Effects of structure, morphology and heparin(-like) coatings on the tissue reaction to poly(ethylene terephthalate)

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Appendices
ABBREVIATIONS

Ab  Antibody
BARE  Uncoated PET
BSA  Bovine Serum Albumin
Coll III  Collagen type III
DSC  Differential Scanning Calorimetry
ECM  Extracellular Matrix
EtO  Ethylene Oxide
FBGC  Foreign Body Giant Cell
FBR  Foreign Body Reaction
FCS  Fetal Calf Serum
FCS  Fetal Calf Serum
FGF  Fibroblast Growth Factor
G-CSF  Granulocyte-colony Stimulating Factor
GM-CSF  Granulocyte Macrophage Colony Stimulating Factor
HEP  Heparin-coated PET
HP  Hydroxylysyl Pyridinoline
HRP  Horseradish Peroxidase
Hyl  Hydroxylsine
Hyp  Hydroxyproline
IgG  Immunoglobulin G
IL4  Interleukin 4
IL6  Interleukin 6
IL8  Interleukin 8
IL13  Interleukin 13
LP  Lysyl Pyridinoline
MIP1α  Macrophage Inflammatory Protein 1 alpha
MMP  Matrix metalloproteinase
MNC  Mononuclear Cells
PBS  Phosphate Buffered Saline
PDGF  Platelet Derived Growth Factor
PEI  Poly(ethylene imine)
PEO  Poly(ethylene oxide)
PET  Poly(ethylene terephthalate)
PMMA  Poly(methyl methacrylate)
pNPP  Alkaline Phosphate Yellow
PP  Poly(propylene)
PTFE  Poly(tetrafluoro ethylene)
PVC  Poly(vinyl chloride)
RGD  Arginine – Glycine – Aspartic acid
RG-PET  Rough Goodfellow PET
RGTA  ReGeneraTing Agent
SG-PET  Smooth Goodfellow PET
TBS  Tris Buffered Saline
TMB  Tetramethyl Benzidine
TGA  Thermogravimetric Analysis
TGFβ  Transforming Growth Factor beta
TH  Collagen Triple Helix
TMB  Tetramethyl Benzidine
TNFα  Tumor Necrosis Factor alpha
VEGF  Vascular Endothelial Growth Factor
WCA  Water Contact Angle
WS-PET  Woven Sefar PET

COLOR FIGURES

Figure 3. Pg. 30. Chapter 3.
Visiopharm-mediated histological analysis
Slides were scanned and analyzed using Visiopharm software. First, background (white areas), muscle, fat (F), artifacts (*) and the PET implant were excluded from analysis. These areas are marked by horizontal lines. Then, blood vessels (V) were defined, as shown in red. Dark blue stained cells were defined, as shown in black (C). The relative surface area of both the blood vessels and cells was calculated.

Figure 4. Pg. 32. Chapter 3.
Thermogravimetric analysis results
The x-axis displays the increasing temperature in °C at which the different samples were weighed. The y-axis displays the weight change in % that occurred as a result of increasing temperature. A) 5G-PET and WS-PET show a clean weight drop at the same temperature. B) SB-PET has less residual weight than 5G-PET.
**Figure 8.** Pg. 34. Chapter 3.
**ED1 Immunostaining of SG-PET, RG-PET and WS-PET**
Micrographs (40x) taken from SG-PET, RG-PET and WS-PET. Red immunostaining marks ED-1 positive macrophages. The PET implants are indicated by P; macrophages are indicated by M; giants cells are indicated by G.

**Figure 9.** Pg. 35. Chapter 3.
**Col III Immunostaining of SG-PET, RG-PET and WS-PET**
Micrographs (40x) taken from SG-PET, RG-PET and WS-PET. Dark red immunostaining marks collagen type III. The PET implants are indicated by P.

**Figure 10.** Pg. 37. Chapter 3.
**Cellular density in the tissue surrounding SG-PET and SB-PET**
The acute inflammatory reaction against SG-PET and SB-PET was evaluated up to 10 days after implantation. Inflammation around SB-PET was less than around SG-PET. **p < 0.01.**
The bottom part of the figure displays micrographs (10x) taken from SG-PET and SB-PET at day 10. Red immunostaining marks ED-1 positive macrophages. The PET implants are indicated by P.

**Figure 2.** Pg. 60. Chapter 5. **Visiopharm-mediated histological analysis**
Slides were scanned and analyzed using Visiopharm software. Firstly, background (white areas), artifacts and the biomaterial (PET) were excluded from analysis. Then, blood vessels (BV) were defined, as shown in red. Dark blue cell nuclei were defined, shown in black. The relative surface area of both the blood vessels and cells was calculated. **A** shows a representative section before digital analysis, **B** shows the same section after analysis.
A) After plasma treatment, acrylamide and acrylic acid are polymerized onto the PET, resulting in a poly(acrylamide) graft, providing amide and carboxyl groups for further treatment. B) Poly(ethylene imine) is coated onto the acrylamide graft, after which the RGTA is covalently bound through random ring opening chemistry.

Figure 1. Pg. 58. Chapter 5. RGTA coating of poly(ethylene terephthalate) (PET)

Figure 7. Pg. 65. Chapter 5. Collagen deposition in the tissue surroundings at day 10
Micrographs (20x) taken from picrosirius red stained slides of explants at day 10. Examples of red-colored collagenous areas are indicated by arrows. Examples of PET fibers are indicated by double arrowheads. RGTA-S showed less collagen deposition compared to BARE-S. In addition, BARE-R showed less collagen than both BARE-S and BARE-H. Lastly, RGTA-R showed less collagen than any of the other samples.

Figure 8. Pg. 66. Chapter 5. Collagen deposition between the PET fibers at day 21
Micrographs (20x) taken from picrosirius red stained slides of explants at day 21. Examples of red-stained collagen sections are indicated by arrows. Examples of PET fibers are indicated by double arrowheads. RGTA-S, RGTA-H and RGTA-R showed less collagen between the PET fibers than any of the BARE samples. RGTA coating therefore prevents collagen deposition.
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