How agarose gels surrounding plga implants limit swelling and slow down drug release

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Research article

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Abstract
The aim of this study was to better understand to which extent and in which way the presence of an agarose gel (mimicking living tissue) around a PLGA [poly(lactic-co-glycolic acid)] implant affects the resulting drug release kinetics. Ibuprofen-loaded implants were prepared by hot melt extrusion. Drug release was measured upon exposure to phosphate buffer pH 7.4 in Eppendorf tubes, as well as upon inclusion into an agarose gel which was exposed to phosphate buffer pH 7.4 in an Eppendorf tube or in a transwell plate. Dynamic changes in the implants' dry & wet mass and dimensions were monitored gravimetrically and by optical macroscopy. Implant erosion and polymer degradation were observed by SEM and GPC. Different pH indicators were used to measure pH changes in the bulk fluids, gels and within the implants during drug release. Ibuprofen release was bi-phasic in all cases: A zero order release phase (~20 % of the dose) was followed by a more rapid, final drug release phase. Interestingly, the presence of the hydrogel delayed the onset of the 2nd release phase. This could be attributed to the sterical hindrance of implant swelling: After a certain lag time, the degrading PLGA matrix becomes sufficiently hydrophilic and mechanically instable to allow for the penetration of substantial amounts of water into the system. This fundamentally changes the conditions for drug release: The latter becomes much more mobile and is more rapidly released. A gel surrounding the implant mechanically hinders system swelling and, thus, slows down drug release. These observations also strengthen the hypothesis of the "orchestrating" role of PLGA swelling for the control of drug release and can help developing more realistic *in vitro* release set-ups.

Key words: PLGA implant; release mechanism; swelling; ibuprofen; hydrogel

Highlights

- Agarose gels around PLGA implants limit system swelling and slow down drug release
- Agarose gel set-ups are likely more realistic than bulk fluid set-ups
- Transwell set-ups can provide practical advantages compared to other gel set-ups
- PLGA swelling is playing a key role for the final rapid drug release phase

1. Introduction

Poly(lactic-co-glycolic acid) (PLGA) offers an interesting potential as matrix former for controlled parenteral drug delivery [1–3]. In particular, PLGA microparticles and implants have been proposed in the literature [4–6]. Several drug products are on the market since decades. The great success of this polymer for this type of applications can be attributed to its good biocompatibility [7], complete biodegradability (avoiding the removal of empty remnants upon drug exhaust) and the possibility to provide desired drug release kinetics during flexible periods of time [8–11]. The resulting drug release kinetics depend on a variety of factors, including the type of PLGA (e.g., average polymer molecular weight, type of end groups), composition of the system as well as the type of manufacturing method and parameters used during processing[12–14].

Despite the great practical importance of PLGA-based drug delivery systems, yet the underlying mass transport mechanisms controlling drug release are generally not fully understood. This can be explained by the potential complexity of the involved physico-chemical processes [15,16], including for example water penetration into the systems, polymer degradation [17], drug dissolution, drug diffusion, polymer – drug interactions [18], water – polymer interactions [19], the creation of a local acidic micro-environment *within* the dosage form [20–22] causing autocatalytic effects [23], the closure of surface pores [24], substantial system swelling [25], limited drug solubility effects and system disintegration. The relative importance of these phenomena can strongly depend on the specific composition and inner & outer structure of the delivery system. For instance, the extent of local drops in the micro-pH can be altered by the addition of basic excipients or by varying the initial device porosity (determining at which rate acids and bases can diffuse into and out of the system).

Often, mono-, bi-, or tri-phasic drug release profiles are observed from PLGA-based drug delivery systems, irrespective of their geometry and size (e.g., implants, microparticles and films) [26–28]. In the case of tri-phasic drug release, the following phases can generally be distinguished: An initial burst release phase (frequently limited to the first few hours or 1-2 days) is followed by a "zero order drug release phase" (with an about constant release rate), and a final, again rapid release phase leading to complete drug exhaust. It has recently been proposed that PLGA swelling might play a decisive, "orchestrating" role for these different release phases [29–31], although PLGA swelling is often neglected in the literature for the explanation of the observed drug release patterns.

The initial burst release might be attributable to the release of drug particles which come into direct contact with water once the system is exposed to an aqueous fluid, because they are located directly at the system's surface, or very close to it. Eventually, this contact is assured via tiny pores with direct surface access or via an interconnected drug particle network. Since drug dissolution is rapid (as well as diffusion through short, water-filled pores), this release phase is often ending during the first 1-2 days. Limited PLGA swelling might also close initially existing surface pores, contributing to the termination of the initial burst release phase [32]. Eventually, if the drug is able to diffuse through the intact PLGA matrix, also drug molecules *dissolved* in the polymer and being located close to the system's surface can contribute to the initial burst release phase. Please note that water penetration into PLGA-based drug delivery systems is relatively fast, so that the entire device is rapidly wetted and polymer degradation occurs *throughout* the system ("bulk erosion") [33]. However, the contribution of this phenomenon to the initial burst release phase is likely limited.

The possible root causes for the second (zero order release) phase are often less well understood. It has been suggested that in the case of microparticles consisting of a PLGA matrix in which tiny diprophylline crystals are dispersed, the continuous growth of a highly swollen surface layer plays a crucial role [34]: Since the PLGA at the system's surface is in contact with

very high amounts of water, polymer degradation can be expected to be accelerated in this region. Upon ester bond cleavage shorter chain acids and alcohols are generated, rendering especially the matrix in the outmost layer more and more hydrophilic. At a certain time point, the latter undergoes substantial swelling. With time its thickness increases, since also "deeper" polymer layers get exposed to very high water concentrations. As long as a diprophylline particle is surrounded by a dense PLGA matrix, it can hardly dissolve (lack of water) and diffuse out (lack of mobility). However, once the steadily growing, highly swollen surface layer reaches the particle, the latter can much more easily dissolve, and the dissolved drug molecules can rather rapidly diffuse through the highly swollen PLGA "gel". This phenomenon was evidenced for *single* microparticles, releasing parts of the drug at random time points: Each particle has "its own structure" and "its own way" to release the drug. Since the drug in that study was *homogeneously* distributed throughout the system, the numerous *occasional* individual partial drug release events summed up to an about constant drug release rate observed from the ensemble of microparticles.

Different hypothesis have been described to explain the final, again rapid drug release phase (3rd phase) observed with many PLGA microparticles and implants. One hypothesis is that the onset of substantial system swelling (occurring after a lag time) is the root cause. Due to PLGA degradation, the entanglement of the polymer chains decreases with time and the concentration of water-soluble degradation products increases (creating a continuously increasing osmotic pressure inside the device). In addition, the system becomes more and more hydrophilic (due to the newly created -COOH and -OH end groups). At a certain time point, the mechanical stability of the macromolecular network becomes insufficient and substantial amounts of water penetrate into the *entire* device, allowing for drug particle dissolution and significantly increased drug mobility *throughout the system*. The PLGA implant or microparticle is transformed into a highly swollen gel, in which drug dissolution and diffusion are facilitated.

In certain cases, only *mono*- or *bi*-phasic drug release patterns are observed from PLGA-based drug delivery systems. This might for instance be due to the fact that no drug has direct initial access to the system's surface (absence of a burst release); or all drug is already released before the substantial entire system swelling sets on (absence of a 3rd drug release phase).

If the hypothesis of an "orchestrating role" of PLGA swelling for the control of drug release is correct, the presence of a hydrogel surrounding the device might have a non-negligible effect on drug release, since it might mechanically hinder the substantial system swelling. *In vivo*, the living tissue surrounding the device can be expected to have a similar impact. However, in most cases, experimental set-ups used for *in vitro* drug release measurements from PLGA-based drug delivery systems expose the dosage form directly to a bulk fluid. In the literature only relatively few studies address the potential impact of the presence of a gel. For example, in an interesting recent report, the group of Lamprecht measured the release of flurbiprofen, lidocaine and risperidone from ethylcellulose- or PLGA-based films, microparticles and cylindrical implants in the presence and absence of a surrounding gel [35]. In many cases, the presence of the gel led to slower drug release from PLGA-based devices. However, in the case of certain flurbiprofen-loaded films, the release rate became faster during most of the release period, the gel hindering the films to deform (the deformation causing a decrease in the surface area available for drug release). And for some other systems, the impact of the presence of a surrounding gel was negligible. The same group proposed the use of components of muscle tissue to better mimic intramuscular environments and addressed the potential role of lipids for drug release from a variety of controlled release microparticles [36]. Allababidi and Sha [37] investigated the release of cefazolin from *glycerol monostearate*-based implants into an agar gel or phosphate buffer pH 7.4 bulk fluid, and did not observe major differences. Furthermore, the release profiles of ciprofloxacin hydrochloride and vancomycin hydrochloride from

different types of *hydroxyapatite* implants functionalized with hydroxypropyl-β-cyclodextrin were measured into agarose gels or well agitated phosphate buffer pH 7.4. Drug release was much faster in the agitated bulk fluid, which was at least in part attributed to accelerated matrix erosion. Hydrogels have also been proposed by the group of Ostergaard as surrogates for subcutaneous tissue when studying controlled release implants [38,39]. Furthermore, Exner and co-workers [40,41] suggested acrylamide-based hydrogel phantoms for a more realistic *in vitro* characterization of *in situ forming* PLGA implants. Importantly, they could demonstrate that the use of such hydrogels allowed for a better prediction of the *in vivo* behavior of the implants (in rats), compared to standard drug release measurements in well agitated bulk fluids.

