Review

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Engineering microenvironment of biodegradable polyester systems for drug stability and release control

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Polymeric systems made of poly(lactic acid) or poly(lactic-co-glycolic acid) are widely used for long-term delivery of small and large molecules. The advantages of poly(lactic acid)/poly(lactic-co-glycolic acid) systems include biodegradability, safety and a long history of use in US FDA-approved products. However, as drugs delivered by the polymeric systems and their applications become more diverse, the significance of microenvironment change of degrading systems on long-term drug stability and release kinetics has gained renewed attention. In this review, we discuss various issues experienced with acidifying microenvironment of biodegradable polymer systems and approaches to overcome the detrimental effects of polymer degradation on drug stability and release control.

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One of the main goals in controlled drug delivery is to provide an active drug at a therapeutic concentration to the intended part of the body for weeks to months, even to years. The extended drug release allows to reduce the frequency of drug administration, minimize the experience of peak and trough blood levels, and improve patient's life quality and adherence to the regimen. For this purpose, drugs are encapsulated in polymeric matrices that degrade over the desired period and are implanted in the body by minor outpatient procedures. Poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) are biodegradable polyesters and one of the most widely used polymers in controlled-release formulations. More than 20 long-acting-release (LAR) products based on PLA and PLGA have been approved by the US FDA (Table 1, [1–4]), with several others in clinical trials (Table 2, [5]). The popularity of these polyesters comes from the favorable FDA track record, based on biodegradability, biocompatibility, commercial availability and a wide range of choices. Therefore, PLA and PLGA are often the go-to polymers in developing LAR drug products. However, controlling the drug release kinetics by PLA or PLGA systems means more than choosing a specific polymer from a catalog, because the microenvironment of degrading polymer matrices can play complex roles. The microenvironment of biodegradable polyester systems and its effects on drug stability and release kinetics have been extensively studied in the protein delivery arena in the past decade [6]. With the increasing diversity of drugs encapsulated in LAR products, there has been renewed interest in the effects of polymeric microenvironment on the encapsulated drugs in a broad range of molecular size and chemical identity. This review discusses the significance of the microenvironment of PLA or PLGA-based products in drug release control, how it interacts with a drug and/or affects its physicochemical status, known methods to evaluate the microenvironment change, and the existing approaches to modify the microenvironment of PLA- or PLGA-based products.

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Table 1. FDA-approved long-acting-release products based on poly(lactic acid) or poly(lactic-co-glycolic acid).

Formulation Type	Polymers	Product name	Drug	Duration	Regimen	Indications	Company	FDA approval
Microparticles	PLGA (LA/GA 50/50); PLA	Lupron Depot®	Leuprolide Acetate	1, 3, 4, 6 months	7.5 mg/month; 22.5 mg/3 months	Advanced prostatic cancer	Abbvie	1989, 1996, 1997, 2011
Microparticles	PLGA	Sandostatin® LAR	Octreotide acetate	1 month	10–30 mg/month	Acromegaly	Novartis	1998
Microparticles	PLGA	Nutropin Depot®	Somatotropin	1 month	13.5 mg/month	Growth hormone deficiency	Genentech	1999
Microparticles	PLGA	Trelstar®	Triptorelin pamoate	1, 3, 6 months	3.75 mg/month	Advanced prostate cancer	Allergen	2000, 2001, 2010
Microparticles	PLGA	Somatuline® Depot	Lanreotide	1 month	60 mg/month	Gastrointestinal & pancreatic neuroendocrine tumors	Ipsen	2000
Microparticles	PLGA	Arestin®	Minocycline HCl	2 weeks	0.5 mg/week	Pathogenic progression of periodontitis	OraPharma	2001
Microparticles	PLGA (75/25)	Risperidal® Consta®	Risperidone	2 weeks	12.5 mg/week	Schizophrenia; Bipolar I disorder	Janssen	2003
Microparticles	PLGA (75/25)	Vivitrol®	Naltrexone	1 month	380 mg/month	Alcohol dependence	Alkermes	2006
Microparticles	PLGA (50/50)	Bydureon®	Exenatide	1 week	2.0 mg/week	Type 2 diabetes	AstraZeneca	2012
Microparticles	PLGA	Lupaneta Pack™	Leuprolide acetate	3 months	3.75 mg/month	Endometriosis	Abbvie	2012
Microparticles	PLGA	Signifor®	LAR Pasireotide	1 month	20–60 mg/month	Acromegaly	Novartis	2014
Microparticles	PLGA	Bydureon Bcise®	Exenatide	1 week	2.0 mg/week	Type 2 diabetes	AstraZeneca	2017
Microparticles	PLGA	Zilretta®	Triamcinolone acetoamide	3 months	32 mg/3 months	Osteoarthritis	Flexion	2017
Microparticles	PLGA	Triptodur Kit®	Triptorelin pamoate	6 months	22.5 mg/6 months	Central precocious puberty	Arbor	2017
Intravitreal implant	PLGA	Ozurdex®	Dexamethasone	3 months	0.23 mg/month	Retinal diseases	Allergan	2009
Solid implant	PLGA	Zoladex®	Goserelin acetate	1, 3 months	3.6 mg/month	Advanced breast cancer; endometriosis; and prostate cancer	AstraZeneca	1989, 1996
Solid implant	PLGA	Propel®	Mometasone furoate	1 month	0.37 mg/month	Chronic sinusitis	Intersect ENT	2011
In situ gel	PLGA (50/50) carboxyl end groups	Eligard®	Leuprolide	1, 3, 4, 6 months	7.5 mg/month	Advanced prostate cancer	Tolmar	2002
In situ gel	PLGA	Sublocade®	Buprenorphine	1 month	100, 300 mg/month	Moderate to severe opioid use disorder	Indivior	2017
In situ gel	PLGA	Perseris™	Risperidone	1 month	90, 120 mg/month	Schizophrenia	Indivior	2018
In situ gel	PLA	Atridox	Doxycycline hyclate	1 week	50 mg	Chronic adult periodontitis	CollaGenex Pharmaceuticals; Atrix Laboratories	1998

LAR: Long-acting-release; PLA: Poly(lactic acid); PLGA: Poly(lactic-co-glycolic acid).

Drug release control by PLA & PLGA systems

PLA consists of lactic acid (LA), present in two enantiomeric forms, L-(+)-LA and D-(-)-LA [7]. PLGA is synthesized by random copolymerization of LA and glycolic acid (GA) [8]. These polyesters are degraded by random hydrolysis of ester linkages along the polymer backbone [9], where a single ester linkage is hydrolyzed to produce one hydroxyl and one carboxylic acid moieties. The scission of long polymer chains generates low molecular weight polymers, which degrade to water-soluble fragments, either removed via the kidney or further hydrolyzed into their monomers subject to the tricarboxylic acid cycle [10]. The degradation rate of PLA and PLGA depends on the molecular weight distribution and monomer composition [11]. Polymers with low molecular weights degrade relatively fast [12]. The lactide stereoisomeric composition (L-lactide or D, L-lactide) influences the solid-state of PLA (amorphous vs.

Table 2. Poly(lactic-co-glycolic acid) implant formulations in clinical trials.

