



## How bulk fluid renewal can affect in vitro drug release from PLGA implants: Importance of the experimental set-up<sup>☆</sup>

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### ARTICLE INFO

#### Keywords:

PLGA  
Implant  
Controlled drug delivery  
Experimental set-up  
Ibuprofen

### ABSTRACT

The aim of this study was to better understand the potential impact of partial vs. complete renewal of the bulk fluid during drug release measurements from poly (lactic-co-glycolic acid) (PLGA)-based implants. A “standard experimental set-up”, in which the implants were directly exposed to well agitated phosphate buffer pH 7.4 was used, as well as set-ups, in which the implants were embedded within agarose hydrogels (mimicking living tissue). The gels were exposed to well agitated phosphate buffer pH 7.4. Ibuprofen-loaded implants were prepared by hot melt extrusion. The systems were thoroughly characterized before and during drug release by optical and scanning electron microscopy, gravimetric analysis, pH and solubility measurements as well as gel permeation chromatography. The bulk fluid was either completely or partially replaced by fresh medium at each sampling time point. In all cases, sink conditions were provided in the agitated bulk fluids throughout the experiments. Interestingly, the agarose set-ups did not show any noteworthy impact of the bulk fluid sampling volume on the observed drug release patterns, whereas complete fluid renewal in the “standard set-up” led to accelerated drug release. This could be explained by the considerable fragility of the implants once substantial polymer swelling set on, transforming them into PLGA *gels*: Complete fluid renewal caused partial disintegration and damage of the highly swollen systems, decreasing the lengths of the diffusion pathways for the drug. The mechanical stress is very much reduced at low sampling volumes, or if the implants are embedded within agarose gels. Thus, great care must be taken when defining the conditions for in vitro drug release measurements from PLGA-based implants: Once substantial system swelling sets on, the devices become highly fragile.

### 1. Introduction

Poly (D,L lactic-co-glycolic acid) (PLGA)-based implants offer an interesting potential for parenteral controlled drug delivery (Shah et al., 1993; Nair and Laurencin, 2007; Grund et al., 2011). This is because: (i) PLGA is biocompatible and completely biodegradable (avoiding the need to remove empty remnants after drug exhaust) (Shah et al., 1993; Nair and Laurencin, 2007; Dorta et al., 2002). (ii) Drug release can be controlled during periods ranging from a few days up to several months (Ochi et al., 2021). (iii) A variety of manufacturing processes can be applied for their preparation, including for example hot melt extrusion (McConville et al., 2015), compression (Maturavongsadit et al., 2020) and 3D printing (Guo et al., 2018).

The underlying mass transport mechanisms controlling drug release from PLGA implants can be very complex (Ochi et al., 2021; Fredenberg et al., 2011; Siepmann et al., 2005). The following are examples for

phenomena which can be involved. Their relative importance can very much depend on the composition and structure of the implant (and, hence the manufacturing procedure), as well as on the experimental conditions chosen for the in vitro release measurements: Upon implantation, water penetrates into the implants. Generally, the entire system is rapidly wetted. However, the amounts of water at this stage are often limited, because the commonly used PLGA grades are rather hydrophobic and the polymeric matrix is dense. The presence of the water initiates ester bond cleavage throughout the device (“bulk erosion”) (von Burkersroda et al., 2002). In addition, the drug can dissolve in the water and becomes mobile (although often only to a limited extent, since the amounts of water in the system are still low). Consequently, the dissolved drug molecules/ions diffuse out into the surrounding environment, due to concentration gradients (Fredenberg et al., 2011; Siepmann and Siepmann, 2020). Depending on the system size and initial inner structure, the pH might *locally* significantly drop (Ding

<sup>☆</sup> Submitted to the special IJP/IJPC issue “In honor of Professor Ian Tucker” (for IJPC).

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et al., 2006; Chen et al., 2017; Ford Versypt et al., 2013). This is because ester bond cleavage generates shorter chain acids, which might accumulate, especially at the center of the implant: The diffusion of these acids out of the device takes time (as well as the diffusion of bases from the environment into the system), and the rate at which the acids are generated can be higher than the rate at which they are neutralized (Antheunis et al., 2010). Since ester bond hydrolysis is catalyzed by protons, this can lead to autocatalytic effects (Siepmann et al., 2005; Ford Versypt et al., 2013; Antheunis et al., 2010). Consequently, polymer degradation can be accelerated and the drug release rate increases. Furthermore, limited drug solubility effects within the PLGA implants as well as within the surrounding bulk fluid might play a major role (Hu et al., 2011; Faisant et al., 2006). Also, drug diffusion through water-filled pores might be of importance. If the pores exist from the beginning, this might cause a high initial drug release rate. The pores might then be closed by (limited) PLGA swelling, terminating the related burst release phase (Huang et al., 2015; Kim et al., 2006).

One of the key reasons for the potential complexity of the drug release mechanisms from PLGA-based implants is polymer degradation, which has multiple consequences over time: (i) The macromolecular network becomes more and more hydrophilic, since the cleavage of each ester bond generates two new hydrophilic end groups: an -OH and a -COOH group. (ii) The mechanical stability of the polymeric matrix decreases, since the macromolecular chain length decreases, resulting in less intense polymer entanglement. (iii) Water-soluble degradation products accumulate (being poorly mobile in the initially dense polymeric system, similar to the drug), creating a steadily increasing hydrostatic pressure in the implant. Thus, the implants become more and more water-loving with time, get less resistant to dimensional changes and more and more actively attract water. Consequently, at a certain time point, substantial implant swelling sets on (Bode et al., 2019a; Tamani et al., 2019; Gasmir et al., 2015; Gu et al., 2016). Increases in system wet mass up to 1000% have been reported (Bode et al., 2019a; Bode et al., 2019b). This tremendous device swelling fundamentally changes the conditions for drug release: The polymer network becomes much less dense, the dissolved drug molecules/ions become much more mobile. Hence, the final, rapid drug release phase sets on (Fredenberg et al., 2011; Brunner et al., 1999; Matsumoto et al., 2005).

Unfortunately, yet no compendial method has been described for the measurement of drug release from parenteral controlled drug delivery systems. So, great care must be taken when comparing drug release kinetics obtained under different conditions. Frequently, the implants are directly exposed to a well agitated bulk fluid, such as phosphate buffer pH 7.4 (Ghalanbor et al., 2013; Gosau and Müller, 2010; Li et al., 2018). At pre-determined time points, samples are withdrawn from the release medium and often replaced by fresh bulk fluid. It is well known that the sampling frequency and volume likely affect the observed drug release patterns, if limited drug solubility effects in the bulk fluid are of importance. Also, since PLGA degradation is catalyzed by protons, temporary drops in the pH of the *bulk fluid* can lead to accelerated polymer chain cleavage and faster drug release (Siepmann et al., 2005; Ford Versypt et al., 2013; Blasi et al., 2007). However, little is yet known about potential further impacts of the sampling schedule on drug release.

