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DESIGN OF DEGRADABLE POLYPHOSPHOESTER NETWORKS WITH TAILOR-MADE STIFFNESS AND HYDROPHILICITY AS SCAFFOLDS FOR TISSUE ENGINEERING

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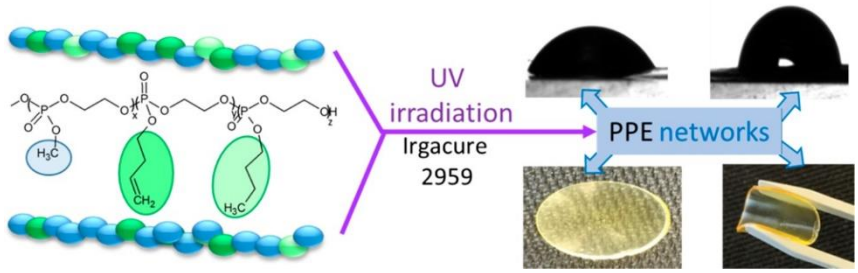
* *Supporting Information*



ABSTRACT

In the recent decades, biodegradable and biocompatible polyphosphoesters (PPEs) have gained wide attention in the biomedical field as relevant substitutes for conventional aliphatic polyesters. These amorphous materials of low glass transition temperature offer promise for the design of soft scaffolds for tissue engineering. Advantageously, the easy variation of the nature of the lateral pendant groups of PPEs allows the insertion of pendent unsaturations valuable for their further crosslinking. In addition, varying the length of the pendent alkyl chains allows tuning their hydrophilicity. The present work aims at synthesizing PPE networks of well-defined hydrophilicity and mechanical properties. More precisely, we aimed at preparing degradable materials exhibiting identical hydrophilicity but different mechanical properties and vice versa. For that purpose, PPE copolymers were synthesized by ring-opening copolymerization of cyclic phosphate monomers bearing different pendent groups (e.g., methyl, butenyl, and butyl). After UV irradiation, a stable and well-defined cross-linked material is obtained with the mechanical property of the corresponding polymer films controlled by the composition of the starting PPE copolymer. The results demonstrate that cross-linking density could be correlated with the mechanical properties, swelling behavior, and degradation rate of the polymers network. The polymers were compatible to human skin fibroblast cells and did not exhibit significant cytotoxicity up to 0.5 mg mL^{-1}. In addition degradation

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products appeared nontoxic to skin fibroblast cells and showed their potential as promising scaffolds for tissue engineering.

Introduction

The rapid development of regenerative medicine triggers the need for more and more demanding tissue scaffolds for the formation of dedicated and viable tissue. Making available degradable and soft polymer materials[1] mimicking soft tissues exhibiting well-defined mechanical properties such as hydrophilicity[2] fitting the modern prerequisite for tissue engineering still remains challenging. Varying only one parameter of a scaffold such as stiffness while keeping identical all the other ones including hydrophilicity is not straightforward but would allow better understanding of their impact on stem cell growth and differentiation. In that framework, tailor-made scaffolds with precisely adjustable chemical and physical properties are highly desirable. Among them, biodegradable scaffolds are especially of interest when an in vivo implantation is foreseen. Most of the synthetic polymers that are used in biomedical applications for their biodegradability are synthetic aliphatic polyesters, i.e., poly(ε-caprolactone) (PCL), poly(lactide) (PLA), poly(glycolide) (PGA), and their copolymers.[3] They have been thoroughly investigated for biomedical applications as surgical sutures, drug delivery carrier, and tissue engineering scaffolds. However, adjusting their physicochemical properties, such as increasing their hydrophilicity, remains limited by their quite tedious chemical functionalization.[4]

Polyphosphoesters (PPEs) presenting phosphoester linkages in the backbone are also biodegradable and have emerged as a promising class of novel biomaterials.[5,6] In contrast to polyesters, they are generally amorphous and exhibit a low glass transition temperature. The pentavalency of the phosphorus atom offers a large diversity of structures and therefore a wide range of properties for these materials. PPEs can be prepared by different synthetic routes such as ringopening polymerization (ROP),7 polycondensation, transesterification, and enzymatic polymerization. Tuning of their chemical structure is easily achieved by simple modification of pendent groups. This task can be achieved by the implementation of the ROP of cyclic phosphoesters prepared by the esterification reaction of 2-chloro-1,3,2-dioxaphospholane 2-oxide (COP) with the appropriate alcohol (Scheme 1). This strategy was already followed in order to adapt the hydrophobicity of the polyphosphoester block of amphiphilic copolymers by increasing the length of the alkyl side-chain.8,9 Besides, introducing an unsaturation as a side-chain allows the efficient photo-cross-linking of the PPEs as reported by Yilmaz et al.10 Considering this distinctive characteristic, PPEs have gain wide attention in the field of biomedical applications including drug delivery, tissue engineering, and many more.11 Recently, PPEs have been combined with polyethylene glycol (PEG),12 polysaccharide,13 or catechol14 in order to produce degradable hydrogels. In this context, we aim at taking profit of the attractive properties of PPEs to prepare degradable and soft scaffolds of well-defined stiffness and hydrophilicity exclusively based on PPE. The ultimate goal of this research was to develop a set of degradable materials varying their hydrophilicity and keeping unchanged their stiffness and vice versa (Scheme 2).

