Studies on the lipase induced degradation of lipid based drug delivery systems

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A B S T R A C T

The question whether lipid based – especially triglyceride based – depot systems can undergo biodegradation is despite many in vivo studies still unanswered. In this paper we studied biodegradation processes in vitro by incubating these lipid based systems in buffer media containing lipases. The main degradation product the free fatty acids (FFA) were isolated from the drawn samples and after derivatization analyzed with RP-HPLC. Lipid microparticles showed a rapid biodegradation whereas the complete degradation of compressed implants would take several months or years. For these two systems surface degradation can be stated. Surprisingly lipid based extrudates changed their structure dramatically upon lipase incubation resulting in a breakdown of the lipid matrix and formation of small lipid particles in the µm-range. This sort of bulk-degradation may enable the use of lipid based extrudates for the long term delivery of drugs. However additional in vivo experiments will be necessary to fully characterize these degradation processes.

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1. Introduction

In recent years great efforts have been made in the development of lipid based parenteral drug delivery devices. Although approved polymers like poly(D,L-lactide-co-glycolide-acid) (PLGA) are still state of the art for the preparation of parenteral drug delivery devices, depot systems based on lipid materials arouse much interest among protein formulators since biocompatibility, release and effect on the stability of the entrapped bioactive drug appear advantageous.

Since the late 70s lipids i.e. fatty acids, triglycerides, cholesterol and lecithin have been used as excipients for the preparation of implants designed for the sustained delivery of drugs like steroids and morphine derivates [1,2]. In the late 80s Wang et al. prepared implants for the controlled delivery of insulin consisting of fatty acids or cholesterol. Implants made of fatty acids showed erosion in vivo; however it was reported that the administration of fatty acids as matrix material leads to inflammation and formation of blisters in the animal tissue. Further on, the use of fatty acid-monoglycerides is limited due to the fast erosion of these systems [3–5]. In 2001 Reithmeier et al. prepared glyceryltripalmitate microparticles and PLGA-based microparticles and compared their biocompatibility after implanting both formulations in mice. After 7 days the amount of PLGA-microparticles as well as the lipid microparticles at the site of implantation was reduced most probably due to degradation in the subcutaneous tissue [3]. In 2003 Vogelhuber et al. produced cylindrical matrices consisting of triglycerides or triglyceride/cholesterol mixtures which allowed a release of pyranine, a model compound, for more than 120 days. In vitro no erosion was detectable [4]. In 2006 Mohl et al. developed a tristearin implant for the controlled delivery of rh-INF over 1 month. In further studies the stability of the entrapped protein in the lipid matrix could be verified [5,6]. Stability and bioactivity of the encapsulated protein was also confirmed by Appel regarding the release of insulin from tripalmitate cylinders in the cell culture [7]. In 2006 Guse et al. conducted an animal experiment with the aim to investigate on the biocompatibility of glyceryltripalmitate and on the effect of cholesterol and distearoyl-phosphatidyl-choline (DSPC) on the erosion behaviour of the lipid matrices. It could be shown that DSPC is quickly eroded leading to a residual porous lipid structure consisting of triglycerides [8]. In order to prove the biocompatibility and to investigate on the in vivo in vitro correlation of the rh-INF release from the tristearin implants an experiment with rabbits as animal model was conducted by Schwab et al. [9] In this study, once again, triglyceride implants showed a good biocompatibility but signs of bioerosion couldn’t be detected.

In summary, depot systems based on lipids – especially triglycerides – are a valuable alternative to the already approved polymeric systems. Especially for long term drug delivery lipids seem to be superior to the commonly used polymers, due to their ability to maintain the stability of the entrapped proteins for a long period of time and due to their excellent biocompatibility. However, the question whether parenteral lipid drug delivery devices – especially implants – are biodegradable is still unanswered as there is no data available from the application in humans. Biodegradation of the lipid depot systems surely is one major parameter for the future success of these materials concerning an approval for their use in human medicine.

Therefore we decided to systematically study the degradation of various lipid drug delivery devices incubated in lipase solution under
physiological conditions. As lipases are present in human subcutaneous fat tissue [10–12] in muscle tissue [13] as well as in serum fluids [14] they therefore exhibit the possibility to hydrolyze lipid based drug delivery systems. Our aim was to investigate whether lipid based depot systems would undergo degradation in the presence of lipases. Further on, we wanted to elucidate the influence of the lipid composition, the size and the manufacturing process of the implants on the degradation kinetics.