To more realistically mimic the conditions experienced by the dosage forms upon implantation into human tissue, hydrogels have been proposed (Kožák et al., 2021; Klose et al., 2008; Leung et al., 2017). The idea is to minimize convective mass transport (as in well agitated bulk fluids) and place the system in a semi-solid environment. It has recently been shown that the presence of such a hydrogel can limit substantial implant swelling and, thus, slow down drug release (Bassand et al., 2022a). The group of Ostergaard published some very interesting reports on how drug transport in a surrounding hydrogel can be monitored using UV analysis from a controlled release implant (Ostergaard, 2018; Jensen et al., 2016; Sun et al., 2017). This type of results can be very helpful to better understand which phenomena are decisive for drug

transport into the environment surrounding the implant.

The aim of this study was to better understand how sensitive the experimentally measured drug release profiles from PLGA-based implants might be on the renewal of the release medium (providing sink conditions in the agitated bulk fluids in all cases). Ibuprofen-loaded PLGA implants were prepared by hot melt extrusion. Three experimental set-ups were used: The implants were either directly exposed to well agitated phosphate buffer pH 7.4, or embedded within agarose gels, which were exposed to phosphate buffer in Eppendorf tubes or transwell plates (Bassand et al., 2022a). The systems were thoroughly characterized before and during drug release by optical and scanning electron microscopy, gravimetric analysis, pH and solubility measurements as well as gel permeation chromatography, to explain the observed results.

## 2. Materials and methods

### 2.1. Materials

Poly (D,L lactic-co-glycolic acid) (PLGA, 50:50 lactic acid: glycolic acid; Resomer RG 503H; Evonik, Darmstadt, Germany); ibuprofen (BASF, Ludwigshafen, Germany); agarose (genetic analysis grade; Fisher Scientific, Janssen Pharmaceutical, Geel, Belgium); potassium dihydrogen orthophosphate and sodium hydroxide (Acros Organics, Geel, Belgium); tetrahydrofuran (HPLC grade) (Fisher Scientific, Illkirch, France); acetonitrile (VWR, Fontenay-sous-Bois, France); sodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ; Panreac Quimica, Barcelona, Spain); aqueous 10 N lactic acid solution and 0.05 N aqueous sodium hydroxide solution (Sigma Aldrich, Saint-Louis, Missouri, USA).

### 2.2. Implant preparation

Appropriate amounts of polymer [milled for  $4 \times 30$  s with a grinder (Valentin, Seb, Ecully, France)] and drug powders were blended for 5 min at 20 rpm in a Turbula T2C Shaker-Mixer (Willy A Bachofen, Basel, Switzerland). Three hundred mg of the mixture were filled into a 1 mL syringe (Henke Sass Wolf, Tuttlingen, Germany), followed by heating to 105 °C for 15 min in an oven (FP115, Binder, Tuttlingen, Germany). The molten blend was manually extruded using the syringe. The extrudate was cut with a hot scalpel into cylindrical implants of approximately 5 mm length.

### 2.3. Optical macroscopy and implant dimensions

Pictures of implants before exposure to the release medium were taken using a SZN-6 trinocular stereo zoom macroscope (Optika, Ponteranica, Italy), equipped with an optical camera (Optika Vision Lite 2.1 software). The lengths and diameters of the implants were determined using the software ImageJ (US National Institutes of Health, Bethesda, Maryland, USA).

### 2.4. Practical drug loading

Implants were dissolved in 5 mL acetonitrile, followed by filtration (PVDF syringe filters, 0.45  $\mu\text{m}$ ; Agilent Technologies, Santa Clara, USA) and drug content determination by HPLC-UV analysis using a Thermo Fisher Scientific Ultimate 3000 Series HPLC. The latter was equipped with an LPG 3400 SD/RS pump, an autosampler (WPS-3000 SL) and a UV-Vis detector (VWD-3400RS) (Thermo Fisher Scientific, Waltham, USA). A reversed-phase column C18 (Gemini 5  $\mu\text{m}$ ; 110 Å; 150  $\times$  4.6 mm; Phenomenex, Le Pecq, France) was used. The mobile phase was a mixture of 30 mM  $\text{Na}_2\text{HPO}_4$  pH 7.0: acetonitrile (60:40, v:v). The detection wavelength was 264 nm, and the flow rate 0.5 mL/min. Ten microliter samples were injected. Each experiment was conducted in triplicate, mean values  $\pm$  standard deviations are reported.

## 2.5. Drug solubility measurements

Excess amounts of ibuprofen were exposed to 20 mL phosphate buffer pH 7.4 USP 42 in glass flasks, which were horizontally shaken (80 rpm) at 37 °C in an incubator (GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). Optionally, the pH was adjusted using aqueous solutions of 10 N lactic acid or 0.05 N NaOH (final pH values at equilibrium are reported). At pre-determined time points, samples were withdrawn, immediately filtered (PVDF syringe filter, 0.45 µm; Agilent, Santa Clara, USA) and diluted. The drug contents of the samples were determined by HPLC-UV, as described above. Samples were withdrawn until equilibrium was reached. Each experiment was conducted in triplicate, mean values  $\pm$  standard deviations are reported.

## 2.6. In vitro drug release

Three experimental set-ups were used to measure ibuprofen release from the PLGA implants:

### 2.6.1. In well-agitated bulk fluids

Implants were placed in 5 mL Eppendorf tubes (1 implant per tube), filled with 5 mL phosphate buffer pH 7.4 USP 42 (an aqueous solution of 0.05 M monophasic potassium phosphate, 0.0391 M sodium hydroxide) (Fig. 1A). Metal baskets avoided that the implants sank to the bottom of the tubes (resulting in potentially limited contact with the bulk fluid). The mesh size (250 µm) was sufficient to allow for convective flow and rapid medium exchange “inside – outside” the basket. The tubes were placed in a horizontal shaker (80 rpm, 37 °C; GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At predetermined time points, the entire bulk fluid (5 mL), or 3 mL or 1 mL of the bulk fluid was replaced by fresh release medium. The withdrawn samples were filtered (PVDF syringe filter, 0.45 µm; Agilent) and analyzed for their ibuprofen contents by HPLC-UV, as described above.

