Rapid Cooling and Neuroprotection: Intranasal Vanilloid Nanodrugs for Acute Brain Injuries

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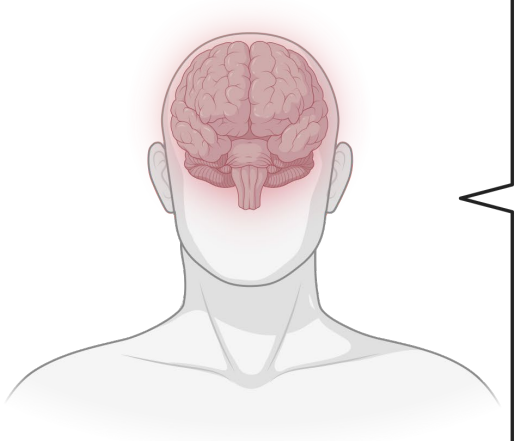
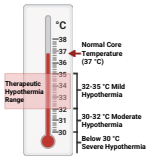
Challenge: Acute Trauma

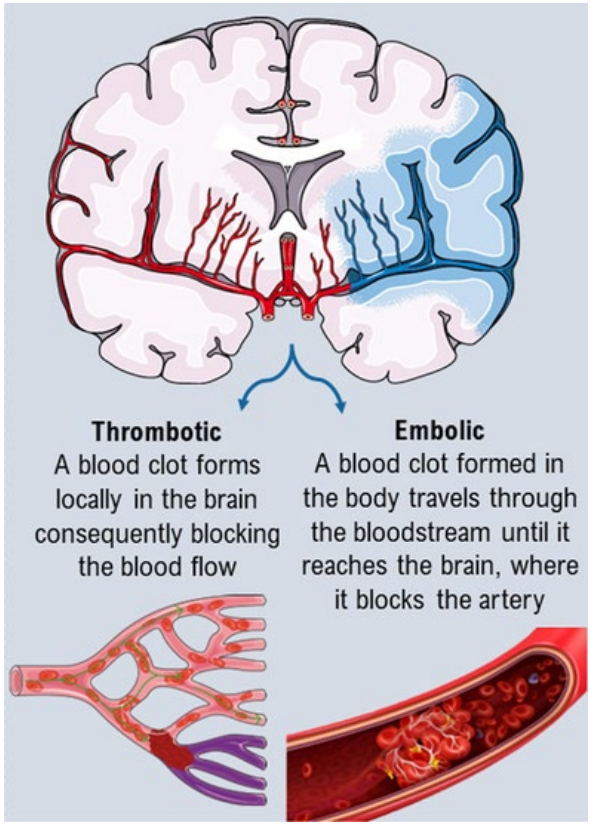
Common Causes of Traumatic Brain Injury



- Falls
- Unintentional trauma
- Assaults
- Vehicle crashes

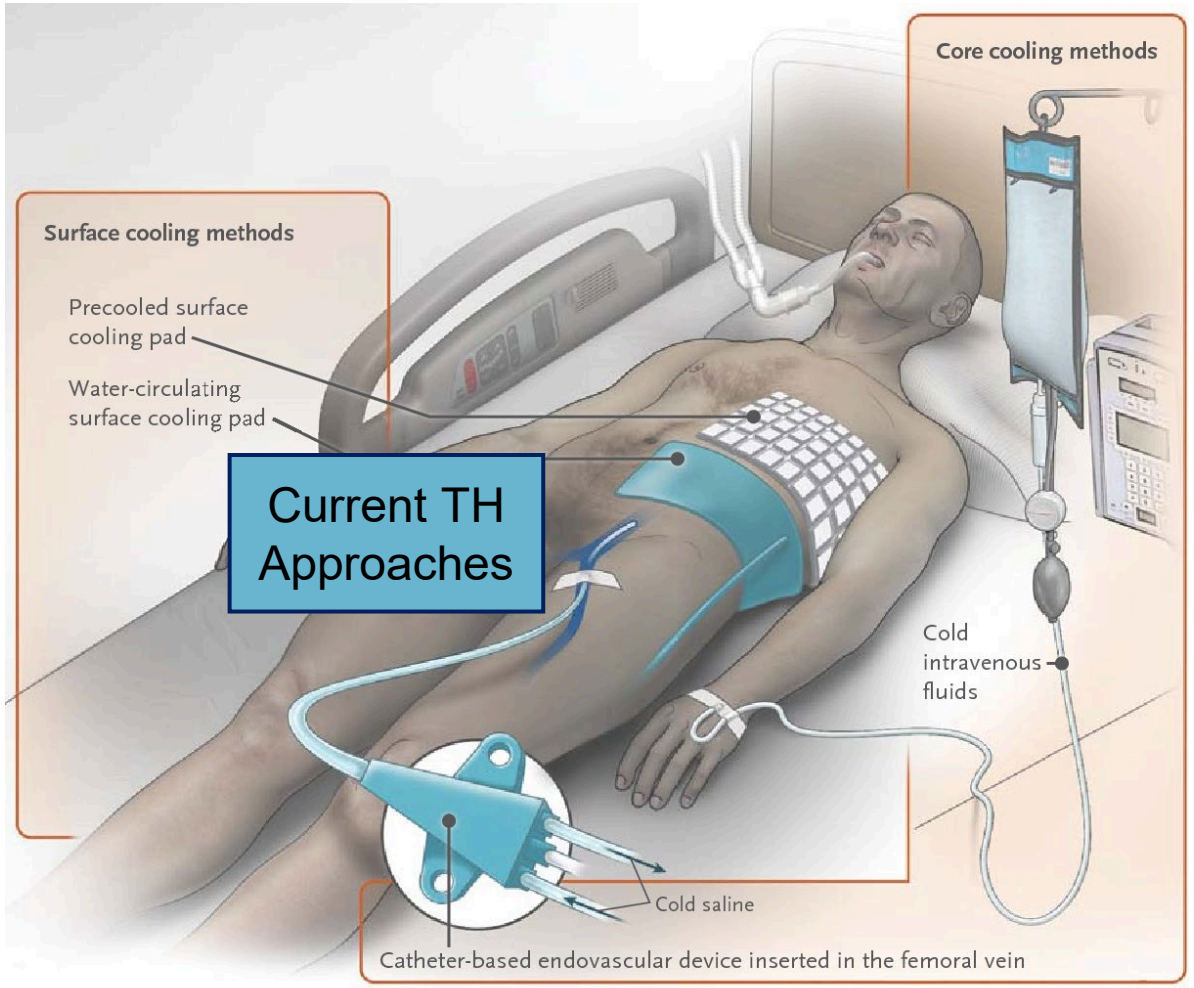


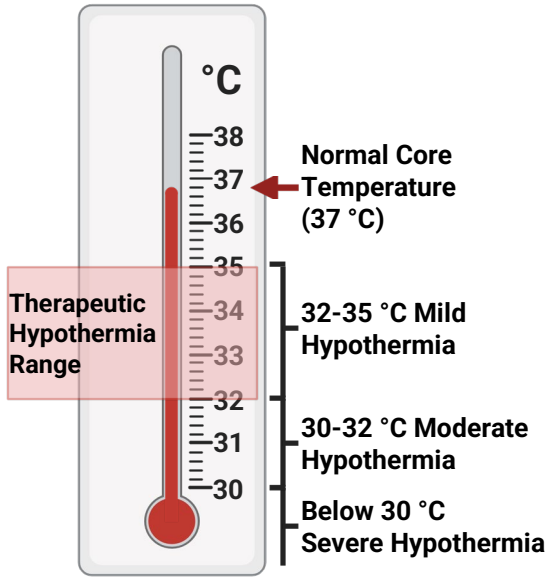
Ischemic Stroke Subtypes



