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

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CHAPTER 7

General Discussion
INTRODUCTION

PET is a chemically inert, non-degradable implantable material that is used in a broad range of medical implantable devices such as annuloplasty rings, mesh materials for intraperitoneal applications and vascular grafts. PET is applied as a smooth, tightly woven, or open weave (knitted) structure. Although chemically inert materials usually do not trigger a strong tissue reaction, knitted PET triggers a foreign body reaction (FBR), characterized by the formation and persistent presence of foreign body giant cells (FBGCs). In addition, knitted PET triggers the formation of a thick fibrous capsule, which may mechanically restrict the function of implanted devices, such as annuloplasty rings and artificial heart valves. Both the chronic FBR and thick fibrous encapsulation can intervene with implant functions and need to be avoided. Therefore, we investigated the extent to which physical, chemical and morphological aspects determine the FBR against PET. Furthermore, we applied heparin(-like) surface modifications in order to investigate whether this ongoing FBR could be avoided.

INFLAMMATION AND FORMATION OF FOREIGN BODY GIANT CELLS

Bellon et al. has shown that the tissue reaction to a non-degradable biomaterial largely depends on the physical and chemical characteristics. Furthermore, the structure and morphology of the material contribute to the development of the tissue reaction. However, research into the effect of structure and morphology is largely limited to the effects of (nano)structuring of smooth metal and polymeric biomaterials. The development of a tissue reaction to a foreign material involves a complex sequence of biochemical and cellular events. Adsorption and denaturation of (plasma) proteins to the material during and after implantation allows recognition of the material by inflammatory cells. The composition of the protein interface that forms on the material is dependent on the chemical and physical characteristics and determines the nature of the acute inflammatory response. In particular, the presence of the fibrinogen p1/p2 epitope on an implant surface activates neutrophils, monocytes and macrophages and, therefore, is believed to play a detrimental role in the nature of the acute inflammatory response against the material. The fibrinogen conformational change that leads to exposure of the normally occult p1/p2 epitope is associated with the hydrophobicity of the material. Being a hydrophobic material, PET therefore can be expected to trigger a stronger inflammatory response compared to hydrophilic materials. We indeed showed that an acute inflammatory response develops against smooth film PET implants, as measured by the presence of mononuclear phagocytes surrounding the implant at five days only. We also showed that a reduction of fibrinogen p2 epitope on PET coupons leads to a reduction in monocyte adhesion, leukocyte activation and tissue interaction in vitro. More importantly, this is in agreement with the nature of the in vivo inflammatory response, at least up to 10 days after implantation.

Having a larger surface area, the roughened PET film showed a slightly stronger acute inflammation. This is probably due to the presence of more fibrinogen p1/p2 epitopes on the surface. Importantly, and in contrast to knitted PET, neither smooth nor roughened PET film triggered an ongoing FBR and the formation of FBGCs. This implies that the structural difference of knitted PET apparently plays an important role in the development of the ongoing FBR. We observed that giant cells were surrounding the individual fibers of the knitted PET material, while the surrounding tissue was not activated and appeared comparable to smooth and roughened PET film. This indicates that the flexible structure and the mesh-like morphology of knitted PET may be responsible for a change in the local intra-fibrillar microenvironment. The major difference between the PET films and knitted PET that are described in this thesis is flexibility of the material. We speculate that this flexible nature of knitted PET causes the intra-fibrillar tissue to be constantly mechanically agitated, triggering infiltration of macrophages and the formation of FBGCs.

The persistent presence of FBGCs at the material interface requires that the FBGCs adhere to the implanted PET. As described, fibrinogen is an important interfacial protein that facilitates activation and adherence of inflammatory cells e.g. neutrophils, monocytes and macrophages. However, formation of FBGCs by fusion of macrophages may require the presence of different interfacial proteins. McNally et al. showed that FBGCs strongly adhere to adsorbed vitronectin. Furthermore, Vroman et al. described that interfacial proteins can be displaced in time, causing the material-protein interface to become dynamic. We postulate that the mechanical action of knitted PET may also be responsible for allowing interstitial fluid components such as vitronectin to continuously access the material surface and to adsorb on it.

The mechanical action of the flexible, knitted PET could continuously reinitiate the events that define the initial phase of implantation, i.e. tissue damage, material-protein interface formation, activation of inflammatory cells (Figure 1). Supportive evidence for this hypothesis could be obtained in future studies by using immunoassays to quantify adherent vitronectin and other interfacial proteins on knitted PET explants over time.