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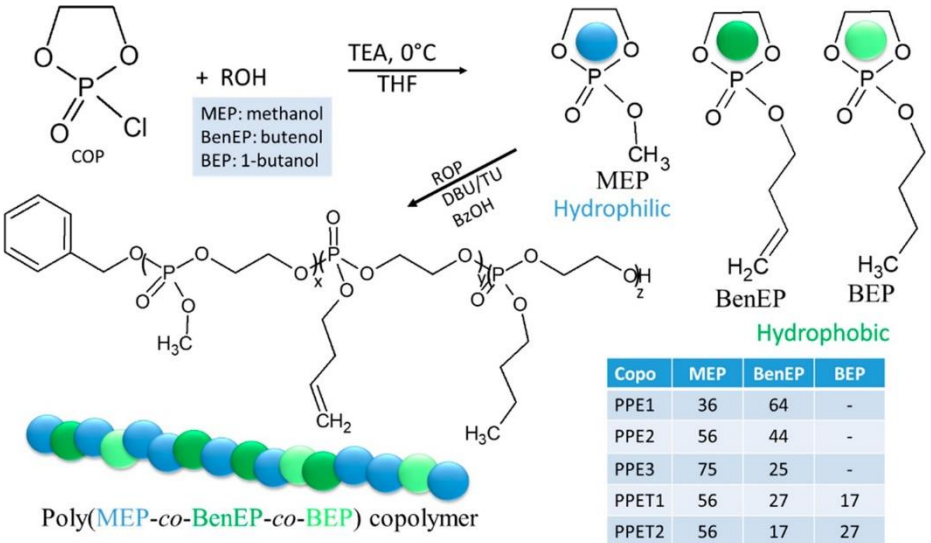
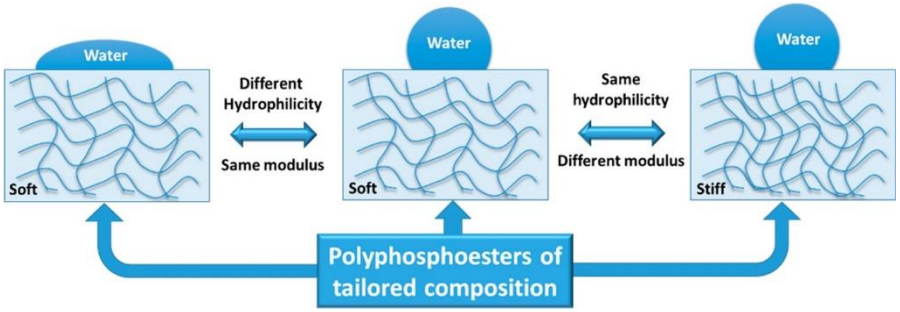
Scheme 1. *Synthesis Path of the Random Polyphosphoester Copolymers and Targeted Compositions*



Copo	MEP	BenEP	BEP
PPE1	36	64	-
PPE2	56	44	-
PPE3	75	25	-
PPET1	56	27	17
PPET2	56	17	27

Scheme 2. *Targeted Degradable Soft Scaffolds of Well-Defined Hydrophilicity and Stiffness Obtained by Cross-Linking of Polyphosphoester Copolymers of Various Compositions*



For that purpose, PPE copolymers were synthesized by organocatalyzed ROP from mixtures of 2 or 3 comonomers, i.e., methyl-phosphoester to impart hydrophilicity, butenylphosphoester to provide cross-linking groups, and butylphosphoester to adjust hydrophobicity. The organocatalyzed homopolymerization of these monomers has already been described.[7,8,10] Therefore, similar conditions have been applied for the copolymer synthesis. These copolymers were then photo-cross-linked to form the soft matrix. Cross-linking efficiency of the PPEs was investigated for the various compositions by the swelling experiments. The stiffness of these networks was determined by measuring the Young modulus in a tensile test performed in the dry and water swollen state. Their hydrophilicity was characterized by measuring the water contact angle on the surface. In vitro hydrolytic degradation was performed in phosphate-buffered saline (PBS) for cross-linked films and in Milli-Q water for polymers, respectively, and assessed by SEC. In vitro cytotoxicity of polymers was also investigated.

