The Fabrication of Electrosprayed Minocycline-Loaded PLGA Nanoparticles for the Treatment of Glioblastoma

Sebastian Flores[1], Jorge De Leon[1], Florestella Rivera[1], Manoj Chhetri[2], Karen S. Martirosyan Ph.D.[2], Sue Anne Chew Ph.D.[1]

[1]Department of Health and Biomedical Sciences, One West University Blvd., Brownsville TX 78520

[2]Department of Physics and Astronomy, One West University Blvd., Brownsville TX 78520

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

Introduction

I. 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 12 to 18 months after diagnosis [2].

II. A derivative of tetracycline, minocycline (Mino) has been shown to have antiangiogenic qualities that reduce tumor development, making it an effective therapy for GBM. [3].

III. The FDA has authorized polylactic-co-glycolic acid (PLGA). Poly lactic-co-glycolic acid (PLGA) is an FDA approved biomaterial that is used for its ability to encapsulate drugs via electrospraying and for its biodegradability, biocompatibility, and versatility [5].

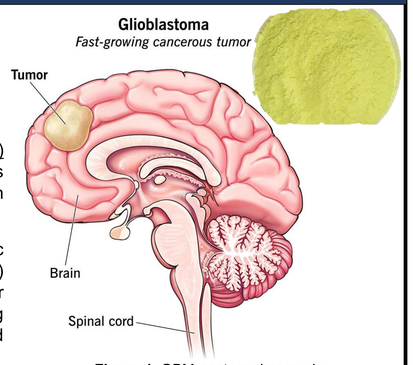


Figure 1. GBM anatomy image via https://my.clevelandclinic.org/health/diseases/17032-glioblastoma; Minocycline Hydrochloride, an antiangiogenic powder used for drug loading on nanoparticles.

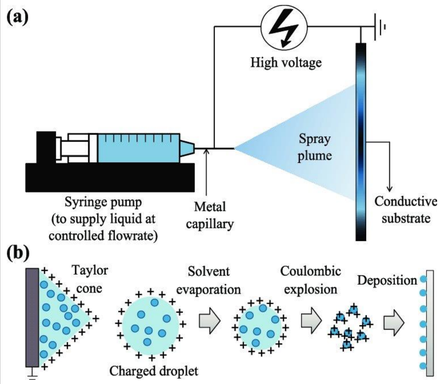


Figure 2. Electrospraying set up for nanoparticles fabrication, with focus on Taylor cone presence, image via https://www.researchgate.net/figure/a-Basic-electrospraying-setup-and-b-Electrospraying-mechanism_fig1_320132877

IV. Electrospraying is an effective method of fabricating particles due to its affordability, high scalability, and high entrapment efficiency [6].

V. Promising areas of application include drug delivery and reducing drug degradation. Due to their small size, nanoparticles interact more efficiently with cells and can be readily taken up through endocytosis, making them effective carriers for delivering drugs to specific cells or tissues within the body. The particles also prolong drug release, increasing cytotoxicity. [7].

Objective and Hypothesis

Objective: To optimize spraying parameters for electrosprayed minocycline-loaded poly lactic-co-glycolic acid (PLGA) nanoparticles with optimal collection and morphology for the treatment of glioblastoma.

Hypothesis: Electrospraying parameters such as the needle-to-collector distance, voltage, flow rate will influence nanoparticle collection and morphology.

Methods

1. Prepare drug/polymer solution and load into glass syringe

BLANK	MINO-LOADED
Minocycline: 0 mg Poly Lactic-co-Glycolic Acid (PLGA): 25 mg Solvent: Dichloromethane: Methanol (700 μL: 300 μL)	Minocycline: 3.5 mg Poly Lactic-co-Glycolic Acid (PLGA): 25 mg Solvent: Dichloromethane: Methanol (700 μL: 300 μL)

2. Spray solution using corresponding parameters onto foil/copper plate
3. Incubate for 36 hours and begin collection process
4. Utilizing spatula, gently lift nanoparticles with round-circle techniques
5. Freeze particles for a day and image using scanning electron microscope (SEM)

