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Research Article

Dissolution Chamber for Small Drug Delivery System in the Periodontal Pocket

Wei Ren,[1] Michael Murawsky,[1] Terri La Count,[1] Apipa Wanasathop,[1] Xin Hao,[1] Gary R. Kelm,[1] Darby Kozak,[2] Bin Qin,[2] and S. Kevin Li[1,3]

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Abstract. Existing dissolution chambers have relatively large volume compared to the size of the periodontal pocket. A small volume dissolution method that simulates the physiological release environment for periodontal drug delivery is needed. The objectives were to construct a small, more physiologically relevant, dissolution chamber and investigate the properties of the new dissolution chamber for the assessment of sustained drug release systems in periodontal delivery. Flow-through dissolution chambers were constructed using three-dimensional (3D) printing. Drug release experiments were performed using the dissolution chamber and a commercially available long-acting periodontal insert product, PerioChip®. Similar experiments were performed under more traditional larger volume bulk solution conditions for comparison. Computer simulations and experimental results showed that drug clearance from the dissolution chamber was fast compared to drug release from the periodontal product. Drug clearance from the flow-through dissolution chamber and drug release from the sustained release product in the chamber were related to the dissolution medium flow rate and chamber volume. Drug release in the flow-through chamber was slower than that observed in bulk solution, but approached it when the medium flow rate increased. The presence of trypsin in the dissolution medium enhanced drug release from the product. A flow-through dissolution system was constructed that could evaluate drug release from a sustained release product in a small dimension environment by modifying the flow rate and composition of the dissolution medium.

KEY WORDS: chlorhexidine; dissolution study; drug release; *in vitro* release testing (IVRT); periodontal pocket.

INTRODUCTION

Dissolution testing provides information for drug performance evaluation and quality control (1–3). A dissolution method is useful not only to monitor batch-to-batch consistency during manufacturing, but also provides information on the physicochemical characteristics of the dosage form. This information may be used to support equivalence determination between a proposed generic product and the reference listed drug (RLD) (4,5). In addition to being able to discriminate differences in the product formulation and manufacturing process, taking the *in vivo* release mechanism and biological environment into consideration to develop a dissolution testing method can facilitate *in vitro-in vivo* correlations. This is particularly important when the drug product is a sustained release dosage form for local drug delivery such as long-acting periodontal drug formulations. Previous *in vitro* testing of drug release from periodontal formulations varied with respect to the apparatus design, size of the dissolution chamber, volume and composition of the dissolution medium, stirring condition, and other factors (6–13). There is no standard testing condition developed for the evaluation of drug release from these periodontal delivery systems.

Among the standard dissolution methods to evaluate drug products and characteristic differences caused by differences in manufacturing, the most common method is United States Pharmacopeia (USP) dissolution testing. The conventional methods of USP dissolution testing employ the USP apparatus: USP dissolution Apparatus 1–7 (14,15). These USP dissolution methods all have relatively large dissolution chambers compared to the size of the periodontal pocket. The large dissolution chamber volume in a USP apparatus is to provide a sink condition to simulate the dissolution environment likely encountered in the gastrointestinal (GI) tract due to the large volume and surface area in GI absorption. To be more specific, these dissolution methods

[1] Division of Pharmaceutical Sciences, James L Winkle College of Pharmacy, University of Cincinnati, 231 Albert Sabin Way, MSB # 3005, Cincinnati, Ohio 45267-0514, USA.
[2] Office of Research and Standards, Office of Generic Drugs, U.S. Food and Drug Administration, Silver Spring, Maryland 20993, USA.
[3] To whom correspondence should be addressed. (e–mail: kevin.li@uc.edu)

are not expected to provide the appropriate testing conditions for periodontal drug products because a sink condition may not occur in the periodontal pocket due to its small volume. This key difference between the dissolution of drug products in the USP systems and in the periodontal pocket illustrates the need of a robust dissolution method for periodontal drug product testing. Periodontal drug products also encounter a small gingival crevicular fluid flow in the periodontal pocket. Although USP Apparatus 4 is a flow-through cell, the objective of this flow-through system is to provide a sink condition for the study of poorly soluble drugs and the advantage is its ability to change the flow medium and flow rate within the dissolution testing (14), which is not designed to mimic a flow-through environment of small flow volume. Therefore, the existing dissolution methods likely cannot provide reliable information of periodontal drug products *in vivo* because of the different dissolution conditions *in vitro* and *in vivo*. The development of a bio-relevant dissolution method for sustained release periodontal products would require a new flow-through dissolution system that could resemble the periodontal pocket hydrodynamics, dissolution medium consisting of an enzyme to mimic the enzymatic degradation environment in the pocket, and a model periodontal product for the testing of *in vitro-in vivo* correlation.

The objectives of this study were to fabricate small dissolution chambers that could better resemble the size of the periodontal pocket and investigate the properties of the new dissolution chambers for the assessment of sustained release systems designed for small tissue compartments. Dissolution chambers were constructed using three-dimensional (3D) printing and characterized to determine the influencing factors for drug release in the chambers. Computer simulations of a diffusion/convection transport model were used to understand the 3D drug concentration profiles in the dissolution chambers under different conditions. PerioChip®, an approved and marketed product in the United States (16), was the model sustained release periodontal drug product in this study to test the dissolution chambers and conditions. Drug release from PerioChip® was determined in the dissolution chambers and compared with drug release in the traditional larger volume bulk solution (denoted by "bulk solution" experiments in this paper). The results in this project could assist pharmaceutical scientists to design dissolution experiments and determine the dissolution behavior of drug delivery systems in small tissue compartments such as long-acting periodontal drug products.

METHODS

Materials

The dissolution media in the bulk solution drug release experiments were phosphate buffered saline (PBS) or sodium chloride (NaCl) solution. PBS, pH 7.4, consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, was prepared using PBS tablets (MP Biomedicals, LLC, Solon, OH) and deionized water (DI water). For PBS of pH 8.0 and 8.7, the pH of PBS was adjusted to the desired pH using concentrated sodium hydroxide (NaOH) solution. NaCl solution at pH 8.0 was prepared by adjusting the pH of 0.15 M NaCl in DI water using concentrated NaOH solution. The dissolution media in the drug release experiments of flow-through dissolution chambers were simulated saliva with and without an enzyme. Simulated saliva, pH 8.0, consisting of 0.137 M sodium chloride, 0.0014 M potassium phosphate monobasic, 0.017 M sodium phosphate dibasic (Fisher Scientific, Fair Lawn, NJ), was prepared in DI water. Trypsin (from porcine pancreas, lyophilized powder, 1000–2000 BAEE units/mg solid) was purchased from Sigma-Aldrich (St. Louis, MO). Pepsin A (powder for biochemistry) was purchased from Acros Organics (Janssen Pharmaceuticalaan, Geel, Belgium). PerioChip® (chlorhexidine gluconate, 2.5 mg) was manufactured by Drexel Pharma Technologies Ltd. (Yokneam, Israel). Chlorhexidine digluconate (20% solution in water) and chlorhexidine (CHX) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium phosphate monobasic, trimethylamine (HPLC grade), and o-phosphoric acid were purchased from Fisher Scientific (Fair Lawn, NJ). Acetonitrile and methanol (HPLC grade) were from Pharmaco-AAPER (Shelbyville, KY). Materials were used as received.

Flow-Through Dissolution Chamber System

Flow-through dissolution apparatuses with chamber volumes of 0.03–0.24 mL were constructed using photo-initiated acrylic polymer clear resin and stereolithography 3D-printing process (Formlabs, MA). To fit the drug product dimensions, and for simplicity in 3D printing, the dissolution chamber design was rectangular in shape. Table I lists the dimensions of the dissolution chambers and PerioChip®. Figure 1 shows the schematic diagram and representative images of the flow-through dissolution chamber. In the open flow-through dissolution system, the dissolution chamber in the apparatus was connected to a syringe pump (Model NE-300, New Era Pump Systems, Farmingdale, NY) through tubing (Intramedic PE-50 or PE-10, Becton Dickinson, Parsippany, NJ). The syringe infusion pump provided a continuous flow of fresh dissolution medium (infusion volume flow rates of 0.6 to 20 μL/min; up to ± 30% error at 0.6 and 1.5 μL/min flow rates during the experiments due to the intrinsic variability of the infusion pump at low flow rates) across the dissolution chamber from a reservoir, and samples were collected at the outlet of the chamber (the end of 1.5 to 3.5 cm tubing extended from the chamber). Before the experiments, the solution flow rate from the infusion pump was calibrated by the weight of the solution (water in this case) using an analytical balance.

