A Novel Thermoresponsive Gel as a Potential Delivery System for Lipoxin

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Abstract

The objective of this study was to evaluate a novel thermoresponsive polyisocyanopeptide (PIC)–based hydrogel as an injectable carrier for local drug delivery for periodontal applications. Three formulations of PIC gels, 0.2%, 0.5%, and 1% w/w, were prepared. As controls, commercially available poloxamer 407 (P407) gels of 20% and 26% w/w were used. Lipoxin A_4 (LXA_4), a proresolving drug, was suspended into the gel solutions. The systems were evaluated regarding dynamic mechanical properties, injectability and stability, release and bioactivity of LXA_4, and cytocompatibility. Results showed that the gelation temperatures of PIC and P407 gels were around 13°C to 23°C. PIC gels were less viscous and mechanically weaker than P407 gels due to the low polymer concentrations. However, PIC gels kept gel integrity for at least 2 wk when incubated with phosphate-buffered saline, whereas P407 gels were disintegrated totally within 1 wk. LXA_4 was chemically stable in both neutral and alkaline medium for over 1 mo. The release of LXA_4 from either 1% PIC or 26% P407 gels depicted an initial burst release followed by a sustained release for around 4 d. The extent of burst release was negatively correlated to the polymer concentration. LXA_4 remained bioactive after release from PIC gels. No cytotoxicity was observed for 1% PIC gel. However, 26% P407 inhibited periodontal ligament cell and gingival epithelial cell growth. In conclusion, the thermoresponsive PIC gel is a potential candidate for periodontal drug delivery.

Keywords: periodontitis, hydrogel, poloxamer, Anti-inflammatory agents, control release, phagocytosis

Introduction

Periodontitis is initiated by microbial biofilms, which in turn elicit a host response and result in the destruction of the periodontal tissue (Van Dyke and Serhan 2003). Uncontrolled inflammation and failed resolution of the inflammation are considered the underlying mechanism of periodontitis (Van Dyke and Serhan 2003; Serhan et al. 2007; Medzhitov 2010; Serhan 2010). In literature, robust evidence has shown that an endogenous anti-inflammatory and proresolving process can depress chronic inflammation and activate wound healing with tissue regeneration (Serhan et al. 2007; Serhan et al. 2008; Ortega-Gómez et al. 2013). This active process is orchestrated, in part, by specialized endogenous proresolving lipid mediators (SPMs), including lipoxins, resolvins, protectins, and maresins (Levy et al. 2012; Kamaly et al. 2013; Ortega-Gómez et al. 2013). SPMs can actively reduce neutrophil infiltration into inflamed tissues, enhance efferocytosis and bacterial phagocytosis by monocytes and macrophages, and simultaneously inhibit inflammatory cytokine production (Lee et al. 2016).

Topical usage of resolvin E1 and lipoxin A_4 (LXA_4) has already been proven to prevent periodontal bone loss and activate the periodontal regeneration in rabbits and swine, without the multiple side effects ascribed to traditional anti-inflammatory antagonist-based treatments (Hasturk et al. 2006; Hasturk et al. 2007; Van Dyke et al. 2015; Lee et al. 2016). We hypothesize that topical application of SPMs without an appropriate carrier may not be able to achieve an effective retention in the periodontal pocket and a drug carrier system would be advantageous. For instance, Van Dyke et al. (2015) delivered membrane-shed vesicles incorporating LXA_4 in swine, resulting in significant regeneration of bone and connective tissues and reestablishment of the periodontal organ. The commonly used carriers for sustained drug release include microspheres, polymer chips, fibers, and hydrogels (Joshi et al. 2016). Among all the carriers, thermoresponsive hydrogels are particularly attractive, because they are liquid at low temperature and thus can be easily injected into deep pockets, and then, they can form gels under body temperature to facilitate sustained drug release (Vyas et al. 2000; Maheshwari et al. 2006; Ji et al. 2010; Nasra et al. 2017). The commercially available poloxamer 407 (P407) has been studied for this purpose. P407 exhibits a thermoresponsive manner at a concentration above 20% w/w, but it is easy to be eroded after gelation under the influence of surrounding liquid (Zhang et al. 2002). Recently, another thermoresponsive hydrogel based on polyisocyanopeptide (PIC) has been developed, which can form a gel at

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extremely low polymer concentrations (~0.5%) and displays strain-stiffening properties comparable to the natural extracellular matrix (Kouwer et al. 2013; Das et al. 2016). This gel system may have the advantages of 1) low viscosity, allowing the drug solution to perfuse periodontal pockets fully, and 2) low toxicity induced by the polymers.