Neves, L. T., et al. (2024). "Environmental Enrichment in Stroke Research: an Update." Translational Stroke Research **15(2): 339-351.**

Possible Solution: Therapeutic Hypothermia



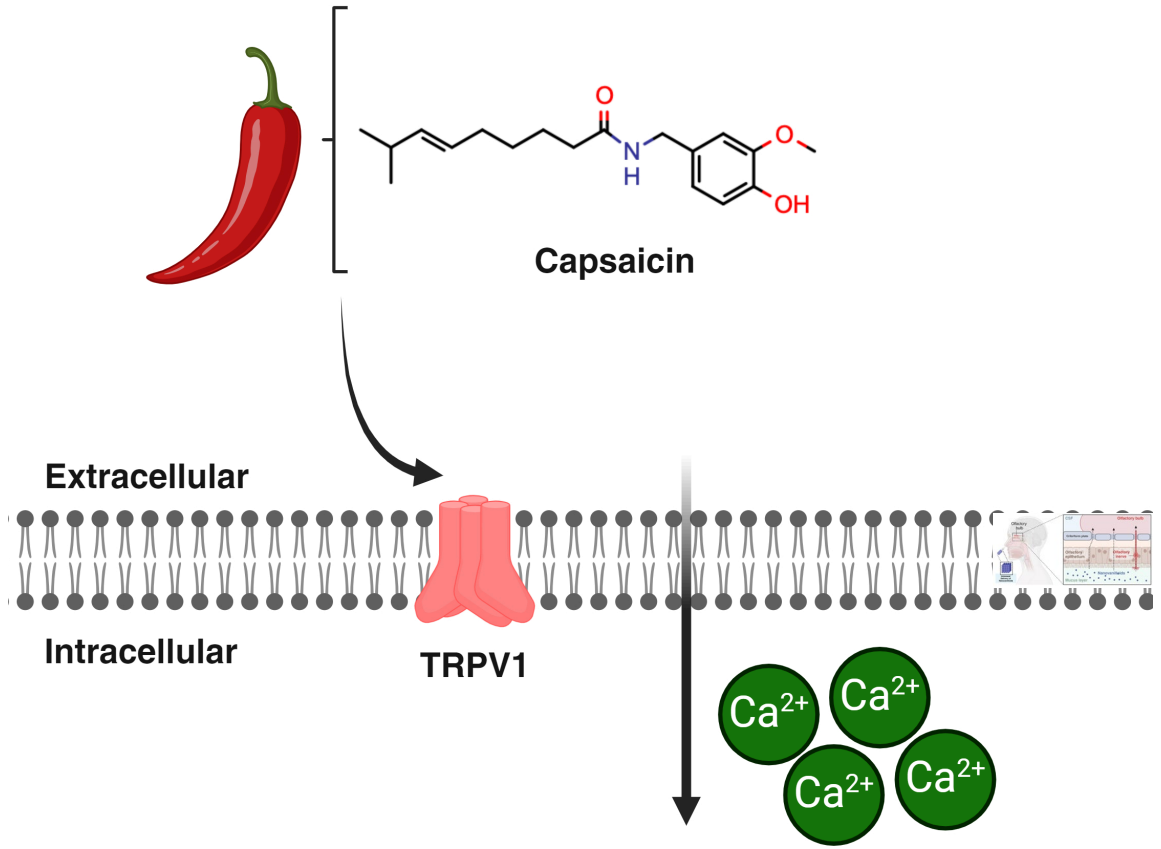


Neuroprotection

1. Excitotoxicity reduction
2. Anti-inflammatory effects
3. Inhibition of apoptosis
4. Oxidative stress reduction
5. Blood-brain barrier (BBB) stabilization

Holzer, M. (2010). "Targeted temperature management for comatose survivors of cardiac arrest." N Engl J Med 363(13): 1256-1264.

