under 10.7 kpa (80 mmHg), 16.0 kpa (120 mmHg) and 225 kpa (170 mmHg), and the stress - strain curve.

SEM observation of the ultrastructure of carotid arterial cross section

SEM was performed to observe the ultrastructure of the carotid arterial cross section in young and elderly groups: randomly selected 1 sample from the young and elderly groups, respectively, cut into pieces of 5 mm, pre-fixed with 4% glutaraldehyde and post- fixed with 1% osmium tetroxide, then dehydrated in acetone and dried at the critical point, coated with gold film under vacuum, then the field emission SEM (Carl Zeiss Company, Jena, Germany) was performed to observe the morphology of carotid artery wall.

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using SPSS 11.0 statistical software. Single-factor randomized group design was used for analysis, and Dunnett's T3 test was applied to all pair-wise comparisons. $P < 0.05$ was considered as statistically significant.

RESULTS

Sample number analysis

The total sample number of carotid artery tensile test in the young and elderly groups was 40, all included in the results analysis, without any loss value. The 2 carotid artery tissue morphology samples of young and elderly groups were all included in the final analysis.

Longitudinal tensile results of carotid artery specimens

The experimental data of longitudinal tensile test were shown in Table 1, the data of the two groups were curve fitted with a computer, and the stress - strain curves were shown in Figure 1.

The tensile test results showed that the maximum stress of the elderly samples was 227.2 ± 7.3 kpa, maximum strain was 67.7 ± 5.0%, and the elastic limit strain was 29.3 ± 2.6%, the elastic modulus at 13.3 kpa stress was 98.5 ± 1.8 kPa, 102.5 ± 2.3 kPa at 16.0 kpa stress and 106.1 ± 1.5 kPa at 22.5 kpa stress, while towards the young specimens, the maximum longitudinal tensile stress was 254.8 ± 13.1 kPa, the maximum strain was 90.6 ± 10.6%, the elastic limit strain was 39.5 ± 2.8%, the elastic modulus was 93.9 ± 5.6 kPa at 13.3 kpa stress, 96.3 ± 1.4 kPa at 16.0 kpa stress and 98.4 ± 1.1 kPa at 22.5 kpa stress. The maximum stress, maximum strain and elastic limit strain of young group were larger than those of the elderly groups ($P < 0.05$), while the elastic modulus at 13.3 kpa, 16.0 kpa and 22.5 kpa stress were less than the elderly group ($P < 0.05$).

In Figure 1, the stress was the ordinate, and the strain was the abscissa. It could be concluded that when the stress was 16.0 kpa, the two stress - strain curves exhibited exponential relationship, the stress and strain of young group were greater than those of elderly group. When the stress exceeded 16.0 Kpa, the strain of both young and elderly groups increased rapidly, along with the increasing stress-strain curve, slope increased, the stress-strain relationship exhibited disproportionate change, the continuous force loading would result in the increaseing deformation of the vascular wall until the sample was damaged.

Establishment of stress-strain functional relations

From the measured data, the dependent relations and the total deviation among variables were investigated for the creation of the stress-strain functional relations:

Young group: $\sigma(\varepsilon) = 0.0000\ e^5 - 0.0051\ e^4 + 0.8803\ e^3 + 2.6361\ e^2$

Elderly group: $\sigma(\varepsilon) = -0.0000\ e^5 + 0.0054\ e^4 + 0.7930\ e^3 + 5.1093\ e^2$

Histological analysis of longitudinal section on young and elderly groups

In order to analyze the tissue morphology of longitudinal section in both young and elderly groups, SEM was performed to observe the morphological changes of longitudinal sections. The longitudinal optical micrographs of carotid artery in young and elderly groups were shown in Figures 2 and 3, respectively.

The morphology observation results showed that the carotid arterial intimal thickening of elderly group was apparent, the proliferation of a large number of smooth muscle cells (SMC) could be seen under the intima, with visible lipid plaque and the accumulation of foam cells, the internal elastic lamina and the elastic lamina degenerated, fractured and disintegrated, a lot of plaques and inflammatory cell infiltration could be seen in intima and tunica media, the tissue morphology exhibited the characteristics of atherosclerosis (Figure 3). While the structures of carotid artery wall layers in young group were normal, without atherosclerotic plaque, the intimal was smooth, and the elastic fibers in the tunica media were normal (Figure 2).

