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Mechanical properties and osteocompatibility of novel biodegradable alanine based polyphosphazenes: Side group effects

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ABSTRACT

The versatility of polymers for tissue regeneration lies in the feasibility to modulate the physical and biological properties by varying the side groups grafted to the polymers. Biodegradable polyphosphazenes are high-molecular-weight polymers with alternating nitrogen and phosphorus atoms in the backbone. This study is the first of its kind to systematically investigate the effect of side group structure on the compressive strength of novel biodegradable polyphosphazene based polymers as potential materials for tissue regeneration. The alanine polyphosphazene based polymers, poly(bis(ethyl alanato) phosphazene) (PNEA), poly((50% ethyl alanato) (50% methyl phenoxy) phosphazene) ($PNEA_{50}mPh_{50}$), poly((50% ethyl alanato) (50% phenyl phenoxy) phosphazene) ($PNEA_{50}PhPh_{50}$) were investigated to demonstrate their mechanical properties and osteocompatibility. Results of mechanical testing studies demonstrated that the nature and the ratio of the pendent groups attached to the polymer backbone play a significant role in determining the mechanical properties of the resulting polymer. The compressive strength of $PNEA_{50}PhPh_{50}$ was significantly higher than poly(lactide-*co*-glycolide) (85:15 PLAGA) ($p < 0.05$). Additional studies evaluated the cellular response and gene expression of primary rat osteoblast cells on PNEA, $PNEA_{50}mPh_{50}$ and $PNEA_{50}PhPh_{50}$ films as candidates for bone tissue engineering applications. Results of the in vitro osteocompatibility evaluation demonstrated that cells adhere, proliferate, and maintain their phenotype when seeded directly on the surface of PNEA, $PNEA_{50}mPh_{50}$, and $PNEA_{50}PhPh_{50}$. Moreover, cells on the surface of the polymers expressed type I collagen, alkaline phosphatase, osteocalcin, osteopontin, and bone sialoprotein, which are characteristic genes for osteoblast maturation, differentiation, and mineralization.

1. Introduction

Biodegradable polymers form an integral part of scaffold-based tissue engineering, and have been used as replacements for connective tissue in the past [1]. They are now currently used to assist or guide the regeneration of tissues or organs [2]. Biodegradable polymers used for tissue regeneration should be biocompatible and not elicit immunological or foreign body response, should be degradable with a controlled rate of degradation that matches the rate of new tissue formation, the degradation products should be non-toxic and metabolized by the body, they should allow cellular attachment, proliferation, and differentiation and finally have suitable mechanical properties [2–4].

Natural polymers (such as collagen, fibrin, chitosan) and synthetic polymers (such as polyesters, polyanhydrides, polyphosphazenes) have been developed for various biomedical applications. Biodegradable polyphosphazenes are a novel class of polymers that have received a great deal of attention recently as potential biomaterials due to their synthetic flexibility, high biocompatibility, and predictable degradation rate [5,6]. Polyphosphazenes are high-molecular-weight polymers with an alternating nitrogen and phosphorus backbone and with each phosphorus atom attached to two organic groups. The general structure of

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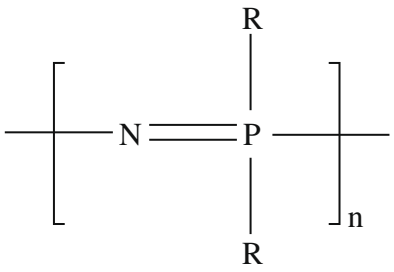

Fig. 1. General structure of polyphosphazenes, where R can be either an alkoxy, or arloxy, or amino group.

polyphosphazenes is shown in Fig. 1 where R can be an alkoxy, aryloxy, or amino group.

The synthetic flexibility of polyphosphazenes allows a wide range of polymers to be synthesized using a highly reactive macromolecular intermediate, poly(dichlorophosphazene). Polyphosphazenes are synthesized via a two-step reaction process (Fig. 2). The first step involves the thermal ring opening polymerization of hexachlorocyclotriphosphazene (1) to form poly(dichlorophosphazene) (2) [7]. In the second step the reactive chlorine atoms in the macromolecular intermediate (2) are replaced by any of a broad range of organic side groups (3, 4).

