Synthesis and Characterization of Novel Poly[(organo)phosphazenes] with Cell-Adhesive Side Groups

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There is a need to develop new scaffold materials with controlled surface properties for tissue engineering applications. For that purpose novel biodegradable poly[(organo)phosphazenes] were synthesized. A cell-binding molecule, galactose, was introduced via a spacer, either 6-aminohexanol (AH) or poly(ethylene glycol) (PEG; $M_w = 3400$). Some polymers were substituted with an additional PEG chain of different molecular weights ($M_w = 750$ or 5000). The polyphosphazene derivatives were characterized by ^{1}H NMR. T_g and T_m were determined using differential scanning calorimetry. A detailed surface analysis of the polymers using X-ray photoelectron spectroscopy (XPS), secondary ion mass spectroscopy (SIMS), and dynamic contact angle (DCA) measurements was performed. Typical backbone and side chain fragments were detected by SIMS and confirmed the polymer composition. Compared to that of the reference polymer (having only amino acid ester side groups), an increased value of the specific ether carbon groups from PEG confirmed the enrichment of PEG at the surface of PEG-Gal polymers. However, the values were lower than expected. DCA studies showed that the galactose moieties were present at the surface after exposure to an aqueous environment. XPS results confirmed the similarity between experimental and theoretical values for the AH-Gal polymers. This indicated the presence of galactose moieties at the surface, which was confirmed by the DCA data because the contact angles were low compared to those of the other polymers.

Introduction

Tissue engineering may be defined as the application of principles of engineering and life science toward the development of biological substitutes that restore, maintain, or improve tissue function.[1] This involves the use of cells, growth factors, and scaffolds, alone or in various combinations.[2,3] Scaffolds can be fabricated from natural or synthetic materials. Natural materials can closely mimic the cellular environment as they are often extracellular matrix components.[4] However, they can induce unwanted immune responses, and they often suffer from batch to batch variations. Synthetic materials have the advantage that material properties such as biocompatibility, strength, degradation time, functionality, surface chemistry, porosity, and microstructure can be better controlled.[5] However, synthetic materials generally lack cell recognition signals. Since these two categories still have their limitations, researchers are now trying to combine the best of both worlds to design a new generation of materials. These biomimetic materials are capable of triggering specific cellular responses and guiding the formation of new tissue by specific interactions.[6] This selectivity can be obtained by modifying polymers with well-defined cell-adhesive peptides[7] or carbohydrates.[8] Since the goals of tissue engineering have increased in complexity, there is a need to develop novel polymer structures that enable cell-to-surface interactions to be tuned. For example, the ability to block nonspecific interactions and promote the adhesion of a specific cell type could be desirable in the early stages of tissue formation.

In this paper we describe the synthesis and evaluation of novel polyphosphazene derivatives having a galactose moiety. The literature suggested that the introduction of galactose would lead to the facilitation of hepatocyte adhesion due to hepatocyte expression of the asialoglycoprotein receptor (ASGP-R), which binds galactose in a unique fashion.[9,10] Polyphosphazenes were chosen because of their biocompatible properties[11−17] and because they can be made biodegradable by introducing appropriate side chains such as amino acid esters.[18−22] Poly[(organo)phosphazenes] are polymers with an inorganic backbone, consisting of alternating nitrogen and phosphorus atoms linked by alternating single and double bonds. Each phosphorus bears two side groups (R); R can be an alkoxy, aryloxy, amino, alkyl, heterocyclic ring, inorganic, or organometallic unit (Figure 1).[23] The synthesis of poly[(organo)phosphazenes] has been extensively studied by Allcock and co-workers.[18,19,24−26] The polyphosphazene derivatives were prepared by nucleophilic replacement of chlorine from a precursor polymer, poly[(dichloro)phosphazene].[27] The polyphosphazenes were characterized by ^{1}H NMR, and the thermal properties were investigated using differential scanning calorimetry (DSC). Surface characteristics of polymers for biomedical applications are very important. The orientation of the substituents at the

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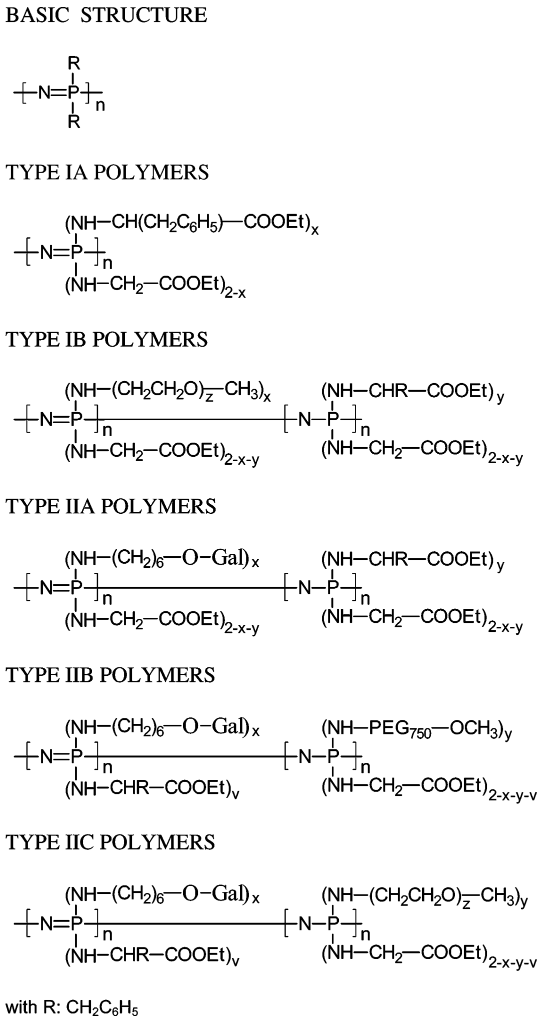


Figure 1. Poly[(organo)phosphazenes].

surface plays an important role in the adsorption of proteins and cells present in the surrounding medium. The polyphosphazene surfaces were characterized by means of X-ray photoelectron spectroscopy (XPS), secondary ion mass spectroscopy (SIMS), and dynamic contact angle (DCA) measurements.

Materials and Methods

Materials. The monomer $Cl_3PNP(O)Cl_2$ was a kind gift from Professor R. De Jaeger (University of Lille, France). Phosphonitrilic chloride trimer (99.99+%), palladium on carbon, 6-amino-1-hexanol, and silica gel (230−400 mesh, 60 Å, grade 9385) were purchased from Sigma-Aldrich. Phenylalanine ethyl ester hydrochloride and glycine ethyl ester hydrochloride were obtained from Acros. They were dried before use in a vacuum cabinet over P_2O_5 at 60 °C for 8 h. β-D-Galactose pentaacetate, phosphorus tribromide, and α-hydroxy-ω-methoxypoly(ethylene glycol), $M_w = 750$, 2000, and 5000, were purchased from Fluka, and α-hydroxy-ω-methoxypoly(ethylene glycol) was dried before use in a vacuum over P_2O_5 at 60 °C for 8 h. α-Amino-ω-hydroxypoly(ethylene glycol), $M_w = 3400$, was obtained from Nektar. All other products and solvents were obtained from Acros Chemicals. All solvents were dried and distilled prior to use.

Synthesis of Poly[(dichloro)phosphazene]. The starting product poly[(dichloro)phosphazene] ($M_w = 79700$, $M_n = 42900$) used for the synthesis of type IA, IB, and IIC polymers was prepared following the method of D'Halluin−De Jaeger et al.,[28] which was also used before by Lemmouchi et al.[29] The precursor polymer for type IIA and IIB polymers was prepared using the following method:[16,24] Phosphonitrilic chloride trimer, purified by sublimation, was heated at 250 °C under vacuum for 3 h to yield poly[(dichloro)phosphazene]. After removal of the residual monomer by sublimation, the resulting polymer was dissolved in anhydrous toluene ($M_w = 201000$, $M_n = 20500$).

Synthesis of Poly[(phenylalanine ethyl ester)-*co*-(glycine ethyl ester)phosphazene] (Type IA). This product was prepared following the procedures described by Allcock et al.[18] and Crommen et al.[20] Yield: 76%. The molar ratio of Phe to Gly was calculated from the integration ratio of the aromatic protons of Phe (expressed per proton) and the ethyl protons of phenylalanine ethyl ester (PheOEt) and glycine ethyl ester (GlyOEt) (subtracting three protons of Phe and expressed per proton) at $\delta = 7.0-7.2$ ppm and $\delta = 0.5-1.35$ ppm, respectively.

Synthesis of Poly[(poly(ethylene glycol))-*co*-(phenylalanine ethyl ester)-*co*-(glycine ethyl ester)phosphazene] (Type IB). α-Amino-ω-methoxypoly(ethylene glycol) was prepared following the procedure described by Loccufier et al.[30] The preparation of the polymers has been described by Vandorpe et al.[31] Yield: 67%. The molar ratio of PEG to Phe to Gly was calculated from the integration ratio of the protons at $\delta = 2.5-4.8$ ppm (subtracting 4H of Gly and 5H of Phe and expressed per proton: PEG_{750}, $[(750/44) \times 4] + 3 = 71H$; PEG_{5000}, $[(5000/44) \times 4] + 3 = 458H$), the aromatic protons of Phe at $\delta = 7.0-7.2$ ppm (expressed per proton), and the ethyl protons of PheOEt and GlyOEt (subtracting three protons of Phe and expressed per proton) at $\delta = 0.5-1.35$ ppm.

Synthesis of Z-Protected Poly(ethylene glycol) 2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranoside. *Selective Deacetylation at the Anomeric Position of Galactose Pentaacetate.* This product was synthesized according to the method of Excoffier et al.[32] Briefly, to a stirred solution of 2.98 g (7.64 mmol) of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranoside in DMF (15 mL) was added 844 mg (1.2 equiv, 9.17 mmol) of hydrazine acetate at 50 °C. The mixture was stirred for 10 min, cooled, and diluted with 100 mL of EtOAc. The organic layer was washed twice with a saturated NaCl solution, dried ($MgSO_4$), and evaporated in vacuo to give 2,3,4,6-tetra-*O*-acetylgalactopyranoside. The reaction product is a mixture of both anomeric forms (α and β). Yield: 98%. TLC: eluent $CHCl_3$/acetone, 9/1, $R_f = 0.29$. ^1H NMR ($CDCl_3$): $\delta = 1.98-2.2$ ppm ($3 \times CH_3CO-$), $\delta = 3.2$ ppm (−OH, β-Gal), $\delta = 3.6$ ppm (−OH, α-Gal), $\delta = 3.95$ ppm (H_5, α-Gal), $\delta = 4.05-4.2$ ppm (H_5, β-Gal, H_6, α-Gal, H_6, β-Gal), $\delta = 4.45$ (H_1, β-Gal), $\delta = 4.7$ ppm (H_1, α-Gal), $\delta = 5.05$ ppm (H_4, α-Gal), $\delta = 5.15$ ppm (H_3, β-Gal), $\delta = 5.35-5.6$ ppm (H_2, α-Gal, H_2, β-Gal, H_3, α-Gal, H_4, β-Gal).