Formulation Type	Drug	Indications	Status	NCT identifier
Subconjunctival insert	Latanoprost	Glaucoma	Phase 1 (terminated, 2014)	NCT01180062
Suspension	Bupivacaine	Acute pain	Phase 1 (completed, 2017)	NCT02982889
Intravitreal implant	Dexamethasone	Exudative retinal detachment and uveal melanoma	Phase 1 (recruiting, 2020)	NCT04082962
Bone screw	Ciprofloxacin	Ankle fracture	Phase 2 (completed, 2014)	NCT01729195
Intravitreal implant	Triamcinolone acetonide	Diabetic macular edema	Phase 2 (completed, 2016)	NCT02221453
Microspheres	Doxycycline	Chronic periodontitis	Phase 2 (completed, 2017)	NCT02726646
Microspheres	Triamcinolone	Rotator cuff disease	Phase 3 (recruiting, 2020)	NCT04094298
Microspheres	Doxycycline	Periodontal disease	Phase 4 (completed, 2015)	NCT02487186
Drug eluting stent	Rapamycin	Cardiovascular disease	Unknown	NCT01681381
Stent implant	Everolimus	Ischemic heart disease	Unknown	NCT03401216

semi-crystalline) [13]. Amorphous poly(D, L-lactide) degrades faster than semicrystalline poly (L-lactide) due to enhanced water penetration [14,15]. PLGA with a relatively high lactic acid to glycolic acid ratio degrades more slowly, due to the high content of relatively hydrophobic LA, which delays the hydration of the polymer. For example, PLGA with a LA/GA ratio of 50/50 degrades faster than PLGA with a similar molecular weight but a LA/GA ratio of 75/25 [16]. The end group chemistry of PLGA also affects its degradation. For example, PLGA with an ester end group degrades slower than PLGA with a carboxylic acid end group [17]. PLA and PLGA polymers with different molecular weights, lactic acid to glycolic acid ratios and end cap groups are commercially available (Table 3, [18–21]).

PLA and PLGA have been used for sustained delivery of vaccines, peptides, proteins or nucleic acids, as microparticles, implants or gels [22–28]. The drug release is controlled by carrier degradation, which depends on the polymer properties mentioned above as well as the dimension of the carrier structure, porosity [12,29], acidity or basicity of the loaded drugs and/or additives, and sterilization methods [30]. The location of carriers in the body can also affect the *in vivo* degradation because the polymer degradation may be accelerated by enzymes such as esterase [31,32].

Several PLGA/PLA systems have been approved by the FDA (Table 1) or in clinical trials (Table 2) for long-term drug delivery. For example, Sandostatin® LAR is octreotide acetate-loaded PLGA microparticles approved for long-term treatment of acromegaly and diarrhea associated with metastatic carcinoid tumors or vasoactive intestinal peptide tumors, administered intramuscularly at 4-week interval [33]. Ozurdex®, an intravitreal implant consisting of PLGA and dexamethasone, is indicated for ocular diseases such as macular edema and uveitis [34]. Administered by intravitreal injection, a single dose of Ozurdex (dexamethasone 0.7 mg) provides a local ant-inflammatory effect lasting up to three months [34]. Zoladex® is a PLGA implant loaded with goserelin acetate, a gonadotropin-releasing hormone agonist, indicated for prostate cancer, endometriosis and breast cancer [35]. It is administered at 4-week interval by subcutaneous injection for long-term palliative treatment of the diseases. Perseris® is a PLGA gel system for sustained delivery of risperidone, an antipsychotic for schizophrenia treatment [36]. The drug is mixed with PLGA/N-methyl-pyrrolidine (NMP) solution prior to use and injected subcutaneously to form a gel-like PLGA depot *in situ* after NMP is exchanged with interstitial fluid. Perseris® is administered at 90 or 120 mg once a month, providing an effect equivalent to 3 mg/day or 4 mg/day oral risperidone, respectively [36]. Similarly, Atridox®, doxycycline hyclate mixed with PLA/NMP, releases the drug in the periodontal pocket for 7 days for local treatment of periodontitis [37].

Significance of microclimate pH of PLA or PLGA-based products in drug release control

PLA or PLGA-based systems generate acidic products in degrading matrices, reducing the pH inside of the polymeric structure (microclimate pH, μpH) [38–40]. The acidifying PLA or PLGA matrices can cause significant issues in release control, stability and activity of the loaded drugs [41,42], in particular acid-labile protein drugs [29,43–45].

The detrimental effect of acidic microclimate in PLGA microspheres was demonstrated with bovine serum albumin (BSA) as a model drug. BSA encapsulated in PLGA (LA/GA 50/50, 0.61 dl/g) implants underwent unfolding, peptide bond hydrolysis and aggregation in the acidifying microclimate (Figure 1A & B) [46], showing no more than 20% release of the total loaded over 28 days in PBS containing Tween 80 (PBST) at 37°C (Figure 1C) [45].

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Table 3. Commercially available poly(lactic acid) and poly(lactic-co-glycolic acid).

Manufacturer	Product name	Chemical name	Molecular weight (kDa)	End group	Inherent viscosity (dl/g)
Evonik	Resomer R 203S	Poly(D,L-lactide)	18 -28	Ester	0.25–0.35
	Resomer R 203H	Poly(D,L-lactide)	18 -24	Acid	0.25–0.35
	Resomer R 202S	Poly(D,L-lactide)	10 -18	Ester	0.16–0.24
	Resomer R 202H	Poly(D,L-lactide)	10 -18	Acid	0.16–0.24
	Resomer RG 756S	Poly(D,L-lactide-co-glycolide) 75/25	76–115	Ester	0.7–1.0
	Resomer RG 752H	Poly(D,L-lactide-co-glycolide) 75/25	4–15	Carboxylic acid	0.14–0.22
	Resomer RG 504	Poly(D,L-lactide-co-glycolide) 50/50	38–54	Ester	0.45–0.60
	Resomer RG 503	Poly(D,L-lactide-co-glycolide) 50/50	24–38	Ester	0.32–0.44
	Resomer RG 503H	Poly(D,L-lactide-co-glycolide) 50/50	24–38	Carboxylic acid	0.32–0.44
Lactel	DL-PL	Poly (D,L-lactide)		Ester	0.26–0.54 0.55–0.75
	L-PL	Poly (L-lactide)		Ester	0.15–0.35 0.90–1.20
	DL-PL	Poly (D,L-lactide)		Carboxylic acid	0.16–0.25
	85:15 DL-PLG	Poly (D,L-lactide-co-glycolide) 85/15		Ester	0.55–0.75 0.76–0.85
	75:25 DL-PLG	Poly (D,L-lactide-co-glycolide) 75/25		Ester	0.55–0.75 0.80–1.20
	75:25 DL-PLG	Poly (D,L-lactide-co-glycolide) 75/25		Carboxylic acid	0.70–0.90
	65:35 DL-PLG	Poly (D,L-lactide-co-glycolide) 65/35		Ester	0.55–0.75 0.83–0.93
	50:50 DL-PLG	Poly (D,L-lactide-co-glycolide) 50/50		Ester	0.15–0.25 0.26–0.54 0.55–0.75 0.76–0.94 0.95–1.20
	50:50 DL-PLG	Poly (D,L-lactide-co-glycolide) 50/50		Carboxylic acid	0.15–0.25 0.55–0.75
Corbion	PURASORB PDL 02	Poly (D,L-lactide)		Ester	0.2
	PURASORB PDL 02A	Poly (D,L-lactide)		Carboxylic acid	0.2
	PURASORB PDL 04	Poly (D,L-lactide)		Ester	0.4
	PURASORB PDL 04A	Poly (D,L-lactide)		Carboxylic acid	0.4
	PURASORB PDL 05	Poly (D,L-lactide)		Ester	0.5
	PURASORB PDLG 8503	Poly (D,L-lactide-co-glycolide) 85/15		Ester	0.3
	PURASORB PDLG 8505A	Poly (D,L-lactide-co-glycolide) 85/15		Carboxylic acid	0.5
	PURASORB PDLG 7502	Poly (D,L-lactide-co-glycolide) 75/25		Ester	0.2
	PURASORB PDLG 7502A	Poly (D,L-lactide-co-glycolide) 75/25		Carboxylic acid	0.2
	PURASORB PDLG 7504	Poly (D,L-lactide-co-glycolide) 75/25		Ester	0.4
	PURASORB PDLG 7504A	Poly (D,L-lactide-co-glycolide) 75/25		Carboxylic acid	0.4
	PURASORB PDLG 7507	Poly (D,L-lactide-co-glycolide) 75/25		Ester	0.7
	PURASORB PDLG 5002	Poly (D,L-lactide-co-glycolide) 50/50		Ester	0.2
	PURASORB PDLG 5002A	Poly (D,L-lactide-co-glycolide) 50/50		Carboxylic acid	0.2
	PURASORB PDLG 5004	Poly (D,L-lactide-co-glycolide) 50/50		Ester	0.4
	PURASORB PDLG 5004A	Poly (D,L-lactide-co-glycolide) 50/50		Carboxylic acid	0.4
	PURASORB PDLG 5010	Poly (D,L-lactide-co-glycolide) 50/50		Ester	1.0

