force, moment of friction, temperature in the friction loop and number of operation cycles.

## Conclusions

The structure of the SBT.03.1 simulator for the friction and wear testing of the intervertebral disc endoprostheses allows a reflection of the scope of mobility and loads occurring in a real spine. This provides an opportunity for preclinical determination of the tribological properties of the friction loop of the intervertebral disc endoprosthesis as regards a short, medium and long period of time. During the testing the endoprosthesis is immersed in a greasing and cooling liquid, which simulates bodily fluids. The presently published papers relating to the issues connected with the wear of the friction elements of the intervertebral disc endoprosthesis are mostly limited to determining of the changes in heights of the individual components. Insufficient attention has been devoted to determining of such parameters as the values of moment of friction, the friction force and the friction coefficient.

**FIG. 1. SBT-03.1 simulator.**

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# CYTOTOXICITY OF HYDROXYAPATITE COATINGS MODIFIED WITH SILVER NANOPARTICLES

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## Abstract

*Application of nanosized particles with antibacterial properties is of great interest in the development of new biocompatible products. Due to the excellent antibacterial activity Ag NPs and Ag NPs containing composites are widely used in many bactericidal applications. The objective of the present research is to characterize the cytotoxity properties of plasma sprayed hydroxyapatite coating (HA) modified by sedimentation of Ag NPs on its' surface. The present research was carried out in the frame of European FP7 NANOMINING Project "Development of New Nanocomposites Using Materials from Mining Industry" (NMP4-CP-2011-263942).*

***Keywords**: plasma spraying, hydroxyapatite, silver nanoparticles, cytotoxicity*
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## Introduction

The percentage of knee, hip and elbow infections is estimated as 0.5-12%; 1-2%; 1-2.5% and 7-9% respectively [1]. Approximately a million people are operated and have implants inserted annually. A small number of the infections may cause serious complications in dozens of patients. An implant surface is susceptible to infections for two main reasons: the formation of a biofilm on the implant surface and a reduced resistance on the implant and tissue interface [2]. The infections are caused mainly by Staphylococus ureus and Staphylococus epidermidis. The bacteria create a biofilm on the implant surface and become extremely resistant to antibiotics. The infection may cause an exhausting pain and long-term disability for the patients.

Presently, hydroxyapatite is widely used in orthopedics, as it shows perfect biocompatibility owing to its similar chemical properties and the bonelike crystallographic structure. The porous structure of hydroxyapatite causes that a strong chemical bonding is created with the natural bone. Moreover, hydroxyapatite is bioactive, which means that it becomes reabsorbed after some time and is replaced by the natural bone. However, low mechanical properties of HA limit its application in implants under high loads [3]. On the other hand, commonly used titanium and its alloys have good biocompatibility, high strength and low density. Apart from that, it shows low resistance to friction wear and release of titanium alloy particles to the surrounding tissues. The combination of HA and its good biotolerance with very good mechanical properties of the titanium seems to be a good solution, which enables a creation of near-perfect biomaterials [4].

Silver-doped HA (Ag+HA) coatings show decreasing toughness with increasing quantities of silver ions. Silver is known for its strong bactericidal properties. Due to the toxicity, compounds based on silver have been widely used in numerous bactericidal applications. The composite of Ag+HA may improve antibacterial properties of the hydroxyapatite coat and support the regeneration of diseased tissues. Antimicrobial influence of silver or its compounds is proportional to the quantity of the released bioactive silver ions ($Ag^{+}$) and their ability to interact with bacterial or fungal cells. Silver shows low toxicity to human body. Chemical properties of silver have not been fully documented and accurate data relating to the relative degrees of silver ionization are not available. The mechanisms depend on numerous factors [5].

This paper aims on preliminary determination of cytotoxicity properties of plasma-sprayed hydroxyapatite coatings with embedded Ag NPs.