Synthesis of 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl Trichloroacetimidate. A stirred solution of 2.61 g (7.5 mmol) of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranoside in dry CH_2Cl_2 (30 mL) and 5.26 mL of trichloroacetonitrile (52.5 mmol) was treated with DBU (1.12 mL, 7.5 mmol) at room temperature and then left for 1 h. The solvent was evaporated and the product purified by column chromatography (eluent $CHCl_3$/acetone, 9/1, $R_f = 0.68$) to give 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate. Yield: 80%. ^1H NMR ($CDCl_3$): $\delta = 1.98-2.2$ ppm ($4 \times CH_3CO-$), $\delta = 4.05$ ppm (H_6, Gal), $\delta = 4.15$ ppm ($H_{6'}$, Gal), $\delta = 4.45$ ppm (H_5, Gal), $\delta = 5.35$ ppm (H_3, Gal), $\delta = 5.45$ (H_2, Gal), $\delta = 5.55$ ppm (H_4, Gal), $\delta = 6.6$ ppm ($J_{1,2} = 3.3$ Hz, H_1, Gal), $\delta = 8.65$ ppm (NH).

Synthesis of Z-Protected α-Amino-ω-hydroxypoly(ethylene glycol). To a stirred solution of 1 g (0.29 mmol) of H_2N-PEO-OH in 5 mL of CH_2Cl_2 were added 82.75 μL (2 equiv, 0.58 mmol) of benzyloxycarbonyl chloride (Z-Cl) and 1 mL of triethylamine (TEA). A 50 mL volume of CH_2Cl_2 and 50 g of ice were added after reaction overnight. The organic layer was washed three times with 0.5 M HCl, dried ($MgSO_4$), and evaporated in vacuo. The residue was redissolved in water and extracted three times with diethyl ether and three times with CH_2Cl_2. The organic layer was dried ($MgSO_4$), and the solvent was evaporated. Yield: 58%. ^1H NMR ($CDCl_3$): $\delta = 3.35$ ppm (Z-$NHCH_2-$), $\delta = 3.65$ ppm (methylene protons of PEG), $\delta = 5.1$ ppm (benzylic protons of Z), $\delta = 7.35$ ppm (aromatic protons of Z).

Synthesis of Z-Protected Poly(ethylene glycol) 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranoside. A solution of 0.6 g (17.66 mmol) of Z-NH-PEO-OH and 90.5 mg (18.37 mmol) of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside trichloroacetimidate in dry CH_3CN (7 mL) was stirred in the presence of 200 μL of trimethylsilyl triflate and 4 Å molecular sieves. The reaction was followed by TLC (eluent $CHCl_3$/MeOH, 9/1) until the spot at $R_f = 0.83$ disappeared. The reaction was quenched by addition of TEA. The solvent was removed, and the residue was redissolved in CH_2Cl_2. The solution was extracted twice with 0.5 M HCl and once with a NaCl solution. The organic layer was dried ($MgSO_4$), and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (eluent $CHCl_3$/MeOH, 9/1, $R_f = 0.34$). Yield: 77%. ^{1}H NMR ($CDCl_3$): $\delta = 1.98$, 2.04, 2.06, and 2.16 ppm (4 × CH_3CO-, Gal), $\delta = 3.45$ ppm (Z-$NHCH_2-$), $\delta = 3.9-4.15$ ppm (H_5, Gal, H_6, Gal, $H_{6'}$, Gal, $-CH_2O$-Gal), $\delta = 4.53$ ppm (H_1, Gal), $\delta = 5.01$ (H_3, Gal), $\delta = 5.10$ ppm (benzylic protons of Z), $\delta = 5.19$ ppm (H_2, Gal), $\delta = 5.39$ ppm (H_4, Gal), $\delta = 7.35$ ppm (aromatic protons of Z).

Synthesis of Poly[(poly(ethylene glycol) *β*-D-galactopyranoside)-*co*-(poly(ethylene glycol))-*co*-(phenylalanine ethyl ester)-*co*-(glycine ethyl ester)phosphazene] (Type IIC). The preparation of a polyphosphazene containing 0.1% PEG-Gal, 13.1% PEG ($M_w = 750$), 56.8% PheOEt, and 30% GlyOEt is given as an example.

First Part: Synthesis of Polyphosphazenes with Poly(ethylene glycol) 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranoside, Poly(ethylene glycol), and Amino Acid Ester Side Chains. Z-NH-PEG-O-Gal$(OAc)_4$ (80 mg, 0.02 mmol) was deprotected by hydrogenation in MeOH (1 mL) in the presence of Pd/C (40 mg) as a catalyst. The reaction was stirred overnight. The Pd/C catalyst was removed by filtration, and the solvent was evaporated in vacuo. To a solution of 0.8 g (6.9 mmol) of poly[(dichloro)phosphazene] in 50 mL of dry toluene, cooled to 0 °C, was added poly(ethylene glycol) 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside (51 mg, 0.01 mmol) dissolved in 2 mL of dry toluene and 5.77 μL (0.04 mmol) of TEA. Stirring was continued for 2 h at 0 °C. Then 1.36 g (1.81 mmol) of α-amino-*ω*-methoxypoly(ethylene glycol) dissolved in 30 mL of dry toluene and 0.76 mL of (5.43 mmol) TEA was added to the poly[(chloro)-*co*-(PEG-Gal)phosphazene] solution. Stirring was continued for 5 h at 0 °C and at room temperature for 15 h. Meanwhile, dried PheOEt·HCl (2.70 g, 11.75 mmol) was refluxed for 4 h in the presence of 50 mL of dry toluene and 4.91 mL (35.25 mmol) of TEA. After removal of the insoluble salts, the PheOEt solution, together with 4.91 mL (35.25 mmol) of TEA, was added dropwise to a cooled (0 °C) poly[(chloro)-*co*-(PEG-Gal)-*co*-(PEG)-phosphazene] solution. The reaction was stirred for an additional 5 h at 0 °C and at room temperature for 15 h. A 1.93 g (13.79 mmol) portion of dried GlyOEt·HCl was refluxed for 4 h in the presence of 60 mL of dry toluene and 5.77 mL (41.37 mmol) of TEA. The mixture was allowed to cool and was filtered. The poly[(chloro)-*co*-(PEG-Gal)-*co*-(PEG)-*co*-(PheOEt)phosphazene] solution was cooled to 0 °C, and the GlyOEt solution with 5.77 mL (41.37 mmol) of TEA was added dropwise. The solution was stirred for 5 h at 0 °C and an additional 15 h at room temperature. After removal of the insoluble salts by centrifugation, the polymer solution was concentrated under reduced pressure. The polymer was precipitated in dry pentane and reprecipitated from dry THF into pentane. Yield: 41%. ^{1}H NMR ($CDCl_3$): $\delta = 0.85$ ppm (CH_3-, Phe), $\delta = 1.1$ ppm (CH_3-, Gly), $\delta = 2.5-4.8$ ppm ($-NHCH$, Phe, $-CH_2Ph$, Phe, $-NHCH_2$, Gly, $-CH_2OC(O)-$, Phe and Gly), $\delta = 3.35$ ppm (CH_3O-, PEG), $\delta = 3.60$ ppm ($-OCH_2CH_2O-$, PEG), $\delta = 7.0-7.2$ ppm (aromatic protons of Phe). The characteristic peaks of galactose are not visible in the spectra because the concentration (mol %) of galactose incorporated is too low.

Second Part: Synthesis of Polyphosphazenes with Poly(ethylene glycol) β-D-Galactopyranoside, Poly(ethylene glycol), and Amino Acid Ester Side Chains. To a solution of 0.21 g of poly[(PEG_{3400}-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside)-*co*-(PEG_{750})-*co*-(PheOEt)-*co*-(GlyOEt)phosphazene] in dry MeOH (3 mL) was added 0.44 mg (1 equiv, $8.23.10^{-3}$ mmol) of NaOMe. The reaction mixture was stirred overnight at room temperature. The reaction was quenched with phosphoric acid. After removal of the solvent, the polymer was redissolved in a minimal amount of dry THF. The polymer was precipitated in dry pentane and reprecipitated from dry THF into pentane (three times). Yield: 70%.

Since polyphosphazenes containing 0.02% PEG-Gal$(OAc)_4$, 1.2% PEG ($M_w = 5000$), 56.8% PheOEt, and 30% GlyOEt side chains are not soluble in MeOH, a different method was used: A 0.47 mg (1.45×10^{-3} mmol) portion of tetrabutylammonium bromide, 1.3 μL (1.2 equiv, $1.45.10^{-3}$ mmol) of NaOMe, and 1.5 mL of dry MeOH were stirred for 10 min at room temperature. Meanwhile 0.2 g of poly[(PEG_{3400}-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside)-*co*-(PheOEt)-*co*-(GlyOEt)phosphazene] was dissolved in 1.5 mL of dry THF. The MeOH solution was added to the polymer solution, and the mixture was stirred overnight. The reaction was quenched with phosphoric acid. After removal of the solvent, the polymer was redissolved in a minimal amount of dry THF. The polymer was precipitated in dry pentane and reprecipitated from dry THF into pentane (three times). Yield: 70%.

