Release Mechanisms from Gentamicin Loaded Poly(lactic-*co*-glycolic Acid) (PLGA) Microparticles

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ABSTRACT: To provide local gentamicin delivery for 1 week based on a biodegradable system, poly(lactic-*co*-glycolic acid) (PLGA) microparticles were developed utilizing a 50/50 blend of Resomer® RG 502H, an uncapped variety of 13.5 kD, and Resomer® RG 503, an endcapped polymer of 36.2 kD. The liberation mechanism was investigated by analysis of morphological changes and thermal analysis focusing on the polymer glass transition temperature (T_g) and the mechanical properties. The release of gentamicin was related to a structural breakdown of the particles reaching a critical molecular weight. A T_g of $< 37°C$ in the hydrated state was not indicative of collapse and agglomeration of the particles because the mechanical strength of the polymer structures in the rubbery state may still render sufficient support. As the gap between incubation temperature and T_g widened, the mechanical stability of the PLGA microparticles decreased and became decisive. Particles prepared with RG 502H show a lower ability to bear mechanical stress than RG 503 and 50/50 RG 502H/RG 503 microparticles. © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 91:845–855, 2002

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INTRODUCTION

In the treatment of infections, systemic therapy with antibiotics is accompanied by the risk of side effects and may be ineffective in cases of deficient local blood flow (e.g., at bony sites) inadequate tissue penetration, or bacterial resistance. This dilemma can be resolved by local delivery of antimicrobial agents that ideally show a low allergization quota, tissue compatibility, bacteri-cidal activity, a low rate of bacterial resistance, broad spectrum activity, a low resorption rate, no interference with wound healing, and chemical stability in the presence of biological material such as pus or fibrin.[1] Gentamicin (GM), a powerful member of the aminoglycoside group of antibiotics, fulfills these requirements for the most part. Because of its polar nature, the oral resorption rate and the tissue penetration of GM are poor, and it is largely excluded from most cells.[2] Consequently, tissue concentrations are lower than the corresponding plasma levels.[3] High tissue concentrations are only found in the renal cortex and in the endolymph and perilymph of the inner ear, and the incidence of nephro- and ototoxicity has been attributed to this accumulation.[4]

The collagen and PMMA carrier systems currently used for local treatment with GM show disadvantages in their pharmacokinetics because they either release too quickly, within hours,[5] or too slowly, over several months.[6,7] Consequently, biodegradable poly(lactic-*co*-glycolic acid) (PLGA) microspheres were developed using a w/o/w multiple emulsion technique. The encapsulation of water-soluble drugs by this manufacturing process is generally limited by the solubility of the drug in the two aqueous phases of the microparticles preparation system. [8,9] The encapsulation efficiency can be manipulated by varying the polymer type, the organic solvent, and/or the emulsification process, by the addition of salts or buffers, by the use of copolymers, by additional coating, or by modification of the solvent evaporation method.[8]

The drug release profiles from PLGA microspheres are characterized by a burst caused by initial dissolution of drug located near the microspheres surface, followed by a period of slow release that is attributed to degradation of the microspheres and diffusion of the drug out of the sphere and a third phase as increased solubilization and erosion of the polymeric matrix results in a secondary burst.[8] Specifically for highly water soluble drugs, a high initial burst has been reported.[10,11] Rapid dissolution of drug loosely bound to the microparticle surface occurs, resulting in a porous wall that makes the drug more available to the fluid. Consequently, release has been shown to be affected by particle size,[12] porosity reflecting internal droplet volume,[8,13] or additional polymer coating.[14] PLGA microparticles have been shown to control the release of gentamicin over 1 week, with an initial burst of $< 20\%$, and the desired release profile could be adapted by mixing of Resomer® RG 503 and the more hydrophilic uncapped variety RG 502H.[15]

The study presented here was designed to characterize the morphological changes and thermal behavior of microparticles in an attempt to understand the release mechanisms from GM-loaded PLGA microparticles, especially focusing on this polymer blend.

MATERIALS AND METHODS

Materials

Polymers used were Resomer® RG 502H [lot 270604; RG 502 H; poly(D,L-lactic-*co*-glycolic acid),

50:50; uncapped; weight-average molecular weight (M_w): 13,500 Da; number-average molecular weight (M_n): 6700 Da; polydispersity index: 2.0; glass transition temperature (T_g): 46°C] and RG 503 [lot 34032; RG 503; poly(D,L-lactic-*co*-glycolic acid), 50:50; end-capped; M_w: 36,200 Da; M_n: 16,300 Da; polydispersity index: 2.2; T_g: 48°C]. All polymers were provided by Boehringer Ingelheim, Ingelheim, Germany, and stored desiccated and protected from light at 2–8°C. Gentamicin sulfate was obtained from Innocoll GmbH, Saal/Donau, Germany, and throughout this paper, the term gentamicin (GM) describes the sulfate form.

Methods

Preparation of Microparticles

Microparticles (RG 502H: 266 ± 100 μm; RG 503: 553 ± 207 μm; 50/50 RG 502H/RG 503: 367 ± 130 μm diameter) were prepared as previously described using a w/o/w solvent evaporation technique.[16]

Release Studies

One-hundred-milligram microparticles were incubated in 5 mL of phosphate buffered saline (PBS; pH 7.4; 5.2 g/L K_2HPO_4 and 1.415 g/L KH_2PO_4) containing 0.2% sodium azide in screw-topped test tubes at 37°C in a shaking bath at 50 strokes/min. At designated time points, 3 mL of the release medium was exchanged. The studies were performed in triplicate. Samples were centrifuged at 5000 rpm for 5 min, and the GM concentration of the supernatant was determined.[17] Briefly, two 500-μL samples were each mixed with 300 μL of isopropanol and 200 μL of labeling reagent (50 mg of *o*-phthaldialdehyde, 50 μL of thioglycolic acid, 1.25 mL of methanol, and 11.2 mL of 0.4 M borate buffer, pH 9.5). After incubation for 45 min at room temperature, samples were analyzed at 332 nm. For morphological studies, particles were removed at designated time points, frozen in liquid nitrogen, and freeze dried.

