JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **14** (2003) 705–711

Nanoparticle systems for the targeted release of active principles of proteic nature

R. SOLARO*, F. CHIELLINI, F. SIGNORI, C. FIUMI, R. BIZZARRI, E. CHIELLINI
Department of Chemistry and Industrial Chemistry, University of Pisa, via Risorgimento 35, 56126 Pisa, Italy
E-mail: rosola@dcci.unipi.it

The preparation and characterization of nanoparticles based on biodegradable/bioerodible polymers is reported. They have been designed for the controlled-targeted release of proteic drugs such as α-interferon and for the release of active principles in tissue engineering. The amenability of some of the prepared polymeric matrices to be used in the fabrication of micro and nano patterned scaffolds is also described.

Introduction

In the last decades, the design of polymeric materials for biomedical applications has been extensively investigated, thus leading to new concepts in the treatment of human diseases. The original uses of polymers in clinical medicine as essential components of permanent prosthetic devices are being replaced by the development of new strategies.

Advances in the production of new protein and nucleic-acid drugs has lead to the development of polymer-based controlled drug delivery and gene therapy [1]. Controlled drug delivery technology represents one of the most rapidly advancing areas of science in which polymer and pharmaceutical scientists are contributing to biomedical research field [2]. Delivery systems offer many advantages as compared to conventional dosage forms, including improved efficacy, reduced toxicity, improved patient compliance, and cost effective therapeutic treatment [3]. In particular, controlled release is strongly required for unconventional drugs, such as proteins and oligopeptides [4]. Indeed, conventional systemic administration of drugs is often characterized by unspecific body distribution, which gives rise to negative therapeutic index and unwanted side effects. If surface modification is possible, targeted delivery is achievable, resulting in enhancement of the therapeutic efficacy of the dosage forms and lowering of toxic effects [5].

The concept of tissue engineering, in which polymers are used to assist regeneration of 3D tissue and complex organ structures (bioartificial organs and bioreactors), stems from the major advances gained in cell and developmental biology combined with polymer science and engineering. The strategy to restore the structure and function of mammalian tissues by seeding cells into a supporting polymeric matrix poses however several problems. Mainly, cells in tissues and organs are arranged in distinct patterns, and the cell orientation depends on the purpose of the tissue [6]. During organ development, cues are given to the proliferating cells that dictate their final position and orientation. These cues can be chemical in nature, as in the presence of adhesion proteins or gradients of specific growth factors, or they can be purely physical such as a well-defined topography [7, 8]. At the same time, it is very well documented that topographical cues generated by the extracellular matrix may have significant effects upon cellular behavior [7, 9]. Indeed, the construction of fully biomimetic matrices containing all of the chemical and topographical factors required for perfect cell differentiation and morphogenesis and able to sustain time and space dependent variations in the concentration of the chemical cues is not yet achieved. In this respect, scientists are focusing on the design of new implantable materials in which both topographical features and chemical cues act synergistically for cell attachment and proliferation.

In the present paper, a study aimed at the formulation of protein-loaded nanoparticles as drug delivery systems and at the development of a soft-lithography technique for the fabrication of micropatterned scaffolds for tissue engineering applications is reported. Preliminary results will be also presented on the preparation of biomimetic matrices able to undergo topographical surface modification as well as to support a sustained release of tissue specific morphogens that will constitute the subject of a forthcoming paper.

Polymeric nanoparticles as drug delivery systems

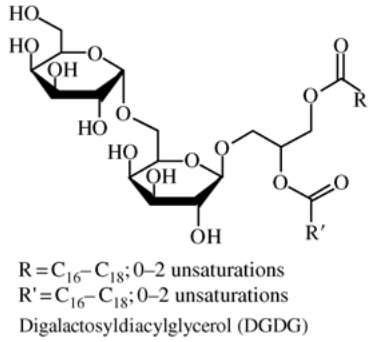
The possibility of achieving time and space controlled release of drug is a main challenge in drug delivery that drives much of current innovation in biomaterial science. Controlled delivery systems are generally diffusion-based release systems intended for systemic circulation or for localized drug administration. An alternative approach is to achieve drug release via degradation of the

*Author to whom all correspondence should be addressed.

polymer by using bioerodible polymers for nanoparticle formulations, which also should be structurally tailored for their physiological excretion after drug depletion. The choice of the polymer is pivoted and constrained by potential drug-polymer interactions, especially in the case of protein drugs [1]. Moreover, body distribution and targeting attitude of nanoparticles are dramatically affected by the size and surface characteristics of the particles in terms of hydrophilic–hydrophobic balance, surface charge, and presence of site-specific components [10]. Nanoparticles can be obtained by several techniques, starting either from monomers or preformed polymers, depending on the nature of the polymeric material and on the characteristics of the drug to be loaded [11].