Model Simulation and Clearance Kinetics in Dissolution Chambers

Simulations, based on diffusion/convection transport and Fick's second law (Eq. 1), were performed in finite-element analyses using Comsol Multiphysics software (Burlington, MA):

$$\frac{\partial C}{\partial t} = \nabla \cdot (D \nabla C - vC) \tag{1}$$

where ∇ is the vector differential operator, C and D are the concentration and diffusion coefficient of the drug,

Table I. Dimensions of Dissolution Chambers and PerioChip®

	Chamber				PerioChip®[a]
Volume (mL)[b]	0.03	0.04	0.06	0.24	~ 0.007
Length (mm)	5	6.5	5	10	5
Width (mm)	4	4	4	8	4
Height (mm)	1.5	1.5	3	3	0.35
Inlet/outlet hole diameter (mm)	0.6	0.6	1[c]	1	
Inlet/outlet hole cross-sectional area (mm^2)	0.28	0.28	0.785[c]	0.785	
Position of the inlet/outlet (mm from the chamber base)	0.5	0.5	1	1	

[a] Information of PerioChip® (17)
[b] Small volume chambers (≤ 0.06 mL) had up to ± 20% volume errors due to 3D printing and the thickness of the glue used to seal the top and bottom halves of the chambers (see half-chambers in Fig. 1a)
[c] Outlet diameter of 1.6 mm (area of 2.0 mm^2) *was also used*

respectively, v is flow velocity vector in the periodontal pocket, and t is time. Incompressible fluid and laminar flow were assumed in the analyses. The settings in Comsol program were general physics, extra fine mesh, transport of diluted species, and constant diffusion coefficient. A 3D structure was constructed in the computer program using the dimensions of the dissolution chambers. The boundary conditions and initial conditions were set according to the conditions in the chambers. As a first approximation, the diffusion coefficient of the drug in the dissolution chamber was assumed to be close to that of free aqueous diffusion, and a range of diffusion coefficients (10^{-6} to 10^{-5} cm^2/s) representing drugs of different molecular sizes was used. Time-dependent drug concentration profiles in the chamber were generated and the volume integral of drug concentration in the chamber was calculated. Drug clearance in the

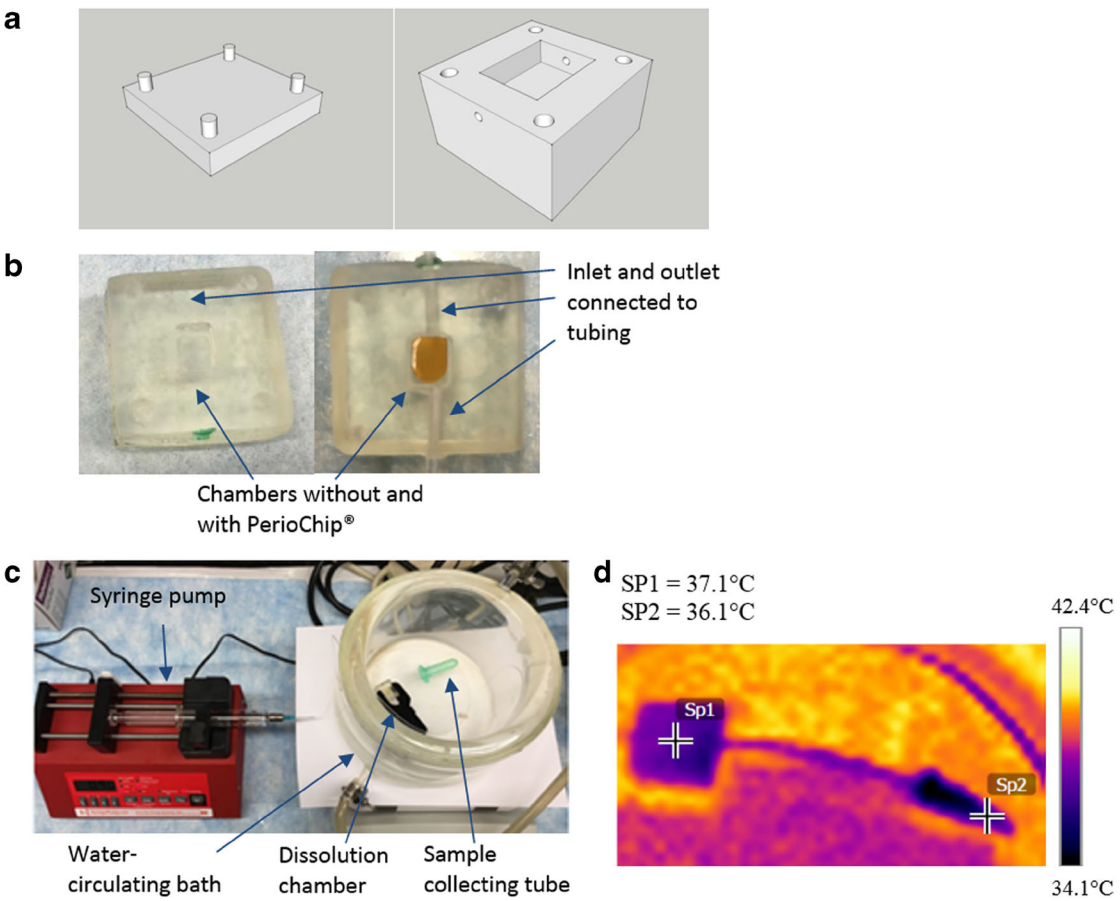

Fig. 1. Flow-through dissolution chambers constructed with 3D printing and dissolution method setup. **a** Schematic diagram of the 3D-printed dissolution chamber. **b** Dissolution chambers with and without PerioChip® (left and right images, respectively). **c** Dissolution method setup including the infusion syringe pump, water-circulating bath, sealed and clamped dissolution chamber, tubing, and sample-collecting tube. **d** Temperatures of the dissolution chamber (SP1) and sample-collecting micro-centrifuge tube (SP2) in the water-circulating bath

outflow fluid from the chamber was determined using the volume integral values (total amounts of drug in the chamber) and mass balance. Changing the mesh size setting in the computer program from extra fine to fine mesh did not significantly affect the results.

The kinetics of drug clearance in the flow-through dissolution chamber was measured using the 0.06 mL dissolution chamber and infusion pump (Fig. 1) at room temperature. Three conditions were examined: (a) 10 μL/min flow across the dissolution chamber, (b) 0.6 μL/min flow across the dissolution chamber, and (c) 0.6 μL/min flow across the dissolution chamber containing an inert 0.02-mL flat rectangular object to mimic a swollen PerioChip®. The rectangular object was constructed using commercial grade rubber (pure gum rubber, CG-40A; Rubber-Cal, Santa Ana, CA). The dissolution chamber was first loaded with a dye solution (0.3 to 1.3 mg/mL acid blue #9) or 0.5 mg/mL CHX. The infusion pump provided a continuous flow of DI water across the chamber at a rate of 0.6 or 10 μL/min and samples were collected at the outlet of the chamber. The samples were diluted by appropriate volume of DI water, and the concentration of the dye or CHX in the samples was determined using a UV/Vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu Scientific Instruments, Inc., Addison, IL) at 628 and 239 nm, respectively. To examine possible errors from evaporation and other factors, the infusion of a dye solution into the chamber preloaded with the same dye solution was used to check for concentration change and as a control. The tubing and chamber surfaces were also inspected physically for surface staining by the dye (e.g., adsorption) after the equilibration of the materials with the dye, and no staining was detected. In addition, the absorbance of the dye solution was checked at pH 6–8 and in DI water, and was found to remain relatively constant (within 5% difference) in this pH range.