Background: TRPV1



Binding of vanilloids such as capsaicin activates TRPV1, and triggers an increase in intracellular Ca^{2+}

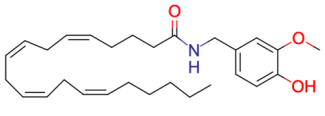
What is the Transient Receptor Vanilloid Potential Vanilloid 1 (TRPV1) Receptor?

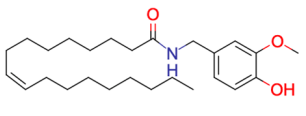
Thermoreceptor. Ion channel.

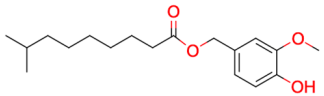
Activated by heat (> 43°C), acidity, and ligands (e.g., capsaicin, endocannabinoids).

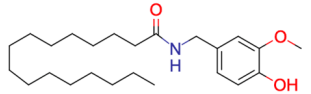
Thermoregulation, pain perception, and inflammation.

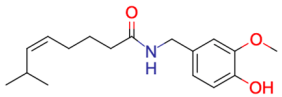
Non-pungent TRPV1 agonists (below) activate TRPV1 without causing pain or inflammation, inducing hypothermia without external cooling, and serving as pharmacological agents for therapeutic hypothermia*.


Arvanil


Olvanil


Dihydrocapsiate


Palvanil


Rinvanil

* Hurley, J. D., Akers, A. T., Friedman, J. R., Nolan, N. A., Brown, K. C., & Dasgupta, P. (2017). Non-pungent long chain capsaicin-analogs arvanil and olvanil display better anti-invasive activity than capsaicin in human small cell lung cancers. *Cell adhesion & migration*, *11*(1), 80–97. https://doi.org/10.1080/19336918.2016.1187368
*Chu, K. M., Ngan, M. P., Wai, M. K., Yeung, C. K., Andrews, P. L., Percie du Sert, N., & Rudd, J. A. (2010). Olvanil: a non-pungent TRPV1 activator has anti-emetic properties in the ferret. Neuropharmacology, 58(2), 383–391. https://doi.org/10.1016/j.neuropharm.2009.10.002