Nanoparticles based on bioerodible polymer matrices

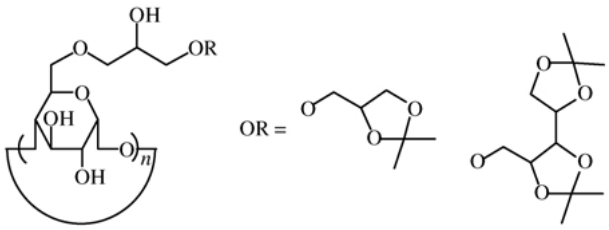
In the framework of a long-standing research [12–18] the formulation of hybrid nanoparticles based on synthetic polymer-protein matrices for the targeted release of α-interferon (IFNα), a powerful antiviral drug was undertaken [19]. Nanoparticles were prepared by using a combination of synthetic polymers, human serum albumin (HSA), a steric stabilizer, and a targeting moiety. A series of alkyl hemiesters of alternating copolymers of maleic anhydride with alkyl vinyl ethers of oligo(ethylene glycol) (PAM) was chosen as the synthetic component (Scheme 1). These materials displayed a high versatility to combine with proteins in different proportion and to provide hybrid bioerodible matrices without any adverse effect on the structure and activity of proteins [12]. Alternating copolymers of maleic anhydride with alkyl vinyl ethers hemiesterified with oligo(ethylene glycol) (VAM) were also prepared. Cytotoxicity assays carried out on 3T3 mouse embryo fibroblasts showed a fairly low toxicity of the synthetic polymers (IC_{50} 0.5–0.7 g/l). In addition, their combinations with human serum albumin/α-interferon mixtures did not give any negative response in both *in vitro* and *in vivo* biocompatibility tests, including platelet aggregation, complement activation, acute toxicity, and acute thromboembolic potential [20]. Moreover, since IFNα is being used in the therapy of myeloproliferative diseases and acute viral hepatitis [21, 22], the asialofetuin receptor present on liver hepatocytes [23] was selected as the target site of choice. Accordingly, a coating of digalactosyl diacyl glycerol (DGDG) (Formula 1), a natural glycolipid selectively recognized by this receptor was used for the targeting of hepatocytes.



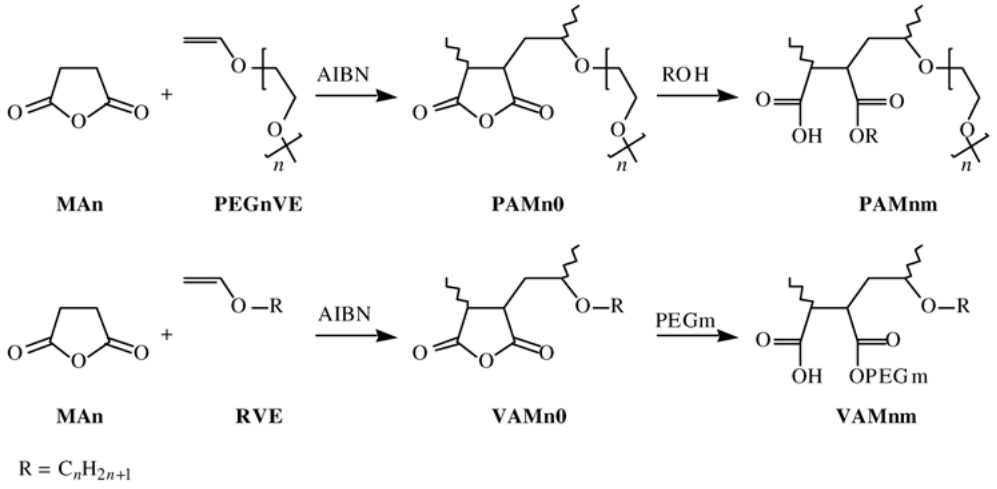
Formula 1 Digalactosyldiacylglycerol (DGDG).

The choice of the nanoparticle stabilizer stemmed from the necessity of coating the hybrid formulates by a strongly hydrophilic shell in order to minimize opsonization by blood proteins. In this respect, modified β-cyclodextrins grafted with the glycidyl ethers of protected polyols (*iso*propylideneglycerol and di*iso*propylidenepentitols) (Formula 2) appeared rather promising components for their amphiphilic character, connected to the presence of an hydrophobic pocket and an external hydrophilic shell with an amplified number of hydroxyl groups [13].

Regarding the nanoparticles formulation, a new proprietary method, based on co-precipitation technique, was developed during the fulfillment of a research project funded by the European Community under the



Formula 2.



Scheme 1 Synthesis of hemiesters of alternating copolymers of maleic anhydride with alkyl vinyl ethers.