### 2.6.2. In agarose gels in Eppendorf tubes

Implants were embedded into agarose gels in 5 mL Eppendorf tubes, as illustrated in Fig. 1B (1 implant per tube). An agarose dispersion (0.5% w:v) in phosphate buffer pH 7.4 (USP 42) was heated to 100 °C under magnetic stirring (250 rpm) until a clear solution was obtained. The latter was cooled to 47 °C and continuously stirred (to prevent gelation). 0.5 mL of the solution was placed into the bottom of an Eppendorf tube and cooled in a refrigerator for 5 min to allow for

gelation. An implant was carefully placed on top of the gel, and covered with second layer of 0.5 mL agarose solution (47 °C), followed by cooling in a refrigerator for 5 min. Four mL phosphate buffer pH 7.4 USP 42 were added on top of the gel, and the tubes were placed in a horizontal shaker (80 rpm, 37 °C; GFL 3033). Thus, an implant was exposed to 1 mL agarose gel and (indirectly) 4 mL bulk fluid: In total 5 mL “release medium” (gel + bulk fluid). This is the same volume of “release medium” as used for the drug release measurements in “well-agitated bulk fluid only” (described above). At predetermined time points, the entire bulk fluid (4 mL), or 3 mL or 1 mL of the bulk fluid was replaced by fresh release medium. The withdrawn samples were treated as for the drug release measurements in well agitated bulk fluids.

### 2.6.3. In agarose gels in transwell plates

Implants were embedded in agarose gels in transwell plates (1 implant per insert, membranes: 1.13 cm<sup>2</sup>, 11 µm, 0.4 µm pore size; Nunc, Roskilde, Denmark), as illustrated in Fig. 1C. The agarose gels were prepared as described above, and the implants included accordingly (placed between 2 “layers” of 0.5 mL gel). The well plates were filled with 4 mL phosphate buffer pH 7.4, covered with lids and Parafilm to minimize evaporation, and placed in a horizontal shaker (80 rpm, 37 °C; GFL 3033). So, again, the total volume of “release medium” (gel + bulk fluid) was 5 mL. At predetermined time points, the entire bulk fluid (4 mL), or 3 mL or 1 mL of the bulk fluid was replaced by fresh (pre-heated) release medium. The withdrawn samples were treated as for the drug release measurements in well agitated bulk fluids.

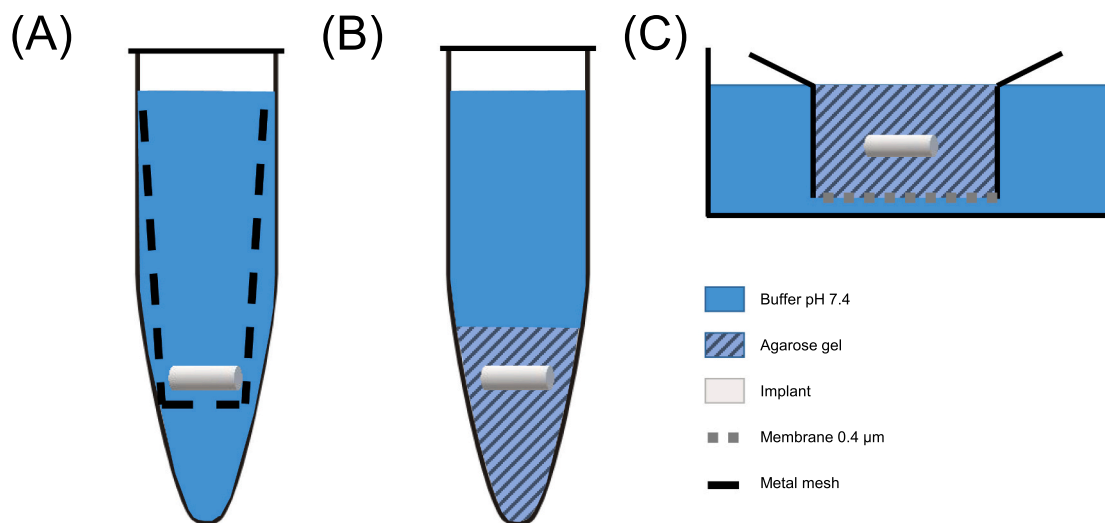
### 2.6.4. For all set-ups the following conditions applied

In all cases, sink conditions were provided in the agitated bulk fluids throughout the observation periods. All experiments were conducted in triplicate. Mean values  $\pm$  standard deviations are reported.

Furthermore, the pH of the bulk fluids was measured at pre-determined time points using a pH meter (InoLab pH Level 1; WTW, Weilheim, Germany). Mean values  $\pm$  standard deviations are reported.

## 2.7. Implant swelling

Implants were treated as for the in vitro drug release studies described above. At pre-determined time points:



**Fig. 1.** Schematic presentations of the experimental set-ups used to monitor drug release from the PLGA-based implants: (A) In well-agitated release medium in Eppendorf tubes, (B) In agarose gels in Eppendorf tubes, the gels being exposed to well-agitated release medium, (C) In agarose gels in transwell plates, the receptor compartment containing well-agitated release medium. Details are given in the text.

- (i) Pictures of the implants were taken with a SZN-6 trinocular stereo zoom microscope (Optika), equipped with an optical camera (Optika Vision Lite 2.1 software). The lengths and diameters of the implants were determined using the software ImageJ (US National Institutes of Health). Dynamic changes in the systems' volume were calculated considering cylindrical geometry.
- (ii) Implant samples were withdrawn and excess water was carefully removed using Kimtech precision wipes (Kimberly-Clark, Rouen, France) and weighed [*wet mass* (*t*)]. The *change in wet mass (%) (t)* was calculated as follows:

$$\text{change in wet mass } (\%) (t) = \frac{\text{wet mass } (t) - \text{mass } (t = 0)}{\text{mass } (t = 0)} \times 100\% \quad (1)$$

where *mass* (*t* = 0) denotes the implant mass before exposure to the release medium.

All experiments were conducted in triplicate. Mean values +/- standard deviations are reported.

### 2.8. Implant erosion and PLGA degradation

Implants were treated as for the in vitro drug release studies described above. At pre-determined time points, implant samples were withdrawn and freeze-dried (freezing at -45 °C for 2 h 35 min, primary drying at -20 °C/0.940 mbar for 35 h 10 min and secondary drying at +20 °C/0.0050 mbar for 35 h; Christ Alpha 2-4 LSC+; Martin Christ, Osterode, Germany).