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Experimental section

MATERIALS.

2-Chloro-3-oxo-1,3,2-dioxaphospholane (COP, Acros), 2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure2959, Aldrich), and calcium hydride (CaH_2, Aldrich) were used as received. Tetrahydrofuran (THF, VWR), toluene (VWR), dichloromethane (CH_2Cl_2, VWR), 3-buten-1-ol (Aldrich), 1-butanol (Aldrich), benzyl alcohol (Aldrich), triethylamine (TEA, Aldrich), methanol (MeOH, Acros), and 1,8 diazobicyclo[5.4.0]undec-7-ene (DBU, Aldrich) were dried over calcium hydride at room temperature, followed by distillation under reduced pressure just before use and stored on molecular sieves 3 and 4 Å, respectively. TU (thiourea) was synthesized according to a described method[15] and dried overnight under vacuum before use. Ultrapure water (18 MΩ cm) was acquired by means of a Milli-Q water filtration system, Millipore Corp. (St. Charles, MO). PBS buffer (pH 7.4) was prepared according to the literature. Cyclic phosphate monomers, i.e., MEP, BenEP, and BEP, were obtained by already described methods.[7] The followed protocol is detailed in the Supporting Information.

COPOLYMER SYNTHESIS AND CHARACTERIZATION.

The poly(MEPco-BenEP) bipolymers and poly(MEP-co-BenEP-co-BEP) terpolymers were synthesized by ring-opening copolymerization of a mixture of MEP/BenEP monomers and MEP/BenEP/BEP, respectively. The synthesis conditions were adapted from the publication of Clement et al.[7] Typically, the polymerization of the monomer mixture was initiated by benzylic alcohol in CH_2Cl_2 in the presence of a DBU/TU organocatalytic system at 0 °C. After 2 h, a conversion of 95% is determined by ^{31}P NMR. The detailed protocol is described in the Supporting Information, and the applied conditions to obtain the five copolymers used in this study are summarized in Table 1.

^{1}H NMR spectra (Figure S1, Supporting Information) and ^{31}P NMR spectra (Figure S2, Supporting Information) were used to determine the average molar mass (M_n) and the composition of the final copolyphosphoesters and SEC (Figure S3, Supporting Information) to determine the dispersity. These data are summarized in Table 1.

All of these polyphosphoester copolymers are amorphous with a low $T_g \sim -50$ °C (determined by DSC) and present an oily/waxy aspect at room temperature.

UV PHOTO-CROSS-LINKING.

A copolymer solution (50 wt % in CH_2Cl_2) containing 5 wt % of I2959 photoinitiator was transferred in a Teflon mold (11 mm diameter and 0.5 mm of thickness) in order to obtain round shaped films. The solvent was then slowly evaporated under vacuum. The polymer film was then exposed under UV irradiation (Omni Cure Series 2000, 200 W, 365 nm) for 1 h at room temperature. The cross-linked film was then collected.

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CHARACTERIZATION.

Structural Characterization.

FTIR measurements were carried out on a Nicolet IS5 spectrometer (Thermo Fisher Scientific) equipped with a diamond attenuated transmission reflectance (ATR) device; 32 scans were recorded for each sample over the range 4000−500 cm^{-1} for the polymer with the different monomer feed with and without UV irradiation.

RHEOLOGICAL MEASUREMENTS.

The cross-linked films were characterized by shear rheology in which the storage modulus (G′) and loss modulus (G″) were examined to reflect the cross-linking strength of the polymers.[16,17] The copolymers were mixed with 5 wt % of I2959 photoinitiator and then tested on an ARES G2 rotational rheometer (TA Instruments) with the parallel plate geometry with and without UV irradiation, at a frequency of 1 Hz and strain of 1%. The dynamic oscillatory frequency sweep test was applied to the cross-linked 3D network with the parallel plate geometry. Then, after the viscoelastic regime was determined by the oscillatory strain sweep test at a frequency of 1 Hz, the storage modulus (G′) and loss modulus (G″) of cross-linked films were characterized as a function of frequency at 1% strain set based on elastic region.

Gel Content and Swelling of the Networks.

The network formation was characterized by the measurement of the swelling ratio and gel content in $CHCl_3$. After irradiation, the samples were weighted (W_i) and then left to swell in chloroform until reaching a constant weight (W_s). Then, the samples were dried by evaporating the $CHCl_3$ (W_d). Gel content and swelling were then calculated according eqs 1 and 2, respectively.

$$\text{gel content (\%)} = \frac{W_d}{W_i} \times 100 \quad (1)$$

$$\text{swelling (\%)} = \frac{W_s - W_d}{W_d} \times 100 \quad (2)$$

Water Swelling.