TABLE I Preparation of nanoparticle suspensions

Polymer[a]		Modified β-cyclodextrin[b]		HSA[b,c]
Type	(mg)	Type	(mg)	(mg)
PAM14	156	GDA-βCD	389	25
PAM14	50	GDA-βCD	125	10
PAM14	156	GDX-βCD	389	25
PAM14	50	GIG-βCD	125	10
PAM14	50	GDA-βCD	125	10
PAM14	78	GDX-βCD	194	13
PAM14	50	GIG-βCD	125	10
PAM14	50	GDA-βCD	125	10
PAM14	78	GDX-βCD	194	13
PAM14	50	GIG-βCD	125	10
VAM41	117	GDA-βCD	292	15
VAM41	25	GDX-βCD	63	5
VAM42	78	GDA-βCD	194	10
VAM42	25	GDX-βCD	62	5
VAM43	25	GDA-βCD	63	5
VAM43	39	GDX-βCD	97	6
DP75	10	—	—	10
DP75	10	GIG-βCD	50	10
DP75	20	GIG-βCD	50	10
DP75	50	GIG-βCD	250	20

[a]In 4 : 1 ethanol/water mixture (5 or 7.78% solution); DP75 = poly(malic acid-*co*-propiolactone). 75 : 25.
[b]In distilled water (5% solution);
GDA = glycidyldi*iso*propylidenarabitolyl-β-cyclodextrin,
GDX = glycidyldi*iso*propylidenxylitolyl-β-cyclodextrin,
GIG = glycidyl*iso*propylidenglyceryl-β-cyclodextrin.
[c]Human serum albumin.

Brite-Euram Program [24, 25]. This technique does not imply the use of chlorinated solvents and of strong shear mixing, which are both known to cause appreciable protein denaturation [26, 27]. The co-precipitation method is based on the dropwise addition of a solution of the synthetic polymer in water-miscible organic solvents to an aqueous protein solution, under gentle magnetic stirring. The progressive interaction between the water insoluble polymer and the protein gives rise to nanoparticles formation. The glycolipid is then added as an aqueous dispersion to the resulting suspension. No sedimentation was observed after several weeks of storage at room temperature. Dimensional analysis of the prepared nanoparticle suspensions, performed by a Coulter size analyzer, indicated that their average diameter was 130–150 nm with 0.1–0.3 polydispersity index (Table I). Purification of nanoparticles (Fig. 1) was carried out by repeated centrifugation and washing with water.

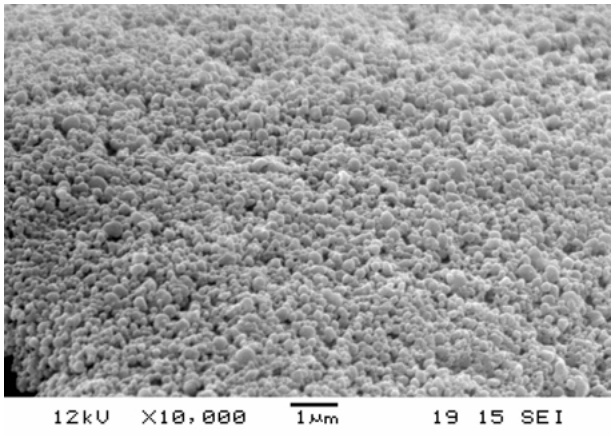


Figure 1 SEM micrograph (10 000 ×) of lyophilized nanoparticles.

TABLE II Inhibition of erythrocyte agglutination by ricine in the presence of the centrifugation products of microsphere suspensions

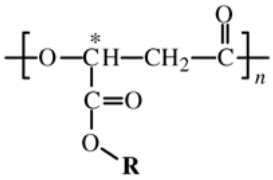
Sample	Fraction	Dilution[a]			
		Human erythrocytes		Guinea pig erythrocytes	
		1 UA	4 UA	1 UA	4 UA
Sample 1	1st supernatant	< 1 : 2	1 : 2	> 1 : 2	> 1 : 2
	2nd supernatant	1 : 4	1 : 2	1 : 8	1 : 8
	3rd supernatant	1 : 2	1 : 2	1 : 4	1 : 4
	pellet	1 : 8	1 : 8	1 : 32	1 : 32
Sample 2	1st supernatant	1 : 16	> 1 : 8	1 : 128	1 : 64
	2nd supernatant	1 : 8	1 : 2	1 : 48	1 : 32
	3rd supernatant	> 1 : 2	> 1 : 2	1 : 6	1 : 4
	pellet	1 : 2	> 1 : 2	1 : 6	1 : 4
Sample 3	1st supernatant	> 1 : 2	> 1 : 2	1 : 4	1 : 2
	2nd supernatant	> 1 : 2	> 1 : 2	1 : 2	> 1 : 2
	3rd supernatant	> 1 : 2	> 1 : 2	1 : 2	> 1 : 2
	pellet	1 : 32	1 : 4	1 : 256	1 : 32

[a]Maximum dilution still able to inhibit the hemoagglutination of erythrocytes in the presence of either 1 or 4 UA of ricine.