The *dry mass (%) (t)* was calculated as follows:

$$\text{dry mass } (\%) (t) = \frac{\text{dry mass } (t)}{\text{mass } (t = 0)} \times 100\% \quad (2)$$

where *mass* (*t* = 0) denotes the implant's mass before exposure to the release medium. All experiments were conducted in triplicate. Mean values +/- standard deviations are reported.

The average polymer molecular weight (*M<sub>w</sub>*) of the PLGA was determined by gel permeation chromatography (GPC) as follows: Freeze-dried implant samples were dissolved in tetrahydrofuran (at a concentration of 3 mg/mL). One hundred µL samples were injected into an Alliance GPC (refractometer detector: 2414 RI, separation module e2695, Empower GPC software; Waters, Milford, USA), equipped with a PLgel 5 µm MIXED-D column (kept at 35 °C, 7.8 × 300 mm; Agilent). Tetrahydrofuran was the mobile phase (flow rate: 1 mL/min). Polystyrene standards with molecular weights between 1480 and 70,950 Da (Polymer Laboratories, Varian, Les Ulis, France) were used to prepare the calibration curve. All experiments were conducted in triplicate. Mean values +/- standard deviations are reported.

### 2.9. Scanning electronic microscopy (SEM)

The internal and external morphology of the implants before and after exposure to the release medium was studied using a JEOL Field Emission Scanning Electron Microscope (JSM-7800F, Japan), equipped with the Aztec 3.3 software (Oxford Instruments, Oxfordshire, UK). Samples were fixed with a ribbon carbon double-sided adhesive and covered with a fine chrome (Cr) layer. In the case of implants which had been exposed to the release medium before observation, the systems were treated as described for the in vitro release studies described above. At predetermined time points, implant samples were withdrawn, optionally cut with a scalpel and freeze-dried (as described in section 2.8. *Implant erosion and PLGA degradation*).

## 3. Results and discussion

During in vitro drug release measurements from PLGA implants, often parts of the release medium are withdrawn at pre-determined time points and replaced with fresh medium. This procedure might affect the

dosage form and alter the release rate. The aim of this study was to investigate the potential importance of the replaced bulk fluid volume, using the 3 experimental set-ups illustrated in Fig. 1. The bulk fluid (phosphate buffer pH 7.4) was either in direct contact with the implants, or in contact with a hydrogel in which the implants were embedded. The bulk fluid was either entirely replaced (100%), or to a major extent (60–75%), or to a minor extent (20–25%). In all cases, sink conditions were provided in the bulk fluids throughout the experiments. Ibuprofen-loaded implants were prepared by hot melt extrusion. The drug loading was about 8%, the implants were cylindrical in shape (5.7 mm length, 2.7 mm diameter) and had a homogeneous appearance (optical macroscopy picture in Table 1). A PLGA with a "50:50 lactic acid: glycolic acid" ratio (Resomer RG 503H) was used as a model PLGA.

### 3.1. In vitro drug release and implant swelling

The diagrams at the top of Fig. 2 show the observed ibuprofen release kinetics from the PLGA implants. The experiments were conducted in triplicate, mean values +/- standard deviations are reported. An implant was:

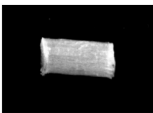
- directly exposed to 5 mL phosphate buffer pH 7.4 in an Eppendorf tube (Fig. 1A). The latter was horizontally shaken at 80 rpm at 37 °C. A metal basket avoided that the implant could sink to the bottom of the tube. Its mesh size (250 µm) was sufficiently large to allow for convective flow and rapid medium exchange between the fluid inside and the outside of the basket.
- embedded within an agarose gel (1 mL), which was exposed to 4 mL phosphate buffer pH 7.4 in an Eppendorf tube (Fig. 1B). The latter was horizontally shaken at 80 rpm at 37 °C.
- embedded within an agarose gel (1 mL), located in the donor compartment of a transwell plate (Fig. 1C). The acceptor compartment was filled with 4 mL phosphate buffer pH 7.4. The transwell plate was horizontally shaken at 80 rpm at 37 °C.

The bulk fluids were completely renewed at each sampling time point (4 or 5 mL), or only 3 or 1 mL of it (green, orange and blue curves). As it can be seen, the volume of the renewed bulk fluid did not have a noteworthy impact on drug release from the implants embedded within an agarose gel. In contrast, in the case of direct exposure to the phosphate buffer (standard set-up), ibuprofen release was faster when the entire release medium was renewed, compared to only partial replacement (Fig. 2, diagram at the top on the left-hand side). To better understand the reasons for this set-up dependent sensitivity, the implants were thoroughly characterized, monitoring the dynamic changes in the systems' dimensions, dry and wet mass, PLGA polymer molecular weight, inner & outer morphology as well as pH of the bulk fluids.