Possible drug binding to the dissolution chamber and tubing was investigated by two methods: (a) passing CHX solutions (20 and 300 μg/mL) across the dissolution chamber by the infusion pump and examining CHX concentration in the solution from the outlet, and (b) equilibration of the dissolution chamber and tubing with the CHX solutions for 12 h (from 0–12 h and 12–24 h). Both methods showed minimal CHX binding to the dissolution chamber and tubing (< 15% and 4% of CHX loss in 20 and 300 μg/mL solution, respectively).

Drug Release in Bulk Solution

Drug release from PerioChip® was evaluated in 10 mL PBS at pH 7.4, 8.0, and 8.7, and 0.15 M NaCl at pH 8.0 in a vial under stirring. PerioChip® was placed in a miniature plastic mesh basket with the basket submersed in the dissolution medium in a 20-mL glass vial (borosilicate glass scintillation vial; Fisher Scientific, Fair Lawn, NJ). The basket was suspended in the dissolution medium above the stir bar with continuous stirring (700 RPM) at 37°C controlled by a water-circulating bath. The dissolution medium volume and stirring speed were selected to allow adequate sensitivity in the HPLC assay and stirring with minimal stirring speed effect on drug release (18). At appropriate time intervals (e.g., 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 312 h), the basket with PerioChip® was removed from the glass vial and transferred to a new vial containing fresh dissolution medium. Samples were taken from the glass vials after the removal of PerioChip®, and the concentrations of CHX in the samples were quantified by HPLC. At the end of the drug release experiments, pepsin digestion was performed in 2 mL 0.5% pepsin solution at pH 3.5 and 37°C under stirring in a vial to dissolve PerioChip® and determine the residual drug content in the chip. Complete digestion of PerioChip® was observed within 12 h. The 2 mL mixture was then diluted and filtered, and samples were collected for HPLC assay. A control experiment using 1 mg/mL CHX in 0.5% pepsin solution that went through the same experimental condition and dilution showed 90% recovery using this pepsin digestion method.

Drug Release in Dissolution Chamber

Drug release from PerioChip® was evaluated using the dissolution chambers, infusion pump, and water-circulating bath at 37°C as described in "Flow-through dissolution chamber system" above (Fig. 1). The temperature of the infusing dissolution medium was warmed in the coiled tubing in the water-circulating bath, and the temperatures of the dissolution chamber and tubing were monitored using an IR thermal camera (FLIR-E63900, FLIR Systems, Sweden). The glass water-circulating bath was covered with Parafilm (Parafilm M wrapping film) to seal the chamber in the bath. Dissolution chamber volumes of 0.04, 0.06, and 0.24 mL and dissolution medium flow rates of 0.6, 1.5, 3, 10, and 20 μL/min were evaluated. 0.6 μL/min was close to the upper value of gingival crevicular fluid flow rate (19). Flow rates higher than 0.6 μL/min were also investigated because the flow velocity was related to the volume of the chamber, and despite that the gingival crevicular fluid flow in the periodontal pocket is small, the flow velocity can be fast due to the small volume of the periodontal pocket *in vivo* (e.g., ~ 0.4–1.5 μL) (20). The smallest flow-through dissolution chamber investigated was 0.04 mL in the drug release study of PerioChip®. The use of dissolution chamber volume smaller than 0.04 mL (e.g., 0.03 mL in Table I) would result in flow blockage of the dissolution medium in the chamber due to the size and swelling of PerioChip® in the drug release experiment.

In the experiments, PerioChip® was placed in the dissolution chamber and the chamber was sealed with Super Power hot glue (Arrow Fastener Corporation, Mayhill, NJ) and secured with a pinch clamp. The infusion pump provided a continuous flow of simulated saliva as the dissolution medium across the chamber, and samples were collected at the outlet of the chamber at predetermined time intervals (e.g., 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 h). The samples were then quantified for CHX using HPLC. The micro-centrifuge tubes used to collect the samples were weighed before and after sample collection to check the sample volume.

Effect of Enzyme

The dissolution medium with enzyme was prepared by adding trypsin into the simulated saliva to final concentration of 0.05–0.3% trypsin. Enzymatic digestion of PerioChip® was first examined in 2 and 100 mL dissolution medium of 0.05–0.3% trypsin in a vial under stirring at 37°C. The effect of

enzymatic digestion on drug release from PerioChip® was then examined using 0.3% trypsin in the dissolution medium and flow-through dissolution chambers. Dissolution experiments were performed as described in "Drug release in dissolution chamber" in the presence of the enzyme. Due to enzymatic digestion, PerioChip® degraded in the flow-through dissolution chamber and small fragments broke off from the drug product. These fragments could block the dissolution chamber outlet. Two methods were investigated to prevent the outlet blockage. The first method used a 3 mm × 4 mm filter (Whatman filter paper grade No. 1) that was placed inside the dissolution chamber lining the chamber wall of the outlet. The second method was to widen the outlet orifice (to 1.6 mm diameter) of the dissolution chamber and increase the diameter of the outlet tubing (to Tygon tubing of 1.2 and 1.6 mm inner and outer diameters, respectively). A preliminary experiment showed that the first method was not effective to mitigate this problem, so the second method was selected in the present study. The second method was also more bio-relevant similar to the periodontal pocket opening to the oral cavity *in vivo*.

HPLC Assay

CHX concentrations in the samples were quantified using a Shimadzu HPLC system (Shimadzu Scientific Instruments, Inc., Addison, IL) at room temperature. The HPLC system consisted of two pumps (LC-20 AT), a variable wavelength UV absorbance detector (SPD-20A), an auto injector (SIL-20A), and a Microsorb-MV100-5 C18 column (15 cm × 4.6 mm, 4.6 μm, Varian, Lake Forest, CA). The mobile phase was prepared by 0.23 mol sodium phosphate monobasic in 1.3 L water, mixed with 10 mL 0.5% trimethylamine in water, adjusted to pH 3.0 with o-phosphoric acid and volume to 1.4 L with water, and mixed with 0.6 L acetonitrile to a final mixture of 70:30 water/acetonitrile. The flow rate was 1.5 mL/min. The injection volume was 50 μL, and the detection wavelength was 239 nm. Standard solutions of 1–50 μg/mL CHX were prepared in simulated saliva to construct the calibration curve.

Data Analysis

The data obtained in the present study generally are presented as means and standard deviations (SD). Statistical analyses were performed using Student's *t*-test with Microsoft Excel (Redmond, WA) and one-way ANOVA with GraphPad (La Jolla, CA), and a difference of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Drug Clearance in Dissolution Chamber

The flow rate of dissolution medium and dimensions of a dissolution chamber can affect the hydrodynamics of fluid flow in the dissolution chamber and hence drug clearance from the chamber. Therefore, model simulations were performed to investigate the flow velocity and 3D concentration profiles in the dissolution chamber, the volume average concentration of the drug in the chamber, and the 1st to 3rd clearance half-lives as a function of dissolution properties (1st, 2nd, and 3rd half-lives were the times for the average concentration decrease from 100 to 50%, 50 to 25%, and 25 to 12.5%, respectively). Figure 2 shows the effects of dissolution chamber sizes, flow rates, and drug diffusion coefficients on the half-lives. At 10 and 20 μL/min flow rates, drug clearance from the dissolution chambers was fast and the half-lives were in the order of minutes (Fig. 2c, d). The 1st half-lives from the model simulations are comparable to that of 1st-order clearance that assumes a well-stirred chamber (Eq. 2) (i.e., ~ 2 and 4 min for 0.06 mL at 20 and 10 μL/min and ~ 1 and 2 min for 0.03 mL at 20 and 10 μL/min, respectively).

