Poster Number 11T0900

Application of USP 4 Dissolution Apparatus to Assess Dissolution of Microparticles for Periodontal Disease

Sravan Kumar Patel[1], Ashlee Greene[1], Sam Rothstein[2], Yuan Zou[3], Stephanie Choi[3], Andrew Glowacki[1], Riccardo Gottardi[1], Charles Sfeir[1], Steven Little[1], Lisa Rohan[1]
[1]University of Pittsburgh, [2]Qrono Inc., [3]U.S. Food and Drug Administration

2016 AAPS ANNUAL MEETING AND EXPOSITION
NOVEMBER 13–17, 2016
COLORADO CONVENTION CENTER, DENVER
aaps

PURPOSE

Local delivery of antimicrobials has been shown to be advantageous in the treatment of periodontal disease. Arestin® is a microparticle formulation consisting of minocycline hydrochloride, marketed for local application in periodontal disease. Currently, there is no compendial level dissolution method for periodontal microparticles that can differentiate performance of different formulations and demonstrate bioequivalence. In an effort to develop a robust dissolution method, a range of comparators that mimic Arestin® were prepared and their dissolution profiles were determined using a modified USP 4 apparatus (Fig. 1).[1]

The ultimate goal of this work is to develop a dissolution method that can delineate the effect of processing parameters and formulation changes on product dissolution and can be utilized for quality control and bioequivalence testing of formulations for delivery to the periodontal pocket.

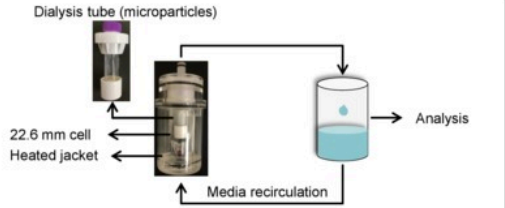

Fig. 1. Schematic of modified USP 4 dissolution set up for microparticle analysis. A single cell insert with dialysis tube is shown. Microparticles dispersed in dissolution medium are placed in the dialysis tube.

METHODS

- Acid terminated PLGA microparticles containing minocycline hydrochloride were prepared by a single emulsion method.
- Using a fractional factorial design, six microparticle products were formulated that varied in the type of organic solvent (ethyl acetate vs. dichloromethane), stirring speed (500, 1000, and 1500 rpm), and PLGA to solvent ratio (0.1, 0.0625, and 0.025).
- The resulting products were evaluated for size, morphology, and drug content.

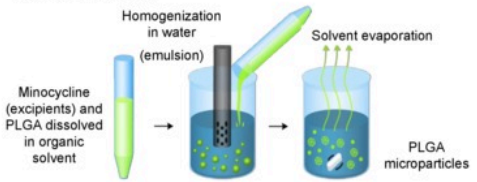

Fig. 2. Single emulsion fabrication method

METHODS

Dissolution

- Dissolution was carried out in a modified USP 4 flow-through apparatus under closed loop configuration (Fig. 1). In order to separate particles from media, dialysis tubes with cellulose membrane (50 kDa MWCO) were loaded with microparticles dispersed in phosphate buffered saline (PBS, 1X). Using 22.6 mm cells that would accommodate dialysis tubes, dissolution studies were performed at 37 °C, 10 mL/min flow rate in PBS. The released drug was analyzed online using a UV-Vis spectrophotometer.
- Proprietary software, QronoMetrics™ (Qrono Inc.), was used to predict drug release based on the physicochemical properties of the drug, polymer, and particles.
- Towards developing a biologically relevant media for assessing periodontal products' dissolution for in vitro-in vivo correlation, a gingival crevicular fluid (GCF) simulant was developed.

