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Acid-Containing Tyrosine-Derived Polycarbonates: Wettability and Surface Reactivity

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Summary: Tyrosine-derived polycarbonates having carboxylic acid pendant groups were characterized by water contact angle and X-ray photoelectron spectroscopy (XPS). A pronounce decrease of receding angle as well as contact angle hysteresis as a function of acid composition strongly indicated that the acid groups are more accessible at the water/polymer interface after hydration. pH dependent contact angle confirmed an existence of carboxylic acid groups in the surface region. The receding angle transition appearing in the pH range of 4-6 was a consequence of hydrophilicity change due to interconverting from carboxylic acid (-COOH) to carboxylate ion ($-COO^-$). The surface compositions of imidazole-labeled polymers as analyzed by XPS were consistent with the bulk stoichiometry of the polymers. Reactivity of acid groups towards chemical reaction at the surface was also investigated. The acid groups at the surface of polymers were capable of adsorbing a significant amount of calcium ion from simulated body fluid and being activated by a reaction with *N*-hydroxysuccinimide.

Keywords: biodegradable; carboxylic acid; surface characterization; tyrosine; wettability

Introduction

Desaminotyrosyl-tyrosine ethyl ester (DTE) has been used as a non-toxic, biocompatible monomer for the preparation of tyrosine-derived polycarbonates such as poly(DTE carbonate).[1] Due to its favorable mechanical and thermal properties and processability, poly(DTE carbonate) can be used as a material for orthopedic implants. Due to its slow rate of degradation, poly(DTE carbonate) is not suitable for some biomedical applications such as

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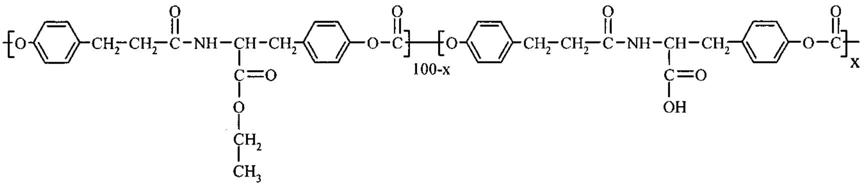
drug delivery, tissue regeneration scaffold.[2] To overcome this limitation, a new series of tyrosine-derived polycarbonates has been developed based on the rationale that the degradation can be improved by incorporating carboxylic acids as pendent groups.[3] The increased hydrophilicity of the polymer is believed to accelerate the polymer degradation via hydrolysis. The carboxylic acid groups can also serve as versatile precursors for a wide range of chemical modifications, including the attachment of amino acids or peptides. This new series of polycarbonates containing both desaminotyrosyl-tyrosine ethyl ester (DTE) and desaminotyrosyl-tyrosine (DT) is referred to as poly(DTE-co-x%DT carbonate) where x is the mole percentage of the comonomer having a free acid pendent chain.



Scheme 1. Structure of poly(DTE-co-x%DT carbonate).

In order to understand the biological properties of a material, it is necessary to know the surface properties since biological systems such as proteins and cells interact first with the surface of the material. In general, a polymer containing two or more functionalities with distinctly different surface free energy in the repeat unit has a strong tendency to exhibit a specific surface composition that may not be consistent with its bulk composition. The fact that poly(DTE-co-DT carbonate) consists of both hydrophobic ester groups and hydrophilic carboxylic acid groups leads to the possibility of surface reconstruction under specific conditions. Such behavior may have significant influences on surface reactivity as well as biological responses.

We have previously reported on the surface characterization of a series of tyrosine-derived polycarbonates containing ethyl, butyl, hexyl and octyl esters as pendent groups.[4] As indicated by contact angle analysis, the degree of surface hydrophobicity was related to the length of the alkyl pendent chain. Elemental composition at the surface as determined by XPS was consistent with stoichiometry of the polymer in the bulk. In this report, the wetting behavior of poly(DTE-co-DT carbonate) is determined by contact angle measurements. The amount of carboxylic acid is qualitatively identified by surface titration and ATR-IR. The surface composition of polymer is characterized by XPS analysis of imidazole-labeled polymer surfaces. The reactivity of carboxylic acid in the surface region is assessed by the determination of adsorbed calcium ions from simulated body fluid and the reaction with *N*-hydroxysuccinimide.

