Tuned polyurethanes for soft tissue regeneration

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Chapter 7

Summary

Most of the tissue engineering based regenerative therapies in order to treat damaged or lost soft tissues depend on the use of biodegradable scaffolds. A functional scaffold should provide a structural support whilst remaining transient until functional restoration is achieved. Tissue restoration and function recovery can only be achieved if biomaterial degradation, physiological turnover and new tissue formation are balanced.
Tunable mechanical properties, ease of processing and relatively good biocompatibility render segmented polyester urethanes (SPEUs) attractive as biodegradable biomedical materials. SPEUs based on polycaprolactone (PCL) soft segment have been thoroughly investigated as biodegradable scaffolds for biomedical applications (*e.g.* meniscus replacement). Although proven beneficial as long term implants, these materials degrade very slowly and are therefore not suitable in applications in which scaffold support is needed for a shorter time. Tunability of SPEUs allowed us to increase susceptibility of these SPEUs to hydrolysis via chemical modification of either polyester soft segment or by introducing more reactive (and hydrolyzable) hard segment. The aim of this thesis was to design biocompatible SPEU scaffolds for soft tissue regeneration that would gradually degrade faster than commercially available SPEUs evoking only a mild tissue response. Cyto- and haemocompatibility of the novel SPEUs presented in this thesis were assessed *in vitro*. Furthermore, we prepared porous scaffolds via a combined salt leaching/thermally induced phase separation method and evaluated their biocompatibility *in vivo*.

Chapter 1 provides an introduction into soft tissue restoration therapies and focuses more specifically on the soft tissue regeneration by use of biodegradable polymers. In this chapter, we also describe scaffold processing methods and degradation mechanisms of biomaterials. Furthermore, we give an overview of the aspects involved in the interaction of biomaterials with the surrounding tissue - biomaterial surface properties related to cell adhesion and haemocompatibility, and foreign body reaction to biomaterials. Finally, structure-property relationship characteristic for segmented biomedical polyurethanes as the best candidates for soft tissue regeneration scaffolds are discussed.

We hypothesized that the hydrolysis of PCL-based SPEUs can significantly be improved by the introduction of a faster hydrolyzable hard segment derived from terephthaloyl diisocyanate (TPHDI). In **Chapter 2** we described the hydrolytic degradation of TPHDI-containing polyacylurethanes (PAUs) *in vitro*. PAUs were shown to degrade via both bulk and surface erosion mechanisms. Fourier Transform Infra Red (FTIR) spectroscopy was successfully applied to study the extent of PAUs microphase separation during *in vitro* degradation. Due to a faster hydrolyzable hard segment based on TPHDI and lower

degree of microphase separation, PAUs were found to degrade much faster *in vitro* than comparable PUs based on 1,4-butanediisocyanate (BDI) with the same polyester (PCL) soft segment. Predominant chain scission at the surface led to different surface properties of PAUs with respect to the bulk. Surface erosion and increased chain mobility at the surface resulted in the increase of both soft and hard segment crystallinity upon degradation. Generation of polar groups upon hydrolysis and the increase of the HS content at the surface probably led to the increase in hydrophilicity, which further rendered PAUs potentially cell adhesive. PAU1000 (molar mass of the oligocaprolactone soft segment = 1000 g/mol) can be recommended as a potential scaffold material to be used in regenerative medicine due to its optimal degradation behavior *in vitro*. In **Chapter 3** the potential to use PAUs as scaffolds for tissue engineering is further investigated by assessing cell adhesion and haemocompatibility of these materials. PAU1000 exhibited excellent haemocompatibility *in vitro*. In addition, PAU1000 supported both adhesion and proliferation of vascular endothelial cells. The contact angle of PAU1000 decreased in biological fluids. In endothelial cell culture medium the contact angle reached 60°, which is optimal for cell adhesion. Taken together, these results support the application of PAU1000 in the field of soft tissue repair as a temporary degradable scaffold.

In order to design biomedical SPEUs that would hydrolyze faster than PCL-based SPEUs, we chemically modified the soft segment too. In **Chapter 4** we describe the synthesis of *γ*-butyrolactone (*γ*-BL) and *ε*-caprolactone (*ε*-CL) co-polyesters (PBCL-PUs) by 1,4-butanediol initiated ring-opening polymerization (ROP), catalyzed by *Candida Antarctica Lipase B* (CAL-B; Novozyme 435). γ-Butyrolactone does not polymerize readily via conventional synthetic pathways. Enzymatic catalysis allowed for the incorporation of up to 26 mol% of *γ*-BL in the oligodiols with relatively good product yield. Introduction of hydrophilic *γ*-BL disturbed PCL crystallinity and resulted in enhanced hydrolytic degradability of PBCL-PUs. Modification of the PCL-soft segment with introduction of *γ*-BL resulted in twice as high hydrolysis rate of the SPEUs when compared to the SPEUs with unmodified soft segment. The proposed manufacturing route to obtain oligodiols for the synthesis of biomedical PUs is attractive due to the employment of the low-cost monomers (*γ*-BL and *ε* -CL), efficiently and easily removable catalyst and low reaction temperatures.

Soft segment modified BDI-based SPEUs were further processed into porous scaffolds and implanted subcutaneously in rats for 21 days (**Chapter 5**). Tissue remodeling and scaffold turnover was associated with a mild tissue response. This foreign body response was characterized by extensive vascularization throughout the interconnected pores,

with low numbers of macrophages and giant cells on the edges and extracellular matrix components formation inside the pores of the implants. The tissue ingrowth appeared to be related to the extent of microphase separation of the SPEUs and foam morphology. At day 21, all of the implants were highly vascularized also confirming the superior quality of the interconnected pore structure. At this time point, biodegradation of P(CL/D,LL)-PU was already observed while the other two polyurethanes remained unaffected in this relatively short period. The degradation of P(CL/D,LL)-PU proceeded without affecting the newly formed tissue This polyurethane could be employed as a short-term biodegradable scaffold in soft tissue (*e.g.* blood vessel) remodeling.

Finally, in **Chapter 6** the morphological and surface properties of the novel SPEUs are discussed with respect to their biological performance. We demonstrate that the *in vitro* hydrolysis of the novel SPEUs is enhanced by the choice of soft and hard segment building blocks. To fully characterize the *in vivo* degradation which is suspected to be enzymatically catalyzed (by *e.g.* esterases, MMPs and other proteolytic enzymes), we further suggest performing long-term (*i.e.* at least up to a year) *in vivo* study relevant to a potential biomedical application. In **Chapter 6** we also discuss how the SPEUs described in this thesis could be further improved to actively support and guide soft tissue regeneration through delivery of instructive factors and hosting progenitor cells relevant to a particular application site. In this way, instead of acting only as a mare support mash, new generation of "smart" SPEU scaffolds would actively induce and guide specific soft tissue regeneration.