The aim of this study was to investigate ibuprofen-loaded PLGA implants using 3 different experimental set-ups: (i) Upon exposure to well agitated bulk fluid in Eppendorf tubes, (ii) Upon embedding in agarose gels, which are exposed to bulk fluid in Eppendorf tubes, and (iii) Upon embedding in agarose gels, which are exposed to bulk fluid using transwell plates. The gel-transwell set-up has the advantage to allow for easier sampling compared to the gel-Eppendorf set-up. The observed drug release kinetics were to be explained based on the monitoring of dynamic changes in the systems' wet and dry mass (gravimetrically), average polymer molecular weight (GPC), inner and outer morphology (optical and scanning electron microscopy) as well as pH measurements in the bulk fluids, gels and implants. In particular, the relative importance of potential substantial implant swelling for the control of drug release was to be elucidated. So far, relatively little information is available on this key feature of PLGA. This is rather surprising, because it can play an "orchestrating role" for the various involved physico-chemical phenomena.

2. Materials and methods

2.1. Materials

Poly (D,L lactic-co-glycolic acid) (PLGA, 50:50 lactic acid: glycolic acid; Mw = 39.1 +/- 0.3 kDa, as determined by GPC analysis described in *section 2.6*.; Resomer RG 503H; Evonik, Darmstadt, Germany); ibuprofen (BASF, Ludwigshafen, Germany); agarose (broad separation range for DNA/RNA/genetic analysis grade: 500bp to 25kb, DNase- and RNase-free, gel strength: 1200g/cm²min, gelation temperature: 34.5 to 37.5°C, Fisher BioReagents BP1356-500, Nacres classification code: NA.25, CAS: 9012-36-6), bromocresol green (BCG), bromothyol blue (BTB), bromophenol blue (BPB), phenol red (PR) and tetrahydrofuran (HPLC grade) (Fisher Scientific, Illkirch, France); monophasic potassium phosphate, sodium hydroxide and ethanol (96 % technical grade) (Acros Organics, Geel, Belgium); acetonitrile (VWR, Fontenay-sous-Bois, France); sodium hydrogen phosphate (Na_2HPO_4; Panreac Quimica, Barcelona, Spain).

2.2. Implant preparation

PLGA was milled for 4 x 30 s in a grinder (Valentin, Seb, Ecully, France). Appropriate amounts of PLGA 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 mixture were filled into a 1 mL syringe (Henke Sass Wolf, Tuttlingen, Germany), followed by heating: 105 °C for 15 min in an oven (FP115, Binder, Tuttlingen, Germany). The molten blend was manually extruded using the syringe. The obtained extrudate (2.6 +/- 0.2 mm in diameter) was cut with a hot scalpel into cylindrical implants of approximately 5 mm length.

2.3. Practical drug loading

Implants were dissolved in acetonitrile (1 implant in 5 mL), followed by filtration (PVDF syringe filters, 0.45 µm; Agilent Technologies, Santa Clara, USA) and drug content determination by HPLC-UV analysis using a Thermo Fisher Scientific Ultimate 3000 Series HPLC, equipped with a LPG 3400 SD/RS pump, an auto sampler (WPS-3000 SL) and a UV-Vis detector (VWD-3400RS) (Thermo Fisher Scientific, Waltham, USA). A reversed phase column C18 (Gemini 5 µm; 110 A°; 150 x 4.6 mm; Phenomenex, Le Pecq, France) was used. The mobile phase was a mixture of 30 mM Na_2HPO_4 pH 7.0: acetonitrile (60:40, v:v). The detection wavelength was 264 nm, the flow rate was set at 0.5 mL/min. The retention time was about 5.8 min. Ten microliter samples were injected.

2.4. In vitro drug release

Ibuprofen release from the PLGA implants was measured using the following 3 experimental set-ups:

<u>In well agitated bulk fluids</u>

Implants were placed in metal baskets in 5 mL Eppendorf tubes (1 implant per basket/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) (**Figure 1A**). Optionally, one of the following pH indicators was added: bromocresol green, bromothyol blue, bromophenol blue or phenol red (0.0025 % w:v). The tubes were placed (and maintained vertically) in a horizontal shaker (80 rpm, 37°C; GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At predetermined time points, the entire bulk fluid was replaced by fresh (pre-heated) 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 in *section 2.3 Practical drug loading*.

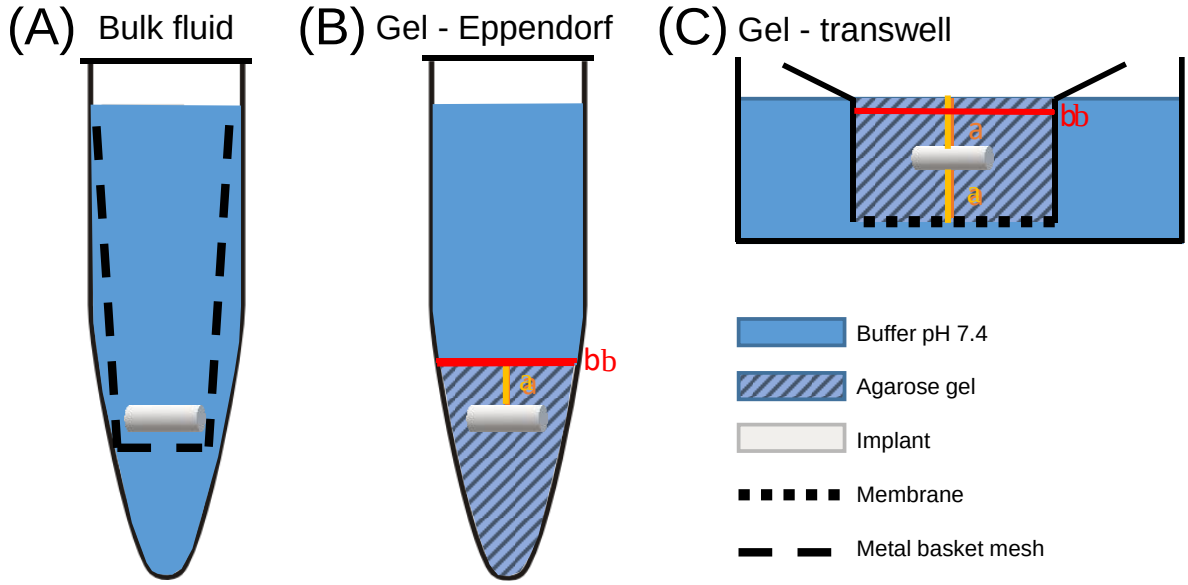

Figure 1. *Schematic presentations of the experimental set-ups used to monitor drug release from PLGA-based implants in: A) well agitated phosphate buffer pH 7.4 (in metal baskets) in Eppendorf tubes, B) agarose gels in Eppendorf tubes, the gels being exposed to well agitated phosphate buffer pH 7.4, C) agarose gels in transwell plates, the receptor compartment containing well agitated phosphate buffer pH 7.4. Please note that the schemes are not up to scale (for visibility), "a = 0.44 cm" and "b = 1.2 cm" indicate the distances. In all cases, sink conditions were provided throughout the experiments in the well agitated bulk fluids. Optionally, pH indicators were added to the phosphate buffer. Details are described in the text.*

In agarose gels in Eppendorf tubes

Implants were embedded in agarose gels in 5 mL Eppendorf tubes, as illustrated in **Figure 1B** (1 implant per tube). The gel was prepared with 0.5 % w:v agarose and 1 mL phosphate buffer pH 7.4 USP 42 (optionally containing 0.0025 % w:v bromocresol green, bromothyol blue, bromophenol blue or phenol red as pH indicator). The concentration of 0.5 % agarose was chosen based on the results obtained by Klose et al. [42], who did not observe a significant impact of the agarose concentration in the range of 0.2-0.6 % (indicating that the polymer network does not significantly hinder drug diffusion, as also observed by NMR analysis). An agarose dispersion in the respective buffer solution 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 at 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 (optionally containing a pH indicator) were added on top of the gel, and the tube was placed in a horizontal shaker (80 rpm, 37°C; GFL 3033). At predetermined time points, the entire bulk fluid was replaced by fresh (pre-heated) release medium. The withdrawn samples were treated as in the case of drug release measurements in well agitated bulk fluids.