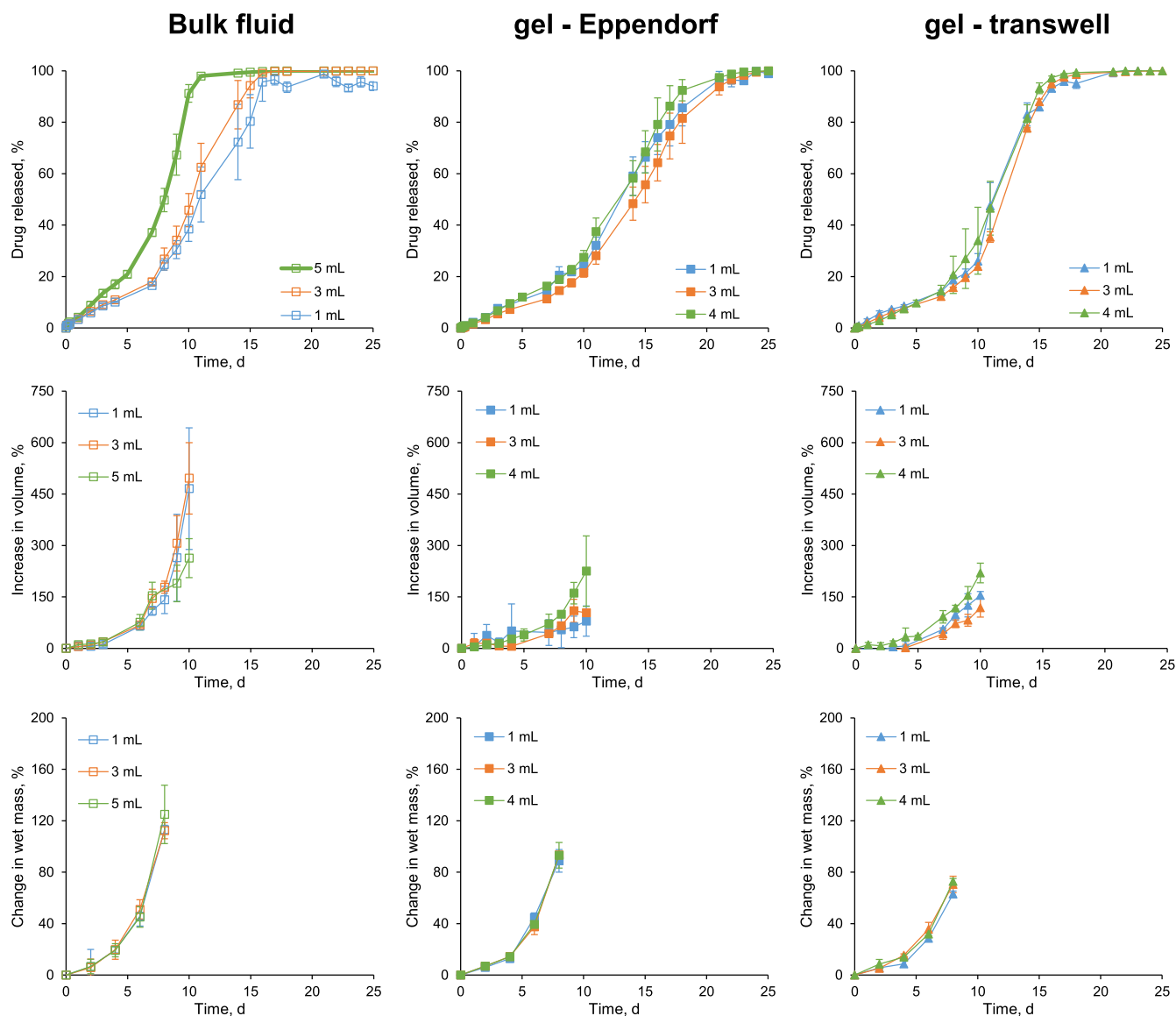
Furthermore, as it can be seen in the top row of Fig. 2, ibuprofen release was bi-phasic in all cases: irrespective of the type of experimental set-up and renewed bulk fluid volume. No noteworthy burst release was observed in any case. A zero-order release phase (with an about constant drug release rate) started from the beginning. After about 7–10 d, the

**Table 1**

Key properties of the investigated ibuprofen-loaded PLGA implants before exposure to the release medium (T<sub>g</sub>: glass transition temperature, T<sub>m</sub>: melting temperature). Mean values ± standard deviations are indicated (*n* = 3).

Practical drug loading (%)	Weight (mg)	Length (mm)	Diameter (mm)	Macroscopic picture
8.0 ± 0.9	30.9 ± 3.5	5.7 ± 0.3	2.7 ± 0.2	





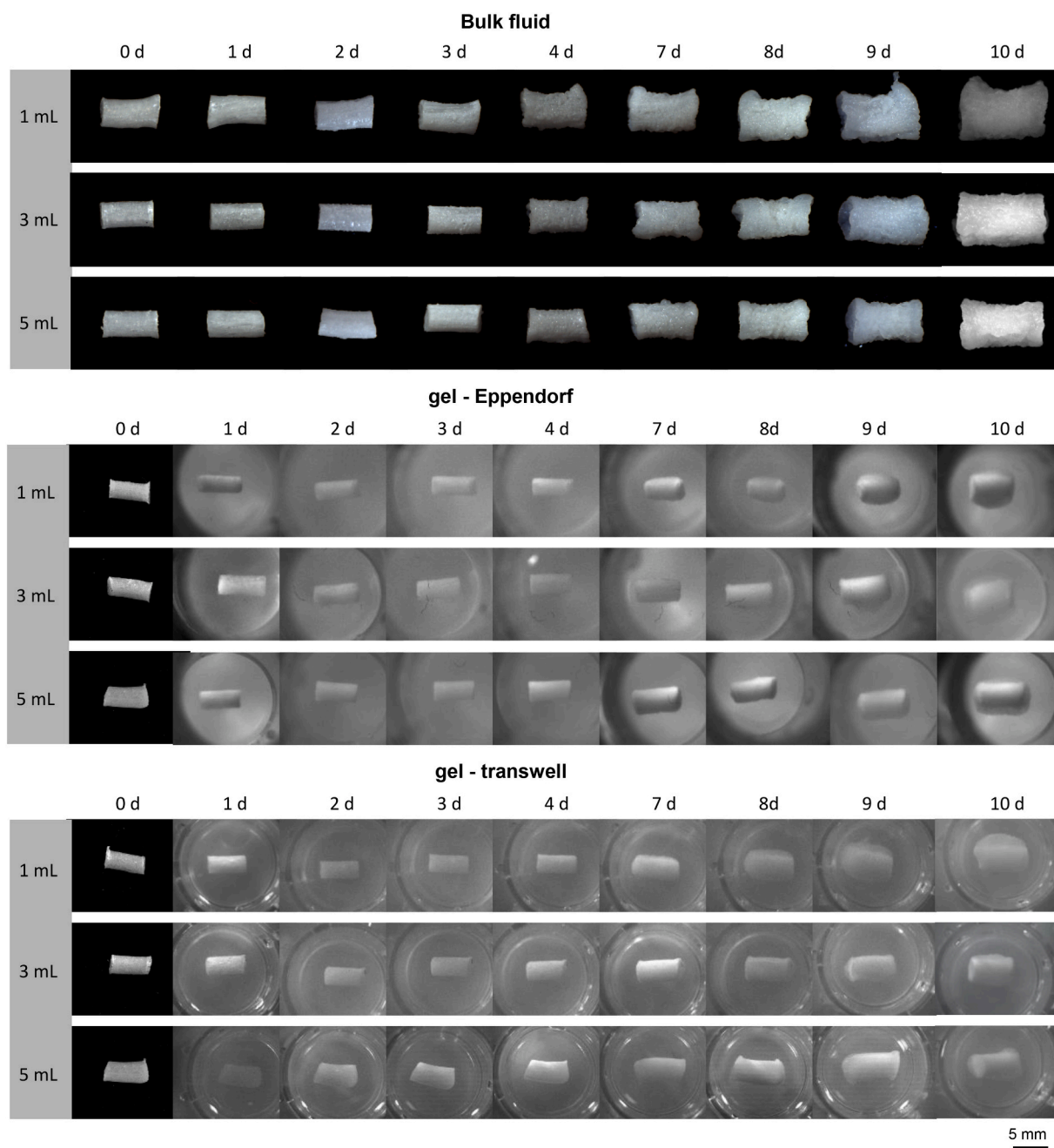
**Fig. 2.** Drug release, increase in volume and change in wet mass of ibuprofen-loaded PLGA implants upon exposure to phosphate buffer pH 7.4 using the 3 experimental set-ups. The implants were placed in well agitated bulk fluids in Eppendorf tubes, in agarose gels exposed to well agitated bulk fluid in Eppendorf tubes, or in agarose gels in transwell plates (the receptor compartment containing well-agitated bulk fluid). At pre-determined time points, the entire bulk fluid, or 3 or 1 mL thereof, was renewed (as indicated).