$$VdC/dt = -QC \tag{2}$$

where V is the volume of the dissolution chamber and Q is the fluid flow rate. However, unlike conventional 1st-order clearance, the half-lives were not constant in the model simulations (1st half-life < 2nd half-life < 3rd half-life) when the clearance mechanism changed from convective flow dominant to diffusion dominant transport, i.e., when the majority of the drug in the center of the chamber was removed by the convective flow and drug diffusion into the center of the chamber was the rate-determining process for further drug removal. A decrease in the flow rate or an increase in the size of the dissolution chamber increased the 1st half-lives proportionally. Drug diffusion coefficients did not affect the 1st half-lives under convective solvent transport dominant clearance, but diffusion became an important factor for the 2nd and 3rd half-lives. The larger the volume of the dissolution chamber and slower the flow rate, the larger was the contribution of diffusion to the 2nd and 3rd half-lives (larger diffusion coefficient effects in the figure). At 0.6 and 1 μL/min flow rates, the effect of diffusion coefficients was not as clear compared to those at the higher flow rates (10 and 20 μL/min), partly due to the more significant contribution of diffusion to clearance for all half-lives under the slow flow rate conditions (Fig. 2e). Similar to the higher flow rate conditions, the 1st half-lives from the model simulations are comparable with those estimated using Eq. 2 (i.e., ~ 20 and 30 min for 0.03 mL at 1 and 0.6 μL/min, respectively).

Figure 3 presents the experimental results of clearance in the 0.06 mL dissolution chamber using dye or CHX solutions preloaded in the chamber under the following conditions: (a) clearance of the dye and CHX from the chamber at 10 μL/min flow rate, (b) clearance of the dye from the chamber at 0.6 μL/min flow rate, and (c) clearance of the dye from the chamber that contained a small inert object at 0.6 μL/min flow rate. At 10 μL/min flow rate, the clearance data are consistent with those of the model simulations, and the clearance half-lives were in the order of minutes (Fig. 3a). With convective transport as the dominant mechanism for drug clearance and the molecular weights of the dye and CHX (747 and 505 g/mol, respectively), there was no significant difference between the clearance of CHX and the dye from the dissolution chamber. At 0.6 μL/min flow rate, the clearance data (without the object) are comparable with those of the model simulation, and the experimental half-life was ~ 60 min (Fig. 3b). The effect of placing a small object of similar volume as a

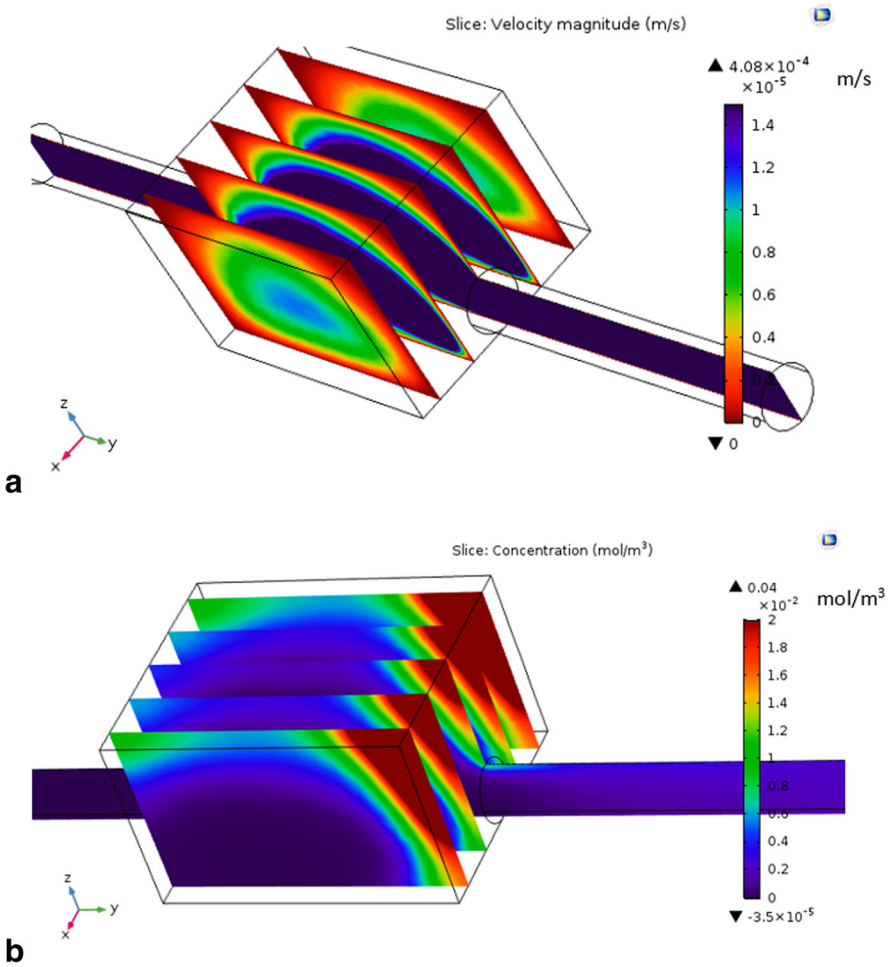

Fig. 2. Representative **a** steady-state flow velocity and **b** drug concentration profiles at 60 min in the 0.06 mL dissolution chamber with 10 μL/min flow rate and diffusion coefficient of 5×10^{-6} cm^2/s. Half-lives ($t_{1/2}$) of drug in the **c** 0.03 mL and **d** 0.06 mL flow-through dissolution chamber at infusion flow rates of 10 μL/min (solid) and 20 μL/min (striped/dotted) and in the **e** 0.03 mL flow-through dissolution chamber at flow rates of 0.6 μL/min (solid) and 1.0 μL/min (striped/dotted) with diffusion coefficients of 10^{-6} cm^2/s (dark), 5×10^{-6} cm^2/s (gray), and 10^{-5} cm^2/s (white/dotted white) obtained from model simulations. 1st half-life is the time from 100 to 50%, 2nd half-life is from 50 to 25%, and 3rd half-life is from 25 to 12.5%

swollen PerioChip® in the dissolution chamber, a situation more representative of the flow dynamics and clearance from the chamber in the drug release study, was then investigated. With the object mimicking the presence of the drug product in the dissolution chamber, the experimental data are comparable with the model simulation of the 0.03 mL dissolution chamber (dash dot curve in Fig. 3b). As expected, the addition of the object in the dissolution chamber reduced the effective volume of the chamber and resulted in faster clearance than that of the 0.06 mL chamber. The experimental half-live was ~ 30 min with the object in the chamber. Although these clearance half-lives are significantly longer than those at 10 μL/min, they are still relatively fast compared to drug release from PerioChip® observed later in the present study (see data from PerioChip® experiments below). For both flow rate conditions, the difference between the experimental data and model simulation results could be attributed to the assumptions used in the model simulations, limitations of the finite-element analysis calculations, and experimental errors. For example, the experiment to examine possible errors from evaporation and an experiment on the effect of tubing length (tubing volume) suggest that approximately 15% and 1 min errors could be introduced due to the small sampling volume and short sampling intervals in the experiments (data not shown).

