

# In Vitro Assessment of Zinc-silver Alloys as a Promising Bioabsorbable Material in Fracture Treatment

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**INTRODUCTION:** In orthopedics and trauma surgery, many fractures are treated surgically using hardware such as screws and nails. The implanted material brings a risk of foreign body reactions and inflammation [1]. With bioabsorbable implants entering the market, there is no need for the implant removal and the patient gains a healed bone structure thoroughly consisting of autologous tissue. In recent years, zinc alloys have attracted attention as a potentially bioabsorbable material due to their excellent mechanical properties and promising biocompatibility [2]. By alloying zinc with other materials such as silver, the tensile strength increases up to 287 MPa [3] and thus gives the material the necessary stability for clinical use. Silver is also known to have antibacterial properties by inhibiting the adherence of bacteria on surfaces [4]. As one of the most common minerals in the human body, phosphorus is involved in vital processes. In previous studies, phosphating of metallurgical samples has been shown to alter the corrosion rate [5] and potentially have other effects on the material and its properties. The objective of this study was to assess the biocompatibility of zinc-silver alloys containing 3.3 wt% silver (ZnAg3) on human osteoblasts (hOb). ZnAg3 alloys with two different surface properties were examined, in which one was phosphated and the other was non-phosphated.

**METHODS:** Round disks with a thickness of 1 mm and a diameter of 6 mm were examined. The samples were characterized by measuring the surface roughness with a 3D-laser scanning microscope and by determining the exact ratio of zinc and silver contained in both alloys with energy dispersive X-ray (EDX) spectroscopy. To assess the biocompatibility, three independent cell culture assays were performed for both alloys. The sample preparation for the cell culture trials included sterilization and incubation in tubes containing medium for 24 h at 37 °C and 5% CO<sub>2</sub> saturation to acquire a homogeneous oxidized surface on all samples. Additionally, this step was important to obtain eluates for the eluent trials. All assays were conducted with 10 000 cells/40 µl using hOb isolated from bone material obtained through surgical therapy such as total knee arthroplasty (Ethics vote FREEZE 418/19 of the ethics commission of Freiburg University Medical Center). For all assays, the samples were tested in direct contact trials and eluent trials using eluates in the dilutions 1 to 6, 1 to 10 and 1 to 15. For investigating the cell viability, the Live/Dead assay was conducted and evaluated under the microscope after 3, 7 and 10 days. The WST assay was used to assess cell proliferation over a 1, 3 and 7 day period. Thirdly, the LDH assay was performed to investigate the cell toxicity and was evaluated after 1, 2 and 3 days. During the cell culture experiments, the pH value was measured for both alloys on day 3, 7 and 10.

**RESULTS SECTION:** The measurements of the surface roughness showed an average roughness (Sa) of  $0.52 \pm 0.11$  µm for the non-phosphated samples and  $0.57 \pm 0.10$  µm for the phosphated samples. The quantitative analysis of the EDX revealed an Ag wt% of 3.93 for the non-phosphate sample, which is a negligible higher amount of Ag than expected. The phosphate sample contained 3.37 wt% of Ag. The results of the Live/Dead assay are presented as percentages of living, unhealthy and dead cells. Unhealthy cells are cells that cannot be clearly identified as living cells and do not show signs of certain death, which would be a cell nucleus stained red. The best cell viability was found in dilution 1 to 15 (Fig. 1A) with more than 94% of the cells living. The dilution 1 to 6 (Fig. 1B) showed a percentage of living cells between 80% and 90%. The direct contact trials (Fig. 1C) displayed a poorer cell viability with living cells between 20% and 30% and an increased amount of unhealthy cells with percentages ranging from 40% to 60%. Over time, the percentage of dead cells decreased significantly with 23% to 30% on day 3 and 10% to 15% on day 10. The WST assay showed a slightly positive proliferation tendency for both alloys. For the LDH assay, both alloys started on day 1 with approximately 25% toxicity in all trials and reached the point of no detectable toxicity by day 3. The pH value was ranging between 7.7 and 8.1 for day 3, 7 and 10.

**DISCUSSION:** The Live/Dead assay displayed a high biocompatibility of both ZnAg3 alloys towards the human osteoblast cells, while all eluent trials showed that the proportion of living cells was over 80% at all dilutions and for each time period. In comparison, the direct contact trials revealed a higher proportion of unhealthy and dead cells, most likely due to the ratio of material and exposed cells. Nonetheless, a significant decrease in the counted dead cells could be observed over a period of time. The WST assay revealed a low proliferation rate, however, hOb are known to take up to 6 weeks after passaging to reach a confluent plate in cell culture. Therefore, it was not to be expected that cell growth would be much higher. The results of the LDH assay revealed no deleterious toxicity of ZnAg3 on hOb and supported the assumption that both alloys were well tolerated by the cells. The pH values measured during the cell culture experiments exceeded 7.36, which is the physiological pH value and, as such, should be kept within a narrow range. However, materials such as magnesium and its alloys are known to increase the pH value even more drastically. Since the cell culture is a rather simulated environment for the cells lacking the buffering systems provided by the human body, the measured pH values should not be considered harmful. Since a material is considered cytotoxic according to ISO 10993 if the reduction in cell viability is greater than 30%, the results do not indicate cytotoxicity. Overall, the conducted trials confirmed the assumed biocompatibility of ZnAg3 alloys and did not show any significant difference between the two surface properties.

**SIGNIFICANCE/CLINICAL RELEVANCE:** The assessment of ZnAg3 alloys revealed a promising biocompatibility, thereby qualifying these materials for further investigation. Prospectively, bioabsorbable implants might improve fracture treatment and reduce the complication rate after surgical therapy.

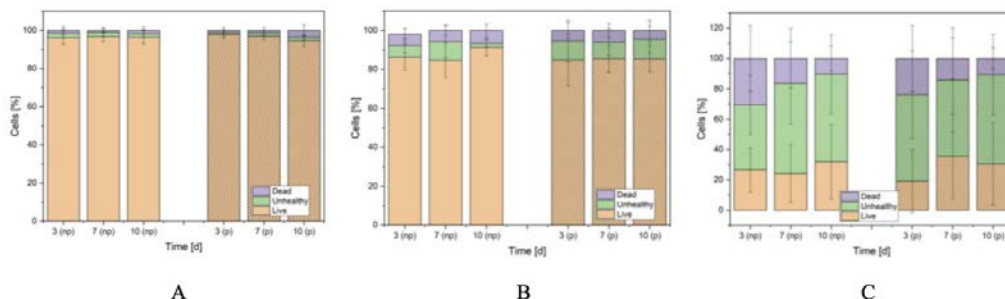


Fig. 1: Live/dead assay for dilution 1 to 15 (A), dilution 1 to 6 (B) and direct contact trial (C) with dead, unhealthy and living cells [%] on day 3, 7 and 10 for non-phosphated (np) and phosphated (p) alloy

**REFERENCES:** [1] Walley, K.C. et al. (2016) Foot Ankle Spec.; [2] Hagelstein, S. et al. (2022) Materials; [3] Sikora-Jasinska, M. et al. (2017) Mater. Sci. Eng.; [4] Qu, X. et al. (2021) Bioact. Mater.; [5] Ashassi-Sorkhabi, H. et al. (2007) J. Iran. Chem. Soc.