This study aimed at evaluating the thermoresponsive PIC hydrogel loaded with LXA$_4$ as a representative SPM for the periodontal application. To this end, 0.2% to 1% w/w PICs were prepared. Then, the physical stability and dynamic mechanical properties of the gels were tested, in comparison with 20% and 26% w/w P407 controls. LXA$_4$ release profiles from PICs or P407s were further investigated. The efficacy of the released LXA$_4$ was tested by a phagocytic test. Finally, the cytocompatibility of the gel systems was tested using periodontal ligament cells (PDLCs) and gingival epithelial cells (GECs).

Materials and Methods

Preparation of Thermoresponsive Gels

PIC polymer (molecular weight ~ 635 kDa) was provided by Dr. Paul H. J. Kouwer (Institute for Molecules and Materials, Radboud University, the Netherlands). PIC or P407 (Sigma-Aldrich) polymer was dissolved in cold phosphate-buffered saline (PBS). Three PIC solutions at 0.2, 0.5, and 1% w/w and 2 P407 solutions at 20% and 26% w/w were prepared. The pH of the prepared solutions was determined at 4°C.

Physical Characterization of the Gels

Dynamic mechanical properties of gels were tested by a rheometer with a temperature sweep program (see Appendix for method). The sol-gel transition temperature (T$_{sol-gel}$) was determined as the crossing point of storage (G′) and loss (G″) moduli during temperature sweep measurements. The values of G′ and G″ of each gel at 37°C were recorded after a 2-min equilibration. Injectability of gels was tested with Milli-Q water as a control (see Appendix for method).

Physical stability of hydrogels, in an aqueous solution, was tested by a gravimetric method (Zhang et al. 2002). Briefly, 0.5 mg gel solution was injected into the bottom of an Eppendorf tube with a known weight (W$_0$/mg) and gelled at 37°C. Three aqueous buffers at different pH were prepared: 1) PBS (pH 7.4), 2) 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; pH 8.5), and 3) citrate acid (pH 5). All buffers had an ionic strength of 154 mM. Afterward, 1 mL buffer was carefully laid over the surface of the gel. All samples were incubated at 37°C with gentle agitation at 120 rpm/min. At each predetermined time point, all the supernatant was carefully removed, and the Eppendorf tubes were weighted again (W$_t$/mg). Then, 1 mL fresh buffer was refilled, and samples were incubated until the next time point. The erosion profiles were obtained by plotting the percentage remaining for each gel formulation ([W$_t$ − W$_0$]/0.5 × 100%) against the incubation time (n = 3).

In Vitro Release of LXA$_4$

To detect the stability of LXA$_4$ in aqueous solutions, 100 μg/mL 15(R)-LXA$_4$ in ethanol (Cayman Chemical) was diluted to 1 μg/mL using the 3 above aqueous buffers at different pH. After dilution, the samples (n = 3) were incubated at 37°C and the amount of LXA$_4$ in the buffers at predetermined time points was detected by reverse-phase high-performance liquid chromatography (RP-HPLC; Hitachi) (for details, see Appendix).

Release studies were performed at 37°C by a membraneless dissolution method (Zhang et al. 2002). Briefly, 200 μL of a PIC or P407 solution was mixed with 1 μg LXA$_4$ on ice and then gelled in an Eppendorf at 37°C (n = 4). Subsequently, 1 mL PBS prewarmed at 37°C was laid over the gels. Samples were then incubated at 37°C with agitation at 200 rpm/min. At each predetermined time point, 900 μL supernatant was withdrawn carefully and 900 μL fresh PBS was refilled. The amount of released LXA$_4$ in the supernatant was detected by RP-HPLC.

To further investigate the interactions between LXA$_4$ and hydrogel carriers and influential factors of LXA$_4$ release, the release study was also performed in media with detergent of 0.1% v/v Tween 20 in PBS (n = 4) and in buffers with different pH (n = 4): 1) PBS (pH 7.4), 2) HEPES (pH 8.5), and 3) citrate acid (pH 5) (Song et al. 2015).

In Vitro Efficacy Test

The efficacy of the released LXA$_4$ was assessed by the ability to stimulate macrophage phagocytosis. The phagocytic activity of RAW264.7 macrophages was examined using a fluorescent bead internalization assay (Prescott and McKay 2011). Cells were seeded in 96-well plates at a density of 2×10^4 cells/cm^2 in α–minimum essential medium eagle containing 1% penicillin-streptomycin (P/S) and 10% fetal bovine serum (FBS) and left for 24 h for attachment prior to experimentation. Thereafter, each macrophage was exposed to 100 latex beads labeled with Nile red fluorescence (D = 2 μm; Invitrogen). Then, 50 μL LXA$_4$ releasate from gels (concentration calculated by HPLC results) or freshly prepared LXA$_4$ (positive control) was added into the culture to reach a final LXA$_4$ concentration of 100 nM. The same volume of ethanol, the drug vehicle, was used as a negative control. After incubation for 4 h, the fluorescence of unphagocytic beads was quenched with 0.4% trypan blue. The fluorescence intensity of each well was read in a Synergy HTX multimode reader (BioTek Instruments), with the excitation/emission wavelength at 535/575 nm. The wells without beads were assessed to determine background fluorescence. Data were expressed as a percent change in fluorescence in treated versus control wells. Phagocytosis results were also visualized by fluorescent microscopy (see Appendix for method).