Little is known about the activity of lipases and their ability to degrade exogenous lipids brought in the body by injection or infusion [15] since research is mostly concentrating on the relationship of lipase activity – i.e. hormone sensitive lipase, lipoprotein lipase and serum hepatic lipase – and lipid metabolism in the context of obesity [10,12,14,16–23]. In general one can state that the average lipase activity in the human serum and subcutaneous fat tissue has approximately an activity of 0.01 U/ml. In these studies higher lipase activities were chosen with respect to the analytical sensitivity and time frame of the experiments. It is therefore understood that the results of the described experiments should be viewed with some caution as the employed lipase levels are 5000–15,000 times higher than the activities present in human tissue.

2. Materials and methods

2.1. Materials

All lipids (glyceryltriblimate, -trilaurinate, -trimyristate -trilaurate, lipid mixture E85, lipid mixture H12) used in this study were purchased from Sasol GmbH, Witten, Germany.

Lipases (lipoprotein lipase from pseudomonas sp. and lipase from Rh. Oryza) and all other chemicals were purchased from Sigma-Aldrich, Deisenhofen, Germany.

2.2. Methods

2.2.1. Preparation techniques

2.2.1.1. Preparation of microparticles. Placebo lipid microparticles either consisting of glyceryltriblimate or glyceryltriblurate were produced resulting in 2 different particle sizes (10 µm and 100 µm mean particle size).

The production techniques are described as followed:

2.2.1.2. Lipid microparticles preparation. Placebo lipid microparticles were produced using 2 different preparation techniques widely used for the preparation of polymeric or lipid based microparticles [3,24]:

2.2.1.3. Solvent evaporation method. 300 mg lipid powder was dissolved in 1.0 ml organic solvent (e.g. methylene chloride). The resulting solution was further emulsified at 40 °C into a small volume (3.0 ml) of a 1% (w/v) aqueous PVA solution using an Ultra Turrax® (IKA Werke GmbH & Co. KG Staufen, Germany). Particle size was controllable by adjusting the rotation speed of the emulsification tool. The emulsion was poured into a larger volume (150 ml) of an ice-cooled (5 °C) aqueous phase (PVA 0.1% (w/v)) and stirred with a propeller stirrer to allow for the evaporation of the organic solvent. The hardened microparticles were separated from the aqueous phase by filtration, rinsed with 40 ml of water and vacuum dried overnight at room temperature.

2.2.1.4. Melt dispersion method. With this preparation technique, a lipid melt was used instead of the solution of the lipid in an organic solvent. The lipid melt (300 mg lipid powder heated above its melting point) was poured into the PVA solution and emulsified as described for the solvent evaporation method. The following steps were the same as for the solvent evaporation method. The stirring time was reduced from 30 min to 5 min since this method is solvent free.

2.2.1.5. Implant manufacturing. Implants were prepared by using a 5 ton hydraulic press (Maassen, Eningen, Germany).

The implant component, i.e. tristearate powder was ground either with or without pore builder and compressed with a pressure of 2 t for 30 s. The obtained implants had an average weight of 50 mg a diameter of 5 mm and an average height of approximately 2.3 mm.

2.2.2. Preparation of twin-screw extrudates (tsc-extrudates)

Lipid based tsc-extrudates were prepared using a MiniLab® Micro Rheology Componuer (Thermo Haake GmbH Karlsruhe, Germany). Extrusion temperature was set to 43 °C to 48 °C which was slightly above the melting temperature of the respective low melting triglyceride. As pore forming agent PEG 6000 was used either alone or in combination with hydroxypropyl-β-cyclodextrin lyophilisate (ilyo). After application of the lipid powder mixture into the extruder barrel and subsequent softening of the mixture, extrusion was performed through the extruder outlet dye (diameter 2.0 mm). The rotation speed of the extruder screws was set to 40 rpm and the bypass channel was closed to inhibit material circulation within the extruder.

2.2.3. Degradation studies setup

In this study, solid lipid drug depot formulations i.e., microparticles, compressed implants, and extruded rods were incubated at 37 °C in PBS (2.0 ml isotonic 0.01 M phosphate buffer pH 7.4) containing the lipases mentioned above in different activities (100, 200 and 300 U/ml respectively). After 3 days of incubation the buffer media with the lipases was exchanged with fresh medium. FFA were isolated from the drawn samples and, after derivatization, analyzed.

