Summary

Introduction.The gold standard of orthopedics as well as maxillofacial surgery is reconstruction with the use of titanium implants. Titanium is believed to be a biocompatible material, but is it an ideal material? Despite its many advantages, titanium also has disadvantages, the greatest of which is poor friction resistance, which results in damage / loss of the passive layer on its surface. The passive layer is formed by titanium oxides (mainly TiO2), and its formation is spontaneous, in contact with the surrounding environment of titanium. In the production of medical implants, the enhancement of the protective properties of the passive layer is achieved through the use of electrochemical oxidation, the so-called anodizing. The test results show that this layer, despite the anodizing process, is damaged, which initiates corrosion on the implant surface.,and corrosion contribute to a phenomenon known as metallosis. Metallosis is the deposition of metallic particles in the tissues surrounding the implant. These particles stimulate the body's immune system, undergo phagocytosis, which results in the release of cytokines and free oxygen radicals (ROS). This causes inflammation around the implant. ROS are responsible for the oxidative damage of proteins, lipids and nucleic acids. They affect the structure and activity of cells. Moreover, by influencing bone remodeling, they contribute to disturbing healing processes.

Goals of the work. The research I conducted and their analyzes were aimed at:

1. Comparison of the cytotoxicity of titanium discs with the passive layer with type II anodizing to the cytotoxicity of titanium discs without the passive layer and with the standard anodized layer in the culture of human, non-genetically modified, primary fibroblasts ATCC-PCS-201-018.
2. Assessment of the effect of titanium discs with a passive layer with type II anodizing on the total antioxidant potential as well as enzymatic and non-enzymatic antioxidant systems of human, genetically unmodified primary fibroblasts ATCC-PCS-201-018 by comparing the obtained results with the results obtained in the culture of the above-mentioned fibroblasts exposed to titanium discs without a passive layer and with an anodized layer as standard.
3. Comparison of oxidative damage and redox imbalance measured by the concentration of oxidative products of protein and lipid modification, as well as the total oxidative potential

in cellshuman, non-genetically modified, primary fibroblasts ATCC-PCS-201-018exposed totitanium discs with a passive layer with type II anodizingand in the above-mentioned cellsfibroblasts exposed to titanium discs without a passive layer and with a standard anodized layer.

4. Assessment of pro-oxidative factors, i.e. NADPH oxidase activity and peroxynitrite concentrationin cellshuman, non-genetically modified, primary fibroblasts ATCC-PCS-201-018 exposed totitanium discs with a passive layer with type II anodizing by comparing the obtained results with the results obtained inthe above-mentioned cellsfibroblasts exposed to titanium discs without a passive layer and with a standard anodized layer.

5. Comparison of the functioning of the respiratory chain enzymes and the activity of the apoptosis marker - caspase-3 in human mitochondria, non-genetically modified primary fibroblasts ATCC-PCS-201-018exposed totitanium discs with a passive layer with type II anodizingand in the above-mentioned cellsfibroblasts exposed to titanium discs without a passive layer and with a standard anodized layer.

6. Assessment of the concentration of growth factors FGF-2 and VEGF-Ain a medium taken from above the cellshuman, non-genetically modified, primary fibroblasts ATCC-PCS-201-018exposed totitanium discs with a passive layer with type II anodizing by comparing the obtained results with the results obtained in the collected mediumabove the abovefibroblasts exposed to titanium discs without a passive layer and with a standard anodized layer.

7. Evaluation of the release of titanium, aluminum and vanadium ions to the culture medium from the surface of titanium discs with a passive layer with type II anodizing by comparing the obtained results with those obtained in the medium collected from above the titanium discs with the standard anodized layerand abovediscs without a passive layer during the cultivation processcellshuman, non-genetically modified, primary fibroblasts ATCC-PCS-201-018.

Materials and methods. The research was carried out on breedinghuman, non-genetically modified, primary fibroblasts(Human Primary Gingival Fibroblasts, ATCC-PCS-201-018) purchased from ATCC. In the experiment, titanium discs (Ti6Al4V) made to individual order by ChM were used, differing in the type of coating: hard anodized, standard anodized and non-anodized discs (without coating). The control group consisted of polystyrene discs of the same diameter and thickness as the titanium discs.