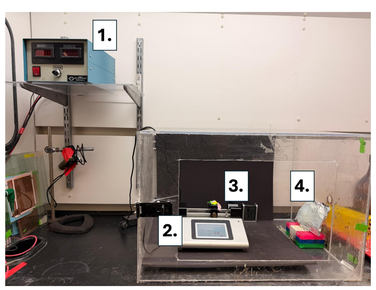


Figure 3. Horizontal Electrospraying Set-Up
1.Voltage
2. Pump
3. Syringe
4. Collection Plate: Foil/copper plate

Results

Blank Nanoparticles

Blank Nanoparticle Spraying Parameters					
Mino (mg)	PLGA (mg)	Solvent Type	Flow Rate (mL/hr, mL/min)	Voltage (kV)	Distance (cm)
0	25	DCM:Methanol (70:30)	0.48 mL/hr, 0.008 mL/min	10	12

Figure 4. Blank nanoparticle electrospraying parameters.

SEM imaging

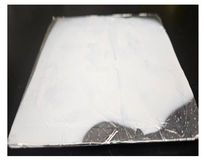


Figure 5. Electrosprayed blank solution onto foil/copper plate

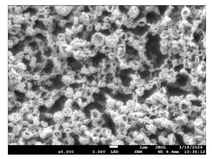


Figure 6. SEM images of electrosprayed Blank nanoparticles.

MINO-Loaded Nanoparticles

Initial: MINO-Loaded Nanoparticle Spraying Parameters					
Mino (mg)	PLGA (mg)	Solvent Type	Flow Rate (mL/hr, mL/min)	Voltage (kV)	Distance (cm)
3.5	25	DCM:Methanol (70:30)	0.48 mL/hr, 0.008 mL/min	10	12

Figure 7. Initial: MINO-loaded nanoparticle electrospraying parameters.

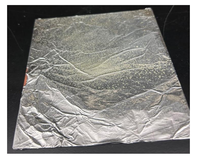


Figure 8. Electrosprayed MINO-loaded solution onto foil/copper plate: 10kV

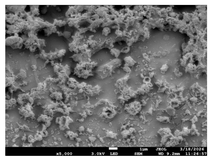


Figure 9. SEM images of electrosprayed nanoparticles loaded with MINO drug.

Voltage

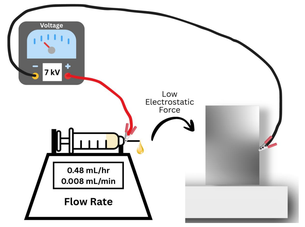


Figure 10. Diagram demonstrating effects of ***low voltage (10kV)*** for troubleshooting of MINO-loaded nanoparticle fabrication.

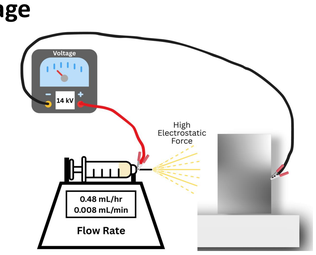


Figure 11. Diagram demonstrating effects of ***high voltage (14 kV)*** for troubleshooting of MINO-loaded nanoparticle fabrication.

Distance

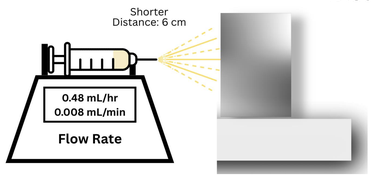


Figure 12. Diagram demonstrating effects of ***shorter distance (6 cm)***for troubleshooting of MINO-loaded nanoparticle fabrication.

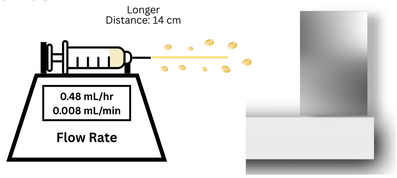


Figure 13. Diagram demonstrating effects of ***longer distance (14 cm)*** for troubleshooting of MINO-loaded nanoparticle fabrication.

Flow Rate

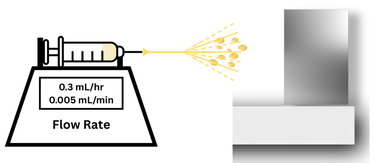


Figure 14. Diagram demonstrating effects of ***slower flow rate (0.3 ml/hr)*** for troubleshooting of MINO-loaded nanoparticle fabrication.

