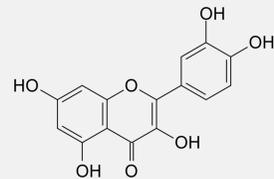


Introduction: This project aims to increase quercetin bioavailability and antiviral activity against cytomegalovirus (CMV). CMV is the leading viral cause of birth defects in the U.S. Ganciclovir, the current treatment for CMV infection, exhibits cytotoxicity in pregnant women and other immunocompromised populations. Quercetin is a naturally derived compound with antioxidant, anti-cancer, antiviral and anti-inflammatory properties and exhibits anti-CMV activity (Cotin, 2012). Low solubility and rapid metabolic breakdown result in low bioavailability, however (Thilakarathna, 2013). Pluronic-F127 capped poly(lactic-co-glycolic acid) (PLGA), and chitosan-capped alginate microbeads are investigated as microcarriers to provide time-release antiviral treatment for congenital CMV. Quercetin, PLGA, chitosan, alginate, and Pluronic F-127 are generally recognized as safe (GRAS) compounds by FDA standards, making them viable alternatives to Ganciclovir during pregnancy and in newborns.

Objectives

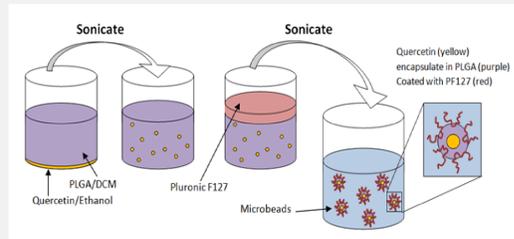
- Create PLGA and alginate microbeads to encapsulate and deliver quercetin at therapeutic levels
- Test the effectiveness of these carriers at loading quercetin and inhibiting CMV in a mouse cell model.



Molecular Structure of quercetin

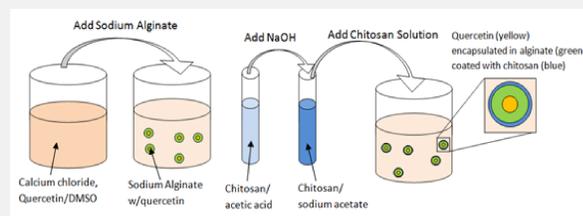
Materials and Methods

PLGA Bead Preparation



Quercetin-loaded P-F127/PLGA beads were synthesized using a double-emulsion process (Tefas, 2015). These beads increase solubility as well as bioavailability by protecting quercetin against metabolic breakdown.

Alginate-Chitosan Bead Preparation



Quercetin-loaded alginate/ chitosan beads were synthesized by a critical concentration gelling method then capped by chitosan to provide stability and particle strength (Li, 2008).

Release Kinetic Test

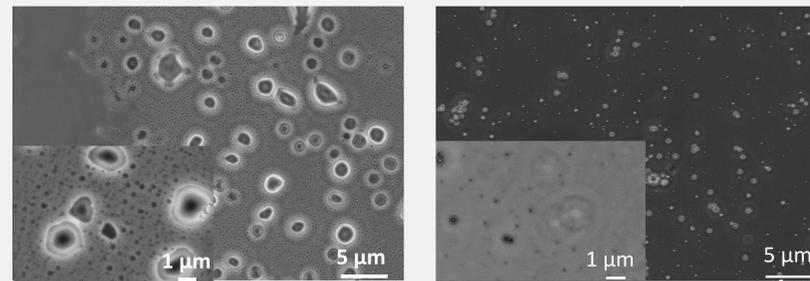
PLGA and alginate beads were separated from free quercetin using centrifugation. The beads were then re-suspended in PBS (Phosphate Buffered Saline.) The release rate of quercetin was determined by taking samples of the supernatant and using UV/Vis absorbance to determine the concentration of quercetin. The process was then repeated at regular time intervals.

Viral Assay

A viral assay was performed to compare cytotoxicity and viral inhibition for quercetin, Ganciclovir, quercetin-loaded microcarriers, and unloaded microcarriers. Varying concentrations of each treatment were tested against virally infected cells, post infection. After 48 hours infected and healthy cells were counted to determine viral inhibition. Cells, without virus were also challenged by each treatment in varying amounts. After 48 hours a viable cell count was done to determine the cytotoxicity of each treatment.