DISCUSSION

In this study, the experimental methods towards both young and elderly corpse carotid artery specimens, namely sampling, sample preservation, sample pre-tune treatment, experimental ambient temperature, experimental speed, and sample interventions for tissue morphological observation specimens, were all consistent, so the experimental results were reliable. Results of this study show that, there are obvious morphology and mechanical property changes of carotid artery in elderly group due to atherosclerosis, with significant difference from young group, which is consistent with expectation.

This study found that, when the stress did not exceed the normal diastolic pressure (16.0 kpa), the carotid arterial strain increased rapidly in both two groups, when the stress exceeded 16.0 kpa, the strain rose slowly. At the beginning, the strain exhibited fast change speed, indicating the inherent expansion pressure inside the common carotid artery was less than the partial pressure, leading to faster water-spilling-out speed, and with continuous fluid outflow from the carotid artery, the difference between swelling pressure and partial pressure decreased, so the changes slowed down in the later stage. The feature, that the deformation of carotid artery wall under the outer load changed slowly instead of rapidly with the increasing of stress, could play a protective role when in emergency, there was no unusual, nor any clinical symptoms in the cerebral artery while the blood pressure increased. The morphology observation of both young and elderly groups showed that in the elderly group, carotid arterial intimal thickening was apparent, the proliferation of a large number of SMC could be seen in the intima, with visible plaques and the accumulation of foam cells, the internal elastic lamina and the elastic lamina degenerated, fractured and disintegrated, a lot of plaques and inflammatory cell infiltration could be seen in intima and tunica media, the tissue morphology exhibited the characteristics of atherosclerosis. While the structures of carotid artery wall layers in young group were normal, without atherosclerotic plaque, the intimal was smooth, and the elastic fibers in the tunica media were normal. The analysis believed that the carotid artery of the elderly group would appear atherosclerosis and proliferation of a large number of SMC under the arterial intima might because of the aging period of elderly body, the internal elastic lamina and the elastic lamina degenerated, fractured and disintegrated, leading to the changes of content and orientating direction of the elastic fibers and collagen fibers, therefore the flexibility and toughness decreased, while the hardness increased, namely the elastic modulus was improved. Long-term high blood pressure and high stress-induced carotid artery remodeling in elderly people led to the changes of blood vessels functions, therefore the arterial compliance decreased, and the mechanical properties of the vascular wall changed. CA has a very close relationship with cerebral infarction, and is the risk factor for cerebral infarction. After the formation of carotid artery plaques, the stress of the arterial wall would increase and the impact of the unstable blood would rupture plaque in hypertensive state, then activate the platelets, started the fibrinolytic system, which would gradually develop into cerebral infarction. The experimental results supported the view that the mechanical property changes of the vascular wall were one of the pathogenesis of atherosclerosis and cerebral infarction.

In this study, the maximum strain of carotid artery in human cadaver was 90.6 ± 10.6%, less than the pig carotid maximum tensile strain by Clerin (17), indicating that there were differences in tensile mechanical properties of the common carotid artery among different species. In the past, the mechanical properties of the common carotid artery studies mainly used the animal corpses carotid artery, and mostly focused on 1D longitudinal stress - strain relations, with the speed of longitudinal tensile experiments was faster than 0.5 mm/mim. In this study, the difference from previous studies was that the test speed was 0.5 mm/mim, with this speed, the maximum stress, strain data, the elastic modulus data at 13.3 kpa, 16.0 kpa and 17.8 kpa stress and stress - strain curve of young and elderly groups were obtained, and regression analysis was used to develop the stress - strain relation expression, quantitatively compared the mechanical properties of the two groups, and discussed the relations of atherosclerosis and cerebral infarction from the biomechanical view, providing the biomechanical basis of the pathogenesis of atherosclerosis and cerebral infarction. There was innovation in the experimental methods and data processing methods.