In order to evaluate the bioactivity and targeting efficacy of the prepared nanoparticles, a set of *in vitro* and *in vivo* experiments was carried out. The presence of exposed galactosyl residues on the particle surface was evidenced by *in vitro* hemoagglutination inhibition test, based on the agglutination of red blood cells induced by ricine, a lectine from *Ricinus communis* that is characterized by a strong affinity toward galactose [28]. Tests were carried out on the pellets and supernatants obtained by centrifugation of nanoparticle dispersions containing DGDG (Sample 1), galactosylated serum albumin (Sample 2), and asialofetuin (Sample 3) as targeting moiety (Table II). Experiments were performed by using either guinea pig or human erythrocytes and ricine solutions at concentrations of 1 and 4 UA, where 1 UA is the minimum ricine concentration required for the agglutination of 1% red cell suspension. The agglutination unit (1 UA) resulted to be 1 : 128 and 1 : 512 for human and guinea pig erythrocytes, respectively. Galactosyl groups present on the surface of purified nanoparticles dispersions containing DGDG or asialofetuin effectively inhibited the hemoagglutination process by competitively interacting with ricine receptors (Table II). Furthermore, in order to test the ability of galactose-labeled particles to actively target hepatocytes, some preliminary experiments were carried out by flow cytofluorimetry, by using primary cultures of rat hepatocytes and nanoparticles prepared with fluoresceinated human serum albumin. Preliminary information on the *in vivo* biodistribution of radio labeled nanoparticles, obtained from experiments carried out on rabbits [29] nicely confirmed the selective liver uptake. In particular, results indicated an almost quantitative, fast liver uptake of labeled nanoparticles followed by rather slow accumulation of the radioactivity in kidneys and bladder. This could be a consequence of the metabolic degradation of the nanoparticles which allows labeled albumin or its by-products to enter again the vascular compartment where it is degraded and then excreted via the kidneys.

Nanoparticles based on biodegradable polymer matrices

Biodegradable polymers represent a valuable option for the formulation of drug delivery systems, since they address the severe problem of the removal of the releasing device after drug depletion. In addition, most biodegradable polymers originate from natural and semi-natural monomeric precursors, thus improving the biocompatibility of the initial polymeric material and its degradation products. Among biodegradable materials, poly(malolactonate)s (Formula 3) represent interesting materials for biomedical uses, since they are biocompatible [30], degrade to non-toxic malic acid under physiological conditions [31], and contain reactive groups. The side-chain carboxyl moieties can be functionalized to obtain a large set of polymers and copolymers with different hydrophobic/hydrophilic balances [32, 33] that already proved useful for the realization of biocompatible devices [34–38]. The chiral center in the poly(malolactonate) chains may be further exploited to tune polymer properties and bioactivity. Convenient procedures for the preparation of both malolactone monomers and the corresponding polymers have been developed starting from commercially available natural products. Significantly, preparation of nanospheres with diameters below 500 nm from poly (alkyl malolactonate)s was recently reported in literature [38]. In the perspective of the preparation of functional biodegradable nanospheres, a series of polyesters with different hydrophilic–hydrophobic balance were prepared by anionic ring opening homo and copolymerization of the synthesized β-malolactonates [39].



Formula 3.

