THE SOFT-TISSUE RESPONSE TO IMPLANTS OF CHITOSAN FOILS

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Abstract

The objective of our in vivo study was to investigate the biocompatibility of polymer foils enriched with chitosan.

***Methods**: three types of chitosan: non-modified (jp), and modified with montmorillonite K5 and montmorillonite K10 (p2-p3) were prepared.*

The in vivo studies were carried out using the rat soft tissues as a model. Small pieces of foils (2mm x 4mm) used in the experiment were sterilized by UV radiation. Next they were implanted under sterile conditions into the gluteus muscle of adult male outbred Wistar rats. Each animal received two implants: the modified foil into the right muscle, and the unmodified foil used as a control into the left one. All procedures were conducted in sterile conditions and under anaesthesia. Animals were anesthetized with intraperitoneal injection of xylazine and ketamine (Biowet Puławy, Poland). Skin in the site of surgery was shaved and disinfected, and a small incision was made in the gluteus muscle. Equal pieces of foils were inserted into the such created pouch. The muscle and skin wounds were closed with 5/0 PDS II (polydioxanone) monofilament absorbable sutures (Ethicon Ltd., UK). All animals survived the surgery. No wound healing complications were observed after the surgery. The animals were maintained under standard conditions with free access to food and water. After 7, and 30 days from the surgery, at each time point 12 animals were sacrificed. Tissue specimens containing the implanted materials were excised and immediately frozen in liquid nitrogen. Next they were cut into 8 μm thick slides in a Shandon cryostat (Thermo-Scientific, UK) at -22°C. Obtained slides were investigated through histological and histochemical methods to estimate the intensity of inflammation, production of collagen, and metabolic activity of connective and muscle tissues surrounding implant. In order to estimate the effect of the implants on the metabolic state of surrounding tissues, activities of the marker metabolic enzymes: cytochrome c oxidase, and NADH dehydrogenase were examined. The activity of acid phosphatase was used to assess the extent of inflammation around the implants. The presence and thickness of fibrous capsule around the implants were estimated on slides stained by van Gieson's method.

***Results:** Differences between non-modified and modified with chitosan materials were not manifested in short, 7- and 30 days series. The activities of mitochondrial oxidative enzymes, cytochrome c oxidase and NADH dehydrogenase, in muscle fibres in close proximity to the implants were slightly lower than in those further away. Probably one month was too short period to obtain the whole recovery after the surgery. At the same time the process of regeneration seemed to be intense: numerous regenerating muscle fibres infiltrated the granulation tissue around the implanted foils were observed The inflammation response was visibly lower in 30 days series compared to 7 days ones what indicate that the inflammation was evoked in higher degree by surgery than by the presence of the implanted materials. The fibrous capsule around foils was thin or not present at all – there were places where pieces of foils were in direct contact with the muscle tissue. After one month experiment there were no signs of degradation of materials. The experiment has been continued to compare abilities of biomaterials in long term series.*

***Conclusion:** The regeneration and enzymic activity of muscle tissue together with the lack of continuous fibrous capsule suggest that the materials used in our study are biocompatibile and are suitable for the treatment of tissue injury.*

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