Similarly, PLGA (LA/GA 85/15, 0.86 dl/g) microparticles encapsulating formaldehyde-detoxified tetanus toxoid protein (150 kDa) released 12% of the total protein in 30 days, partly due to acid-induced protein unfolding [44]. Insulin was also subject to acid-induced degradation in PLGA (LA/GA 50/50, 53 kDa) microparticles, with 1% release in 7 days in pH 7.4 Tris buffer [47]. Ovalbumin loaded in PLGA hot-melt extruded implants showed a typical incomplete release, likely due to ionic interactions between the acidic polymer degradation products and the protein facing pH below the isoelectric point [48]. In addition to the structural change, proteins can undergo chemical

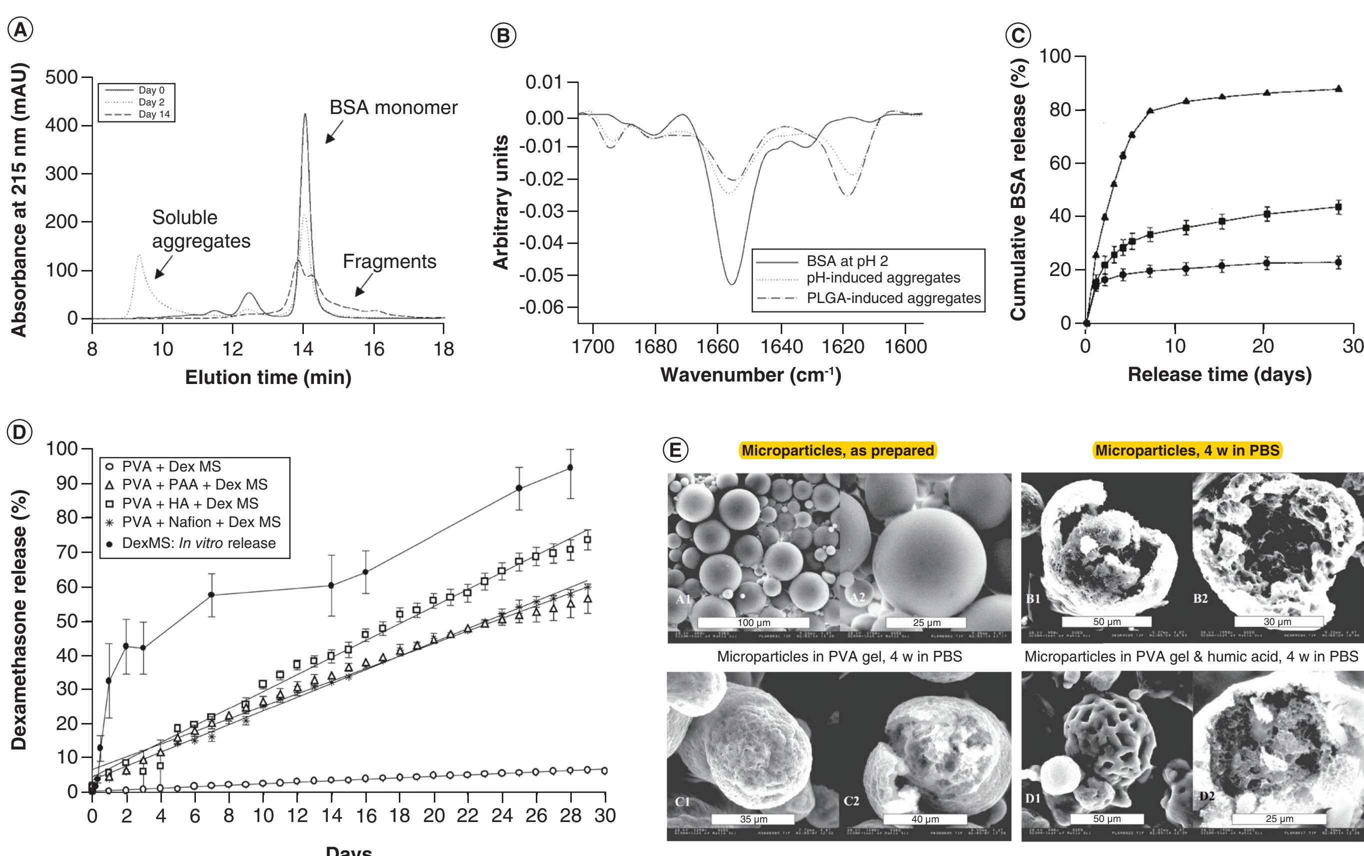

Figure 1. Effects of acidic microclimate in PLGA microparticles on drug stability and release control. (A) Representative size-exclusion HPLC chromatograms showing the degradation of bovine serum albumin (BSA) at pH 2 during incubation at 37°C. BSA monomer, soluble aggregates and peptide fragments are indicted. The protein sample was prepared in high-ionic strength buffer at a protein concentration of 40 mg/ml. **(B)** FTIR analysis of BSA aggregates from pH and poly(lactic-co-glycolic acid) (PLGA) induced stresses. Aggregates extracted from PLGA millicylinder after 2 weeks of incubation at 37°C in release media compared with BSA gel produced from incubation at pH 2 for 7 days at 37°C (100 mg/ml and high-ionic strength buffer). BSA prepared at pH 2, 40 mg/ml and high-ionic strength buffer, is also shown for comparison. **(C)** BSA release kinetics from PLGA implants at 37°C in PBS containing Tween 80. Millicylinders were loaded with 15% BSA and 0% (circle), 0.5% (square) and 3.0% (triangle, facing down) $Mg(OH)_2$. **(D)** Cumulative release of dexamethasone (Dex) from PLGA microspheres (MS); and MS incorporated into the poly(vinyl alcohol) (PVA) hydrogel matrix: with no additives; and in presence of PAA; HAs; and Nafion. **(E)** Environmental scanning electron microscopy photomicrographs of PLGA microspheres: (A1, A2) as-prepared, nondegraded, no-gel; (B1, B2) after 4 weeks submersion in PBS; (C1, C2) embedded in PVA gel, no additives after 4 weeks submersion in PBS; and (D1, D2) embedded in PVA gel with humic acids after 4 weeks submersion in PBS. **(A & B)** Reprinted with permission from [46] © Elsevier (2006) **(C)** reprinted with permission from [45] © Springer Nature (1969); **(D & E)** reprinted with permission from [53] © Springer Nature (2005).

reactions such as acylation in the acidifying µpH, where polymer degradation products serve as substrates [49,50]. The acidifying µpH of PLGA (LA/GA 50/50, 13.7 kDa) microparticles was shown to decrease the stability of nucleic acids, especially linear high molecular DNA [51]. PLGA microparticles loaded with double-stranded (ds) DNA showed a biphasic release of total DNA in PBS (pH 7.4), mirroring polymer degradation kinetics. However, they released very little dsDNA over 45 days, indicating the degradation of encapsulated dsDNA in the acidifying microclimate of PLGA particles [51]. Small molecules can be affected by the acidic µpH of degrading polymer matrix as well. A recent study showed that nifedipine, with a pKa value of 2.69, precipitated as crystals in a degrading PLGA (LA/GA 50/50, 7–17 kDa) implant, facing a greater challenge in drug release with a higher drug loading content, due to the increased chance of drug crystallization [52].

