Controllable Surface Modification of Poly(lactic-co-glycolic acid) (PLGA) by Hydrolysis or Aminolysis I: Physical, Chemical, and Theoretical Aspects

Tristan I. Croll, Andrea J. O’Connor, Geoffrey W. Stevens, and Justin J. Cooper-White*

Department of Chemical and Biomolecular Engineering, The University of Melbourne, Victoria 3010 Australia

Received August 18, 2003; Revised Manuscript Received October 30, 2003

While biodegradable, biocompatible polyesters such as poly (lactic-co-glycolic acid) (PLGA) are popular materials for the manufacture of tissue engineering scaffolds, their surface properties are not particularly suitable for directed tissue growth. Although a number of approaches to chemically modify the PLGA surface have been reported, their applicability to soft tissue scaffolds, which combine large volumes, complex shapes, and extremely fine structures, is questionable. In this paper, we describe two wet-chemical methods, base hydrolysis and aminolysis, to introduce useful levels of carboxylic acid or primary and secondary amine groups, respectively, onto the surface of PLGA with minimal degradation. The effects of temperature, concentration, pH, and solvent type on the kinetics of these reactions are studied by following changes in the wettability of the PLGA using contact angle measurements. In addition, the treated surfaces are studied using X-ray photoelectron spectroscopy (XPS) to determine the effect on the surface chemical structure. Furthermore, we show using XPS analysis that these carboxyl and amine groups are readily activated to allow the covalent attachment of biological macromolecules.

Introduction

Soft tissue engineering offers a number of unique challenges not seen in other tissue engineering applications such as bone, cartilage or skin. For many soft tissue applications, scaffolds must be large (e.g. breast reconstruction1), very highly porous, and soft, yet have enough strength to resist the contractile forces generated by growing tissue. Scaffolds produced from poly(lactic-co-glycolic acid) (PLGA) using thermally induced phase separation (TIPS)2,3 are able to meet these goals. However, it is well-known that the surface properties of PLGA are not ideal for cell growth.4–7 PLGA is relatively hydrophobic compared to the natural extracellular matrix (ECM), is unable to interact specifically with cells, and does not possess any functional groups for the attachment of biologically active molecules.

Since the mechanical and degradative properties of PLGA are seemingly ideal, and given that PLGA is approved by the U.S. Food and Drug Administration (FDA) and other regulatory bodies for implantation into humans, a promising approach appears to be the surface modification of PLGA scaffolds post-formation. Ideally, this gives useful surface characteristics to the polymer, without changing the properties of the bulk.

Many approaches have been taken to modify the surface of PLGA to date; however, very few of these appear promising for soft tissue applications. Plasma treatment, although very popular,8–11 appears unable to penetrate more than a few millimeters into the pores of a scaffold. Surface

Experimental Section

Materials. Poly(D,L-lactic-co-glycolic acid) (PLGA) with a lactic acid:glycolic acid ratio of 75:25 and an inherent viscosity in chloroform of 0.69 dL/g (molecular weight approximately 100 kDa) was purchased from Birmingham
Polymers Inc. Ammonia (LR, 25% aqueous solution), hydrogen peroxide (LR, 30% aqueous solution), chlorotrimethylsilane (CTMS) (>99%), toluene (HPLC grade), acetone (HPLC), ethanol (AR, >99.7%), n-hexane (HPLC), dichloromethane (HPLC), and 2-propanol (iso-propyl alcohol, IPA) were obtained from Merck. N-Aminooethyl-1,3-propanediamine (AEPDA) (AR), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC), and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich. Acetic acid (AR), ethylenediamine (ED) (AR), and triethanolamine (AR) were obtained from BDH. 2-(N-Morpholino)ethanesulfonic acid (MES) (AR) was obtained from ICN Biomedicals Inc. (Ohio). Low molecular weight chitosan (viscosity @ 0.5% w/v = 5–20 cP, >80% deacetylated, Wako Fine Chemicals Inc.) was kindly donated by Professor Yoshinari Baba of Miyazaki University. All reagents were used as supplied, and all water used was purified using a MilliPore Simplicity unit to a resistivity of ≥18.2 MΩ·cm. All glassware was washed sequentially in toluene, acetone, and ethanol and dried using compressed air filtered through activated carbon immediately prior to use.

