INITIAL IN VIVO BIOCOMPATYBILITY EVALUATION OF BIOMATERIALS USED IN THE NEW BIOMIMETIC HEART VALVE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 81]

Introduction
The new polyurethane valves were designed for extracorporeal ventricular assist device ReligaHeart® EXT in order to guarantee athrombogenic construction, by reduction of turbulence and sufficient valve wall washing [1,2].
The single-leaflet inflow valve and double-leaflet outflow valve prototypes, consisting of very thin titanium – polyurethane composite flexible leaflets were manufactured. The titanium surface was modified with a-C:H carbon surface layer to improve the surface mechanical, physical and biological properties.
After first biomaterial blood compatibility in vitro evaluation [3], the initial in vivo biocompatibility investigation was performed.

Materials and Methods
Titanium Grade 2 surface was modified with a-C:H carbon layer with the thickness of 110nm using magnetron sputtering. Biomaterial flat samples, 10mm diameter and 1,5mm thick, sterilized with ETO, were initially investigated according to biocompatibility standard for medical devices, including tests for: irritation and skin sensitization (ISO 10993-10), local effects after implantation (ISO 10993-6) and systemic toxicity (ISO 10993-11). All in vivo tests were performed with the utilization of New Zealand white rabbits, both sexes, weighing more than 2 kg.
In the irritation and skin sensitization test the biomaterial extracts were injected in animals (n = 3). Directly after injection and then after 24, 48 and 72 hours the detail evaluation of animal skin was performed in order to detect edema or erythema - signs of skin irritation.
In the local effects and systemic toxicity after implantation tests 6 animals had been subcutaneously biomaterial implanted (2 implants for every animal) as well as 6 animals as control group had been operated (only surgical procedure, no biomaterial implanted). The observation period included 28 days. Every day the post-operative scar macroscopic evaluation was carried out (healing level, tissue status around the implant location). General animal behaviour and condition were observed. Before the implantation as well as before euthanasia the blood samples were collected for haematological and biochemical evaluation; the animals were weight. The macroscopic evaluation of post-operative scar and tissues around biomaterial implants were done. After the experiment vitals samples (heart, thymus, liver, spleen, kidneys and lungs) were collected for histopathological examination.

Results and Discussion
a-C:H carbon layer on Titanium Grade 2 surface extract did not induce any skin irritation. Biomaterial implantation revealed no signs of abnormal local inflammatory reaction as well as no signs of systemic toxicity. No statistically significant difference was observed for any tested haematological and biochemical parameters.
Clinical signs - no mortality, behavioural changes, treatment-related adverse clinical signs or signs of physical self-mutilation indicating localized or neurological toxicity were observed during the post-operative examinations, or at the time of euthanasia in any of the groups.
Body weights - all the groups showed gradually weight increasing related to animals' feeding.
Food consumption - all groups showed normal food consumption initially following the postsurgical period.
Histological studies after subcutaneous biomaterial implantation in rabbits showed no pathological changes in the adjacent to the implant tissues and in other organs.

Conclusions
Standardized tests to detect skin sensitization and irritation, local tissue reaction and systemic toxicity, demonstrated the safety and biocompatibility of evaluated a-C:H carbon layer on Titanium Grade 2 surface, in accordance with the ISO-10993 requirements.
The performed studies together with in vitro biocompatibility evaluation carried out before, confirmed that the biomaterial can be safety used as a construction material for the new biomimetic heart valve in extracorporeal heart prostheses for the period of 28 days. Further biological investigation of ReligaHeart® EXT devices equipped with the new polyurethane valves will be continued as well as biocompatibility study will be complemented for long-term clinical utilisation.

Acknowledgments
The authors would like to thank NCBIR (Grant bioVALVE in M-Era.Net programme) for providing financial support to this project.

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