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A Novel Biodegradable and Biocompatible Ceramer Prepared by the Sol–Gel Process

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A biomaterial is a nonliving material used in a medical device which is intended to interact with a biological environment.[1] It may be "bioinert", "bioactive", or "resorbable".[2] Biomaterials are commonly based on one of the traditional construction materials, metal, ceramic, or organic polymer, that have distinct property advantages and equally distinct limitations. For example, most organic polymers have an opposite set of characteristics as compared with ceramics. A possible answer to this dilemma is to design, synthesize, and produce entirely new materials that combine properties of at least two traditional materials. In this respect, the sol–gel process provides a unique opportunity of creating hybrid materials that intimately associate both inorganic and organic constitutive components into a two-phase morphology, under mild processing condition. Depending on the constitutive organic and inorganic components, the phase morphology and the possible covalent bonds between the phases, a large range of properties can be made available from elastomeric to high-modulus materials.

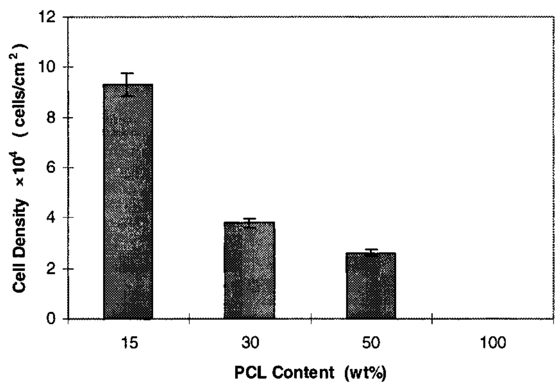

Figure 1. Density of fibroblasts for ceramers of different PCL contents (20 h culture).

Poly(ε-caprolactone) (PCL) is well-known for a unique set of properties, i.e., biocompatibility, permeability, and biodegradability. The range of these properties is advantageously increased by copolymerization with lactides and glycolide, which accounts for widespread applications in medicine, such as biodegradable sutures, artificial skin, resorbable prostheses, and containers for sustained drug release.[3] Depending on the polymerization mechanism, PCL can be end-capped with a

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(2) Hench, L. L. *J. Am. Ceram. Soc.* **1992**, *74*, 1487.
(3) Pitt, C. G.; Marks, T. A.; Schindler, A. *Biodegradable Drug Delivery Systems based on Aliphatic Polyesters: Application of Contraceptives and Narcotic Antagonists*, in *Controlled Release of Bioactive Materials*; Baker, R., Ed.; Academic Press: New York, 1980.