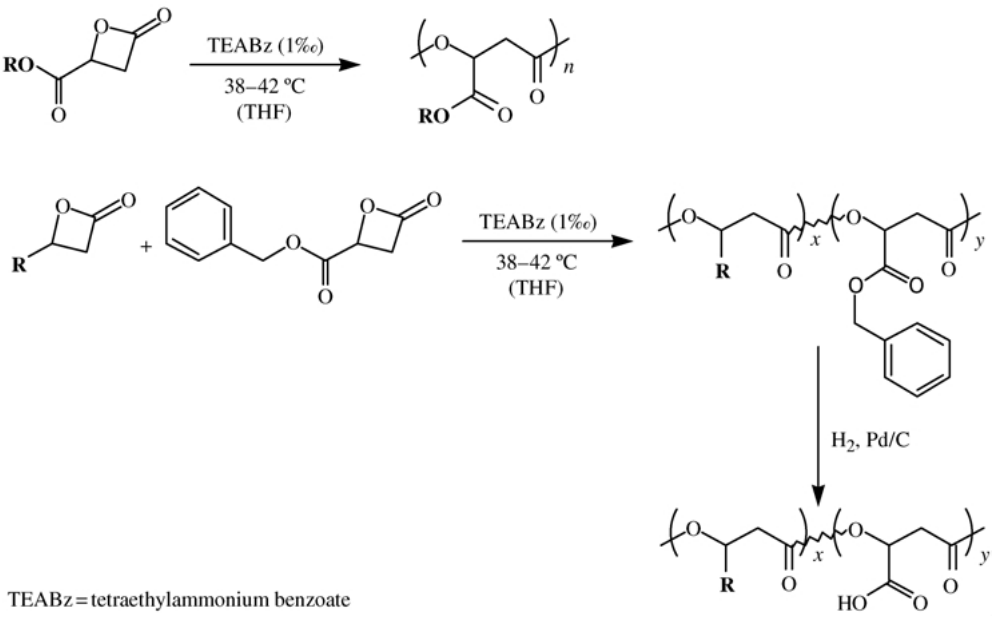
Copolymers of benzyl malolactonate with commercially available β-lactones, such as β-propiolactone, were also prepared (Scheme 2). It is worth noting that, throughout all the copolymerization experiments, benzyl malolactonate was kept as first comonomer, due to the possibility of selective hydrogenolytic removal of side benzyl groups, thereby leading to polycarboxylate structures. The *in vitro* cytotoxicity of the malolactone copolymers resulted to be remarkably low (IC_{50} 1.5–3.0 g/l) [40]. A further series of nanoparticle suspensions was prepared by using malolactonate polymers of different hydrophilic–hydrophobic balance as synthetic polymer component. Also in this case, experiments were performed by the co-precipitation technique. In most cases, formation of 100–150 nm nanopheres was observed.

Polymeric hydrogels for tissue engineering

Synthetic polymeric materials are used in tissue engineering for a wide variety of applications, including physical supports for the creation of functional tissues. In order to encourage a successful tissue growth, scaffolds must incorporate bioactive molecules such as growth factors that are released during tissue development. They must also be able to drive cell adhesion, orientation, and migration thanks to an appropriate surface topography. Among the different classes of biomedical materials, polymeric hydrogels have gained increasing attention thanks to their ascertained biocompatibility [41], and one of their most popular application dates back in the 1960s with the use of 2-hydroxyethyl methacrylate (HEMA) hydrogels as basic materials for contact lenses [42].

Surface microstructured HEMA based hydrogels

Fundamental knowledge of cell-substrate interactions is a key point in tissue engineering, since it may explain differences in cell behavior observed *in vivo* and *in vitro*. Independent of biochemistry, topographical cues gener-



Scheme 2 Synthesis of β-lactone polymers and copolymers.

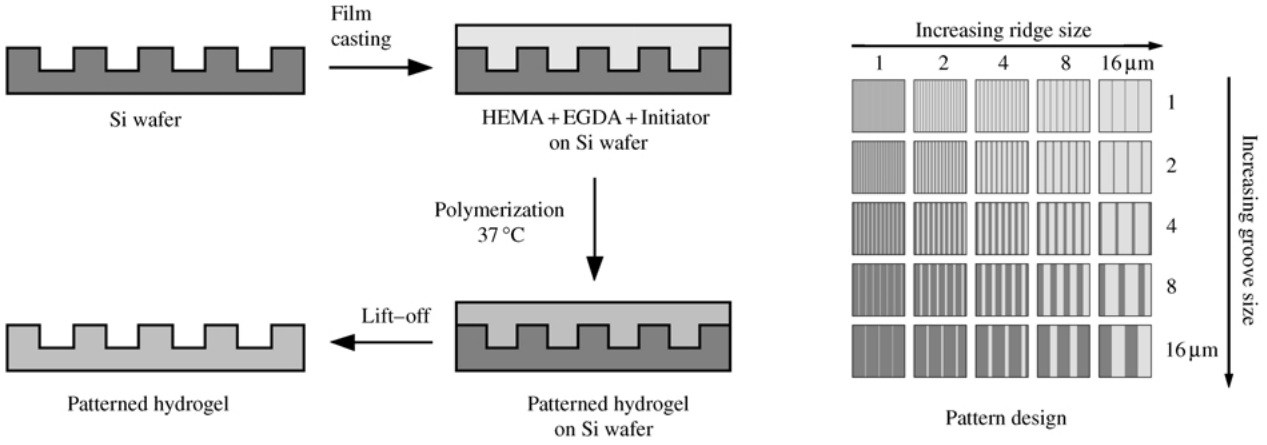


Figure 2 Schematic representation of hydrogel patterning by soft lithography.