In summary, the experimental results with the dye and CHX support the conclusion in the model simulation study that clearance from the dissolution chambers was relatively fast, as such conditions close to sink conditions are expected to be maintained in these dissolution chambers. Together with the model simulation results, these data suggest that diffusion and clearance in the dissolution chamber (in minutes, up to 30 min) was not a significant factor to drug release from a long-acting sustained drug release system (in hours) in the flow-through dissolution chamber. Although the model simulations and clearance experiments did not cover all the conditions later used in the present drug release studies, which could have different flow dynamics in the dissolution chamber, the conclusion would likely remain the same because clearance from the dissolution chamber was at

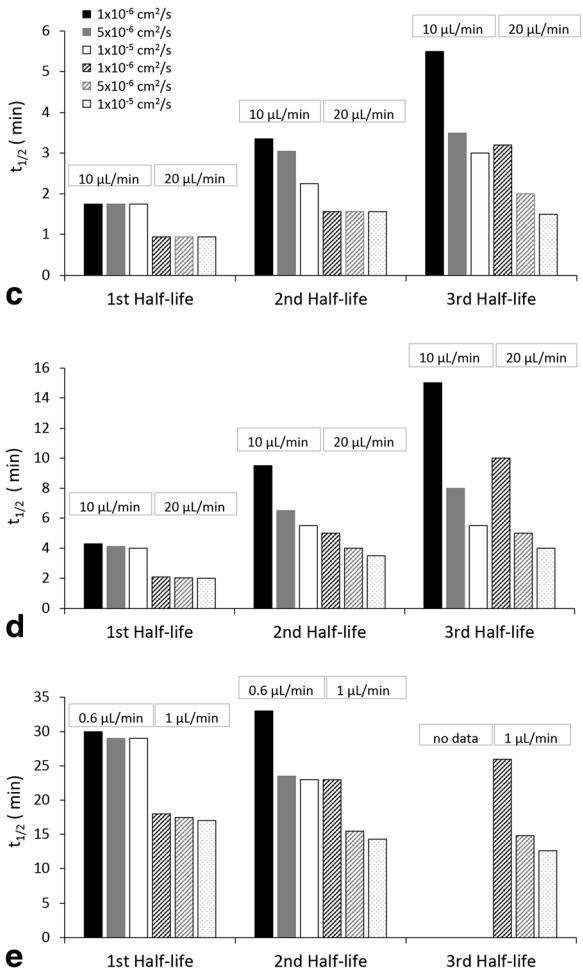

Fig. 2. continued.

least an order of magnitude faster than drug release from PerioChip®, the sustained release product examined in the present study, when compared at the same flow rate (see data from PerioChip® experiments below).

Drug Release in Dissolution Chamber

PerioChip® was selected as the model sustained release delivery system to examine the dissolution chamber designed in the present study. PerioChip® weighs approximately 6.9 mg and contains 2.5 mg CHX gluconate, which is equivalent to 1.4 mg CHX in a biodegradable matrix of hydrolyzed gelatin cross-linked with glutaraldehyde. As per the drug label, PerioChip® is administered once every 3 months to provide sustained release of CHX in the periodontal pocket as an adjunct to scaling and root planning procedures for patients with periodontitis (16). It is one of three currently marketed sustained release periodontal drug products that are administered directly into the periodontal pocket for the drug indication. As such, a dissolution chamber developed for PerioChip® may be utilized for other sustained release periodontal formulations.

Figure 4 shows the cumulative amounts of drug release from PerioChip® in bulk solution of PBS at pH 7.4, 8.0, and 8.7. Drug release in saline (0.9% NaCl) at pH 8.0 was also evaluated because of possible interactions between CHX and phosphate ions in PBS (21). Although CHX can precipitate with chloride ions (22), no precipitation was observed in the present study, possibly due to the CHX concentration encountered and the presence of gluconate during drug release from PerioChip®. The results of PerioChip® swelling (total weight) in these experiments are also presented in Fig. 4. After dissolution in the bulk solution for 24 h, more than 70% of CHX was released and the gelatin matrix swelled by more than twice its initial weight. The gelatin matrix was still intact at 7 days in the vial in the dissolution experiment. No significant difference in drug release and swelling was observed under the pH conditions investigated in the present study. In addition, the similar drug release and swelling profiles in PBS *vs.* 0.9% NaCl suggest no significant effect of phosphate ions.

The flow velocity across the surface of PerioChip® can affect the thickness of the aqueous boundary layer (unstirred layer) (23,24) in the flow-through dissolution chamber, which can affect drug release from the sustained delivery system. When drug release is controlled by the gelatin matrix of PerioChip®, the effect of flow rate on drug release is expected to be minimal. A significant effect of flow rate on

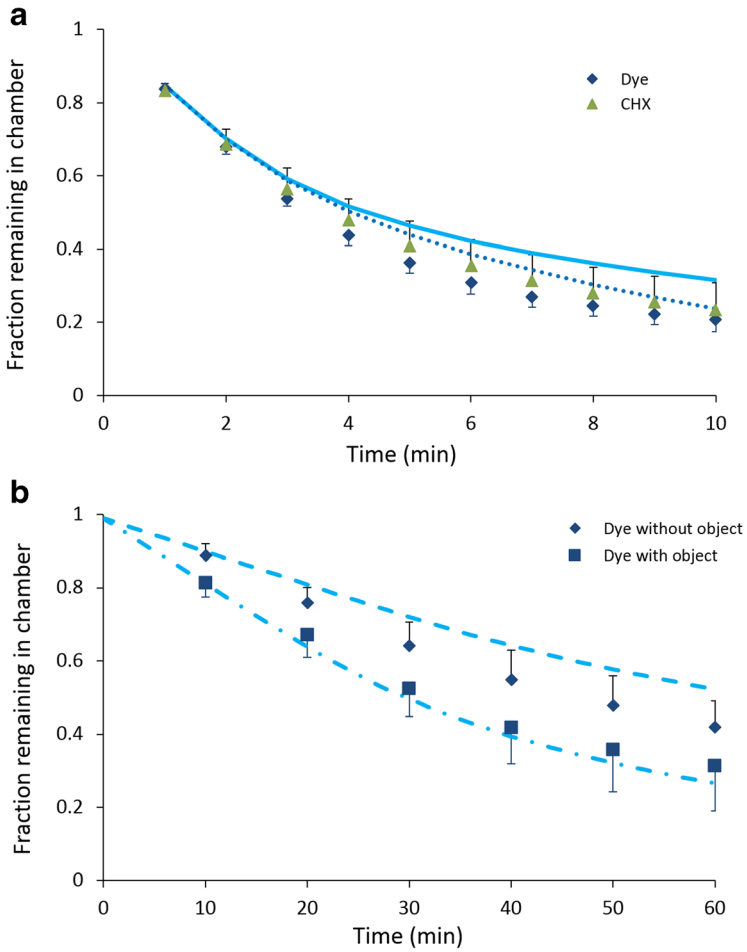

Fig. 3. Clearance from the 0.06 mL dissolution chamber as the fraction remaining in the chamber over time. **a** Clearance of the dye (diamonds) and CHX (triangles) at flow rate of 10 μL/min. **b** Clearance of the dye from the chamber at flow rate of 0.6 μL/min with and without an object in the chamber mimicking a drug product (squares and diamonds, respectively). The curves represent the results from model simulations using the 0.06 mL dissolution chamber at 10 μL/min flow rate assuming diffusion coefficient of 10^{-6} cm^2/s (solid curve) and 10^{-5} cm^2/s (dotted curve) and at 0.6 μL/min flow rate assuming diffusion coefficient of 10^{-6} cm^2/s (dashed curve) and the 0.03 mL dissolution chamber at 0.6 μL/min flow rate assuming diffusion coefficient of 10^{-6} cm^2/s (dash dot curve). Mean ± SD ($n = 3$–6)

drug release would indicate that the aqueous boundary layer is a significant barrier to drug release from PerioChip®. Figure 5a presents the CHX release profiles at different flow rates in the 0.06 mL dissolution chamber. There was minor difference between the release profiles at 10–20 μL/min, suggesting that the contribution of the aqueous boundary layer to drug release under these flow velocity conditions was small in the chamber. At 0.6 and 1.5 μL/min, drug release was significantly reduced relative to that at 3–20 μL/min, suggesting that when the flow rates (and corresponding flow velocities) decreased to ~ 1.5 μL/min, the aqueous boundary layer became a barrier to drug release from PerioChip® that drug release was no longer controlled only by the gelatin matrix. The discontinuity between 1.5 and 3 μL/min could also suggest the existence of a critical shear stress above which the flow across the PerioChip® surface would affect drug release. Figure 5b shows the effects of the dissolution chamber systems (bulk solution and dissolution chambers of different sizes) on drug release from PerioChip®. Drug release in the bulk solution was faster than that in the 0.06 mL dissolution chamber at 20 μL/min. The 0.06 and 0.24 mL dissolution chambers provide similar drug release profiles at 0.6 μL/min except for the time lag due to the larger void volume in the 0.24 mL chamber (turnover time = volume/flow rate = 6 h). The lack of a significant effect with the considerably larger dissolution chamber volume (0.24 mL) compared to the periodontal pocket suggests that the chamber volume is not an important factor on drug release from the sustained release product under the conditions studied. The difference between drug release in the bulk solution *vs.* that in the flow-through dissolution chamber could be attributed to (a) the restricted swelling of PerioChip® in the small volume dissolution chamber, (b) drug release from one PerioChip® surface in the chamber *vs.*