Synthesis of 6-((Benzyloxycarbonyl)amino)hexyl 2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranoside. *Synthesis of 6-(((Benzyloxy)carbonyl)amino)-1-hexanol.* A 23.59 g (2 equiv, 0.17 mol) portion of K_2CO_3 and 18.3 mL (1.5 equiv, 0.13 mol) of Z-Cl were added to a stirred solution of 10 g (0.09 mol) of 6-amino-1-hexanol in distilled water (130 mL). After being stirred for 18 h, the reaction mixture was filtered. 6-((Benzyloxycarbonyl)amino)-1-hexanol was obtained as a white crystalline product. The product was washed with pentane. Yield: 92%. TLC: eluent EtOAc, $R_f = 0.51$. ^{1}H NMR ($CDCl_3$): $\delta = 1.34$ ppm ($-NHCH_2CH_2\mathit{CH_2CH_2}CH_2CH_2OH$), $\delta = 1.52$ ppm ($-NHCH_2\mathit{CH_2}-$), $\delta = 1.55$ ppm ($-\mathit{CH_2}CH_2OH$), $\delta = 3.18$ ppm ($-OC(O)NHCH_2-$), $\delta = 3.61$ ($-CH_2OH$), $\delta = 4.75$ ppm ($-OC(O)NH$), $\delta = 5.07$ ppm ($-OCH_2Ph$), $\delta = 7.37$ ppm (aromatic protons of Z).

Synthesis of 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl Bromide. To a stirred and cooled solution of 1,2,3,4,6-penta-*O*-acetyl-*β*-D-galactopyranoside (11.3 g, 28.97 mmol) and PBr_3 (10 mL) in acetic anhydride (55 mL) was added water (18 mL) dropwise during a period of 90 min. The reaction was monitored by TLC (eluent $CHCl_3$/acetone, 9/1, $R_f = 0.66$). After dilution with 300 mL of diethyl ether/pentane (1/1), the solution was washed twice with ice-cold water and finally with an ice-cold 10% $NaHCO_3$ solution. The solvent was removed after drying ($MgSO_4$). Yield: 71%. ^{1}H NMR ($CDCl_3$): $\delta = 2.02$, 2.07, 2.10, and 2.16 ppm (4 × $CH_3C(O)-$), $\delta = 4.15$ ppm (H_6, Gal, $H_{6'}$, Gal), $\delta = 4.49$ ppm (H_5, Gal), $\delta = 5.05$ ppm (H_2, Gal), $\delta = 5.41$ (H_3, Gal), $\delta = 5.52$ ppm (H_4, Gal), $\delta = 6.69$ ppm ($J_{1,2} = 3.8$ Hz, bromide in the α position).

Synthesis of 6-((Benzyloxycarbonyl)amino)hexyl 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranoside. To a solution of 13.5 g (53.78 mmol) of 6-((benzyloxycarbonyl)amino)-1-hexanol in $CH_3CN/CHCl_3$ (17/18 mL) was added 5.8 g (22.96 mmol) of $Hg(CN)_2$. The mixture was stirred at room temperature and treated with a solution of 8.4 g (20.44 mmol) of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in 15 mL of dry $CHCl_3$. The reaction was stirred overnight and diluted with 200 mL of $Na_2S_2O_3$, 100 mL of diethyl ether, 50 mL of EtOAc, and 50 mL of CH_2Cl_2. The solution was washed with a 2 M $Na_2S_2O_3$ solution and three times with a 2 M NaCl solution. The organic layer was dried (Na_2SO_4 and $Na_2S_2O_3$) and evaporated to give a syrup, which was purified by column chromatography (gradient elution, diethyl ether/pentane, from 10% to 50% diethyl ether, containing 5−10% CH_2Cl_2). A second gradient elution was needed (acetone/CH_2Cl_2, from 0% to 5% acetone). Yield: 58%. ^{1}H NMR ($CDCl_3$): $\delta = 1.35$ ppm ($-NHCH_2$-$CH_2\mathit{CH_2CH_2}CH_2CH_2O$-Gal), $\delta = 1.50$ ppm ($-NHCH_2\mathit{CH_2}-$), $\delta = 1.60$ ppm − $\mathit{CH_2}CH_2O$-Gal), $\delta = 1.98$, 2.04, 2.06, and 2.16 ppm (4 × CH_3C-(O)−), $\delta = 3.18$ ($-OC(O)NHCH_2-$), $\delta = 3.47$ and 3.88 ppm ($-CH_2O$-Gal), $\delta = 3.9$ ppm (H_5, Gal), $\delta = 4.11$ ppm (H_6, Gal), $\delta = 4.18$ ppm ($H_{6'}$, Gal), $\delta = 4.45$ ppm ($J_{1,2} = 8.0$ Hz, *β*-anomer, H_1, Gal), $\delta = 4.8$ ppm ($-OC(O)NH$), $\delta = 5.01$ ppm (H_3, Gal), $\delta = 5.10$ ppm ($-OCH_2$-Ph), $\delta = 5.19$ (H_2, Gal), $\delta = 5.39$ ppm (H_4, Gal), $\delta = 7.35$ ppm (aromatic protons of Z).

Synthesis of Poly[(6-aminohexyl *β*-D-galactopyranoside)-*co*-(phenylalanine ethyl ester)-*co*-(glycine ethyl ester)phosphazene] (Type

IIA). The preparation of a polyphosphazene containing 5% AH-Gal (AH = 6-aminohexanol), 65% PheOEt, and 30% GlyOEt is given as an example.

First part: Synthesis of Polyphosphazenes with 6-Aminohexyl 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranoside and Amino Acid Ester Side Chains. Z-NH$(CH_2)_6$O-Gal$(OAc)_4$ (0.6 g, 1.03 mmol) was deprotected by hydrogenation in MeOH (7.5 mL) in the presence of Pd/C (0.03 g) as a catalyst. The reaction was followed by TLC. After 7 h the deprotected product was obtained. To a solution of 0.7 g (6.03 mmol) of poly[(dichloro)phosphazene] in 40 mL of dry THF, cooled to 0 °C, was added 6-aminohexyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside (0.27 g, 0.6 mmol) dissolved in 4 mL of dry THF and 0.31 mL (1.8 mmol) of diisopropylethylamine (DIPEA). Stirring was continued for 2 h at 0 °C. Then a suspension of dried PheOEt·HCl (2.70 g, 11.77 mmol) in 50 mL of dry THF and 12.3 mL (70.62 mmol) of DIPEA was added to the poly[(chloro)-*co*-(AH-Gal)phosphazene] solution in a drybox. This mixture was stirred for 5 h at 0 °C and for an additional 2 days at room temperature. Finally, a suspension of 1.68 g (12.07 mmol) of dried GlyOEt·HCl in 30 mL of dry THF and 12.6 mL (72.42 mmol) of DIPEA was added to the poly[(chloro)-*co*-(AH-Gal)-*co*-(PheOEt)phosphazene] solution in a drybox. This mixture was again stirred for 5 h at 0 °C and for 2 days at room temperature. After removal of the salts by centrifugation, the polymer solution was concentrated under reduced pressure. The polymer was precipitated in dry pentane and reprecipitated from dry THF into pentane (three times). Yield: 78%. ^{1}H NMR ($CDCl_3$): $\delta = 0.85$ ppm (CH_3-, Phe), $\delta = 1.1-1.35$ ppm (CH_3-, Gly, $-NH(CH_2)_2(CH_2)_2(CH_2)_2O$-Gal), $\delta = 1.50$ ppm ($-NHCH_2CH_2-$, Gal), $\delta = 1.60$ ppm ($-CH_2CH_2O$-Gal), $\delta = 1.98$, 2.04, 2.06, and 2.16 ppm ($4 \times CH_3C(O)-$, Gal), $\delta = 2.5-4.8$ ppm ($-NHCH_2$, Gal, $-NHCH$, Phe, $-CH_2Ph$, Phe, $-NHCH_2$, Gly, $-CH_2OC(O)-$, Phe and Gly), $\delta = 3.47$ and 3.88 ppm ($-CH_2O$-Gal), $\delta = 3.9$ ppm (H_5, Gal), $\delta = 4.11$ ppm (H_6, Gal), $\delta = 4.18$ ppm ($H_{6'}$, Gal), $\delta = 4.45$ ppm (H_1, Gal), $\delta = 5.01$ ppm (H_3, Gal), $\delta = 5.19$ (H_2, Gal), $\delta = 5.39$ ppm (H_4, Gal), $\delta = 7.0-7.2$ ppm (aromatic protons of Phe).

Second Part: Synthesis of Polyphosphazenes with 6-Aminohexyl β-D-galactopyranoside and Amino Acid Ester Side Chains. A 114 mg (0.35 mmol) portion of tetrabutylammonium bromide, 325 μL (1.2 equiv, 0.35 mmol) of NaOMe, and 1.5 mL of dry MeOH were stirred for 10 min at room temperature. Meanwhile 0.23 g of poly[(6-aminohexyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside)-*co*-(PheOEt)-*co*-(GlyOEt)phosphazene] was dissolved in 1.5 mL of dry THF. The MeOH solution was added to the polymer solution, and the mixture was stirred overnight. The reaction was quenched with phosphoric acid. After removal of the solvent, the polymer was redissolved in a minimal amount of dry THF. The polymer was precipitated in dry pentane and reprecipitated from dry THF into pentane (three times). Yield: 95%. ^{1}H NMR ($CDCl_3$): $\delta = 0.85$ ppm (CH_3-, Phe), $\delta = 1.1-1.35$ ppm (CH_3-, Gly, $-NH(CH_2)_2(\mathit{CH_2})_2(CH_2)_2O$-Gal), $\delta = 1.50$ ppm ($-NHCH_2$-$\mathit{CH_2}-$, Gal), $\delta = 1.60$ ppm ($-\mathit{CH_2}CH_2O$-Gal), $\delta = 2.5-4.8$ ppm ($-NH\mathit{CH_2}$, Gal, $-NH\mathit{CH}$, Phe, $-CH_2Ph$, Phe, $-NH\mathit{CH_2}$, Gly, $-CH_2$-$OC(O)-$, Phe and Gly), $\delta = 3.5$ ppm (H_2, Gal), $\delta = 3.6-3.65$ ppm (H_3, H_5, H_6, Gal), $\delta = 3.7-3.8$ ppm ($H_{6'}$, $-CH_2O$-Gal), $\delta = 3.9$ ppm (H_4, Gal, $-CH_2O$-Gal), $\delta = 4.3-4.4$ ppm (H_1, Gal), $\delta = 7.0-7.2$ ppm (aromatic protons of Phe).

Synthesis of poly[(6-aminohexyl *β*-D-galactopyranoside)-*co*-(poly(ethylene glycol))-*co*-(phenylalanine ethyl ester)-*co*-(glycine ethyl ester)phosphazene] (Type IIB). The preparation of a polyphosphazene containing 5% AH-Gal, 15% PEG ($M_w = 750$), 50% PheOEt, and 30% GlyOEt is given as an example.