The amount of FFA released during the incubation was used to compare the different formulations in terms of biodegradation. Degradation of the lipid mass was calculated as percentage of the total amount of saponifiable lipid mass.

2.2.4. Microparticles

10 mg of microparticles were incubated at 37 °C in 2 ml PBS pH 7.4 containing 100 U of both lipases. After 3 days 100 µl of the supernatant were drawn and the volume was replenished with fresh lipase solution. FFA were isolated from the drawn samples and, after derivatization, analyzed.

2.2.5. Compressed lipid implant study

In order to investigate on the degradation behaviour of compressed lipids tristearate implants were prepared from tristearate powder applying compression forces used for the preparation of the implants established in previous experiments [5,9].

The relationship between the lipase activity and the FFA-rate was analyzed using 3 different lipase activities (100, 200 and 300 U/ml respectively).

To analyse the influence of pores in the lipid implants tristearate implants were produced with increasing amount of pore builder (PEG 6000). The amount of pore builder used was 0, 20, 30, 40 and 50%. Prior to the start of the degradation experiments the implants were incubated in PBS for 4 weeks to allow the pore building agent to dissolve.

2.2.6. Lipid extrudate degradation study

10 different formulations of lipid based extrudates have been produced for this study. The prepared lipid extrudates always consist of a low melting and a high melting lipid component. Glyceryltripalmitate (D118), glyceryltripalmitate (D116) and glyceryltrimyristate (D114) served as high melting lipid component, whereas glyceryl trilaurate (D112) and two triglyceride-mixtures (H12 and E85) served as low melting component. H12 and E85 are synthetically produced triglycerides consisting of different fatty acids (lauric, myristic and palmitic acid) esterified in different ratios with glycerol. Due to the
specific composition H12 has a melting point of approx. 35 °C and E 85 melts at 41 °C.

In Table 1 the different formulations of the produced lipid extrudates are listed.

The aim of this study was to investigate on the effect of the high melting lipid composition, the effect of the low melting lipid composition, the effect of the ratio of the lipid composition and the effect of the different amounts of pore builder on the degradation behaviour.

The results of the preliminary experiments (data not shown) showed that the presence of PEG can interfere with the lipase induced lipolysis in an unpredictable manner therefore lipid extrudates have been incubated in 2 ml PBS pH 7.4 for 4 weeks to allow the pore builder to dissolve. These extrudates were used for the lipase incubation. A second batch of extrudates has been incubated in PBS for 2 months without the addition of the lipase solution to investigate on the stability under non lipolytic conditions.

2.2.7. Characterization methods

2.2.7.1. FFA extraction method. FFA were extracted from the lipase activity assay samples and samples drawn in the degradation experiments. Extraction provided quick analysis time and avoidance of derivatization interference with buffer components. The extraction method is based on the Dole and Meinertz extraction procedure [25]. The extraction solvent was prepared by mixing isopropanol–heptane–phosphoric acid (2 M) (40:10:1, v/v) and was thoroughly stirred before use. Extraction solvent (2.5 ml) was added and the tubes were thoroughly vortexed. The tubes were then immersed in a sonicator water bath (Branson Ultrasonic, Danbury, CT, USA) and the samples were sonicated in 30 s intervals for 2 min. Care was taken to avoid heating the samples during sonication. The samples were then vortexed vigorously and allowed to incubate at room temperature for 10 min. Heptane (1 ml) and water (1.5 ml) were added and the tubes were thoroughly vortexed and sonicated again for one min. Tubes were centrifuged at 1000 g for 10 min at 4 °C. After centrifugation the top organic layer was seen to separate cleanly from the aqueous layer. A 1.5 ml aliquot (88% of the total organic layer of 1.7 ml) of the top layer was transferred carefully using a pipette to 1.5 ml HPLC vials and dried under a stream of warm nitrogen. These samples were then used for derivatization.