In Vitro Cytocompatibility Test

Cytocompatibility of LXA$_4$/PIC and LXA$_4$/P407 was tested using human PDLCs and GECs. Cells were harvested from extracted third molars with the informed consent of the patients

Table. In Vitro Characterization of PIC or P407 Gels with Different Polymer Concentrations ($n = 3$): pH, $T_{\text{sol-gel}}$ Sol-Gel Transition Temperature, and Storage (G′) and Loss (G″) Moduli at 37°C.

Gel	Concentration (w/w)	pH	$T_{\text{sol-gel}}$ (°C), Mean ± SD	G′ (Pa), Mean ± SD	G″ (Pa), Mean ± SD
PIC	0.2%	7.4	16.5 ± 0.8[a]	334 ± 62[a]	33 ± 3[a]
	0.5%	7.4	16.2 ± 0.4[a]	1,050 ± 77[a]	45 ± 11[a]
	1%	7.4	12.9 ± 1.0[b]	4,964 ± 353[b]	195 ± 42[a]
P407	20%	7.4	22.9 ± 0.4[c]	16,101 ± 289[c]	787 ± 121[b]
	26%	7.4	18.2 ± 0.4[a]	28,422 ± 1,585[d]	859 ± 121[b]

Different superscript letters in each column indicate significant difference between different groups.

(all ~20 y old). Cell culture methods are shown in the Appendix. PDLCs or GECs were seeded on 24-well plates or Thermanox coverslips at a density of 1×10^4 cells/cm^2 for 24 h before the assays.

Then, 200 μL cold gel solution with or without LXA$_4$ was injected in Corning Transwell cell culture inserts (pore size = 8 μm) and gelled at 37°C. Next, the transwells were inserted separately into the wells with PDLCs or GECs. The final concentration of LXA$_4$ for the LXA$_4$-loaded groups was 100 nM calculated according to the whole liquid volume in each well. The cytocompatibility of gels was tested by alamarBlue, DNA assay, and live/dead staining at days 2 and 4 (see Appendix for methods).

Statistical Analysis

All data were reported as a mean ± standard deviation and analyzed using 1-way analysis of variance by GraphPad Prism 7.0 (GraphPad Software). Differences were considered statistically significant at $P < 0.05$.

Results

Physical Characterization of the Gels

As shown in the Table, the pH of all gel groups was 7.4 and same with PBS. $T_{\text{sol-gel}}$ of all formulations was between 13°C and 23°C. With the increase of PIC or P407 concentration, $T_{\text{sol-gel}}$ slightly dropped, whereas, G′ and G″ increased. Both G′ and G″ of PICs were lower than those of P407s. Overall, PIC solutions were less viscous than P407 solutions. All the ice-chilled solutions were easily pushed through a 22-gauge needle comparable to water (Appendix Fig. 1).

All PIC and P407 gels stayed at the bottom of the Eppendorf without detachment. Regardless of buffers, P407, 20% and 26% w/w, disintegrated completely within 5 and 7 d, respectively (Fig. 1). In contrast, even the weakest 0.2% PIC remained ~70% after 15 d. As expected, the rate of gel disintegration decreased as the concentration of gel increased. There was almost no weight loss for 1% PIC gel up to 15 d. pH did not show any significant influence on gel integrity for both PIC and P407.

In Vitro Release of LXA$_4$

Chemical stability of LXA$_4$ results (Fig. 2A) revealed that over 80% of LXA$_4$ remained intact in 1 wk under various pH
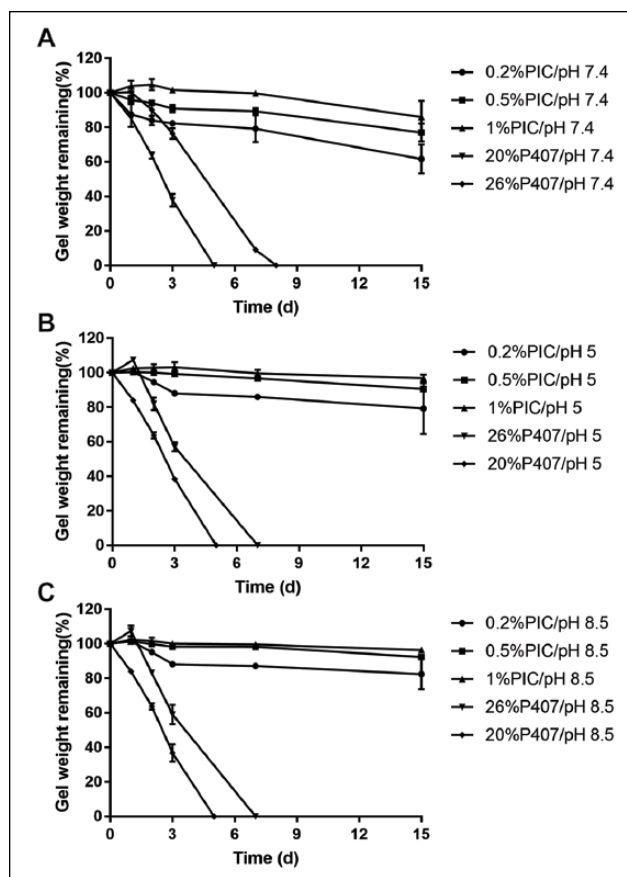