Experimental

Materials and Methods

All chemicals were purchased from Aldrich and used as received. Films of poly(DTE-co-DT carbonate) (Mw ~80,000-100,000) were prepared by solution casting from 10% (w/v) polymer in methanol/methylene chloride (1/10 v/v). Contact angle measurements were made with a Rame'-Hart telescopic goniometer and a 24-gauge flat-tipped needle. Water purified using a Millipore Milli-Q system and buffer solutions (Metrepak, Micro Essential Laboratory) were used as probe fluids. Dynamic advancing (θ_A) and receding (θ_R) angles were recorded while the probe fluid was added and withdrawn from the drop, respectively. X-ray photoelectron spectra (XPS) were obtained with a Kratos XSAM-8000 instrument with a monochromatic MgKα radiation. Spectra were taken at a takeoff angle of 45° between the plane of the sample surface and the entrance lens of the detector optics. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was performed using a Mattson Cygnus 100 Spectrometer equipped with ZnSe crystal. All spectra were obtained from 200 scans at 2 cm^{-1} resolution. The spectra were corrected for atmospheric water and carbon dioxide absorption, then resolved into gaussian components by means of an iterative least-squares method (GRAMS 386 software; Galactic Industries)

Labeling of Carboxylic Acid Groups *via* Formation of Carbonyl Imidazolide

To a nitrogen-purged Schlenk flask containing polymer films, a solution of 1,1'-carbonyldiimidazole (CDI) (0.15 g, 0.93 mmol) in 30 mL THF/diethyl ether (1/4 v/v) was introduced. The reaction proceeded at ambient temperature. After 8h, the films were rinsed sequentially with three aliquots of THF/diethyl ether (1/4 v/v) and hexane and dried under vacuum for 24h.

Adsorption of Ionic Species from Simulated Body Fluid

A polymer film was incubated in a small glass vial containing 10 mL freshly prepared simulated body fluid (SBF) from 0.071 g Na_2SO_4, 0.174 g K_2HPO_4, 0.353 g $NaHCO_3$, 0.368 g $CaCl_2$, 0.305 g $MgCl_2.6H_2O$, 7.99 g NaCl, 0.224 g KCl, 6.06 g Tris and 45 mL HCl (1.0N) at 37°C. After the desired incubation time, the film was removed and rinsed thoroughly by two aliquots of SBF followed by three aliquots of water. The film was dried under vacuum for 24h.

Reaction with *N*-hydroxysuccinimide

To a nitrogen-purged Schlenk flask containing polymer films, a solution of *N*-hydroxysuccinimide (NHS) (0.0955 g, 0.5 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI) (0.0575 g, 0.5 mmol) in 10 mL ethanol was added. The reaction proceeded at ambient temperature to yield *N*-hydroxysuccinimide ester on the surface. After the desired reaction time, the films were rinsed thoroughly with ethanol and dried under vacuum for 24h.

Results and Discussion

Advancing/receding water contact angles (θ_A/θ_R) as a function of acid content are shown in Figure 1. The advancing angle of polymers containing up to 50%DT was not significantly changed as %DT increased. The angles were only varied in the range of 77±2°. Even though θ_A was reduced to ~71° for poly(DT carbonate)(100%DT), the value was not as low as what can be expected from the surface bearing such hydrophilic acid groups. This data implied that the hydrophobic parts of the polymer (main chain & side chain) are the dominating species at the polymer/air interface especially when %DT is below 50%. The carboxylic acid groups preferably reside in the subsurface in order to minimize the surface free energy. It is also possible that the density of carboxylic acid group is quite low considering a relatively large overall dimension of the polymer repeat unit so that they do not contribute significantly to the wetting of polymer. Upon hydration, the carboxylic acid side groups can expose themselves to the polymer/water interfaces such that a significant reduction of the receding angle was observed; 47° for poly(DTE carbonate) to 20° for poly(DT carbonate). Apparently, the receding angle can be a better indication of the change in surface hydrophilicity than the advancing angle as a function of %DT. In general, functional groups at polymer/air and polymer/liquid interfaces arrange themselves in such ways that the surface energy at the interfaces are minimized as long as the rotational energy barrier of functional groups in the surface region can be overcome. The rising of contact angle hysteresis (θ_A- θ_R) is also evidenced. In this particular study, the heterogeneous nature of the copolymers due to functional group variation is regarded as a major factor causing contact angle hysteresis. We do not take surface roughness into consideration since an unusually abrupt change of contact angle was not observed.

