Biocompatibility study of a nickel-free medical TiNbZr shape memory alloy

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Abstract. Biocompatibility of a Ti - (20-30) Nb - 5Zr shape memory alloy was studied in vitro in standard cell medium. The number of cells, the mitotic index, the number of dead cells were estimated. All samples were biocompatible in vitro. Mitochondrial activity and good cell survival were observed. The Ti-28Nb-5Zr alloy has the best characteristics.

1. Introduction
The biological compatibility of the implanted material, i.e. the absence of its general toxic effect on the body, inflammatory reaction of surrounding tissues, mutagenic and carcinogenic activity, etc., is one of the most important characteristics of such objects. In this connection, they try to produce the materials of introduction from biologically inert components. For example, research is being actively carried out on the replacement of elements in shape memory alloys endowed with a set of mechanical characteristics necessary for operation in the body (superelasticity, low Young's modulus, delayed response to exposure) [1-3].

The most famous of them is NiTi [4–5]. At the same time, there is a significant dependence of the properties of the material on the state of the surface, which is determined by the method of production, certain difficulties in their processing by traditional methods [6-7], and, most importantly, the toxic properties of nickel contained in a high concentration and the likelihood of corrosive destruction of the material (product damage in the working environment) [8-11], which determines the need to change the composition. The shape memory effect is observed in titanium alloys with content of niobium, tantalum, molybdenum 20-40%, zirconium less than 8 at. % [12-14].

Thus, these alloys can be considered as promising materials for medical production. This work was aimed at studying the biocompatibility of the Ti-Nb-Zr alloy of several compositions.

2. Materials and methods
In the in vitro biocompatibility study, SH-SY5Y neuroblastoma cells cultured in DMEM medium (Gibco) supplemented with 10% bovine serum (FBS, Gibco) and 15 μg / ml gentamicin were used. The suspension was evenly distributed over the plate surface and placed in a CO2 incubator (95% air,
5% CO₂, 100% humidity) for cell attachment. After 30 min, the unattached cells were washed off, and the volume of the culture medium was increased so that the column of the culture medium rose 2 mm above the sample with cells. The culture was left in a CO₂ incubator (95% air, 5% CO₂, 100% humidity) for 72 hours.

Cells were stained with fluorescent dyes Hoechst 33342 (Hoe, 5 μM) - blue nuclei, stains living cells, Propidium Iodide (PI, 3 μM) - red, dead cells, MitoTracker Deep Red FM (MTDR, 0.3 nM) - stains mitochondria, those chromosome carriers, in green, for MI determination - 20 min in Hanks medium supplemented with 20 mM HEPES, pH = 7.36, then washed for 10 min in Hanks medium without dyes and placed in a measuring cell. The studies were carried out using a Leica DMI6000 B inverted fluorescence microscope with a Leica HCX PL APO 63x / 1.40 OIL objective (Leica Microsystems GmbH, Wetzlar, Germany). In each sample, at least 3 areas were randomly selected.

The stained nuclei were counted in the ImageJ program using the Cell Counter plugin. The number of cells in an area of 1 mm² (by the number of nuclei stained with Hoechst 33342, which penetrates the plasma membrane and binds to DNA in the nucleus), the percentage of cells in a state of division (mitotic index, MI, is determined by the presence of visible chromosomes), the number of dead cells (according to the fluorescence of Propidium Iodide, which does not penetrate viable cells, but stains DNA in the nuclei of dead cells and cells with impaired plasma membrane permeability) were estimated. The total number of cells in three fields of view was counted. Statistical data were obtained for three samples of the same type.

### 3. Results and discussion

Data on cell density, mitotic index, and a number of non-viable cells in the presence of the obtained wire are presented in Table 1. Figures 1-5 show the results of fluorescence microscopy in several areas of the sample. Mitochondrial activity and good cell survival were observed.

The mitotic index is an indicator showing the percentage of dividing cells out of the total number of cells analyzed. This index can be calculated by counting cells with visible chromosomes in the field of view and dividing it by the total number of cells in the field of view. Shows the intensity of division by the presence of cells in the growth phase (dividing cells). The higher the value, the more intense the process of cell division and vice versa. It was shown that the proliferative activity of SH-SY5Y cells, when grown on glass, is approximately 1.6% (mitotically active cells).

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In some cases, morphologically abnormal cores were observed, characterized by a strongly elongated, angular shape. Sometimes cells with impaired plasma membrane permeability were observed, as evidenced by the staining of the PI nuclei. This is usually seen in necrosis or the late stages of apoptosis (but in this case, chromatin is condensed and fragmented). In this case, even cells in the process of division are well stained with PI (figure 5).

All samples except pure Nb are biocompatible. The superficial analysis revealed no obvious compositional dependencies. The alloy had no short-term toxic effect on cells overgrowing de novo surfaces.

| Table 1. Cellular activity |
|---------------------------|
| Composition   | Cells/mm² | Dead cells, % | MI, % | N* |
| Ti-30Nb-5Zr   | 1414      | 3.3           | 0.8   | 430 |
| Ti-28Nb-5Zr   | 1230      | 2.4           | 1.4   | 374 |
| Ti-25Nb-5Zr   | 1072      | 3.7           | 0.3   | 326 |
| Ti-20Nb-5Zr   | 1253      | 3.7           | 1.7   | 381 |
| Ti-23Nb-5Zr   | 1691      | 4.9           | 0.8   | 210 |
| TiNi          | 1401      | 4.7           | 1.9   | 426 |
| Nb            | 291       | 31.7          | 0     | 83  |
| Zr            | 1299      | 3.7           | 1.4   | 395 |
Table

| Ti  | 1023 | 14,3 | 1.1 | 311 |

* N – the total number of cells counted for a given sample

**Figure 1.** Cell culture on a Ti-30Nb-5Zr alloy sample. Channel overlay (Hoe fluorescence - blue nuclei of living cells, PI fluorescence - red dead cells, MTDR fluorescence - green cell mitochondria)

**Figure 2.** Cell culture on a Ti-28Nb-5Zr alloy sample. Channel overlay (Hoe fluorescence - blue nuclei of living cells, PI fluorescence - red dead cells, MTDR fluorescence - green cell mitochondria)
**Figure 3.** Cell culture on a Ti-25Nb-5Zr alloy sample. Channel overlay (Hoe fluorescence - blue nuclei of living cells, PI fluorescence - red dead cells, MTDR fluorescence - green cell mitochondria)

**Figure 4.** Cell culture on a Ti-23Nb-5Zr alloy sample. Channel overlay (Hoe fluorescence - blue nuclei of living cells, PI fluorescence - red dead cells, MTDR fluorescence - green cell mitochondria)
Figure 5. Cell culture on a Ti-20Nb-5Zr alloy sample. Channel overlay (Hoe fluorescence - blue nuclei of living cells, PI fluorescence - red dead cells, MTDR fluorescence - green cell mitochondria)

4. Conclusions

All samples were biocompatible in vitro. Mitochondrial activity and good cell survival were observed. The Ti-28Nb-5Zr alloy has the best characteristics. Thus, a new functional medical material was obtained, which is most adapted to work in a living organism.

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