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# Histological Evaluation of the Local Soft Tissue Reaction After Implanting Resorbable and Non-resorbable Monofilament Fibers

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A - research concept and design; B - collection and/or assembly of data; C - data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

### **Abstract**

**Background.** The development of technologies and scientific disciplines connected with medical implantation devices is dynamically affecting modern treatments by contemporary medicine and veterinary medicine; it also entails a need to monitor their impact on living organisms.

**Objectives.** The aim of the study was to conduct a comparative histological evaluation of the response of soft tissues after implanting monofilament fibers from resorbable glyconate and from non-resorbable polypropylene (PP) and polyamide (PA) in rats. **Material and Methods.** Non-resorbable polyamide-based fibers were applied to skin anastomoses in rats. Macroscopic and histological evaluations were performed on the 7<sup>th</sup>, 14<sup>th</sup> and 30<sup>th</sup> days. Non-resorbable polypropylene fibers and resorbable glyconate fibers (composed of 72% glycolide, 14% trimethylene carbonate and 4% caprolactone) were implanted in muscle tissue for periods of 7, 14, 30 and 90 days.

**Results.** A semi-quantitative and qualitative histological evaluation found different dynamics and degrees of intensification of cell and tissue response around the resorbable and non-resorbable fibers being tested. The resorption process of the glyconate threads caused a prolonged inflammatory cellular response compared to the non-resorbable threads; it passed, however, without the participation of giant cells. Around the non-resorbable threads the observed cellular response was less intensified, with the formation of single polymorphonuclear macrophages around the PP threads, along with a stronger degree of fibrosis and the presence of fatty infiltrate.

**Conclusions.** During the early period, moderately intensified inflammatory cell response with the presence of single giant cells was observed around the non-degradable PA and PP fibers. In the late period, a band of fibrous connective tissue was present around the PP threads. Glyconate fibers underwent fragmentation and the process of resorption, which was associated with a weakly intensified inflammatory process lasting up to 90 days after implantation (**Polim. Med. 2016, 46, 2, 135–143**).

Key words: histological evaluation, biocompatibility, resorbable and non-resorbable fibers.

The development of technologies and scientific disciplines connected with medical implantation devices is dynamically affecting modern treatments in contemporary medicine and veterinary medicine; it also entails a need to monitor their impact on living organisms [1–3]. In evaluating the biological effect of implantation devices, not only preliminary *in vitro* studies are necessary, but also studies of interactions *in vivo*, including the degree of biocompatibility and biofunctionality [2, 4–6]. Histological evaluation of the local tissue response after implantation is an important tool in the evaluation of biocompatibility *in vivo* to allow traceability of the im-

plant healing process [7, 8]. It lets the degree of reaction after implanting biomaterial be determined, including intensification of the cell response (the participation of granulocytes, lymphocytes, plasma cells, macrophages and giant cells) as well as the tissue reaction, including the degree of connective tissue growth, fibrosis, fatty infiltrate, necrotic lesions and the degree of resorption of the material. The implants produced today are improved in terms of technological processes, structure, composition and surface properties, which increases their biofunctionality and biocompatibility. Medical procedures still create a demand for both resorbable

and non-resorbable polymer fibers, including surgical meshes, suturing materials, etc. [1, 9-13]. Suturing products from resorbable copolymer - i.e., glyconate containing glycolide, ε-kaprolacton and trimethylene carbonate - are widely regarded as advantageous in soft tissue surgery. Anastomoses of soft tissues, including intradermal anastomoses, can be performed both with resorbable and non-resorbable sutures [1, 14-17]. Polyamide (PA) and polypropylene (PP) have an important position among the non-resorbable materials. Suturing fibers and articles from PP and PA are characterized by minimal friction and preferred strength parameters compared with resorbable materials [1]. It is assumed that monofilament threads may cause less tissue resistance and less intensified reactions in comparison with multi-fibrillary threads [1, 18]. The aim of the study was a comparative histological evaluation of the response of soft tissues after implantation of monofilament fibers from resorbable glyconate and from non-resorbable polypropylene and polyamide in rats.