For the study of swelling behavior (water content), the cross-linked films were further dried in an air circulating oven at 50 °C for 24 h until the sample reached a constant weight and was weighed as W_d. The polymer films were then immersed in Milli-Q water. The swollen polymer network was taken out at regular time intervals. After wiping off the water on the surfaces with moist filter papers, the cross-linked films were weighed and recorded as W_s. The swelling ratio (EWC) was then defined according eq 2.

WATER CONTACT ANGLE MEASUREMENT.

The wettability of the PPE-based polymer films was measured with an OCA-20 apparatus (Data Physics Instrument GmbH) in the sessile drop configuration by deposition of a 5 μL droplet of Milli-

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Q water. Each sample was measured before and after 3 min. All the experiments were performed at room temperature.

TENSILE PROPERTIES.

Rectangular shaped polymer films were prepared using Teflon molds with the following dimensions: length of 25 mm, width of 5 mm, and thickness of 0.5 mm. Tensile properties were determined using an Instron 5594 tensile machine at a speed of 10 mm min^{-1} with a load capacity of 500 N. The E-modulus, tensile strength (stress), and elongation at break (strain) were estimated by the average values of 3 repeated samples. The Young modulus (Elastic modulus) of the individual samples was calculated according to eq 3.

$$\text{Young's modulus} = \frac{\text{stress}}{\text{strain}} \tag{3}$$

IN VITRO HYDROLYTIC DEGRADATION.

Degradability of the copolymers and networks was examined at 37 °C in Milli-Q water and PBS buffer (pH = 7.4), respectively. The concentration of polymers was set at 10 mg mL^{-1} and incubated in the Milli-Q water with continuous stirring (50 rpm). At a selected time interval, a sample of the solution was taken out and freeze-dried for SEC analysis. For the cross-linked films, the samples were immersed in water or PBS buffer at 37 °C with the continuous stirring (50 rpm). At regular periods of time, the samples were taken out and rinsed with deionized water, lyophilized, and weighted as W_f. PBS solutions were refreshed every 5 days, and three independent measurements were averaged for each sample. The percentage weight loss was calculated according to eq 4.

$$\text{weight loss (\%)} = \frac{W_d - W_f}{W_d} \times 100 \tag{4}$$

CELL CULTURE AND CYTOTOXICITY TEST.

The cytotoxicity of the polymer was evaluated in comparison to polyethylene glycol with a concentration of 1 mg mL^{-1} in Milli-Q water. Polymer solution was sterilized and filtered with the help of a Millipore filter unit with a pore size of 0.22 μm and PES (polyethersulfone) membrane. Human skin fibroblasts cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Lonza) supplemented with 5% fetal bovine serum (FBS, Gibco), 1% nonessential amino acids (NEAA, Gibco), 1% penicillin and streptomycin (Lonza), and 0.2% amphotericin B (Gibco) and were incubated in 5% CO_2 at 37 °C.

The cytotoxicity of the polymer solutions was evaluated on cultured fibroblasts. The cells were seeded in 96-well culture plates (Falcon) at a fixed number of 7500 cells per well. After 24 h, the medium was replaced by the culture medium with the polymer solution at concentrations ranging from 0 to 1 mg mL^{-1}. After an exposure time of 48 h at 37 °C, the WST-1 assay was performed following the manufacturer's instructions (Roche). The absorbance was recorded in a multiwell microplate (FilterMAX F5, Molecular Devices) at 450 and 620 nm. The amount of formazan dye formed directly

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correlates to the mitochondrial activity of the cells. The viability is expressed as the percentage of the negative control.

Table 1. *Applied Conditions for the Synthesis of the Used Polyphosphoester Copolymers and Their Macromolecular Characteristics*

sample	catalyst DBU/TU (mmol)	$[MEP]_0/[BenEP]_0/[BEP]_0$[a] (mol %)	time[b] (min)	M_n NMR[c] (g mol^{-1})	$[MEP]_{exp}/[BenEP]_{exp}/[BEP]_{exp}$[d] (mol %)	Đ[e] (SEC)
PPE1	0.8/0.4	36/64/0	120	11 400	37/63/0	1.2
PPE2	0.8/0.4	56/44/0	120	21 000	60/40/0	1.2
PPE3	0.8/0.4	75/25/0	120	26 000	74/26/0	1.3
PPET1	0.8/0.4	56/27/17	120	41 000	56/26/18	1.3
PPET2	0.8/0.4	56/17/27	120	52 000	60/16/24	1.2

[a]Monomer ratio in the comonomer feed. [b]Polymerization time at 0 °C. [c]Average molar mass of the polyphosphoester copolymer determined by 1H NMR as follows: *M*n = DP_{BenEP} 178 + DP_{MEP} 138 + DP_{BEP} 180. [d]Composition of the PPE copolymers determined by ^{1}H NMR. [e]Molecular weight distribution (*M*w/*M*n) determined by SEC.