Production of Thin Films. PLGA films with a thickness of <2 μm and an RMS roughness of <2 nm measured via contact-mode AFM imaging over a 10 μm × 10 μm area were prepared on clean, hydrophobically treated glass coverslips by a dip-coating method similar to that used by others. Coverslips (22 mm × 22 mm, 100 μm thick) were cleaned in a boiling mixture of 9 parts neat hydrogen peroxide solution and 1 part neat ammonia solution. These were rinsed in three washes of water, placed individually into 23 mm internal diameter glass vials so that they stood vertically, and dried on a hotplate at 80 °C. The coverslips were then made hydrophobic by treatment in a freshly prepared solution of CTMS in n-hexane (10% v/v) for 10 min and rinsed with three washes of fresh n-hexane, and allowed to dry by evaporation. These were dipped in a solution of PLGA in dichloromethane (5% w/v) at 0 °C and withdrawn slowly to leave a thin film of polymer solution on the glass surface. Most of the solvent evaporated immediately, leaving a translucent white polymer film on the glass. The coverslips were then returned to their vials, loosely capped with aluminum foil and placed horizontally in an oven at 70 °C for 24 h to remove the residual solvent and anneal the polymer film. At this point the films were entirely transparent and almost indistinguishable from the coverslips, except for the presence of refraction fringes, indicating a very low surface roughness on the order of nanometers.

Hydrolysis and Aminolysis of Films. Films to be hydrolyzed were immersed in a solution of aqueous sodium hydroxide (NaOH) at suitable concentrations and allowed to react at 20 °C for the desired time period, as described elsewhere. Films to be aminolized were immersed in ED or AEPDA at various concentrations in either water or IPA and allowed to react at either 20 or 0 °C for the desired time period. Treated films were washed three times with ice cold water, followed by soaking for a further hour in fresh water on ice, dried under vacuum over silica gel for 12 h, and stored in a desiccator over silica gel until further use.

Activation of Films with Cross-Linking Reagents. Hydrolyzed films were treated in 10 mL of a freshly made solution of 4 mM EDAC and 100 mM NHS in 10 mM MES buffer at pH 6.1 for 1 h at room temperature. Aminolysed films were treated in freshly prepared 40 mM DMA in 10 mM triethanolamine buffer at pH 8.0 for 1 h at room temperature. Activated films were rinsed thoroughly with water and used immediately.

Binding of Chitosan to Films. Chitosan was dissolved in 0.1 M acetic acid to a concentration of 10 mg/mL. This solution was filtered through a 0.25 μm syringe filter to remove insoluble particles. A 1 mL aliquot of this solution was made up to 90 mL with water, and 10 mL of 100 mM triethanolamine, 1.4 M NaCl at pH 8.0 or 9.0 (adjusted with 1 M NaOH) was added. At this point, the solution turned slightly milky, indicating the formation of a stable colloidal suspension of 100 μg/mL chitosan with a physiological salt concentration.

Preactivated surfaces or nonactivated hydrolyzed or aminolysed controls were incubated in 10 mL of this chitosan suspension at pH 8.0 (aminolyzed surfaces) or 9.0 (hydrolyzed surfaces) for 1 h. The coated surfaces were then washed twice with 0.1 M acetic acid to remove all weakly bound chitosan, followed by three washes with water.

Scanning Electron Microscope (SEM) Analysis. Samples to be analyzed via SEM were sputter-coated with gold under vacuum in an argon atmosphere using a sputter current of 60 mA (DynaVac Mini Coater, Dynavac USA). SEM analysis was carried out with a Philips XL30 SEM using an LaB6 electron gun with an accelerating voltage of 20 kV.

Contact Angle Measurement. Equilibrium advancing (θa) and receding (θr) air/water contact angles were measured with a contact angle goniometer and drop shape analysis software (First Ten Angstroms, Inc) using the captured (sessile) drop method on PLGA coated glass coverslips at a temperature of 22.5 °C. Water was pumped onto the surface of the coverslip through a 23-gauge stainless steel needle at a rate of 2 μL/s until the drop diameter was greater than 4 times the diameter of the needle. At this point, the drop was allowed to stand for 2 min before measuring θa. Water was then removed from the drop at a rate of 2 μL/s until the interface began to recede. A further 2 min relaxation time was allowed before θr was measured. During the first and second relaxation periods, contact angles decreased or increased by 3–5° respectively; the contact angles changed by <0.5° in the next 3 min. Three drops (six interfaces) were imaged for each surface; values reported are the mean ±1 standard deviation.

