The Development of Minocycline-Loaded Microparticles and Injectable Alginate Scaffolds Composites to Prolong Release for The Treatment of Glioblastoma

Samantha C. Davila[1], Dorina A. Madrid[1], Florestella Rivera[1], Jorge De Leon [1], Sebastian Flores[1], Serenade Trevino[2] , and Sue Anne Chew [1]

Department of Health and Biomedical Sciences, University of Texas Rio Grande Valley, One West University Blvd., Brownsville TX 78520

The University of Texas Rio Grande Valley — Department of Health & Biomedical Sciences

Introduction

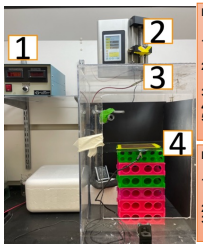
- Glioblastoma multiforme (GBM), also known as a Grade IV astrocytoma, is a highly common and aggressive malignant brain tumor [1] with poor prognosis, on average <15 months after diagnosis.
- GBM tumors supply themselves through angiogenesis – the process developing new blood vessels for growth (↑ recurrence rate) [1].
- Current treatment include tumor resection, chemotherapy, radiotherapy [2].
- Minocycline, a commonly used antibiotic, has anti-angiogenic properties and can be used to prevent GBM reoccurrence [3].
- Electrospraying is an effective method of fabricating microparticles due to its affordability, high scalability, and high entrapment efficiency [5]
- Alginate is a biocompatible polymer that can be utilized to fabricate injectable scaffolds for non-invasive delivery of drugs at tumor site [2].

Objective: This study investigates the release kinetics from MINO-loaded microparticles (MPs), MINO-loaded alginate scaffolds, and MINO-loaded MPs in alginate scaffolds for their potential for GBM treatment.

Hypothesis: The combination of microparticles and injectable alginate scaffolds will prolong the release of the therapeutic agents and allow better control and tailoring of the release kinetics compared to using only one biomaterial.

Methods

Microparticle Fabrication and Studies



Electrospraying

1. **Prepare drug/polymer solution** and load into glass syringe
2. **Spray solution** using corresponding parameters and collect in glass plate
3. **Incubate** (37°C)glass plates for 72 hrs.
4. **Collect and freeze** particles for 24 hrs.
5. **Ready** for next studies.

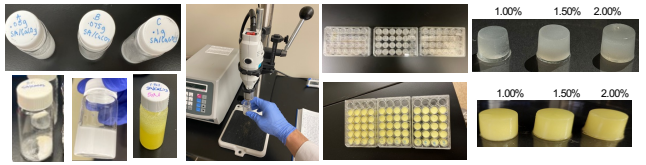
Drug Loading and Loading Efficiency

1. **Weigh triplicate** samples for each microparticle group (2mg/vial)
2. **Add 1ml of DMSO** to each vial and vortex
3. **Plate** 200 µL/well in triplicates and **read absorbance** in spectrophotometer (350nm Mino)

Cell Viability

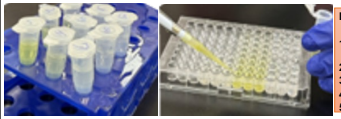
1. **Plate** a 96-well plate and prepare the treatments.
2. **Treat the cells**, with the specific calculations for every percentage and incubate for 72 hrs.
3. **Add MTT solution** after aspirating.
4. **Incubate the cells** with MTT solution for 3 hours to form formazan crystals.
5. **Add DMSO** to each well after aspirating to dissolve crystals.
6. **Read the plate** in spectrophotometer at 570nm.