The acid-induced changes of the encapsulated drugs can cause adverse effects on long-term drug delivery, such as inflammatory tissue responses. An example is lysozyme encapsulated in PLGA (LA/GA 50/50, 8 kDa) microparticles [54]. The particles showed a burst release (50%) and an incomplete release of lysozyme with ever-decreasing bioactivity, which is partially attributed to acidifying microenvironment. Notably, subcutaneously

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injected lysozyme-loaded PLGA microparticles induced a sign of local inflammation, characterized by a moderate foreign body reaction and abundant neutrophils in Sprague-Dawley rats, suggesting a potential pro-inflammatory effect of the destabilized protein [54]. The stability and release kinetics of encapsulated lysozyme as well as tissue responses to the microparticles were improved by the inclusion of chondroitin sulfate A, which forms an ionic complex with lysozyme to reduce the exposure of the protein to the deleterious environment of the particles [54].

Conversely, the acidic μpH can accelerate drug release. Dexamethasone-incorporated PLGA (LA/GA 50/50, 60 kDa) microspheres were embedded in physically cross-linked poly(vinyl alcohol) (PVA) hydrogels for 1-month delivery of dexamethasone [53]. In PBS (pH 7.4), dexamethasone-loaded PLGA microspheres (without PVA gel) released 40% of the loaded drug as an initial burst release in 2 days and the remainder in 28 days (Figure 1D). Embedded in PVA hydrogels, the PLGA microspheres showed an extremely slow release: 6% of the loaded drug in 30 days. Here, PVA hydrogels served as a reservoir of acidic oligomeric degradation products of PLGA and made the oligomers unavailable for acid-catalyzed degradation of PLGA. Adding polyacids (such as polyacrylic acid, humic acids and Nafion) to the PVA gels helped release dexamethasone from microspheres (60–75% release for 1 month), as the polyacids accumulated on the microsphere surface to increase the acidity, thereby restoring the acid-catalyzed PLGA degradation (Figure 1E). This example shows how acidic degradation products help accelerate drug release. The controlled acidification of PLGA matrix may be applicable to drugs that are not sensitive to acidity.

Methods to monitor microenvironment pH of PLA or PLGA-based systems

Indirect measurement

The simplest way of observing the acidic environment of degrading polymer devices is to measure the pH of the aqueous medium in which the devices are located. For example, PLA or PLGA discs (2 × 10 × 15 mm) were placed in PBS (pH 7.4) at 37°C with periodic measurement of the medium pH over 2 years [55]. The pH change was minimal: with PLGA (LA/GA 85/15, 114 kDa) discs, the medium pH decreased by 1.5 units in 40 weeks and PLGA (LA/GA 96/4, 100 kDa) discs showed <1 unit of pH change in 90 weeks. The limitation of this method is that the acidification occurs more intensely in the central part of the sample than on the surface, and the internal pH change is not fully reflected in the medium, especially when it is buffered. With this method, there is a chance that the internal pH change is substantially underestimated [38,56].

Potentiometry

A potentiometric method uses standard glass pH electrodes, which are coated with PLGA films and measure the zero-current potential (the potential in the electrochemical cell in an equilibrium state) of an aqueous layer present between the PLGA coating and the electrode with respect to a reference electrode (Figure 2A) [57]. This technique assumes that the aqueous layer adjacent to the electrode mimics the microenvironment of the aqueous pores inside the polymer matrix. The μpH is determined from the measured cell potential after correcting for the diffusion and interfacial potentials. This method was used to determine the μpH developed in thin polymer films with varying thicknesses. The μpH was declined to <3 in 30–100 μm-thick PLGA (LA/GA 50/50) films after 24 h incubation in PBS (37°C) and remained acidic for 4 weeks, whereas a ∼7 μm-thick film showed a slight decrease in pH (4.3) followed by a steady increase to pH 7 in 1 week, reflecting the difference in the diffusion of acidic degradants. The potentiometric method is quick and straightforward but not applicable to systems with different geometries, such as microspheres and nanospheres.

For measurement of internal pH of degrading microparticles with pH electrodes, microparticles are dissolved in a water-miscible organic solvent such as acetonitrile and diluted with distilled water to make an organic solution mixture [39]. The pH measured by glass electrodes are corrected for the change in hydrogen activity in the organic solution. With this method, the initial pH of PLGA (LA/GA 50/50, 0.15–0.20 dl/g) microparticles incubated in PBST at 37°C was as low as 3.3 immediately and increased to 4.2 by 7 days with the loss of oligomers and monomers upon hydration, followed by a decrease to 2.8 in 4 weeks.

Nuclear magnetic resonance spectroscopy

^{31}P Nuclear magnetic resonance (NMR) was used to study the internal environment in PLGA (LA/GA 50/50, 0.15 dl/g) microparticles [58]. This method monitors the diffusion of naturally occurring inorganic phosphate (^{31}P) in the external medium (sheep serum) into the microparticles and determines the change in μpH based on the variation of ^{31}P chemical shifts in the particles relative to the external phosphate population. ^{31}P NMR determined that the pH in PLGA microparticles dropped to 6.4 in 45 days at 37°C. However, the permeability