Scanning Electron Microscopy (SEM)

Samples were sputtered with gold in a sputter chamber and investigated with an Amray scanning electron microscope (model 1810 T; Amray Inc., Bedford, MA).

Differential Scanning Calorimetry (DSC)

The measurements were carried out on a DSC machine with a low temperature feature (Polymer Laboratories Inc., Loughborough, UK). Samples of 10 ± 5 mg were sealed in 40 µL aluminum containers and heated at 5°C/min for the dry samples and 10°C/min for wet samples starting at 0 and −30°C, respectively. Studies were performed in triplicate.

Microscopic Investigation

A stereological microscope and a microscope equipped with a heating stage (Hertel & Reuss, Kassel, Germany) were used for particle characterization. Approximately 20 mg microparticles were dispersed in 1 mL of PBS at 25°C. A 100 µL sample was placed on a microscope slide protected with a cover slip and heated at 1°C/min while images were taken at $100 \times$ magnification approximately every minute. The tests were repeated three times.

Dynamic Mechanical Thermal Analysis (DMTA)

Testing was performed on a dynamic mechanical spectrometer (DMTA Mark IV; Rheometrics, Bensheim, Germany). Individual particles were placed between the two cylindrical metal plates, which were approached to initial contact. Time- or temperature-dependent analysis (heating rate: 5°C/min for the dry samples and 10°C/min for wet samples) was performed at 1 Hz and 0.02% amplitude for five particles.

Fourier Transform-Infrared Spectroscopy (FT-IR)

FT-IR spectra were obtained on a Magna-IR 550 (Nicolet, Madison, WI) using 100 µm polymer films prepared by tape casting of a 300 mg polymer solution in 660 µL of CH_2Cl_2. Samples were heated at a rate of 1°C/min in a temperature-controlled CaF_2 cell.

RESULTS AND DISCUSSION

The release of GM from RG 502H particles was characterized by a 60% burst within the first hours (Fig. 1) and subsequent liberation of the remaining GM within 4 days. In contrast, RG 503 particles released only 10% within 4 h. After a 2-week lag phase with an additional 10% GM liberation, substantial drug release set in and was completed after 4 weeks, reflecting the bulk
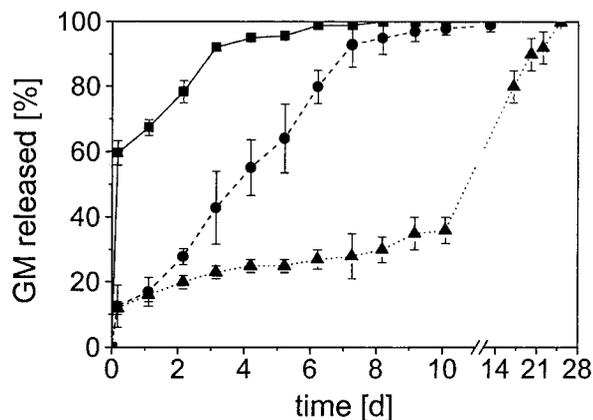

Figure 1. GM release from microparticles prepared from RG 502H/RG 503 blends 100/0 (—■—), 50/50 (−−●−−), and 0/100 (···▲···); each data point represents the mean and Sd of three runs.

erosion process of the polymer.[8] Whereas only marginal drug diffusion occurs during the first phase, drug release becomes substantial as a critical PLGA molecular weight of $\sim$15,000 Da is reached with ongoing ester hydrolysis.[18,19] Microparticles prepared from PLGA blends result in intermediate-release profiles.[20,21] Accordingly, RG 502H/RG 503 particles prepared from a 50/50 mixture showed a reduced initial GM release, and liberation occurred over a period of 1 week without a marked lag phase.

Morphological Changes

To clarify the release mechanism, particle morphology during the course of release testing was studied by SEM. The image series of RG 503 microparticles over time illustrated no major increase in particle diameter during the first days due to water penetration and swelling (Fig. 2a). After 2 weeks, particle deformation was observed. At that time, substantial erosion started and led to polymer mass loss and disintegration of the particles over the next 2 weeks. This phase correlated with massive water penetration and approach of the critical molecular weight[16] as well as GM release, which can be ascribed to accessibility of new surfaces and dissolution of internal antibiotic nests resulting from the primary w/o emulsion.[22] RG 502H microparticles incubated in PBS showed progressive changes in surface morphology, deformation, and aggregation within 24 h, and, by day 3, the particles had turned into an unstructured mass because the initial molecular weight was already below the critical level (Fig. 2b). Swelling, deformation, and progressive

breakdown corresponded to massive water uptake[16] and GM release within the first days. Subsequently, the water content decreased as the polymer particles collapsed, resulting in separation of a dense polymer phase from the surrounding incubation medium. The structural changes of 50/50 RG 502H/RG 503 particles took a course similar to the ones for the individual poly(α-hydroxy acid) spheres but occurred within a different time window (Fig. 2c). The surface roughness of the particles increased until day 3. At that point, the polymer structures started to collapse, leading to strong deformation of the individual particles at day 6, with simultaneous release of GM. The breakdown continued, and large polymer aggregates could be identified at