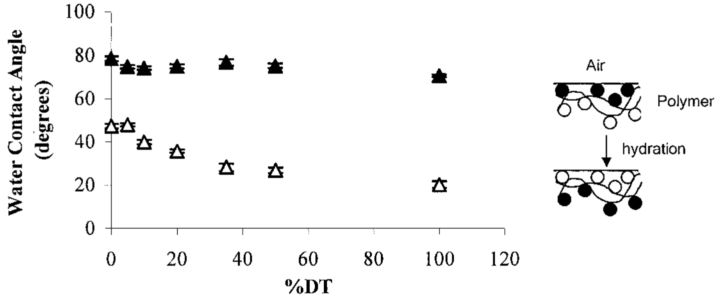


Figure 1. Water contact angles of poly(DTE-co-DT carbonate) as a function of %DT in the bulk: θ_A(▲), θ_R(△). ● and ○ represent ethyl ester side groups and carboxylic acid side groups, respectively.

To verify that an increased hydrophilicity was originated from ionizable carboxylic acid groups in the surface region, pH dependent contact angle analysis or surface titration was performed using buffered solutions. The method has been developed for characterizing the ionizable functional groups.[5] In the case of a surface having carboxylic acid groups, a water contact angle is expected to decrease as a consequence of –COOH being transformed to –COO^- after exposure to a basic solution. Without carboxylic acid groups, poly(DTE carbonate) did not exhibit advancing or receding contact angle transition. According to Figure 2, such a transition can be seen on both advancing and receding angle for poly(DT carbonate). Since the acid groups are only accessible at polymer/water interfaces in the cases of polymers containing up to 50%DT, the transition was observed on the receding angles, not on the advancing ones (as shown in Figure 3). It is quite interesting to see that the transition shifts towards lower pH when the acid content is increased.

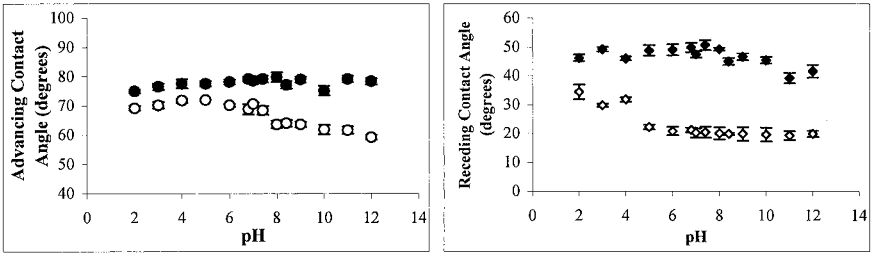


Figure 2. Water contact angles of poly(DTE carbonate) : θ_A(●), θ_R(○) and poly(DT carbonate): θ_A(♦), θ_R(◊) as a function of pH.

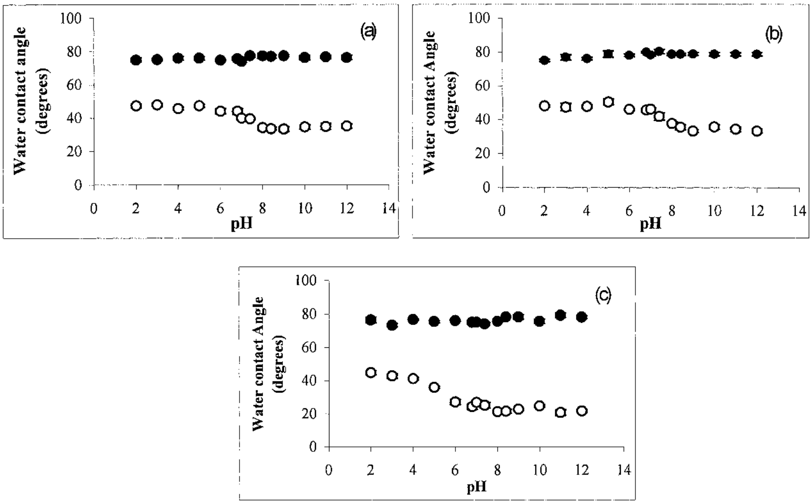


Figure 3. Water contact angles of poly(DTE-co-x%DT carbonate) as a function of pH when (a) x = 10, (b) x = 20 and (c) x = 50 : θ_A(•), θ_R(○).