2.2.7.2. Free fatty acid determination. As free fatty acids (FFA) are the major cleavage product of lipase induced triglyceride degradation, a quantifiable analysis was based on the FFA release upon lipid degradation. Phenacyl esters of fatty acids were prepared by the method described by Wood and Lee [26]. Briefly, samples were placed in HPLC vials with closed silicone/PTFE screw caps (VWR International, Darmstadt, Germany), then 25 ml of a phenacyl bromide solution (10 mg/ml in acetone) and 25 ml of a triethylamine solution (10 mg/ml in acetone) were added, capped under N2 and heated in a boiling water bath for 5 min. The excess of the phenacyl bromide was reacted with acetic acid (40 µl of a 2 mg/ml solution in acetone) and, after the evaporation of the solvents under a stream of N2 at a laboratory temperature, the derivatization products were reconstituted in methanol. FFA solutions of known concentrations (5–200.0 µg/ml) in methanol were used to generate calibration curves. Samples were analyzed using a Thermo Separation Products HPLC system (Thermo Fisher Scientific, Inc. Waltham, U.S.A.) equipped with a LiChrosphere RP C18 column (4.6 mm i.d.×250 mm, 5 µm) (Merck, Darmstadt, Germany). The mobile phase was a mixture of methanol–acetonitrile–water (80:10:10, v/v/v). Elution of phenacyl esters was monitored by absorbance at 254 nm. The detection limit was about 5 µg FFA per ml, accounted for stearic acid, i.e. approx. 20 nmol of fatty acid.

2.2.7.3. Lipase activity assay. In order to determine the activity and the stability of the used lipase enzyme activity assays were carried out. For each assay a trioleate emulsion was prepared by mixing 4.5 ml polyvinyl alcohol solution (2% w/v) with 1.5 ml triolein. This mixture was then emulsified at 0 °C with an Ultra Turrax® for 2 min at 15,000 rpm. 500 µl of this emulsion and 400 µl PBS buffer 0.01 M pH 7.4 then were incubated in a 2 ml Eppendorf cap for 10 min at 37 °C in a water bath. 100 µl of lipid solution was added and the mixture was incubated again at 37 °C. After 10 min the reaction was stopped by adding 1 ml of a methanol:acetone:methylene chloride mixture (1:1:1 v/v). Before derivatization and HPLC analysis the FFA produced in the triglyceride cleavage were extracted using the method described below.

1 U of enzyme activity was defined as the release of 1 µmol FFA per min at 37 °C.

2.2.7.4. Scanning Electron Microscopy (SEM). To investigate whether degradation of lipid drug delivery devices is visible SEM measurements were carried out. Samples were analyzed using a Field Emission Scanning Electron Microscope Joel JSM-6500F (Joel Inc., Peabody, USA). Therefore samples were put on adhesive carbon tape (BAL-TEC AG, Balzers, Principality of Liechtenstein) and attached to a custom made brass stub, carbon-sputtered and analyzed.

2.2.7.5. Particle size distribution. Particle size was determined using a Horiba LA 950 laser diffraction particle size distribution analyzer (Retsch Technology GmbH, Haan, Germany).

2.2.7.6. Determination of lipase adsorption on lipid surface. To analyse the amount of lipase adsorbed on the surface of the lipid devices, i.e. the amount of lipase which is hydrolytically active at the lipid–water interface, adsorption studies were carried out using a modified method after Brockmann [27]. Briefly, after the incubation of the lipid implants in lipase solutions for a defined amount of time at 37 °C, implants were washed in water and then incubated for 15 min in PBS pH 7.4 containing 0.15% Brij 35 to desorb bond lipase. Aliquots of the resulting solution were assayed for lipase concentration determination via fluorescence spectroscopy.

A calibration curve of known lipase concentrations was prepared for each adsorption test.

3. Results and discussion

3.1. Microparticles

Samples drawn from the incubated microparticles revealed a steady generation of free fatty acids due to the lipase activity (Fig. 1).