Nanodrugs & Intranasal Delivery

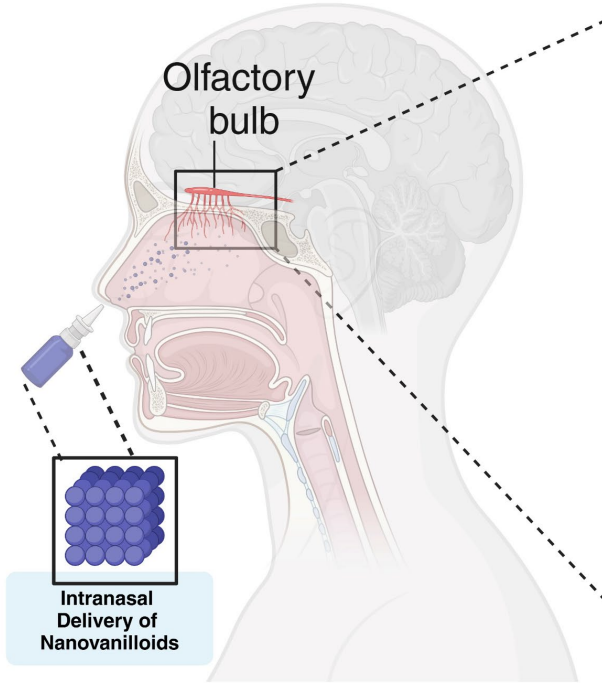
Bypasses blood-brain barrier

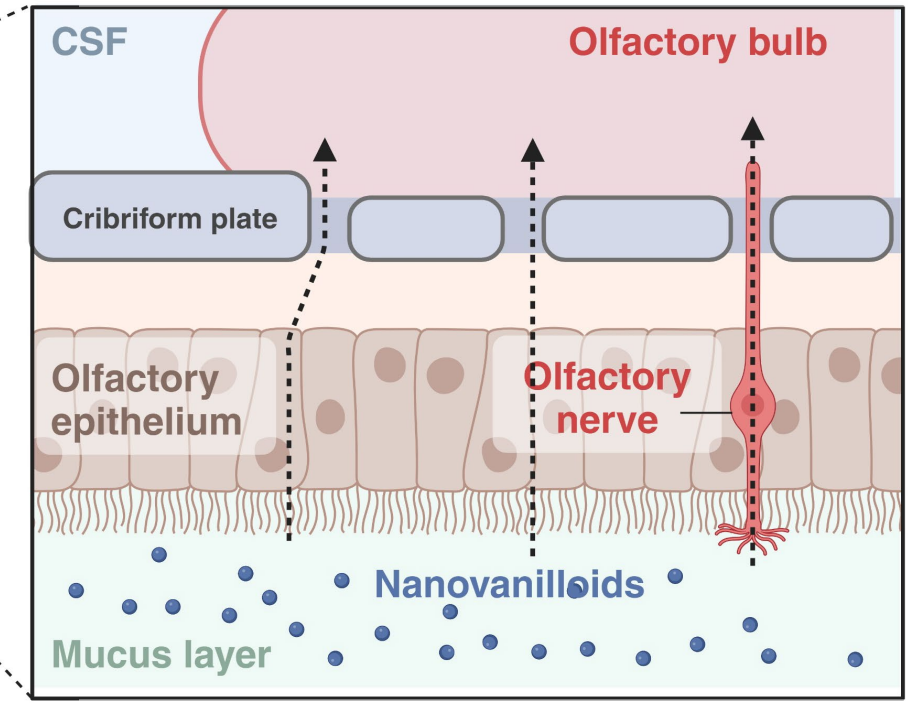
Non-invasive

Improved aqueous solubility

Increased biocompatibility

No organic solvents in the final solution