Results and discussion

NETWORKS FORMATION BY UV-LIGHT IRRADIATION.

Polymer networks were formed by UV irradiation of the different copolymers (PPE1 to PPET2 in Table 1) bearing unsaturations from the BenEP comonomer available for cross-linking. These copolymers are obtained by already described protocols (see the Supporting Information), and their composition is reported in Table 1. As determined by ^{31}P NMR, after 2 h of copolymerization of the comonomer mixtures, the conversion is above 95%. Therefore, the copolymer composition is close to that of the starting comonomer mixture. Recent data from the literature[18] report that MEP favors transesterification reactions during the polymerization. The occurrence of these transesterification reactions was also made evident here by the broadening of the SEC traces for polymerization time above 30 min (Figure S3a). Since networks are targeted here, the investigation of the copolymer microstructure was not carried out further by determining the reactivity ratios. The average composition of the five copolymers was precisely determined by ^{1}H NMR (Figure S1) and ^{31}P NMR (Figure S2) and the dispersity by SEC (Figure S3, Supporting Information). Before cross-linking, the copolymers are purified by dialysis in MeOH in order to remove DBU/TU catalyst residues, known to be toxic.[19] When biomedical applications are targeted, I2959 (5 wt %) is generally selected as the photoinitiator for the crosslinking of polymers because of its limited toxicity.[20] The intensity of the UV light was between 3 and 5 mW cm^{-2}, which is enough to form a polymer network. Indeed, upon irradiation, the strength of the obtained polymer network increased while increasing the UV irradiation time. After 1 h, the complete disappearance of the polymer unsaturation was observed by IR spectroscopy as shown in Figure 1.

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After UV irradiation, the absorption band at 1640 cm^{-1}, corresponding to the double bond of the BenEP unit pendant group, completely vanished due to the efficient coupling reaction induced by the UV treatment of the polyphosphoester copolymer, confirming the cross-linking.

In order to determine the required time to reach a high cross-linking level, the measurement of the swelling ratio in chloroform vs irradiation time was performed (Figure 2).

Figure 1. *ATR-FTIR spectra of poly(MEP-co-BenEP) before and after UV irradiation.*

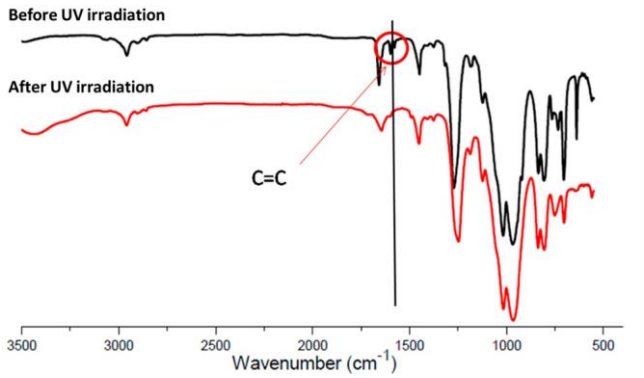
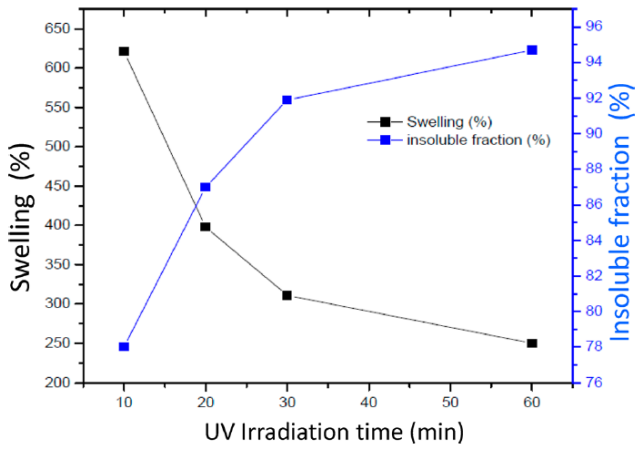


Figure 2. *Swelling ratio and insoluble fraction in chloroform of poly(MEP-co-BenEP) for increasing UV irradiation time.*



As expected, the swelling ratio decreased with irradiation time, whereas the insoluble fraction increased. These results confirmed that a UV irradiation time of a minimum of 60 min is required to limit the insoluble fraction and reach optimal cross-linked density. An irradiation time of 60 min was then selected and applied for the cross-linking of all the copolymers used in this work.