Figure 1. The erosion profile of polyisocyanopeptide (PIC) or poloxamer 407 (P407) gels with different polymer concentrations in phosphate-buffered saline (**A**), citrate buffer (**B**), and HEPES (**C**) at 37°C versus time. Results are shown as the percentage of gel-remaining weight. Data are expressed as mean ± standard deviation ($n = 3$). The sign of standard deviation may not visible when it is smaller than the data symbol.

conditions. After 1 mo, LXA$_4$ was relatively stable in neutral and alkaline solution but fully degraded in acidic buffer.

The release profile displayed a burst release of 40% to 60% LXA$_4$ in the first 8 h (Fig. 2E–G) and a sustained release thereafter for around 4 d for all groups (Fig. 2B–G). Polymer concentration had a minor effect on the duration of LXA$_4$ release but considerably decreased the initial burst release. The 26% P407 formulation possessed a similar release pattern to 1%
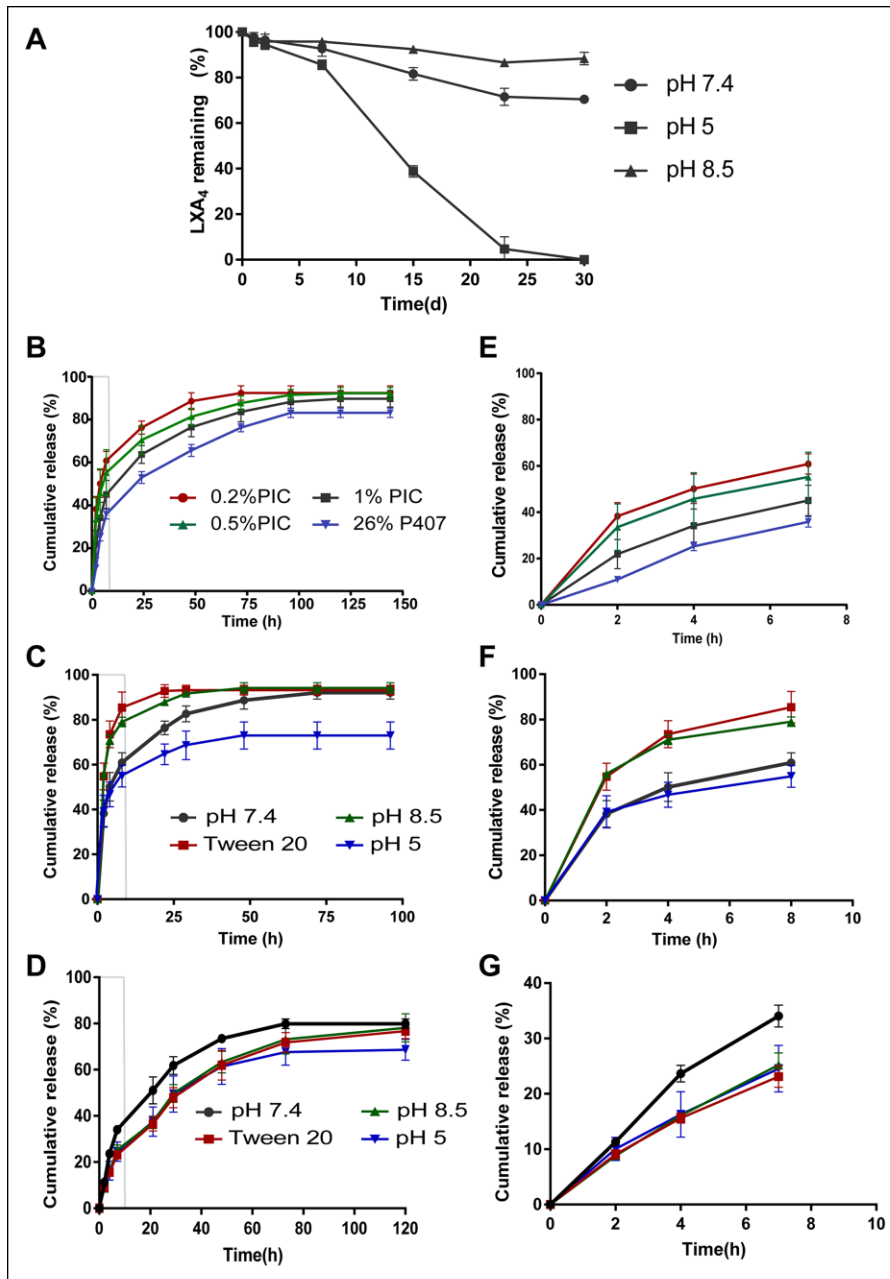
Figure 2. Lipoxin A_4 (LXA$_4$) stability and release curves. (**A**) LXA$_4$ chemical stability in media with different pH and (**B–D**) the cumulative release profiles of LXA$_4$ from different gels with different release media. (A) Eighty percent of LXA$_4$ remained intact after 1 wk under various pH conditions. After 1 mo, around 70% of LXA$_4$ was left in the neutral solution and more than 90% in the alkaline buffer. However, no LXA$_4$ could be detected in the acidic buffer. (B) LXA$_4$ release from 0.2%, 0.5%, 1% w/w polyisocyanopeptide (PIC) and 26% w/w poloxamer 407 (P407) gels in phosphate-buffered saline release medium at 37°C. (C) Detergent and pH influence of LXA$_4$ release from 0.2% PIC gel. (D) Detergent and pH influence of LXA$_4$ release from 26% P407 gel. The partial enlargement of release pattern of first 8 h in B, C, and D are shown in E, F, and G, respectively. The sign of standard deviation may not visible when it is smaller than the data symbol.