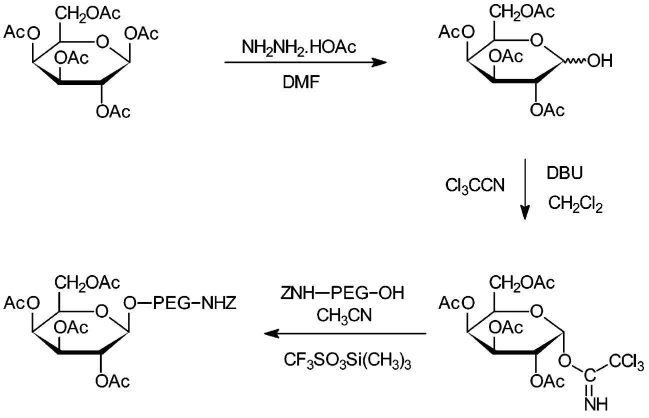
First Part: Synthesis of Polyphosphazenes with 6-Aminohexyl 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranoside, Poly(ethylene glycol), and Amino Acid Ester Side Chains. Z-NH$(CH_2)_6$O-Gal$(OAc)_4$ (0.6 g, 1.03 mmol) was deprotected by hydrogenation in methanol (6 mL) in the presence of Pd/C (0.03 g) as a catalyst. The reaction was followed by TLC. After 7 h the deprotected product was obtained. To a solution of 0.7 g (6.03 mmol) of poly[(dichloro)phosphazene] in 40 mL of dry THF, cooled to 0 °C, was added 6-aminohexyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside (0.27 g, 0.6 mmol) dissolved in 4 mL of dry THF and 0.31 mL (1.8 mmol) of DIPEA. Stirring was continued for 2 h at 0 °C. Then 1.36 g (1.81 mmol) of α-amino-*ω*-methoxypoly(ethylene glycol) dissolved in 20 mL of dry THF and 0.95 mL (5.43 mmol) of DIPEA was added to the poly[(chloro)-*co*-(AH-Gal)phosphazene] solution. The solution was stirred for 5 h at 0 °C and an additional 15 h at room temperature. Then a suspension of dried PheOEt·HCl (2.08 g, 9.05 mmol) in 50 mL of dry THF and 9.45 mL (54.3 mmol) of DIPEA was added to the poly[(chloro)-*co*-(AH-Gal)-*co*-(PEG)phosphazene] solution in a drybox. This mixture was stirred for 5 h at 0 °C and for 2 days at room temperature. Finally, a suspension of 1.68 g (12.07 mmol) of dried GlyOEt·HCl in 30 mL of dry THF and 12.6 mL (72.42 mmol) of DIPEA was added to the poly[(chloro)-*co*-(AH-Gal)-*co*-(PEG)-*co*-(PheOEt)phosphazene] solution in a drybox. This mixture was again stirred for 5 h at 0 °C and for 2 days at room temperature. After removal of the salts by centrifugation, the polymer solution was concentrated under reduced pressure. The polymer was precipitated in dry pentane and reprecipitated from dry THF into pentane (three times). Yield: 65%. ^{1}H NMR ($CDCl_3$): $\delta = 0.85$ ppm (CH_3-, Phe), $\delta = 1.1-1.35$ ppm (CH_3-, Gly, $-NH(CH_2)_2(CH_2)_2(CH_2)_2O$-Gal), $\delta = 1.50$ ppm ($-NHCH_2CH_2-$, Gal), $\delta = 1.60$ ppm ($-CH_2$-CH_2O-Gal), $\delta = 1.98$, 2.04, 2.06, and 2.16 ppm ($4 \times CH_3C(O)-$, Gal), $\delta = 2.5-4.8$ ppm ($-NHCH_2$, Gal, $-NHCH$, Phe, $-CH_2Ph$, Phe, ($-NHCH_2$, Gly, $-CH_2OC(O)$, Phe and Gly), $\delta = 3.47$ and 3.88 ppm ($-CH_2O$-Gal), $\delta = 3.35$ ppm (CH_3O-, PEG), $\delta = 3.60$ ppm ($-OCH_2$-CH_2O-, PEG), $\delta = 3.9$ ppm (H_5, Gal), $\delta = 4.11$ ppm (H_6, Gal), $\delta = 4.18$ ppm ($H_{6'}$, Gal), $\delta = 4.45$ ppm (H_1, Gal), $\delta = 5.01$ ppm (H_3, Gal), $\delta = 5.19$ (H_2, Gal), $\delta = 5.39$ ppm (H_4, Gal), $\delta = 7.0-7.2$ ppm (aromatic protons of Phe).

Second Part: Synthesis of Polyphosphazenes with 6-Aminohexyl β-D-Galactopyranoside, Poly(ethylene glycol), and Amino Acid Ester Side Chains. To a solution of 0.2 g of poly[(6-aminohexyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside)-*co*-(PEG_{750})-*co*-(PheOEt)-*co*-(GlyOEt)phosphazene] in dry MeOH (3 mL) was added 6.2 mg (1 equiv, 0.12 mmol) of NaOMe. The reaction mixture was stirred overnight at room temperature. The reaction was quenched with phosphoric acid. After removal of the solvent, the polymer was redissolved in a minimal amount of dry THF. The polymer was precipitated in dry pentane and reprecipitated from dry THF into pentane (three times). Yield: 92%. ^{1}H NMR ($CDCl_3$): $\delta = 0.85$ ppm (CH_3-, Phe), $\delta = 1.1-1.35$ ppm (CH_3-, Gly, $-NH(CH_2)_2(CH_2)_2(CH_2)_2O$-Gal), $\delta = 1.50$ ppm ($-NHCH_2$-CH_2-, Gal), $\delta = 1.60$ ppm ($-CH_2CH_2O$-Gal), $\delta = 2.5-4.8$ ppm ($-NHCH_2$, Gal, $-NHCH$, Phe, $-CH_2Ph$, Phe, ($-NHCH_2$, Gly, $-CH_2$-$OC(O)-$, Phe and Gly), $\delta = 3.35$ ppm (CH_3O-, PEG), $\delta = 3.5$ ppm (H_2, Gal), $\delta = 3.6-3.65$ ppm ($-OCH_2CH_2O-$, PEG, and H_3, H_5, H_6, Gal), $\delta = 3.7-3.8$ ppm ($H_{6'}$, $-CH_2O$-Gal), $\delta = 3.9$ ppm (H_4, Gal, $-CH_2O$-Gal), $\delta = 4.3-4.4$ ppm (H_1, Gal), $\delta = 7.0-7.2$ ppm (aromatic protons of Phe).

Characterization of the Polymers. ^{1}H NMR spectra were recorded on a 360 or 500 MHz Brucker apparatus. Glass transition and melting temperatures were measured by differential scanning calorimetry in modulated and standard mode (Thermal Analysis Instruments, DSC 2920, modulated DSC instrument). The heating rate was 10 °C/min for the standard mode and 2 °C/min with a modulation of ±0.32 °C every 60 s for the modulated mode. T_g was determined at half-height and T_m at the peak top.

Surface Analysis. *XPS.* Polymer samples were prepared by spin-coating on an aluminium substrate (type I polymers) or a glass slide (type II polymers) from 0.5% (w/v) polymer solutions in $CHCl_3$ or THF, respectively. The samples were washed with hexane to remove poly(dimethylsiloxane) (PDMS). Spectra were obtained using a Vacuum Generators XPS instrument with a CLAM2 analyzer working in constant analyzer energy mode. Samples were run at a base pressure of around 1×10^{-8} mbar or better. The X-ray source was unmonochromated and used a magnesium anode, Mg Kα (1253.6 eV). A wide scan (0−1100 eV) and a narrow scan (region of carbon 1s, 283−302 eV) were

Scheme 1. Synthesis of Z-Protected Amino-PEG with a Galactose End Group



obtained. Spectra were acquired using Spectra 6 software, SCIENTA ESCA 300 analysis.

SIMS. Time-of-flight secondary ion mass spectroscopy experiments were performed using a Cameca TOF-SIMS IV (Cameca Instruments). The primary $^{69}Ga^+$ ion pulsed beam was incident at the sample with an energy of 25 keV. The time-averaged pulsed primary ion current of 0.25 pA was rastered over a sample area of 100 μm × 100 μm. The samples were prepared by spin-coating on a silicon substrate from 1% (w/v) polymer solutions in $CHCl_3$. The samples were washed with hexane to remove PDMS.

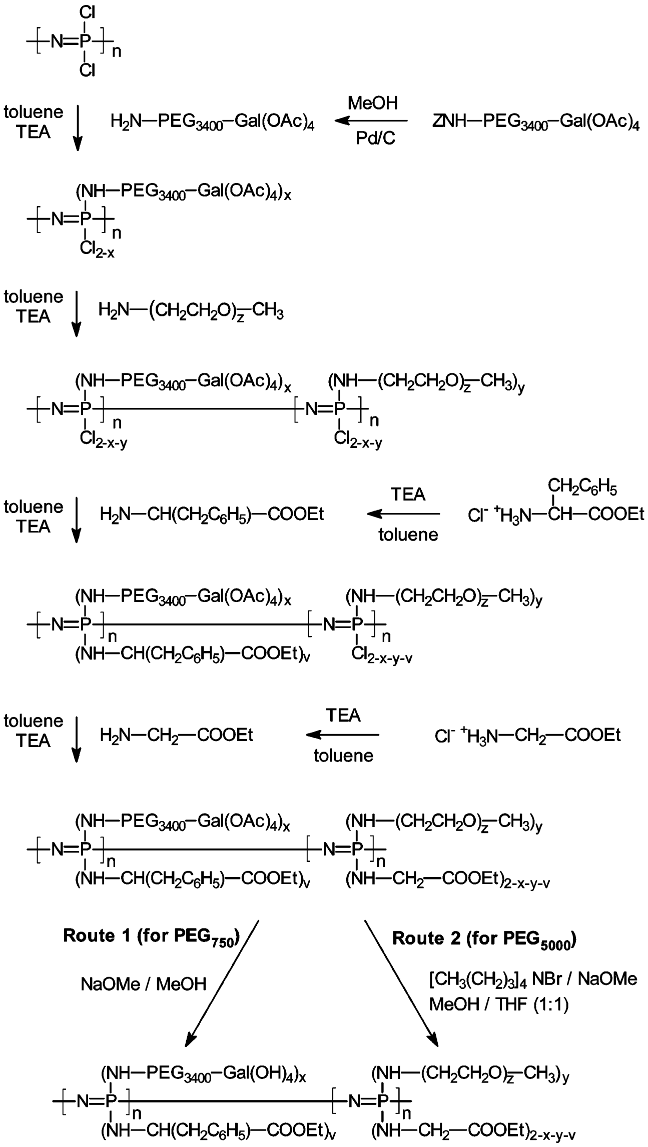
DCA. Advancing and receding contact angles were determined using a Cahn DCA analyzer 322 (Cahn Instruments). Stainless steel substrates were polished, rinsed with acetone and pentane, and three times dip-coated in 2% (w/v) polymer solutions in $CHCl_3$ or THF. The samples were washed with hexane to remove silicon. Three samples of each polymer were analyzed at 20 °C. Doubly distilled water (MilliRo, MilliPore) was used as an immersion liquid. The velocity was 40 μm/s. Two cycles of immersion were performed, the first one from 0 to 15 mm and the second one from 3 to 20 mm.