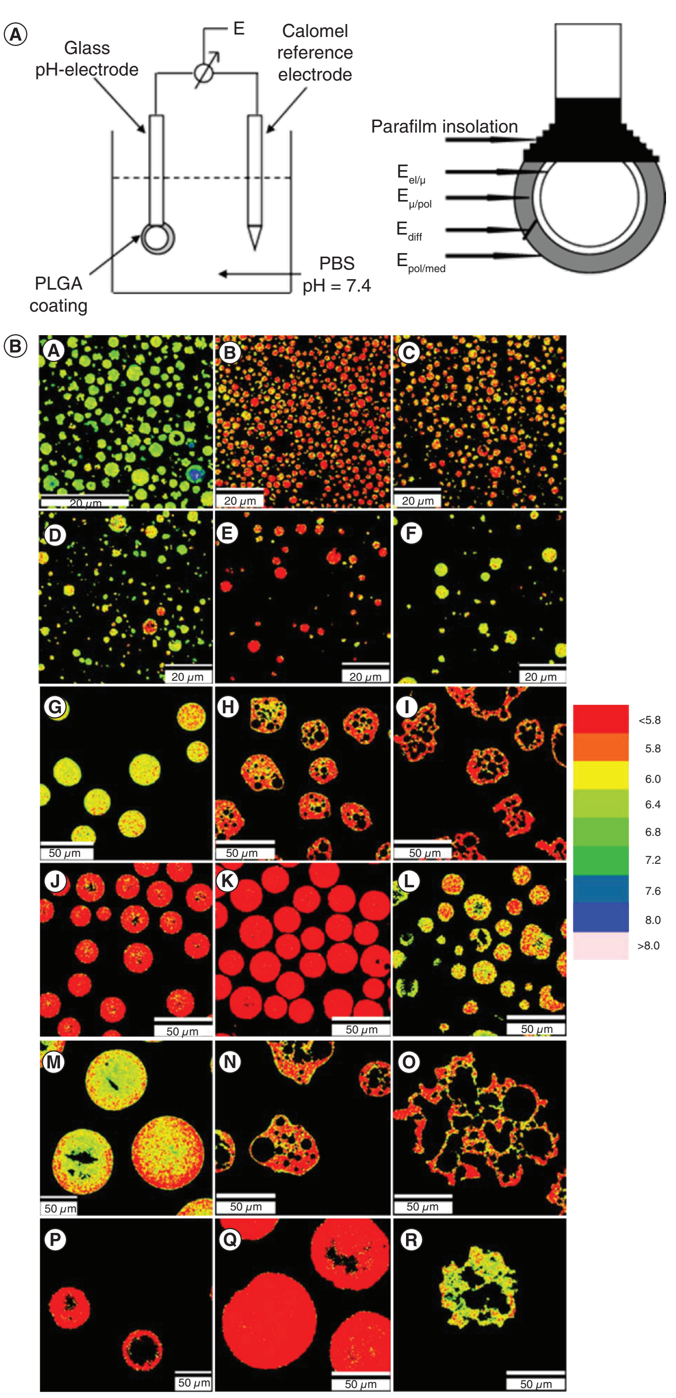

Figure 2. Methods to monitor microclimate pH of PLGA systems. (A) Schematic representation of the experimental setup for potentiometric µpH measurement. Left: A glass electrode was coated with a poly(lactic-co-glycolic acid) (PLGA) film and a zero-current cell potential (*E*) was measured with respect to a calomel reference electrode in PBS containing Tween 80 at 25°C. Right: Potentials expected to be formed during the measurement include the interfacial potential between aqueous microclimate and electrode ($E_{el/\mu}$), the interfacial potential between microclimate and polymer phase ($E_{\mu/pol}$), the diffusion potential in the polymer (E_{diff}) and the interfacial potential between polymer and the media ($E_{pol/med}$). The measured potential $E = E_{el/\mu} - E_{\mu/pol} + E_{diff} + E_{pol/med} - E_{ref}$. **(B)** pH map of small PLGA 50/50 microspheres with and without base at different incubation times (2 h, 7 days and 28 days). (A, B & C) Small-size microspheres with base, (D, E & F) small-size microspheres without base, (G, H & I) medium-size microspheres with base, (J, K & L) medium-size microspheres without base, (M, N & O) large-size microspheres with base and (P, Q & R) large-size microspheres without base. **(A)** Reprinted with permission from [57] © American Chemical Society (2004); **(B)** Reprinted with permission from [56] © Elsevier (2005).

of external phosphate ions into the polymer phase was limited; thus, the measured pH in this study was likely underestimated. In a modified version of this method, sodium phosphate salts were encapsulated in PLGA (LA/GA 50/50, 0.16 dl/g) microparticles serving as porogens and phosphate probes. ^{31}P NMR of microparticles incubated in fetal calf serum at 37°C revealed that the internal pH decreased to 2–4 in 2 days with little additional pH change through day 14 [59].

Electron paramagnetic resonance spectroscopy

Electron paramagnetic resonance (EPR) spectroscopy employs imidazole-derived spin probes or spin probe-labeled agents, encapsulated in the PLGA systems [60]. The protonation of nitrogen in position 3 of imidazoline results in a decrease of spin density at nitrogen in position 1, which can be quantified as a hyperfine splitting constant and correlated with pH [60]. This method was used to monitor a pH change in PLGA implants in vivo [60] and PLGA microspheres in vitro [61,62]. The PLGA (LA/GA 50/50, Mw 51 kDa) tablets implanted in Balb/c mice showed pH 4 in 7 days, which continued to decrease to pH 2 in the next two weeks [60]. PLGA (LA/GA 50/50, Mw 8 kDa, nonend-capped) microspheres showed a pH value of <4.7 from the beginning to 13 h, due to free carboxylic acid groups of the polymer [61]. However, the sensitivity of this method deteriorates with time as the polymer degradation intensifies, with increasing microviscosity that impairs the mobility of spin probes [62] or with the loss of encapsulated probes [61].

Confocal microscopy

Confocal laser scanning microscopy allows for direct visualization of spatial μpH distribution within microparticles. The use of confocal microscopy in measuring μpH in PLGA systems was first reported by Shenderova *et al.* [39]. It uses pH-sensitive fluorescent dyes such as fluorescein, which shows a decreasing fluorescence emission intensity with a decreasing pH, as an indicator of the μpH. A fluorescent dye (as a conjugate to high molecular dextran to prevent premature leaching out) is encapsulated in microparticles, and a confocal microscope is used to measure the emission intensity in the microparticles, which is converted to pH according to a calibration curve of pH versus fluorescence intensity (Figure 2B).

Fu *et al.* used two fluorescent dye-dextran conjugates (SNARF-dextran and NERF-dextran) for probing pH change in PLGA microparticles [38]. They used the ratio of fluorescence intensities at two respective emission wavelengths instead of the fluorescence intensity at a fixed wavelength to eliminate the dependence on dye concentration. A 2:1 mixture of the two dyes showed a linear relationship between the pH vs. the ratio of emission intensities (I_{580}/I_{535}) over a pH range of 1.5–3.5. Encapsulated in PLGA (LA/GA 50/50, 25 kDa) microparticles, the two dyes located the acidifying region in the particles as a function of time and particle size, indicating that relatively larger particles developed more acidic pH (∼1.5) from the center, which diminished with a prolonged incubation (>15 d) due to the efflux of degradation products. The range of detectable pH varies with the choice of fluorescence dye. SNARF-dextran was used to map μpH in the range of 5.8–8.0 with BSA-loaded PLGA microparticles (2, 40, 137 μm), showing that smaller particles had a more neutral pH due to the diffusion of acidic degradation products and that co-encapsulated $MgCO_3$ helped resist acidification [56]. Lysosensor yellow/blue® Dextran conjugate was used for probing an intermediate pH range (2.8–5.8) [40].

The advantage of this technique is the ability to visualize the spatiotemporal pH distribution within degrading microparticles. However, a caveat is that the fluorescence intensity is affected not only by the pH but also by the amount and type of encapsulated drugs, requiring a careful correction to reduce the interference [63]. Another limitation is that the pH range each fluorescence dye covers is rather narrow and may not be effective in observing a broad pH change.

Approaches to modify microenvironment of PLA or PLGA-based products

With the recognition of damaging effects of acidifying μpH of PLA- and PLGA systems on drug stability and release kinetics control, various formulation approaches have been employed to counteract the acidifying μpH, aiming to neutralize by basic excipients and/or delay the acidification by facilitating the diffusion of acidic products.

Inorganic bases

Inorganic bases, such as $Mg(OH)_2$, $MgCO_3$ and $ZnCO_3$, were coencapsulated in PLGA systems to neutralize the acidifying μpH and protect the stability of encapsulated drugs [43–45,64,65]. The rationale of this approach is that the

bases neutralize the acidic pH, reducing the acid-catalyzed polymer degradation, and also react with low molecular weight polymer degradants to form salts, creating osmotic pressure to enhance water influx [45].

The improvement of drug stability and release control by inorganic bases was demonstrated with several protein drugs. With the coencapsulation of 3% $Mg(OH)_2$, BSA in PLGA millicylinders avoided aggregation, fragmentation and alterations in the secondary or ternary structure and released 80% of the total loaded in 28 days (as opposed to 20% from the counterpart without $Mg(OH)_2$) (Figure 1C) [45]. Likewise, recombinant human basic fibroblast growth factor and bone morphogenetic protein-2 encapsulated in PLGA millicylinders were released continuously only after the coencapsulation of 3% $Mg(OH)_2$ and 15% BSA (used to mask protein interaction with polymer matrix) [45]. $Mg(OH)_2$ or $MgCO_3$ helped increase the release of ovalbumin encapsulated in hot-melt extruded PLGA implants, albeit incompletely, from 25% (without additives) to 35–46% over 5–7 weeks, in a similar mechanism [48].

The effectiveness of inorganic bases varies with their basicity and water solubility, as well as the dimension and geometry of the polymer system. For example, $Ca(OH)_2$, a strong base relative to $Mg(OH)_2$, increased the pH excessively, generating free thiols to induce disulfide formation in the encapsulated BSA [45]. Conversely, a weaker base $ZnCO_3$ was insufficient to bring the pH above the threshold pH for protein stability (e.g., pH 3 for BSA) and resulted in relatively high protein aggregation compared with $Mg(OH)_2$ (10% for 3% $ZnCO_3$ vs 2% for 3% $Mg(OH)_2$) [45]. The dimension and geometry of the polymer system are relevant to the effectiveness of inorganic bases due to the heterogeneity of internal μpH. In this case, a more water-soluble base such as $MgCO_3$ may help as it diffuses better into the acidic pores in the matrix [45]. However, the use of a water-soluble base or increasing the amount may limit the time frame for release control and thus requires caution [48].