# Material and Methods

Three types of monofilament fibers were studied:

- 1) uncoated glyconate fibers composed of 72% glycolide, 14% trimethylene carbonate, and 14% caprolactone (Aesculap-Chifa Sp. z o.o., Braun Group, Nowy Tomyśl, Poland) with a diameter of 3/0 (USP);
- 2) polypropylene fibers (Yavo Sp. z o.o., Bełchatów, Poland) with a diameter of 3/0 (USP); and
- 3) polyamide fibers (Yavo Sp. z o.o., Bełchatów, Poland) with a diameter of 2/0 (USP), with a homogeneous structure and a smooth surface.

All of the threads tested were sterilized with ethylene oxide.

# **Surgical Procedures**

Surgical procedures with the three types of thread were performed in fully aseptic conditions. The tests were performed on Wistar rats. After mechanical depilation the operative field was disinfected with SkinSept (Ecolab Sp. z o.o., Kraków, Poland).

Resorbable glyconate threads from and nonresorbable PP fibers were implanted in muscle tissue for 14, 30 and 90 days. Nonresorbable PA threads were used for skin anastomoses for periods of 7, 14 and 30 days.

The study was approved by the First Local Ethics Committee in Wrocław for research on animals.

## **Macroscopic Studies**

In the postoperative period the animals' condition and the appearance of the post-operative wounds were observed. At the scheduled times the animals were sacrificed and the fiber implantation sites were evaluated, the organs were inspected and tissue sections with the implants were taken for further examination.

## **Histological Studies**

The sections of skin and muscle tissue with implants were fixed for 48 h at room temperature in a 5% aqueous solution of formic formaldehyde with phosphate buffer. Next, they were dehydrated in acetone at 56°C, washed in xylene at room temperature and embedded in paraffin blocks. Sections approximately 4 µm thick were cut on a Leica 2025 rotary microtome (Leica Microsystems, Wetzlar, Germany). The prepared samples were stained with hematoxylin and eosin (HE) or van Gieson's staining (VG), then they were sealed in a mounting medium (CV Mount Medium, Leica Biosystems GmbH, Nussloch, Germany). Histological evaluation and documentation were performed under a light microscope (Olympus BX41, Olympus Corportation, Tokyo, Japan) with a program for analysis and image acquisition (cellSens Software, Olympus Corportation, Tokyo, Japan).

A semi-quantitative evaluation of the intensification of the reaction was carried out based on the standard ISO biological evaluation of medical devices [7]; the degree of intensification of each trait of the response was evaluated on a scale from 1 to 5 (Tables 1 and 2).

<b>Table 1.</b> Evaluation of inflammatory cells				
Cell type/response				

Cell type/response	Score					
	0	1	2	3	4	5
Granulocytes	0	1-5*	5-10*	10-20*	heavy infiltrate	packed
Lymphocytes	0	1-5*	5-10*	10-20*	heavy infiltrate	packed
Plasma cells	0	1-5*	5-10*	10-20*	heavy infiltrate	packed
Makrofagi	0	1-5*	5-10*	10-20*	heavy infiltrate	packed
Giant cells	0	1-5*	5-10*	10-20*	heavy infiltrate	sheets
Necrosis	lack	minimal	mild	moderate	severe	extensive

<sup>\*</sup> for large magnification ×400

<b>Table 2.</b> Eval	luation o	f tissue	response
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Response	Score							
	0	1	2	3	4	5		
Growth of connective tissue	0	minimal capillary proliferation 1–3 buds	groups of 4–7 capillaries with supporting fibroblastic structures	groups of 8–20 capillaries	broad bands of capillaries with supporting structures	extensive bands of capillaries with supporting fibro- blastic structures		
Fibrosis	0	minimal band	narrow band	moderately thick band	thick band	extensive band		
Fatty infiltrate	0	minimal amount of fat associated with fibrosis	several layers of fat and fibrosis	from several to over a dozen layers of fat	elongated and broad bands of accumulated fat tissues about the implant site	extensive fat com- pletely surroun- ding the implant		

