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BIORESORBABLE HYDROGELS PREPARED FROM POLYLACTIDE/ POLY(ETHYLENE GLYCOL) BLOCK COPOLYMERS

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[Engineering of Biomaterials, 47-53,(2005),2-3]

Introduction

The delivery of drugs to a human body can be achieved through oral, transdermal, topical and parenteral administrations. A great deal of work has been done during the past two decades to develop controlled drug delivery systems (DDS) adapted to these various routes.Hydrogels are of growing interest for applications as DDS because of their excellent biocompatibility due to the presence of large amounts of water [1-3]. Bioactive molecules can be physically entrapped in a hydrogel or chemically attached to the polymeric network. Hydrogels are usually formed by a hydrophilic polymer matrix crosslinked chemically through covalent bonds or physically through hydrogen bonds, crystallized domains or hydrophobic interactions.They are particularly interesting for the release of poorly soluble drugs, proteins, genes or nucleic acids [4-6].
In this work, polylactide/poly(ethylene glycol) block copolymers were prepared by ring opening polymerization of L- or D-lactide in the presence of PEG, using non toxic zinc powder as co-initiator. Hydrogels were prepared from aqueous solutions containing both PLLA/PEG and PDLA/PEG copolymers. The rheological properties and drug release behaviors of the hydrogels were investigated.

Experimental

Copolymers were synthesized by ring opening polymerization of L- or D-lactide in the presence of dihydroxyl PEG with Mn of 10000, 12000 and 20000 or monomethoxy poly(ethylene glycol) (mPEG) with Mn of 5000 by using zinc metal as catalyst.
Hydrogels were prepared by mixing predetermined amounts of PLLA/PEG and PDLA/PEG copolymers in 2 ml of distilled water. Gelation was allowed to proceed at predetermined temperatures for various periods of time. Hydrogels containing bovine serum albumin (BSA) were prepared under similar conditions, BSA being mixed in the aqueous solution before gelation.
In vitro release experiments were realized at 37°C by immersing 2 ml of BSA-containing hydrogel in 4 ml of phosphate buffered saline (PBS). The release was regularly monitored by U.V. at 277 nm, using calibration curves obtained from standard solutions.
H Nuclear magnetic resonance (NMR) spectra were recorded at room temperature with a Bruker spectrometer operating at 250 MHz by using DMSO-d_6 as solvent [1]. Rheological properties were determined on a Carri-Med CSL2 Rheometer of TA Instruments. The release of BSA was monitored by a Lambda 15 Perkin Elmer UV-Vis spectrophotometer. Circular dichroism (CD) spectra were registered with a Jobin Yvon CD6 instrument.

Results and discussion

Acronym	Structure	Initiator	Monomer	EO/LA	DP_{PEG}	DP_{PLA}	M_n
1L	$L_{19}EO_{227}L_{19}$	PEG10000	L-lactide	6.1	227	38	12700
1D	$D_{20}EO_{227}D_{20}$	PEG10000	D-lactide	5.6	227	40	13700
2L	$L_{20}EO_{273}L_{20}$	PEG12000	L-lactide	6.8	273	40	14900
2D	$D_{19}EO_{273}D_{19}$	PEG12000	D-lactide	7.3	273	38	14700
3L	$L_{21}EO_{454}L_{21}$	PEG20000	L-lactide	11.0	454	42	23000
3D	$D_{22}EO_{454}D_{22}$	PEG20000	D-lactide	10.5	454	44	23100
4L	$L_{28}EO_{113}$	mPEG5000	L-lactide	4.1	113	28	7000
4D	$D_{27}EO_{113}$	mPEG5000	D-lactide	4.2	113	27	6900

TABLE 1. PLA/PEG block copolymers obtained by ring opening polymerization of L(D)-lactide in the presence of PEG or mPEG

PLA-PEG-PLA triblock copolymers were synthesized by ring opening polymerization of L(D)-lactide in the presence of dihydroxyl PEG, while PLA-PEG diblock copolymers were synthesized by using mPEG5000 as initiator. Non-toxic Zn powder was used as catalyst instead of stannous octoate which can be more or less cytotoxic. TABLE 1 presents the molecular characteristics of the triblock and diblock copolymers used in this work. The molar ratio of ethylene oxide/ lactyl (EO/LA) repeating units was in the range of 4 to 11 for the water solubility of the copolymers.
FIG.1A shows the evolution of storage modulus (G') and loss modulus (G") of a 14% 1L/1D solution as a function of time at 25°C and at a frequency of 1 Hz. Initially, G" was higher than G', the solution behaving as a viscoelastic solution. Both G' and G" slightly decreased at first and remained constant during the first 60 min. Beyond, the moduli increased continuously, G' increasing faster than G". A crossover point was observed at 7 h. After that, G' became higher than G" and a hydrogel was formed. FIG.1B shows the changes of storage and loss moduli of the 14% 1L/1D sample as a function of frequency at t=0 and t=24h. Both moduli increased with increasing frequency. At t=0, the storage modulus G' was higher than the loss modulus G", and both moduli increased almost linearly with frequency, which is characteristic of a viscoelastic liquid-like state. In contrast, at t=24h, G' became higher than G", and both moduli tended towards a plateau at high frequency, which can be assigned to formation of a tridimensional network.
Bovine serum albumine (BSA) was retained as a model drug for release studies. The protein was mixed with the copolymer solution before gel formation. Various BSA-containing hydrogels were prepared under different gelation conditions in order to elucidate the drug release behaviors. Figure 2A shows the BSA release profiles of 20% 2L/2D hydrogels obtained after 90 h at 37°C or after 24 h at 50°C. The release rate appeared almost constant and there was almost