Results and Discussion

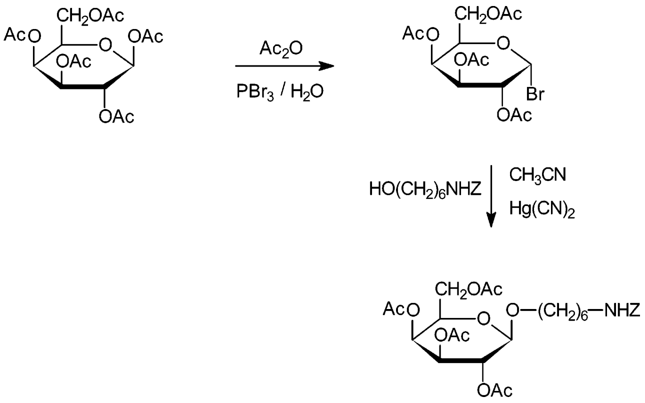
Cellular recognition of specific ligands on polymer surfaces has received much attention in tissue engineering applications. The literature suggests that the introduction of galactose on the polyphosphazenes will lead to the facilitation of hepatocyte adhesion.[9,10] Galactose was linked to the end of a PEG spacer (M_w = 3400). Heterobifunctional PEG (H_2N-PEG-OH) was used, and the reaction is shown in Scheme 1. In a first step the anomeric position of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranoside was selectively deacetylated by reaction with hydrazine acetate in dimethylformamide.[32] Subsequent treatment of the resulting hemiacetal with an excess of trichloroacetonitrile[33] in the presence of DBU[34] as a catalyst for 1 h at room temperature resulted in the formation of the thermodynamically more stable α-trichloroacetimidate. The corresponding β-anomer was not detected by NMR. The chloroacetimidate derivative was then reacted with the Z-protected PEG in the presence of trimethylsilyl triflate and molecular sieves in acetonitrile,[35] which resulted in the formation of the β-derivative. The stereochemistry was confirmed by NMR spectra. Z-protected PEG was obtained by reaction of H_2N-PEG-OH with Z-Cl in dichloromethane.

A number of phosphazene polymers containing PEG-galactose, monomethoxy-PEG, phenylalanine ethyl ester, and glycine ethyl ester side groups were synthesized (type IIC). The general procedure for the preparation of these polymers is given in Scheme 2. They were prepared by reacting amino-terminated PEG 2,3,4,6-tetra-*O*-acetyl-galactopyranoside with poly[(dichloro)phosphazene]. In a second step, PEG (M_w = 750 or 5000) was added, and finally, the remaining chlorine atoms were replaced by PheOEt and GlyOEt. Because of the steric hindrance of the bulky PheOEt groups, it was not possible to achieve 100% substitution; hence, GlyOEt was used.[18] The synthesis of these types of polymers caused unforeseen problems; a part of the polyphosphazene started cross-linking after the addition of PEG-Gal. This was probably due to the impurity of the commercially available H_2N-PEG-OH (97% purity); small amounts of the diamine will cross-link the poly[(dichloro)phosphazene]. The synthesized polyphosphazenes should be non-water-soluble, but since cross-linking occurred, the amount of incorporated PEG was higher than anticipated. This led to the formation of a few water-soluble derivatives. The amounts of the pendent groups were determined by ^1H NMR. After reaction of poly[(dichloro)phosphazene] with PEG-Gal(OAc)$_4$, a sample was taken out of the mixture and reacted with GlyOEt to substitute all the remaining chlorines. The molar ratio of PEG-Gal to Gly was determined from the integration ratio of the methylene protons of PEG (δ = 3.6 ppm, expressed per proton) and the ethylene protons of GlyOEt (δ = 1.1 ppm, expressed per proton). The concentration (mol %) of Phe and Gly was calculated from the NMR of poly[(PEG-Gal)-*co*-(PEG)-*co*-(PheOEt)-*co*-(GlyOEt)-phosphazene] (aromatic protons at δ = 7.0−7.2 ppm and

Scheme 2. Synthesis of Type IIC Polymers



Scheme 3. Synthesis of Z-Protected 6-Aminohexanol with a Galactose End Group



ethylene protons of PheOEt and GlyOEt at $\delta = 0.5-1.35$ ppm). Equation 1 was used to determine the amount of PEG_{750} or

$$y = \frac{(\text{integration of } \delta = 2.5 \text{ to } 5.4 \text{ ppm} - 5\text{H of Phe} - 4\text{H of Gly})}{71} - \frac{316x}{71} \quad (1)$$

PEG_{5000} (for PEG_{5000} replace 71 with 458). Equation 2 expresses the concentration (mol %) of PEG-Gal. It is possible to calculate

$$\frac{x}{\text{integration Phe expressed per proton} + \text{integration Gly expressed per proton} + x + y} = \text{mol \% PEG-Gal} \quad (2)$$

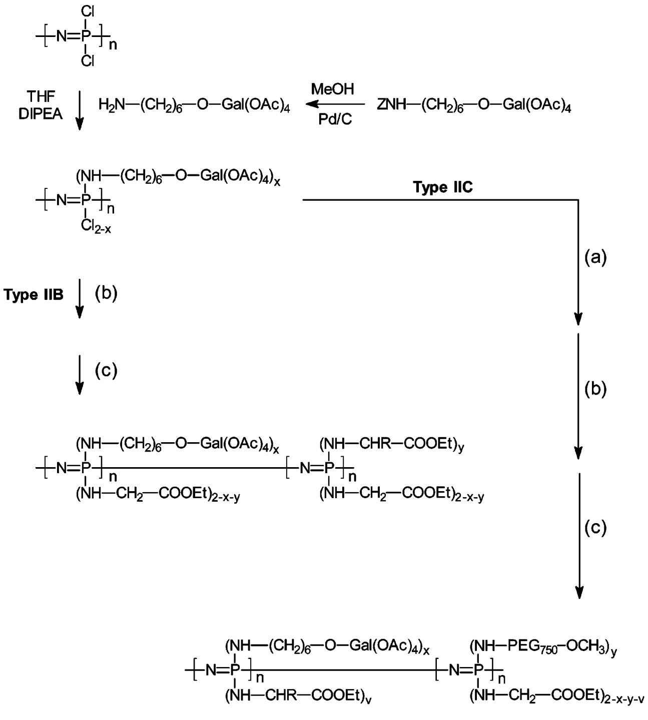
x (integration of PEG-Gal expressed per proton) by substituting y in eq 2 with eq 1 because the concentration (mol %) of PEG-Gal was determined from the NMR of poly[(PEG-Gal)-*co*-(GlyOEt)phosphazene]. y can be calculated by replacing x in eq 1, and hence, the concentration (mol %) of PEG can be determined.

In a final step the acetate esters of polyphosphazenes having PEG-Gal, PEG_{750}, PheOEt, and GlyOEt side groups were hydrolyzed with NaOMe in MeOH (route 1 in Scheme 2). The polymer having PEG-Gal, PEG_{5000}, PheOEt, and GlyOEt side chains however was not soluble in MeOH; consequently, a solution of tetrabutylammonium bromide and NaOMe in MeOH was added to the polymer solution in THF (route 2 in Scheme 2).

The use of AH as a spacer instead of PEG did not cause cross-linking. The synthesis of Z-protected 6-aminohexanol with a galactose end group is shown in Scheme 3. Glycosylation of 6-((benzyloxycarbonyl)amino)hexanol using the Koenigs−Knorr[36] donor 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide and mercury(II) cyanide as a promoter in acetonitrile[37] led to the formation of the 1,2-*trans*-galactoside.[38] This was confirmed by ^{1}H NMR. Previously, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide was made by a selective attack of a bromide nucleophile, which is formed in situ by the reaction of phosphorus tribromide and water, on 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranoside.[39]

Type IIA polyphosphazenes were substituted with AH-Gal and amino acid ester side chains. The synthesis is shown in Scheme 4. Poly[(dichloro)phosphazene] was treated with a selected amount of 6-aminohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside, and then an excess of the hydrophobic PheOEt was added to yield partly substituted copolymers.[18] The remaining chlorine atoms were replaced by GlyOEt units, and in a final step the acetate esters were hydrolyzed by adding tetrabutylammonium bromide and NaOMe in MeOH to a polymer solution in THF. The molar ratio of AH-Gal to Phe to Gly was calculated from the integration ratio of the acetate protons at $\delta = 1.98-2.16$ ppm (expressed per proton), the aromatic protons of Phe at $\delta = 7.0-7.2$ ppm (expressed per proton), and the ethylene protons of PheOEt and GlyOEt at $\delta = 0.5-1.35$ ppm (subtracting 3H of Phe and 4H of AH-Gal and expressed per proton).