Conflict of interest

The authors declare that they have no conflict of interest

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Table: Longitudinal tensile results of carotid artery specimens of young and elderly groups.

Index	Young group	Elderly group
Maximum stress (KPa)	254.8 ± 13.1	227.2 ± 7.3[a]
Maximum strain (%)	90.6 ± 10.6	67.7 ± 5.0 [a]
Elastic limit strain at 16.0 Kpa	39.5 ± 2.8	29.3 ± 2.6[a]
Elastic limit strain at 13.3 Kpa	93.95 ± 5.6	98.5 ± 1.8 [a]
Elastic modulus at 16.0 Kpa	96.3 ± 1.4	102.5 ± 2.3 [a]
Elastic modulus at 22.5 Kpa	98.4 ± 1.1	106.1 ± 1.5[a]

Note: The data were expressed as x ± s, the group comparison was performed using Sceffe France, [a]$P < 0.01$, *vs* young group.

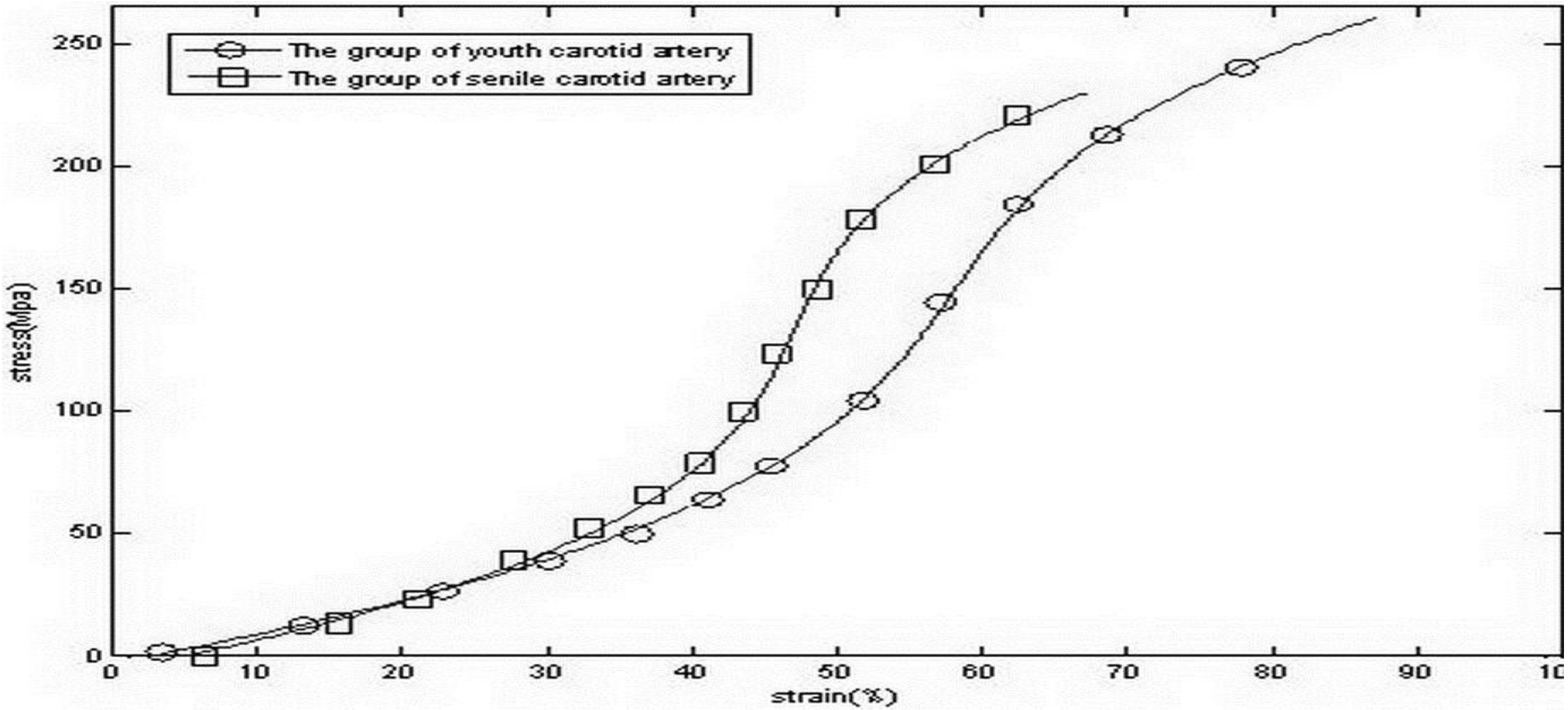

Fig.1: Stress-strain curve of longitudinal tensile in both young and elderly groups.