FIBROUS ENCAPSULATION

Another aspect of the tissue reaction is the formation of a fibrous capsule around the implant. The formation of a thick fibrous capsule was detected around knitted PET, compared to smooth and roughened PET film. In concurrence with the persistent presence of FBGCs, we postulate that the formation of a thick fibrous capsule is a consequence of the inherent flexibility and consequent mechanical action of subcutaneously implanted knitted PET. Non-exclusive hypotheses that could
support this postulation include: 1) The PET mechanical action directly and continuously triggers fibroblast-myofibroblast transdifferentiation and results in a profibrotic fibroblast phenotype; and 2) Continuous re-activation of inflammatory cells indirectly triggers the formation profibrotic fibroblasts through secretion of profibrotic cytokines, such as TGFβ.

HEPARIN(-LIKE) COATINGS
To study modification of the tissue reaction against knitted PET, surface coatings were applied. At first, a heparin surface modification was applied to knitted PET. Surface modifications can be used to alter the physicochemical characteristics of a biomaterial, but may also add biological functionality to the material surface. Heparin is a natural bioactive molecule that acts as a cofactor for a number of proteins. Besides its anticoagulant action, heparin is well known to enhance the function of heparin-binding growth factors and is shown to have anti-inflammatory properties. A heparin surface modification makes PET more hydrophobic, reducing the amount of adherent fibrinogen p1/p2 epitope, as well as the initial surface interaction with inflammatory cells, as we demonstrated in our in vitro experiments. However, after subcutaneous implantation in rats we only observed a slight beneficial effect of heparin during the acute tissue response. Furthermore, the heparin biological functionality was believed to alter the biochemical microenvironment, such that inflammation would be inhibited. After three weeks, however, we did not see any difference between the tissue reaction to heparin coated and uncoated knitted PET surfaces. Being a natural biomolecule, heparin is susceptible to degradation by heparinase enzymes. We speculated that, at least in part, heparinase action could have degraded the heparin coating, thereby diminishing its effect in vivo. Therefore, we subsequently studied a PET coating based on RGTA, a heparinase-resistant, synthetic heparin analog. We observed that RGTA coating on PET improved the tissue reaction up to three weeks after implantation, indicating that RGTA remains present during this period of time. Most importantly, RGTA coating inhibited the inflammatory response and minimized FBGC formation. In addition, only a thin fibrous capsule was formed around RGTA-coated PET. Although these results are promising, long-term implantation studies should be conducted to prove feasibility of this coating. Nevertheless, the potency of the RGTA coating appears to be sufficient to overcome the strong pro-inflammatory stimuli that would otherwise result in the ongoing presence of FBGCs.

CONCLUSION
In conclusion, because (roughened) PET film did not trigger a chronic FBR, mechanical movement between fibers of knitted PET may be responsible for the ongoing FBR, the formation of FBGCs and the formation of a thick fibrous capsule. This fiber movement may cause intra-fibrillar tissue disruptions that lead to changes in the tissue microenvironment. These changes probably include biochemical signals that reinitiate the acute events of the tissue reaction, including formation (modulation) of the material-protein interface as well as re-induction of an inflammatory response. This continuous cycle probably triggers the formation of FBGCs and results in the development of a chronic FBR against the non-degradable biomaterial. By coating PET with the synthetic heparin-like biomolecule RGTA, this continuous cycle can potentially be avoided. The use of an RGTA coating is, therefore, promising for future applications that require the use of knitted PET. Long-term studies should, however, be performed in the future to prove feasibility.

Figure 1: Modulation of the tissue reaction against knitted PET
During the acute events after subcutaneous implantation of knitted PET (1), an inflammatory response will be induced (2) and a material-protein interface will form (3), allowing activation and adherence of inflammatory cells. These events characterize the acute tissue reaction i.e. the foreign body reaction (FBR) (4), which also includes the formation of giant cells (5). Movement between the fibers of the knitted PET (6) could cause continuous intra-fibrillar tissue disruptions (7). This may continuously reinitiate the events that define the acute tissue reaction (8), leading to the development of an ongoing FBR (9). However, RGTA coating prevents adverse remodeling of the material-protein interface and affects the biochemical microenvironment such that the formation of giant cells and the chronic FBR is prevented.