Scaffold Fabrication and Studies



Dissolve SA 2.00% w/v at a 1:1 SA:Ca2+ molar ratio with GDL prepared at a GDL:CaCO3 molar ratio of 1.5 in 1 mL ddH2O → Homogenize before & after adding GDL and drug if needed in SA/CaCO3 → Pipette homogeneous scaffold solution to their respective molds → Allow scaffolds to gel

Scaffold + Microparticle Studies



Drug Release Kinetics

1. **Add 1x DPBS media** to the drug loaded groups (MP's, Scaffold, S+MP's) tubes in **and incubate**
2. **Centrifuge** all tubes at respected timepoints (1,2,3,7,10,14)
3. **Pipette triplicates** of samples into 96-well plate
4. **Read absorbance** using spectrophotometer
5. **Change DPBS**, and incubate

Results

Previous Studies- Microparticles

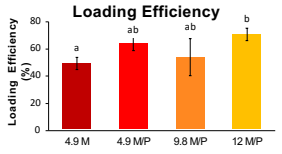


Figure 1. Loading Efficiency of microparticles fabricated with different drug amount and presence of PEG. Groups with different lowercase letters indicate significant difference from one another, p-value < 0.05.

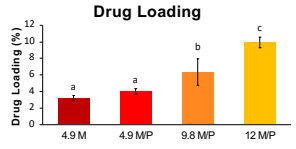


Figure 2. Drug loading of microparticles fabricated with different drug amount and presence of PEG. Groups with different lowercase letters indicate significant difference from one another, p-value < 0.05.

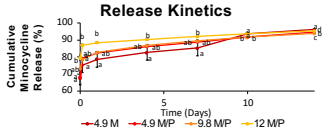


Figure 3. Release kinetics of microparticles fabricated with different drug concentrations and presence of PEG. Groups with different lowercase letters indicate significant difference from one another. P value < 0.05.

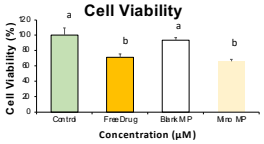


FIGURE 4. Cells viability of 4 groups: Control , Free drug Mino, Blank MP Mino and Mino MP. Groups that showed no significance to the control were free drug and mino MP Groups with different lowercase letters indicate significant difference from one another, p-value < 0.05.

Previous Studies- Scaffolds

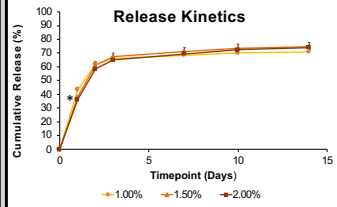


Figure 5. Minocycline cumulative drug release of scaffolds with different SA concentrations (1.00, 1.50 and 2.00% w/v) at a 1:1 SA:Ca2+ molar ratio with GDL prepared at a GDL:CaCO3 molar ratio of 1.5. Results are demonstrated as mean ± standard deviation for n=3. Groups with * denotes significantly different from 1.00

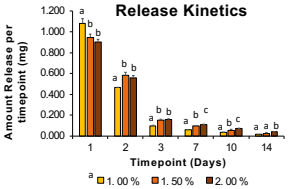


Figure 6. Amount of minocycline drug release of scaffolds with different SA concentrations (1.00, 1.50 and 2.00% w/v) at a 1:1 SA:Ca2+ molar ratio with GDL prepared at a GDL:CaCO3 molar ratio of 1.5. Results are demonstrated as mean ± standard deviation for n=3. Groups with different lowercase letters (i.e. a vs. b vs. c) are significantly different (p< 0.05) from one another

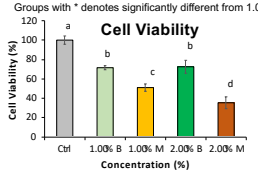


Figure 7. Cell viability of Scaffolds with different SA concentrations (1.00, and 2.00% w/v) at a 1:1 SA:Ca2+ molar ratio with GDL prepared at a GDL:CaCO3 molar ratio of 1.5. Results are demonstrated as mean ± standard deviation for n=12. Groups with different lowercase letters (i.e. a vs. b vs. c vs. d) are significantly different (p< 0.05) from one another