Scheme 4. Synthesis of Type IIA and Type IIB Polymers



(a): THF/DIPEA and $H_2N\text{-}PEG_{750}\text{-}OCH_3$

(b): THF/DIPEA and $Cl^{-}\,{}^{+}H_3N\text{-}CHR\text{-}COOEt$ with R: $CH_2C_6H_5$

(c): THF/DIPEA and $Cl^{-}\,{}^{+}H_3N\text{-}CH_2\text{-}COOEt$

For tissue engineering applications it is necessary to balance the desire for active biological interactions with the need to prevent unfavorable passive interactions. This can be achieved by the inclusion of PEG, a known protein-repellent polymer.[40−47] Type IIB polymers contain a certain amount of PEG ($M_w = 750$) next to AH-Gal. The residual chlorine atoms were again replaced by PheOEt and GlyOEt (Scheme 4). The amounts of AH-Gal, Phe, and Gly were determined as described for type IIA polymers. The amount of PEG_{750} was calculated from the integration at $\delta = 2.5-4.8$ ppm (subtracting 5H of Phe, 4H of Gly, and 8H of AH-Gal and expressed per proton). In a final step the acetate esters of the galactose were hydrolyzed with NaOMe in MeOH. Two types of reference polyphosphazenes were made, one having two amino acid ester side groups (PheOEt and GlyOEt) (type IA) (Figure 1) and one containing monomethoxy-PEG ($M_w = 750$ or 5000), PheOEt, and GlyOEt side chains (type IB) (Figure 1). The polymers were synthesized following the procedure of Allcock et al.[19] and Vandorpe et al.,[31] respectively.

All polymers were characterized by means of ^{1}H NMR and DSC (Table 1). Poly[(dichloro)phosphazene] has a T_g of −66 °C; the flexible nature of the P−N backbone is responsible for this low value.[48] Torsion of a P−N bond can bring the nitrogen p orbital into an overlapping position with any of the five 3d orbitals; therefore, the torsional barrier is much smaller than in a $p_\pi-p_\pi$ double bond. The T_g of poly[(organo)phosphazenes] can vary depending on the introduced side chains. Type IA polymers were amorphous, and the T_g corresponded to values

Table 1. Concentrations (mol % and wt %) of the Different Side Groups and Glass Transition and Melting Temperatures of the Prepared Polymers

	^{1}H NMR				DSC			
polym (type)	concn [mol % (wt %)]				T_g (°C)	T_m (°C)	$\Delta H_{m,PP}$ ($\Delta H_{m,PEG}$) (J/g)	deg of crystallization[a] (%)
IA-1			PheOEt 57.3 (70)	GlyOEt 42.7 (30)	13			
IA-2			PheOEt 69.4 (80)	GlyOEt 30.6 (20)	12			
IB-1		PEG_{750} 8.5 (30)	PheOEt 49.6 (47)	GlyOEt 41.8 (23)	−61/−4			
IB-2		PEG_{750} 22.8 (56)	PheOEt 49.4 (33)	GlyOEt 27.8 (11)	−60	19	20 (32)	23
IB-3		PEG_{5000} 1.8 (34)	PheOEt 63.0 (50)	GlyOEt 35.2 (16)		47	36 (106)	61
IIA-1	AH-Gal 2.8 (7)		PheOEt 55.1 (64)	GlyOEt 42.1 (29)	17			
IIA-2	AH-Gal 5.8 (14)		PheOEt 56.7 (62)	GlyOEt 37.6 (24)	24			
IIB-1	AH-Gal 4.3 (7)	PEG_{750} 16.6 (44)	PheOEt 47.5 (35)	GlyOEt 31.6 (14)	−52	24	9 (20)	15
IIC-1	PEG-Gal 0.3 (4)	PEG_{750} 14.9 (41)	PheOEt 51.9 (40)	GlyOEt 32.9 (15)	−60	23	14 (34)	24
IIC-2	PEG-Gal 0.1 (1)	PEG_{5000} 1.5 (29)	PheOEt 67.9 (55)	GlyOEt 30.5 (14)		50	25 (63)	48

[a] The degree of crystallization (%) is calculated from the contribution of $\Delta H_{m,PEG}$: 100% for PEG_{750} is 140 J/g, and 100% for PEG_{5000} is 174 J/g.

Table 2. Comparison of the Experimental and Theoretical Elemental Compositions (atom %)

polym	C 1s, exptl (theor)	O 1s, exptl (theor)	N 1s, exptl (theor)	P 2p, exptl (theor)	Si 2p, exptl (theor)
IA-1	61 (67)	19 (17)	9 (11)	3 (4)	9 (0)
IA-1[a]	68 (67)	16 (17)	12 (11)	4 (4)	0 (0)
IB-3	65 (67)	19 (21)	9 (8)	3 (3)	4 (0)
IIA-1	71 (66)	14 (17)	11 (12)	4 (4)	
IIA-2	67 (66)	16 (18)	13 (12)	4 (4)	
IIB-1	65 (62)	20 (23)	11 (10)	4 (5)	
IIC-1	64 (63)	20 (22)	11 (10)	4 (5)	
IIC-2	63 (64)	19 (20)	13 (10)	5 (5)	
IIC-3WS[b]	65 (65)	21 (27)	10 (6)	4 (3)	

[a] After washing with hexane. [b] Water-soluble polyphosphazene with 0.2 mol % PEG-Gal, 19.8 mol % PEG_{2000}, 51% PheOEt, and 29% GlyOEt.

found in the literature.[49] Large and inflexible groups impose their own restrictions on the flexibility. The T_g increased when additional AH-Gal side groups were introduced, and an even higher value was obtained when the amount of AH-Gal was increased. DSC results revealed two T_g values for IB-1, indicating phase separation of the PEG and amino acid ester domains. This also suggested that PEG, when possible, was able to crystallize. When the polyphosphazene derivatives had a melting point, only one T_g was observed (IB-1 and IIC-1). These polymers crystallized during heating, a phenomena called “cold crystallization”, indicating that PEG_{750} chains had trouble crystallizing. The T_g for IB-3 and IIC-2 could not be determined; this was an indication that these polymers had a high degree of crystallinity. The area under the melt endotherm per unit sample weight is a measure of the degree of crystallinity (Table 1). Normalizing for the PEG content gave values lower than those measured for the free PEG (750, 140 J/g; 5000, 174 J/g). Evidence for crystallization of linear oligo(ethyloxy) side chains on polyphosphazenes was detected earlier in connection with cation transport membranes. Those results suggest that side chain crystallization occurs when five or more ethyleneoxy units are present in the side chains.[50]

XPS spectra of the polyphosphazene derivatives confirmed the presence of phosphorus (2p, 132 eV; 2s, 189.5 eV), carbon (1s, 285 eV), nitrogen (1s, 398 eV) and oxygen (1s, 532.5 eV). Type I polymers were also characterized by the presence of silicon peaks (2p, 100 eV; 2s, 150 eV); this was due to the contamination of the polymers with PDMS. The contaminant could be removed by a hexane wash. The spectra of type IIA polymers showed additional bromine peaks (3d, 67 eV; 3p, 181 eV); these were due to NaBr salts. The starting material, poly[(dichloro)phosphazene], was fully functionalized; no chlorine peaks were visible. The elemental composition of the polymers (Table 2) was, except for the ones containing PDMS, in good agreement with the theoretical values. A narrow scan of C 1s revealed the presence of three carbon group types, 285 eV (C−C (1)), 286.5 eV (C−N/C−O (2)), and 289 eV (C═O (3)). This confirmed the chemical structure of the polymers (Table 3). Carbon peak deconvolution spectra of IIA-1 and IIC-1 are presented in Figure 2. Type IA polymers had a higher experimental value of C−C compared to the theoretical one (Table 3), indicating the presence of more PheOEt than GlyOEt side chains at the air/solid interface. This also explained the lower value of C−O/C−N and C═O, since not all the side groups were present at the surface. Compared to that of the type IA polymer, an increased value of the specific ether carbon groups of PEG confirmed the enrichment of PEG at the surface of type IB polyphosphazenes (Figure 2). The experimental

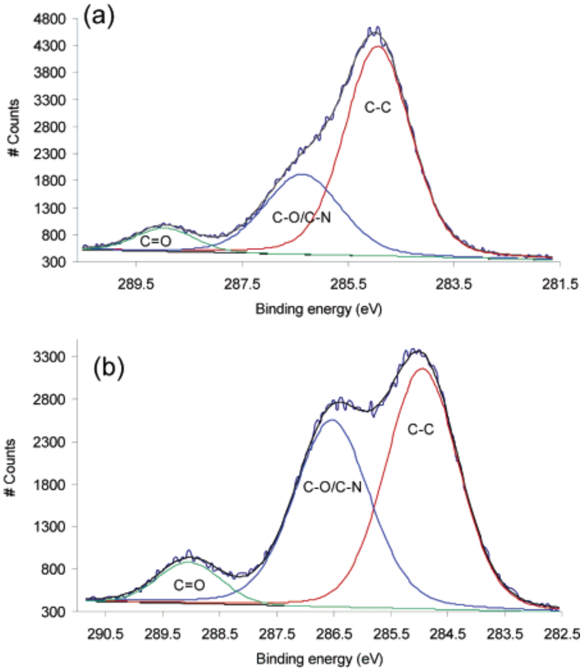
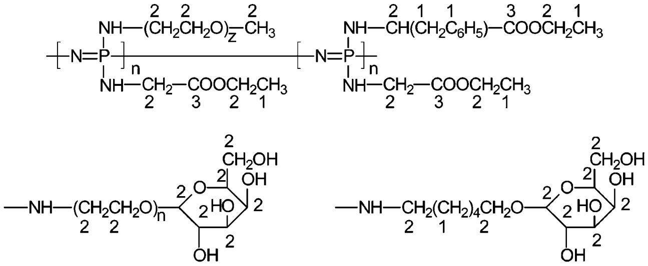


Figure 2. Deconvolution of C (1s) for (a) IIA-1 and (b) IIC-1 (charge corrected to 285.0 eV for C-C).

Table 3. Contribution (%) of the Three Different Carbon Types



polym	C−C (1) (285 eV), exptl (theor)	C−O/C−N (2) (286.5 eV), exptl (theor)	C=O (3) 289 (eV), exptl (theor)
IA-1	72 (63)	21 (25)	7 (12)
IB-3	64 (34)	29 (49)	7 (8)
IIA-1	66 (61)	28 (27)	6 (12)
IIA-2	61 (61)	32 (28)	7 (11)
IIB-1	46 (33)	47 (60)	7 (6)
IIC-1	51 (33)	42 (54)	7 (6)
IIC-2	61 (46)	31 (46)	8 (8)
IIC-3WS	40 (17)	54 (80)	6 (3)

values were lower than expected, indicating that the hydrophilic component was preferentially orientated to the bulk of the polymer. Similar observations were made by other researchers.[51,52] Type IIC polymers gave similar results. The contribution of C−O/C−N (from PEG-Gal and PEG) in the series IIC-2/IIC-1/IIC-3WS was increased from 31% to 42% to 54%; the values were again lower than the theoretical ones. The experimental and theoretical values of type IIA polymers were similar, and there was an increased contribution from C−N/C−O (286.5 eV) compared to that of type IA polymers. As the amount of 6-aminohexyl *β*-D-galactopyranoside increased, the percentage of C−O/C−N increased from 28% to 32%. Type IIB polymers, which had an extra PEG_{750} chain, showed the same results as type IB or type IIC polymers; the value of the ether carbon increased to 47% but was still lower than expected.