It has to be pointed out that using this set-up, *a part* of the drug which has been released from the implant at a given time point is not detected "as released" in the well agitated bulk fluid, because it is diffusing through the agarose gel. Thus, the determined drug release rate is underestimated. To evaluate the relative importance of this error, a worst case situation was identified: A time point was selected at which the amount of ibuprofen in the bulk fluid was particularly high. Hence, also the amount of ibuprofen diffusing through the agarose gel "at this moment" can be expected to be particularly high. Under these conditions, the agarose gel was withdrawn and dissolved in a 50:50 (v:v) distilled water:ethanol mixture. The solution was filtered (PVDF syringe filter, 0.45 µm; Agilent) and its ibuprofen content was determined by HPLC-UV as described above. The experiment was conducted in triplicate. The mean value +/- standard deviation is reported.

In agarose gels in transwell pates

Implants were embedded in agarose gels in transwell plates (1 implant per insert, 1 mL gel, membranes: 1.13 cm^2, 11 µm, 0.4 µm pore size; Nunc, Roskilde, Denmark), as illustrated in **Figure 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 USP 42 (optionally containing 0.0025 % pH indicator, as described above), covered with lids and Parafilm to minimize evaporation, and placed in a horizontal shaker (80 rpm, 37°C; GFL 3033). At predetermined time points, the entire bulk fluid was replaced by fresh (pre-heated) release medium. The withdrawn samples were treated as in the case of drug release measurements in well agitated bulk fluids.

In all cases, the pH of the release medium was measured at pre-determined time points using a pH meter (InoLab pH Level 1; WTW, Weilheim, Germany). Furthermore, in all cases, sink conditions were provided throughout the experiments in all agitated bulk fluids.

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

2.5. Implant swelling

Implants were treated as for the *in vitro* drug release measurements described in *section 2.4.* At pre-determined time points:

(i) Pictures of the implants were taken with an a SZN-6 trinocular stereo zoom macroscope (Optika, Ponteranica, Italy), equipped with an optical camera (Optika Vison Lite 2.1

software). Cross-sections were obtained by cutting with a scalpel. The lengths and diameters of the implants were determined using the ImageJ software (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). The samples were weighed [*wet mass (t)*], and the *change in wet mass (%) (t)* was calculated as follows:

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

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.

2.6. Implant erosion and PLGA degradation

Implants were treated as for the *in vitro* drug release studies described in *section 2.4.* 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, 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:

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

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.

The average polymer molecular weight (Mw) of the PLGA was determined by gel permeation chromatography (GPC) as follows: Freeze-dried implant samples were dissolved in tetrahydrofuran (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 Phenogel 5 µm column (kept at 35°C, 7.8 × 300 mm; Phenomenex). Tetrahydrofuran was the mobile phase (flow rate: 1 mL/min). Polystyrene standards with molecular weights between 5,120 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.

2.7. Differential scanning calorimetry (DSC)

DSC thermograms of PLGA (raw material) and implants were recorded using a DCS1 Star System (Mettler Toledo, Greifensee, Switzerland). Approximately 5 mg PLGA and around 10 mg implant samples were heated in perforated aluminum pans as follows: from -70 to 120 °C, cooling to -70 °C, re-heating to 120 °C (heating/cooling rate = 10 °C/min). The reported glass temperatures (Tgs) were determined from the 1st heating cycles in the case of implants (the thermal history being of interest), and from the 2nd heating cycle in the case of the PLGA raw material (the thermal history not being of interest). All experiments were conducted in triplicate. Mean values +/- standard deviations are reported.

2.8. 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 layer. In the case of implants which had been exposed to the release medium, the

systems were treated as described for the *in vitro* release studies in *section 2.4*. At predetermined time points, implant samples were withdrawn, optionally cut using a scalpel and freeze-dried (as described in *section 2.6*).

3. Results and discussion

The image in Table 1 shows an optical macroscopy picture of an ibuprofen-loaded PLGA implant before exposure to the release medium. The practical drug loading was 6.6 +/- 0.3 %. The theoretical loading was 10%, the main reason for drug loss was the preferred ibuprofen particle adherence to the recipients used for mixing. The glass transition temperature (Tg) of the implants was determined to be 34.5 +/- 0.3 °C, while the Tg of the PLGA raw material was 47.1 +/- 0.1 °C. This difference can be explained by the fact that ibuprofen acts as a plasticizer for PLGA and by the decrease in polymer molecular weight during implant preparation (from 39.1 +/- 0.3 to 36.9 +/- 0.4 kDa).

Table 1. *Physical key properties of the investigated ibuprofen-loaded PLGA implants (Tg: glass transition temperature). Mean values ± standard deviations are indicated (n=3).*

Practical loading (%)	Weight (mg)	Length (mm)	Diameter (mm)	Tg (°C)	Picture
6.6±0.3	33.7±4.4	5.1±0.3	2.6±0.2	34.5±0.3	 2 mm

3.1. In vitro release set-ups

The schemes in **Figure 1** illustrate the 3 experimental set-ups, which were used to monitor ibuprofen release. The idea was to evaluate and understand the potential impact of the presence of a hydrogel around the implants (mimicking living tissue) on the resulting drug release kinetics.

Figure 1A shows the "Bulk fluid" set-up, in which an implant is placed into a metal basket in an Eppendorf tube filled with 5 mL phosphate buffer pH 7.4. The tubes are placed into a horizontal shaker (80 rpm) and kept at 37 °C. At pre-determined time points, the entire bulk fluid is replaced by fresh (pre-heated) release medium. The metal basket assures that the implant does not sink to the bottom of the tube. Its meshes are sufficiently large (250 µm) to allow for convective flow and rapid medium exchange between the liquid inside and outside the basket.

Figure 1B illustrates the "gel – Eppendorf" set-up: In this case, an implant is surrounded by a 0.5 % agarose gel prepared with 1 mL phosphate buffer pH 7.4. Four mL phosphate buffer pH 7.4 are carefully added on top of the gel, and the Eppendorf tube is placed into a horizontal shaker (80 rpm) at 37 °C. At pre-determined time points, the entire bulk fluid is replaced by fresh (pre-heated) phosphate buffer pH 7.4, and the drug content in the withdrawn samples is measured. Thus, to be "detected as released", drug molecules/ions released from the implant also have to cross the gel. As a first evaluation of the impact of the presence of this additional mass transport step on the observed ibuprofen release kinetics, the following reference experiment was conducted: Drug release from a 0.5 % agarose gel, which was prepared with a 200 µg/mL *solution* of ibuprofen in phosphate buffer pH 7.4, was measured with the same set-up. The black circles in **Figure 2** show the obtained results: More than 50 % of the drug was released after 10 h. This is rapid compared to the ibuprofen release rate from the investigated

PLGA implants (which was of the order of 10 d in bulk fluids). This also indicates that the exact position of the implants within the agarose gel is not expected to substantially impact the observed results.

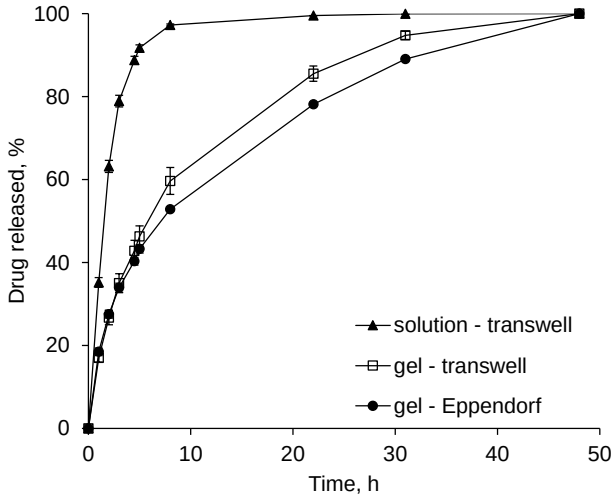

Figure 2. *Ibuprofen release from a drug solution (200 µg/mL) using the transwell set-up, from an agarose gel containing a drug solution (200 µg/mL) using the transwell set-up, and from an agarose gel containing a drug solution (200 µg/mL) using the Eppendorf tube set-up. Please note that the time is plotted in hours on the x-axis.*

The "gel – transwell" set-up is schematically shown in **Figure 1C**. In this case, an implant is placed in the donor compartment of a transwell plate, being embedded in a 0.5 % agarose gel prepared with 1 mL phosphate buffer pH 7.4. Four mL phosphate buffer pH 7.4 are placed into the acceptor compartment. The transwell plate is horizontally shaken (80 rpm) at 37 °C. At pre-determined time points, the entire bulk fluid in the acceptor compartment is replaced by fresh (pre-heated) release medium. From a practical point of view, this is easier than replacing the release medium in the "gel – Eppendorf" set-up (the risk of gel damage being reduced). The drug content in the withdrawn samples is determined by HPLC-UV analysis. Thus, also in this case, the presence of the agarose gel can be expected to slow down ibuprofen release to a certain extent. In addition, the presence of the membrane of the transwell plate might impact the rate at which the ibuprofen reaches the acceptor compartment. To evaluate the relative importance of these 2 phenomena, the following reference experiments were conducted: (i) The "release rate" of an ibuprofen solution in phosphate buffer pH 7.4 (200 µg/mL) from the donor compartment (free of agarose gel) into the acceptor compartment was measured. The black triangles in **Figure 2** show that the entire drug amount was released in less than 10 h under these conditions. This is very rapid compared to the release periods from the investigated implants in bulk fluids in this study (>= 10 d). Thus, the impact of the transwell plate membrane can likely be neglected. (ii) Ibuprofen release from a 0.5 % agarose gel, prepared with an ibuprofen *solution* in phosphate buffer pH 7.4 (200 µg/mL) was measured using this set-up. The open squares in **Figure 2** illustrate the obtained results, indicating that the impact of the presence of the gel was similar to the impact of the gel in the "gel – Eppendorf" set-up. This is fully sound, since the compositions of the gels are identical and the distances to be overcome similar (Figure 1). Hence, again, the observed delay in drug release due to ibuprofen transport through the agarose gel is relatively small compared to the much longer release periods from the investigated PLGA implants. Furthermore, this indicates that the exact position of the implants within the agarose gel is not expected to substantially impact the observed results (as discussed for the gel – Eppendorf set-up above).