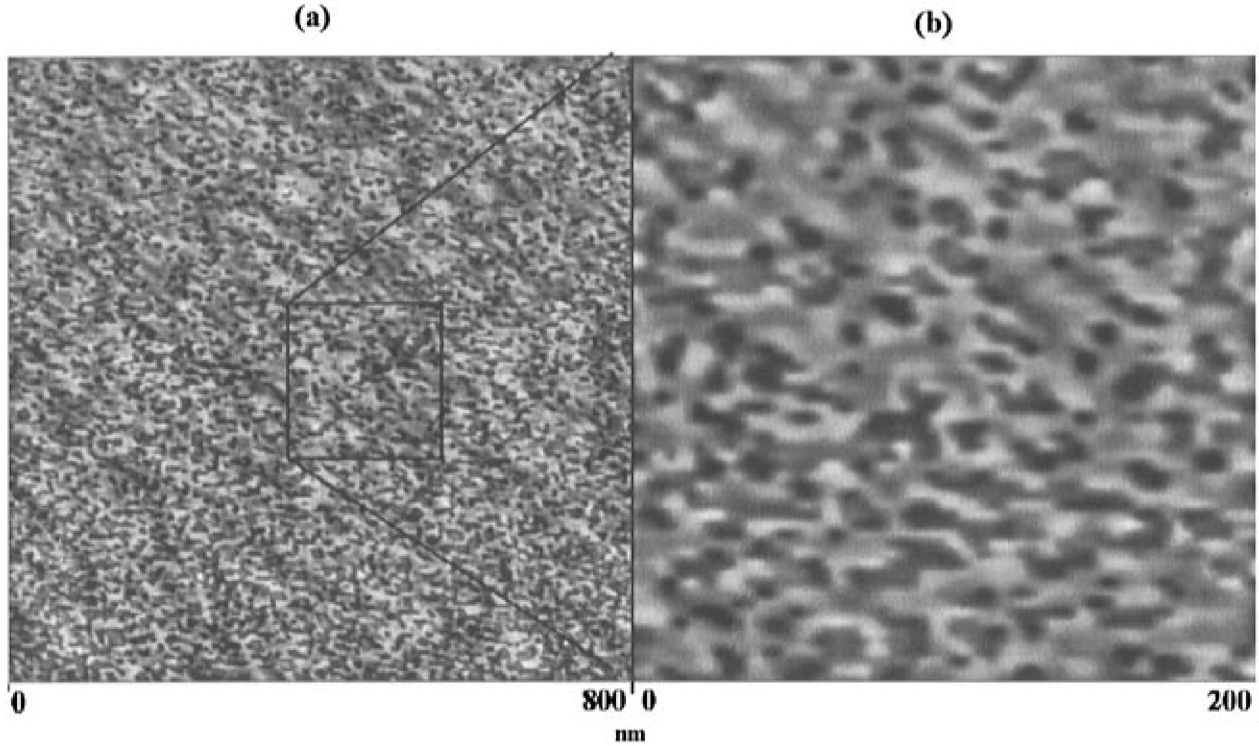


Figure 2. (a) 800 × 800 nm^2 tapping mode AFM picture of ceramer containing 50 wt % PCL. (b) 200 × 200 nm^2 zoom area of (a).

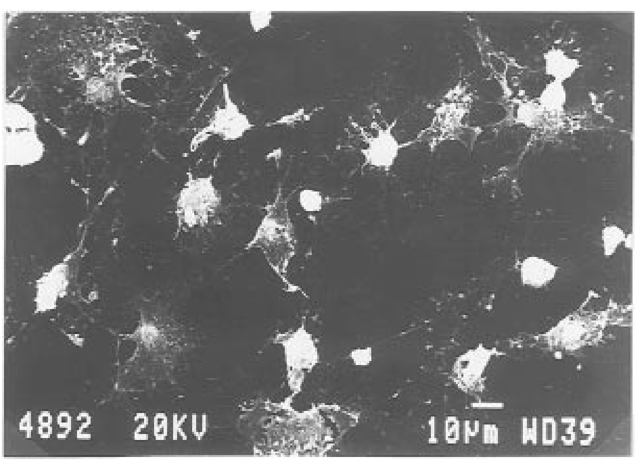


Figure 3. Scanning electron microscope image. Typical morphology of fibroblasts cultured on inorganic-organic hybrid ceramer for 20 h.

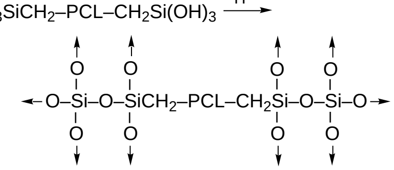
hydroxyl group at both ends, which is reactive toward alkoxysilane. The sol−gel process has thus the potential of combining valuable properties into a novel type of ceramer, such as nontoxicity for living organisms, resorption after an appropriate period of implantation time, and good ultimate mechanical properties. We have recently reported on a new family of ceramers by changing the tetraethoxysilane-based sol−gel process by the addition of α,ω-hydroxyl (or triethoxysilane) end-capped PCL.[4] Equations 1−3 (eq 3 is unbalanced) are an oversimplified view of the reaction pathway envisioned for the synthesis of biodegradable and biocompatible ceramer.[4,6] The arrows are symbols for the growth of a three-dimensional silica network.