Cross-linking occurring upon UV irradiation was followed by an oscillatory shear rheometer. As shown in Figure 3, UV light has been shined on the sample in the rheometer through a UV-light transparent plateau. Then, the storage modulus (G') and loss modulus (G") were measured during irradiation. As expected for a network formation, the G' crosses G" at the socalled "gel point" traducing the formation of the network. The starting viscous copolymers become an elastic network

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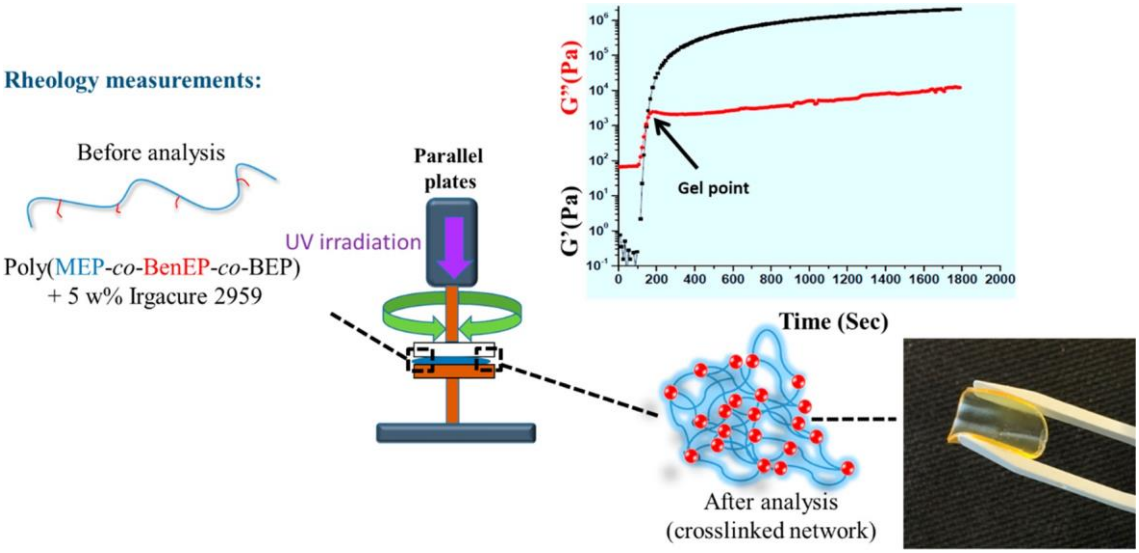
upon photoinduced cross-linking. In the rheometer, the gel point appeared between 100 and 500 s depending on the copolymer (Figure 3 and Figure S4, Supporting Information). These times are quite different from the 1 h determined by the swelling experiments which is due to the different configuration of irradiation in the rheometer (small amount of material, focused light, reflection of the light, ...). If the gel point time is thus not indicative, this experiment clearly proves the network formation thanks to irradiation. In addition, after these rheological experiments, the samples are converted in a soft film (Figure 3).

Figure 3. *Network formation upon UV irradiation as made evident by rheology for PPET1.*



From the plateau reached at the end of irradiation, the elastic modulus (G′) has been measured to be around 3.5 MPa for the PPE1 copolymer. As summarized in the Table 2, the value of G′ measured by frequency sweep tests for the different networks significantly decreases with the decrease of the double bond content in the copolymer. In addition, frequency sweep tests also show that G′ was almost constant independent of the frequency, indicating that the photo-cross-linked covalent networks possess an elastic behavior.

Table 2. *Hydrophilicity and Mechanical Properties of the PPE Networks*

sample	CA[a] (deg)	EWC[b] (%)	G′[c] (MPa)	E_{Young} dry[d] (MPa)	E_{Young} wet[e] (MPa)
PPE1	82 ± 5	44 ± 2	3.50	14.5 ± 1.5	9.8 ± 0.3
PPE2	65 ± 3	49 ± 1	2.20	8.9 ± 0.3	3.8 ± 0.8
PPE3	40 ± 2	88 ± 3	0.50	3.7 ± 0.5	2.2 ± 0.5
PPET1	68 ± 5	50 ± 1	0.45	6.5 ± 0	4.1 ± 0.3
PPET2	70 ± 2	56 ± 3	0.60	3.9 ± 0.3	2.6 ± 0.4

[a]Contact angle (CA) of water on the polymer networks. [b]EWC equilibrium water content. [c]Elastic modulus of the copolymer network measured by shear rheometer. [d]Young's modulus of the networks in the dry state. [e]Young's modulus of the networks in the swollen state.