no burst release in both cases. On the other hand, BSA release appeared faster from the hydrogel obtained at 37°C than from the one obtained at 50°C, even though the gelation time was longer for the former than for the latter. Nearly

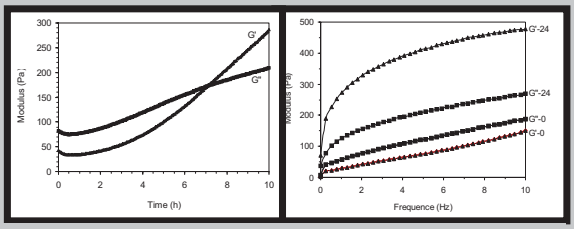

FIG. 1. A) Time-dependent changes of storage modulus (G') and viscous modulus (G") of a 14% 1L/1D sample at 25°C and at 1Hz; B) Changes of storage modulus (G') and loss modulus (G") of a 14% 1L/1D solution as a function of frequency at t=0 and t=24h at 25°C.

38% and 24% of BSA were released after 360 h, respectively. The difference can be assigned to the fact gelation at 50°C led to much more consistent hydrogel structure than at 37°C, which disfavored drug diffusion.

Circular dichroism (CD) was used to determine whether BSA molecules were denatured after the gelation procedure. Figure 2B shows the CD spectra of original BSA and of BSA released from the hydrogel obtained at 50°C. The two spectra appeared almost identical, indicating that the gelation procedure at 50°C did not denature BSA proteins.

Conclusion

Bioresorbable hydrogels were prepared from aqueous solutions containing both PLLA/PEG and PDLA/PEG block

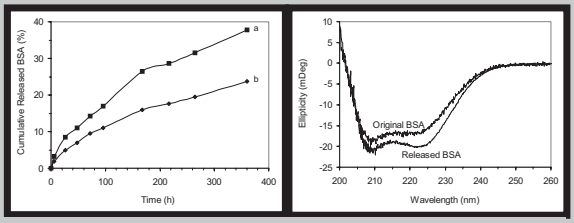

FIG. 2. A) drug release profiles of 20% 2L/2D hydrogels containing c.a. 40mg of BSA: a) gelation for 90h at 37°C, b) gelation for 24h at 50°C; B) Circular dichroism (CD) spectra of original BSA and of BSA released from the hydrogel obtained at 50°C.

copolymers due to interactions and stereocomplexation between PLLA and PDLA blocks. Rheological studies showed that both storage and loss moduli depend not only on the polymer properties such as the molar mass and EO/LA ratio, but also on the factors such as the concentration, temperature, time and frequency. The gelation process is time- and temperature-dependent and the hydrogel is a dynamic and evolutive system because of continuous formation/destruction of crosslinks and degradation. Drug release studies show that the release rate can be adjusted by changing the gelation conditions and factors such as drug load, polymer concentration and molar masses.

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ADHESION OF BONE AND VASCULAR CELLS ON CARBON FIBRE-REINFORCED CARBON COMPOSITES COATED WITH A FULLERENE LAYER

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Abstract

Carbon fibre-reinforced carbon composites (CFRC), i.e. materials promising for hard tissue surgery, were coated by a fullerene layer in order to strengthen the material surface and create its nanostructure pattern which is known to be attractive for colonization with bone cells. The fullerene layer was relatively resistant to wear, at least swabbing with cotton, rinsing with liquids and exposure to cells and proteolytic enzymes. Both human osteoblast-like MG 63 cells and rat vascular smooth muscle cells (VSMC) in 1- and 2-day-old cultures adhered to these surfaces in lower numbers in comparison with the control uncoated material and tissue culture polystyrene. In addition, the VSMC on the fullerene-coated surfaces were less spread. The lower cell adhesion was probably due to a relatively high hydrophobicity of fullerenes. On the other hand, the spreading of MG 63 cells was comparable to that observed on the control surfaces, and these cells also assembled dot-like vinculin-containing focal adhesion plaques and relatively rich fine filamentous beta-actin cytoskeleton. We suppose that the cell adhesion may be enhanced by derivatization of fullerenes with specific chemical functional groups or peptidic ligands for cell adhesion receptors.
[Engineering of Biomaterials, 47-53,(2005),3-6]

Introduction

Fullerenes are spheroidal molecules made exclusively of carbon atoms (e.g., C_{60}, C_{70}). Similarly as carbon nanotubes, and nanodiamonds, fullerenes are considered