Table 1

<table>
<thead>
<tr>
<th>Batches</th>
<th>High melting lipid component (HMLC)</th>
<th>Low melting lipid component (LMLC)</th>
<th>Ratio of lipid components HMLC:LMLC</th>
<th>Amount/type of pore builder</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>D118</td>
<td>H12</td>
<td>80:20</td>
<td>0% PEG</td>
</tr>
<tr>
<td>E2</td>
<td>D118</td>
<td>H12</td>
<td>80:20</td>
<td>20% PEG</td>
</tr>
<tr>
<td>E3</td>
<td>D118</td>
<td>H12</td>
<td>80:20</td>
<td>40% PEG</td>
</tr>
<tr>
<td>E4</td>
<td>D118</td>
<td>H12</td>
<td>90:10</td>
<td>10% lyo, 10% PEG</td>
</tr>
<tr>
<td>E5</td>
<td>D118</td>
<td>H12</td>
<td>80:20</td>
<td>10% lyo, 10% PEG</td>
</tr>
<tr>
<td>E6</td>
<td>D118</td>
<td>H12</td>
<td>70:30</td>
<td>10% lyo, 10% PEG</td>
</tr>
<tr>
<td>E7</td>
<td>D118</td>
<td>E85</td>
<td>80:20</td>
<td>10% lyo, 10% PEG</td>
</tr>
<tr>
<td>E8</td>
<td>D116</td>
<td>D112</td>
<td>80:20</td>
<td>10% lyo, 10% PEG</td>
</tr>
<tr>
<td>E9</td>
<td>D116</td>
<td>H12</td>
<td>80:20</td>
<td>10% lyo, 10% PEG</td>
</tr>
<tr>
<td>E10</td>
<td>D114</td>
<td>H12</td>
<td>80:20</td>
<td>10% lyo, 10% PEG</td>
</tr>
</tbody>
</table>
For D118 microparticles a distinctive differentiation can be made concerning the degradation velocity of microparticles produced with different preparation techniques: microparticles produced with the solvent evaporation technique show faster degradation than microparticles prepared with the melt dispersion technique. Regarding the influence of the particle size on the FFA release from the microparticles it was found that it has a bigger influence for longer chain length triglycerides than for shorter chain lengths. For the particles made from D116 no such distinction can be made. All particle batches showed comparable degradation velocities. After 48 days the degradation of the total lipid mass reached 80% for D116 whereas D118 microparticles showed a degradation ranging from 45 to 65%. Over the time degradation velocity was decreasing in both studies probably due to the lipases inhibition by the cleavage products, i.e. by the accumulation of fatty acids in the incubation tubes as reported in the literature [28].

To investigate whether the degradation of the lipid drug delivery devices was visible SEM measurements were carried out. SEM photographs revealed strong differences in the surface morphology prior and after the degradation period. As seen in Fig. 2a–b the surface of the microparticles before lipase incubation was smooth whereas microparticles incubated with lipase changed their morphology.
towards a rough and uneven surface. However cracks or pores couldn’t be detected indicating that the degradation is only happening at the lipid water interface.

3.2. Implants degradation study

Compressed tristearate implants showed a constant and linear FFA release during lipase incubation. After 60 days of incubation only 0.7% of the total lipid mass was degraded by the enzymes. Compared to the fatty acid release from the microparticles, degradation kinetics of the implants were very slow most probably due to the small surface area exhibited by the implants. When extrapolating the applied lipase activity to a level found in human tissue (approx. 0.01 U/ml) the total degradation of these systems would surely exceed the average life span of a human being (>100 years). Macroscopically analyzed implants showed no change in surface morphology. However, SEM photographs did show changes in surface morphology but revealed no cracks or holes in the lipid matrix (Fig. 2c–d). Typical lipid crystals were visible after lipase incubation in high magnification. As expected, these findings confirm the interfacial activity of the lipases [29–31] resulting in a strict surface degradation and erosion of the compressed lipid implants. In this study we can assume the substrate concentration, i.e. the lipid, to be very low and the enzyme being not saturated, thus resulting in linear fatty acid release over several days. To analyze whether an increase in surface area could increase lipid hydrolysis porous implants were prepared.

With increasing amount of pore builder fatty acid generation was increased in a linear manner. Implants with 50% of pore builder partially collapsed upon lipase incubation probably due to the physically unstable porous structure. These fragments additionally increased the surface area presented to the lipase thus increasing the total degradation rate up to 1.8% after 30 days of incubation (Fig. 3a). The relationship between degradation rate and lipase activity was analyzed in another experiment. In this setup tristearate implants were incubated with 100, 200, 300 and 400 U/2 ml lipase respectively. As seen in Fig. 3b the results indicate that, after an initial a plateau phase, a linear relationship between the lipase activity and the degradation velocity can be assumed over a wide range of the lipase activity. To investigate on the amount of lipase involved in the lipid cleavage lipase adsorption studies have been conducted as described above.

3.3. Lipase adsorption studies

Results of these studies show that only a minor percentage of the total lipase used in these experiments is involved in the cleavage of the triglyceride. It can be presumed that less than 0.25% of the total lipase content is bound on the lipid surface of a compressed implant when applying a lipase activity of 100 U (Fig. 4a). Another experiment confirmed the linear relationship between lipase adsorption and presented lipid surface (Fig. 4b).
3.4. Extrudates degradation study

10 formulations of lipid based extrudates exhibited different degradation rates. Compared to the degradation rates of implants extrudate degradation was almost 10 times faster reaching up to 4% after 30 days of incubation. In our study we focused on the influence of the low melting lipid component, the high melting lipid component, the effect of the ratio of the different lipid components and the effect of pore builder on the degradation behaviour.