day 9 when the drug load was completely released. Again, the water content decreased from day 3 on as the microspheres fell in and subsequently amassed, which led to separation of incubation medium from water-filled pores and channels. The molecular weight of the 50/50 RG 502H/RG 503 blend started out at 27,000 Da. Because water penetration is more pronounced due to the hydrophilic RG 502 H fraction, polymer hydrolysis occurred at the same rate as for RG 502H particles and, at day 3, a molecular weight of 15,000 Da was measured.[16] The process of swelling, erosion, and structural collapse identified by SEM corresponded to the time course of water uptake by the microparticles and the GM release profiles.
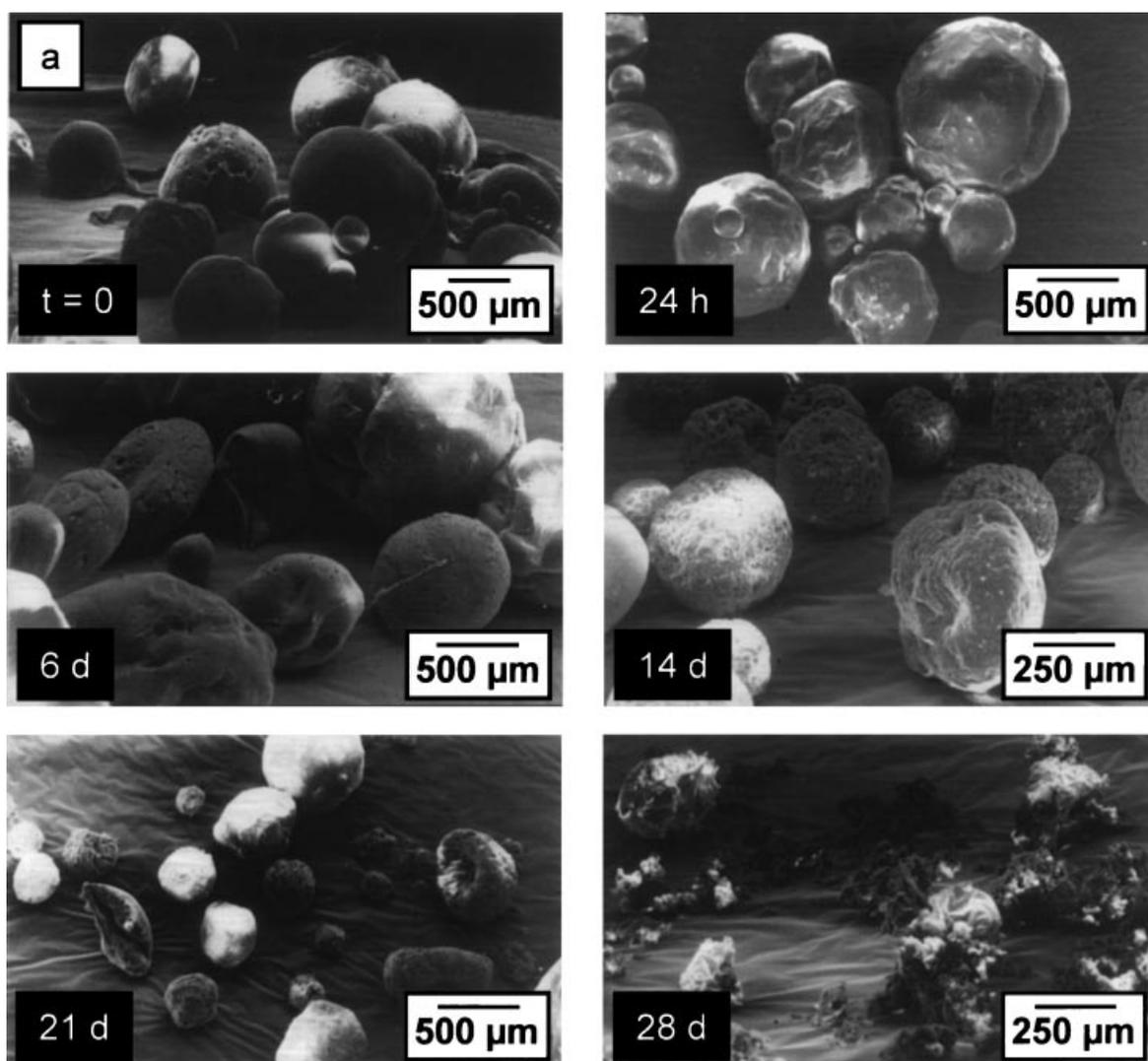

Figure 2. SEM of microparticles prepared from (a) RG 503, (b) RG 502H, and (c) 50/50 RG 502H/RG 503 during incubation in PBS.