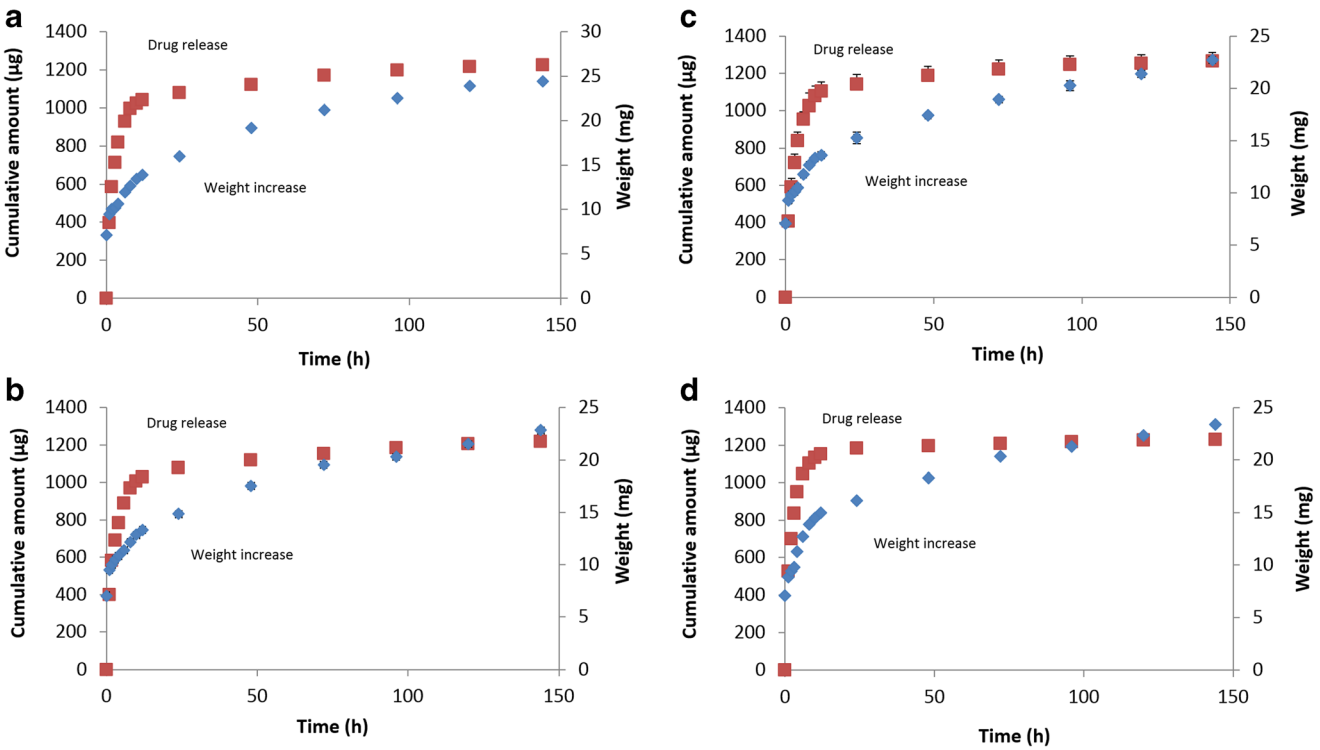

Fig. 4. Profiles of cumulative amounts of drug release (squares) and swelling in total weight (diamonds) of PerioChip® in bulk solution of **a** PBS, pH 7.4; **b** PBS, pH 8.0; **c** PBS, pH 8.7; and **d** 0.15 M NaCl, pH 8.0. Mean ± SD ($n = 3$)

two surfaces in the bulk solution, and (c) differences in fluid dynamics and aqueous boundary layers in the dissolution chamber and stirred vial. For example, different swelling of PerioChip® was observed at the end of the drug release studies in the 0.06 mL flow-through dissolution chamber (1.5–10 µL/min flow rate, ~ 2.6× swelling; and 0.6 µL/min flow rate, ~ 2.3×) and in bulk solution in a vial (~ 3.6× swelling). The restricted swelling of PerioChip® could affect drug release from PerioChip®. It should be noted that the gelatin matrix was still intact at the end of the flow-through dissolution experiment, and an enzyme is required to digest the gelatin matrix as those observed when PerioChip® is used in clinical practice *in vivo*.

Effect of Enzyme on Drug Release

Drug release from a formulation with a biodegradable matrix is often affected by the rate of matrix degradation. The degradation of a drug matrix can be pH dependent and/or enzyme specific at the site of drug administration. Gelatin, the polymer matrix of PerioChip®, can be hydrolyzed by the enzymes in the gingival crevicular fluid such as collagenases and elastases (proteases) (25–27). The present study examined the effects of trypsin, a digestive protease enzyme, on drug release from PerioChip®. Trypsin was selected as the model enzyme because it is effective at neutral pH, well studied, and readily available to be used in dissolution testing to induce a similar matrix degradation effect. In addition, the use of other biological enzymes can be cost prohibitive.

Preliminary studies showed that including trypsin at 0.05% in the simulated saliva dissolution media only deformed the PerioChip® structure when it was placed in a vial with 2 mL media under constant stirring for 7 days at 37°C. Increasing the concentration of trypsin to 0.3% could partially digest the gelatin matrix of PerioChip® in 2 mL dissolution medium over 7 days. With larger volume of 100 mL, the same dissolution medium (0.3% trypsin) completely digested PerioChip® after 4 days. In the flow-through dissolution chamber (10 µL/min flow rate), PerioChip® was completely digested by 0.3% trypsin enzyme in 4 days. This flow rate and time correspond to a total volume of ~ 60 mL dissolution medium that flowed through the dissolution chamber. The results suggest faster PerioChip® digestion under the larger volume conditions. The volume effect could be related to the concentration of degradation products in the dissolution medium and/or the total amount of enzyme required for complete gelatin digestion. Both enzyme concentration and dissolution medium volume (or amount of enzyme used) could impact the digestion of the gelatin matrix of PerioChip®.

Figure 6a presents the drug release data of PerioChip® and the dissolution medium with 0.3% trypsin in the 0.06 mL flow-through dissolution chamber at 0.6 µL/min flow rate and 0.04 and 0.06 mL chambers at 10 µL/min flow rate. Drug release data under the same conditions without trypsin are also presented in the figure for comparison. The gelatin matrix showed signs of fragmentation in the dissolution chamber beginning at 12–24 h and drug release from the matrix was significantly enhanced in the presence of the enzyme. At 0.6 µL/min, drug release was ~ 1.5–2× faster (1.5–2× increase in the cumulative amount release *vs*. time slope) with trypsin than that without over the first 2 days of the dissolution study. When the flow rate increased to 10 µL/min, the release profiles with and without trypsin overlapped in the