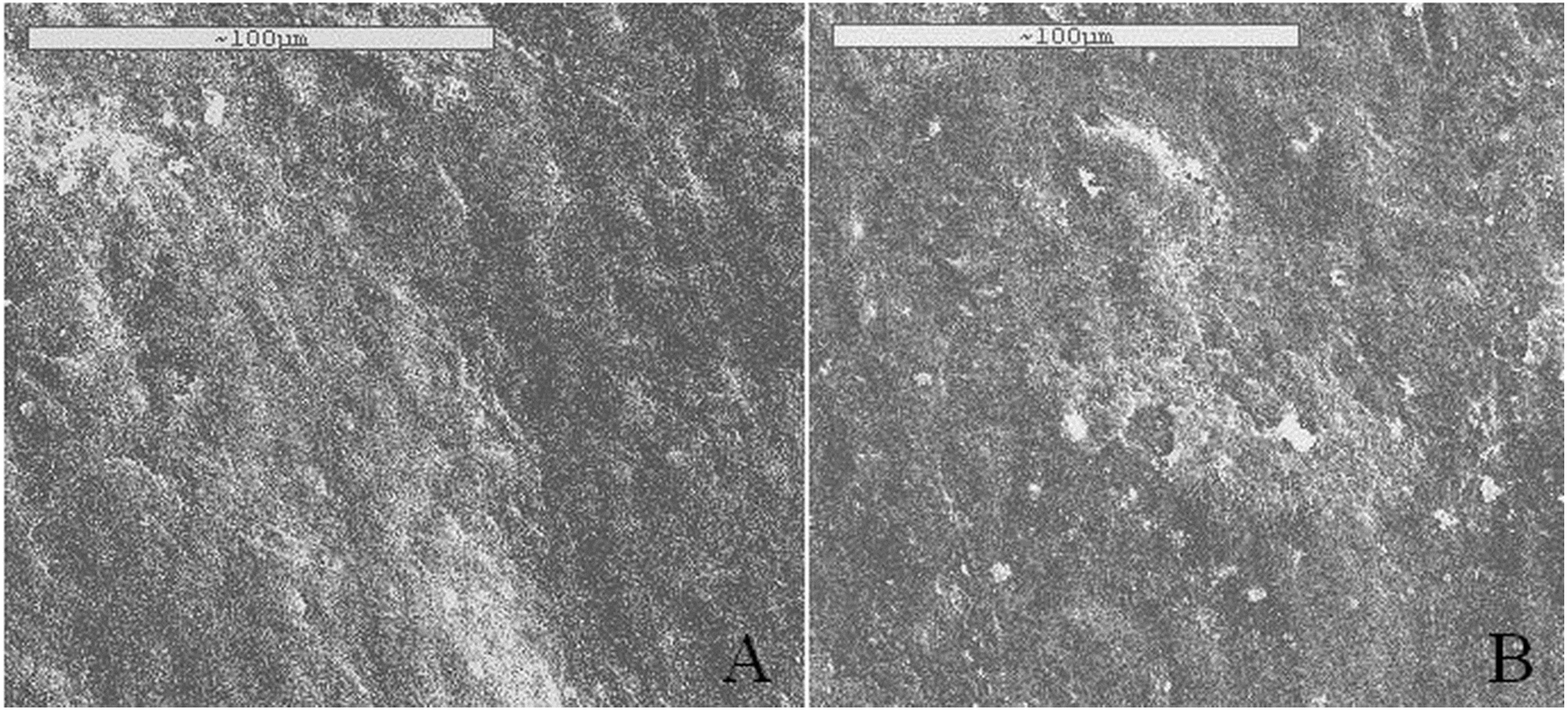

Fig.2: Longitudinal optical micrograph of young group.

