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 implant 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-caprolacton 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);

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 Corporation, Tokyo, Japan) with a program for analysis and image acquisition (cellSens Software, Olympus Corporation, 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).

**Table 1. Evaluation of inflammatory cells**

<table>
<thead>
<tr>
<th>Cell type/response</th>
<th>Score</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Granulocytes</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0</td>
</tr>
<tr>
<td>Plasma cells</td>
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<tr>
<td>Makrofagi</td>
<td>0</td>
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<tr>
<td>Giant cells</td>
<td>0</td>
</tr>
<tr>
<td>Necrosis</td>
<td>lack</td>
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</tbody>
</table>

* for large magnification ×400
Histological Evaluation After Implantation

**Results**

**Macroscopic Studies**

On the 7th 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 14th 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).

Table 2. Evaluation of tissue response

<table>
<thead>
<tr>
<th>Response</th>
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<tr>
<td>Growth of connective tissue</td>
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<td>1</td>
<td>2</td>
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<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
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<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Fatty infiltrate</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

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

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

Fig. 2. Macroscopic image of rat thigh muscle 14 days after implantation of resorbable glyconate thread
Histological Studies
Polyamide Suture Material

On the 7th 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 14th day skin wounds were covered wholly or partially 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 30th 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)
Glyconate Suture Material

On the 14th 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 30th 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).
On 90th 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 mononuclear inflammatory cells was visible, which encroached on the area of the reduced implant; in individual cases, it completely filled the implant (Fig. 10).

**Polypropylene Fibers**

On the 14th 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 30th 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 90th 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...
Histological Evaluation After Implantation

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>
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<th>Term /days/</th>
<th>G</th>
<th>L</th>
<th>P</th>
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<th>KO</th>
<th>MR</th>
<th>R</th>
<th>ZW</th>
<th>NT</th>
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<td>7</td>
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<td>PP</td>
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<td>1</td>
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</table>

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 ×100)

Table 3. Semi-quantitative microscopic histological evaluation of soft tissue response after the use of glyconate (G), polypropylene (PP) and polyamide (PA) fibers
period, in some places with the participation of eosinophils, 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 subsided, 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.

References


[7] PN-EN ISO 10993-6 Biological evaluation of medical devices part 6 Local effect after implantation


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