The positive and negative ion spectra, generated by SIMS analysis, contained secondary ion fragments from the phosphazene backbone as well as from the side chains. The characteristic ion fragments are listed in Table 4 and were assigned according to the literature data.[53,54] The SIMS technique proved to be especially useful for detecting contamination products at the surface. In some cases silicones were detected (Figure 3). The characteristic masses of 73 ($Si(CH_3)_3^+$) and 147 ($Si_2O(CH_3)_5^+$) were found in the positive spectrum and could be removed by a hexane wash.

The hydrophobic/hydrophilic nature of the polymer surfaces was investigated by DCA. This technique is based on the Wilhelmy plate method;[55] the principles, conditions, and limits of this system have been described.[56] The results are given in Figure 4 and indicate that type IA polymers are very hydrophobic. A quasi thermodynamic hysteresis, due to surface roughness, was observed for these polymers. The receding contact angle was reduced when hydrophilic groups (PEG) were incorporated. A slight decrease in the first advancing contact angle (θ_{A1}) of type IB polymers was observed; in the series IB-1 → IB-2, where the fraction of PEG was enlarged, an increased θ_{A1} was observed (93.5° → 95.9°), although the opposite was expected. The advancing curves of these polymers were characterized by an irregular line (Figure 5); this slip/stick behavior[57−59] was due to the nonuniform movement of a water drop. An energy barrier exists for the liquid front, resulting in sticking of the water, which results in a higher θ. As more liquid is supplied into the drop, the liquid front will possess enough energy to overcome the energy barrier, resulting in slipping, which implies a lower θ value. An additional explanation could be that spreading of a liquid on a solid may cause deformation.[60,61] If the solid is viscoelastic, a fraction of the energy stored in the deformation is dissipated; consequently, the liquid spreading slows. An increased energy dissipation results in an increased advancing contact angle. This phenomenon disappeared in the second cycle, because the contact angle was measured on a wet surface; θ_{A2} decreased (90° → 65°). When these materials were brought into an aqueous environment, the hydrophilic chains were able to reorientate to the surface (kinetic hysteresis). This surface reorganization was induced to minimize the interfacial free energy.[52] The mobility was influenced by the crystallinity of the polymer, because the uptake of the solvent was slowed by the crystal structure. DSC results showed a high degree of crystallinity (61%) for IB-3; no reorientation was observed. IIC-2 had a lower θ_{A1} than IIC-1, because of the more hydrophilic nature of IIC-2. A reduction of θ_{A2} was observed for IIC-1 (65°) and IIC-2 (81.7°); this was attributed to the rearrangement of the PEG chains. IIC-1 contained 41 wt % PEG_{750}, which was between the values for IB-1 (30 wt %) and IB-2 (56 wt %); nevertheless, θ_{A2} had the

Table 4. Characteristic Ion Fragments of the Different Polymers Observed in the SIMS Spectra

polym	positive ion fragments
type IA	$CH_3CH_2^+$ (29), $NH_2^+{=}P^-{=}NH_2^+$ (63), C_6H_5 (77), $C_6H_5CH_2^+$ (91), $NH_3^+CH_2COOCH_2CH_3$ (104), $NH_2^+{=}CHCH_2C_6H_5$ (120), $P{\equiv}N^+CH{=}CHC_6H_5$ (148), $NH_3^+CH(CH_2C_6H_5)COOH$ (166), $NH_3^+CH(CH_2C_6H_5)COOCH_2CH_3$ (194)
type IB	the same ion fragments from the reference + specific ones from PEG: $^+CH_2CH_2OCH_3$ (59), $^+(CH_2CH_2O)_2CH_3$ (103), and $^+(CH_2CH_2O)_3CH_3$ (147)

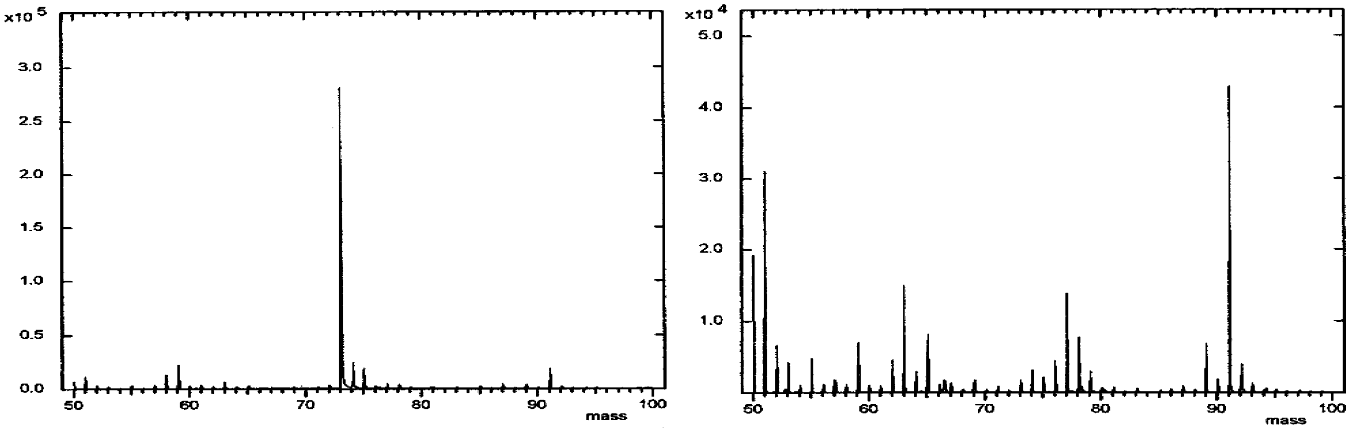


Figure 3. Positive SIMS spectra (mass 50−100) of IB-1 (left) and IB-1 after a hexane wash (right).

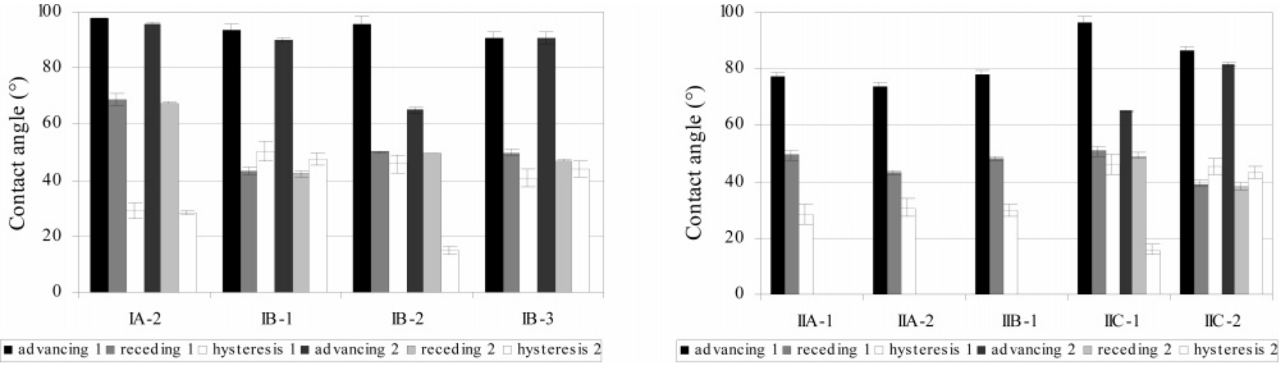


Figure 4. Dynamic contact angle measurements of type I polymers (left) and type II polymers (right).

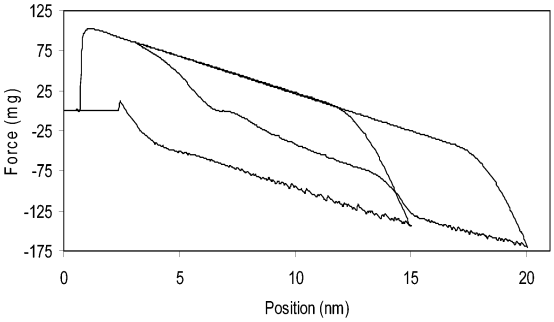


Figure 5. Kinetic hysteresis of IB-2 (slip/stick behavior).

same value as IB-2. IIC-2 contained 30 wt % PEG_{5000}, which was similar to the value for IB-3 (34 wt %), which showed no reorientation. This suggested that the galactose moieties were able to reorientate to the surface, hence lowering the contact angle. The lowest θ_A values were observed for type IIA (77.5° and 73.9°) and type IIB (78.2°) polymers. In this instance the galactose groups were present at the surface from the beginning. This was in good agreement with the XPS results.

Conclusions

In this paper, the synthesis and surface characterization of novel biodegradable poly[(organo)phosphazenes] were described. The cell binding molecule, galactose, was introduced via a spacer, either 6-aminohexanol or poly(ethylene glycol) ($M_w = 3400$). Some polymers have an additional PEG side chain of different molecular weights ($M_w = 750$ or 5000). The surface composition was studied using XPS and SIMS. The hydrophobic/hydrophilic nature was determined by DCA. XPS and SIMS analysis confirmed the polymer composition. It was demonstrated by XPS that the hydrophilic component, PEG, was preferentially orientated to the bulk of the polymer, which resulted in lower C−N/C−O values. DCA studies showed that the galactose moieties of type IIC were able to reorientate to the surface, hence lowering the contact angle. The galactose moieties from type IIA and IIB polymers were present at the surface, which was confirmed by XPS and DCA.

Hepatocyte adhesion on type II polymers should be promoted, since the galactose moieties were exposed on the surface of these polyphosphazenes. This has been evaluated and will be reported in a subsequent paper.

