

THE EFFECT OF NITRIDING TITANIUM ALLOY UNDER GLOW DISCHARGE CONDITIONS AND STERILIZATION PROCESSES ON BIOCOMPATIBILITY

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INTRODUCTION: Nitrided surface layers produced on titanium alloys exhibit good biocompatibility and improved corrosion and wear resistance [1]. Testing of sterilization effects on material used in contact with the human body is required since it has been reported that surface modifications occur under sterilization conditions [2,3], which in turn affect biocompatibility [3]. The aim of the study was to study the effect of steam autoclaving, gas, or plasma sterilization techniques on the biocompatibility of TiN surface layers.

MATERIALS & METHODS: Specimens of the Ti-1Al-1Mn alloy in the shape of discs, 8mm diameter, were subjected to plasma nitriding under glow discharge conditions for 4 h at a temperature of 850°C and 4hPa pressure. Nitrided and untreated samples cleaned in ethanol and washed in deionized water were exposed to sterilization in a steam autoclave (steam, 134°C, 1400hPa, 30 min), gas (ethylene oxide, 70°C, from -1 to +0.5hPa, 4h), or plasma-sterrad*100 (hydrogen superoxide, 54°C, 7hPa, 60min) in one or ten cycles. Human skin fibroblasts retrieved from a primary culture were plated on the upper surface of individual samples at a density of $7.5 \times 10^4 / 1 \mu\text{l}$ medium, and cultured in Dulbecco's medium containing 15% fetal bovine and 1% antibiotic-antimycotic solution for 12 days. At term, the number of growing cells and dead cells, and content of metals ions in the cells and in the incubation medium were investigated. Statistical comparisons between the groups were carried out by variance analysis.

RESULTS: Investigations of fibroblast proliferation and its viability showed the significance of the sterilization method used on cell behaviour. Ten cycles of every variant of sterilization essentially decreased the cell population growing on samples and the viability of the cells, particularly in cultures on samples sterilized by autoclave (fig.1). X-ray microanalysis of the cells and the medium from the cell culture from all experiments detected no Ti ions.

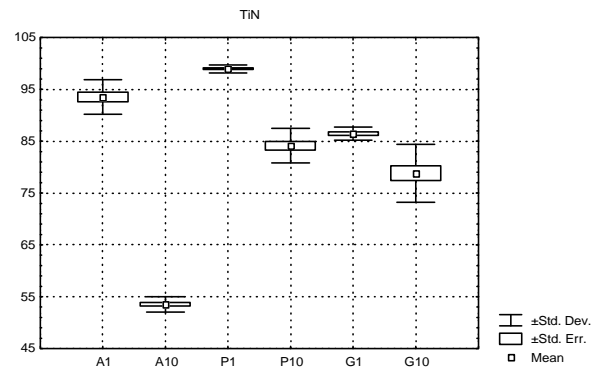


Fig.1 Viability of fibroblasts (expressed as % of living cells in population) cultured on TiN surface layers exposed on autoclave (A), plasma (P) or gas (G) sterilisation in one cycle (1) or ten cycles (10). $p < 0.001$

DISCUSSION & CONCLUSIONS: The fact that there were significant differences in cell proliferation and viability on the sterilized surface points to chemical and topographic changes resulting from sterilisation treatment which has also been noticed by other authors [2,3]. This study indicates that particularly repeated autoclaving has deleterious effects on the biocompatibility of surfaces. Further work is required to assess the effect of these modifications on adhesion and membrane receptor activity to determine the most appropriate methods to sterilize TiN surface layers. The absence of release of metal ions from the samples due to sterilisation under biological conditions, confirms the high quality of TiN surface layers produced under glow discharge conditions.

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ACKNOWLEDGMENTS: These study are supported by the Polish State Committee for Scientific Research – project 7T08C01918