Importantly, all 3 experimental set-ups guaranteed sink conditions throughout the experiments in this study in the agitated bulk fluids. Furthermore, no signs for agarose gel erosion were observed throughout the experiments.

3.2. Drug release and implant swelling

Figures 3A and B show the experimentally measured ibuprofen release kinetics from the investigated PLGA implants using the 3 different set-ups (3B is a zoom on the first 10 d). As it can be seen, drug release was faster when using the "bulk fluid" set-up compared to the "gel – Eppendorf" and "gel – transwell" set-ups. For instance, complete released was observed after about 11 d versus 17 d and 21 d. This can only to a minor extent be explained by the additional drug transport step through the agarose gels, as discussed above. As a second experimental evaluation of the relative importance of the amount of drug, which is released from the implant, but not detected in the "gel – Eppendorf" set-up (only drug in the well stirred bulk fluid is measured, not the drug diffusing through the agarose gel), the following experiment was conducted: After 10 d exposure of an implant in the "gel – Eppendorf" set-up (when the amount of drug detected in the bulk fluid was highest for the "bulk fluid" set-up, **Figure 3**), the agarose gel was withdrawn and its ibuprofen content determined. Under these conditions, 3.6 +/- 0.4 % drug (referred to the total drug amount) was detected in the gel. This is only a minor amount compared to the difference in ibuprofen release observed between the "bulk fluid" and "gel" set-ups (about 60 %) at this time point.

Interestingly, all release profiles shown in **Figure 3** (for all set-ups) were "bi-phasic": A zero order release phase (with an about constant release rate) was followed by a more rapid drug release phase, leading to complete drug exhaust. No noteworthy "burst release" was observed, irrespective of the experimental set-up. This can probably be explained by the fact that the implants were prepared by hot melt extrusion, leading to a non-porous surface, as also evidenced by SEM (e.g. pictures at the top on the left hand side of **Figure 4**. Furthermore, in contrast to small PLGA microparticles, the release of minor *absolute* amounts of drug from surface-near regions of a "large" implant at early time points is negligible from a *relative* point of view (the 100 % reference value being considerably higher).

Figures 3C and D show the dynamic changes in the wet mass and volume of the PLGA implants upon exposure to the release medium when using the 3 different set-ups. Clearly, all systems started to fundamentally swell after a "lag phase". Importantly, as in the case of drug release, there was a clear impact of the presence of the agarose gel surrounding the PLGA implant: The onset of substantial system swelling was delayed by several days. The optical macroscopy pictures in **Figure 5** illustrate this behavior: At the top, images of surfaces are shown, at the bottom images of cross-sections. The implants were exposed to the release medium for up to 10 d using the 3 different set-ups (as indicated). In all cases, system swelling was limited during the first few days, followed by the onset of substantial PLGA swelling. When looking at the 3 diagrams on the right hand side of **Figure 3** (showing drug release as well as changes in the systems' wet mass and volume during the first 10 d), it can be seen that the onset of important system swelling coincided with the onset of the final, rapid drug release phase in all cases. The observed ranking orders for the "lag time" for substantial system swelling and for the onset of the final rapid drug release phase were the same: "bulk fluid" < "gel -transwell" ≈ "gel -Eppendorf" set-up.

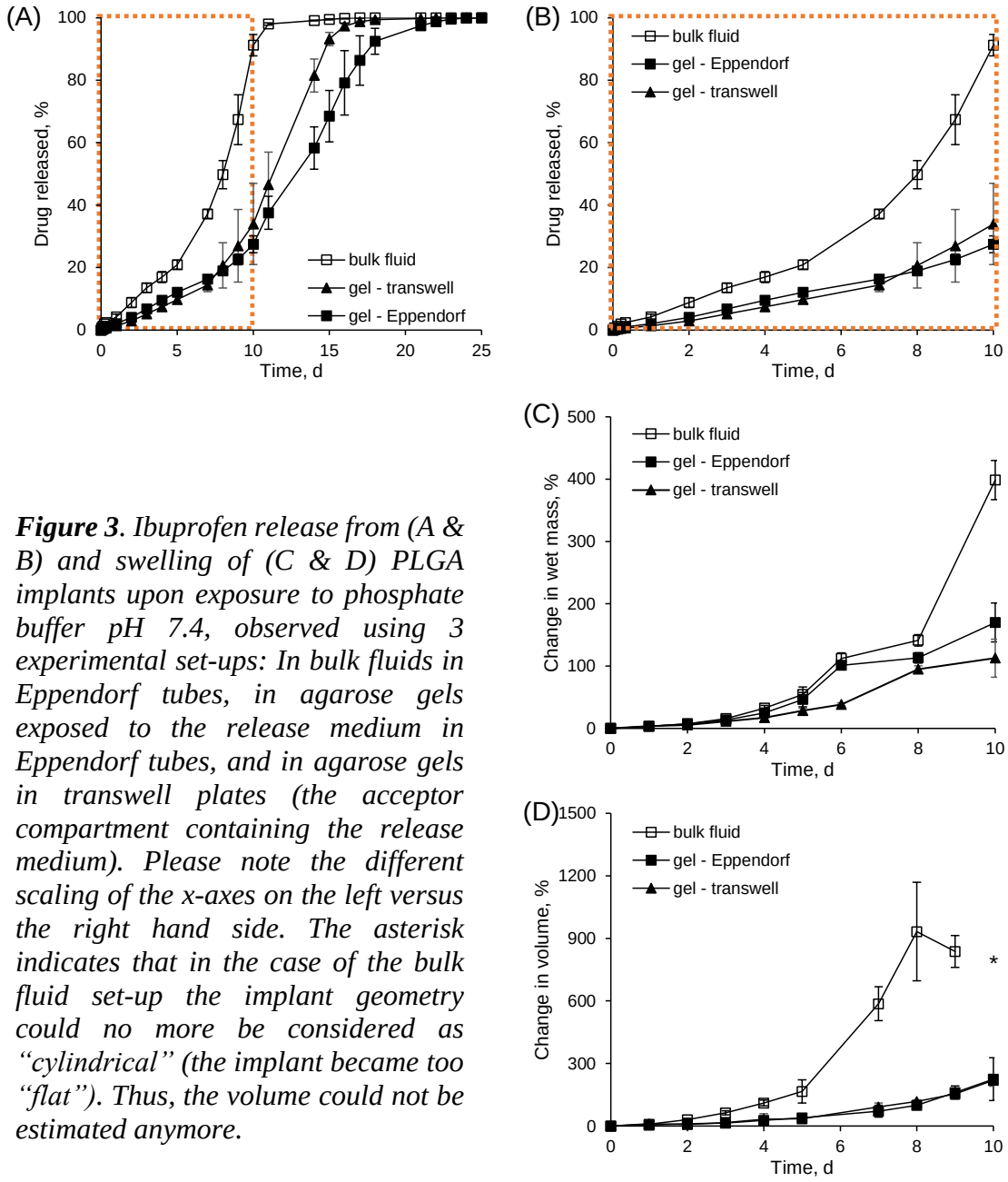

***Figure 3**. Ibuprofen release from (A & B) and swelling of (C & D) PLGA implants upon exposure to phosphate buffer pH 7.4, observed using 3 experimental set-ups: In bulk fluids in Eppendorf tubes, in agarose gels exposed to the release medium in Eppendorf tubes, and in agarose gels in transwell plates (the acceptor compartment containing the release medium). Please note the different scaling of the x-axes on the left versus the right hand side. The asterisk indicates that in the case of the bulk fluid set-up the implant geometry could no more be considered as "cylindrical" (the implant became too "flat"). Thus, the volume could not be estimated anymore.*