X-ray Photoelectron Spectroscopy (XPS) Analysis. XPS analysis was performed using a AXIS HSi spectrometer (Kratos Analytical Ltd) equipped with a monochromatic Al Kα source at a power of 300 W. The total pressure in the main vacuum chamber during analysis was typically 2 × 10−8 mbar. Elements present were identified from survey spectra. For further analysis, high-resolution spectra were recorded from individual peaks at 40 eV pass energy. Atomic concentrations of each element were calculated by determining the relevant integral peak intensities (using a linear type background) and applying the sensitivity factors supplied by
Activity of the newly formed groups toward covalent binding, in Scheme 1. Chitosan was used as a label to indicate the PLGA via hydrolysis or aminolysis is shown schematically to correct for charging of specimens under irradiation.

It is generally agreed that the reaction is bulk, rather than surface hydrolysis.22 Acid hydrolysis of (PLLA) that hydrolysis in aqueous solution at pH 2 leads to negatively charged end groups and solvated hydroxide ions, which are consumed during the hydrolysis reaction, in the process generating negatively charged carboxylic acid groups on the polymer surface. The electrostatic repulsion between these negatively charged end groups and solvated hydroxide ions, combined with the fact that the hydroxide ion is somewhat bulkier than the proton and is not regenerated, acts to reduce the rate of diffusion of hydroxide ions into the polymer. Thus, under these conditions surface-oriented, rather than bulk, hydrolysis is favored. Once again, this is borne out by experimental evidence in the literature both on PLLA22 and on poly(ethylene terephthalate) (PET) used for textile manufacture.24,25 On 50 μm thick PLLA films hydrolyzed in aqueous sodium hydroxide solution at pH 12,22 weight loss showed a linear profile indicating surface erosion, reaching 70% loss after 150 days, which equates to an erosion rate of approximately 5 nm per hour on each surface of the double-sided films, compared to a sigmoidal weight-loss profile at pH 2.0, typical of bulk degradation. Base hydrolysis of PLGA for the purposes of surface modification has been reported in the past;15,26 however, the authors used very high concentrations of sodium hydroxide that led to high levels of surface erosion, with a concomitant increase in roughness.

Base hydrolysis of model esters such as ethyl acetate and methyl formate has been found to be first-order in hydroxide ion concentration,27–29 with a very high activation energy (~105 kJ/mol in the case of ethyl acetate27,28). Thus, the rate of hydrolysis is strongly dependent on both solution pH and temperature: a pH change of one unit causes a 10-fold change in reaction rate, whereas in the case of ethyl acetate, there is a further 10-fold difference in the rate between 0 and 20 °C.

Aminolysis. Aminolysis is a technique which has been used in the textile industry for many years to improve the dyeability, wettability characteristics, and “feel” of synthetic polyesters such as PET.24,30–33 However, its applicability to surface modification of biodegradable polyesters for tissue engineering has only recently been noticed by our group and others.34–36

A large amount of work has been carried out in the past to elucidate the mechanism of aminolysis of model esters.29,37–44 The reaction proceeds via nucleophilic attack on the carbonyl carbon to form a positively charged tetrahedral intermediate. Under acidic or neutral conditions, the amine leaving group (R−NH₂) is strongly preferred over the alcohol leaving group (RC−O⁻), and hence, the reaction does not proceed. However, under basic conditions, the tetrahedral intermediate is deprotonated, leading to the extremely unfavorable R−NH⁻ leaving group (pKₐ > 30), and hence, the reaction proceeds to the formation of an amide and an alcohol. Therefore, aminolysis is generally carried out either in basic aqueous solution (pH > pKₐ of the amine)29,37,40,42 or in an aprotic, polar solvent with a high degree of π basicity, such as an alcohol.41, 43, 44

Since both the polymer and the amine modifying species are uncharged under optimal aminolysis conditions, the reaction is not inherently self-limiting as in the case of alkaline hydrolysis. It has been shown, for example, that small, mobile amines such as methylamine and N-propylamine are able to diffuse readily into PET fibers, causing significant cracking.24,30,35 In addition, weight loss profiles24,30 showed a lag phase after the beginning of treatment typical of a bulk degradation process.
In contrast to base hydrolysis, the overall activation energy for the aminolysis reaction is very low and in organic solvents is often negative, leading to a low or inverse dependence of the reaction rate on temperature. In aqueous solutions, the reaction rate typically reaches a plateau at pH values just above the pKₐ of the amine, dropping sharply at pH values below this. It has been found from numerous experiments that in general the log of the aminolysis rate increases with the pKₐ of the amine to the 0.8th power.