The addition of Tween 20 significantly increased the release rate of LXA$_4$ with 90% of cumulative release within 1 d in PIC groups (Fig. 2C). Especially, in the first 8 h, around 20% more LXA$_4$ was released compared to that in the PBS. The LXA$_4$ release in alkaline medium showed the same pattern as that in Tween 20. In acidic medium, the release rate was low, but in total, only around 70% of LXA$_4$ was released. For P407 groups, the LXA$_4$ release was not greatly affected by detergent and alkaline medium in velocity, duration, and cumulative amount (Fig. 2D). Similarly, less LXA$_4$ amount was finally released from the P407 in the acidic buffer.

In Vitro Efficacy and Cytocompatibility Test

Phagocytotic activity of RAW264.7 is shown in Figure 3. Both LXA$_4$ released from PIC and fresh LXA$_4$ enhanced the phagocytosis activity of RAW264.7. However, no phagocytic enhancement was detected for the LXA$_4$ released from P407 or in the groups without LXA$_4$ (Fig. 3C). Phagocytic images illustrated that more fluorescent particles were internalized in PIC/LXA$_4$ groups compared to PIC without LXA$_4$ groups (Fig. 3A and B).

Results of cytocompatibility test are shown in Figure 4. The response of PDLCs and GECs to different gel systems was similar. One percent PIC and PIC/LXA$_4$ showed good cell viability, while 26% P407 and P407/LXA$_4$ hindered cell growth, similar to 5% dimethyl sulfoxide (DMSO) (Fig. 4A and C). The DNA assay results corroborated that cells cultured with PICs had significantly higher amounts of DNA than those with P407s (Fig. 4B and D). The live/dead staining (Fig. 4E and F) displayed that most cells were viable in PIC groups, and dead cells
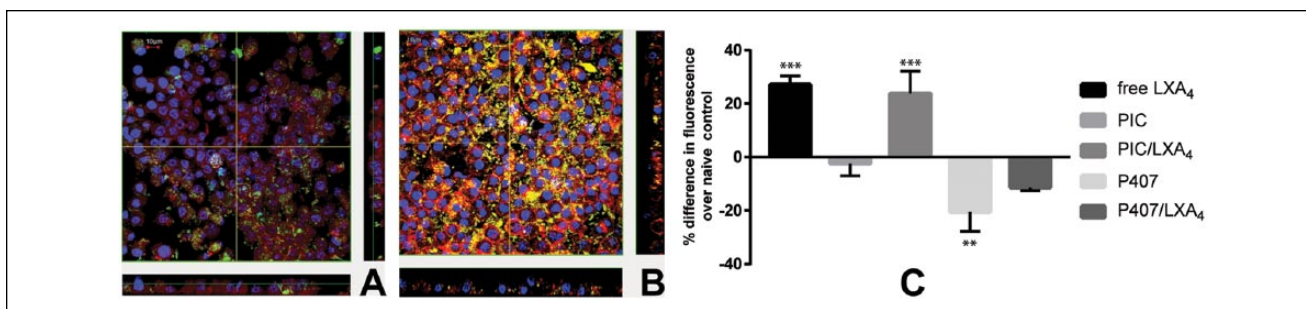
PIC, but the gel dissolved fast during the release test. Finally, the cumulative release amounts reached more than 90% for PICs, in contrast to around 80% for the P407s.

were seldom found. However, a considerable number of dead cells and less live cells could be seen in the P407 groups.