Cells were grown in 12-well plates in the manufacturer's recommended environment. After reaching the appropriate confluence,Titanium disks, each in 6 replications, were applied to the plates. After application of titanium disks, cells were cultured for 24 h, 7, 14 and 21 days. At the indicated time intervals:

1) the viability of the cells was assessed by means of the MTT test.

2) mitochondria were isolated and the concentration of total protein, caspase-3 (CAS-3) activity and the functioning of the respiratory chain: activity of complex I and II, cytochrome c oxidase (COX) and citrate synthase (CS) were determined by colorimetric methods.

3) the fluid above the cells was collected, in which the concentrations were determined by: colorimetric methods:total protein (BCA), malonyldialdehyde (MDA), disulfide groups (SS), peroxynitrite (ONOO-),3-Nitrotyrosine (3-NT) and total oxidative status (TOS) and total antioxidant capacity (TAC) were assessed; by fluorimetric methods: content of end products of protein oxidation (AOPP) and end products of advanced glycation of proteins (AGE); by ELISA methods: concentration of fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF-A) and 4-hydroxynonenal adducts with proteins (4-HNE adducts).

4) in the cell lysate, the concentrations of total protein (BCA), malonyldialdehyde (MDA), disulfide groups (SS), peroxynitrite (ONOO-) were determined by colorimetric methods,3-Nitrotyrosine (3-NT), reduced glutathione (GSH), activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), NADPH oxidase (NOX) and total oxidation status (TOS) and total antioxidant capacity (TAC): by fluorimetric methods: content of protein oxidation end products (AOPP) and advanced glycation end products (AGE); ELISA method: concentration of 4-hydroxynonenal adducts with proteins (4-HNE adducts).

The metal content in the medium was assessed after 3, 6, 15, and 21 days using the ICP-MS method.

The statistical analysis of the obtained results was performed with the use of the Statistica 12.0 program, using the following tests: ANOVA with the HSD Tukey post-hoc test.The correlation analysis was performed using the Pearson method with $p < 0.05$ as the level of significance.

Results. Due to the number of obtained results, I present only statistically significant results.

After 24h I showed:

- significantly higher activity of GPx in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly higher TOS for alloy V as compared to the control and in relation to TOS in fibroblast cells exposed to V (st) and V (t) feet.
- significantly higher NOX activity in cells for alloy V as compared to the control, as well as in relation to the activity of this enzyme in cells exposed to rates V (st) and V (t).
- significantly higher concentration of AOPP in cells for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of disulfide groups in cells for alloy V, compared to the control and alloys V (st) and V (t).
- significantly lower activity of complex I in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower activity of complex I in fibroblast mitochondria for alloy V compared to alloys V (st) and V (t),
- significantly higher TOS in the medium for alloys V, V (st), V (t) compared to the control.
- significantly higheradduct concentration 4-HNE in medium for alloys V, V (st) and V (t) compared to control.

After 7 days, I showed:

- significantly lower concentration of total protein in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly higher SOD activity in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly higher SOD activity in fibroblast cells for alloys V (st), V (t) compared to V.
- significantly higher activity of GPx in cells for alloys V, V (st), V (t) compared to the control.
- significantly lower GR activity in fibroblast cells for alloy V compared to the control.
- significantly higher GR activity for alloys V (st), V (t) compared to the activity of GR in fibroblast cells exposed to alloy V.
- significantly higher GSH concentration in cells for alloys V (st), V (t) in relation to GSH concentration in fibroblast cells exposed to alloy V.
- significantly higher GSH concentration for alloy V (t) as compared to alloy V (st).
- significantly lower TAC for alloys V and V (st) compared to the control.
- significantly higher TOS in cells for alloys V, V (st), V (t) compared to the control.