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References and Notes

(1) Langer, R.; Vacanti, J. P. *Science* **1993**, *260* (5110), 920−926.
(2) Bonassar, L. J.; Vacanti, C. A. *J. Cell. Biochem. Suppl.* **1998**, *30/31*, 297−303.
(3) Ruano-Ravina, A.; Diaz, M. *J. Osteoarthritis Cartilage* **2006**, *14* (1), 47−51.
(4) Vacanti, J. P.; Langer, R. *Lancet* **1999**, *354* (Suppl. 1), SI32−SI34.
(5) Kim, B. S.; Mooney, D. J. *Trends Biotechnol.* **1998**, *16* (15), 224−230.
(6) Lee, C.-T.; Huang, C.-P.; Lee, Y.-D. *Biomacromolecules* **2006**, *7* (*7*), 2200−2209.
(7) Pierschbacher, M. D.; Ruoslahti, E. *Nature* **1984**, *309*, 30−33.
(8) Novikoff, P. M.; Cammer, M.; Tao, L.; Oda, H.; Stockert, R. J.; Wolkoff, A. W.; Satir, P. *J. Cell Sci.* **1996**, *109*, 21−32.

(9) Griffith, L. G.; Lopina, S. *Biomaterials* **1998**, *19* (11−12), 979−986.
(10) Yoon, J. J.; Nam, Y. S.; Kim, J. H.; Park, T. G. *Biotechnol. Bioeng.* **2002**, *78* (1), 1−10.
(11) Cohen, S.; Baño, M. C.; Cima, L. G.; Allcock, H. R.; Vacanti, J. P.; Vacanti, C. A.; Langer, R. *Clin. Mater.* **1993**, *13* (1−4.), 3−10.
(12) Laurencin, C. T.; Norman, M. E.; Elgendy, H. M.; El-Amin, S. F.; Allcock, H. R.; Pucher, S. R.; Ambrosio, A. A. *J. Biomed. Mater. Res.* **1993**, *27* (7), 963−973.
(13) Langone, F.; Lora, S.; Veronese, F. M.; Caliceti, P.; Parnigotto, P. P.; Valenti, F.; Palma, G. *Biomaterials* **1995**, *16* (5), 347−353.
(14) Aldini, N. N.; Fini, M.; Rocca, M.; Martini, L.; Giardino, R.; Caliceti, P.; Veronese, F. M.; Lora, S.; Maltarello, M. C. *J. Bioact. Compat. Polym.* **1997**, *12* (1), 3−13.
(15) Aldini, N. N.; Fini, M.; Rocca, M.; Giavaresi, G.; Giardino, R. *Int. Orhop.* **2000**, *24* (3), 121−125.
(16) Veronese, F. M.; Marsilio, F.; Lora, S.; Caliceti, P.; Passi, P.; Orsolini, P. *Biomaterials* **1999**, *20* (1), 91−98.
(17) Passi, P.; Marsilio, Z. A.; Lora, S.; Caliceti, P.; Veronese, F. M. *J. Mater. Sci.: Mater. Med.* **2000**, *11* (10), 643−654.
(18) Allcock, H. R.; Fuller, T. J.; Mack., P.; Matsumura, K.; Smeltz, K. M. *Macromolecules* **1977**, *10* (4), 824−830.
(19) Allcock, H. R.; Pucher, S. R.; Scopelianos, A. G. *Biomaterials* **1994**, *15* (8), 563−569.
(20) Crommen, J.; Vandorpe, J.; Schacht, E. *J. Controlled Release* **1993**, *24* (1−3), 167−180.
(21) Ruiz, E. M.; Ramirez, C. A.; Aponte, M. A.; Barbosa-Canovas, G. V. *Biomaterials* **1993**, *14* (7), 491−496.
(22) Conconi, M. T.; Lora, S.; Menti, A. M.; Carampin, P.; Parnigotto, P. P. *Tissue Eng.* **2006**, *12* (4), 811−819.
(23) Heyde, M.; Schacht, E. In *Phosphazenes: A Worldwide Insight*; Gleria, M., De Jaeger, R., Eds.; NOVA Science Publishers: New York, 2004; Chapter 15, pp 367−398.
(24) Allcock, H. R.; Kugel, R. L. *J. Am. Chem. Soc.* **1965**, *87* (18), 4216−4217.
(25) Ambrosio, A. M. A.; Allcock, H. R.; Katti, D. S.; Laurencin, C. T. *Biomaterials* **2002**, *23* (7), 1667−1672.
(26) Ambrosio, A. M. A.; Sahota, J. S.; Runge, C.; Kurtz, S. M.; Lakshmi, S.; Allcock, H. R.; Laurencin, C. T. *IEEE Eng. Med. Biol.* **2003**, *22* (5), 18−26.
(27) Mark, J. E.; Allcock, H. R.; West, R. *Inorganic Polymers*; Prentice Hall: Upper Saddle River, NJ, 1992; Chapter 3.
(28) D'Halluin, G.; De Jaeger, R.; Chambrette, J. P.; Potin, Ph. *Macromolecules* **1992**, *25* (4), 1254−1258.
(29) Lemmouchi, Y.; Schacht, E.; Dejardin, S. *J. Bioact. Compat. Polym.* **1998**, *13* (1), 4−18.
(30) Loccufier, J.; Crommen, J.; Vandorpe, J.; Schacht, E. *Makromol. Chem., Rapid Commun.* **1991**, *12* (3), 159−165.
(31) Vandorpe, J.; Schacht, E. *Polymer* **1996**, *37* (14), 3141−3145.
(32) Excoffier, G.; Gagnaire, D.; Utille, J. P. *Carbohydr. Res.* **1975**, *39* (2), 368−373.
(33) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25* (3), 212−235.
(34) Numata, M.; Sugimoto, M.; Koike, K.; Ogawa, T. *Carbohydr. Res.* **1987**, *163* (2), 209−225.
(35) Lindhorst, T. K.; Kötter, S.; Kubisch, J.; Krallmann-Wenzel, U.; Ehlers, S.; Kren, V. *Eur. J. Org. Chem.* **1998**, *8*, 1669−1674.
(36) Koenigs, W.; Knorr, E. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 957−981.
(37) Helferich, B.; Zirner, J. *Chem. Ber.* **1962**, *95* (11), 2604.
(38) Bochkov, A. F.; Zaikov, G. E. *Chemistry of the O-glycosidic Bond: Formation and Cleavage*; Pergamon Press: New York, 1979; p 17.
(39) Kempen, H. J. M.; Hoes, C.; Vanboom, J. H.; Spanjer, H. H.; Delange, J.; Langendoen, A.; Van Berkel, T. J. C. *J. Med. Chem.* **1984**, *27* (10), 1306−1312.
(40) Lea, A. S.; Andrade, J. D.; Hlady, V. *Colloids Surf., A* **1994**, *94*, 349−357.
(41) Sharma, S.; Johnson, R. W.; Desai, T. A. *Langmuir* **2004**, *20* (2), 348−356.
(42) Jon, S. Y.; Seong, J. H.; Khademhosseini, A.; Tran, T. N. T.; Laibinis, P. E.; Langer, R. *Langmuir* **2003**, *19* (24), 9989−9993.
(43) Sharma, S.; Popat, K. C.; Desai, T. A. *Langmuir* **2003**, *18* (23), 8728−8731.
(44) Black, F. E.; Hartshorne, M.; Davies, M. C.; Roberts, C. J.; Tendler, S. J. B.; Williams, P. M.; Shakesheff, K. M. *Langmuir* **1999**, *15* (9), 3157−3161.
(45) Vansteenkiste, S. O.; Corneillie, S. I.; Schacht, E. H.; Chen, X.; Davies, M. C.; Moens, M.; Van Vaeck, L. *Langmuir* **2000**, *16* (7), 3330−3336.
(46) Snellings, G.; Vansteenkiste, S.; Corneillie, S.; Davies, M. C.; Schacht, E. H. *Adv. Mater.* **2000**, *12* (24), 1959−1962.
(47) Snellings, G.; Vansteenkiste, S.; Schacht, E. *Polym. Med. Surg.—PIMS* **2000**, 3−12.
(48) Allcock, H. R. *Chemistry and Applications of Polyphosphazenes*; Wiley: New York, 2003; p 744.
(49) Goedemoed, J. H.; De, Groot, K. *Makromol. Chem. Symp.* **1988**, *19*, 341−365.
(50) Allcock, H. R.; O'Connor, S. J. M.; Olmeijer, D. L.; Napierala, M. E.; Cameron, C. G. *Macromolecules* **1996**, *29*, 7544−7552.
(51) Shard, A. G.; Davies, M. C.; Li, Y. X.; Vollard, C.; Kissel, T. *Macromolecules* **1997**, *30* (10), 3051−3057.
(52) Takahara, A.; Takahashi, K.; Kajiyama, T. *J. Biomater. Sci., Polym. Ed.* **1993**, *5* (3), 183−196.
(53) Groenewold, G. S.; Cowan, R. L.; Ingram, J. C.; Appelhans, A. D.; Delmore, J. E.; Olsen, J. E. *Surf. Interface Anal.* **1996**, *24* (12), 794−802.
(54) Shard, A. G.; Davies, M. C.; Schacht, E. *Surf. Interface Anal.* **1996**, *24* (12), 787−793.
(55) Johnson, R. E.; Dettre, R. H. In *Surface Colloid Science*; Matijevic, E., Ed.; Wiley: New York, 1969; Vol. 2, p 85.
(56) Andrade, J. D.; Smith, L. M.; Gregonis, D. E. In *Surface and Interfacial Aspects of Biomedical Polymers vol 1 Surface Chemistry and Physics*; Andrade, J. D., Ed.; Plenum Press: New York; 1985; p 249.
(57) Kwok, D. Y.; Lam, C. N. C.; Li, A.; Neung, A.; Neumann, A. W. *Langmuir* **1998**, *14* (8), 2221−2224.
(58) Kwok, D. Y.; Neumann, A. W. *Adv. Colloid Interface Sci.* **1999**, *81*, 167−249.
(59) Kwok, D. Y.; Gietzelt, T.; Grundke, K.; Jacobasch, H. J.; Neumann, A. W. *Langmuir* **1997**, *13*, 2880−2894.
(60) Tomasetti, E.; Rouxhet, P. G.; Legras, R. *Langmuir* **1998**, *14* (12), 3435−3439.
(61) Shanahan, M. E. R.; Carré, A. *Langmuir* **1994**, *10*, 1647−1649.

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