Surface Characterization. It is well-known that surface roughness on the micron scale has a strong effect on measured contact angles, as well as cell–surface interactions. This can be a confounding factor when attempting to quantify the effect of surface treatments on these parameters, a fact which appears to have been largely ignored in much of the literature on surface modification. SEM and AFM images of many PLGA or polylactic acid (PLA) films produced by solvent casting from dichloromethane described in the literature display very high levels of surface roughness, mostly due to the formation of microscopic bubbles of escaping solvent vapor. By comparison, the thin films used in this work (Figure 1) are typically 1–2 μm thick, with an RMS roughness of <2 nm measured by AFM imaging over a 100 μm² area. As can be seen from Figure 1, the surface undulates slightly over larger areas; however, these undulations are smoothly varying over relatively large length scales. Evidence from the literature indicates that it is sharp changes in topology that have the most effect on contact angles and cell responses. These very thin, smooth films allow much more reliable quantification of surface characteristics such as contact angle and cell adhesion properties than is achievable using solvent-cast films.

Contact Angle. As a surface-specific lysis reaction such as hydrolysis or aminolysis proceeds, the number of active end groups present at the surface initially increases. However, eventually an equilibrium is reached at which the rate of removal of soluble oligomeric fragments is equal to the rate of generation of new end groups. The density of functional groups at this point is likely due to a number of factors such as the initial molecular weight of the polymer, the molecular weight at which oligomers become soluble, and the surface organization of the polymer.

As may be expected from the mechanism of modification, the θₑ and θᵣ of hydrolyzed or aminolyzed films appeared to decrease to equilibrium values via a first-order decay profile. Consequently, first-order decay models were fitted simultaneously to θₑ and θᵣ profiles for each treatment method. The fitted equilibrium advancing (θₑ,eq) and receding (θᵣ,eq) contact angles and the decay half-time (t₁/₂) are reported in Table 1.

For surfaces treated in 0.01 or 0.05 N NaOH (Figure 2), equilibrium levels of modification were reached extremely quickly, with decay half-times on the order of seconds. As expected, the NaOH concentration had very little effect on the final level of modification; the equilibrium values of θₑ and θᵣ were not significantly different for surfaces treated in 0.01 N NaOH or 0.05 N NaOH (P > 0.5). Films treated for up to 24 h in 0.01 N NaOH had slightly reduced θₑ and θᵣ values of 69.7 ± 0.7° and 47.4 ± 0.7°, respectively. Films

Table 1. Fitted First-Order Decay Parameters for Treated Surfaces (Treatment Temperature = 20 °C)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>θₑ,eq (°)</th>
<th>θᵣ,eq (°)</th>
<th>t₁/₂ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>74.9 ± 0.6</td>
<td>54.7 ± 0.5</td>
<td>N/A</td>
</tr>
<tr>
<td>0.01 N NaOH</td>
<td>71.7 ± 0.8</td>
<td>49.1 ± 1.1</td>
<td>≤ 1</td>
</tr>
<tr>
<td>0.05 N NaOH</td>
<td>71.6 ± 0.8</td>
<td>48.7 ± 0.4</td>
<td>≤ 0.2</td>
</tr>
<tr>
<td>0.05 M ED (aq)</td>
<td>70.8 ± 0.7</td>
<td>45.9 ± 0.5</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>0.05 M ED (IPA)</td>
<td>72.9 ± 0.8</td>
<td>50.2 ± 0.9</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>0.05 M AEPDA (aq)</td>
<td>70.1 ± 0.6</td>
<td>44.3 ± 0.5</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>0.05 M AEPDA (IPA)</td>
<td>71.9 ± 0.8</td>
<td>45.8 ± 0.7</td>
<td>7.6 ± 0.5</td>
</tr>
</tbody>
</table>

Figure 1. SEM image of a fractured PLGA-coated coverslip. The thickness of the film here is approximately 1–2 μm. G = glass substrate, P = polymer film. Bar = 10 μm.

Figure 2. Relationship between treatment time and air/water contact angle for PLGA surfaces treated in (a) 0.01 N NaOH and (b) 0.05 N NaOH at 20 °C. Diamonds = θₑ, squares = θᵣ. Lines = fitted first-order decay profiles.
treated in 0.05 N NaOH were etched through to the glass substrate in places after approximately 3 h.

Surfaces treated in 0.05 M ED or AEPDA (Figure 3) displayed somewhat more interesting behavior. The limiting values of $\theta_a$ and $\theta_r$ for surfaces treated with AEPDA in IPA were significantly lower than those surfaces treated with ED in IPA ($P < 0.05$ advancing, $P < 0.01$ receding). Contact angles on surfaces treated with aqueous AEPDA were also slightly lower than on surfaces treated with aqueous ED ($P = 0.1$ advancing, $P < 0.05$ receding). Furthermore, the limiting contact angles for either treatment in water were significantly lower than the corresponding values for hydrolysis ($P < 0.1$ for ED advancing, $P < 0.01$ for ED receding and AEPDA). These decreases in contact angle are likely to be due to extra added hydrophilic groups per reaction site, rather than a higher overall modification density. While hydrolysis yields two hydrophilic species per reaction (a carboxylic acid and an alcohol), ED yields three (amide bond, primary amine, and alcohol) and AEPDA yields four (amide, primary amine, secondary amine, and alcohol).