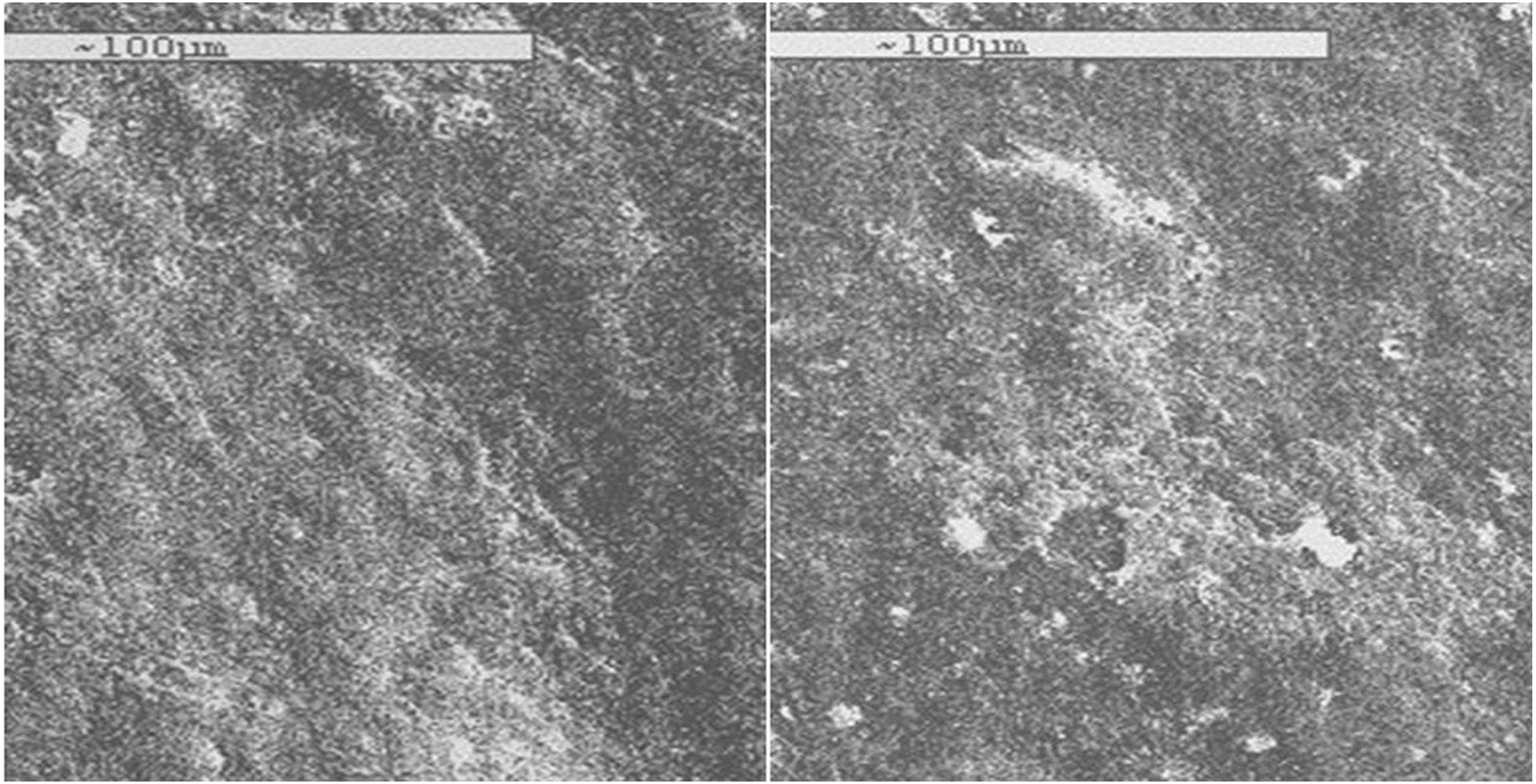

Fig.3: Longitudinal optical micrograph of elderly group.