GCF Simulant Preparation Method

- Mix and dissolve all components except calcium chloride dihydrate
- Adjust pH to 7.0
- Add calcium chloride dihydrate solution (in water)

Table 1. GCF simulant composition

Component	Amount/L
0.1 M citric acid monohydrate	16 mL (0.34 g)
0.1 M trisodium citrate dihydrate	184 mL (5.4 g)
Sodium chloride	9.0 g
Potassium chloride	0.7 g
Bovine serum albumin	0.054 g
Imidurea	0.5 g
Calcium chloride dihydrate	0.3 g

Table 2. Comparison of human GCF and simulant

Component/Attribute	Human[2]	Simulant
Sodium (mEq/L)	174.7±18	172.3
Potassium (mEq/L)	9.54±2.4	9.38
Calcium (mEq/L)	5.41±0.37	5.40
Total protein (g/L)	0.05	0.054
pH	7.2 (inflamed)	7.0
Osmolality (mOsm/L)	-	357

RESULTS

- Particles prepared in ethyl acetate (EA) showed an overall larger particle diameter than those prepared in dichloromethane (DCM) as shown by coulter counter measurements. However, SEM imaging indicated both solvents resulted in particles with similar surface morphology.
- A lower drug content was also observed with particles prepared in EA.

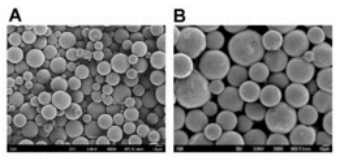

Fig. 3. SEM of a (A) comparator product vs. (B) Arestin®

Table 3. Panel of comparators

PLGA:Solvent	Solvent	Stir speed (rpm)	Particle size (μm)	Drug loading
0.025	DCM	1500	28.05±9.289	0.088
0.025	EA	500	39.05±17.11	0.028
0.0625	DCM	1000	49.57±15.54	0.098
0.0625	EA	1000	51.85±19.61	0.074
0.1	DCM	500	35.65±13.30	0.103
0.1	EA	1500	39.69±15.43	0.081

- Dissolution performed for 3 days showed complete drug release. Arestin® showed a biphasic release with an initial burst release, a lag phase, and a second burst release (Fig. 4).
- Comparator products 1 and 2 prepared in DCM at 500 and 1000 RPM respectively, however, did not exhibit a lag phase.

RESULTS

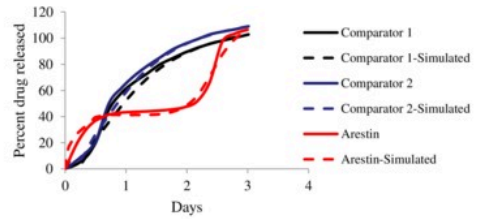

Fig. 4. Dissolution of Arestin® and comparators

- To qualify the application of this dissolution method, proven software for prediction of drug release from PLGA microparticles, QronoMetrics™, was applied. The predicted dissolution differences between Arestin® and two representative comparator products were clearly correlated with the observed dissolution using USP 4 apparatus (Fig. 4).
- Arestin® dissolution in GCF simulant showed lower (~60% in 4.6 days) drug release compared to PBS. Method optimization is in-progress.

CONCLUSIONS

- High similarity was observed between dissolution profiles for minocycline microparticles obtained using a USP 4 apparatus and in silico predictions obtained using the QronoMetrics™ software.
- Future work will focus on quantitative mechanistic models to predict and validate drug dissolution of periodontal microparticles.
- Furthermore, this method will be applied to obtain dissolution profiles for bioequivalence studies based on in vitro-in vivo correlations.

REFERENCES / FUNDING/Disclaimer

1. Bharadwaj, U., Burgess, D.J. A novel USP apparatus 4 based release testing method for dispersed systems. *Int J Pharm.* 388: 287-294, 2010.
2. Bang, J., Cimasoni, G., Rosenbusch, C., and Duckert, A.: Sodium, Potassium and Calcium Contents of Crevicular Exudate: Their Relations to Gingivitis and Periodontitis. *J Periodontol.* 49: 770-774, 1973.

- This work is funded by U.S. Food and Drug Administration (Grant Award 1U01FD005447-01).
- Disclaimer: This poster reflects the views of the authors and should not be construed to represent FDA's views or policies.

Magee-Womens Research Institute

University of Pittsburgh