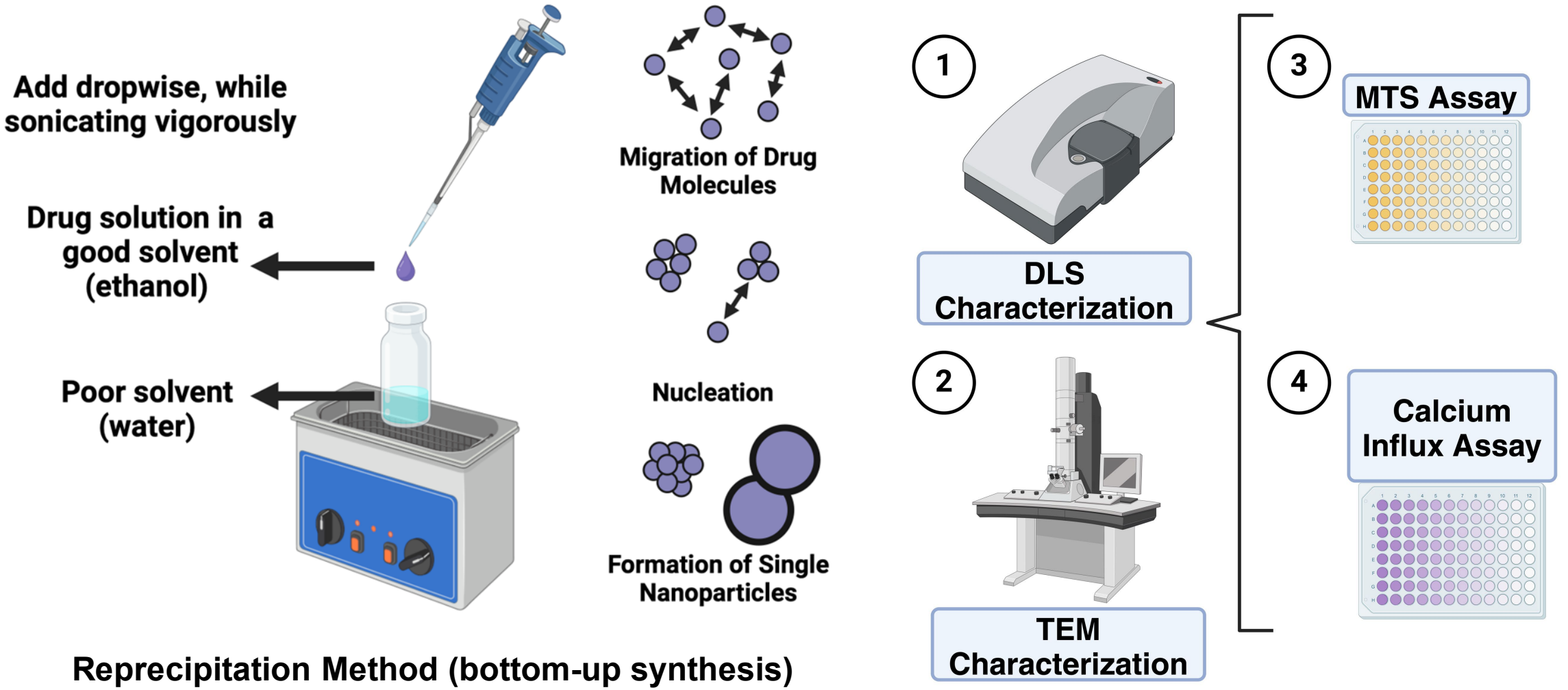




Higher bioavailability

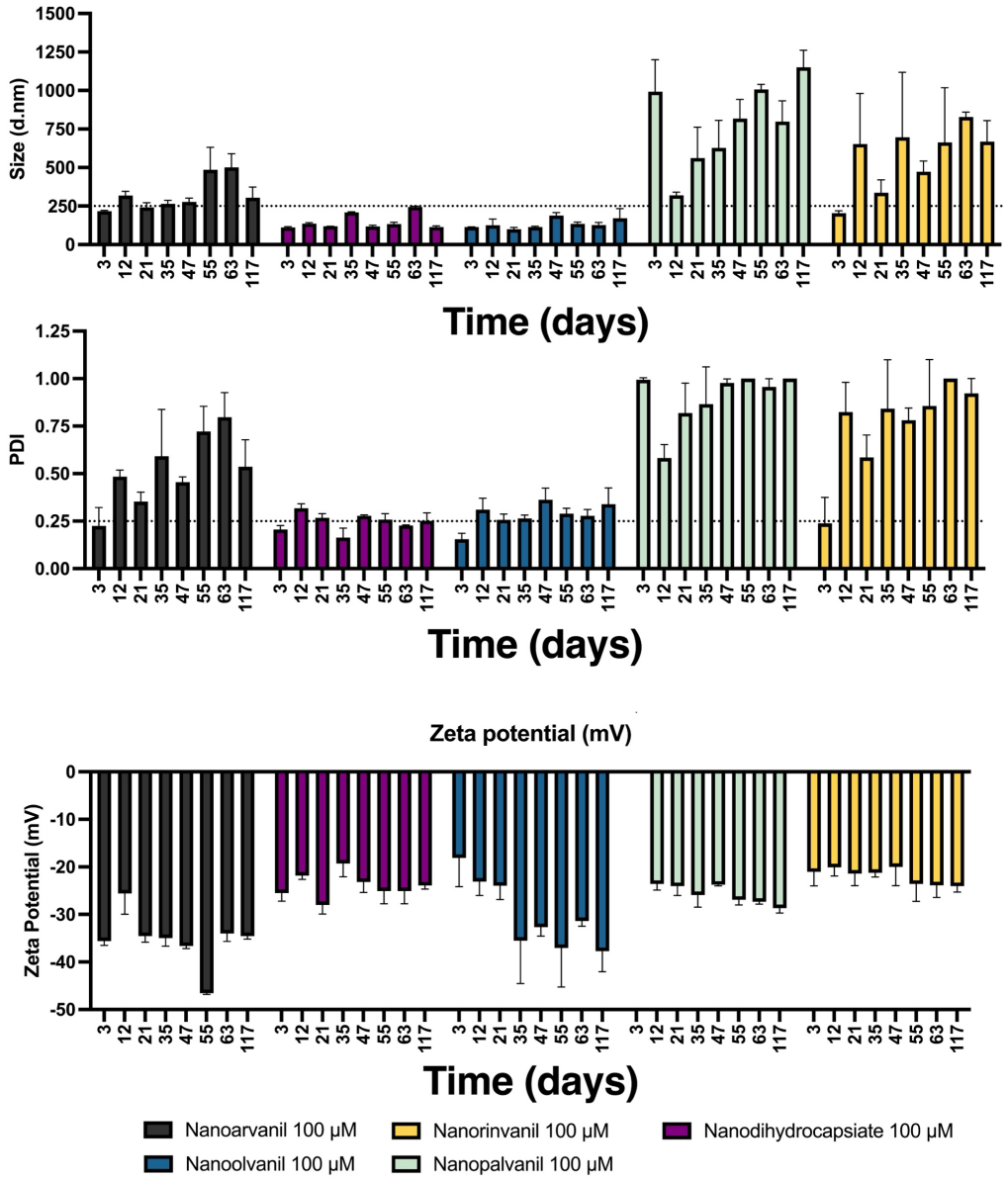
Drug dose reduction

Nanodrug Synthesis



Reprecipitation Method (bottom-up synthesis)

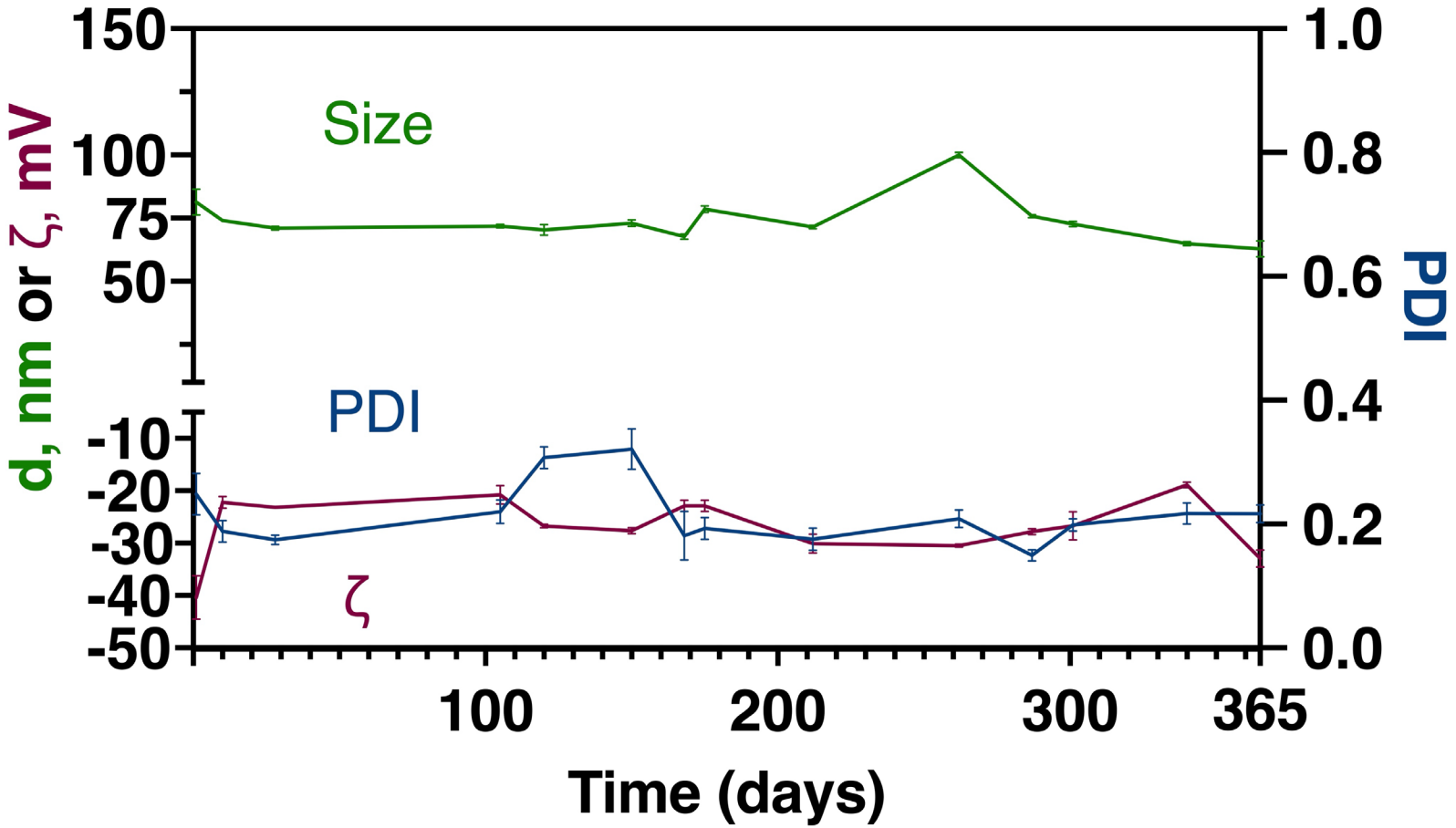
Stability of vanilloid nanodrugs, 100 μM