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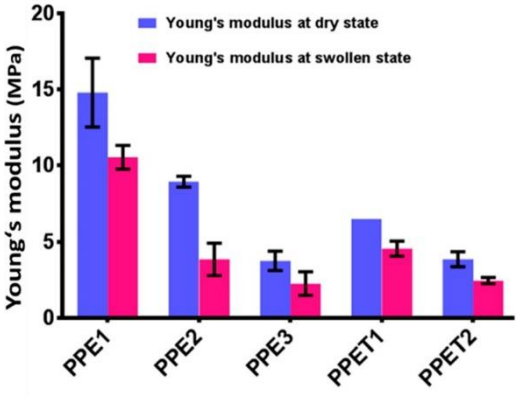
HYDROPHILICITY OF THE NETWORKS.

By increasing the MEP copolymer content from PPE1 to PPE3, the corresponding networks are expected to exhibit increasing hydrophilicity. This was confirmed by water contact angle measurements that decrease from 82° for the most hydrophobic PPE1 to 40° for the most hydrophilic PPE3 (Table 2). The same content of MEP was used in PPE2, PPET1, and PPET2. As expected, these three materials have a quite similar contact angle value around 68°. This experiment confirms that the hydrophilic character of the networks is directly related to the MEP comonomer content in the material.

Being able to prepare networks of different hydrophilicity (PPE1 to PPE3), it is expected that these materials exhibit different swelling in aqueous media, i.e., when placed in cell culture media or in the body. The water swelling of the networks was thus measured in Milli-Q water at 37 °C (Table 2). As expected, the water swelling follows the increase of hydrophilicity from PPE1 (44%) to PPE3 (88%). Interestingly, decreasing the double bond content in copolymers having the same contact angle (PPE2 > PPET1 > PPET2) leads to a slight increase of the water swelling (from 49% to 56%), probably related to the decrease of the cross-linking density and thus to the improved chain segment mobility.

Stiffness of the Networks. The influence of the PPE composition on mechanical properties of the networks was investigated first in the dry state and then swollen in water, by the conventional tensile test. In the dry state, the Young's modulus (E_{Young}) of PPE networks is decreasing with the decrease of the BenEP comonomer content, i.e., the reactive comonomer responsible for the cross-linking reaction (Table 2 and Figure 4). Remarkably, PPE3 and PPET2 have a similar modulus (~3.8 MPa) for different hydrophilicities (40 and 70). Besides, PPE2, PPET1, and PPET2 are networks of similar hydrophilicity exhibiting a decreasing modulus (8.9 > 6.5 > 3.9 MPa).

Figure 4. *Young's moduli of polyphosphoester-based films in dry (blue) and swollen (pink) states.*



This trend is further confirmed by the results of the tensile test at the swollen state. Three individual samples were immersed in PBS (pH 7.4) for 30 min and wiped slightly with tissue paper to remove PBS solution on the surface. All PPE samples underwent the same experiment of the tensile test. A noticeable decrease of the Young's modulus was noted for all PPE networks due to water absorption by the hydrophilic chains which soften the networks. It is worth noticing that the elongation at break falls below 10% for the most hydrophilic sample as depicted in Figure S5 (Supporting Information).

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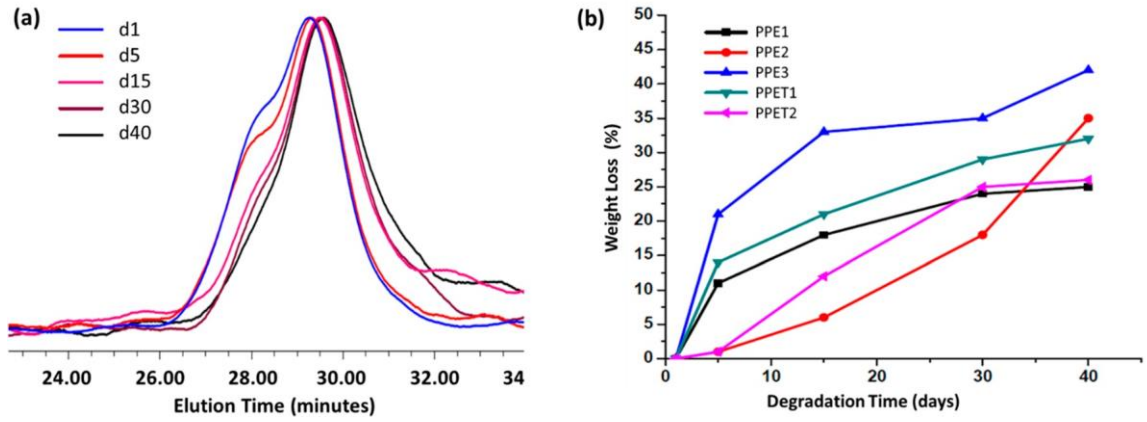
Nevertheless, these results of the Young's modulus of PPE networks in the swollen state and in the dry state expound our main goal of this study to prepare the material with a similar hydrophilicity and various stiffnesses and vice versa.