According to the ATR-IR spectra shown in Figure 4, the O-H stretching of hydrogen-bonded carboxylic groups which appeared as a broad baseline in the region of 3600-2200 cm^{-1} was seen for polymers containing acid side groups. The magnitude of peak broadening corresponded very well with the acid content suggesting that the carboxylic acid functionality can be qualitatively identified by ATR-FTIR analysis.

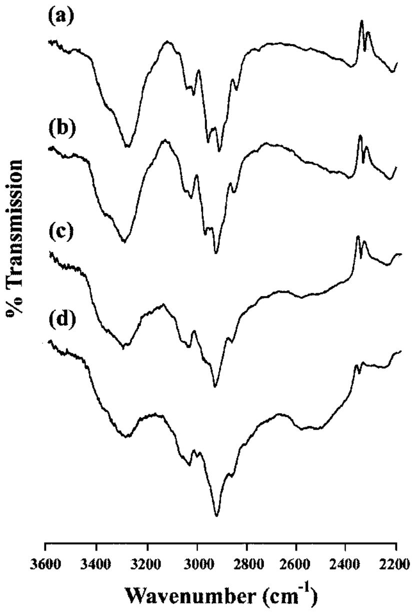
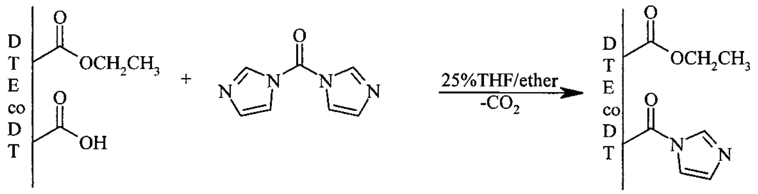


Figure 4. ATR-FTIR spectra of poly(DTE-co-x%DT carbonate) : (a) x = 0, (b) x = 20, (c) x = 50 and (d) x = 100.

The labeling reaction (Scheme 2) was performed by reacting polymer films with CDI in THF/diethyl ether (1:4 v/v).[6] The reaction introduced two additional nitrogen atoms into each DT unit that can be quantified by XPS. The percentage of DT unit in the surface region is equal to (14(%N)-50)/1-(%N/100). % DT tabulated from XPS data was not distinguishable from the bulk composition (analyzed by ^{1}H NMR) for polymers containing 20 and 35% DT, while a slightly underestimated value of 44% DT was detected for poly(DTE-co-50%DT carbonate).



Scheme 2. Labelling reaction of poly(DTE-co-x%DT carbonate) with 1,1'-carbonyldiimidazole.

The reactivity of surface carboxylic acid groups in the form of carboxylate ions (pKa < 6 from surface titration) in physiological pH (~7.4) was assessed by XPS analysis of the polymer films after soaking in SBF. According to Table 1, as high as 0.67% of calcium was detected on poly(DTE-co-50%DT carbonate) after 3-day incubation. The XPS survey spectrum is shown in Figure 5. It should be noted that 0.9% of calcium should be found if there is one calcium ion (Ca^{2+}) binding ionically with every two acid groups. Interestingly, there were no signals from other cationic species such as Na^+, K^+, Mg^{2+}. Due to the limited swelling of poly(DTE-co-20%DT carbonate) in aqueous solution of SBF, 7 days of incubation was required to reach the adsorption equilibrium at ~0.2%. The fact that no calcium signal was observed on poly(DTE carbonate) implied that non-specific physical adsorption did not occur. Due to its rapid degradation, poly(DTE-co-50%DT carbonate) became mechanically unstable after 7-day incubation.

Table 1. Percentage of Ca_{2p} from XPS analysis of polymers after incubation in SBF.

Polymer	Percentage of Ca_{2p} after incubation in SBF		
	1 day	3 days	7 days
Poly(DTE carbonate)	0.00	0.02	0.02
Poly(DTE-co-20%DT carbonate)	0.03	0.05	0.21
Poly(DTE-co-50%DT carbonate)	0.44	0.67	-

