Synthesis and RGD Peptide Modification of a New Biodegradable Copolymer: Poly(lactic acid-*co*-lysine)

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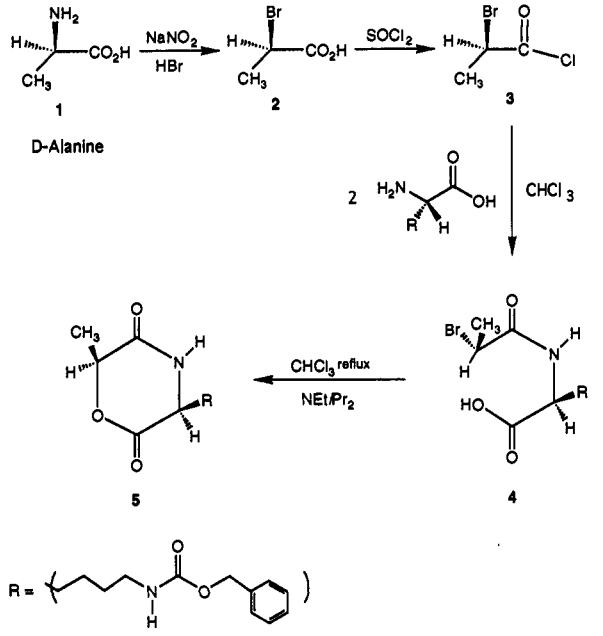
Tissue engineering holds great promise for the millions of patients who suffer tissue loss or organ failure.[1] One approach to replacing lost tissue function is attaching mammallian cells to natural or synthetic matrices and implanting the resulting device. A critical challenge to this approach is developing suitable matrix materials, because cell function and viability are greatly affected by the substrate.[2] Natural substances, e.g., fibronectin, contain information that supports cell adhesion and differentiated function.[3] However, these materials can suffer from batch-to-batch variations and processing and scale-up difficulties. Synthetic matrices can be manufactured on any scale, and properties (e.g., strength, molecular weight) can be tightly controlled. However, cellular function is often compromised since no natural recognition sites are available on the synthetic matrix surface.

Polylactic acid (PLA) is versatile, well characterized, and one of the few degradable materials used clinically (e.g., sutures).[4-6] We describe herein the synthesis of a new degradable polymer, poly(lactic acid-*co*-lysine) and the chemical attachment of the peptide GRGDY to the polymer's lysine residue. By grafting this biologically active peptide to the synthetic polymer, the advantages of synthetic and natural materials are combined.

Several groups have modified synthetic nondegradable polymers with biological moieties.[7-18] However, degradability may be important so that implanted cells can eventually obtain a completely natural environment, thereby eliminating the possibility of long-term detrimental tissue responses. Although several potentially degradable polyesters containing side chains with functional groups have been synthesized,[19-29] we are not aware of any synthetic degradable polymer containing ligands that regulate cell function.

Scheme I. Synthesis of the Cyclic Dimer of L-Lactic Acid and Protected L-Lysine



To produce a lactic acid/lysine copolymer, it was first necessary to synthesize a monomer that could polymerize by the same mechanism as the monomer used for PLA synthesis. An outline of the synthesis of 3-(N_ϵ-benzoxycarbonyl-L-lysyl)-6-L-methyl-2,5-morpholinedione (**5**) is provided in Scheme I.[30] During the final ring closure step, a minor amount of epimerization occurred. However, the diastereomeric purity of **5** was in excess of 95%.

The copolymerization of this new monomer **5**, which contains a protected lysine residue, and L,L-lactide **6** is shown in Scheme II. This reaction's first step is similar to glycine/lactic acid copolymerization.[31] The bulk copolymerization of **6** and **5** yielded number average weights (M_n) of 45 000 g/mol and weight average molecular weights (M_w) of 64 000 g/mol. The copolymer contained 1.3 mol % lysine residues as determined by ^{1}H NMR using the lysine protecting group's phenyl protons. (This quantity of lysine is equivalent to 2.6 mol % of **5** since this monomer contains one lactic acid residue and one lysine residue.) Differential scanning calorimetry yielded a T_g of 56 °C and a T_m of 157 °C. In comparison, PLA has a T_g of 62 °C and a T_m of 169 °C. By increasing the amount of **5** in the copolymerization,