Figure 5. *(a) Evolution of the molecular weight determined by SEC of poly(MEP-co-BenEP-co-BEP) terpolymer from day 1 to day 40 in Milli-Q water. (b) Weight loss of the networks as a function of degradation time in PBS buffer.*



In Vitro Hydrolytic Degradation. From the tissue engineering application point of view, materials exhibiting biodegradability are essential since they will allow the total replacement by regenerated tissue.[21] The key advantage of PPEs in medical applications as compared to other aliphatic polyesters is that the PPE degradation product in aqueous media does not alter the pH that much. In addition, the degradation rate of the PPEs can be adjusted by controlling the nature of the pendent group.[22]

In the present work, the in vitro degradation of copolymers (biopolymers and terpolymers) and their networks was performed at 37 °C in Milli-Q water and in PBS buffer (7.4 pH), respectively. The degradation product was freeze-dried, and the molecular weight (M_n) was analyzed by SEC measurements. As depicted in Figure 5a, the molecular weight is decreasing with the incubation time (up to 40 days). The weight loss (Figure 5b) dropped to 43% for PPE3 in 40 days as compared to PPE1 (25%) and PPE2 (35%) which reflects the faster hydrolytic cleavage of the phosphoester bonds in the backbone in the more hydrophilic PPE3. The weight loss values for PPET1 (32%) and PPET2 (28%) are near each other due to the similar hydrophilic–lipophilic balance.

In Vitro Cytotoxicity. To investigate the potential of such polymers in tissue engineering applications, an in vitro cell cytotoxicity assay was assessed against human skin fibroblast cells. Viability of the cells in the presence of the polymers with different compositions was studied by the live/dead assay. PEG (noncytotoxic) and SDS (cytotoxic) were used for the sake of comparison. As depicted in Figure 6, all the PPEs did not show significant cytotoxicity in the cultured fibroblast cells with the concentration up to 0.5 mg mL^{-1}. At a concentration of 1 mg mL^{-1}, all PPEs become more cytotoxic than the PEG reference, the PPET2 terpolymer appearing as the most cytotoxic with a cell viability of 67% after 48 h of incubation.

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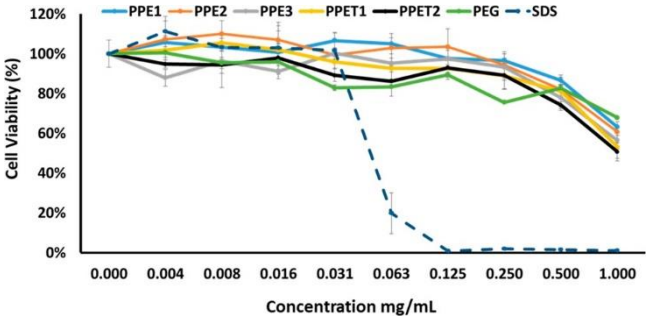
Figure 6. *Cytotoxicity of poly(MEP-co-BenEP) and poly(MEP-coBenEP-co-BEP) copolymers in comparison with PEG and SDS after 48 h of incubation with fibroblasts cells.*



Conclusion

A series of PPE copolymers with various ratios of methyl (hydrophilic), butyl (hydrophobic), and butenyl (reactive) side groups have been synthesized by organo-catalyzed ring opening polymerization of cyclic phosphoester monomers using BzOH initiator and DBU and TU catalysts in a controllable manner. 3D polymer networks were then prepared under UV irradiation with the help of I2959 photoinitiator. These networks contain tunable properties like swelling ratio, wettability, and degradation rate. In the rheological study we found that G' solid deformation (storage modulus) shows clear dependence on the cross-linking density of the polymers. The swelling ratio and the degradation rate are dependent on the hydrophilic character of the networks. The degradation rate of the PPEs films in PBS buffer shows the impact of both hydrophilicity and cross-linking density of the poly(MEP-coBenEP) and poly(MEP-co-BenEP-co-BEP) networks. These materials did not exhibit significant toxicity toward skin fibroblast cells. This work made evident the ability to prepare materials of various stiffnesses in the soft material range with the same hydrophilicity as materials of different hydrophilicity exhibiting a similar stiffness. These networks appear thus as a promising candidate to study cell growth and develop soft implantable medical devices.

Associated content

*$_S$ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.9b01276.

Synthetic procedures and characterization data (^{1}H and ^{31}P NMR spectra and SEC traces) for the starting PPE copolymers, rheological profiles of the copolymers under UV irradiation, and stress−strain curves of the PPE networks in dry and water swollen states (PDF)

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NOTES

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