While inorganic bases have become popular since the first demonstration of the benefit [45], a recent study suggests that the increase of μpH by $Ca(OH)_2$ can be detrimental to drug stability and release control [66]. In this study, the encapsulation of 5% $Ca(OH)_2$ in PLGA (LA/GA 50/50, 2.4–3.8 kDa) microparticles completely neutralized the μpH throughout the incubation period. However, with the neutralization of μpH, polymer degradation and mass transfer between the particles and medium slowed down, increasing the exposure of a model peptide (octreotide acetate) to oligomers (acylation substrate) both in quantity and time, thereby aggravating the peptide acylation and delaying the release.

Porosity enhancers

The porosity of a polymer matrix affects the initial influx of aqueous medium and the efflux of encapsulated drug and polymer degradation products, thereby the acidification of the internal matrix and the drug release kinetics. The porosity can be controlled by additives but also affected by random factors, often unintended, such as manufacturing variabilities, including monomer sequence, type, residual solvents, monomers and oligomers. A recent study illustrates that the porosity can play a more dominant role in drug release control than the molecular weight, which has long been believed to govern the polymer degradation kinetics, hence the drug release kinetics [67]. In this study, risperidone-loaded PLGA (LA/GA 75/25, 86 kDa) microparticles showed a shorter lag phase in drug release kinetics than those with lower molecular weight polymers (LA/GA 75/25, 70 or 57 kDa) (Figure 3). The former had a higher porosity (73%) than the latter (57%, 66%) for unknown reasons, helping to wet the microparticles and access the encapsulated drug. However, the porous particles showed a slower release rate in the secondary release phase, undergoing slower polymer degradation than the less porous particles, likely due to the facile release of acidic degradation products (i.e., reduced acidification), thereby the reduction of acid-catalyzed degradation [67]. This study demonstrates the significance of porosity in controlling the acidification of polymeric drug delivery systems. The following section introduces additives employed to control the porosity of PLA or PLGA matrices.

Polyethylene glycol

Polyethylene glycol (PEG) is an uncharged, linear and hydrophilic polymer that can be blended in polymer matrices due to the solubility in organic solvents. PEG was co-encapsulated in PLA microparticles to enhance BSA release from the particles [65]. The rationale of this approach was that the hydrophilic PEG in the polymer blend would swell and dissolve in the release medium to form aqueous pores, increase fluid ingress, facilitate the efflux of polymer degradation products, thereby reducing the acid-induced protein degradation, and increase the diffusion of the encapsulated protein. PEG was effective as a porogen at a weight content of >20%, releasing 75% BSA in 29 days (Figure 4A & B) and acidic degradation products (Figure 4C) in PBST at 37°C with no sign of aggregation or

(A)

Polymer	Manufacturer	Mw (kDa)	Polydispersity	IV (dL/g)	Tg (°C)
E1	Evonik	70.12 ± 0.26	1.42 ± 0.00	0.64 ± 0.12	49.40 ± 1.01
E2	Evonik	56.55 ± 0.20	1.42 ± 0.02	0.49 ± 0.06	48.82 ± 0.25
P	Polyscitech	86.11 ± 1.14	1.71 ± 0.02	0.78 ± 0.08	45.96 ± 2.84
L	Lactel	103.71 ± 10.29	1.50 ± 0.14	0.91 ± 0.03	45.31 ± 0.88

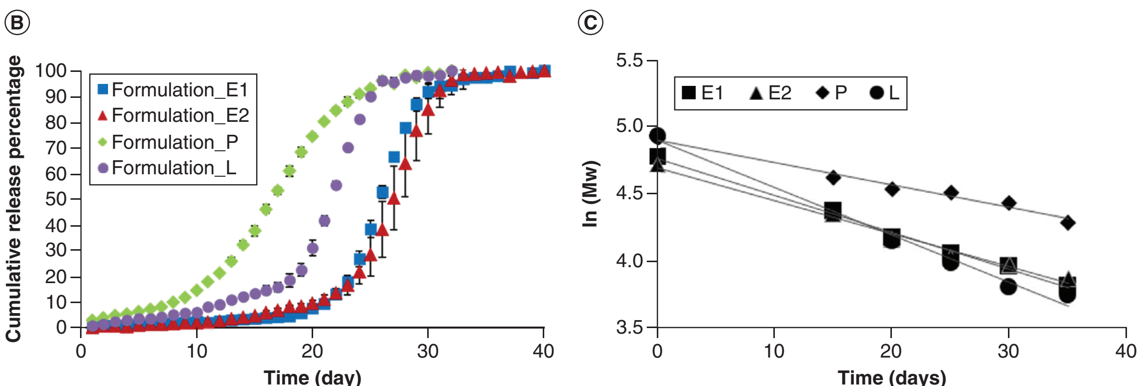

Figure 3. Effect of particle porosity on risperidone release from PLGA microparticles. (A) Physicochemical properties of polymers (mean ± SD., n = 3); **(B)** *In vitro* release profiles of risperidone microsphere formulations obtained using the flow-through cell method and **(C)** changes in molecular weight of PLGA in the prepared risperidone microspheres in 10 mM HEPES (pH 7.4 with sodium chloride, Tween 20 and sodium azide) at 37°C: (■) Formulation_E1, (▲) Formulation_E2, (♦) Formulation_P and (•) Formulation_L. Reprinted with permission from [67] © Elsevier (2020).

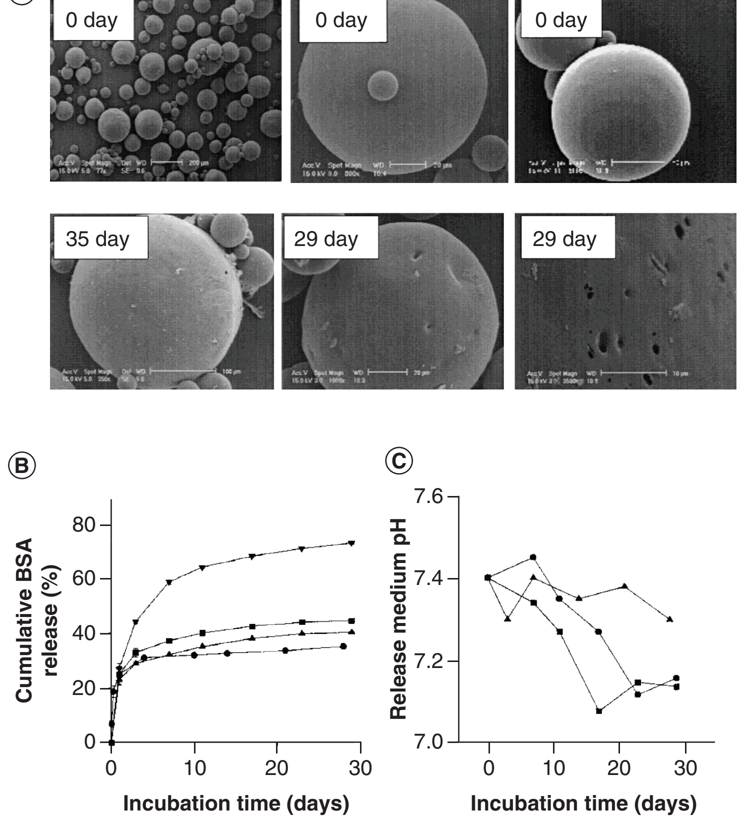

Figure 4. Control of BSA release and microclimate pH of PLA microparticles by PEG. (A) SEM images of poly(lactic acid) (PLA) (i.v. 4 1.07 dl/g) microspheres, PLA/polyethylene glycol (PEG) 35,000 (90/10) microspheres and PLA/PEG 35,000 (80/20) microspheres before and after incubation in the release medium. **(B)** The effect of PEG content in the PLA/PEG blend on the release kinetics of bovine serum albumin. PEG 10,000 content was 0% (circle), 5% (square), 10% (triangle, facing up) and 20% (triangle, facing down). **(C)** pH change of release medium in PLA (triangle), PLA/PEG 35,000 (80/20) (square) and PLA/PEG 10,000 (80/20) (circle) microspheres containing 5% bovine serum albumin when incubated at pH 7.4 PBS containing Tween 80 and 37°C. Reprinted with permission from [65] © Springer Nature (2001).

hydrolysis of the protein [65].