Glass Transition Temperature (T_g)/DSC

The hydrolytic reduction of the polymer molecular weight has not only been shown to be critical for the breakdown of poly(α-hydroxy acid) microparticles due to increased hydrophilicity of the fragments but also leads to a decrease of the T_g.[23] Mobility of compounds in polymeric matrices increases drastically as the T_g is exceeded, and diffusivity of both penetrating water and escaping drug are higher in the rubbery state.[24] Consequently, faster hydrolysis and drug release are found above T_g.[25,26] Addition of low molecular weight PLA to higher molecular weight material can be used to enhance drug release by reduction of T_g. The resulting transition temperatures were found to represent intermediates between the values for each individual polymer, indicating that the two poly(α-hydroxy acid) fractions were miscible at all ratios.[27]

For PLGA polymers, the T_g ranges from 40 to 65°C[28] and increases with rising molecular weight.[21] DSC analysis of microparticles prior to incubation revealed a T_g of 48°C for both RG 503 and 50/50 RG 502H/RG 503 blend and of 46°C for RG 502H. As can be seen in Figure 3a, already after 1 h in PBS, the T_g dropped below the 37°C level for all three preparations because of the plasticizer effect of hydrating water. Thus, the particles exist in the rubbery state with enhanced mobility of the polymer chains. Despite different levels of water uptake,[16] the T_g values varied only
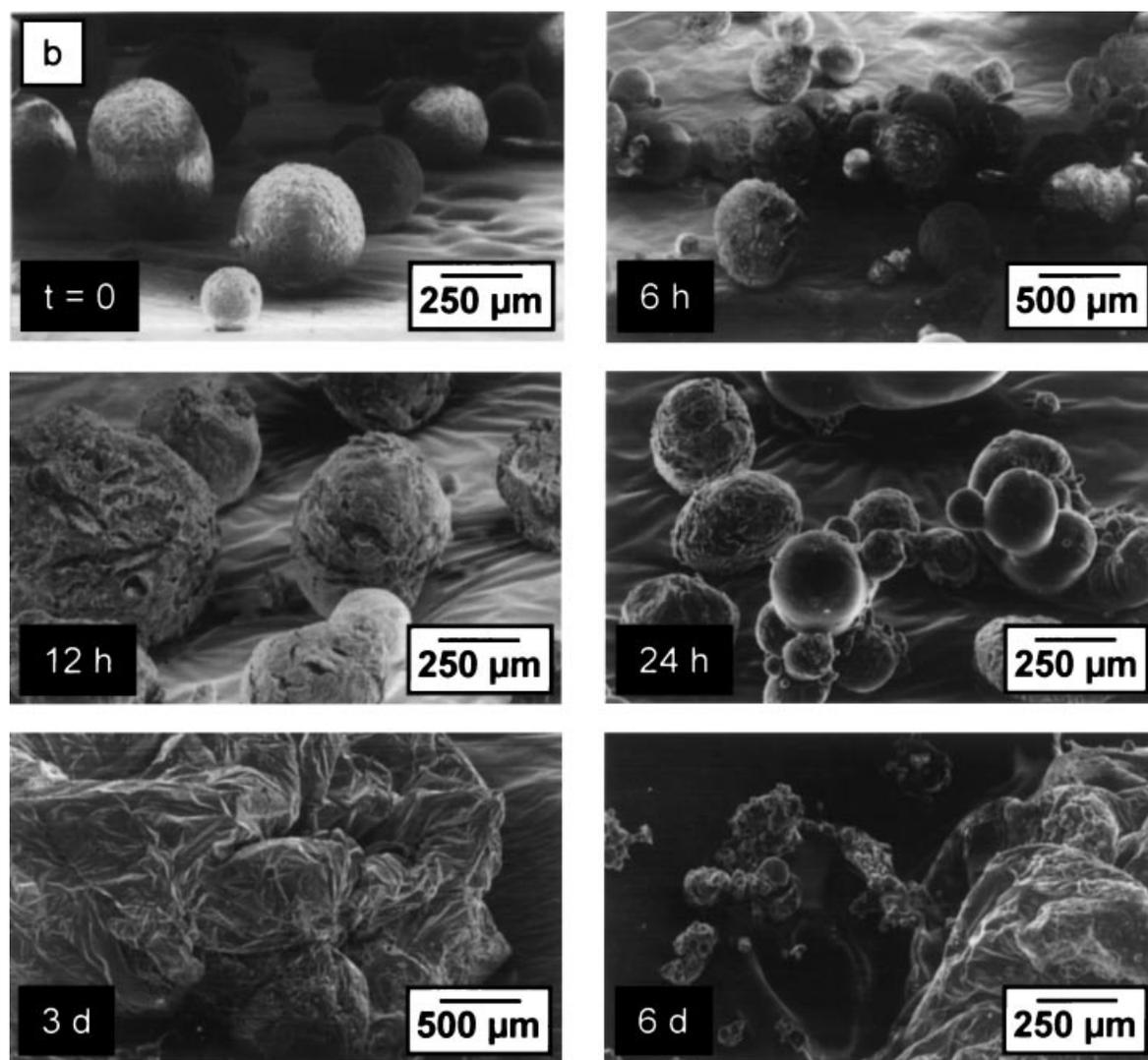