Under aqueous conditions, the limiting contact angles for both aminolysis treatments were significantly lower than those reached by aminolysis in IPA ($P < 0.01$). In IPA solution, aminolysis is the only reaction able to occur at any appreciable rate.37 The fitted $t_{1/2}$ values under these conditions were somewhat higher than those in water. This is likely due to the lower catalytic ability of IPA compared to water.37,38

To probe the relative contributions of hydrolysis and aminolysis in aqueous solution and to maximize the amine yield, PLGA surfaces were treated for 2 h with a series of solutions of 0.10 or 1.0 M ED at various pH values. To test the effect of temperature on the reaction selectivity, surfaces were treated in solutions of identical composition at either 0 or 20 °C.

The rate equation for aminolysis of PLGA by ED in aqueous solution was assumed to be analogous to that proposed by Satterthwait and Jencks42 for the reaction of hydrazine with model esters:

$$
\frac{r_a}{[ED]_{free}[ester]} = \frac{k_1 + k_2[ED]_{free} + k_3[OH^-]}{1 + k_{-1}a_{H^+} + k_{-2}[EDH^+] + k_{-3}}
$$

where $r_a$ is the overall aminolysis rate (M min$^{-1}$), [ED]$_{free}$ and [EDH$^+$] are the concentrations of free and ionized ED, respectively, and $k_a$ are rate constants.

[ED]$_{free}$ and [EDH$^+$] can be calculated from the $K_b$ of ED and the pH via

$$
[ED]_{free} = [ED]_{tot} - [EDH^+] = [ED]_{tot}\left(1 - \frac{K_b}{[OH^-] + K_b}\right)
$$

where [ED]$_{tot}$ is the total concentration of ED in solution.

The competing base hydrolysis reaction is first-order in hydroxide ion,42 thus

$$
\frac{r_h}{[OH^-][ester]} = k_0
$$

where $r_h$ is the hydrolysis rate (M min$^{-1}$). The total rate, $r_t$, is therefore

$$
\frac{r_t}{[ester]} = r_a[ED]_{free} + r_h[OH^-]
$$

and the ratio of amine to carboxylic acid groups on the treated surface can be defined by

$$
\phi = \frac{\gamma}{\gamma + 1}
$$

where

$$
\gamma = \frac{r_a[ED]_{free}}{r_h[OH^-]}
$$

The ratio of the amine group density on the surface, $\rho_a$, to the maximum possible density, $\rho_{a,max}$, is then

$$
\frac{\rho_a}{\rho_{a,max}} = \phi(1 - e^{-kt})
$$

where $t$ is the treatment time and $k = cr/[ester]$ is the surface pseudo-first-order decay constant, in units of min$^{-1}$. The
constant $c$ is used to convert from bulk to surface-specific rates. Although the bulk rate constants are unknown, it was assumed as a starting point that the bulk rate of hydrolysis at 20 °C is approximately equal to that of ethyl formate.\(^4\)

Assuming that the measured change in contact angle is a linear combination of the changes due to hydrolysis and aminolysis

$$\theta_a = \phi(\theta_a^{eq} + \Delta\theta_a e^{-k t}) + (1 - \phi)(\theta_{a,ref} + \Delta\theta_a e^{-k_t}) \quad (8)$$

$$\theta_r = \phi(\theta_r^{eq} + \Delta\theta_r e^{-k t}) + (1 - \phi)(\theta_r^{eq} + \Delta\theta_r e^{-k_t}) \quad (9)$$

where the second subscript on $\theta$ refers to aminolysis (a) or hydrolysis (h), the superscript $eq$ refers to the contact angle after treatment to equilibrium, and $\Delta\theta = \theta^o - \theta^{eq}$ refers to the maximum possible change in contact angle due to hydrolysis or aminolysis.

For each treatment temperature, the above model was fitted simultaneously to the pH-dependent data for both 0.1 and 1 M ED concentration using the least-squares method, with the squared errors weighted by the inverse of the standard deviation at each data point. In addition, the half-time and equilibrium contact angles determined for 0.05 M ED at 20 °C and its native pH (Figure 3a) were used to constrain the 20 °C model fit.