- significantly lower TOS in fibroblast cells for alloys V (st), V (t) compared to TOS in fibroblast cells exposed to alloy V.
- significantly higher NOX activity in fibroblast cells for alloys V, V (st) and V (t) compared to the control.
- significantly higher NOX activity in fibroblast cells for alloy V compared to the activity of this enzyme in cells exposed to V (st) and V (t) feet.
- significantly higher MDA concentration for alloys V, V (st) and V (t) compared to the control.
- significantly higher concentration of MDA for alloys V and V (st) in relation to the concentration of MDA in cellsexposed to alloy V (t).
- significantly higher concentration of 4-HNE adducts in fibroblast cells for alloys V and V (st) compared to the control.
- significantly higher concentration of 4-HNE adducts in cells for alloys V and V (st) compared to the concentration of 4-HNE adducts in cellsexposed to alloy V (t).
- significantly higher AOPP concentration for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of disulfide groups in fibroblast cells for alloy V, compared to the control and alloys V (st) and V (t).
- significantly lower activity of complex I in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower activity of complex I in fibroblast mitochondria for alloy V compared to alloys V (st) and V (t).
- significantly lower activity of complex II in fibroblast mitochondria for alloys V and V (st) compared to the control.
- significantly lower activity of complex II in fibroblast mitochondria for alloy V compared to alloy V (t).
- significantly lower COX activity in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of FGF-2 in the medium for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of VEGF-A in the medium for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of VEGF-A in the medium for alloy V compared to alloys V (st) and V (t).

- significantly lower concentration of VEGF-A in the medium for alloy V (st) compared to alloy V (t).
- significantly higher MDA concentration in the medium for alloy V compared to the control and for alloys V (st) and V (t).
- significantly higher concentration of 4-HNE adducts in the medium for alloys V, V (st) and V (t) compared to the control.
- significantly higher concentration of AGE in the medium for alloys V, V (st) and V (t) compared to the control.

After 14 days, I showed:

- significantly higher cell viability for alloy V (t) as compared to alloy V.
- significantly higher cell viability for alloy V (st) as compared to alloy V.
- significantly lower concentration of total protein in fibroblast cells for alloys V, V (st),
- significantly higher SOD activity in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly higher SOD activity in fibroblast cells for alloys V (st), V (t) compared to V.
- significantly higher CAT activity in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly lower CAT activity in fibroblast cells for alloys V (st), V (t) compared to V.
- significantly lower GR activity in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly higher GR activity in fibroblast cells for alloys V (st), V (t) compared to the activity of GR in fibroblast cells exposed to alloy V.
- significantly higher GR activity in fibroblast cells for alloy V (t) as compared to the GR activity in fibroblast cells exposed to alloy V (st).
- significantly lower concentration of GSH in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly higher GSH concentration in fibroblast cells for alloys V (st), V (t) compared to the concentration of GSH in fibroblast cells exposed to alloy V.
- significantly higher GSH concentration in fibroblast cells for alloy V (t) as compared to alloy V (st).
- significantly lower TAC in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly higher TAC in fibroblast cells for alloys V (st), V (t) compared to TAC in fibroblast cells exposed to alloy V.

- significantly higher TAC in fibroblast cells for alloy V (t) compared to alloy V (st).
- significantly higher TOS in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly lower TOS in fibroblast cells for alloys V (st), V (t) compared to TOS in fibroblast cells exposed to alloy V.
- significantly higher TOS in fibroblast cells for alloy V (st) compared to alloy V (t).
- significantly higher NOX activity in fibroblast cells for alloy V compared to the control, as well as in relation to the activity of this enzyme in cells exposed to feet V (st) and V (t).
- significantly higher MDA concentration in fibroblast cells for alloys V and V (st) compared to the control.
- significantly higher MDA concentration in fibroblast cells for alloys V and V (st) in relation to the concentration of MDA in cells exposed to alloy V (t).
- significantly higher concentration of MDA in fibroblast cells for alloy V in relation to the concentration of MDA wcells exposed to alloy V (st).
- significantly higher concentration of 4-HNE adducts in fibroblast cells for alloys V and V (st) compared to the control.
- significantly higher concentration of 4-HNE adducts in fibroblast cells for alloys V and V (st) as compared to the concentration of 4-HNE adducts in cells exposed to alloy V (t).
- significantly higher concentration of 4-HNE adducts in fibroblast cells for alloy V compared to the concentration of 4-HNE adducts in cells exposed to alloy V (st).
- significantly higher concentration of AOPP in cellsfibroblasts for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of disulfide groups in fibroblast cells for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of disulfide groups in fibroblast cells for alloy V, compared to alloys V (st) and V (t).
- there was a significantly higher concentration of 3-NT in fibroblast cells for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of BCA in fibroblast mitochondria for alloys V, V (st) compared to control and alloy V (t).
- significantly lower activity of complex I in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower activity of complex I in fibroblast mitochondria for alloy V compared to alloys V (st) and V (t).