Figure 2. *(Continued)*

slightly, indicating that surplus unbound water, for example in pores or channels, contributed to the high water contents of RG 502H and 50/50 RG 502H/RG 503 microparticles. Over a period of 10 days, the T_g remained consistently at 30°C for RG 503 despite a reduction in molecular weight.[16] During this time, no major water penetration or drug release occurred. At day 14, when substantial water penetration and GM release started, the T_g was reduced to 26.5°C. After 3 and 4 weeks, the T_g could no longer be detected because of superimposition by the melting of ice crystals. In contrast, the T_g of particles prepared from 50/50 RG 502H/RG 503 decreased only slightly within the first 3 days. However, particle breakdown and GM release coincided with further decrease of T_g to 17°C between day 3 and day 6. For RG 502H

microparticles, a similar drop in the T_g to 18°C occurred after 24–72 h, a time window that correlated with the previous observations of marked drug liberation and morphological changes. Thus, a T_g below 37°C in the wet state did not necessarily reflect collapse and agglomeration of the particles, and the compressive strength of the hydrated polymer structures in the rubbery state may still be sufficient to support the spherical shape. But, as the gap between incubation temperature and T_g widened, the mechanical stability of the PLGA microparticles was further reduced. The particles collapsed and 37°C reflected a critical softening point as the T_g reached ~20°C. Because the method did not allow for detection of a T_g below 10–15°C, additional analysis of the samples after vacuum drying was
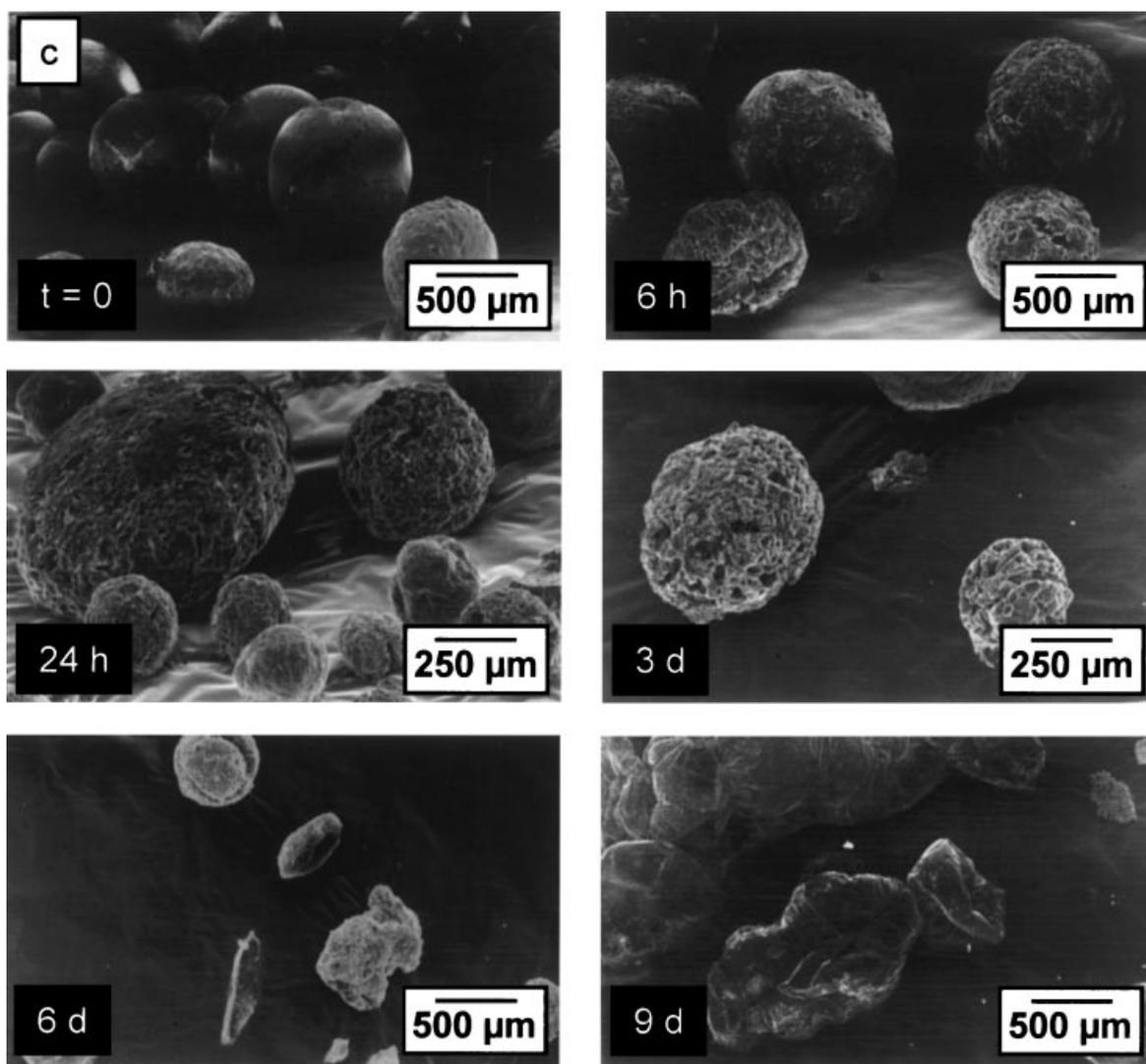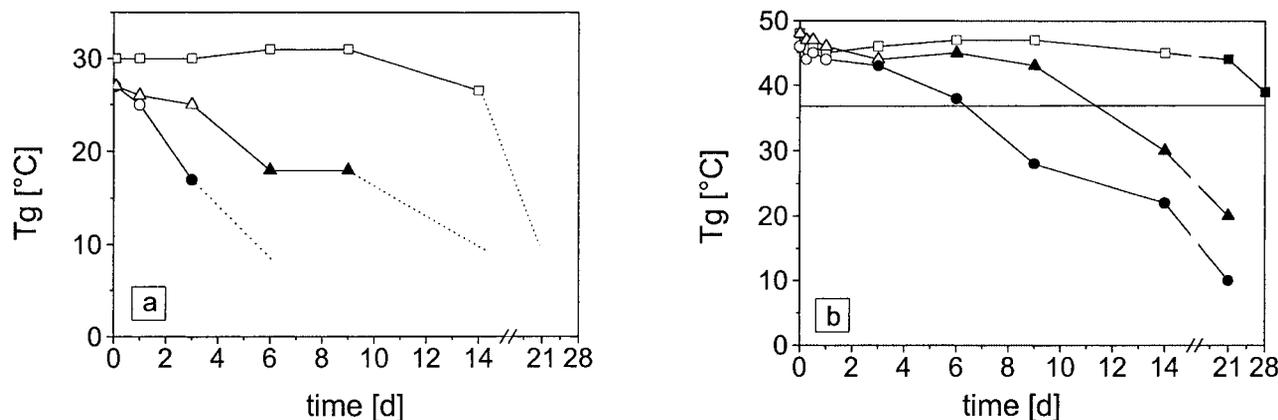