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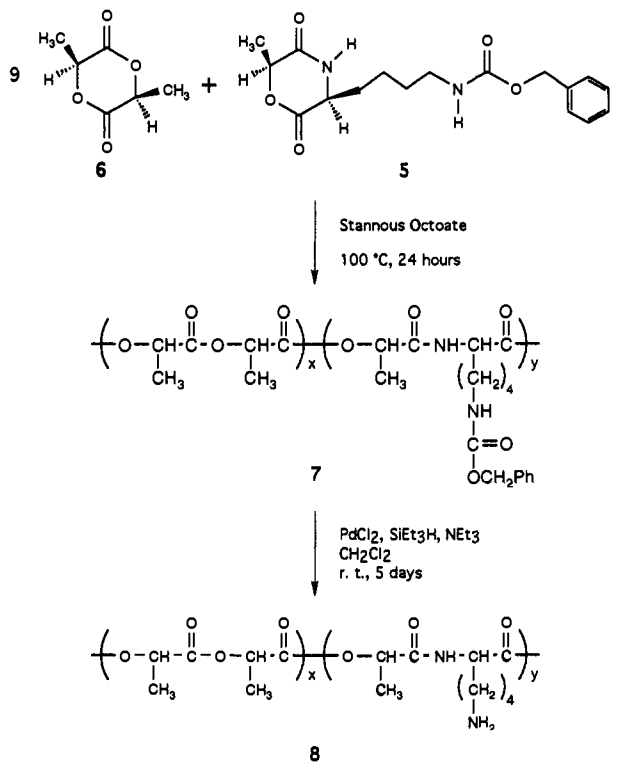
Scheme II. Synthesis of Poly(lactic acid-*co*-lysine)



up to 10 mol % of **5** could be incorporated into the polymer. However, the molecular weights of the lysine-rich polymers were lower. Increasing lysine content also disrupted crystallinity, yielding an amorphous polymer.

Lysine amino group deprotection was accomplished with a palladium chloride catalyst system. The extent of decarbamylation was 75%. During this step, M_w decreased from 64 000 to 40 000 g/mol, possibly due to hydrolysis that could occur over the long reaction times due to trace quantities of water. Amino acid analysis performed before and after deprotection indicated that lysine content decreased by 10–30%. This reduction can be attributed to the purification procedure which preferentially eliminates low molecular weight material. Since lower molecular weight material has been found to contain a slightly greater concentration of lysines, the overall lysine content decreases when lower molecular weight material is removed.

A colorimetric assay for primary amino groups[32] confirmed the presence of 0.35 mol % deprotected lysine residues. This amount of deprotected lysine is equivalent to 48 μmol of NH_2 groups/g of copolymer, yielding a surface density of 4800 fmol/cm^2 assuming a polymer density of 1 g/cm^3 and a 10-Å access layer.

The final deprotected polymer, poly(lactic acid-*co*-lysine), was insoluble in water, methanol, and ethanol; sparingly soluble in acetone, DMSO, DMF, dioxane, and ethyl acetate; and highly soluble in methylene chloride and chloroform. If M_n was above 25 000 g/mol, pliable translucent films could be obtained by solvent casting from chloroform. Poly(lactic acid-*co*-lysine) films degraded to half their original M_w in 5 weeks when exposed to pH 7.1 phosphate-buffered saline at 37 °C with agitation. In comparison, molecular weights of commercial poly(L-lactic acid) films took 15 weeks to degrade to half their original values.

The utility of the copolymer's primary amino groups in the copolymer was demonstrated by coupling a cell adhesion promoting peptide (GRGDY) to the copolymer using 1,1′-carbonyldiimidazole (CDI).[33] A significant challenge was finding a common solvent for the copolymer, the active moiety, and the linking reagent. A solvent that solubilized all three components was dimethyl sulfoxide (DMSO)/CH_2Cl_2.

Amino acid analysis demonstrated that GRGDY was coupled to the copolymer at a concentration of 3.1 μmol/g. However, during peptide immobilization, the primary amino group concentration dropped from 48 to 10 μmol of NH_2 groups/g of polymer. This additional decrease in NH_2 content beyond the decrease attributed to the peptide coupling reaction was postulated to arise from copolymer cross-linking, which is a side product of the coupling reaction. Evidence for cross-linking was obtained in a separate experiment where M_n increased from 27 000 to 40 000 g/mol and M_w increased from 40 000 to 59 000 g/mol. Additional side reactions could occur due to the reaction of CDI with the peptide's carboxylic acids. However, the reactivity of primary amino groups should be greater than that of carboxylic acid groups.

Very small concentrations of active peptides can have dramatic biological affects. A surface density of only 1 fmol/cm^2 of an RGD peptide effectively promotes cell adhesion to an otherwise nonadherent surface.[7] Assuming a density of 1 g/cm^3 and a 10-Å access layer, a film containing 3.1 μmol/g of peptide has a surface concentration of 310 fmol/cm^2 if the surface and bulk are the same. Therefore, by carefully selecting processing conditions, films made from this new degradable material should promote cell adhesion through specific adhesion receptors in the cell membrane. Other ligands possessing other cell regulatory functions could also be attached to poly(lactic acid-*co*-lysine) using this approach.

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Supplementary Material Available: Details of analytical techniques and experimental procedures including spectroscopic data (14 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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