This paper deals with the in vitro testing of these nanocomposites or molecular composites[5] as substrates for cell culture and with studies of in vitro biodegradation.

$$Si(OEt)_4 + 4H_2O \xrightarrow{H^+} Si(OH)_4 + 4CH_3CH_2OH \qquad (1)$$

$$(EtO)_3SiCH_2\text{–}PCL\text{–}CH_2Si(OEt)_3 + 6H_2O \xrightarrow{H^+} (HO)_3SiCH_2\text{–}PCL\text{–}CH_2Si(OH)_3 + 6C_2H_5OH \qquad (2)$$

$$Si(OH)_4 + (HO)_3SiCH_2\text{–}PCL\text{–}CH_2Si(OH)_3 \xrightarrow{H^+}$$



$+ H_2O$ (3)

The in vivo testing of biomaterials is a time consuming method, which however provides the final answer, i.e., a metabolic response to synthetic materials. Cell and/or tissue culture is a valuable alternative to “in vivo” testing, because of rapidity, low cost, and higher sensitivity to cell−surface interactions.

Fibroblasts were used to evaluate the cytotoxicity of the new hybrid materials. Ceramers of various PCL contents (50%, 30%, 15%) and pure PCL were used as substrates for in vitro cell cultures. Fibroblasts were

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(6) PCL/TEOS mixtures of various compositions were dissolved in THF (20 wt %) and hydrolyzed with a stoichiometric amount of water with respect to the alkoxide functions. HCl was used as a catalyst in a 0.05/1 HCl/TEOS molar ratio. A representative synthesis was as follows: 1.5 g of TEOS was added to the α,ω-triethoxysilane PCL (0.5 g, M_n = 2000) solution in THF (10.0 mL) and thoroughly mixed until a homogeneous solution was formed. Then deionized water (0.54 mL), ethanol (0.80 mL), and HCl (0.01 mL) were added under rapid stirring at ambient temperature for ca. 10 min. The clear solution was then cast into a plastic Petri dish and covered with a Parafilm. Based on a preliminary series of gelation experiments it was shown that after a few days depending on the PCL end-groups (hydroxyl or triethoxysilane) the Parafilm was to be removed. The gelified material was then dried under ambient condition for 1 week and finally cured at 100 °C for 2 days prior to testing.