Figure 2. *(Continued)*

Figure 3. T_g of microparticles prepared from RG 503 (■, □), RG 502H (●, ○), and 50/50 RG 502H/RG 503 (▲, △) during incubation in PBS in (a) the wet state and (b) after vacuum drying (full symbols denote macroscopic collapse); each data point represents the mean of three runs.

performed. The results demonstrated a continuing decrease of T_g for RG 502H and 50/50 RG 502H/RG 503 particles with ongoing degradation over 3 weeks, whereas the T_g values for RG 503 started to drop between weeks 3 and 4 (Figure 3b).

Heat Stage Microscopy

The difference between T_g and the collapse temperature could be visualized by microscopy of microparticles incubated in PBS on a heat-stage microscope (Fig. 4). With heating at ~1°C/min, no major change in appearance of RG 503 microparticles was observed up to 40°C despite a T_g of 30°C. At 45°C, minor structural changes at the surface could be identified. In contrast, RG 502H spheres strongly increased in size as water penetration and swelling occurred. On heating, the structures started to collapse at ~37°C and to coalesce at 40°C. Deformation and breakdown had also been identified by the morphological investigations within the first hours of incubation at 37°C (Fig. 2b). Particles prepared from the 50/50 RG 502H/RG 503 blend also demonstrated swelling and surface modifications initiated at ~40°C leading into deformation at 45°C. Thus, the studies demonstrated that a critical softening temperature of the PLGA microparticles was reached at 10–15°C above the T_g of 30 and 27°C, respectively.

Dynamic Mechanical Thermal Analysis

The results obtained by heat stage microscopy and DSC indicate that differences in the mechan-

ical stability of the microparticles contribute substantially to the GM release profile. Consequently, the mechanical properties of the microparticles were further investigated by DMTA. A sinusoidal mechanical stress is applied to induce a strain that lags behind the stress by phase angle δ because of the viscoelastic behavior of the material.[29] T_g is defined as the temperature at which the loss modulus E'' (a measure for the viscous behavior of the material) or the tan δ (reflecting the ratio of energy lost/energy stored per deformation cycle) reach a maximum.[30,31] A T_g of 57°C was determined by DMTA for RG 503 (Fig. 5a) as well as for 50/50 RG 502H/RG 503 microparticles, whereas a T_g of 50°C was obtained for RG 502H microparticles based on the maximum of E''.[32] Thus, the T_g values obtained by DMTA are ~7°C higher than those measured by DSC. This discrepancy can be explained by the inertia of the larger DMTA system with the temperature probe being further away from the sample than in DSC. Approximately 6–10°C higher T_g values obtained with this DMTA setup as compared with DSC have been reported for PLGA material.[33]