G – granulocytes; L – lymphocytes; P – plasma cells; M – macrophages; KO – giant cells; MR – necrosis; R – growth of connective tissue; ZW – fibrosis; NT – fatty infiltrate

# **Results**

# **Macroscopic Studies**

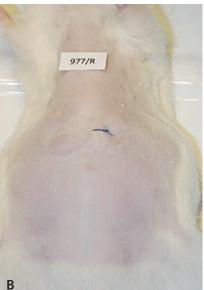
On the  $7^{\rm th}$  day after anastomosis with non-resorbable PA threads, the skin wounds were partially healed by first intention and partially covered with residues of brown masses (scabs). The skin in the immediate vicinity of the seam was normal in appearance. On the  $14^{\rm th}$  day the wounds were completely healed by first intention. In the late period, i.e. after 30 days, the surgical wounds were macroscopically invisible (Fig. 1).

At the sites of implantation of resorbable PP and glyconate fibers the macroscopic images were similar in all respects under consideration. Muscle tissues remained unchanged with the correct color and appearance. The implanted fibers remained difficult to see macroscopically (Fig. 2).



**Fig. 2.** Macroscopic image of rat thigh muscle 14 days after implantation of resorbable glyconate thread







**Fig. 1.** Macroscopic image of the surface of rat skin after anastomosis with polyamide (PA) thread A) after 7 days; B) after 14 days; C) after 30 days

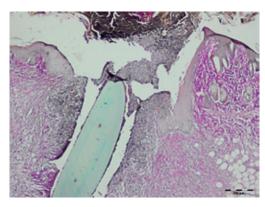
# **Histological Studies**

### **Polyamide Suture Material**

On the 7<sup>th</sup> day the wound was covered with homogeneous masses (scabs), under which strips of stratified squamous epithelium encroaching on the collagen layer of the dermis. In the immediate vicinity of the threads were remnants of the wet phase with inflammatory cells; deeper, the proliferative phase, with fibroblasts and collagen fibers of the dermis, was visible (Figs. 3, 4).

On the 14<sup>th</sup> day skin wounds were covered wholly or partiallly with epithelium. The threads were found adjacent to epithelium strips surrounded by a thin band of rich-cellular connective tissue with single giant cells (Figs. 5, 6).

On the 30<sup>th</sup> day the wounds anastomosed with PA fibers were covered completely with epithelium, and in the dermis cutaneous appendages were recovered. In individual cases, tiny remains of small homogeneous masses (scabs) were preserved, and in these transverse sectioned threads were found (Fig. 7)



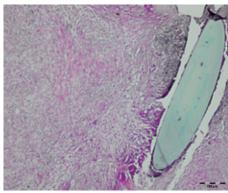
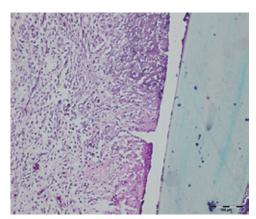


Fig. 3. Microscopic image of rat skin wound 7 days after anastomosis with polyamide (PA) thread. PA fiber visible in the center; emerging strips of epithelium at the top right and left (VG staining, magnification ×40)



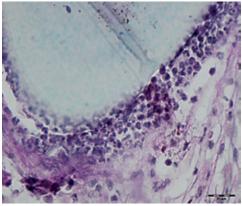
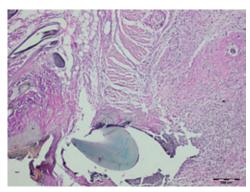


Fig. 4. Microscopic image of rat skin wound 7 days after anastomosis with polyamide (PA) thread. Visible implant/tissue boundary phases (VG staining, magnification ×100 and ×400)



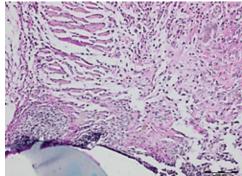
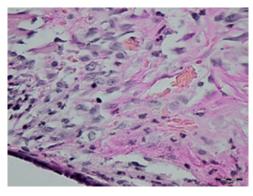