Figure 3. Lipoxin A$_4$ (LXA$_4$) released from polyisocyanopeptide (PIC)/LXA$_4$ upregulated phagocytosis by RAW264.7 macrophages, as visualized by fluorescence microscopy and quantified. (**A**) RAW264.7 cells incubated with released medium from PIC gel without LXA$_4$; (**B**) RAW264.7 cells incubated with 100 nM LXA$_4$ released from PIC/LXA$_4$ (nuclei were stained by 4',6-diamidino-2-phenylindole (DAPI) to blue; actin was stained by phalloidin 568 to red; particles were yellow-green). (**C**) Differences of fluorescence in groups over naive control ($n = 4$, results are expressed as mean $\pm$ SD, **$P < 0.01$, ***$P < 0.001$ compared with naive controls). In positive control (free LXA$_4$), a 27.18% $\pm$ 3.2% increase in RAW264.7 phagocytosis; LXA$_4$ released from PIC gel enhanced the phagocytosis activity by 23.75% $\pm$ 8.4%. No positive effect on phagocytosis could be found for the LXA$_4$ released from poloxamer 407 (P407). As expected, no enhancement of phagocytosis was found for the groups without LXA$_4$ (1% PIC and 26% P407) as well.

Discussion

This study aimed to explore the feasibility of thermoresponsive PIC gel for controlled release of LXA$_4$ and to evaluate their potential for clinical periodontal applications. Three important parameters were considered: 1) the placement and retention of the dosage form at the site of application, 2) the ability to offer controlled drug release, and 3) the cytocompatibility of the gel (Jones et al. 2009). P407 was adopted as the control (Bruschi et al. 2007; Kumari and Pathak 2012). The results revealed that PIC possessed good clinical potential featured by its convenient handling ability and long-term stability, facilitating sustained release of LXA$_4$ and cytocompatibility.

When developing a topically applied drug, the first consideration would be the local physiological conditions. The pH within the periodontal pocket was reported as ranging from 2 to 9, depending on the physiological or pathological conditions (Galgut 2001). The stability results of LXA$_4$ stated its integrity for at least 1 wk regardless of the pH. However, LXA$_4$ quickly degraded or isomerized in 1 mo in the acidic solution, indicating that the drug formulation needs to be carefully designed if a longer treatment period in a low pH environment is desired.

To obtain a sustained release of the intended drug in the periodontal pockets, thermoresponsive gels have unique advantages to easily perfuse the deep or irregular pockets upon injection at low temperature and then form gel immediately to facilitate drug release in the pockets. The average pocket temperature has been reported to be 36.6°C $\pm$ 0.4°C (Haffajee et al. 1992). Both PIC and P407 gels had T$_{sol-gel}$ between 13°C and 23°C; thus, they can be easily injected through thin needles in a second and gel in situ, which contributes to their good handling ability. The process of thermally induced PIC gel formation is attributed to hydrophobic effects caused by the ethylene glycol tails grafted onto its polyisocyanide backbone (Kouwer et al. 2013). For P407 gel, micellar desolvation and swelling give rise to a large cross-linked micelle network when the temperature rises (Veyries et al. 1999). The PIC gels showed a much lower G″ than P407, indicating a superior flowability to fully irrigate deep, irregular pockets. After injection, the long-term retention of the gel in the pockets is the premise of a sustained release. P407 gels disintegrated much faster than PIC gels. This result corroborates the previous report that the orderly packed micelles of P407 dissociated gradually with the influence of surrounding liquid (Dumortier et al. 2006). In contrast, PIC has a stiff helical polyisocyanide backbone stabilized with the hydrogen-bonded dialanyl groups, which enables PIC to form a semiflexible network in water and thus tolerate the microflow. However, this setting of the gel stability test in this study is not comparable to the in vivo complicated condition, for example, a) continuous epithelial turnover at the interface, b) flow of gingival crevicular fluid, c) efflux of immune cells, and d) gravity. Although PIC gel is not biodegradable, it can be removed by cold water irrigation. Several thermosensitive hydrogel products, mostly based on poloxamers, have been developed for periodontal application (Maheshwari et al. 2006; Pandit et al. 2016; Nasra et al. 2017). Poloxamers degrade very slowly (Erlandsson 2002) and preferably should be cleaned by cold rinsing. At the same time, such a procedure would also prevent the (unlikely) possibility that remaining gel would hinder the reattachment of periodontal tissue. To our knowledge, there have been no reports showing that periodontal gels prevented the reattachment; instead, gels incorporated with therapeutic agents displayed a favorable effect on periodontal regeneration (Maheshwari et al. 2006; Nasra et al. 2017). After disintegration, PIC gels may enter the digestive tract before irrigation and may be excreted out of body with feces like PEG gel (Sternebring et al. 2016) or with urine, as shown in the other study of our group (unpublished data). Based on the results of stability and drug release in this study, PIC gel is suggested to be changed in weekly cycles.