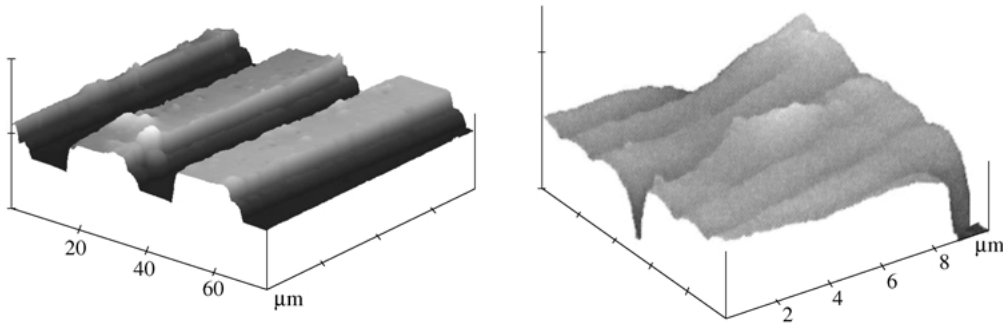


Figure 3 AFM pictures of dry hydrogel patterns: 16 µm grooves and 8 µm ridges (left) and 1 µm grooves and 1 µm ridges (right).

ated by the extracellular matrix may have significant effects upon cellular behavior [9]. To gain insight into these interactions a method to prepare surface microstructured HEMA based hydrogels was investigated. Hydrogels were prepared by radical polymerization of HEMA with ethylene glycol dimethacrylate (EGDMA) as cross-linking agent at 37 °C for 4 h, in the presence of 0.75% of an equimolar water solution of ammonium persulfate/sodium metabisulfite as redox initiator [43]. Hydrogels with microstructured surfaces were obtained by polymerizing the monomer mixture directly onto patterned silicon wafers; pattern designs consisted of 25 square regions of ridges and grooves with increasing size from 1 to 16 µm (Fig. 2) [44]. The lift-off of the polymerized hydrogel from the silicon wafer represented a major problem, as the applied mechanical stresses altered the patterns. Therefore, the silicon surface was modified by soaking the wafer into a 1% solution of 1H,1H,2H,2H-perfluorooctyltrichlorosilane in dichloromethane for 22 h. The primed silicon wafer was attached to a silanized glass plate and covered with a second silanized glass plate separated by a 1 mm thick silicon spacer. The monomers-initiator mixture was injected between the two glass plates and cured at 37 °C for 4 h. At the end of the polymerization, the glass in contact with the polymerized hydrogel was removed and the hydrogel was allowed to swell in water for at least 12 h. The swelling process combined with the highly hydrophobic surface of the primed silicon wafer made possible to lift off the surface patterned hydrogel without the application of even minor mechanical stress.

Surface patterned HEMA hydrogels were analyzed in the dry state by scanning electron microscopy (SEM). This technique gave a nice picture of the size and morphology of the patterned microstructure. However, the exposure of the hydrogel to an electron beam caused deterioration of the soft material during analysis, thus somewhat altering the morphology of the pattern.

A more accurate analysis of the dry patterned hydrogels was carried out by atomic force microscopy (AFM) (Fig. 3). Results of this investigation indicated that grooves in the hydrogels are always 10–20% smaller than those printed on the wafer master. Moreover, the edges of the ridges are round rather than squared, and the surface is usually rough. Nevertheless, the overall morphology is very well preserved. However, patterns containing 1 µm features showed morphology collapse, indicating that the hydrogel structure is not strong enough to preserve features of sub-micron size.

It is worth noting that apart from a 20% size increase, no significant modification of the surface pattern occurred when the hydrogels were swollen in water, as indicated by optical microscopy (Fig. 4).

Concluding remarks

The modern vision in biomedical sciences and technology, that is more and more oriented to profit of autologous transplant and regeneration of damaged tissues and organs, is demanding a close marriage between life sciences and material science.

The present contribution is aimed at highlighting some of the fundamental characteristics that polymeric