Combination of Scaffold and Microparticles

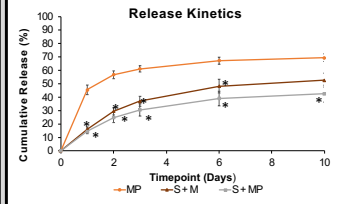


Figure 8. Minocycline cumulative drug release of MP's and 2% scaffolds (drug loaded, drug loaded MP) Results are demonstrated as mean ± standard deviation for n=2. Groups with * denotes significantly different from MP

Discussion

Microparticles

- The addition of PEG to minocycline-loaded microparticles led to higher drug loading and loading efficiency **(Figure 1 & 2)**
- Higher drug loading was observed as more minocycline was added to the microparticles **(Figure 2)**
- The incorporation of PEG increased drug loading, since PEG allows for higher amounts of minocycline to be dissolved
- All four groups of microparticles demonstrated a burst release of minocycline during the 1st hour **(Figure 3)**
- The 12 M/P group showed a to have a statistically higher minocycline release than 4.9 M from the 1-hour timepoint to the 7 days timepoint **(Figure 3)**
- The Blank MP were not toxic to the cells, and the drug-loaded MP was able to result in significantly higher cell death compared to the control and blank MPs, showing that the cell viability is lower when the minocycline is present. **(Figure 4)**

Scaffolds

- At Day 1, 1.00 wt./vol.% scaffolds showed a significantly higher burst release compared to all other groups. (**Figure 5 & 6)**
- The lower crosslinking in 1.00 wt./vol% could have resulted in the increase release.
- From Day 7 to 14, 2.00 wt./vol.% scaffolds showed a significantly higher amount of release compared to 1.00 and 1.50 wt./vol. % scaffolds. **(Figure 5 &6)**
- Minocycline loaded scaffolds resulted in increased cell death compared to the control and blank scaffold release solution treatments with 2.00 wt./vol.% scaffolds being more effective in ↓ cell viability. **(Figure 7)**

Combination of Scaffold and Microparticles

- With a 45% release on Day 1, MP's alone demonstrated the highest initial burst release and the highest cumulative release compared to the, MINO-loaded scaffolds and MINO-loaded MP scaffolds (16%). **(Figure 8)**
- On Day 2, 3, and 6, MINO-loaded scaffolds released significantly more drug compared to MINO-loaded MPs and MINO-loaded MP scaffolds had a trend of having a lower % of drug cumulative release compared to MINO-loaded scaffolds. **(Figure 8)**

Conclusions

- The 9.8 MP group with 3.5 mg of PEG yielded high drug loading and loading efficiency, since PEG allows more minocycline to be dissolved as seen in a solubility study and resulted in cell death compared to control and blank MPs.
- An increase in SA concentration led to more crosslinking interactions resulting in a tighter matrix and lower burst release of the drug.
- Minocycline MPs alone yielded the highest cumulative release compared to both MINO-loaded scaffolds and the MINO-loaded MP scaffolds.
- MINO-loaded MP scaffolds had a trend of being able to better sustain the release of drug compared to MPs alone and MINO-loaded scaffolds.
- The alginate scaffolds combined with drug loaded microparticles may be a promising adjuvant therapy for GBM treatment as they can be used as delivery vehicles for anti-cancer drugs like minocycline with less of a burse release than drug loaded MPs alone.
- Future studies will focus on investigating further the drug release kinetics of the drug loaded MP's scaffolds and completing cell viability studies with a higher concentration of drug.

Acknowledgements

This project is supported by the National Institute of General Medical Sciences of the National Institutes of Health (1T34GM1378540 and 1SC3GM135138) and UTRGV Engaged Scholar and Artist Awards (ESSA).

References

[1] Hanif et al. *Asian Pacific Journal of Cancer Prevention*. (2017).
[2] Yan et al. *Materials Science and Engineering*. (2016).
[3] Tamargo, R J et al. *Cancer research*. (1993).
[4] Maxwell et al. *Society for Biomaterials*. (2022).
[5] Zhang, T et al. Drug Delivery. (2021).