- significantly lower activity of complex II in fibroblast mitochondria for alloys V and V (st) compared to the control.
- significantly lower activity of complex II in fibroblast mitochondria for alloys V and V (st) compared to alloy V (t).
- significantly lower COX activity in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower CS activity in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower CS activity in fibroblast mitochondria for alloys V and V (st) compared to the activity of CS in mitochondria of fibroblasts exposed to alloy V (t).
- significantly higher activity of CAS-3 in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of FGF-2 in the medium for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of VEGF-A in the medium for alloys V, V (st) and V (t) compared to the control.
- significantly higher MDA concentration in the medium for alloys V, V (st) and V (t) compared to the control.
- significantly higher MDA concentration in the medium for alloy V compared to alloys V (st) and V (t).
- significantly higher concentration of 4-HNE in the medium for alloys V, V (st) and V (t) compared to the control.
- significantly higher concentration of AGE in the medium for alloys V, V (st) and V (t) compared to the control.

After 21 days, I showed:

- significantly higher cell viability assessed after 21 days for alloy V (t) as compared to alloy V.
- significantly higher cell viability for alloy V (st) as compared to alloy V.
- significantly lower concentration of total protein in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly higher SOD activity in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly higher SOD activity in fibroblast cells for alloys V (st), V (t) compared to V.

- significantly higher CAT activity in fibroblast cells for alloys V, V (st), V (t) compared to the control.
- significantly lower CAT activity in fibroblast cells for alloys V (st), V (t) compared to V.
- significantly lower GR activity in fibroblast cells for alloy V compared to the control.
- significantly higher GR activity in fibroblast cells for alloys V (st), V (t) compared to the activity of GR in fibroblast cells exposed to alloy V.
- significantly lower concentration of GSH in fibroblast cells for alloy V compared to the control and in relation to the concentration of GSH in fibroblast cells exposed to feet V (st) and V (t).
- significantly lower TAC in fibroblast cells for alloy V compared to the control, and in relation to TAC in fibroblast cells exposed to feet V (st) and V (t).
-significantly higher TOS in fibroblast cells for alloys V, V (st), V (t) compared to the control.
-significantly lower TOS in fibroblast cells for alloys V (st), V (t) compared to TOS in fibroblast cells exposed to alloy V.
- significantly higher TOS in fibroblast cells for alloy V (st) compared to alloy V (t).
- significantly higher NOX activity in fibroblast cells for alloy V compared to the control, as well as in relation to the activity of this enzyme in cells exposed to feet V (st) and V (t).
-significantly higher MDA concentration in fibroblast cells for alloy V compared to control and alloys V (st) and V (t).
- significantly higher concentration of AOPP in fibroblast cells for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of disulfide groups in fibroblast cells for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of disulfide groups in fibroblast cells for alloy V, compared to alloys V (st) and V (t).
- significantly higher AGE concentration in fibroblast cells for alloys V, V (st) and V (t) compared to the control.
- significantly higher concentration of ONOO- in fibroblast cells for alloys V, V (st) and V (t) compared to the control.
- significantly higher concentration of 3-NT in fibroblast cells for V, V (st) alloys compared to the control.
- significantly higher concentration of 3-NT in fibroblast cells for alloys V and V (st) compared to alloy V (t).