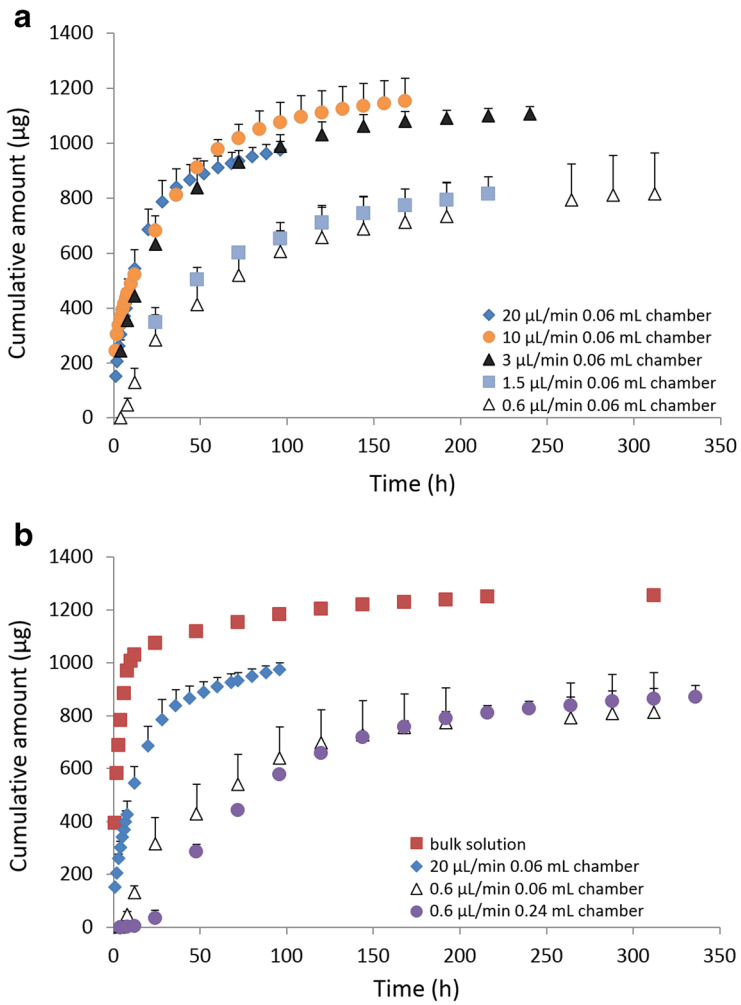

Fig. 5. **a** Effect of flow rates on drug release from PerioChip® in 0.06 mL flow-through dissolution chamber at flow rate 0.6 (open triangles), 1.5 (squares), 3 (closed triangles), 10 (circles), and 20 (diamonds) µL/min. **b** Effect of dissolution system and its volume on drug release from PerioChip®: bulk solution (squares), 0.6 (triangles) and 20 (diamonds) µL/min in the 0.06 mL dissolution chamber, and 0.6 µL/min in the 0.24 mL dissolution chamber (circles). Mean ± SD ($n = 3$–4)

first 12 h, and drug release was ~ 1.5–2× faster between 1 and 2 days. In both cases, trypsin-enhanced drug release approached a plateau value at ~ 4–6 days. With the enzyme, increasing the flow velocity of the dissolution medium by using the 0.04 mL dissolution chamber, which had a smaller cross-section area, resulted in faster drug release than that in the 0.06 mL dissolution chamber. This condition (0.04 mL dissolution chamber and 10 µL/min flow rate) provided the fastest drug release observed in the present flow-through chamber study.

Figure 6b compares the drug release data of PerioChip® in the present study with the *in vivo* data in a canine study (unpublished data (28)) and the data estimated using CHX concentration in the gingival crevicular fluid reported in a human study (29). To calculate the human data, the amounts of drug release were determined using the area under the curve (AUC) of the CHX concentration *vs.* time profile and flow rate under the assumption of a constant fluid flow: i.e., AUC × flow rate. From mass balance, the total amount of CHX release from PerioChip® and the mass of CHX in the product were the same when the flow rate was 0.22 µL/min. Regardless, the percent dose release profile presented in the figure was independent of the flow rate; percent dose release data were calculated from dividing the cumulative amount (cumulative AUC × flow rate) by the total amount (total AUC × flow rate) that the flow rate values in the numerator and denominator cancelled in the calculation. The data in Fig. 6b show that the *in vivo* canine result was comparable to drug release in the bulk solution *in vitro*. The similar drug release profiles observed in the *in vitro* dissolution study of bulk solution and in canine *in vivo* are likely coincidental (see the "Dissolution method for small drug delivery systems" section below). Drug release in the flow-through dissolution chamber at 10 µL/min with trypsin was slower than that in the canine study *in vivo* but faster than that of the *in vivo* human result. The fastest drug release observed in the 0.04 mL dissolution chamber at 10 µL/min with trypsin was still slower than that in bulk solution. At the lower flow rate of 0.6 µL/min with trypsin, the flow-through dissolution chamber data

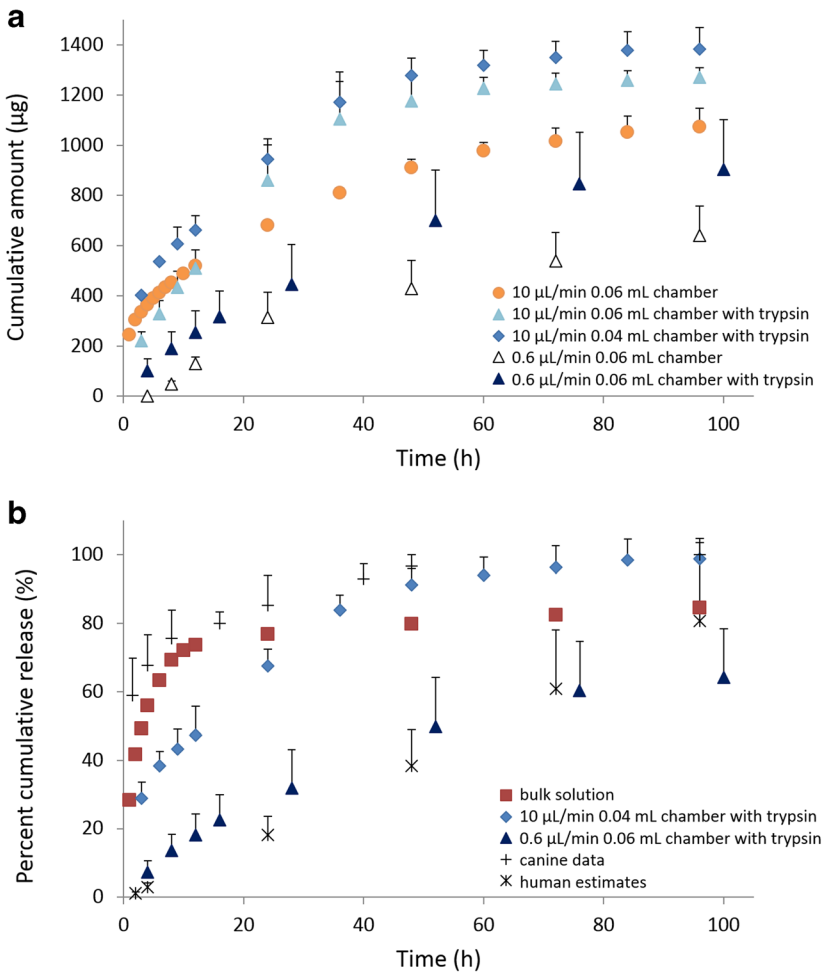

Fig. 6. **a** Effects of enzyme on drug release from PerioChip® in 0.04 (diamonds) and 0.06 mL (closed dark and light blue triangles) dissolution chambers with trypsin at flow rates of 0.6 and 10 µL/min. Drug release data of the 0.06 mL dissolution chamber and flow rates of 0.6 µL/min (open triangles) and 10 µL/min (circles) without trypsin from Fig. 5 are also presented for comparison. **b** Comparison of *in vitro* drug release data of the 0.06 and 0.04 mL dissolution chambers at 0.6 and 10 µL/min with enzyme (triangles and diamonds, respectively) to *in vivo* canine data and human data estimates (crosses and asterisks, respectively). Drug release data of bulk solution (squares) without trypsin from Fig. 5 are also presented for comparison. Mean ± SD ($n = 3$–4). *In vivo* canine data are unpublished data obtained from FDA (28). *In vivo* human data were estimated using gingival crevicular fluid concentration (17,29) and 0.22 µL/min fluid flow rate

were slightly higher and comparable to the *in vivo* human result. Although the comparable results suggest *in vitro-in vivo* correlation for PerioChip® in human, this could be a combination of the effects of the dissolution medium flow rate, enzyme concentration, and fluid dynamics in the dissolution chamber employed in the present study. The observed correlation does not necessarily imply the same fluid flow and enzyme conditions in the flow-through dissolution chamber and periodontal pocket, so the correlation could be product specific.

