Surface Engineering of Poly(lactic acid) by Entrapment of Modifying Species

Robin A. Quirk, Martyn C. Davies, Saul J. B. Tendler, and Kevin M. Shakesheff*

School of Pharmaceutical Sciences, The University of Nottingham, University Park, Nottingham, NG7 2RD, U.K.

Received September 23, 1999

Here, we describe a new method of engineering PLA surfaces based on the entrapment of molecules during the reversible swelling of the polymer surface region. This strategy may also have applications in the surface engineering of other polymeric materials. Such modifications may be required as the chemical properties of biodegradable polymer surfaces determine the nature of many interactions that occur within biological environments.[1,2] Poly(lactic acid) (PLA) and related poly(α-hydroxyacid)s are widely employed in biomaterial applications as resorbable sutures, scaffolds, and drug delivery devices. Recently, related developments in the fields of tissue engineering, biocompatibility, and drug delivery have required the immobilization of biologically active molecules on the surfaces of PLA-based devices.[3] Strategies for achieving this immobilization are limited for PLA because the chemical structure of this polymer lacks any functional groups for the covalent grafting of surface-modifying species.

The motivation for developing a new surface engineering strategy was the limitations inherent in existing strategies employed on PLA (and related polymers). The need to engineer PLA surfaces has been heightened by the exploitation of this polymer in new tissue engineering and drug delivery applications. These applications require the surfaces of the polymer either to present biological molecules that actively promote receptor-mediated interactions with cells[4,5] or to present molecules that change the distribution of protein adsorption in vivo.[6] One general strategy to immobilize the required surface-modifying species is to adsorb the molecule to the polymer surface. However, this approach requires that the surface-modifying species possess suitable surfactant properties and that adsorption does not restrict activity. An alternative strategy is to introduce functional groups, capable of supporting covalent grafting, into the surface chemical structure of the biodegradable polymer. This may be achieved by exposing the polymer to environments that alter the chemical structure of the surface without affecting bulk chemical structure, for example by the partial hydrolysis of the polymer surface to yield higher densities of hydroxyl end groups.[7] Another approach, exemplified by the synthesis of poly(lactic acid-*co*-lysine) (PLAL), is to redesign the polymer backbone so it contains monomer units with reactive side chains.[8,9] Such methods of introducing functional groups can be problematic because they alter the molecular weight of the polymer at the surface, introduce limited densities of the groups, or require the formation of a new polymer type.

* Corresponding author. E-mail: kevin.shakesheff@nottingham.ac.uk. Telephone: +44 115 951 5104. Fax: +44 115 951 5110.

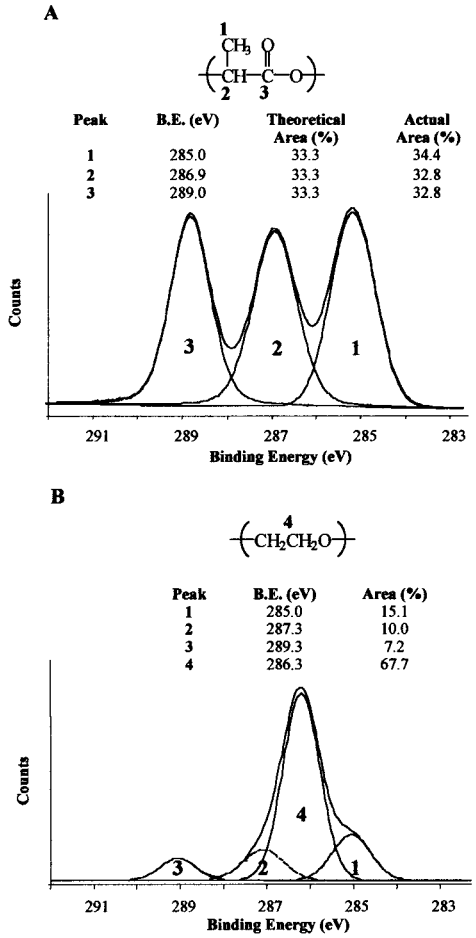

Figure 1. XPS scans of C 1s regions: (A) PLA before treatment; (B) PLA following PEG modification (10% TFE, 50% w/v PEG, 24 h immersion time).

Our strategy for the surface engineering of PLA involves the physical entrapment of a second polymer material and is related to a method devised by Hubbell for the modification of poly(ethylene terephthalate) (PET).[10,11] A similar approach has also previously been reported for the modification of various other nonbiodegradable polymers, for example attachments of A−B block copolymers onto poly(methyl methacrylate), poly(styrene), and poly(vinyl acetate) surfaces.[12] Our method is able to immobilize species that do not adsorb to PLA surfaces and does not require the introduction of new chemical groups at the polymer surface. The strategy uses a miscible mixture of a solvent and a nonsolvent for PLA, with the surface-modifying species being soluble in the mixture and the nonsolvent for PLA. The PLA material is exposed to the solvent/nonsolvent mixture, causing rapid polymer gelation at the surface. This swelling is then reversed by the addition of a large excess of the nonsolvent.