Fig. 5. Microscopic image of rat skin wound 14 days after anastomosis with polyamide (PA) thread. Visible thread in the collagen layer of the dermis (VG staining, magnification ×40 and ×100)



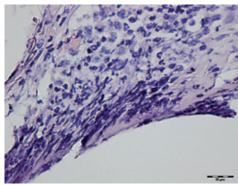
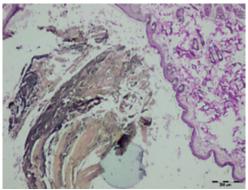


Fig. 6. Microscopic image of rat skin wound 14 days after anastomosis with polyamide (PA) thread. In the immediate vicinity of the anastomosis, a thin band of rich-cellular connective tissue is visible (VG staining, magnification ×400). On the right: a single giant cell (HE staining, magnification ×400)



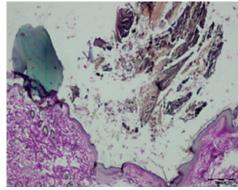
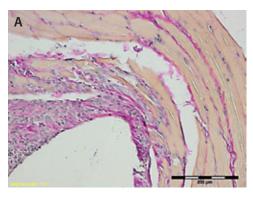


Fig. 7. Microscopic image of skin wounds 30 days after anastomosis with polyamide (PA) thread. Visible skin wounds covered with epithelium. In the homogeneous masses of scab remnants cross-sectioned threads are visible (VG staining, magnification ×40)

### Glyconate Suture Material

On the 14<sup>th</sup> day after implantation, in transversely striated muscles, threads or oval spaces in place of threads, limited with a band of rich-cellular loose connective tissue were visible in the histological preparations (Fig. 8).

On the 30<sup>th</sup> day after implantation, the threads were surrounded by a band of connective tissue with a two-layer structure: fibrous on the muscle side and loose rich-cellular with accumulations of mononuclear inflammatory cells on the material side. This band interdigitated with the area occupied by the defragmented threads (Fig. 9).



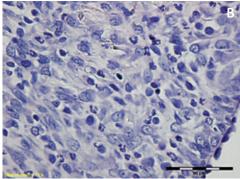
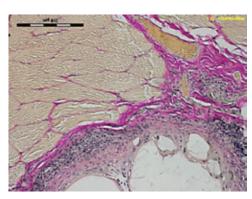


Fig. 8. Microscopic image of muscle tissue 14 days after implantation of absorbable glyconate fiber in rat muscle tissue. A) On the left, visible oval space in place of thread, surrounded by loose connective tissue (VG staining, magnification ×100). B) Rich-cellular connective tissue in the immediate vicinity of the thread (HE staining, magnification ×400)



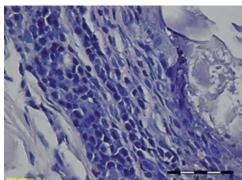


Fig. 9. Microscopic image of rat muscle tissue 30 days after implantation of resorbable glyconate fiber. On the left, threads surrounded by a band of connective tissue with a two-layer structure, fibrous on the muscle side and rich-cellular on the material side (VG staining, magnification ×100). On the right, accumulations of mononuclear inflammatory cells in the immediate vicinity of the thread (HE staining, magnification ×400)

On 90<sup>th</sup> day the implanted threads remained separated from transversely striated muscles with a band of fibrous connective tissue. On the material side, rich-cellular connective tissue with accumulations of mononu-

clear inflammatory cells was visible, which encroached on the area of the reduced implant; in individual cases, it completely filled the implant (Fig. 10).

