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Hydrolytic and oxidative degradation of electrospun supramolecular biomaterials: In vitro degradation pathways

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ABSTRACT

The emerging field of in situ tissue engineering (TE) of load bearing tissues places high demands on the implanted scaffolds, as these scaffolds should provide mechanical stability immediately upon implantation. The new class of synthetic supramolecular biomaterial polymers, which contain non-covalent interactions between the polymer chains, thereby forming complex 3D structures by self assembly. Here, we have aimed to map the degradation characteristics of promising (supramolecular) materials, by using a combination of in vitro tests. The selected biomaterials were all polycaprolactones (PCLs), either conventional and unmodified PCL, or PCL with supramolecular hydrogen bonding moieties (either 2-ureido-[1H]-pyrimidin-4-one or bis-urea units) incorporated into the backbone. As these materials are elastomeric, they are suitable candidates for cardiovascular TE applications. Electrospun scaffold strips of these materials were incubated with solutions containing enzymes that catalyze hydrolysis, or solutions containing oxidative species. At several time points, chemical, morphological, and mechanical properties were investigated. It was demonstrated that conventional and supramolecular PCL-based polymers respond differently to enzyme-accelerated hydrolytic or oxidative degradation, depending on the morphological and chemical composition of the material. Conventional PCL is more prone to hydrolytic enzymatic degradation as compared to the investigated supramolecular materials, while, in contrast, the latter materials are more susceptible to oxidative degradation. Given the observed degradation pathways of the examined materials, we are able to tailor degradation characteristics by combining selected PCL backbones with additional supramolecular moieties. The presented combination of in vitro test methods can be employed to screen, limit, and select biomaterials for pre-clinical in vivo studies targeted to different clinical applications.

1. Introduction

Tissue engineering aims to restore tissue structure and function of diseased or damaged tissues by implantation of specifically designed biodegradable materials, with or without the addition of cells [1–3]. Conventional tissue engineering aims to collect autologous cells from patients, which are utilized for the in vitro generation of new tissues, and are often cultured in bioreactors for several weeks before implantation. A new and promising approach is in situ tissue engineering, in which in vitro culture is omitted and the patient's body is used as a bioreactor [4–7]. New tissue will be regenerated directly in the body by host cells after implantation of, for example, a biodegradable electrospun polymeric scaffold. This makes the overall procedure less demanding in terms of costs, time, and regulatory challenges, and creates off-the-shelf availability.

In situ tissue engineering of load-bearing tissues places high demands on the biodegradable scaffolds, as these scaffolds should be able to provide mechanical stability immediately upon implantation, and for a prolonged period thereafter, until sufficient mature neo-tissue is formed by recruited cells to take over the mechanical function of the scaffold. Various synthetic biodegradable polymers are used for tissue engineering applications, and

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these polymers include aliphatic polyesters (e.g. polylactic acid (PLA), polyglycolic acid (PGA) and polycaprolactone (PCL)), as well as various polyurethanes [8–11]. A new set of synthetic materials are the supramolecular polymers, which are formed by arrays of directed, non-covalent interactions between the building blocks, and can form complex 3D-structures by self assembly [12]. Material properties such as mechanics and resorption rate, which are critical for the success of in situ tissue engineering can be modified by combining or changing ratios of the same building blocks. This potentially allows for a variety of polymers with varying properties to be synthesized in a relatively short time span, thereby accelerating the development process. Monomeric units of the supramolecular polymers possess a relatively low molecular weight, resulting in beneficial processing properties, e.g. easy dissolution in organic solvents. Furthermore, supramolecular polymers may show self-healing properties [13–15], can easily be made bioactive [16,17], and allow for a more controlled way of synthesis, which can result in complex molecular structures [12]. Because of these features, these materials pose excellent candidates for use in in situ tissue engineering. Particularly, we are interested in biomaterials that either have 2-ureido-[1H]-pyrimidin-4-one (UPy) [18–21] or bis-urea (BU) [17] hydrogen bonding supramolecular motifs incorporated into their molecular structure, as these contain elastomeric properties, which makes them suitable candidates for cardiovascular applications. In vitro tests of these materials resulted in satisfactory fatigue properties (unpublished results), which is mainly important for in situ tissue engineering of heart valves, which will be exposed to cyclic loading. In vitro toxicity tests performed by Dankers et al. [16] and Wisse et al. [17] indicated that the UPy- and BU-moieties are not toxic and thus biocompatible. Furthermore, it was shown that solution cast polymer films comprising UPy or BU moieties were shown to be non-toxic after subcutaneous implantations in rats [16,17].