To demonstrate this surface engineering technique, we have modified PLA (Alkermes, MW 12,845, polydispersity 1.57) with high densities of poly(ethylene glycol) (PEG) (Polysciences Inc, MW 18 500, polydispersity 1.72), using 2,2,2-trifluoroethanol (TFE)/water as the solvent/nonsolvent mixture. Modification of PLA with PEG provides a good test of this method because there is negligible adsorption of PEG to the PLA surface (data

10.1021/ma9916133 CCC: $19.00
Published on Web 01/08/2000

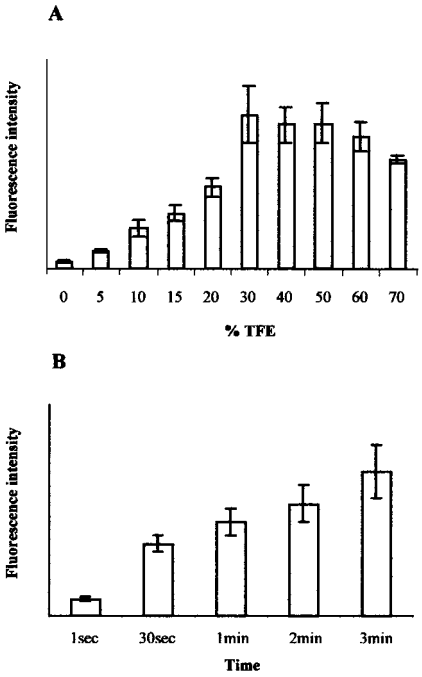

Figure 2. PEG−rhodamine incorporation into PLA films, as measured by fluorimetry: (A) effect of % TFE in solvent mixture (after 1 min exposure); (B) effect of solvent exposure time (20% TFE). (In both cases, $n = 8$.)

not shown); therefore, changes in surface chemistry must be attributed to nonadsorptive mechanisms. The X-ray photoelectron spectroscopy (XPS) (Scienta ESCA300) data in Figure 1 compares the C 1s region data from a PLA surface and a PLA surface after entrapment of PEG from 10% TFE/90% water mixture containing 50% polymer by weight during a 24 h exposure, followed by a 30 min washing in water. The unmodified PLA surface generates the three expected peaks with close to equal peak areas, indicating that this surface contains the three carbon regions of the PLA surface with no detectable contamination. After the surface entrapment procedure has been performed, there is a significant surface chemistry change, with the C 1s data being dominated by the ether peak from the PEG component. The XPS data shown in Figure 1 does not include the perfluorocarbon region (290−295 eV) as there is no evidence of such chemistry at the surface after TFE exposure. From the data, it is possible to estimate the ratio of ethylene glycol units to lactic acid units at the polymer surface. Using the average area of the three PLA peaks, this ratio is 3.1:1, equating to a PEG surface coverage of approximately 75%.

Control over the entrapment process is achieved by variations in either the solvent/non solvent ratio, the time of exposure of the polymer surface to this mixture, and/or the concentration of the surface-modifying agent. To determine the optimal conditions for surface engineering, we quantified the entrapment of fluorescently tagged PEG (PEG−rhodamine). This was accomplished by dissolving 1 cm^2 modified PLA films in TFE and measuring absorbance values with a fluorescence spectrophotometer (Hitachi F-4500). The relationships between both TFE concentration and time of solvent exposure on the amount of PEG incorporated are shown in the graphs in Figure 2. The amount of PEG−rhodamine incorporation increases when the TFE concentration is increased from 0% through to 30%. Further increasing the TFE concentration does not increase

Table 1. XPS Results for PLL-Modified PLA, Shown as the Percentage Nitrogen of Total Elemental Signal (Using 10% w/v PLL)

	% N signal after time in solvent		
% TFE	30 min	3 h	24 h
0	1.8	4.2	4.2
10	4.1	5.1	5.6
20	4.8	6.1	6.7
30	5.2	5.6	5.0
40	5.1	3.7	4.4

entrapment. This may be explained by PLA dissolution dominating the polymer−solvent interaction at TFE concentrations of about 30%.

Measurements of the entrapment of PEG−rhodamine also indicate that a rapid surface engineering process can be achieved, with entrapment occurring with a contact time between the PLA and the surface-modifying solution of only 30 s (see Figure 2b). XPS analysis of PLA surfaces after a short exposure time to the solution indicated that a rapid surface engineering approach may be viable, with an observed ethylene glycol to lactic acid ratio of around 0.6:1 (37% of surface) being achieved (10% w/v PEG, 50% TFE, 2 min contact time). Fluorescence microscopy studies confirm that a thin gel layer (less than 5 μm wide) forms instantly on contact between PLA and the TFE/water mixture and broadens with time as the solvent and fluorescent polymer ingress further into the PLA.