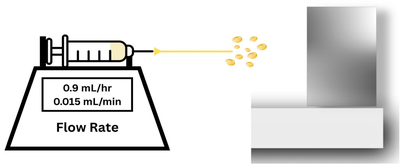


Figure 15. Diagram demonstrating effects of ***faster flow rate (0.9 ml/hr)*** for troubleshooting of MINO-loaded nanoparticle fabrication.

MINO-Loaded Nanoparticles

Final: MINO-Loaded Nanoparticle Spraying Parameters					
Mino (mg)	PLGA (mg)	Solvent Type	Flow Rate (mL/hr, mL/min)	Voltage (kV)	Distance (cm)
3.5	25	DCM:Methanol (70:30)	0.48 mL/hr, 0.008 mL/min	14	12

Figure 16. Final: MINO-loaded nanoparticle electrospraying parameters.



Figure 17. Electrosprayed MINO-loaded solution onto foil/copper plate: 14kV.

Discussion

Electro hydrodynamics

- Minocycline increases solution viscosity and intermolecular interactions (H- bonding with PLGA ester groups).
- This destabilized the jet, resisting droplet breakup and suppressing cloud formation. **(Figure 8)**
- Addition of minocycline resulted in small, wet-droplet deposition, introducing capillary forces and pressing the droplets against the surface. **(Figure 8)**
- Components of solvent (DCM/MeOH) wets the surface if in-flight evaporation is incomplete and spreads out on the foil, maximizing the contact area. This leads to better surface conformity, further strengthening Van der Waals interactions.
- When dissolved PLGA lands during wet deposition, the polymer also conforms to surface roughness, creating mechanical interlocking.
- After wet deposition, the droplets solidify and permanently adhere to the surface, leading to particles that are unable to be collected for future studies without mechanical force or solvent re-dissolution. **(Figure 8)**

Voltage

- Minocycline increases solution conductivity, enhancing charge density but its viscosity reduces charge mobility, making jet formation more difficult. **(Figure 11)**
- Higher voltage (14kV) needed in the minocycline loaded NPs to overcome surface tension and restore jet stability compared to lower voltage (10 kV) of blank nanoparticles. **(Figure 10 & 11)**

Distance

- Shorter distances (6cm) strengthen the electric field and support stability of aerosol formation while larger distances (12 cm) weaken the electric pull and promote jet instability in certain solutions**. (Figure 12 & 13)**
- Blank solution is stable at 12 cm, but minocycline requires shorter distance at the same voltage to reduce wet deposition and strengthen the electrical field enough to compensate for decreased charge mobility. **(Figure 13)**
- This can be adjusted by using the formula E=kV/d. By increasing the voltage (14 kV), E (electric field) can be increased while keeping the distance at 12 cm to ensure proper flight time and induce Coulomb Fission before deposition**. (Figure 16)**

Flow-rate

- At low flow rates (0.005 ml/min), increased evaporation limits deposition, while high flow rate (0.015 ml/min) increase jet diameter and reduce charge density per unit volume, limiting droplet breakup and promoting unstable spray modes (e.g., dripping or multi-jet). **(Figure 14 & 15)**
- Flow rate kept constant (0.008 ml/min) to isolate other parameters effects.
- At this rate, viscous minocycline solution limits droplet breakup, indicating flow rate could be secondary factor affecting aerosol deposition. **(Figure 16)**
- Stable aerosol formation was therefore more strongly governed by voltage than by flow rate under these conditions. **(Figure 16)**

Conclusions & Future Work

- Incorporation of minocycline significantly alters solution properties, increasing viscosity, intermolecular interactions, which disrupt stable cone-jet during electrospraying.
- Increasing voltage partially compensates by improving jet stability and reducing wet deposition but does not store optimal spray behavior.
- Overall, results indicate that drug polymer interactions play a critical role in nanoparticle fabrication and require optimization beyond blank conditions .
- Future studies will investigate
  - Optimize SEM imaging parameters to prevent PLGA melting and to get nanoscale size and spherical morphology confirmation.
  - Optimize electrospraying parameters (Voltage, distance, flow rate) to achieve stable cone jet and uniform aerosol deposition with minocycline.
  - Perform drug loading and encapsulation efficiency studies to evaluate minocycline incorporation into PLGA nanoparticles .

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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).