Results

Bead Sizing and Imaging

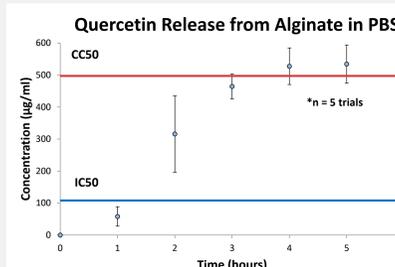


Loaded alginate-chitosan microbeads
Mean diameter 2.57 μm ± 0.34 μm.

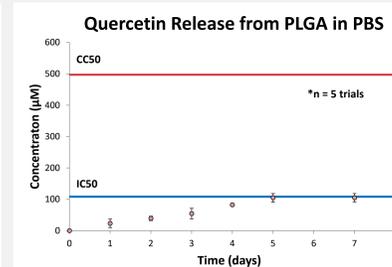
Loaded PLGA microbeads
Mean diameter 243 ± 127 nm

Loading Efficiencies and Kinetic Release Rates

The maximum loading achieved for the PLGA microbeads was 0.3% w/w quercetin to PLGA. The maximum loading of alginate-chitosan microbeads was 8.7% w/w quercetin to alginate-chitosan.



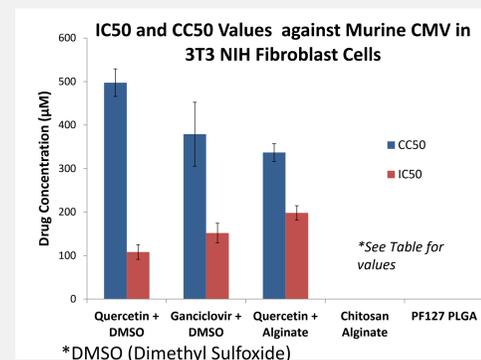
Alginate-chitosan beads release ~50% of the encapsulated quercetin in the first 5 hours. This is supported by the data below.



PF127-PLGA beads release only ~20% of the encapsulated quercetin after 5 days. To inhibit the virus ~10.5 mg/ml of PF127-PLGA beads are needed.

Antiviral and Cytotoxicity Assay

IC50 and CC50 indicate the concentration at which 50% of the virus is inhibited, or 50% of the cells are compromised.



Compound	CC50 (μM)
Quercetin/DMSO	497 ±32
Ganciclovir/ DMSO	379 ±74
Quercetin/Alginate	336 ±20
Alginate-Chitosan	> 1400
PF127-PLGA	> 1400

Compound	IC50 (μM)
Quercetin/DMSO	108 ±17
Ganciclovir/ DMSO	152 ±23
Quercetin/Alginate	198 ±16
Alginate-Chitosan	n/a
PF127-PLGA	n/a

Discussion

The slow release rate and low loading of quercetin in PF127-PLGA microbeads would not allow for an inhibitory concentration of quercetin to be achieved without an unrealistic dosage of PLGA beads (>10 mg/mL). In order for PLGA to be used as viable delivery vehicle, the encapsulation methods would need to be tuned to allow for a greater quercetin to PLGA ratio so that a therapeutic level of quercetin can be achieved without reaching an unrealistic level of PLGA .

Quercetin loaded chitosan-alginate beads exhibited delivery of quercetin at a therapeutic level within hours. These beads will require further tuning to lengthen release time. Such treatment would also require daily doses to maintain a therapeutic level due to the relatively short biological half life of quercetin.

Conclusion

- Congenital cytomegalovirus infection is a significant problem in the US, and the development of polymeric microparticles for the encapsulation of quercetin is a viable treatment option in lieu of currently available antivirals.
- This new form of treatment will be much safer, especially for pregnant women and other immunocompromised groups because it is less toxic than Ganciclovir.
- The use of alginate beads has the potential for a steady, controlled release of quercetin at therapeutic levels after an initial, immediate dose.
- Moving forward, quercetin-loaded PLGA and alginate beads will need to be synthesized that either have a higher loading efficiency, or a more favorable release rate.

Acknowledgements

Albert Park, MD – University of Utah Otolaryngology/Pediatric
 Craig Day, PhD – Institute of Antiviral Research
 Tom Chang, PhD – Utah State University Professor
 USU Undergraduate Students: Jared Madsen, Elizabeth Sherman
 USU Graduate Student: Abul Bashar Giasuddin Shameem

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