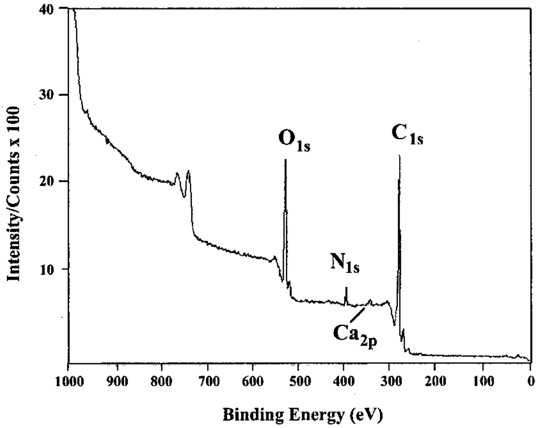
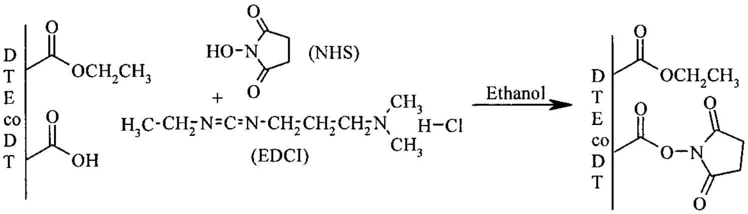


Figure 5. XPS spectrum of poly(DTE-co-50%DT carbonate) after incubation in SBF at 37°C for 3 days.

A coupling reaction with NHS has been recognized as one of effective methods used for converting the carboxylic acid groups into active esters before reacting with amino-containing compounds (Scheme 3). Poly(DTE-co-20%DT carbonate) was chosen as a representative for reactivity assessment. To increase the swelling of poly(DTE-co-20%DT carbonate), the polymer was exposed to ethanol. This pretreatment allowed the reaction to proceed into sufficient depth so that the success of the surface activation could be assessed by ^{1}H-NMR.



Scheme 3. Reaction of poly(DTE-co-x%DT carbonate) with NHS/EDCI.

The extent of reaction was determined from relative peak area at 2.85 ppm corresponding to the protons of *N*-succinimide and those binding to the α-carbon of tyrosine at 4.8 ppm. Data displayed in Figure 6 suggested that the higher the concentration of NHS and EDCI, the higher the extent of the reaction can be achieved. The yields of reaction were 0.5, 2.8 and 8.6% when 10, 20 and 50 mmol (in 10mL ethanol) of NHS and EDCI were used, respectively. It is worth noting that the calculated reaction yields based on ^1H NMR data represent the information from the bulk so reaction yields at the surface should be considerably higher.

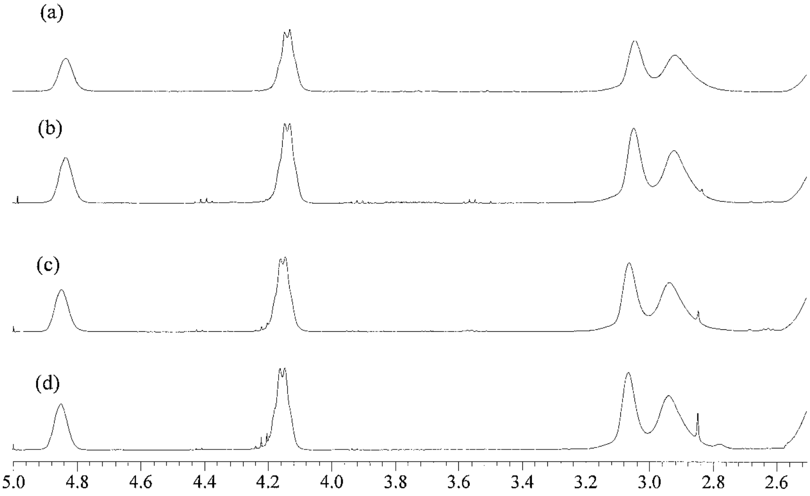


Figure 6. ^1H NMR spectra of poly(DTE-co-20%DT carbonate) after reaction with NHS/EDCI: (a) 0 mmol, (b) 10 mmol, (c) 20 mmol and (d) 50 mmol.

Conclusion

It has been proven that surface hydrophilicity of the tyrosine-derived polycarbonate can be enhanced by an introduction of carboxylic acid as pendant groups. The presence of acid component in the surface region can be effectively identified by pH-dependent contact angle measurements, ATR-FTIR and XPS analyses. The ability to adsorb calcium ions in proportion to the acid content suggested that these acid-containing polymers are very useful for orthopedic applications whose healing process basically involves hydroxyappatite formation. According to the reactivity assessment by a reaction with *N*-hydroxysuccinimide, it can be concluded that the carboxylic acid groups in the surface region are reactive enough for further chemical modification.

Acknowledgements

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