final rapid release phase began (accounting for about 80% drug release), leading to complete ibuprofen exhaust. The onset of this final rapid release phase coincided with the onset of substantial implant swelling, as it can be seen in the diagrams in the middle and at the bottom of Fig. 2. It has previously been reported that initially only limited amounts of water are able to penetrate into PLGA-based drug delivery systems, so that drug mobility remains limited (Bode et al., 2019a). Nevertheless, the entire dosage forms are rather rapidly wetted (by small amounts of water), initiating PLGA degradation throughout the system (“bulk erosion”). Upon hydrolytic cleavage of an ester bond in a PLGA chain, 2 shorter chains are created and 2 new hydrophilic end groups: an -OH and a -COOH end group. Thus, with time, the polymeric system becomes more and more hydrophilic. In addition, the PLGA chains become shorter and, hence, less entangled. Furthermore, water-soluble degradation products create a steadily increasing hydrostatic pressure inside the implant. At a certain time point, these changes result in the penetration of *considerable* amounts of water into the system, e.g. the volume of the investigated PLGA implants increased by about 500% after 10

d upon direct exposure to phosphate buffer pH 7.4 (Fig. 2, diagram on the left hand side in the middle row). This dramatically changes the conditions for drug release: Ibuprofen is much more mobile in a highly swollen PLGA gel compared to the initially “dry” and dense polymer matrix. Consequently, the drug release rate increases.

It has previously been shown that the presence of a hydrogel surrounding a PLGA implant of similar composition substantially limits implant swelling, resulting in slower drug release (Bassand et al., 2022a). This tendency has been confirmed in this study: As it can be seen in the diagrams middle and at the bottom of Fig. 2, system swelling was less pronounced, when the implants were embedded in a gel. The optical macroscopy pictures in Fig. 3 illustrate these phenomena: The implants underwent substantial swelling after about 7–10 d. This process was particularly pronounced upon direct exposure to the bulk fluid (compared to the agarose gel set-ups). This is also in good agreement with other reports on the hindering of PLGA swelling by surrounding hydrogels, e.g. (Kozák et al., 2021).

The SEM pictures at the top of Fig. 4 show surfaces and cross-sections



**Fig. 3.** Optical macroscopy pictures of ibuprofen-loaded PLGA implants after different exposure times to phosphate buffer pH 7.4 using the 3 experimental set-ups. The implants were placed in well agitated bulk fluids in Eppendorf tubes, in agarose gels exposed to well agitated bulk fluid in Eppendorf tubes, or in agarose gels in transwell plates (the receptor compartment containing well-agitated bulk fluid). At pre-determined time points, the entire bulk fluid, or 3 or 1 mL thereof, was renewed (as indicated).

of ibuprofen-loaded implants before exposure to the release medium. As it can be seen, the surface was smooth and non-porous (due to the manufacturing process), which can in part explain the absence of any noteworthy burst release. The other SEM pictures in Fig. 4 show implants which had been directly exposed to the release medium, or embedded within agarose gels for 8 d. Please note that the samples had been freeze-dried before analysis, so artefacts have been created. The pictures of surfaces show highly porous and “folded” structures. These likely result from the drying of highly swollen PLGA gels (Bassand et al., 2022a; Bassand et al., 2022b; Bassand et al., 2022c). The cross-sections indicate that in addition to these highly swollen surface-near regions, much less swollen regions exist at this time point, irrespective of the type of set-up. Please note that the pictures were not systematically taken at

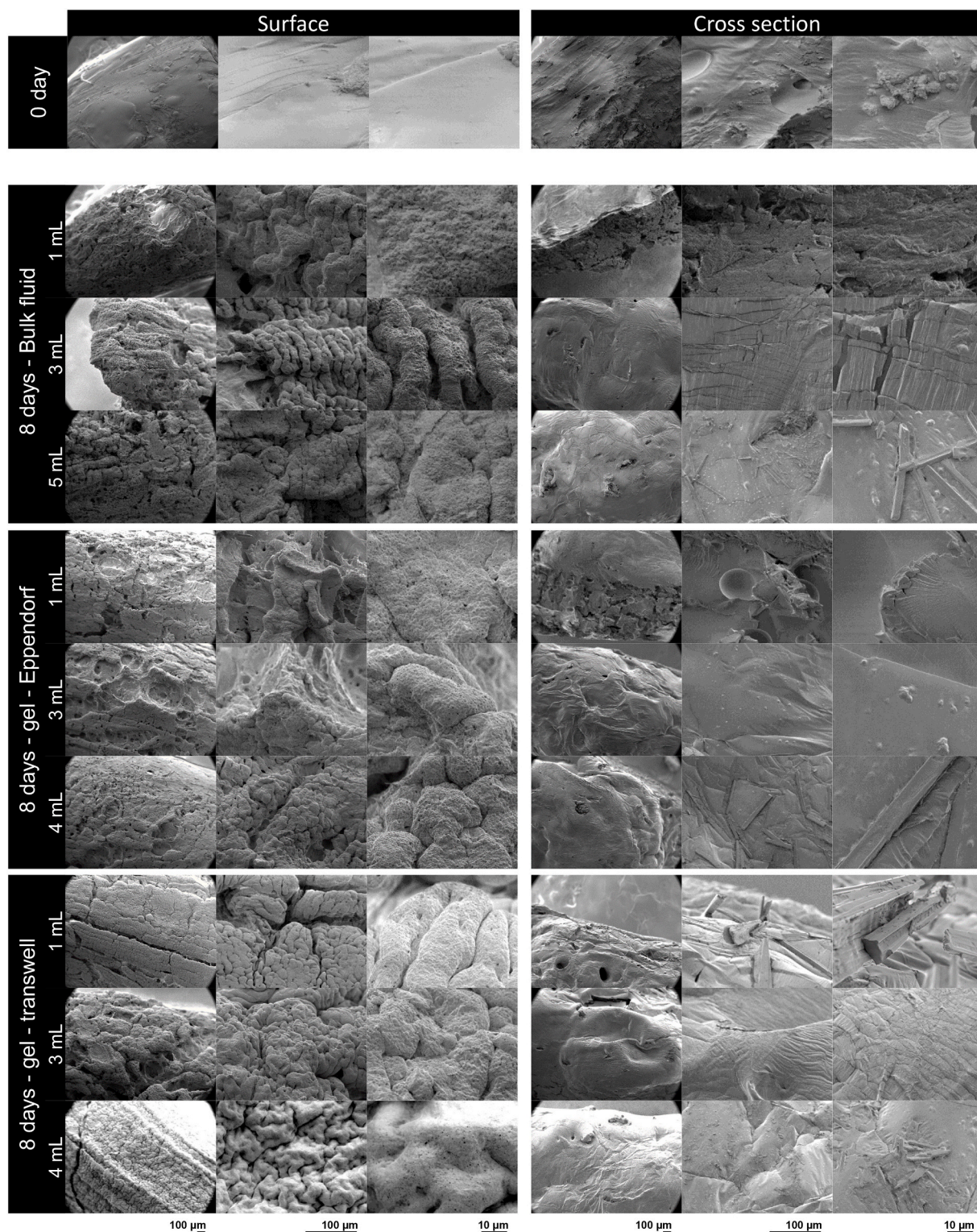
similar positions. Importantly, there was no sign for a significant impact of the volume of the replaced bulk fluid on the dynamic changes of the inner and outer implant morphology.