PEG was also found useful in maintaining stability and facilitating the release of insulin encapsulated in PLGA or PLA microparticles [68]. The pore size increased during the incubation of particles in PBS at 37°C due to the degradation of the polymer and the removal of PEG and insulin. In addition to pore formation, PEG may have helped dissolve insulin in the particle core and generated a high concentration gradient for diffusion, further enhancing insulin release [68]. Since PEG is water-soluble and can be partially removed during the microparticle formation, PEG can be used to form microparticles with pre-formed pores. With PEG at >20 wt% of PLGA (LA/GA 50/50, 48 kDa), microparticles in varying degrees of porosity were produced to control the release of encapsulated protein [69]. A model protein lysozyme was loaded *post hoc* in the porous PLGA microparticles, where the pores were closed by incubation with water-miscible solvents [69]. The pore closed particles reduced the burst release while allowing for sustained protein release in vitro over a month.

Poloxamer (Pluronic) polymers

Poloxamers (also known as Pluronics) are nonionic triblock copolymers composed of one hydrophobic polypropyleneoxide (PPO) block flanked by two hydrophilic polyethyleneoxide (PEO) chains [70]. As amphiphilic polymers, Poloxamers can be incorporated in PLGA matrices to modify the activity and release profile of the encapsulated proteins, playing a similar role as PEG. The bioactivity (stability) and *in vitro* release of a model enzyme urease were increased by 41 and 70%, respectively, by the addition of Poloxamer 407 (15 wt%) to PLGA microparticles. Poloxamer is thought to have protected the protein by entrapping it in the gel structure and preventing its direct exposure to PLGA matrix and enhanced the protein release by facilitating the polymer wetting and the release of acidic degradants [71]. Poloxamers were used in the delivery of tetanus toxoid (TT) via PLGA (LA/GA 50/50, 98 kDa) microparticles [72], BSA, immunoglobulin G [73], fibroblast growth factor-2, platelet-derived growth factor [74] or plasmid DNA encoding a green fluorescent protein from PLGA (LA/GA 50/50, 35 kDa) nanoparticles [75] and lysozyme from PLGA microparticles [76].

Polyvinylpyrrolidone

Polyvinylpyrrolidone (PVP) is a nonionic, water soluble polymer, serving as an emulsifier in preparation of PLGA particles [77]. PVP has been used as a porogen to generate large porous PLGA microparticles for pulmonary drug delivery [78]. The primary purpose of the porogen in this study was to achieve favorable aerodynamic properties for inhalation, but the porosity also helped to control the drug release kinetics. PLGA (LA/GA 50/50, i.v. 0.32–0.44 dl/g) particles carrying cinaciguat, a model hydrophobic drug, were prepared by the single emulsion method with varying contents of PVP. The *in vitro* release of cinaciguat from the particles in 7 days increased from 20 to 60%, 90 and 95% with 10%, 20% and 40 wt% PVP, respectively [78]. The increasing drug release was explained by the increasing contact area with release medium, thereby enhanced PLGA degradation [78]. However, given the short period of time (7 days) relative to the time frame of polymer degradation (<3 months) [21], it is possible that the release kinetics control was a function of particle wetting and the surface area for drug diffusion.

Salts, sugars & their derivatives

Hydrophilic additives such as sucrose, trehalose, cyclodextrins, dextran and salts have been used as porogens to increase water movement and control polymer degradation [79]. Sucrose acetated isobutyrate (SAIB) is a sucrose derivative, soluble in organic solvents and forming a viscous hydrogel in water. Therefore, similar to PEG, SAIB can be blended in PLGA microparticles via common solvents to change the microenvironment and improve the stability of the encapsulated lysozyme [80]. The inclusion of SAIB in PLGA microparticles reduced the burst release (5 vs 35% of no SAIB-PLGA particles) followed by continuous release up to 2 months and improved the lysozyme stability, with delayed PLGA degradation. The authors attributed the slow polymer degradation to the high viscosity of SAIB without specifying the mechanism [80]. Given the prevailing discussions of other porogen-containing PLGA matrices, the polymer degradation may have been slowed down due to the removal of acidic degradants and the reduced acid-catalyzed degradation.

Decomposing agents

Highly porous microparticles can be prepared by porogens that decompose into gas bubbles (e.g., ammonium bicarbonate into CO_2 and NH_3) or water-soluble compounds during particle preparation [81,82]. Sponge-like, porous PLGA (LA/GA 75/25, 0.28 dl/g) microparticles encapsulating progesterone were prepared by a process

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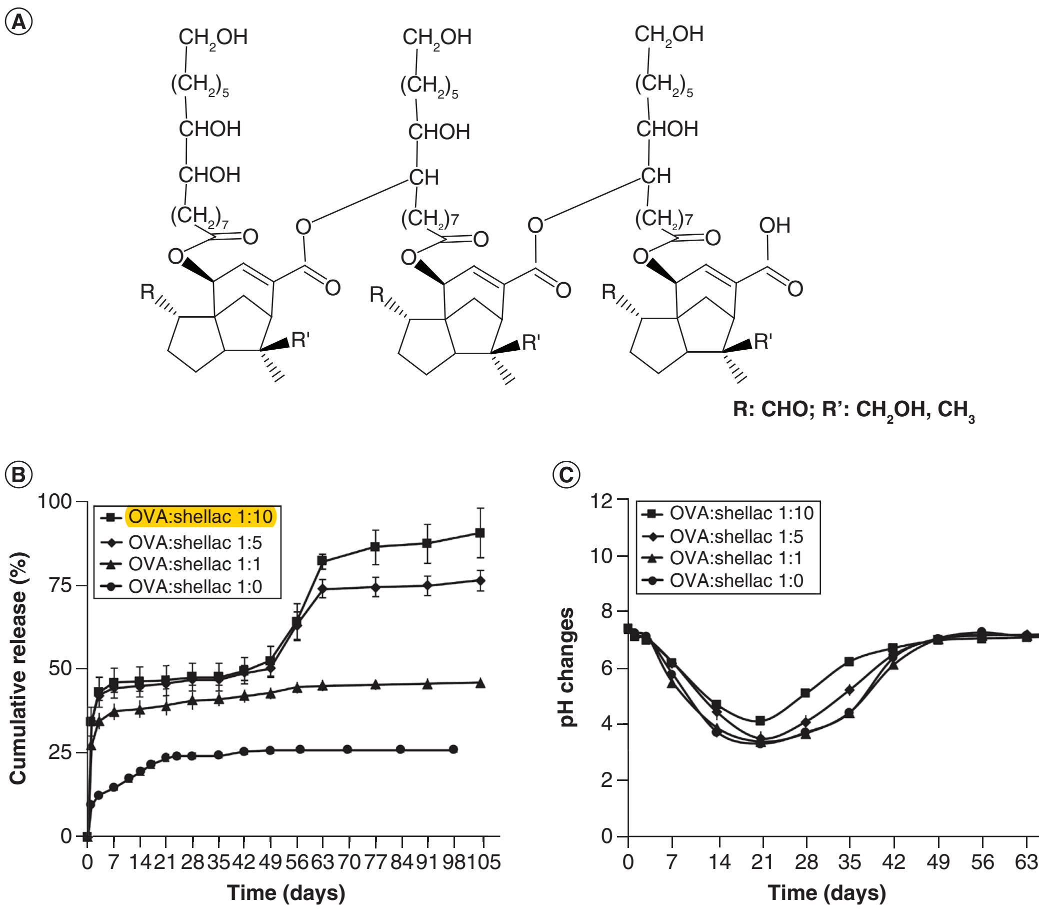