Conditions: 4 ℃, for 3.8 months.
Properties noted day of synthesis

Nanodrug	Average Size (nm)	Average PDI	Average Zeta Potential (mV)
Nanoarvanil	217 ± 5	0.225 ± 0.1	-35.6 ± 0.92
Nanodihydrocapsiate	111 ± 5.5	0.206 ± 0.02	-25.5 ± 1.7
Nanoolvanil	114 ± 1.7	0.155 ± 0.03	-18.13 ± 6
Nanopalvanil	993 ± 207	0.993 ± 0.01	0.2 ± 0.15
Nanorinvanil	194 ± 16	0.238 ± 0.14	-21.03 ± 3

Stability of nanoolvanil at 72 mM

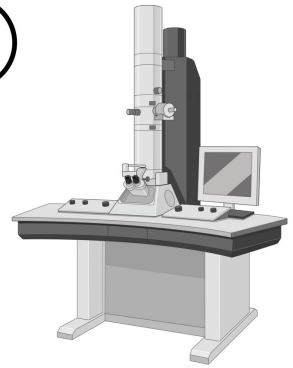


Conditions: 4 ℃, for 12 months.

Average size (nm)	Average PDI	Average Zeta Potential (mV)
73.97 ± 8.9	0.214 ± 0.05	-27.69 ± 5.6

TEM Characterization of Nanodrugs

2

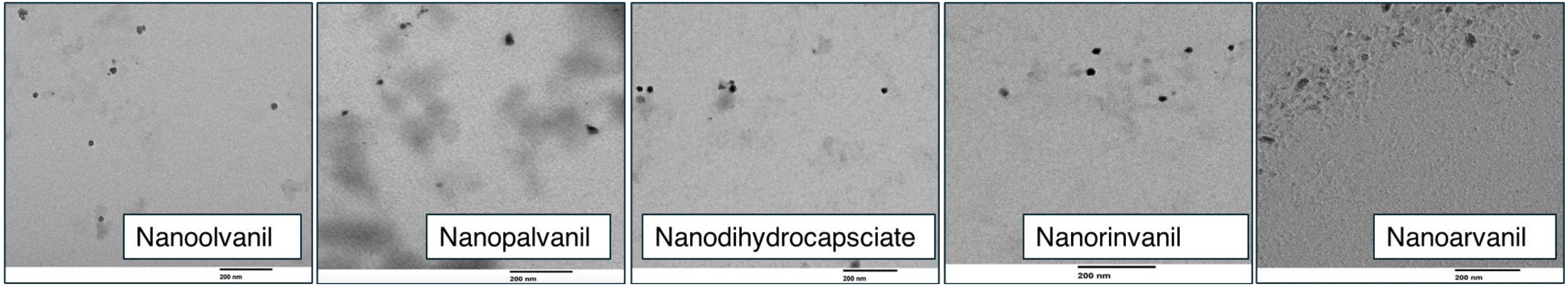


TEM Characterization

TEM allows us to see the actual shape and morphology of our nanodrugs, which is important for confirming their structure, shape, and size.

Nanodrug Uniformity: This helps assess how uniform they are.

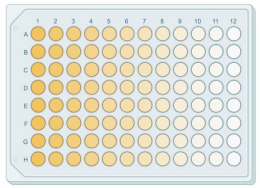
Size: Smaller than DLS measures hydrodynamic radius, not actual dry size.