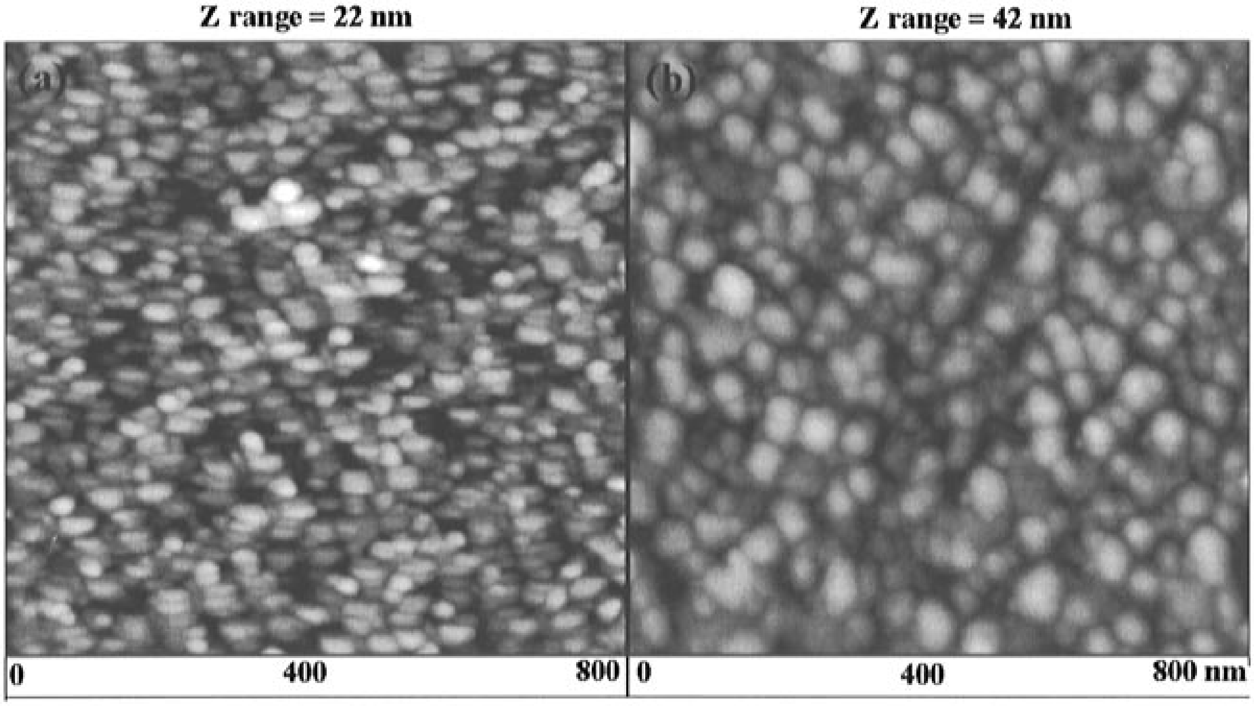

Figure 4. 800 × 800 nm^2 tapping mode AFM image of ceramer containing 50 wt % PCL: (a) after 8 days of enzymatic degradation; (b) after 10 days of enzymatic degradation.

Table 1. Contact Angle (θ) between Water and Ceramers of Various PCL Contents

PCL content	15%	30%	50%	100%
θ	29°	54°	62°	78°

observed to be attached to all the ceramers under examination in contrast to what happened for pure PCL. The extent of the cellular attachment however depends on the PCL content of ceramers. As shown in Figure 1, the density of cells attached to the ceramer surface decreases when the PCL content is increased, as a result of changes in the surface composition and properties with the nominal ceramer composition. Prediction of cell adhesion to a synthetic substrate is hazardous since several physicochemical factors influence the surface properties, such as hydrophobicity, chain mobility, and the presence of specific chemical groups. In the present case, it is obvious that the hydrophobic polymer and silica weight ratio can modify the surface hydrophilicity. Thus different PCL contents can lead to ceramers with different surface tensions. Table 1 shows the dependence of the water/ceramer contact angle on the PCL content as measured by the sessile drop method.[7,8] As suggested, the ceramers hydrophilicity decreases as more PCL is incorporated into ceramers. Clearly, the fibroblast adherence decreases with decreasing of hydrophilicity of the substrate, to the point where only few fibroblasts are attached to pure PCL under the experimental conditions used in this work.

The surface morphology of ceramers of different PCL contents has also been analyzed by atomic force microscopy (AFM). A typical 800 × 800 nm^2 tapping mode AFM image is shown in Figure 2a for 50 wt % PCL ceramer. The surface topography shows a highly dispersed structure made of very small domains (the dark spots of the picture) randomly distributed in a matrix. At a higher magnification (Figure 2b, zoom 200 × 200 nm^2), the size of these domains may be approximated to 10−15 nm. The tapping mode AFM is known to probe the viscoelastic properties of the surfaces[9] since the tip can slightly penetrate a soft surface in contrast to harder domains. It is accordingly proposed that the dark spots in Figure 2 are the softer PCL domains dispersed in the harder silica matrix formed by the sol−gel process. However, when the PCL content of the ceramer is smaller than 40 wt %, the two constitutive components cannot be distinguished from each other anymore, at least by the tapping mode AFM technique.

Typical morphology of fibroblasts cultured on the PCL containing ceramers for 20 h is shown in Figure 3 (e.g., on a 50 wt % PCL containing ceramers). Cells are compact with a weblike attachment to the substrate surface, and cell division is sometimes visible.

"In vitro" biodegradation has been studied at 37 °C by immersing the ceramer (50% PCL content) in a 0.1 M sodium phosphate buffer solution (pH = 8) added with porcine esterase (23 units/mL). At various immersion times, the sample is removed, washed with water, and dried. Initially transparent, ceramers immersed in the porcine esterase solution become translucent. The surface morphology is also changing (Figure 4a,b, to be compared with Figure 2a). After 4 immersion days (data not shown here), the surface morphology has deeply changed since no evidence of two distinct phases is observed anymore, and an irregular granular aspect is now visible. This granular morphology is much more pronounced after 8 days (Figure 4a (800 × 800 nm^2 scan size)). It consists of densely packed grains with quite a

(7) Contact angles (θ) were measured with a home-built equipment according to the Mack indirect method.[8] θ was calculated as $\theta = 2 \arctan(2h/d)$, where d and h are the diameter and height of the water droplet, respectively.