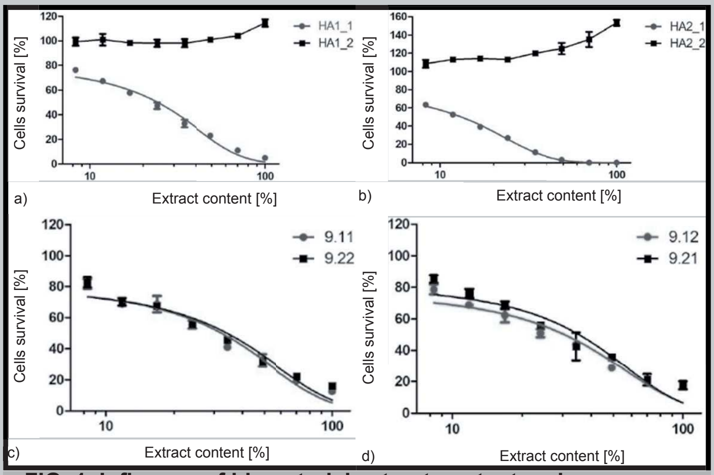


**FIG. 1. Influence of biomaterial extract content on bone cancer cells (SAOS-2) survival for ti-alloy samples with HA and HA+ AgNp coating: a, c- 24h test, b,d-48h test.**

## Materials and methods

Hydroxyapatite coating were plasma sprayed onto flat surfaces of samples made of Ti6Al4V titanium alloy by Projection Plasma System (2PS), a French company, under conditions typical for production of real HA-coated implants. Fabricated samples with HA coating were used in further tests relating to the deposition for sedimentation of silver nanoparticles (Ag NPs) on their surface. The liquid containing Ag NPs with nanoparticle mean size 15 nm (produced by Particular GmbH) was used in sedimentation experiments.The method of Ag NPs sedimentation is relatively simple and it includes an insertion of HA-coated samples in a flask placed on a rotating platform ensuring a maximum rotation speed of 600 rpmThe mixing of the liquid was also realized with the help of magnetic mixer to ensure more uniform distribution of Ag NPs on HA coating surface.

Evaluation of cytotoxicity properties of HA coatings against bone cancer SaOS-2 cells was performed by BioCentrum (Kracow, Poland) both for samples with initial HA coating (Ti6Al4V+HA: HA1_1, HA1_2, HA2_1, HA2_2) and for samples with HA coating modified with Ag NPs (Ti6Al4V+HA+Ag NPs: 9.11, 9.12, 9.21, 9.22).

## Results and discussions

The cytotoxicity test was performed in accordance with PN-EN ISO 10993-5 and PN-EN ISO 1099-12 standards. The influence of the extracts prepared in a culture medium with fetal bovine serum was determined upon 24- and 48-hour incubation by adding tetrazole salt to the solution. Upon the elapse of the above-mentioned incubation periods, the survival rate of the SaOS-2 cells subject to the influence of the extracts of the tested materials was determined. The results were expressed as a percentage of metabolically active/live cells in relation to the control group, i.e. cells that were not subject to the influence of the tested preparation, but only to the influence of a solvent. The obtained results were analyzed with the use of Dose-response-Inhibition/log(inhibitor) vs. normalized response-Variable slope).

The samples were tested in the following subgroups:
1)HA1_1 and AH1_2, incubation period – 24 hours,
2) 9.11 AND 9.22, incubation period – 24 hours,
3) HA2_1 and HA2_2, incubation period – 48 hours,
4) 9.12 and 9.21, incubation period – 48 hours,

Based on the obtained results (FIG.1), it has been confirmed that HA1_1, 9.11, 9.22 and HA2_1, 9,12, 9.21 samples demonstrate cytotoxic effects against the SaOS-2 cells, whereas HA1_2 and HA2_2 samples do not show any cytotoxic effects. In the group of HA+Ag NPs samples, all the tested samples showed a decrease in the survival rate of the cells by more than 30 % both after 24-hour and 48-hour incubation.

## Conclusions

Cytotoxic effect against SaOS-2 bone cancer cells was tested both for both for samples with initial HA coating and for samples with HA coating modified with AgNPs. All the tested samples containing Ag NPs showed a decrease in the survival rate of the cells by more than 30 % both after 24-hour and 48-hour incubation.

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