Besides handling ability and physical stability, a more important aspect is the release of the targeted drugs. Several aspects may affect the release of LXA$_4$ from gels: the
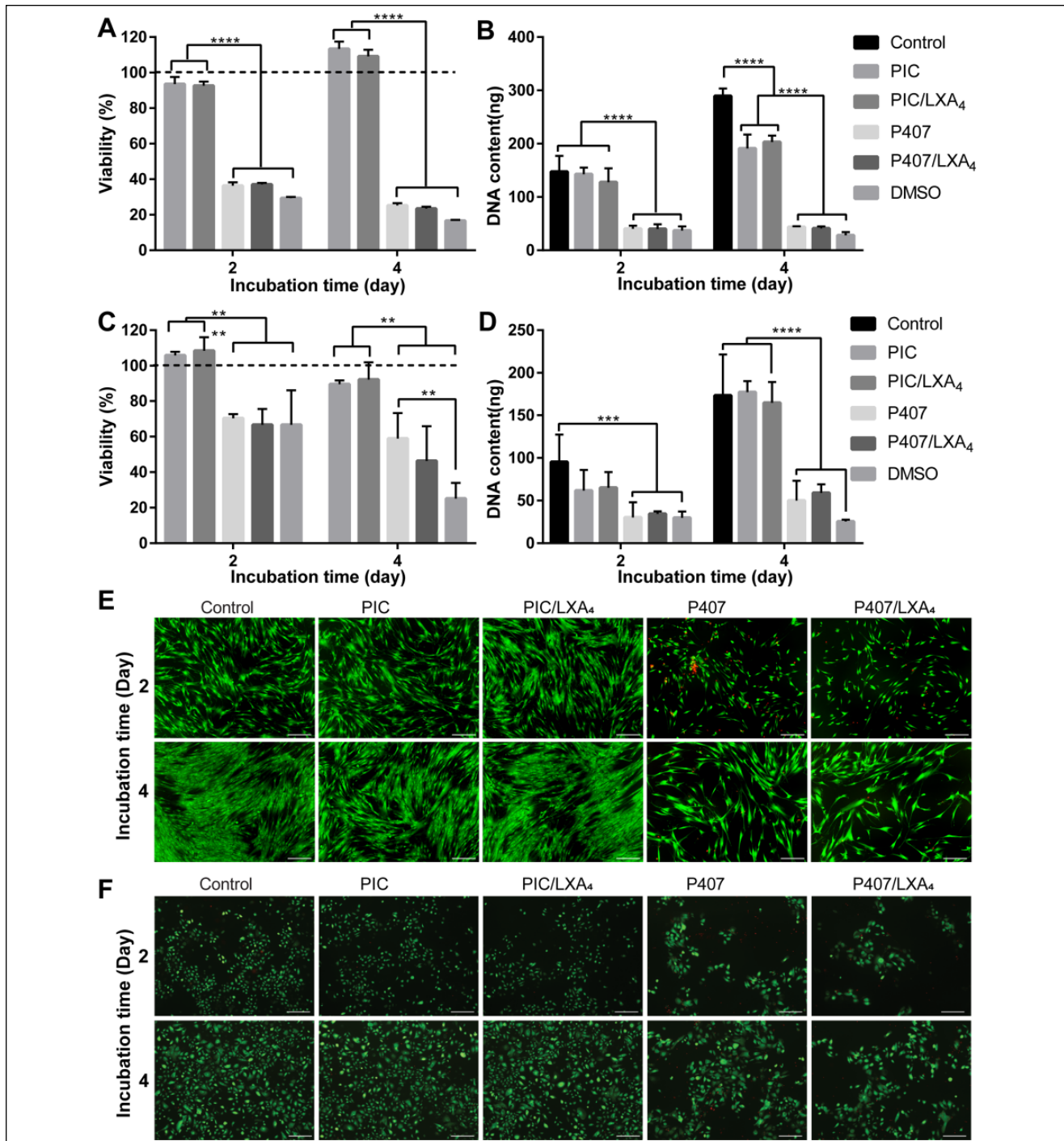
Figure 4. Cytocompatibility of 1% polyisocyanopeptides (PICs) and 26% poloxamer 407 (P407s) with or without lipoxin A$_4$ (LXA$_4$) on periodontal ligament cells (PDLCs) and gingival epithelial cells (GECs). (**A**) PDLC viability test on day 2 and day 4 by alamarBlue. PIC and PIC/LXA$_4$ showed good cell viability comparable to the positive control, while P407 and P407/LXA$_4$ had only around 36% cell viability at day 2 and 25% at day 4, same as 5% dimethyl sulfoxide (DMSO). Cell viability is normalized by control group (cells cultured on cell culture plate without gel); the black dashed line represents the control group with 100% viability. (**B**) Total PDLC DNA content on day 2 and day 4. Cells cultured with PIC gels had significantly higher amounts of DNA than those with P407 gels. ****$P < 0.0001$. (**C**) GEC viability test on day 2 and day 4 by alamarBlue. PIC and PIC/LXA$_4$ showed good viability on GECs comparable to that on PDLCs. P407 and P407/LXA$_4$ were less toxic to GECs than to PDLCs, which is around 70% cell viability at day 2 and 50% at day 4. (**D**) Total GEC DNA content. In the PIC and PIC/LXA$_4$ group, DNA content is comparable to control on days 2 and 4. A significantly lower DNA amount was shown for P407 and P407/LXA$_4$ than that for the control group. Live/dead staining of PDLCs (**E**) and GECs (**F**) culturing with 1% PICs and 26% P407s with or without LXA$_4$ of 2 d and 4 d. A few fibroblasts from the gingival tissues could also be found in GEC culture. Green, live cells; red, dead cells. Scale bar is 200 μm.