(8) (a) Mack, G. L.; *J. Phys. Chem*. **1936**, *40*, 159. (b) Wu, S. *Polymer Interface and Adhesion*; Marcel Dekker: New York, 1982.

(9) Magonov, S. N.; Whangbo, M.-H. In *Surface Analysis with STM and AFM: Experimental and Theoretical Aspects of Image Analysis*; VCH: New York, 1996; pp 37−39.

homogeneous size distribution and a few larger dark areas, thought to be pores formed by the PCL degradation. After 10 days (Figure 4b), the grains appear to be more spherical and their size has further increased. According to the classical techniques of image analysis (autocorrelation function, standard granulometry) the mean size of the particles has increased from 30 nm after 8 days up to 44 nm after 10 days. The distance between the nearest-neighboring particles increases in parallel from 36 nm after 8 days up to 53 nm after 10 days, which indicates that PCL is not merely biodegraded while keeping the originally formed silica particles unchanged. As a rule, biological digestion of the organic material is expected to result in a much more selective degradation compared to the usually more aggressive chemical and/or thermal treatments. So, the selectivity of the enzymatic degradation toward the organic constitutive component (PCL) can account for the conclusion that a two-phase system is no longer observed after 4 days of treatment. Nevertheless, the surface morphology goes on changing beyond 4 days. The enzymatic degradation is expected to release not only hydroxyl-end-capped PCL segments but also silanol groups, which may react further and contribute to the apparent rearrangement of the surface silica (or at least rich in silica) particles into larger ones increasingly distinct from each other. There would thus be a complex interplay of physical (void formation) and chemical (condensation reaction and/or hydrogen bonding[10]) processes. Since the particle size is observed to increase between the eight and the tenth day of immersion, the PCL degradation is not yet completed after 10 days.

The surface composition was analyzed by EDAX. Figure 5 shows that the surface composition (1 μm depth) of the 50 wt % PCL containing ceramer is changing as a result of the enzyme reaction. Indeed, the relative content of C with respect to Si and thus the PCL content decreases with increasing time of immersion in the porcine esterase solution. Moreover, this solution is still active beyond 10 days. It is thus clear that biodegradability of PCL is preserved in the nanocomposites.

In conclusion, the preliminary in vitro cell cultures and biodegradation tests reported in this paper confirm that the new inorganic–organic hybrid ceramers prepared by the sol–gel process are novel biomaterials endowed with biodegradable and biocompatible properties. These preliminary results are not only encouraging but also pave the way to entirely new biomaterials that combine characteristic properties of classical materials, such as ceramics and organic polymers. This strategy has potential in producing a bone-bioerodible polymer composite for skeletal tissue repair. Indeed the ceramer has "bioactive" and "resorbable" properties, since it has proved to be a valuable support for cell culture whereas the slow PCL degradation allows tissue invasion and reconstruction to happen. A similar approach has been proposed in the scientific literature.[11] which relies upon the solvent-casting of a mixture of poly(D,L-lactide-*co*-glycolide) and hydroxyapatite in acetone with the formation of a composite film.

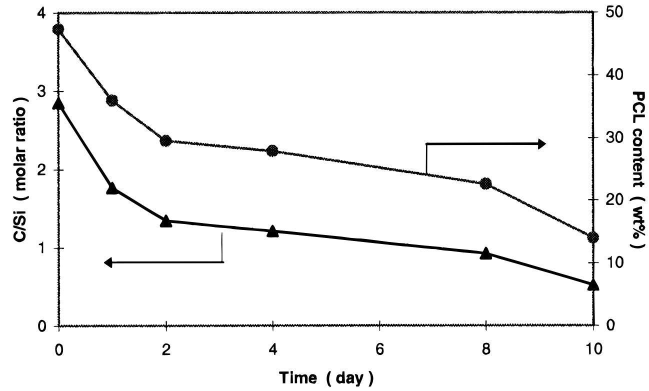

Figure 5. Surface composition of the 50 wt % PCL containing ceramer after immersion in the porcine esterase solution (pH = 8) at 37 °C for different times.

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