The fluorescence and XPS results confirm that PEG entrapment occurs within the gel layer formed by the TFE/water mixture. Low densities of PEG can be immobilized in the thin gel layer formed during short contact periods. Over extended periods of contact, entrapment increases but the modification becomes less surface specific.

In addition to the entrapment of PEG we have demonstrated that we can immobilize relatively high concentrations of poly(L-lysine) (PLL) (Sigma, MW 29 300, polydispersity 1.35). This surface engineering provides functional amine groups onto which biological ligands can be covalently coupled. Although PLL will adsorb to a PLA surface, using the same TFE/water solvent system we have shown that it is possible to enhance PLL surface concentrations. As with the PEG system, the amount of modifying material entrapped may be controlled by varying process parameters. The effect of varying polymer/solvent contact time and solvent/nonsolvent ratio as determined by XPS is shown in Table 1.

At the peak surface PLL level obtained (10% w/v PLL, 20% TFE, 24 h), the PLL to PLA monomer ratio is around 0.25 (20%). We estimate that this would far exceed the amount required for many tissue engineering applications, for example to support grafting of the cell adhesion ligand RGD.[13] The data again indicates that increasing the TFE concentration above 30% reduces entrapment efficiency.

Finally, applications of surface-engineered biodegradable polymers require the homogeneous lateral distribution of modifying species across the polymer surface. We investigated the distribution of PEG or PLL on PLA surfaces using time-of-flight secondary ion mass spectrometry (ToF−SIMS) (Physical Electronics PHI17200) to map the location of PEG-, PLL-, or PLA-specific ions. The resultant images are formed by raster scanning a liquid gallium ion beam across a given sample area, with a sampling depth of approximately 10 nm and a

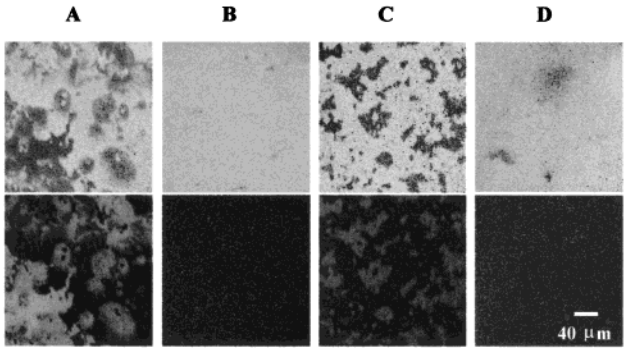

Figure 3. ToF−SIMS images, illustrating surface distribution of PLL on PLA under different conditions. The top row represents PLA (as shown by distribution of O, M − H, M + H, M + O − H and M + OH negative ions), and the bottom row depicts the distribution of PLL-specific ions (Br and CN negative ions). Key: (A) phase-separated control (drop-cast polymer blend, 50:50 PLA:PLL in 66:33 TFE:water); (B) PLL adsorption (10% w/v solution, 5 min); (C) PLL entrapment I (10% w/v PLL, 20% v/v TFE, 5 min); (D) PLL entrapment II (10% w/v PLL, 10% v/v TFE, 5 min).

lateral resolution of around 1 μm. While the entrapment of PEG produced a uniform surface distribution of the polymer (data not shown), this cannot be assumed when immobilizing PLL. The data in Figure 3 show typical PLL distributions following entrapment in PLA under varying conditions. Figure 3a shows a surface that was intentionally prepared to give a heterogeneous surface (prepared from a blend solution) to demonstrate the detection of phase separation on the micron scale. Figure 3b shows that PLL adsorption generates a homogeneous surface distribution. However, under conditions that swell the PLA surface for extended periods, the PLL appears to phase separate (Figure 3c). This phase separation can be inhibited by restricting the time of exposure of PLA to the TFE-containing solution, although this is at the expense of the total quantity of PLL entrapped (Figure 3d).

Therefore, it is possible to use surface entrapment as a method of enriching PLA surfaces with PEG, PLL, and potentially many other surface-modifying species. The surface coverage of the entrapped species are in excess of those required for many applications, and we predict this includes the stimulation of integrin-mediated cell adhesion. However, our results also indicate that some surface-modifying species will phase separate under prolonged engineering procedures, necessitating the validation of spatial distribution to determine the optimal conditions for surface engineering.

Acknowledgment. R.A.Q. thanks the BBSRC for Ph.D. Studentship funding. K.M.S. is an EPSRC Advanced Fellow and acknowledges support for surface engineering studies from the EPSRC.

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MA9916133