- significantly lower concentration of BCA in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of BCA in fibroblast mitochondria for alloys V, V (st) compared to alloy V (t).
- significantly lower activity of complex I in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower activity of complex I in fibroblast mitochondria for alloy V compared to alloys V (st) and V (t).
- significantly lower activity of complex II in mitochondria for alloys V and V (st) compared to the control.
- significantly lower activity of complex II in fibroblast mitochondria for alloys V and V (st) compared to alloy V (t).
- significantly lower COX activity in fibroblast mitochondria for alloys V, V (st) and V (t) compared to the control.
- significantly lower activity of COX in mitochondria for alloys V and V (st) compared to activity of COX in mitochondria of fibroblasts exposed to alloy V (t).
- significantly lower mitochondrial CS activity for alloys V, V (st) and V (t) compared to the control.
- significantly lower CS activity in fibroblast mitochondria for alloys V and V (st) compared to the activity of CS in mitochondria of fibroblasts exposed to alloy V (t).
- significantly higher activity of CAS-3 in mitochondria for alloys V and V (st) compared to the control and in relation to the activity of CAS-3 in mitochondria of fibroblasts exposed to titanium discs with vanadium from alloy V (t).
- significantly lower concentration of FGF-2 in the medium for alloys V, V (st) and V (t) compared to the control.
- significantly lower concentration of VEGF-A in the medium for alloys V and V (st) compared to the control.
- significantly lower concentration of VEGF-A in the medium for alloys V and V (st) compared to alloy V (t).
- significantly higher TOS in the medium for alloys V, V (st), V (t) compared to the control.
- significantly higher TOS in the medium for alloy V compared to alloys V (st) and V (t).
- was significantly higher TOS in the medium for alloy V (st) compared to alloy V (t).
- significantly higher MDA concentration in the medium for alloys V and V (st) compared to the control and alloy V (t).

- significantly higher MDA concentration in the medium for alloy V compared to alloy V (st).
- significantly higher concentration4-HNE adducts in the medium for alloys V, V (st) and V (t) compared to the control.
- significantly higher AGE concentration inmedium for alloys V, V (st) and V (t) compared to the control.

In addition, after 3 days, I showed:

- significantly higher content of titanium in the medium for alloy V (t) compared to alloy V and V (st).
- significantly higher aluminum content in the medium for alloy V (t) compared to alloy V and V (st).
- significantly higher vanadium content in the medium for alloy V (t) compared to alloy V and V (st).

After 6 days, I showed:

- significantly higher content of titanium in the medium for alloy V (t) compared to alloy V and V (st).
- significantly higher content of titanium in the medium for alloy V compared to alloy V (st).
- significantly higher aluminum content in the medium for alloy V (t) compared to alloy V and V (st).
- significantly higher aluminum content in the medium for alloy V compared to alloy V (st).
- significantly higher vanadium content in the medium for alloy V (t) compared to alloy V and V (st).

After 15 days, I showed:

- significantly higher content of titanium in the medium for alloy V (t) compared to alloy V and V (st).
- significantly higher content of titanium in the medium for alloy V compared to alloy V (st).
- significantly higher aluminum content in the medium for alloy V (t) compared to alloy V (st).
- higher aluminum content in the medium for alloy V compared to alloy V (st).
- significantly higher vanadium content in the medium for alloy V (t) compared to alloy V and V (st).
- significantly higher vanadium content in the medium for alloy V compared to alloy V (st).

After 21 days, I showed:

- significantly higher content of titanium in the medium for alloy V (t) compared to alloy V and V (st).
- significantly higher content of titanium in the medium for alloy V compared to alloy V (st).
- significantly higher aluminum content in the medium for alloy V (t) compared to alloy V.
- significantly higher vanadium content in the medium for alloy V (t) compared to alloy V and V (st).