Reference extrudates stored in lipase-free buffer media for 2 months remained stable and showed no signs of erosion.

3.4.1. Effect of the low melting component

As seen in Fig. 5 extrudates containing trilaurate or H12 showed the highest degradation rate, reaching over 3% of lipid mass degradation after 30 days of incubation, whereas extrudates comprising E85 showed a lipid mass degradation of about 1.5%.

Effect of the high melting lipid component: The use of D114 as high melting lipid component leads to the highest degradation rate (3.6%) compared with D116 or D118 based extrudated implants (Fig. 6).

The effect of the mixing ratio of the lipid composition: Extrudated lipid implants were always prepared from mixtures of high and low melting components. As seen in Fig. 7 the composition with a mixing ratio of 80:20 showed a degradation of 3.75% of the total lipid mass after 30 days and therefore seems superior to 70:30 (2.5%) or 90:10 (1.6%).

3.4.2. Effect of the amount of pore builder

As already confirmed by the experiments with porous implants, extrudated lipid rods containing the highest amount of pore builder exhibited the highest degradation rate (3.95%). Extrudates without any pre-built pores reached only 1.65% of total lipid degradation at the end of the degradation study (Fig. 8).

Surprisingly almost all extrudates —except E1, E4 and E7— lost their physical integrity after a few days of lipase incubation resulting in small lipid particles suspended in the buffer media. The extrudates of the control group maintained their structure and morphology after incubation for more than 2 months in buffer media without lipase solution. Laser light diffraction measurements were carried out to analyze the size of the generated particles. As seen in Fig. 9 mean particle sizes of the collapsed lipid extrudates were in the µm size range.

This breakdown of the lipid matrix is rather unexpected and can be considered as a form of bulk erosion. Since polymers typically undergo surface erosion when they are enzymatically degraded [32–34] additional experiments have to be carried out in order to explain the reasons for the lipid extrudate erosion phenomenon and to analyze the mechanism of the lipid matrix breakdown. However, in vivo such degradation behaviour would be highly desirable as the generated small particles can be finally washed away by body fluids or can be eliminated by phagocytosis and therefore would lead to minimized irritation and foreign body reactions.

![Fig. 8. Extrudate degradation study: influence of the PEG content on the degradation behaviour (Average + S.D., n = 3).](image)

![Fig. 9. a) SEM photographs of particles generated during lipid matrix collapse of incubated extrudates (magnification 1000×). b) Surface morphology of particle generated by matrix collapse of lipid based extrudates (magnification 1000×). c) Typical particle size distribution of particles generated during lipase incubation of lipid based extrudates (size distribution of particles from extrudate formulation E6). d) Table of particle size distributions: mean particle size range (Average size [µm]).](image)
5. Conclusion

The presented results lead to the conclusion that depot systems based on single triglycerides and triglyceride–mixtures can be degraded by lipolytic enzymes under physiological conditions. Although lipid cleavage is a rather slow process, our studies clearly demonstrate that this process can be accelerated by using certain materials, implant geometries and preparation techniques. For particulate systems it can be stated that with decreasing particle sizes lipase degradation is increasing and finally capable to fully degrade such microparticles. This finding is consistent with the results of Reithmeier et al. who claimed a reduction of the number of implanted microparticles after 7 days of incubation [3].

Thus, if a short term drug delivery is necessary lipid microparticles may very well fulfill all the requirements for biodegradable controlled release devices. Compared to the microparticles compressed implants show only very slow degradation velocity. Presumably this is a result of the minor adsorption rate of the enzymes on the monolithic compressed lipid system: since microparticles exhibit a higher surface area due to their particulate nature these systems can be degraded quicker as monolithic systems with a relatively small surface area. Our studies indicate that the lipid cleavage is strictly happening on the surface of the implants — a finding concordant with several other publications [31,35,36]. This surface degradation and erosion process prevents behavior dramatically resulting in surface area. Using certain lipid components changes the degradation preparation technique of the depot system and thereby increasing the amount of pore builder as well as by the sort and the ratio of the used lipid system: since microparticles exhibit a higher surface area due to

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