Additionally, time-dependent tests were performed varying the preload at 37°C. RG 502H particles do not withstand a preload of 0.2 or 0.1 N; they become deformed instantaneously. In contrast, particles prepared from 50/50 RG 502H/RG 503 are compressed by 0.2 N load within 400 s (Fig. 5b), and the same preload applied to RG 503 particles results in deformation starting after ~300 s and completed after 1000 s. At 0.1 N preload, RG 503 microparticle compression is further delayed and the particles resist to 0.05 N load.
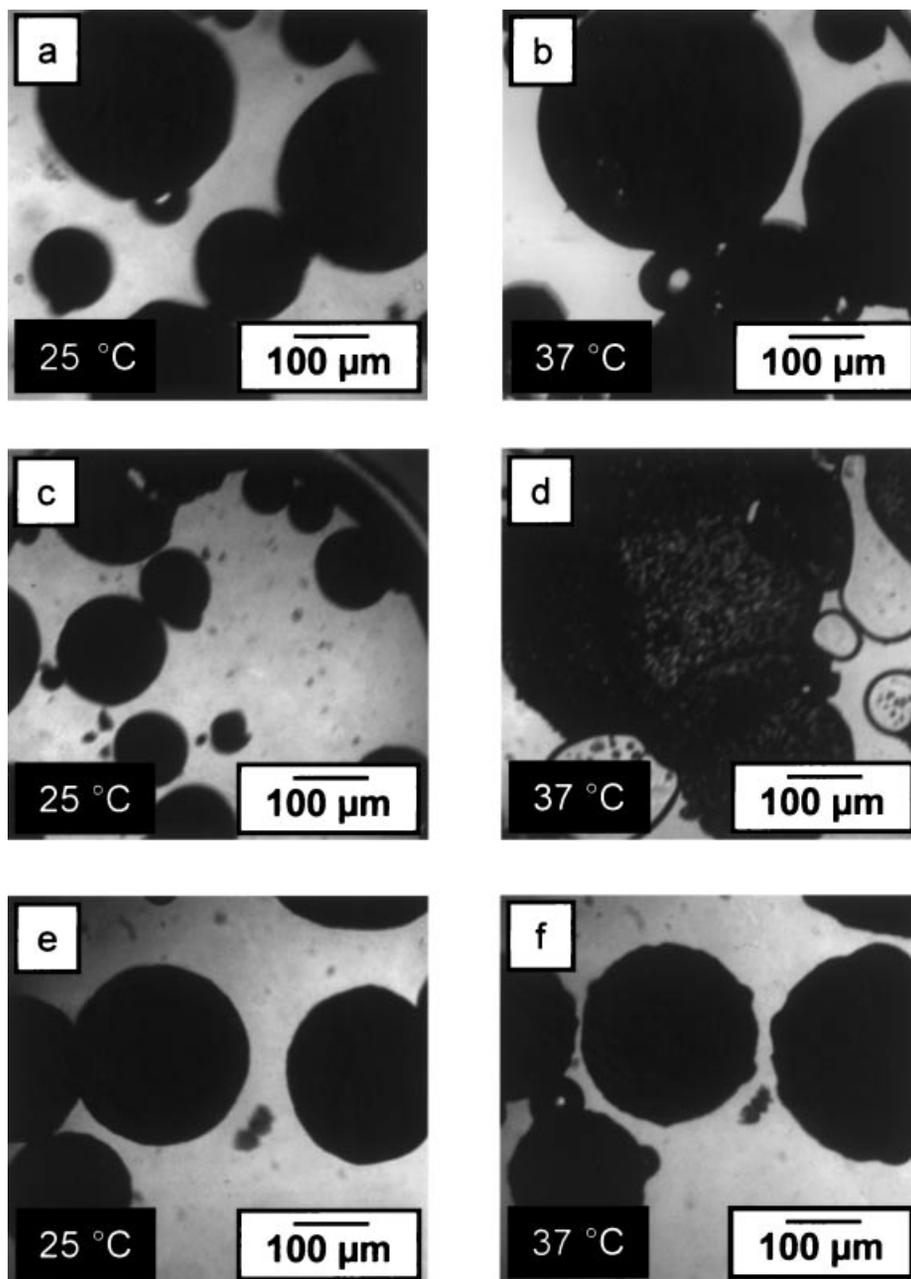
Figure 4. Heat stage microscopy of microparticles prepared from (a, b) RG 503, (c, d) RG 502H, and (e, f) 50/50 RG 502H/RG 503 in PBS at 25 and 37°C.