According to the fitted parameters (Table 2), there was little evidence of catalysis of the reaction by OH\(^-\) ($k_0$) or of inhibition by H\(^+\) (k\(_{i-}\)). Half-times for pure alkaline hydrolysis at pH 12 (0.01 N NaOH) and pH 12.7 (0.05 N NaOH) and 20 °C were calculated using the fitted values of $k_0$ and $c$ to be 1.0 and 0.2 min, respectively, in good agreement with the time-dependent data (Figure 2 and Table 1).

Plots of contact angles versus treatment pH (Figure 4) displayed three distinct regions. At high pH > 12, hydrolysis is the dominating reaction, and hence, the measured contact angles approach those expected from pure hydrolysis. At intermediate pH, a minimum is reached where aminolysis is the dominant reaction, whereas at low pH, the reaction kinetics are too slow to give a significant active group density within the treatment time, and hence, the contact angle remains close to control levels.

Although the hydrolysis rate dropped markedly with decreasing temperature, the aminolysis rate was decreased by a similar amount, giving no useful gain in selectivity (Figure 5).

The maximum achievable amine group density according to the model (Figure 6) was greater than 90% of the maximum possible density for all temperature and concentration conditions tested. At 0 °C, however, both the height and width of the maxima were smaller compared to equivalent amine concentrations at 20 °C, indicating that lowering of the temperature is actually somewhat detrimental. The calculated maximum surface amine density as a function of amine concentration (Figure 7) showed a steep increase at low concentration, leveling out at approximately 0.05 M ED for 20 °C. At 0.1 M ED, the optimal amine density is approximately 97% of the maximum; there appears to be very little gain in increasing the ED concentration further than this.

**XPS Analysis.** In the case of partial surface hydrolysis by NaOH treatment, no detectable changes in surface composition using XPS are expected. Simple calculations show that for every 1% of ester bonds hydrolyzed the amount of oxygen present in the surface should increase by only 0.1 atomic % (at. %). Since the random error in XPS is approximately 1–2%, the measured value for elements present in abundance,\(^5\) this level of modification is undetectable. Moreover, since the number and type of carbon–oxygen bonds are unchanged by hydrolysis (Figure 8), no change in the relative peak areas in the carbon 1s spectrum is expected. Although it is true that alcohols and carboxylic acids display small peak shifts of approximately 0.1 and 0.2 eV relative to ethers and esters respectively,\(^6\) in practice these are inseparable from the existing peaks.

In the case of aminolysis, a new element, nitrogen, is added to the surface, making detection somewhat easier. Once again, however, the expected level of nitrogen present is very small.

The atomic compositions calculated from the XPS wide-scan spectra for untreated, hydrolyzed, and aminolyzed surfaces are detailed in Table 3. The measured composition of untreated films was very close to the theoretical composition of 75:25 PLGA. As expected, hydrolysis or aminolysis induced very little change in the measured compositions, except for a slight increase in the carbon:oxygen ratio, attributed to the CH\(_2\) peak by analysis of the C1s spectrum (Figure 9). This change is not significant, however, given the typical random error in XPS results. The shape of the C1s spectrum for untreated films was very close to the expected shape for 75:25 PLGA calculated from the atomic composition.

PLGA surfaces aminolyzed in aqueous ED or in AEPDA in IPA for 120 min were found to contain approximately 0.5 at. % nitrogen within the XPS sampling depth of about 5 nm. Thus, calculations based on the empirical formulas of PLGA, ED, and AEPDA indicate that approximately 1.2 ED molecules or 0.8 AEPDA molecules were added per 100