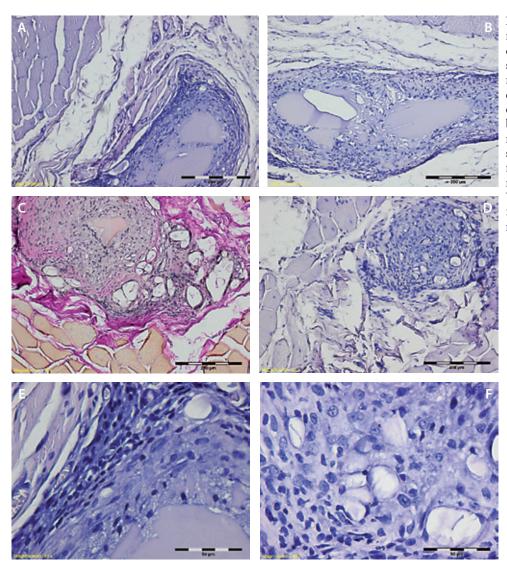


Fig. 10. Microscopic image of rat muscle tissue on the 90<sup>th</sup> day after implantation of resorbable glyconate fiber. Fragmented thread surrounded by connective tissue with two-layer construction is visible, fibrous on the muscle side and rich-cellular on the material side, or residue of the implant filled with rich-cellular connective tissue (A–D) HE and VG staining, magnification ×100; E, F] HE staining, magnification ×400)

# Polypropylene Fibers

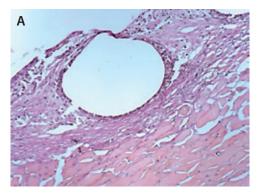
On the 14<sup>th</sup> day after the implantation of polypropylene fibers in muscle tissue, fibers or spaces in place of fibers were visible, limited with a band of newly formed connective tissue with a loose structure and a width corresponding to 1/8 of the diameter of a single fiber of the implant. In the surrounding tissue numerous fibroblasts and fibrocytes were visible, along with eosinophil groupings in places, less numerous macrophages, plasma and lymphoid cells, as well as single neutrophils (Fig. 11).

On the 30<sup>th</sup> day after PP implantation in muscle tissue, cross sections of fibers surrounded by young connective tissue were visible. The width of the connective band was 1/8 of the diameter of a single fiber.

The band consisted of a number of collagen fibers and fibrocytes, fewer fibroblasts, and single inflammatory mononuclear cells and polymorphonuclear macrophages (Fig. 12).

On the 90<sup>th</sup> day after PP implantation in muscle tissue, transversely striated fibers or spaces in place of fibers were visible, surrounded by bands of connective tissue. On the muscle side the connective tissue band was built of densely arranged collagen fibers, while on the implant side it was characterized by a loose rich-cellular structure (Fig. 13).

The results of a semi-quantitative evaluation of the intensification of soft tissue reaction after the use of glyconate, polypropylene and polyamide fibers are



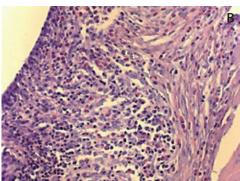
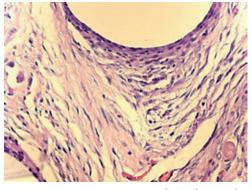
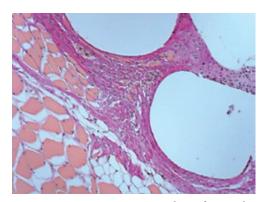


Fig. 11. Microscopic image 14 days after implantation of polypropylene (PP) fibers in rat muscle tissue. A. In the center, an oval space is visible in place of thread, surrounded by a band of young rich-cellular tissue vaguely separated from the muscle tissue (HE staining, magnification ×40). B. A band of rich-cellular connective tissue with a predominance of eosinophils is visible, separating the PP fiber from transversely striated muscles (HE staining, magnification ×400)



**Fig. 12.** Microscopic image 30 days after implantation of polypropylene (PP) fibers in rat muscle tissue. On the top, oval spaces are visible in place of fiber, surrounded by a connective tissue band quite sharply separated from the surrounding tissues (HE staining, magnification ×100)



**Fig. 13.** Microscopic image 90 days after implantation of polypropylene (PP) fibers in rat muscle tissue. In the center, spaces in place of fibers are visible, surrounded by a band of fibrous connective tissue. Around the band are transversely striated muscles (VG staining, magnification  $\times 100$ )

presented in Table 3. The degree of intensification of each response characteristic was evaluated on a scale from 1 to 5.