Applying 1 g of preload resulted in no evidence of structural deformation in any of the spherical samples (e.g., Fig. 5b for RG 502H). The mechanical stability of PLGA particles was decreased in the wet state, and the samples could not bear a 0.2 N preload. At 0.01 N, deformation of 50/50 RG 502H/RG 503 particles wetted with PBS set in immediately—similar to the result obtained for the particles in the dry state at 0.2 N—reaching a plateau faster than RG 503 particles tested (Fig. 5b). These mechanical tests indicated resistance to mechanical load of < 0.2 N applied to a spherical particle, with a marked reduction in the wet state. The increase in mechanical strength from RG 502H to the mixture and RG 503 corresponded to the differences in temperature-dependent behavior observed by DSC and heat stage microscopy.
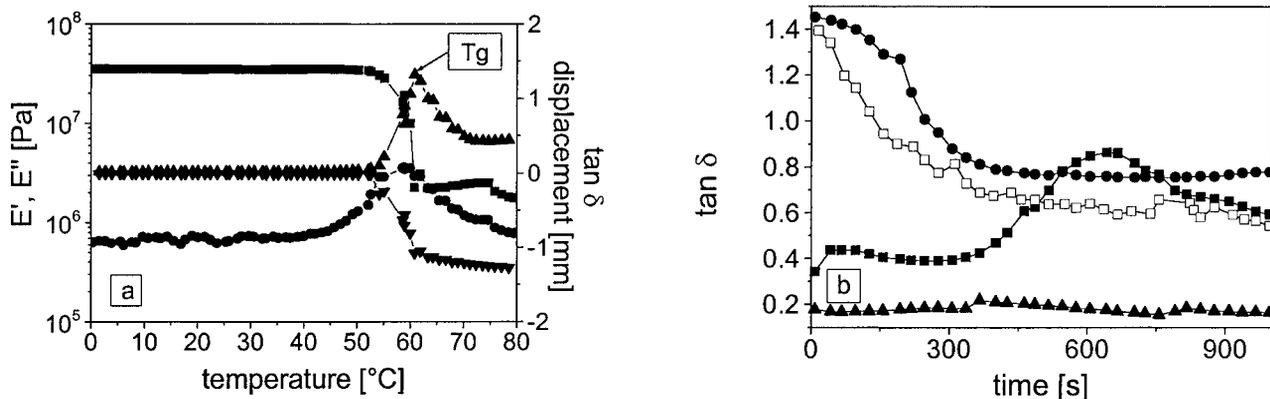
Figure 5. (a) Storage modulus E' (■), loss modulus E'' (●), tan δ (▲), and plate displacement (▼) for microparticles prepared from RG 503 at 10 g preload. (b) Time-dependent tan δ for microparticles prepared from RG 503 at 20 g preload, dry (■); 50/50 RG 503/RG 502H at 20 g preload, dry (●); RG 502H at 1 g preload (▲); and 50/50 RG 503/RG 502H at 1 g preload in PBS (□) at 37°C.
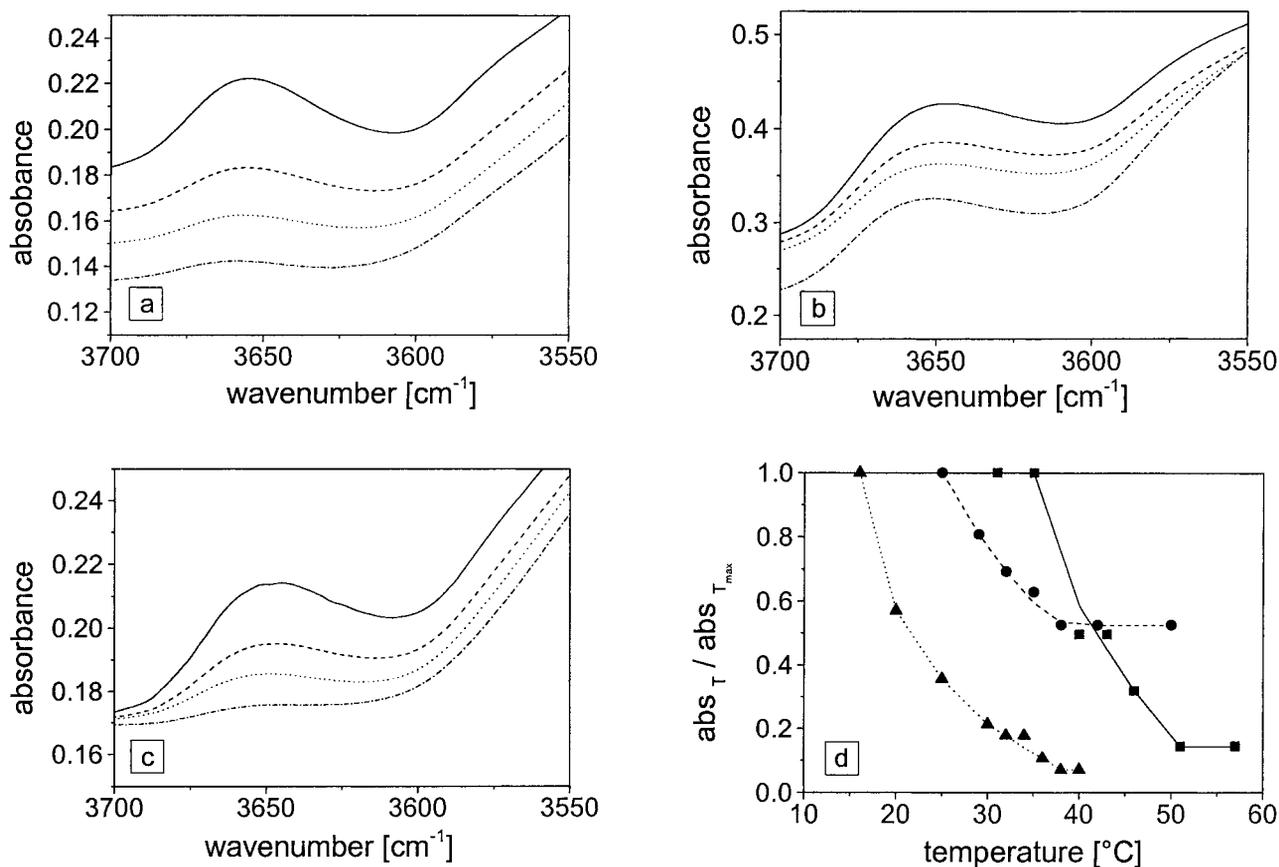

Figure 6. Temperature-dependent FT-IR spectra (3700–3550 cm^{-1}) of polymer films prepared from (a) RG 503 [31°C (—), 40°C (– – –), 46°C (········), 57°C (– – –)]; (b) RG 502H [25°C (—), 29°C (– – –), 32°C (········), 42°C (– – –)); (c) 50/50 RG 503/RG 502H [16°C (—), 20°C (– – –), 25°C (········), 32°C (·—·—·—)]; and (d) relative peak height for RG 503 (■), RG 502H (●), and 50/50 RG 503/RG 502H (▲).

Fourier Transform-Infrared Spectroscopy

Temperature-induced phase transitions in polymers can be characterized by FT-IR spectroscopy investigating molecular mobility. For the poly(α-hydroxy acid)s tested, peaks were shifted to lower wave numbers with increasing temperature. In addition, increasing temperature resulted in the diminution of the peak at 3650 cm^{-1}, the region of the stretching vibrations of hydroxylic groups found in PLGA (Fig. 6).[34] Whereas the decrease in intensity is completed at 50°C for RG 503 (Figs. 6a, 6d), the peak heights for RG 502H and 50/50 RG 502H/RG 503 reach a minimum by 40°C (Figs. 6b, 6c, 6d). The higher absorbance at the maximum temperature for non-endcapped polymer corresponds to the increased number of hydroxylic groups. The difference in the temperature dependence of this molecular change measured at 1°C/min confirms the DSC and DMTA results in the dry state.

Thus, the fate of PLGA microparticles during incubation at 37°C could be explained by combination of different analytical methods. GM release coincided with structural collapse and bulk erosion when a critical molecular weight was reached and a critical softening temperature ($\sim$10–15°C higher than T_g) decreased to the 37°C level. The results substantiate that the mechanical strength of PLGA microparticles is a decisive factor for the degradation and release characteristics. DMTA will be further evaluated as a tool to characterize the mechanical properties of and the drug release from PLGA delivery systems.

ACKNOWLEDGMENTS

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