To better understand the sensitivity of drug release on the sampling volume in the case of direct exposure to the release medium and insensitivity in the case of implants, which were embedded into agarose gels, also the dynamic changes in the PLGA polymer molecular weight, implants’ dry mass and pH of the bulk fluid were studied.

### 3.2. PLGA degradation and implant erosion

The diagrams at the top of Fig. 5 show the pH of the bulk fluids the implants or agarose gels were exposed to, as a function of the exposure

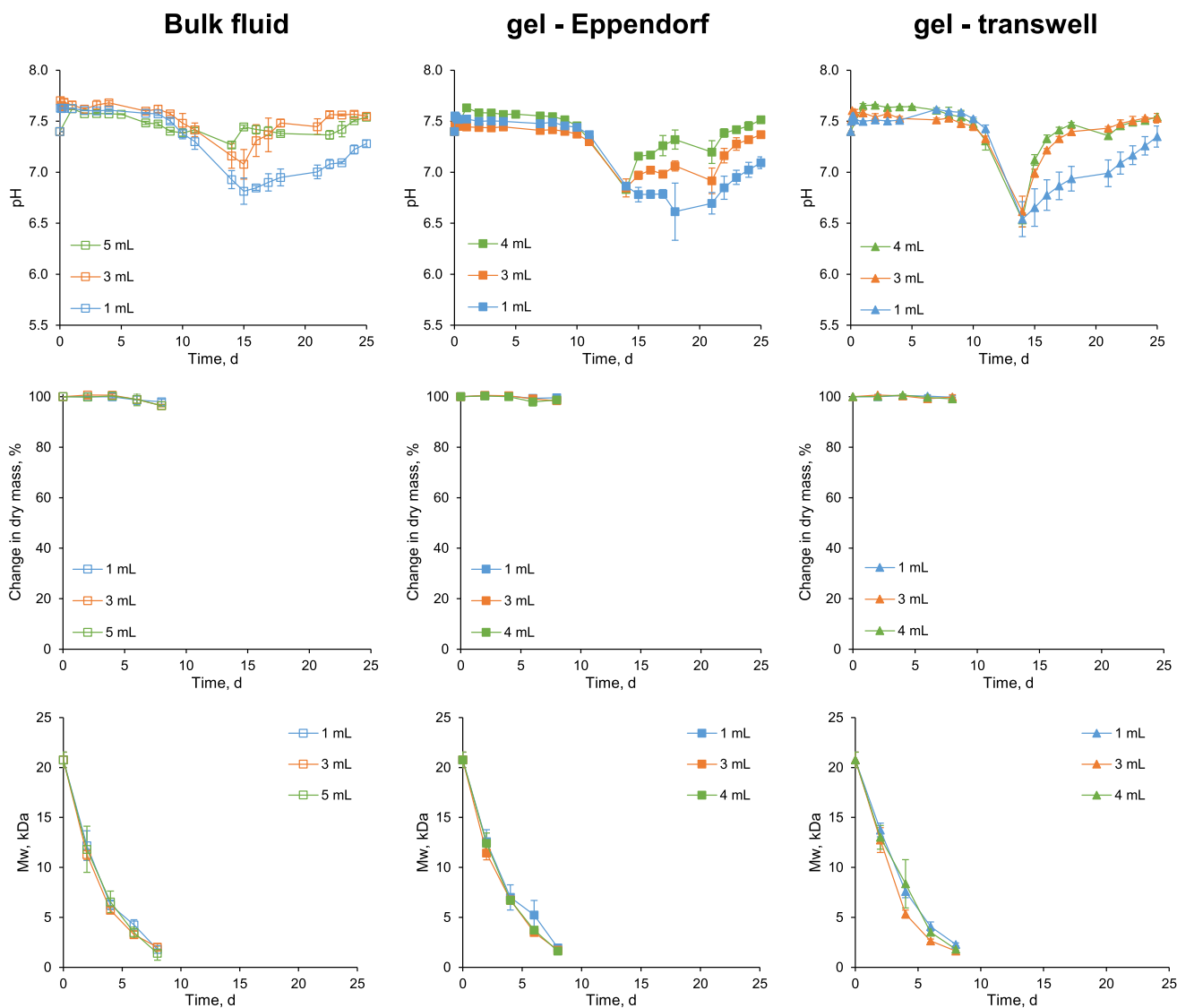




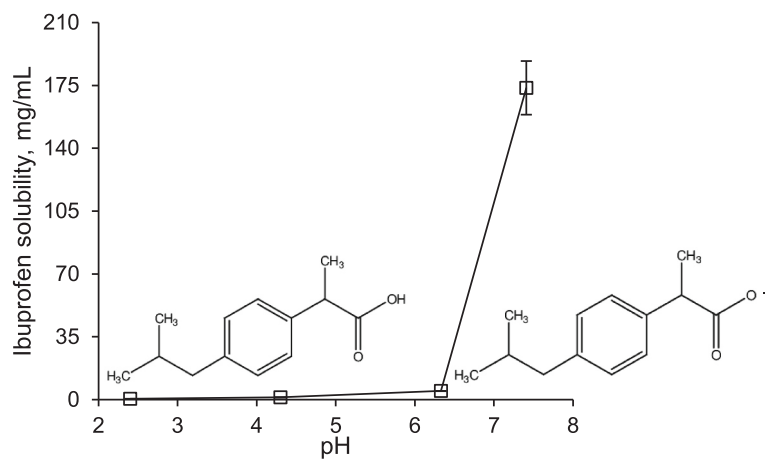
**Fig. 4.** SEM pictures of surfaces and cross-sections of ibuprofen-loaded PLGA implants before and after 8 days exposure to phosphate buffer pH 7.4 using the 3 experimental set-ups. The implants were placed in well agitated bulk fluids in Eppendorf tubes, in agarose gels exposed to well agitated bulk fluid in Eppendorf tubes, or in agarose gels in transwell plates (the receptor compartment containing well-agitated bulk fluid). At pre-determined time points, the entire bulk fluid, or 3 or 1 mL thereof, was renewed (as indicated). Please note that after exposure to the release medium, the implants were freeze-dried prior to analysis. Thus, caution must be paid due to artefact creation.

time (Fig. 1). Green, orange and blue curves indicate complete and partial bulk fluid renewal. As it can be seen, the pH remained about constant during the first 7–10 d, and then temporarily dropped. This can at least partially be explained by the release of short chain acids into the

bulk fluids. As for the drug, the mobility of these water-soluble compounds fundamentally increases once substantial system swelling sets on. In addition, the longer sampling intervals in this period (“week end breaks”) likely contributed to the observed pH drops (Bassand et al.,



**Fig. 5.** Dynamic changes in the pH of the well agitated bulk fluid, in the implants' dry mass and PLGA polymer molecular weight (Mw) upon exposure of ibuprofen-loaded implants to phosphate buffer pH 7.4 using the 3 experimental set-ups. The implants were placed in well agitated bulk fluids in Eppendorf tubes, in agarose gels exposed to well agitated bulk fluid in Eppendorf tubes, or in agarose gels in transwell plates (the receptor compartment containing well-agitated bulk fluid). At pre-determined time points, the entire bulk fluid, or 3 or 1 mL thereof, was renewed (as indicated).



**Fig. 6.** Dependence of the solubility of ibuprofen as a function of the pH at 37 °C. Phosphate buffer pH 7.4 USP 42 served as bulk fluid. Its pH was adjusted using aqueous 10 N lactic acid or 0.05 N NaOH solutions (final pH values are reported).



2022a). The fact that the temporary pH drop is less pronounced in the case of higher bulk fluid volume renewals can be explained by the higher amounts of withdrawn acids and higher amounts of newly added bases. Since ibuprofen exhibits strongly pH dependent solubility (Fig. 6), it might be that pronounced drops in the pH of the release medium lead to non-sink conditions and, thus, slower drug release. However, in this study, sink conditions were provided in all well agitated bulk fluids (even when their pH decreased) in all set-ups during the entire observation periods.