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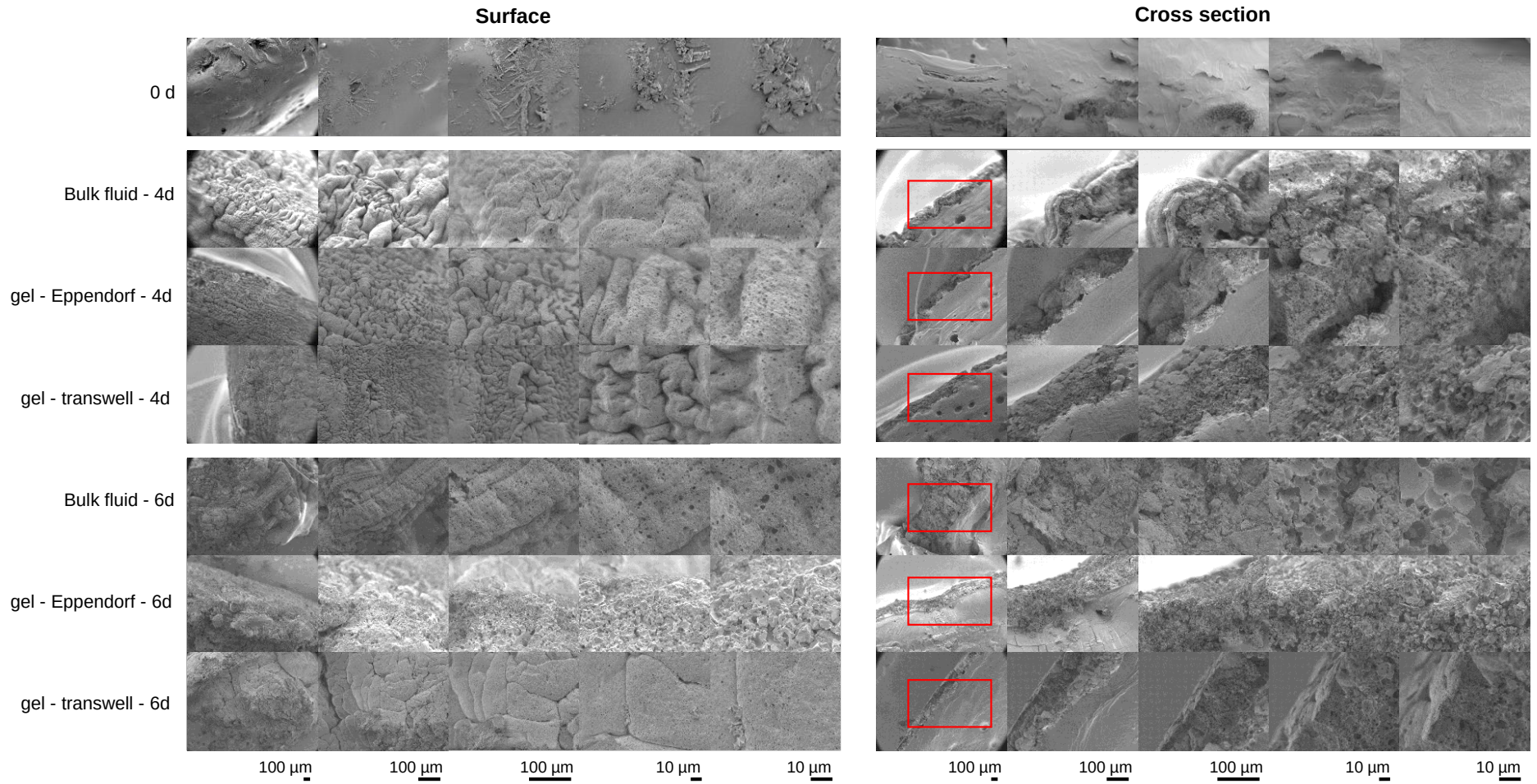

Figure 4. *SEM pictures of surfaces and cross sections of ibuprofen-loaded PLGA implants before and after exposure to phosphate buffer pH 7.4 using the 3 experimental set-ups. The type of set-up and exposure times are indicated on the left hand side. Please note that after exposure to the release medium the implants were freeze-dried prior to analysis. Thus, caution must be paid due to artefact creation.*

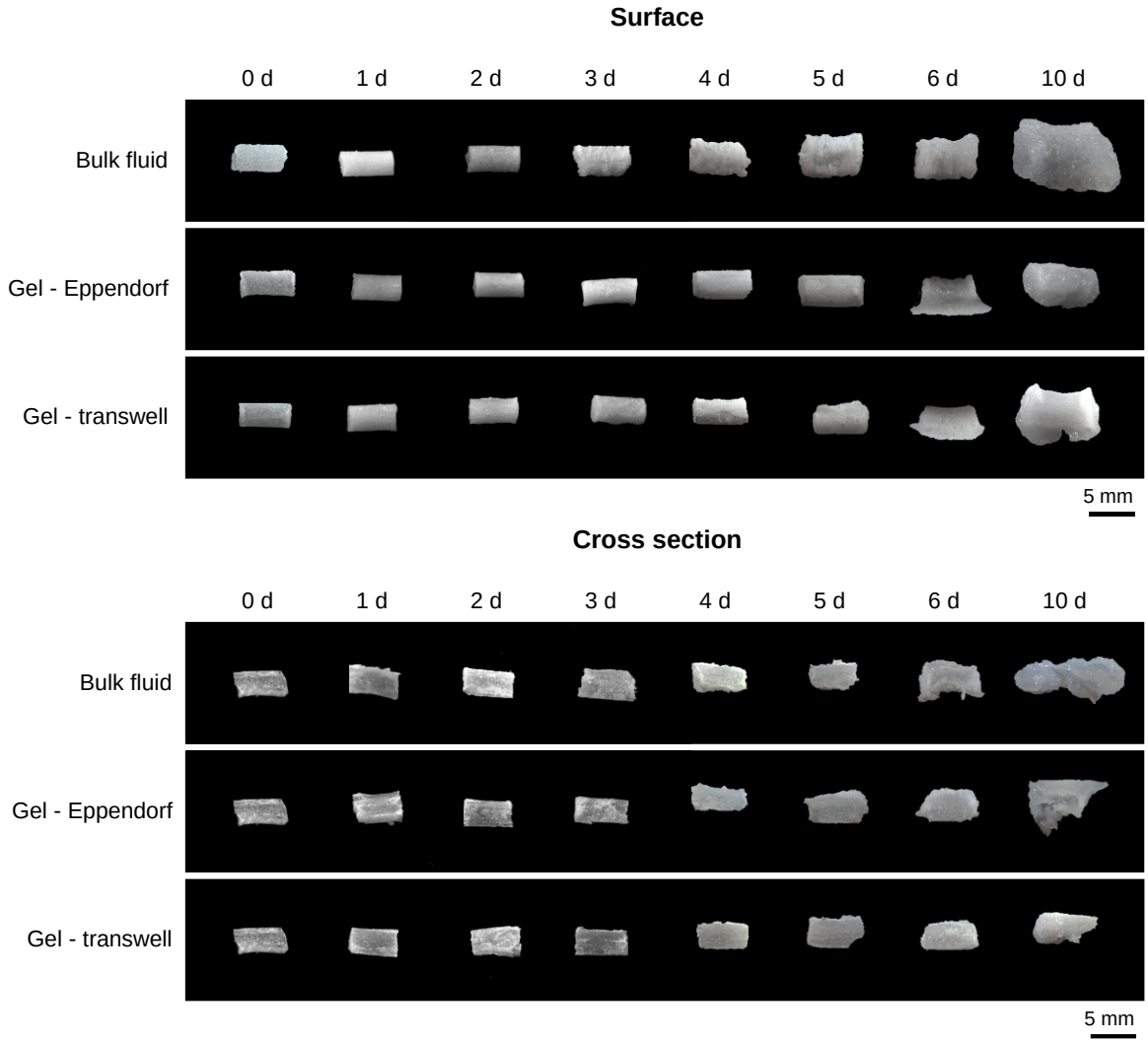

Figure 5. *Optical macroscopy pictures of surfaces and cross sections of ibuprofen-loaded PLGA implants before and after exposure to phosphate buffer pH 7.4 using 3 experimental set-ups: In bulk fluids in Eppendorf tubes, in agarose gels exposed to the release medium in Eppendorf tubes, and in agarose gels in transwell plates (the acceptor compartment containing the release medium). The exposure times are indicated at the top, the type of set-up is indicated on the left hand side.*

The difference in the swelling kinetics of the implants upon exposure to a "bulk fluid" versus "gel" can probably be attributed to the sterical hindrance caused by the agarose matrix: Once the PLGA implants are in contact with the bulk fluid or gel, water penetrates into the system and the entire implant is rather rapidly wetted. Since the investigated PLGA is relatively hydrophobic, the amounts of water diffusing into the implants at early time points remain limited. But these "low" amounts of water are sufficient to initiate polymer degradation throughout the device ("bulk erosion"). Consequently, the macromolecules become shorter and less entangled with time. Also, since the newly created end groups (upon ester bond hydrolysis) are hydrophilic (-COOH and -OH), the polymer matrix becomes more and more hydrophilic. In addition, the concentration of water-soluble degradation products (short chain acids) is steadily increasing, generating a continuously increasing osmotic pressure inside the implant. At a certain time point, the mechanical stability of the initially dense polymeric system becomes insufficient (due to the decreasing degree of macromolecular entanglement) and substantial amounts of water penetrate into the device: driven by the generated osmotic pressure and

hydrophilicity of the degrading implant. The considerable increase in the water content of the implant fundamentally changes the conditions for drug release: Initially, the ibuprofen was effectively trapped within a dense PLGA matrix. After this substantial device swelling, the drug is in contact with considerable amounts of water and surrounded by a highly swollen PLGA "gel" (as it can be seen in the pictures on the right hand side of **Figure 5**). Under these conditions, drug release is very much facilitated. The scheme in **Figure 6** schematically illustrates this hypothesized drug release mechanism (in a simplified manner). Due to the key importance of implant swelling for the control of drug release, its role can be considered as "orchestrating" [31]. Please note that the dissolution of drug particles is probably not playing a role in the investigated implants, since at a practical loading of 6.6 %, the ibuprofen is likely completely dissolved in the PLGA matrix from the beginning ("monolithic solution") [43]. Also, the increase in the length of the diffusion pathways due to system swelling is overcompensated by the tremendous increase in drug mobility. Importantly, the presence of an agarose gel around the implant sterically hinders this phenomenon and delays the onset of substantial system swelling (**Figures 3C and D**: filled versus open symbols).