---

**Table 2.** Fitted Parameters for Kinetic Model of ED Aminolysis in Aqueous Solution

<table>
<thead>
<tr>
<th>parameter</th>
<th>units</th>
<th>value at T = 0 °C</th>
<th>value at T = 20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_0$</td>
<td>M(^{-1}) min(^{-1})</td>
<td>0.33(^a)</td>
<td>4.0(^b)</td>
</tr>
<tr>
<td>$k_{1,a}$</td>
<td>M(^{-1}) min(^{-1})</td>
<td>0.032</td>
<td>0.15</td>
</tr>
<tr>
<td>$k_{2,a}$</td>
<td>M(^{-2}) min(^{-1})</td>
<td>0.019</td>
<td>0.94</td>
</tr>
<tr>
<td>$k_{3,a}$</td>
<td>M(^{-3}) min(^{-1})</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k_{-1,a}$</td>
<td>M(^{-1})</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k_{-2,a}$</td>
<td>M(^{-1})</td>
<td>3.62</td>
<td>7.06</td>
</tr>
<tr>
<td>$k_{-3,a}$</td>
<td>M(^{-1})</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$k_{0,c}$</td>
<td>dimensionless</td>
<td>2 × 10^{-4}</td>
<td>6.4 × 10^{-5}</td>
</tr>
<tr>
<td>$\theta_a^{eq}$</td>
<td>degrees</td>
<td>75.0</td>
<td>75.0</td>
</tr>
<tr>
<td>$\theta_h^{eq}$</td>
<td>degrees</td>
<td>54.8</td>
<td>54.8</td>
</tr>
<tr>
<td>$\theta_{a,h}^{eq}$</td>
<td>degrees</td>
<td>71.6</td>
<td>71.6</td>
</tr>
<tr>
<td>$\theta_{h,a}^{eq}$</td>
<td>degrees</td>
<td>48.8</td>
<td>48.8</td>
</tr>
<tr>
<td>$\theta_{a,h}^{eq}$</td>
<td>degrees</td>
<td>69.1</td>
<td>67.6</td>
</tr>
<tr>
<td>$\theta_{h,a}^{eq}$</td>
<td>degrees</td>
<td>44.9</td>
<td>44.9</td>
</tr>
<tr>
<td>$c$</td>
<td>dimensionless</td>
<td>17.3</td>
<td>17.3</td>
</tr>
<tr>
<td>$r$</td>
<td>min</td>
<td>120(^b)</td>
<td>120(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Fitted. \(^b\) Held fixed. \(^c\) Measured.
monomer units within this depth. Because the C1s binding energies for amines (~285.5) and amides (~288.5) are very close to the binding energies for CHx and C(O)O, respectively, this level of modification cannot be deconvoluted from the C1s spectrum.

The level of silicon detected on the tested surfaces was at or below the detection limit of the XPS instrument, indicating that the PLGA films formed a cohesive layer completely covering the glass substrate both before and after treatment.

To study the availability of the newly created surface groups for covalent binding, low molecular weight chitosan was used as a naturally nonadhesive amine-functional biological macromolecule. Two commonly used cross-linking systems, EDAC/NHS and DMA, were used to preactivate hydrolyzed and aminolyzed surfaces, respectively. Samples were rinsed thoroughly with 0.1 M acetic acid solution after treatment to ensure that only strongly bound chitosan remained attached.

The N 1s spectra for these treated surfaces are shown in Figure 10, and the corresponding nitrogen concentrations are shown in Table 4. Surfaces with adsorbed chitosan (c) were essentially indiscernible from the associated hydrolyzed or aminolyzed surfaces (b), indicating that physical adsorption of chitosan occurred only to a very low degree on all surfaces. Preactivation of the surfaces led to distinctly higher nitrogen concentrations (d). In all cases, the amount of
nitrogen present increased by 0.45–0.6 at. %. The atomic concentrations of carbon and oxygen also increased and decreased, respectively, consistent with the presence of approximately 5 mol % chitosan within the XPS sampling depth.

General Discussion. The thick polymer films currently used by many groups for testing of surface modification techniques are far from an ideal model for most tissue engineering scaffolds. These films, generally with thicknesses ranging from tens to hundreds of microns, are generally 1 or 2 orders of magnitude thicker than the fine structures within such scaffolds. Hence, many treatments which seem promising when tested on such models involve conditions which quickly destroy these structures. In addition, thick films prepared via the popular solvent casting technique with dichloromethane display surface features on a scale which has been shown to strongly influence cell–surface interactions as well as contact angle measurements. The dip-coating/annealing technique described here results in films with a thickness of 1–2 μm and roughness on the scale of a few nanometers. These very smooth films allow much easier determination of the physical and chemical effects of treatments, while their small thickness demands stringent control of conditions similar to those required to avoid structural collapse in a 3-dimensional situation.

Contact angle data showed that the level of modification quickly reached a limiting value, independent of time and concentration of modifying species, as expected given the chain-lysis mechanism of hydrolysis and aminolysis. The smaller drop in contact angle achieved by aminolysis in IPA compared to aminolysis in water can be explained by a lower equilibrium amine group density in the former. This may be due to increased solubility of PLGA fragments in IPA and possibly swelling of the molecular structure at the surface, allowing for easier disentanglement and removal of these fragments.

Using a relatively simple rate equation (eq 1), it was possible to combine all of the collected contact angle data for aqueous ED aminolysis and hydrolysis into a single self-consistent model. The model shows that, due to competition between aminolysis and hydrolysis at high pH and the nonlinear relationship between aminolysis rate and pH, the maximum amine group density for any given ED concentration is achieved at around pH 9.8, where approximately 50% of the amine is ionized (measured pK_a = 9.8 ± 0.05) and hence unreactive. Furthermore, the concentration of ED required to achieve 97% of the theoretical maximum amine group density is only 0.1 M.