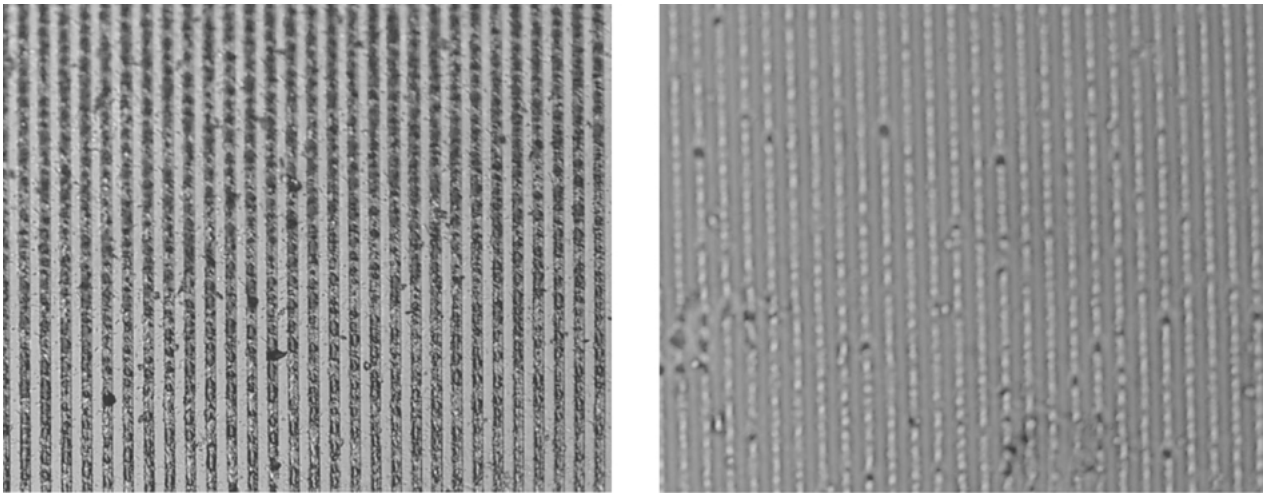


Figure 4 Optical micrographs of the surface pattern (8 μm grooves and 8 μm ridges) of dry (left) and water swollen (right) hydrogel.

materials designed to be classified as biomaterials in the future trend of biomedical applications, must possess.

In particular, an example has been reported of the potential combination of smart delivery systems amenable to nanotargeting of non conventional drugs with the tissue engineering technology, requiring multifunctional biodegradable/bioerodible scaffolds.

Series of bioerodible and biodegradable material with specific structural and functional features have been shown to be suitable for formulations allowing for the fabrication of micro/nano devices for potential modern biomedical applications.

The above mentioned methodology stems from the soft-lithography techniques used in microfabrication. It allows for a straightforward preparation of surface-patterned hydrogels, thus appearing promising for the preparation of micromachined polymeric scaffolds able to reproduce the topographical intricacies typical of living tissues.

Acknowledgments

This work was performed with the financial support by Brite-Euram project BE-7052, BRE2.CT94.0530 and by MIUR Cofin project.