The polyphosphazene platform of macromolecules constitutes more than a 700-member group of different polymers with a broad diversity of properties. The large number of polymers accessible in this system is a consequence of the wide variety of alcohols, phenols, and amines available to obtain polymers with different pendant groups and the fact that two or more different side groups can be incorporated into the same polymer molecule by simultaneous or sequential chlorine replacement reactions [7]. Due to the synthetic versatility of the polyphosphazene, the properties of the polymer can be tuned by an appropriate choice of side groups to suite a specific application. The chemical properties such as hydrophilic or hydrophobic character, solubility, biocompatibility, basicity or acidity, and susceptibility to hydrolysis [7] depend on the nature of the side group. Because the phosphorus–nitrogen backbone in these polymers is highly flexible, material properties such as glass or melting temperatures and the mechanical properties of the polymer depend on the side groups attached to the phosphorus atom. Thus, these unique properties of polyphosphazenes have sparked great interest in their biomedical use [5,8–11].

Amino acid substituted polyphosphazenes with various functional groups have been successfully synthesized [8–15]. The rate of hydrolysis for polyphosphazenes depends on the nature of the group attached to the α-carbon of the amino acid ester side group; bulkier side groups result in less hydrolytically sensitive polymers [16–20]. In the case of co-substituted polyphosphazenes, the nature of the co-substituent determines the rate of degradation.

Our recent studies have been focused on modulating the properties of biodegradable polyphosphazenes by varying the side group chemistry as well as by blending polyphosphazenes with other degradable polymers such as poly(lactic acid-co-glycolic acid) (PLAGA) [21,22]. By blending biodegradable polyphosphazenes with PLAGA, novel polymeric mixtures with varying glass transition temperatures and degradation profiles can be developed [21]. Another versatile process to modulate the properties of biodegradable polyphosphazenes is by varying the side groups. The effects of side groups on various properties of homo- and co-polymers of ethyl alaninato substituted polyphosphazenes have been evaluated. The properties investigated include glass transition temperature, hydrolytic degradation, surface wettability, tensile strength and modulus. The glass transition temperature of the polymers was found to increase with increase in bulkiness of the side groups. The study demonstrated the feasibility of varying the properties of biodegradable polyphosphazenes by varying the bulkiness and hydrophobicity of the side groups [22].

Ethyl alanato derivates of polyphosphazenes have been used as membranes in the treatment of periodontal disease [23], as nerve guide conduits [24] and as scaffolds for orthopaedic applications [19,25]. The objective of this study was to evaluate systematically the effect of side group chemistry on the compressive strength and osteocompatibility of biodegradable polyphosphazenes containing alanine. The mechanical properties and osteocompatibility of these alanine substituted polyphosphazenes, poly(bis(ethyl alanato) phosphazene) (PNEA), poly((50% ethyl alanato) (50% methyl phenoxy) phosphazene) ($PNEA_{50}mPh_{50}$), poly((50% ethyl alanato) (50% phenyl phenoxy) phosphazene) ($PNEA_{50}PhPh_{50}$) were evaluated. These are the first studies of their kind to fully examine these novel alanine based polyphosphazene polymers.

2. Materials and methods

The biodegradable polymers used for mechanical property evaluations in the present study were poly(bis(ethyl alanato) phosphazene) (PNEA), poly((50% ethyl alanato) (50% methyl phenoxy)

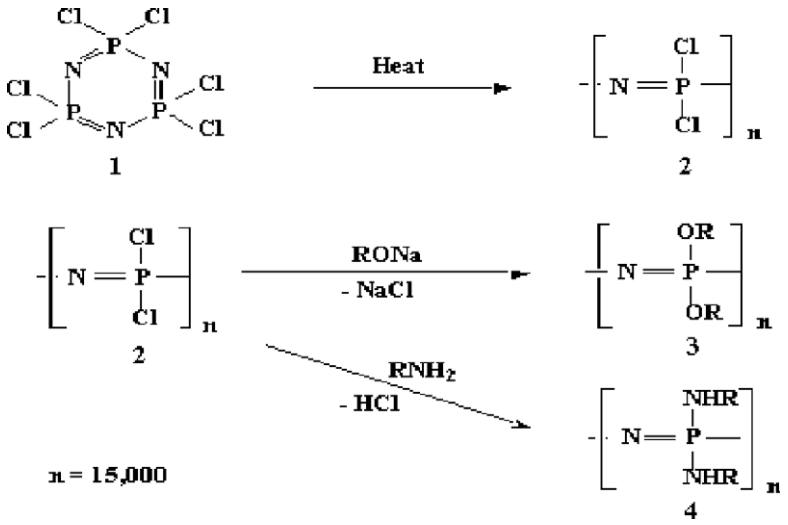

Fig. 2. Macromolecular substitution reaction to form amino acid ester based polyphosphazenes.

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