## Discussion

Implantation studies of synthetic resorbable and non-resorbable monofilament fibers were conducted in rats. Non-resorbable polyamide-based fibers with a diameter of 2/0 (USP) were used in skin anastomoses in rats. Macroscopic and histological evaluations were performed on the 7th, 14th and 30th days. Non-resorbable polypropylene fibers with a diameter of 3/0 (USP) and resorbable glyconate fibers (composed of 72% glycolide, 14% trimethylene carbonate and 14% caprolactone) were implanted in muscle tissue for periods of 7, 14, 30 and 90 days. The results obtained showed the presence of non-resorbable thread of polyamide in the dermis of rats after 14 days. Later, in the process of healing, wound cleansing and epithelialization followed by separation, threads were found in scab residues. This process was accompanied by a weakly intensified inflammatory process (with the formation of single giant cells) leading to the regeneration of the dermis. Mirković et al. reported a similar reaction after the use of polyamide threads in a study of the influence of various types of fiber on mucous membranes [19]. In the present study, a weakly intensified inflammatory process was observed in the muscle tissue around non-resorbable polypropylene fibers in the early pe-

**Table 3.** Semi-quantitative microscopic histological evaluation of soft tissue response after the use of glyconate (G), polypropylene (PP) and polyamide (PA) fibers

	G	L	P	M	КО	MR	R	ZW	NT
Term /days/	G PP PA								
7	2 3 3	2 3 2	2 2 2	2 2 2	0 0 0	0 0 0	1 1 1	1 1 1	0 0 0
14	3 4 3	2 2 2	2 2 2	2 1 2	0 0 1	0 0 0	1 2 1	3 2 2	0 0 0
30	2 2 -	3 2 -	2 2 -	3 1 -	0 1 -	0 0 -	2 2 -	3 3 -	0 0 -
90	3 1 -	3 2 -	2 1 -	2 1 -	0 1 -	0 0 -	2 2 -	3 4 -	0 3 -

riod, in some places with the participation of eosinophiles, connected with irritant action and single polymorphonuclear macrophages. In the late period (i.e., after 90 days) quite a broad band of fibrous connective tissue was present. In a study involving a pig model, Kolb et al. also showed a continuing weakly intensified inflammatory reaction after implanting polypropylene thread, which minimized over in time, and fibrous connective tissue appeared around the thread [20]. In the present study, the presence of a thin band of loose connective tissue was observed around resorbable glyconate threads in muscle tissue in the early period (i.e., up to the 14th day). In the late period (i.e., on the 30th and 90th day after implantation) the connective tissue capsule surrounding the fragmented thread had a two-layer construction. In the immediate vicinity of the threads, rich-cellular tissue was seen with accumulations of mononuclear inflammatory cells associated with the degradation of the material. On the muscle side the implant was separated quite sharply with a band of fibrous connective tissue with a predominance of collagen fibers. In this tissue, no giant cells were present. During the process of healing, gradual fragmentation and reduction of the whole implanted glyconate fiber was observed.

In a semi-quantitative evaluation of the responses to the resorbable and non-resorbable threads being tested, different dynamics and degrees of intensification of cell and tissue response were found. The resorption process of glyconate threads caused a prolonged inflammatory cellular response compared to the non-resorbable threads; it subsideded, however, without the participation of polymorphonuclear macrophages. The cellular response observed around the non-resorbable threads was less intensified, with the formation of single polymorphonuclear macrophages. Around the PP fibers a stronger degree of fibrosis and the presence of fatty infiltrate were observed.

### Conclusions

The results obtained demonstrated the biocompatibility of the tested fibers.

During the early period, a moderately intensified inflammatory cell response with the presence of single polymorphonuclear macrophages was observed around the non-resorbable polyamide and polypropylene threads. In the late period a band of fibrous connective tissue was present around the PP threads.

Glyconate threads underwent fragmentation and resorption, which was associated with a weakly intensified inflammatory process lasting up to 90 days after implantation.

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