To enable the formation of a completely autologous tissue, the scaffold should degrade at the right pace during neo-tissue formation, leaving behind a living implant that is able to remodel and grow. In vivo, degradation of implanted scaffold materials can be accomplished via different pathways that operate at the same time, and that even may affect each other [22–25]. A well-known pathway is hydrolytic degradation, where chemical bonds of the polymer chains are cleaved by reaction with water molecules, forming oligomers and ultimately generating small molecules that can be cleared from the body [22,23]. Previous studies have reported that several enzymes, like proteases and esterases, which are present in human serum or are expressed by macrophages and other activated cells that are in contact with the scaffold, are known to catalytically accelerate this process [22,26,25,27,28]. Another well-described pathway is oxidative degradation, which is mediated by reactive oxygen species (ROS) that are secreted by macrophages, neutrophils and giant cells that are in contact with the scaffold [29,22]. These ROS include hydrogen peroxide (H_2O_2), nitric oxide (NO), hydroxyl radical ($^{\cdot}OH$) and the superoxide anion ($^{\cdot}O_2^-$). Previous studies have investigated that oxidation of polymers is often initiated by abstraction of a hydrogen atom by radicals, resulting in chain scission and/or crosslinking of the polymer [30,31]. Mapping the degradation characteristics of promising (supramolecular) materials for use in in situ tissue engineering approaches, as well as their susceptibility for certain degradation pathways, paves the way for screening and selection of materials for various clinical implantation sites.

The degradation properties of widely used and well-known materials such as polyesters, polycarbonates and polyurethanes have been examined extensively, both in vitro and in vivo [32,33,28,34,35,11,36,37]. In general, results of these studies show that polymers containing ester or anhydride linkages react with water molecules and undergo hydrolysis [33,23,38,39]. The water molecules can access those chemical species more easily, and thus increase the hydrolytic activity, when the polymer is amorphous or contains aliphatic structures [40,23]. Other polymers, including polyethers and polyurethanes, were found to be more susceptible to the oxidative pathway, as these materials contain α-methylene groups adjacent to ether or urethane groups, which are more prone to the formation of carbon centered radicals by abstraction of a hydrogen atom [41,42,23,24,31,43]. Just a few studies reported on the degradation characteristics of various polymers (PCL, polycarbonates, or polyurethane) modified with UPy or BU units. These were performed by incubating the materials in phosphate buffer saline (PBS) or solutions of various lipases at 37 °C [20,16,44,45]. These studies showed that the rates of enzymatic degradation can span a wide range, from less than 1% degradation after 1 month [45] to 90% after only 15 days [16], depending on the types of lipase and polymers used. No hydrolytic degradation, in terms of weight loss, of the UPy containing materials was observed for 126 days when samples were incubated with PBS [20], and a decrease in weight of only 2% after 120 days was observed for BU-containing materials [44].

Although these studies gave some insight into the degradation properties of biodegradable materials, the major part of these studies were performed on films or disks which are quite dense, while degradation rate of electrospun scaffolds, that are more porous and have higher surface to volume ratio, can be different. Studying the degradation properties of electrospun meshes is, from a clinical point of view, more relevant as these are more likely to be implanted as a tissue replacement, rather than a compact, solid construct. Furthermore, most research has focused on a single degradation pathway, while it is of importance to assess either the enzyme-accelerated hydrolytic and the oxidative degradation pathways, since in vivo both pathways may be operative and consequently, both may affect the implanted scaffold.

Here, an in vitro study was designed to investigate both degradation pathways in an accelerated fashion and was used to assess the degradation of several promising supramolecular biomaterials for in situ tissue engineering. We have chosen three previously reported supramolecular biomaterials, in which PCL backbones are combined with either UPy hydrogen bonding groups (materials PCL_{2000}-UPy and PCL_{800}-UPy) [46] or BU hydrogen bonding groups (PCL_{2000}-BU) [17]. High molecular weight PCL, a material frequently used for tissue engineering scaffolds, was added as a benchmark. All materials were electrospun and the resulting scaffold meshes were either exposed to enzymes that catalyze hydrolysis or to oxidative conditions. Degradation was monitored over time by examining the remaining scaffold with respect to weight, molecular weight, fiber diameter, and mechanical properties. Statistical analyses were performed to analyze changes in properties over time of all polymers with the various treatments, as well as to investigate their susceptibility to degradation and its mechanism (surface or bulk erosion). Finally, an explorative feasibility study was performed to show the effect of activated macrophages on the degradation of PCL scaffolds.

2. Materials and methods

2.1. Materials

All reagents, chemicals, materials, and solvents were obtained from commercial sources and were used without further purification, unless otherwise noted. The polycaprolactone based supramolecular biomaterials PCL_{2000}-UPy, PCL_{800}-UPy and PCL_{2000}-BU were synthesized as previously described from polycaprolactone diol building blocks of molecular weights 800 or 2000 [17,46]. These PCL_{2000}-diol and PCL_{800}-diol building blocks are prepared by initiation from diethylene glycol, so they contain