Based on the results obtained and the analyzes performed, I have drawn the following ***conclusions:***

1. The degree of cytotoxicity of the titanium discs with type II anodizing depends on the duration of cell culture of human, non-genetically modified, primary fibroblasts ATCC-PCS-201-018. From the 14th day of the experiment, until its completion, it is lower compared to titanium discs without a passive layer, while the type of anodizing does not affect the cytotoxicity degree of the Ti6Al4V titanium alloy.
2. Changes in the antioxidant defense of human, genetically unmodified primary fibroblasts ATCC-PCS-201-018 exposed to titanium discs without a passive layer significantly differ from the changes in the antioxidant defense of the above-mentioned fibroblasts exposed to anodized titanium discs, with the above-mentioned unfavorable changes being visible in fibroblasts exposed to standard anodized discs.
3. Oxidative modifications of proteins occur in all groups of human, non-genetically modified, primary ATCC-PCS-201-018 fibroblasts exposed to titanium discs, and this process is the least intense in the above-mentioned fibroblasts exposed to titanium discs with type II anodizing. Slight and limited to day 7 of culture of human, non-genetically modified, primary ATCC-PCS-201-018 fibroblasts increase of only one lipid peroxidation marker (MDA) and no changes in concentration4-HNE adducts with proteins vs. The control group proves the reversibility of the lipid peroxidation process and a slight increase in oxidative stress. Oxidative modifications of cellular elements were most pronounced in the group of the above-mentioned fibroblasts exposed to titanium discs without a passive layer.

The production of ROS was increased in all groups of human, non-genetically modified, primary ATCC-PCS-201-018 fibroblasts, but the process in the groups of fibroblasts exposed to titanium discs with a passive layer did not start until the 7th day of the experiment. ROS generation was the highest in the group of the above-mentioned fibroblasts exposed to discs without a passive layer, and the second type of anodizing decreased the degree of ROS production compared to standard anodizing.

4. The increase in NADPH oxidase (NOX) activity was dependent on the duration of the culture of non-genetically modified human primary fibroblasts ATCC-PCS-201-018, it was the lowest in the above-mentioned fibroblasts exposed to anodized titanium discs (vs. fibroblasts exposed to titanium discs without a layer passive), the type of anodizing had no effect on the activity of this enzyme. ONOO- concentration increased significantly in the last day of the experiment (vs.control). This increase seems to be independent of the presence of the passive layer or the type of anodizing.

5. The activity of complex I throughout the experiment in all study groups was significantly reduced (vs.control), with the presence of a passive layer appearing to have a protective effect. The type of anodizing does not affect the activity of this enzyme. Type II anodizing, on the other hand, prevents changes in the activity of complex II (vs. control).

Changes in CS activity depend on the duration of the culture, the reduction in the activity of this enzyme can be observed from the 14th day of culture in all test groups (vs. control). The lowest degree of inhibition of this enzyme occurs in the mitochondria of human, non-genetically modified primary fibroblasts ATCC-PCS-201-018 exposed to titanium discs with type II anodizing (compared to the other two studied groups).

COX activity from day 7 to the last day of culture was significantly decreased in the mitochondria of human, non-genetically modified, primary ATCC-PCS-201-018 fibroblasts exposed to titanium discs (vs. control). In the last phase of cultivation, the presence of type II anodizing reduces the degree of inhibition of this enzyme as compared to the other test groups.

The degree of apoptosis in the mitochondria of human, non-genetically modified, primary fibroblasts ATCC-PCS-201-018 depends on the duration of the culture. In the last phase of cultivation, the presence of type II anodizing prevents apoptosis in the mitochondria of the above-mentioned fibroblasts.

6. The concentration of FGF-2 in the medium from all studied groups decreases from the 7th day of cultivation and remains at a significantly lower levelvs.control until the end of the experiment. The value of this reduction was not determined by the presence of a passive layer or the type of anodizing.

Changes in VEGF-A concentrations depend on the duration of the culture and the type of titanium discs, and on day 21, the presence of a passive layer with type II anodizing prevents disturbances in the secretion of this factor by fibroblasts (vs. control).

7. Throughout the experiment, the release of titanium, aluminum and vanadium ions from titanium discs with a hard-anodized passive layer was higher than from other titanium discs. The degree of ion release from the surfaces of all tested titanium discs decreased with time.