The above described hypotheses are in good agreement with SEM pictures of surfaces and cross sections of the implants obtained after different time periods using the 3 different set-ups. However, please note that great caution should be paid when drawing conclusions from these SEM images, because the implants had to be dried prior to analysis, creating artefacts. The pictures on the left hand side of **Figure 4** show surfaces, those on the right hand side cross sections of implants exposed to the bulk fluid or gels for up to 6 d. As it can be seen, all implant surfaces became wrinkled and highly porous. These structures are clearly artefacts: During drug release, the polymer can be expected to be *highly* swollen (and not wrinkled) in *surface-near* regions, since the latter are in contact with high amounts of water (in contrast to regions deeper inside the implant). The presence of a highly swollen, surface-near matrix layer was also visible in the *optical* macroscopy pictures shown in **Figure 5**, which were obtained without sample drying. The red rectangles in the SEM pictures on the right hand side of **Figure 4** highlight the two zones which can be distinguished: A highly swollen surface-near layer and a "non swollen" layer located below. The thickness of the highly swollen surface-near zone increases with time. This growth is due to the fact that high amounts of water are present in the highly swollen outmost layer and are, thus, in contact with the PLGA in the layer right below. Please note that the scheme in **Figure 6** does not reflect this phenomenon for reasons of simplicity.

Interestingly, the SEM pictures of the cross sections shown in **Figure 4** clearly evidence the impact of the presence of an agarose gel on implant swelling: As it can be seen, the highly swollen surface layer is much thicker in the "bulk fluid" set-up compared to the "gel – Eppendorf" and "gel – transwell" set-ups after 6 d. This can explain the higher release rate observed in the "bulk fluid" set-up compared to the gel set-ups during the zero order release phase (**Figure 3**).

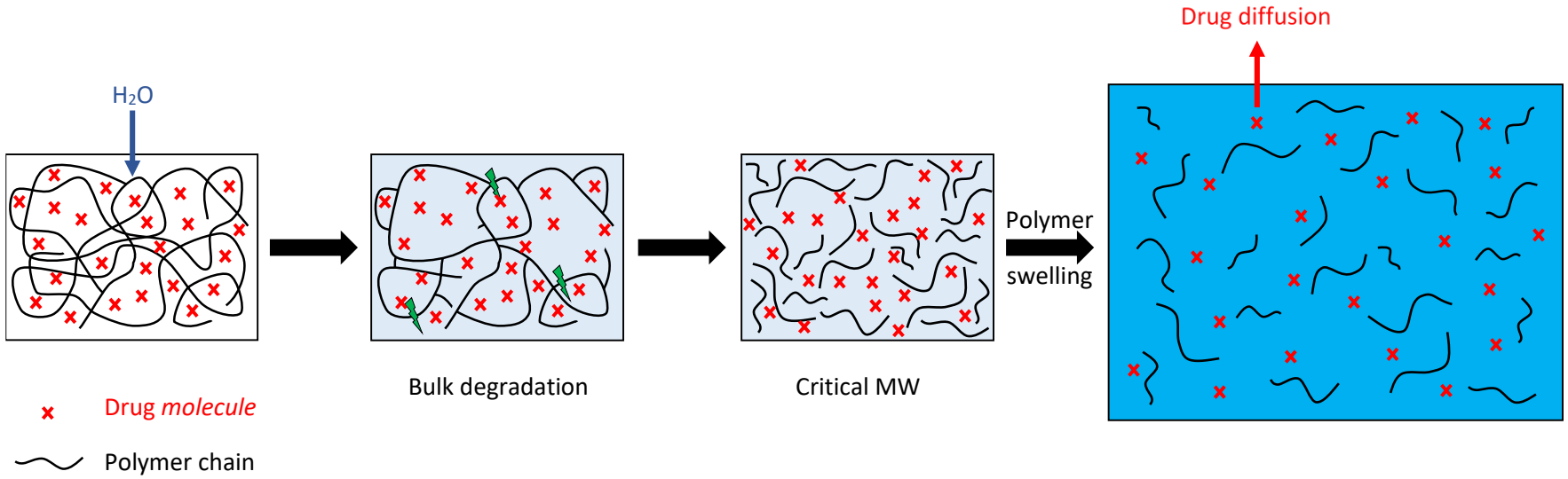

Figure 6. *Simplified schematic presentation of the mass transport mechanisms controlling ibuprofen release from the investigated PLGA implants. Initially, limited amounts of water diffuse into the system, leading to polyester degradation throughout the implants ("bulk erosion"). As soon as a critical polymer molecular weight is reached, substantial amounts of water penetrate into the device, facilitating subsequent drug release. Details are described in the text.*

3.3. Implant erosion and PLGA degradation

Figure 7 shows the dynamic changes in the pH of the agitated bulk fluids in the 3 experimental set-ups (top) as well as the decrease of the dry mass and average polymer molecular weight of the PLGA upon implant exposure to the release media. The diagram on the right hand side at the top shows a zoom on the first 10 d. Importantly, the pH in the bulk fluids remained about constant (neutral) for up to 10 d in all cases. This corresponds to the entire release period of implants studied in the "bulk fluid" set-up. Afterwards, a temporary drop in the pH was observed, the importance of which decreased in the following rank order: "gel – transwell" > "gel – Eppendorf" > "bulk fluid" set-up. This drop can at least partially be attributed to the release of short chain, water-soluble acids (as degradation products of PLGA) into the bulk fluids: As discussed above, once the implants become sufficiently hydrophilic and mechanically instable, substantial system swelling sets on. This does not only fundamentally change the conditions for *drug* release, but also for the release of these water-soluble acids. In addition, released drug (ibuprofen, an acid) can be expected to decrease the pH of the bulk fluid to a certain extent. Please note that this temporary drop in pH can also (in part) be attributed to the longer sampling interval (3 d "week-end gap", compared to daily sampling during the week; at each sampling time point, the entire bulk fluid was renewed). Thus, the water-soluble acids (and released drug) accumulated during the longer sampling interval. However, as it can be seen, the following 3 d "week-end sampling gap" at day 21 led to a much less important decrease in the pH of the bulk fluids.

It has to be pointed out that the pH values shown in **Figure 7** were measured in the agitated *bulk fluids* in all set-ups. In the case of the "gel -Eppendorf" and "gel – transwell" set-ups, the implants were not in direct contact with this bulk fluid. This is why also potential dynamic changes in the pH within the *agarose gels* were monitored during drug release. Three pH indicators [phenol red (PR), bromothymol blue (BTB) and bromocresol green (BCG)] were added to the phosphate buffer pH 7.4, which was used for the preparation of the gels. On the left hand side of **Figure 8**, the pH values are shown at which the indicators change their color. Implant samples were treated as for the *in vitro* drug release studies. After pre-determined exposure periods, optical macroscopy pictures were taken. The dotted red rectangles highlight the most informative images at each time point. In the case of the "gel – Eppendorf" set-up, the pH in the gel surrounding the implant remained above 6.6 during the first week, and then temporarily dropped: to pH 6.0-6.5 on day 10 and to pH 5.4-6.0 on day 14. It subsequently raised again. In the "gel – transwell" set-up a similar behavior was observed. These drops are consistent with the pH drops observed in the agitated bulk fluids used in these set-ups (discussed above). They can mainly be attributed to the release of short chain acids and of ibuprofen from the implants after the onset of substantial system swelling, and (in part) to the accumulation of the acids during the longer (3 d) sampling interval.