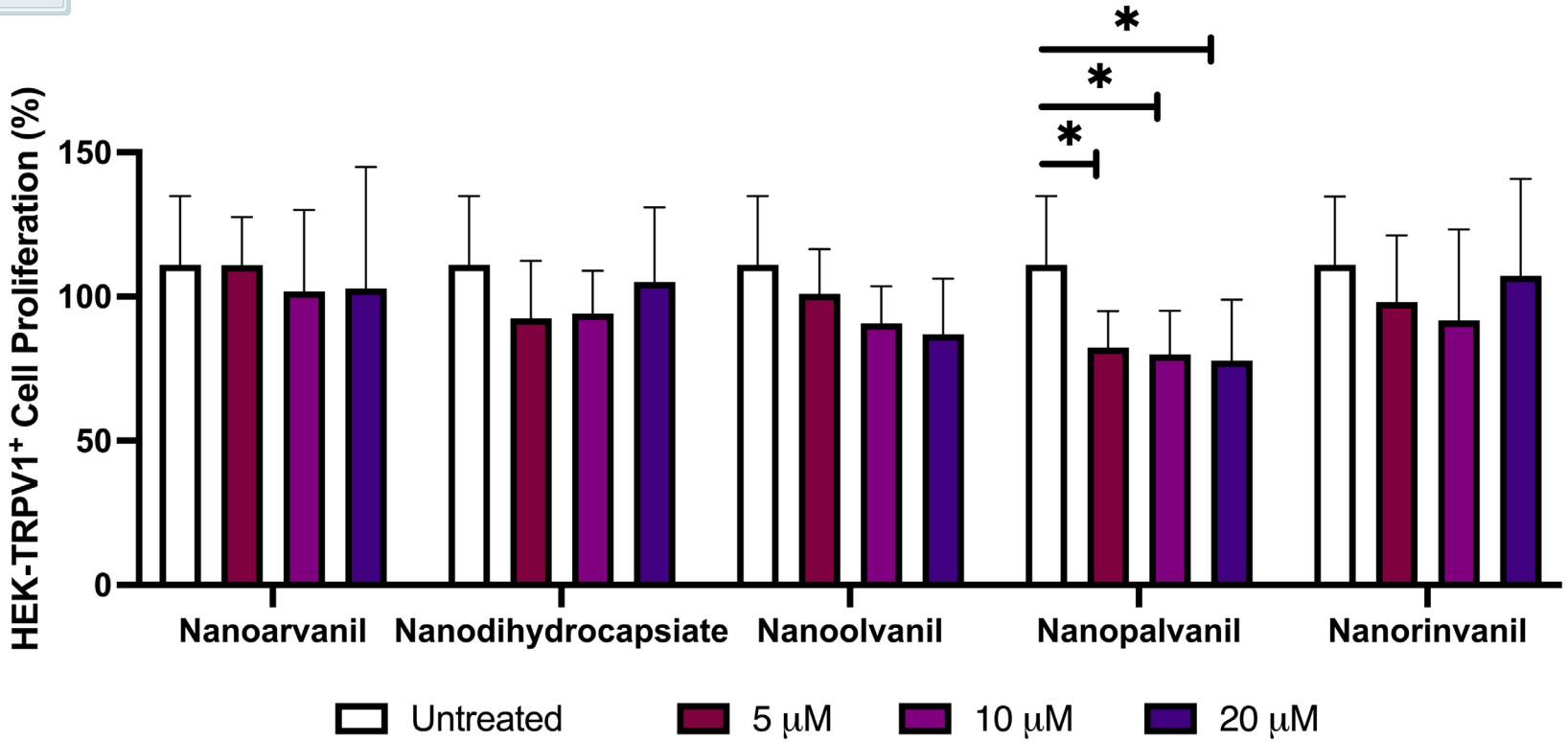
Cytotoxicity Studies

3

MTS Assay



The **MTS assay** is a colorimetric method used to assess **cell viability** and **proliferation** after treating the cells with nanodrugs for 24 hours.



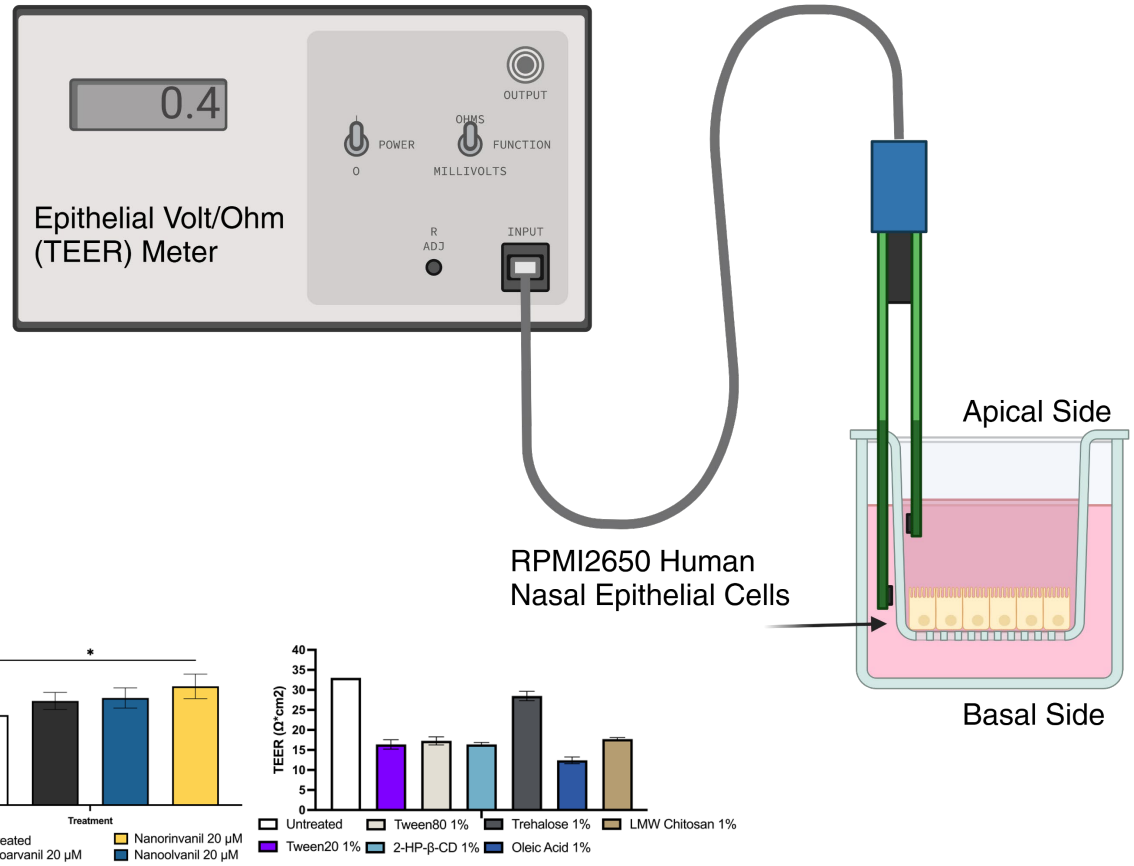
A two-way ANOVA was performed to analyze the data, with results reported as the **Relative Standard Deviation (RSD) of N = 9 replicates**. Results are non-significant unless stated otherwise.

Development of Intranasal Carrier Formulation

- Excipients enhance solubility, stability, nasal absorption, and brain delivery, while prolonging retention and minimizing side effects.