Figure 5. Control of OVA release from PLGA implants by shellac. (A) Chemical structure of Shellac; **(B)** release profile of OVA-loaded poly(lactic-co-glycolic acid-based implants using shellac at different ratios. **(C)** pH changes during OVA release from poly(lactic-co-glycolic acid-based implants using shellac at different ratios. **(A)** Reprinted with permission from [86] © Elsevier (2007); **(B & C)** Reprinted with permission from [48] © Elsevier (2018).

called ammonolysis [83]. PLGA particles were prepared by the single emulsion method with isopropyl formate as an organic solvent. Pores were formed as ammonia added to the continuous phase diffused into the polymer droplets and decomposed (ammonolyzed) the solvent into water-soluble isopropanol and formamide, which helped encapsulate a drug serving as antisolvents and formed porous structure as leaching out of the matrix. The porous particles showed a sustained release of progesterone (∼100% in 16 days) without a lag phase, as opposed to nonporous particles (plateaued at ∼75% release), reflecting a large surface area and a short diffusion path for the drug release of the former [83]. This study illustrates how porous structure can help facilitate the transport of encapsulated drugs. However, excessive porosity can result in a high burst release. For example, highly porous PLGA (LA/GA 50/50, 38–54 kDa) microparticles, produced by ammonium bicarbonate as a porogen, released most of the encapsulated donepezil hydrochloride in 8 h [84]. The pores had to be closed with calcium alginate to control the burst release [84].

pH-responsive polymers

pH-responsive polymers that are water-soluble in alkaline to neutral pH but insoluble in acidic pH have been incorporated in PLGA matrices to protect the encapsulated drug. The rationale of this approach is that the pH-responsive polymers remain soluble in the beginning, when the pH is still neutral, allowing the drug to release from the matrix by diffusion. As μpH drops due to PLGA degradation, the polymers become insoluble, preventing direct exposure of drug molecules in the vicinity to the acidic microenvironment. Drug release resumes when the acidic byproducts diffuse out to restore the neutral μpH. Shellac, a natural polyester consisting of inter- and intraesters of polyhydroxy carboxylic acids (Figure 5A) [85–87], is an acid-insoluble polymer used for enteric coating and has

been used to protect acid-labile ovalbumin from acidic μpH and increase drug release from a PLGA implant [48]. While ovalbumin release from a plain PLGA implant was incomplete (25% after 84 days), the addition of shellac increased the ovalbumin release up to 85% in three months, showing a triphasic release (initial burst due to partial dissolution of shellac, delayed diffusion by insoluble shellac in acidic μpH, and accelerated release following the resumed dissolution of shellac at neutralized μpH) (Figure 5B) with a quicker restoration of release medium pH (Figure 5C) [48].

Alternatively, the harsh effects of acidic byproducts on drug molecules can be mitigated by modifying the microenvironmental viscosity in the degrading PLGA matrix. Glycol chitosan (GC) is an ethylene glycol derivative of chitosan, more hydrophilic than chitosan, and forms a viscous solution [88]. GC was incorporated in PLGA (LA/GA 50/50, 8 kDa) microparticles by the double emulsion method to stabilize a model protein lysozyme. Without GC, the particles showed a high burst release (52%) followed by a plateau at 58% release in PBST for 22 days. When GC was included, the initial burst release was reduced to ∼12%, the total release increased up to 78% in 22 days with a triphasic release curve, and the stability of lysozyme was maintained at >95% for 28 days. The higher stability and improved release are explained by the formation of the viscous GC phase in the particles, which sequestered PLGA degradants by forming ionic complexes while slowing down the lysozyme diffusion. The protective effect was unique to lysozyme (not as effective to BSA) because GC fragments produced by lysozyme were more effective in interacting with acidic byproducts [89].

Alternative biodegradable polyester systems

To avoid issues related to acidification of PLA and PLGA systems, one may consider alternative aliphatic biodegradable polyesters, such as polycaprolactones (PCL), polydioxanones (PDO), polybutyrolactones and polyvalerolactones. In particular, PCL is a slow-degrading polymer (>1 year); thus, the acidity of degrading PCL microenvironment is negligible, unlike PLGA [90]. PCL has been employed in microspheres [91,92], nanoparticles [93–95], implants [96,97] and devices [98]. Since very slow bioresorption is not always desirable, a porous PCL implant was developed to accelerate the polymer degradation and drug release for intraocular dexamethasone delivery [99]. Another study describes a bi-layered composite of PCL and PDO nanofibers as a carrier of two antibacterial agents, titanium dioxide and tetracycline [100]. An inner layer of PDO nanofibers contained the two active ingredients, on which a more hydrophobic layer of PCL nanofibers was overlaid to delay hydration and maintain mechanical stability. *In vitro*, the nanofiber composite released tetracycline in less than 4 days by diffusion, showing a greater release with a higher titanium dioxide content, which helped to solvate the PDO fibers [100].

Conclusion & future perspective

PLA and PLGA-based LAR products develop acidifying microenvironments to varying degrees according to the polymer properties (molecular weight, LA/GA ratio, end group chemistry and stereochemistry) and manufacturing variabilities (monomer sequence, type, residual solvents, monomers and oligomers). The acidic μpH can damage acid-labile drugs or alter drug solubility, resulting in slow and incomplete drug release. Alternatively, the acidic environment can catalyze polymer degradation and accelerate drug release. Therefore, the nature of encapsulated drugs and their interaction with degrading polymers must be considered in choosing polymers and designing delivery systems. Acidification of the polymer matrix may be controlled by various additives, such as inorganic bases, salts, hydrophilic polymers or small molecules. When carefully selected according to their unique mechanisms, these release modifiers can help control drug stability and release kinetics without making drastic changes in the encapsulated drugs or polymeric matrices. The release modifiers also allow for the use of slow-degrading polyesters such as PCL, traditionally considered for long-term (months to years) drug delivery, for sustaining drug release over days to weeks with little concern of matrix acidification. These efforts are leveraged by recent advances in 3D imaging techniques such as 3D X-ray microscopy or micro-computed tomography, which help probe real-time drug distribution and release from degrading polymer matrices.

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Executive summary

Significance of microclimate pH in drug release control by poly(lactic acid) & poly(lactic-co-glycolic acid) systems

- Poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) have been used for sustained delivery of vaccines, peptides, proteins or nucleic acids, as microparticles, implants or gels.
- PLA or PLGA-based systems generate acidic products in degrading matrices, reducing the pH inside of the polymeric structure.
- The acidifying PLA or PLGA matrices can cause significant issues in release control, stability and activity of the loaded drugs.

Methods to monitor microenvironment pH of PLA or PLGA systems

- Internal microenvironment pH of degrading PLA or PLGA systems may be indirectly estimated by measuring the release medium or directly by potentiometry, nuclear magnetic resonance, electron paramagnetic resonance spectroscopy or confocal microscopy.

Formulation approaches to counteract damaging effects of acidifying microenvironment in PLA or PLGA systems

- Inorganic bases, salts, sugars or hydrophilic polymers are blended in the systems to neutralize the acidic pH and/or delay the acidification by facilitating the diffusion of acidic products.
- These release modifiers can help control drug stability and release kinetics without making drastic changes in the encapsulated drugs or polymeric matrices.

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