Please note that since the solubility of ibuprofen is pH-dependent, local acidic environments around the implant might *decrease* drug solubility and, hence, slow down drug release. However, Kozac et al. [35] reported slower release from PLGA-based films and microparticles surrounded by agarose gel compared to agitated bulk fluid also for the *free base* lidocaine, which is *more soluble at acidic pH*. Furthermore, the observed temporary drops in the pH of the agarose gels surrounding the implants are also in good agreement with recently reported results on *in situ* forming PLGA implants formed in an agarose gel [44]. Ostergaard and coworkers studied the key characteristics of PLGA implants forming upon injection of a polymer solution into an agarose gel, mimicking living tissue better than an agitated bulk fluid. Bromothymol blue was used as a pH indicator in that study.

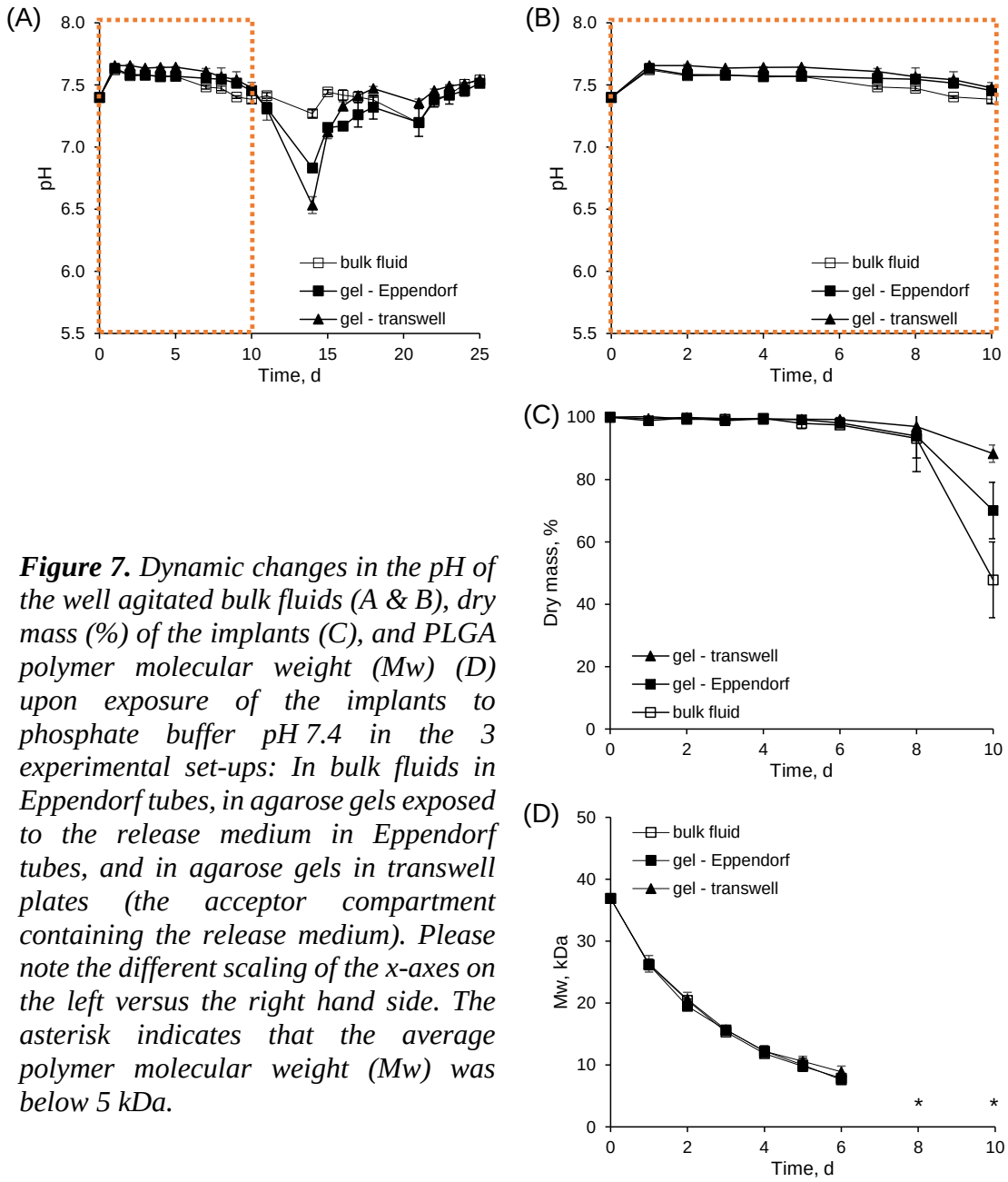

Figure 7. *Dynamic changes in the pH of the well agitated bulk fluids (A & B), dry mass (%) of the implants (C), and PLGA polymer molecular weight (Mw) (D) upon exposure of the implants to phosphate buffer pH 7.4 in the 3 experimental set-ups: In bulk fluids in Eppendorf tubes, in agarose gels exposed to the release medium in Eppendorf tubes, and in agarose gels in transwell plates (the acceptor compartment containing the release medium). Please note the different scaling of the x-axes on the left versus the right hand side. The asterisk indicates that the average polymer molecular weight (Mw) was below 5 kDa.*

In addition to dynamic the pH changes in the agitated bulk fluids and gels, pH changes can also occur *within* the PLGA-based implants during drug release. In an attempt to monitor such events, 4 different pH indicators were added to the bulk fluids and gels in the 3 experimental set-ups: phenol red (PR), bromothymol blue (BTB), bromocresol green (BCG) and bromophenol blue (BPB). The pH values at which they change colors are indicated on the left hand side of **Figure 9**. The idea was that the pH indicators penetrate into the implants (together with the water) and optical macroscopy pictures of surfaces and cross sections of the devices would allow to estimate the pH within the systems at different time points. As it can be seen in **Figure 9**, this strategy allowed to gain some insight in the case the "bulk fluid" set-up (the dotted red rectangles highlight the most informative images): After 10 d, sufficient amounts of the pH indicators penetrated into the implants to allow monitoring a pH value of 4.6-5.4 in regions close to the center of the implants, and pH values in the range of 5.4-6.0 in the rest of

the implants. This is interesting information, because PLGA ester hydrolysis is catalyzed by protons. However, in this case, drug release was already complete when using this set-up at this time point (**Figure 3A**). So, we prefer not to draw conclusions on the potential importance of autocatalytic effects based on these data. Due to the limited degrees of implant swelling at earlier time points, the concentrations of the pH indicators *within* the systems was too low to map the pH (**Figure 9**). When using the gel set-ups, only the BTB indicator penetrated to a sufficient extent into the implants, indicating a pH below 6.0 for the "gel -Eppendorf" set-up (the implants were too fragile to be cut in the case of the "gel – transwell" set-up).

As it can be seen in the middle of **Figure 7**, the implants' *dry mass* remained about constant during the first week, and then decreased, due to the release of water-soluble PLGA degradation products and of the drug. Importantly, the erosion rate was higher in the absence of an agarose gel. These observations are in good agreement with the hypothesized drug release mechanism: During the first few days, the PLGA network is still highly entangled and the small amounts of generated short chain acids and drug are poorly mobile, resulting in negligible dry mass loss. However, once substantial amounts of water are present in the system, drug and water-soluble degradation products are much more rapidly released. Since the presence of the agarose gel delays implant swelling, also the dry mass loss of the implants is delayed compared to the "bulk fluid" set-up.

The diagram at the bottom of **Figure 7** shows that the *decrease in polymer molecular weight* of the PLGA is not significantly affected by the type of experimental set-up: In all cases, the length of the macromolecules exponentially decreased from the beginning, indicating pseudo-first order degradation kinetics. After day 6, the values were too small to be reliably detected by the applied GPC method. These results suggest that the relatively rapid entire implant wetting and subsequent ester bond cleavage during the first couple of days are not substantially affected by the type of experimental set-up. This is consistent with the hypothesized drug release mechanism: The absence or presence of a hydrogel around the implant does not alter the rate at which the limited amounts of water diffuse into the system upon contact with phosphate buffer pH 7.4 to a noteworthy extent. Hence, also PLGA degradation throughout the polymer matrix is not affected during this initial phase.

Importantly, the absence of a noteworthy effect of the type of experimental set-up on PLGA degradation during this early phase strengthens the hypothesis that implant *swelling* (and not PLGA *degradation*) orchestrates drug release: While the decrease in polymer molecular weight is very similar during the first 6 d in the 3 experimental set-ups (**Figure 7** at the bottom), first indications for the hindrance of system swelling by the presence of an agarose gel are visible: as reflected by differences in the wet mass & volume changes (**Figures 3C and D**) as well as by optical macroscopy pictures (**Figure 5**). The differences in system swelling (in the absence vs. presence of a surrounding gel) affect drug mobility and drug release as well as the mobility of the water-soluble PLGA degradation products and, thus, implant erosion (dry mass loss).