References

1. L. G. GRIFFITH, *Acta Mater*. **48** (2000) 263.
2. K. E. UHRICH, S. M. CANNIZZARO, R. S. LANGER and K. M. SHAKESHEFF, *Chem. Rev*. **99** (1999) 3181.
3. R. S. LANGER, *Nature* **392** (1998) 5.
4. A. VILA, A. SANCHEZ, M. TOBIO, P. CALVO and M. J. ALONSO, *J. Controlled Release* **78** (2002) 15.
5. K. S. SOPPIMATH, T. M. AMINABHAVI, A. R. KULKARNI and W. E. RUDZINSKI, *ibid*. **70** (2001) 1.
6. A. S. G. CURTIS and M. RIEHLE, *Phys. Med. Biol*. **46** (2002) R47.
7. C. D. W. WILKINSON, M. RIEHLE, M. WOOD, J. GALLAGHER and A. S. G. CURTIS, *Mat. Sci. Eng*. **19** (2002) 263.
8. W. M. SALTZMAN and W. L. OLBRICHT, *Nature Rev*. **11** (2002) 177.
9. R. G. FLEMMING, C. J. MURPHY, G. A. ABRAMS, S. L. GOODMAN and P. F. NEALEY, *Biomaterials* **20** (1999) 573.
10. J. KREUTER, in ''Colloidal Drug Delivery Systems'', edited by J. Swarbrick (Marcel Dekker Inc., New York, 1994).
11. E. CHIELLINI, E. E. CHIELLINI, F. CHIELLINI and R. SOLARO, *J. Bioact. Compat. Polym*. **16** (2001) 441.
12. E. CHIELLINI, R. SOLARO, G. LEONARDI, D. GIANNASI, R. LISCIANI and G. MAZZANTI, *J. Controlled Release* **22** (1992) 273.
13. R. SOLARO, S. D'ANTONE, L. BEMPORAD and E. CHIELLINI, *J. Bioact. Compat. Polym*. **8** (1993) 236.
14. E. CHIELLINI, L. BEMPORAD and R. SOLARO, *ibid*. **9** (1994) 152.
15. R. SOLARO, E. DOSSI, E. CHIELLINI and G. MAZZANTI, *ibid*. **12** (1997) 27.
16. R. BIZZARRI, R. SOLARO and E. CHIELLINI, *ibid*. **14** (1999) 504.
17. S. MIERTUS, A. C. NAIR, V. FRECHER, E. CHIELLINI, F. CHIELLINI, R. SOLARO and J. TOMASI, *J. Incl. Phenom. Macrom. Chem*. **34** (1999) 69.
18. E. CHIELLINI, R. BIZZARRI, P. BONAGUIDI, P. TALAMELLI and R. SOLARO, *J. Macromol. Sci.-Pure Appl. Chem. A* **36** (1999) 901.
19. E. CHIELLINI, G. LEONARDI, R. SOLARO, R. LISCIANI and G. MAZZANTI, PCT to Istituo Sierovaccinogeno Italiano. Eur. Pat. EP0, 509, 968 (1992).
20. E. CHIELLINI, E. E. CHIELLINI, F. CHIELLINI and R. SOLARO, in ''Polymeric Drugs and Drug Delivery Systems'', edited by R. M. Ottenbrite and K. S. Wang (Technomic Publishing, Lancaster, PA, 2001).
21. V. BOCCI, *Clin. Pharmacokinet*. **21** (1991) 274.
22. C. H. SPIRIDOMIDIS, *Drugs of Today* **27** (1991) 35.
23. G. ASHWELL and J. HARFORD, *Ann. Rev. Biochem*. **51** (1982) 531.
24. A. CARLSSON, E. E. CHIELLINI, F. CHIELLINI, J. COWDALL, J. DAVIES, G. MAZZANTI, M. ROBERTS, E. SÖDERLIND and R. SOLARO, Ital. Pat. IT1293087 (1997); PTC WO9902131 (1999).
25. A. CARLSSON, E. CHIELLINI, E. E. CHIELLINI, J. COWDALL, J. DAVIES, G. MAZZANTI, M. ROBERTS, E. SÖDERLIND and R. SOLARO, Ital. Pat. IT1293088 (1997); PCT WO9902135 (1999).
26. V. H. LEE, in ''Peptide and Protein Drug Delivery'', edited by V. H. Lee (Dekker, New York, 1991).
27. J. L. CLELAND and A. J. S. JONES, US Pat. Appl. US/94/01666; WO Pat. Appl. 94/19020 (1994).
28. H. YOSHIOKA, T. OHMURA, M. HASEGAWA, S. HIROTA, M. MAKINO and M. KAMIYA, *J. Pharm. Sci*. **82** (1993) 273.
29. E. CHIELLINI, E. E. CHIELLINI, F. CHIELLINI and R. SOLARO, *Polym. Prepr*. (ACS) **39** (1998) 182.
30. P. FOURNIÉ, D. DOMORAUD, P. GUERIN, C. BRAUD, M. VERT and R. PONTIKIS, *J. Bioact. Compat. Polym*. **7** (1992) 113.
31. C. BRAUD and M. VERT, *Polym. Bull*. **29** (1992) 177.
32. S. CAMMAS-MARION and P. GUERIN, *Macromol. Symp*. **153** (2000) 167.
33. C. BARBAUD, S. CAMMAS-MARION and P. GUERIN, *Polym. Bull*. **43** (1999) 297.

34. S. CAMMAS-MARION, M. BEAR, A. HARADA, P. GUERIN and K. KATAOKA, *Macromol. Chem. Phys.* **201** (2000) 355.
35. S. CAMMAS-MARION, M. BEAR, L. MOINE, R. ESCALUP, G. PONCHEL, K. KATAOKA and P. GUERIN, *Int. J. Biol. Macromol.* **25** (1999) 273.
36. L. MOINE, S. CAMMAS, C. AMIEL, E. RENARD, B. SEBILLE and P. GUERIN, *Macromol. Symp.* **130** (1998) 45.
37. H. ROSSIGNOL, M. BOUSTTA and M. VERT, *Int. J. Biol. Macromol.* **25** (1999) 255.
38. S. STOLNIK, M. C. DAVIES, L. ILLUM, S. S. DAVIS, M. BOUSTTA and M. VERT, *J. Controlled Release* **30** (1994) 57.
39. R. BIZZARRI, F. CHIELLINI, R. SOLARO, E. CHIELLINI, S. CAMMAS-MARION and P. GUERIN, *Macromolecules* **35** (2002) 1215.
40. F. CHIELLINI, C. FIUMI, F. SIGNORI and C. BOGGI, ''Proceedings of the International Meeting on Advanced Frontiers in Polymer Science'' (Pisa, Italy, 2002) 23.
41. M. S. JHON and J. D. ANDRADE, *J. Biomed. Mater. Res.* **7** (1973) 509.
42. O. WICHTERLE and D. LIM, *Nature* **185** (1960) 117.
43. F. CHIELLINI, F. PETRUCCI, E. RANUCCI and R. SOLARO, *J. Appl. Polym. Chem.* **85** (2002) 2729.
44. F. CHIELLINI, R. BIZZARRI, C. K. OBER, D. SCHMALJOHAN, R. SOLARO and E. CHIELLINI, *Macromol. Rapid Commun.* **22** (2001) 1284.

Received 1 October 2002
and accepted 15 January 2003