Sustained Release of Antiviral Agent GS-441524 from *In Situ* Depots for COVID-19 Treatment

Divya Muthusamy[1,2], Varunaa Hemanth[1], Tao L. Lowe[1,2]

[1]Fischell Department of Bioengineering, A. James Clark School of Engineering, University of Maryland, College Park, MD
[2]Department of Oral and Maxillofacial Surgery, School of Dentistry, University of Maryland, Baltimore, MD

Statement of Purpose: As of October 2021, over 242 million people have been diagnosed with COVID-19 cases and over 4.9 million of those cases resulted in mortality.[1] Vaccination has significantly impacted the COVID-19 epidemic in reducing cases and deaths.[2] However, 60% of the global population is still unvaccinated and at high risk of COVID-19 infection and hospitalization.[1,2] Remdesivir, a nucleoside antiviral agent, has shown effect in reducing ventilation time and hospitalization and has been approved by FDA for emergency use for COVID-19 patients.[3,4] GS-441524, a metabolite of Remdesivir, has similar antiviral effect as Remdesivir with a much lower manufacturing cost.[5,6] However, GS-441524 has a half-life of about 25 h, and thus it is important to develop a drug delivery system to sustain release GS-441524 to treat viral diseases.[3,5] The focus of this work is to develop an injectable *in situ* gelling system consisting of FDA approved biodegradable materials for sustained release of GS-441524 that can be used to treat COVID-19 and other viral diseases.[7]

Materials and Methods:

1) Formulation Preparation: Depots are formulated by dissolving 24 wt% PLGA 50/50, 75/25, 85/15 and a blend of PLA with varying inherent viscosities in N-n-methyl pyrrolidone (NMP) and triethyl citrate (TEC). Subsequently 6 wt% of GS-441524 is dissolved separately combined with each polymer solution to form the final three depot formulations.
2) Viscosity of Formulations: Viscosity of the polymeric formulations were measured using a 20 mm parallel plate on a dynamic hybrid rheometer (DHR30, TA Instruments).
3) GS-441524 Release Study: Depots are formed by injecting 80 µL of the polymeric solution into 4 mL of PBS (pH 7.4), and kept at 37 °C under stirring at 50 rpm for 70 days. Surrounding media is collected daily for the first week, and intermittently afterwards. Following collection, samples are filtered through 0.2 µm syringe filtration and analyzed using RP-UPLC-UV (Waters Acquity UPLC® H-Class and Acquity BEH C18 Column) to quantify the amount of corresponding drug release from each sample. A gradient method was developed with mobile phases 0.1% Formic Acid in Acetonitrile (Fisher Scientific) and 0.1% Formic Acid in DI Water, and detection was determined as 241 nm following UV spectrum scanning (200-400 nm).

Results: The viscosities of the polymer formulations containing PLGA 50/50 75/25 and 85/15 were 1.25, 1.80, and 2.40 Pa*s, respectively. All 3 formulations of depots exhibit sustained release of GS-441524 as shown below for 70 days.

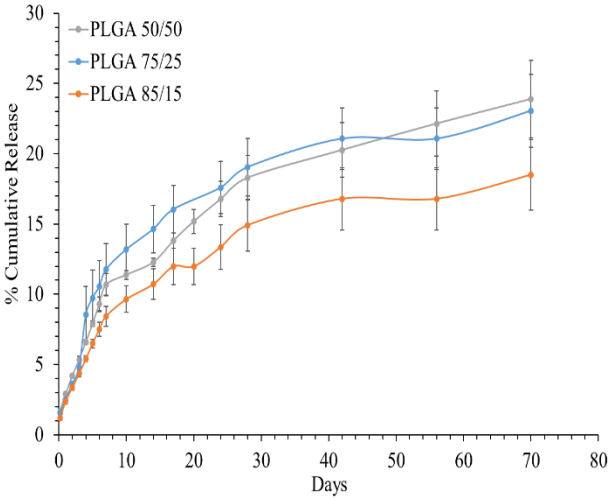

Figure 1. *In-vitro* cumulative release of three depot formulations (n=4) loaded with GS-441524 over 70 days.

Release profiles exhibit zero order release kinetics initially, followed by higher order release kinetics after 1 week. Formulation dependent release kinetics are presented throughout the duration evaluated. PLGA 85/15 exhibits lower cumulative release over time than the other formulations, while PLGA 50/50 and 75/25 exhibit similar cumulative release. Corresponding to viscosity, the formulation with highest viscosity, PLGA 85/15, exhibits the lowest cumulative release thus far.

Conclusions: Herein we demonstrate a sustained release of antiviral agent GS-441524 from our *in situ* gelling system. Future directions include evaluating depot formulations *in vivo* for determining biotherapeutic effect against COVID-19. Successful development of *in situ* depots with GS-441524 and Remdesivir will provide progress towards an accessible treatment option for infected patients and an understanding of therapeutic efficacy of anti-viral therapy in a sustained release dosage form for COVID-19.

References:

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