solubility and diffusion rate of the drug, as well as the erosion of the gels. LXA_4 theoretically possesses 3 hydroxyl residues, 1 carboxyl residue, and 4 double bonds (Serhan et al. 1984), which facilitate binding to the polyisocyanide backbone and ethylene glycol tails of PIC by hydrogen bonds and hydrophobic interactions. It can be inferred that the higher the polymer concentration, the more hydrogen bonds are formed, resulting in a slower and prolonged release, as shown in this study. The addition of detergent significantly increased the release rate, which confirms the hydrophobic interaction (Song et al. 2015). For practical concerns, if enough surfactant in the dentifrices goes inside the pockets, it may accelerate LXA_4 release. Similarly, LXA_4 release accelerates when pH increases, possibly because the carboxyl residues become more ionized in the base, which increases the drug solubility in water (Savjani et al. 2012). Correspondingly, the release rate was slower in acid probably due to decreased drug solubility. Regarding P407, LXA_4 can be trapped inside of its micelles, which act as a barrier to weaken the effect of detergent and base (Dumortier et al. 2006). Because P407 is easily eroded, LXA_4 release relied on the gel erosion besides diffusion (Zhang et al. 2002; Maheshwari et al. 2006). The LXA_4 total release amount diminished in the acidic medium for both PIC and P407 likely due to the degradation induced by acid. Finally, it is mentionable that a cumulative release amount reached around 90% in PICs and 80% in P407s. Not fully releasing may also partially be attributed to the slow degradation of LXA_4.

A phagocytic activity test was used to evaluate the efficacy of LXA_4 released from gels (Prescott and McKay 2011). Only LXA_4 from PIC gels promoted phagocytosis of macrophages. Since there is no obvious chemical reaction between LXA_4 and P407, it is highly unlikely that the presence of P407 leads to deactivation of LXA_4. Therefore, other substances in the P407 releasates may hinder the phagocytosis process, which counteracts the LXA_4 effect. Further cytocompatibility experimentation revealed that P407 could be toxic to PDL and GEC cells, which could explain the low phagocytosis in the P407/LXA_4 group. In practice, w/w ≥20% P407 is widely used (e.g., for oral, rectal, ophthalmic usage) in vivo (Ji et al. 2010). Some studies reported that low concentrations of P407 had no inherent cytotoxicity (Hokett et al. 2000; Exner et al. 2005), but the cytotoxicity at 20% or higher concentration has not been reported yet. Based on our study, 26% P407 has unexpected growth inhibition to PDL cells in transwell culture, which may be due to its easy dissolution to the medium through the transwell pores. Such hindrance of cell growth should be further investigated. In contrast to P407, PIC did not erode to such an extent but remained more stable and cytocompatible. Because metabolic assays (e.g., alamarBlue) could not accurately reflect cellular proliferation rates, a discrepancy was observed between the viability and proliferation results in PIC groups and the control group (Fig. 4A and B) (Quent et al. 2010). A recent study demonstrated that fibroblasts, endothelial cells, adipose-derived stem cells, and melanoma cells did survive, thrive, and differentiate in PIC gels, further confirming its cytocompatibility (Zimoch et al. 2018).

Conclusion

In this study, PIC/LXA_4 gel featured by convenient handling ability was stable for over 2 wk. It was capable of releasing LXA_4 in a sustained manner while preserving biological activity and was cytocompatible. In conclusion, PIC hydrogel was appropriate for the intended periodontal applications, and its clinical potential for treating periodontitis should be further developed.

Author Contributions

B. Wang, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; J. Shao, J.A. Jansen, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript; X. F. Walboomers, F. Yang, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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