Importantly, the sampling volume did not affect the dynamic changes in the implants' dry mass and PLGA polymer molecular weight during the first 8 d upon direct exposure to phosphate buffer pH 7.4 or agarose gels (Fig. 5, diagrams in the middle and at the bottom). However, the implants became very fragile with time: The highly swollen systems were difficult to handle. During bulk fluid removal, the implants were rather well protected when embedded in an agarose gel and the risk of accidental damage during sampling was limited. In contrast, in the case of implants which were directly exposed to the phosphate buffer in Eppendorf tubes, the bulk fluid removal became more and more delicate with time: Especially, if the entire release medium was to be replaced. This accelerated implant disintegration (visual observation) and was the reason why most of the measurements had to be stopped after 8 d. The partial disintegration of the highly swollen PLGA gels led to a decrease in the length of the diffusion pathways to be overcome by the drug to be released. Consequently, ibuprofen release was accelerated. In contrast, the agarose gel effectively protected the implants from such damage. In addition, the presence of the agarose gels slowed down implant swelling (Fig. 2, diagrams in the middle and bottom rows). So, the observed sensitivity of drug release from the investigated PLGA implants to the sampling volume in the case of direct exposure to the release medium can likely be attributed to the mechanical stress experienced by the systems during complete bulk fluid renewal.

#### 4. Conclusions

Great caution has to be paid when defining the conditions for in vitro drug release measurements from PLGA implants: The systems become highly fragile once substantial polymer swelling sets on. The renewal of important parts of the release medium can cause partial damage of the highly fragile polymeric systems, accelerating device disintegration and decreasing the length of the diffusion pathways for the drug to be overcome. Embedding implants into agarose gels and lower sampling volumes can limit these effects. In any case, it must not be forgotten that, once substantial system swelling sets on, the devices become highly fragile. This can also be expected in vivo.

#### CRedit authorship contribution statement

**C. Bassand:** Investigation, Methodology, Validation, Conceptualization, Writing – original draft, Visualization. **L. Benabed:** Investigation. **J. Freitag:** Investigation. **J. Verin:** Investigation, Methodology, Validation. **F. Siepmann:** Conceptualization, Methodology, Supervision, Resources, Writing – review & editing, Visualization, Project administration, Funding acquisition. **J. Siepmann:** Conceptualization, Methodology, Supervision, Resources, Writing – review & editing, Visualization, Project administration, Funding acquisition.

#### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Juergen Siepmann reports financial support was provided by Interreg 2 Seas programme. Juergen Siepmann reports financial support was provided by European Regional Development Fund. The Editor-in-Chief of the journal is one of the co-authors of this article. The manuscript has been subject to all of the journal's usual procedures, including peer

review, which has been handled independently of the Editor-in-Chief.

#### Acknowledgments

This project has received funding from the Interreg 2 Seas programme 2014-2020 co-funded by the European Regional Development Fund under subsidy contract No 2S04-014 3DMed. The authors are very grateful for this support. They would also like to thank Mr. A. Fadel from the "Centre Commun de Microscopie" of the University of Lille ("Plateau technique de la Federation Chevreul CNRS FR 2638") for his valuable technical help with the SEM pictures.

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