Dissolution Method for Small Drug Delivery Systems

The use of 3D printing in the construction of dissolution apparatus provides the flexibility of the dissolution chamber design. This device constructing method is particularly useful for the development of the small flow-through dissolution chambers. 3D printing also allows quick and easy manufacturing of the apparatus devices for testing before using the devices in the evaluations of drug products. Although a simple design was used in the present study, more complex designs can be developed and used.

For the testing of new dissolution chambers, the present study used a computer simulation program of a diffusion/convection transport model to evaluate drug concentration profiles in the dissolution chambers under different conditions. The model simulations described the fluid dynamics and drug concentration profiles in the dissolution chambers. The model results can be used to predict the effects of different factors before experimental studies. Thus, this can provide information to design the experiments. This approach can also assist in the investigation of the influencing factors during dissolution testing in drug product evaluations.

To develop a bio-relevant dissolution method for the periodontal pocket, the physiological environment of drug release from the periodontal product should be considered. Drug release from the product in the periodontal pocket is likely under the following conditions *in vivo* (20,25–27,30,31): local pH 7.0–8.7 depending on the extent of inflammation, gingival crevicular fluid components such as collagenase that could degrade the drug product matrix, gingival crevicular fluid flow rate of ~ 0.3–0.5 μL/min, available volume for dissolution ~ 0.4–1.5 μL, and fluid turnover time (volume divided by flow rate) of ~ 1–3 min. The size of the periodontal pocket varies, depending on the disease conditions. For example, PerioChip® is recommended for periodontal pocket with probing pocket depth of 5 mm or more, and a decrease in pocket depth of 2 mm or more in ~ 20% pockets was shown after 9 months of treatment (16). Therefore, periodontal pocket length of ~ 5 mm or greater with cross-sectional area similar to PerioChip® can be assumed. The dissolution method in the present study used the following approach to simulate these *vivo* conditions *in vitro*. The dissolution medium had pH 7.4–8.7 and the simulated saliva had pH 8.0. An enzyme was used in the dissolution medium to digest the gelatin matrix of PerioChip®. Although the 3D-printed dissolution chambers were small, they could still be larger than the volume available for *in vivo* dissolution in the periodontal pocket. Due to the size and swelling of PerioChip®, the smallest flow-through dissolution chamber that was able to maintain a constant fluid flow through the chamber in the drug release study was 0.04 mL. The focus was therefore to increase the flow rate (up to 20 μL/min) in the flow-through chamber to resemble the turnover time of the fluid (i.e., flow velocity across the PerioChip® surface) in the periodontal pocket *in vivo*. Particularly, the available volume in the 3D-printed dissolution chamber for the dissolution medium was approximated by "chamber volume" – "PerioChip® volume" (dry to swollen PerioChip® volume). The turnover time of 10–20 μL/min medium flow in the 0.04 to 0.06 mL dissolution chamber (available volume divided by flow rate) was close to the turnover time in the periodontal pocket *in vivo* (~ 1–3 min).

The manipulation of controlling factors such as flow velocity and dissolution medium enzyme in the present flow-through dissolution system was shown to affect drug release from a periodontal test product, PerioChip®, and this could be used to establish an *in vitro-in vivo* correlation for the evaluation of periodontal drug products. For *in vitro-in vivo* comparison in the present study, drug release in the dissolution chamber (with the flow velocity in the 0.04 mL chamber and 0.3% trypsin) was slower than that in a previous canine study *in vivo*. This can be attributed to the method used in the canine study and the differences in enzymatic degradation under the *in vivo* and *in vitro* conditions. In the canine study, drug release was evaluated by the removal of PerioChip® from the periodontal pocket, measurement of CHX in the collected drug product fragments, and the determination of CHX release by mass balance (i.e., amount of CHX release = total CHX amount in PerioChip® − CHX amount remaining in PerioChip® collected from the periodontal pocket) (28). The gelatin matrix of PerioChip® disintegrated in 2 days in the canine study: only 50% CHX remained in PerioChip® at 1.5 h after implantation, and no CHX was found by 48 h *in vivo*. This is different from the information provided in the PerioChip® product label, which suggests slower enzymatic degradation of PerioChip® in human *in vivo*. Specifically, the concentration of CHX in gingival crevicular fluid peaked at 4 h and remained relatively constant until 72 h (17). In addition, PerioChip® dislodgement that occurs 7 days after placement is considered a full course of treatment and dislodgement within 2 days requires the insertion of a new insert product (16). The PerioChip® product label also advises patients to avoid dental floss at the site of PerioChip® insertion for 10 days after placement because flossing may dislodge the chip. This information implies that PerioChip® does not completely disintegrate within 10 days in human periodontal pocket. The degradation and drug release data of PerioChip® in the present *in vitro* study (degradation of the gelatin matrix in 4–7 days) resemble more of the descriptions in the PerioChip® product label than those in the canine study. Other factors that could result in the accelerated digestion of the drug product in the canine periodontal pocket include physiological conditions such as animal chewing behavior and high saliva flow. In summary, the differences among the observed significant digestion of PerioChip® within 2 days in the canine study, the slower enzymatic degradation and drug release over 10 days in human (PerioChip® product label), and the degradation and drug release data under the *in vitro* conditions in the present study suggest that enzymatic degradation can be an important factor in this type of studies. For example, in the evaluation of drug release from biodegradable drug products, drug products of similar drug release profiles in dissolution experiments without an enzyme could show different drug release profiles in the presence of the enzyme, e.g., when the drug products have different responses to enzymatic digestion.

CONCLUSION

A flow-through dissolution system was constructed for sustained release drug products that are used in an environment of small space. The influencing factors on drug release in the dissolution system were investigated, so that drug release could be evaluated in the dissolution system by modifying these factors. Drug release experiments were performed using the 3D-printed flow-through chambers (chamber volume as small as 0.04 mL) and a periodontal sustained release product PerioChip®. Drug release from PerioChip® in the flow-through dissolution chambers was affected by chamber volume, dissolution medium flow rate, and enzymatic digestion of the product. Under the conditions studied, dissolution medium flow rate and enzymatic digestion were found to be the major factors. The presence of trypsin (0.3%) in the dissolution medium enhanced drug release from PerioChip® in the flow-through chambers. Dissolution medium flow rate affected both drug clearance from the dissolution chambers and drug release profiles from PerioChip® (e.g., via its effect on the unstirred aqueous boundary layer on the drug product surface). Computer model simulations and experimental results indicated that drug clearance from the dissolution chambers was fast and not a major factor; drug release from PerioChip® was significantly slower than drug clearance from the flow-through chamber. The similar drug release profiles observed at the higher flow rates (e.g., 10 and 20 μL/min) suggest that the effect of aqueous boundary layer was small and drug release from PerioChip® was the rate-determining step under these conditions. However, drug release in

the flow-through chamber at the highest flow rate studied was slower than drug release in bulk solution in a vial. The flow-through chamber provided drug release comparable to or slightly faster than that in human *in vivo* under the conditions studied. On the other hand, drug release in the flow-through chamber was slower than that observed in a previous *in vivo* canine study, which could be due to the difference in enzymatic digestion of PerioChip® *in vivo* and *in vitro*. The present results suggest the importance of enzymatic degradation in the drug release study and the need of an appropriate enzyme in the dissolution medium in the evaluation of biodegradable sustained delivery products in the flow-through dissolution chamber study.

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