Reducing the temperature of the aqueous aminolysis reaction from 20 to 0 °C results in an approximately 10-fold drop in aminolysis rate, and a 12-fold drop in the hydrolysis rate for any given pH. Although this indicates that reduced temperature is not a useful option for surface modification, it indicates that storage in neutral solution at reduced temperature may be a valid option for PLGA scaffolds; the calculated half-time for base hydrolysis at pH 7 and 0 °C is in excess of two years.

Without the use of further labeling techniques, the XPS C1s spectrum is of little or no use in analyzing surface
modifications of this type on ester-based polymers, because the very small changes caused are obscured by the underlying polymer peaks. The slight enrichment of CH found in the surface of most treated samples is not significant enough to draw any conclusions.

The amount of chitosan attached to the surfaces via covalent binding was somewhat lower than that reported using surface entrapment modification of PLLA. However, surface entrapment does not appear to be applicable to fine three-dimensional structures.

Although many groups working on surface modification in the past have aimed for very high levels of surface reactive groups on substrates such as PLGA, a simple analysis of the scale factors involved shows that this may not be necessary. Based on its crystal structure, human serum albumin, a relatively small protein, covers an area of approximately 100 nm², equivalent to roughly 400–600 PLGA monomer units, and hence, only a very low level of primary modification (<1%) is theoretically required to allow the attachment of a complete monolayer of protein via covalent binding. The data presented here shows that the level of surface coverage with active species achieved by hydrolysis or aminolysis is at least 1%, and possibly as high as 5%, which appears more than adequate for the purpose of further functionalization.

**Concluding Remarks**

Creation of active groups allowing covalent attachment of further species on the surface of a polymer such as PLGA is a necessary first step toward the creation of a truly biomimetic polymer-based scaffold for tissue engineering. Various methods for creation of these groups have been proposed in the past; however, these have generally displayed major problems in translating to a 3-dimensional structure of arbitrary size, shape, and structure. Soft tissue scaffolds in particular generally have large volumes, complex shapes and internal wall structures of submicron dimensions.

In this study, we have demonstrated the use of two different mild wet chemical techniques, controlled surface hydrolysis and aminolysis, to activate the surface of poly-(lactic-co-glycolic acid) (PLGA) with either carboxylic acid or primary amine groups. Using contact angle measurements, we have shown that the concentration of these active groups on the surface quickly reaches an equilibrium value independent of the modifying species concentration. It was shown that, in aqueous solutions of ethylenediamine (ED), conditions can be chosen under which the contribution of hydrolysis to the surface modification is negligible, even at remarkably low concentrations of ED.
The amine-functional polysaccharide chitosan is regarded by many as a candidate biomaterial in its own right.53–55 However, we have used it here simply as a normally nonadhesive model biomacromolecule for covalent binding studies. X-ray photoelectron spectroscopy (XPS) analysis showed that carboxyl- or amine-functionalized surfaces were readily available for covalent binding of chitosan using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC)/N-hydroxysuccinimide (NHS) or dimethyl adipimidate (DMA), respectively.

The PLGA films used as model surfaces throughout this study were no more than 2 μm thick, with RMS roughness values of <2 nm on untreated films. Although films treated in 0.01 N NaOH reached equilibrium contact angle values within 5 min, they were still intact after 24 h, indicating an extremely slow etching rate. Hence, these treatments may be carried out within even the most delicate porous structures, with little loss of mechanical strength.

To direct tissue growth into a tissue engineering scaffold, it is not sufficient to simply provide a surface on which cells grow well. Equally important is the retention of the phenotype of the tissue. To control this, the surface of the scaffold should ideally resemble as closely as possible the natural extracellular matrix of the desired tissue. Thus, specific and permanent binding of proteins to the scaffold surface appears to be a necessity. The modifications described here have been shown to allow this, using chitosan as a model, nonadhesive biomolecule. Furthermore, since the reaction rates are tunable over a wide range, there is no theoretical limit to the size or shape of scaffolds that can be treated via this method.

Acknowledgment. The authors thank CSIRO Molecular Science for kindly allowing the use of their laboratory and XPS facilities during this work. Thanks also to Dr. David Steele for his useful discussions and advice and to Roger Curtain for his expert help with the SEM. We also gratefully acknowledge the funding support of the Australian Research Council and the Particulate Fluids Processing Centre.

References and Notes