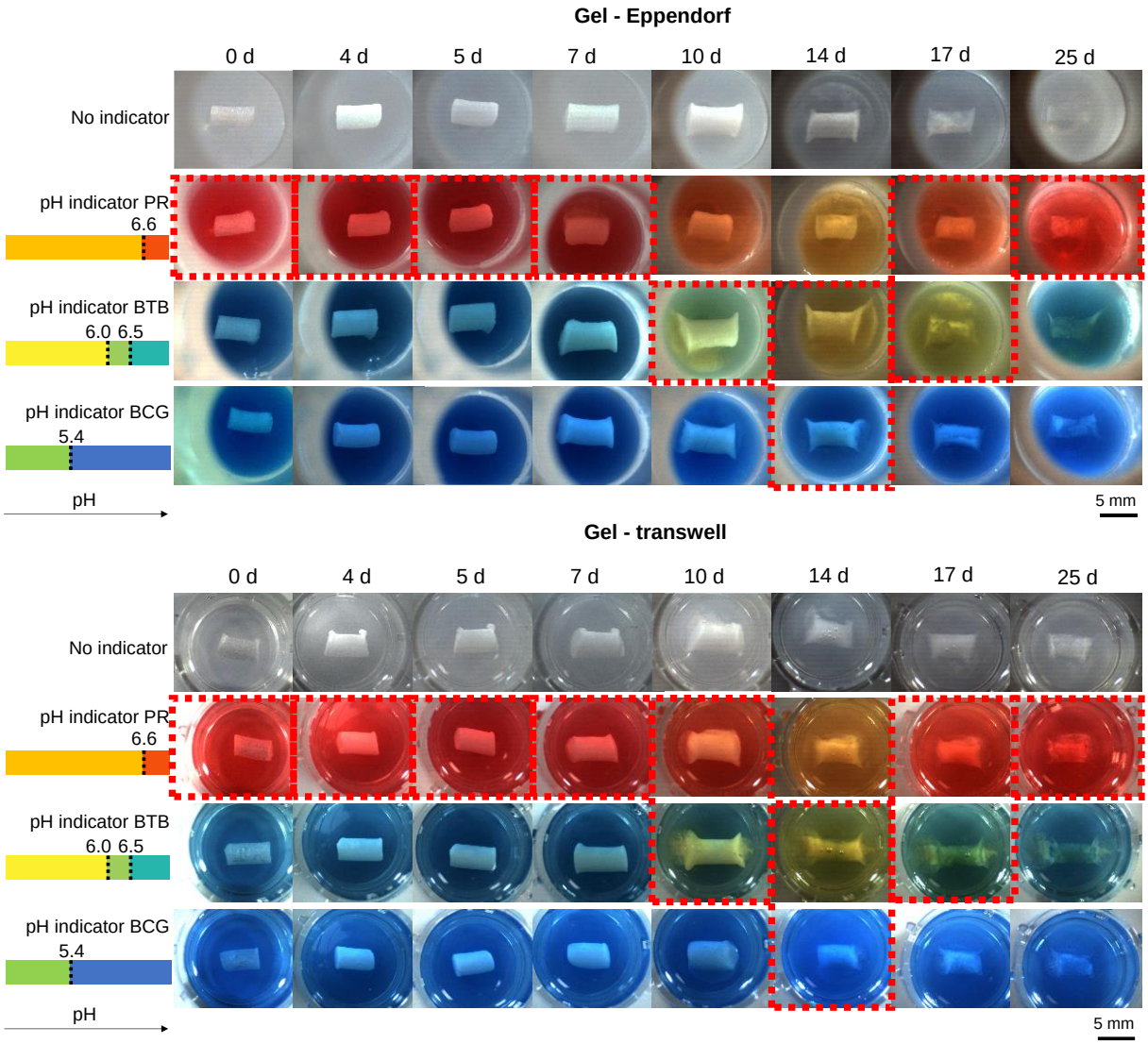

Figure 8. *Optical macroscopy pictures of ibuprofen-loaded PLGA implants embedded in agarose gels ("gel – Eppendorf" and "gel – transwell" set-ups) before and after exposure to phosphate buffer pH 7.4, optionally containing 0.0025 % phenol red (PR), bromothymol blue (BTB), or bromocresol green (BCG), as indicated. The dotted red rectangles highlight the most informative images.*

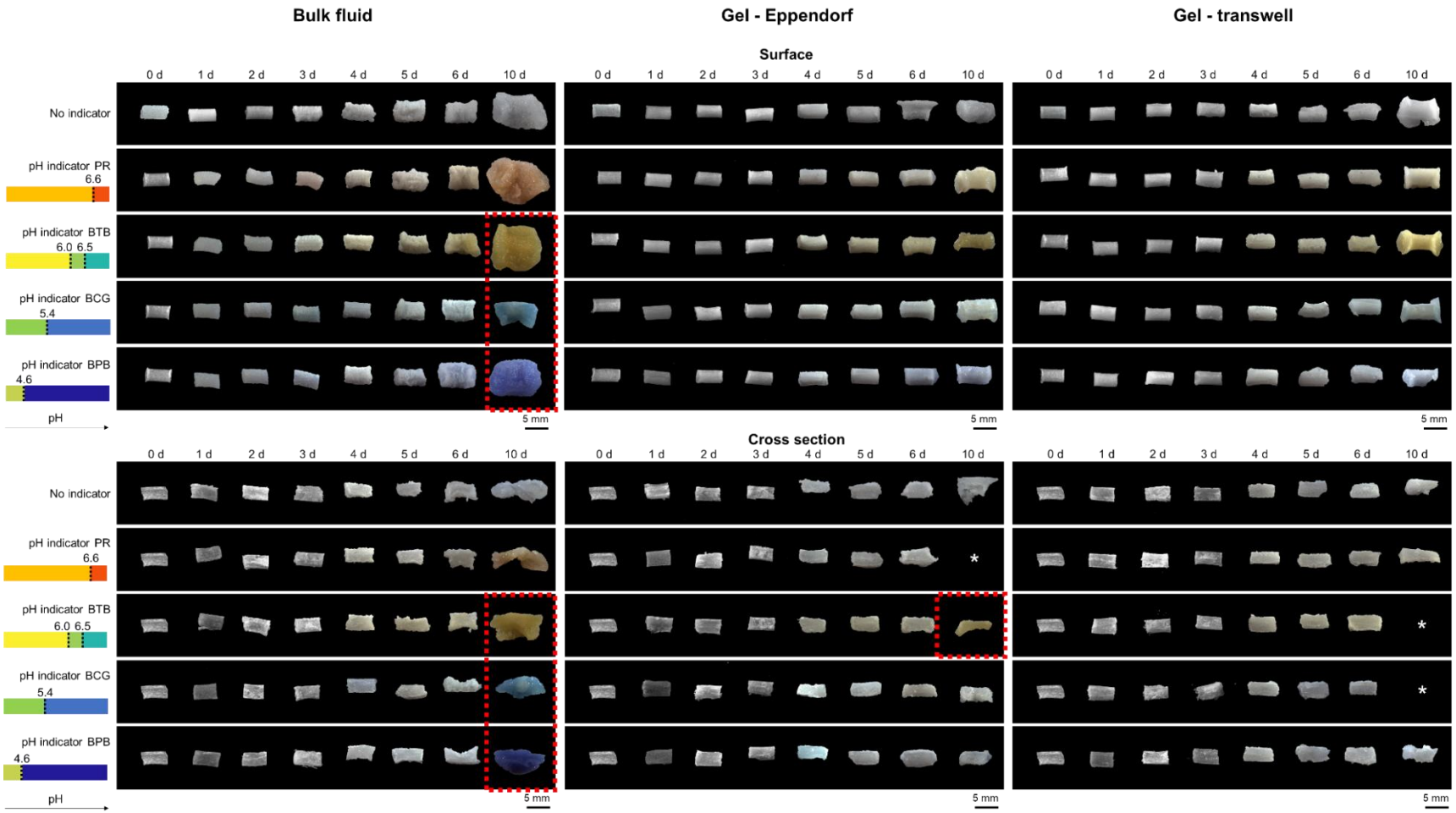

Figure 9. *Optical macroscopy pictures of surfaces and cross sections of ibuprofen-loaded PLGA implants before and after exposure to phosphate buffer pH 7.4 containing 0.0025 % phenol red (PR), bromothymol blue (BTB), bromocresol green (BCG) or bromophenol blue (BPB). Three experimental set-ups were used: Bulk fluids in Eppendorf tubes, agarose gels exposed to the release medium in Eppendorf tubes, and in agarose gels in transwell plates (the acceptor compartment containing the release medium). The type of set-up and exposure times are indicated at the top, the type of pH indicator is given on the left hand side. The asterisk indicates that the respective samples were too fragile to be cut. The dotted red rectangles highlight the most informative images.*

4. Conclusion

The presence of an agarose gel surrounding PLGA implants significantly hinders polymer swelling (sterically) and slows down drug release (due to delayed penetration of substantial amounts of water into the system). *In vivo* it can be expected that surrounding tissue has a similar mechanical effect. However, yet it is unknown how important the impact of mechanical stress caused by body movements (e.g., muscle contractions) is for the fate of a degrading PLGA implant. The results presented in this study can help developing more realistic *in vitro* drug release set-ups for parenteral drug delivery systems. They also strengthen the hypothesis that implant *swelling* plays an orchestrating role for the control of drug release from PLGA-based drug delivery systems.

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