Excipient	Function	Purpose
Tween20	Surfactant, solubilizer, permeation enhancer	Improve solubility, stability, and enhance absorption
Tween80	Surfactant, solubilizer, permeation enhancer	Improve solubility, stability, and enhance absorption
Dodecyl Maltoside	Surfactant, solubilizer, permeation enhancer	Improve solubility, stability, and enhance absorption
2-HP-β Cyclodextrin	Solubilizer	Increase drug solubility
Trehalose	Stabilizer, cryoprotectant	Increase drug stability
Low Molecular Weight Chitosan	Mucoadhesive	Prolong drug retention
Oleic Acid	Stabilizer, permeation enhancer	Enhance absorption

Epithelial Integrity Studies



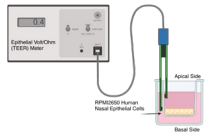
Transepithelial Electrical Resistance (TEER)

- A technique used to measure the **integrity of epithelial barriers** by assessing the electrical resistance across cell layers.
- Evaluate how the chosen excipients affect the nasal epithelial barrier.

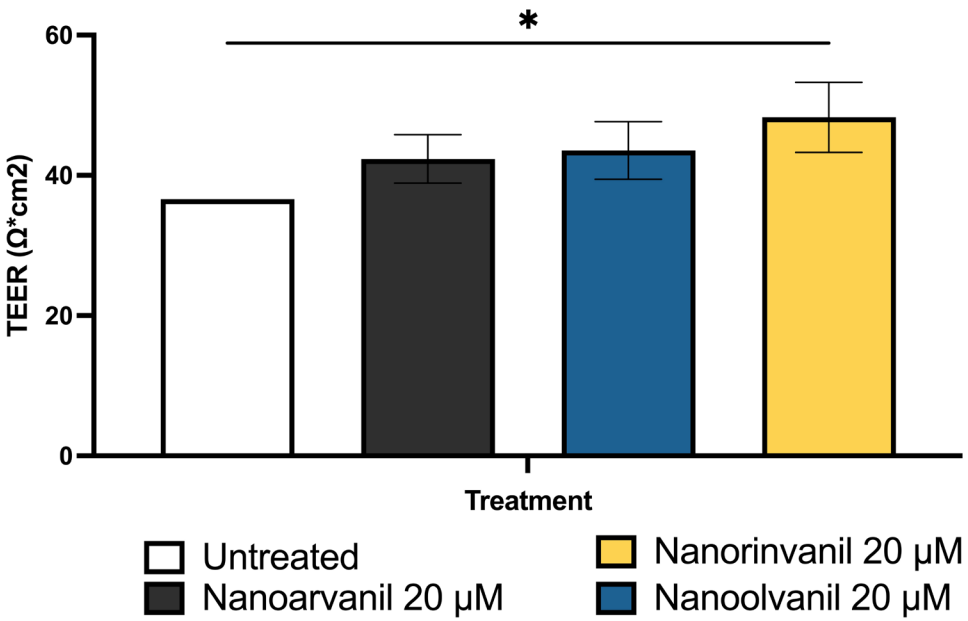
- **Increased TEER:** may potentially reduce drug permeation.
- **Decreased TEER:** may potentially increase drug permeation.

$TEER\ (\Omega\ x\ cm^2)$ = [(measured resistance, Ω) – (blank resistance, Ω)] x (surface area cm^2)

Epithelial Integrity Studies

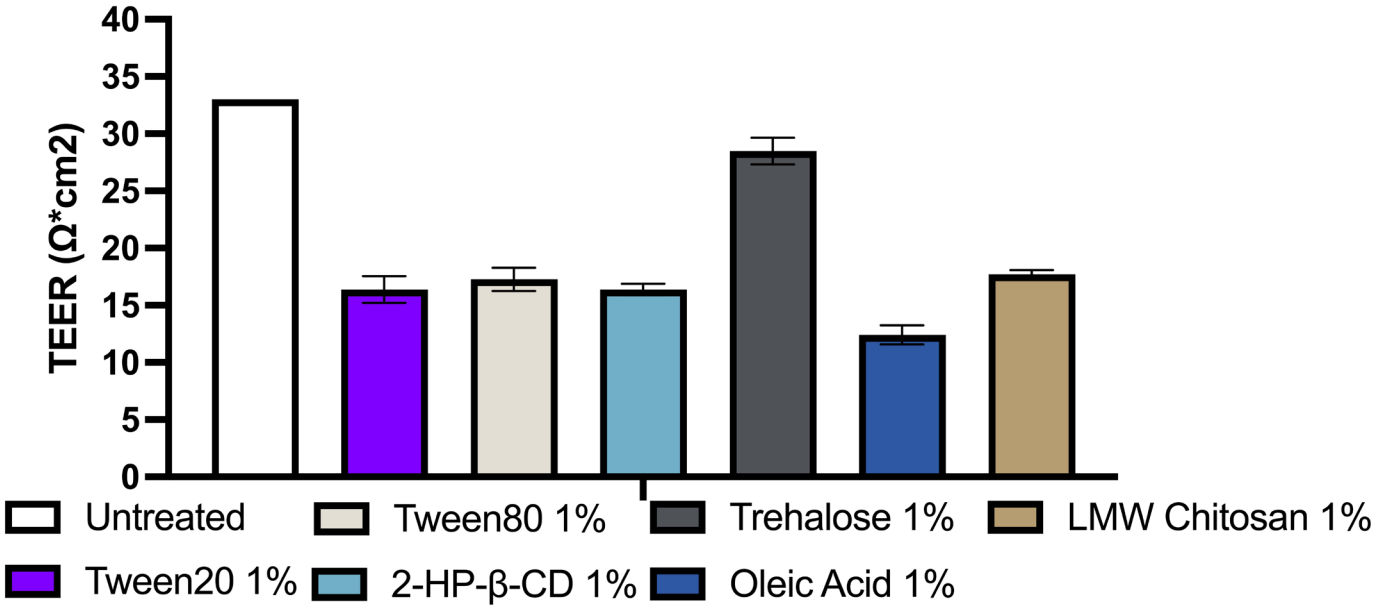


Treatment for 2 hours



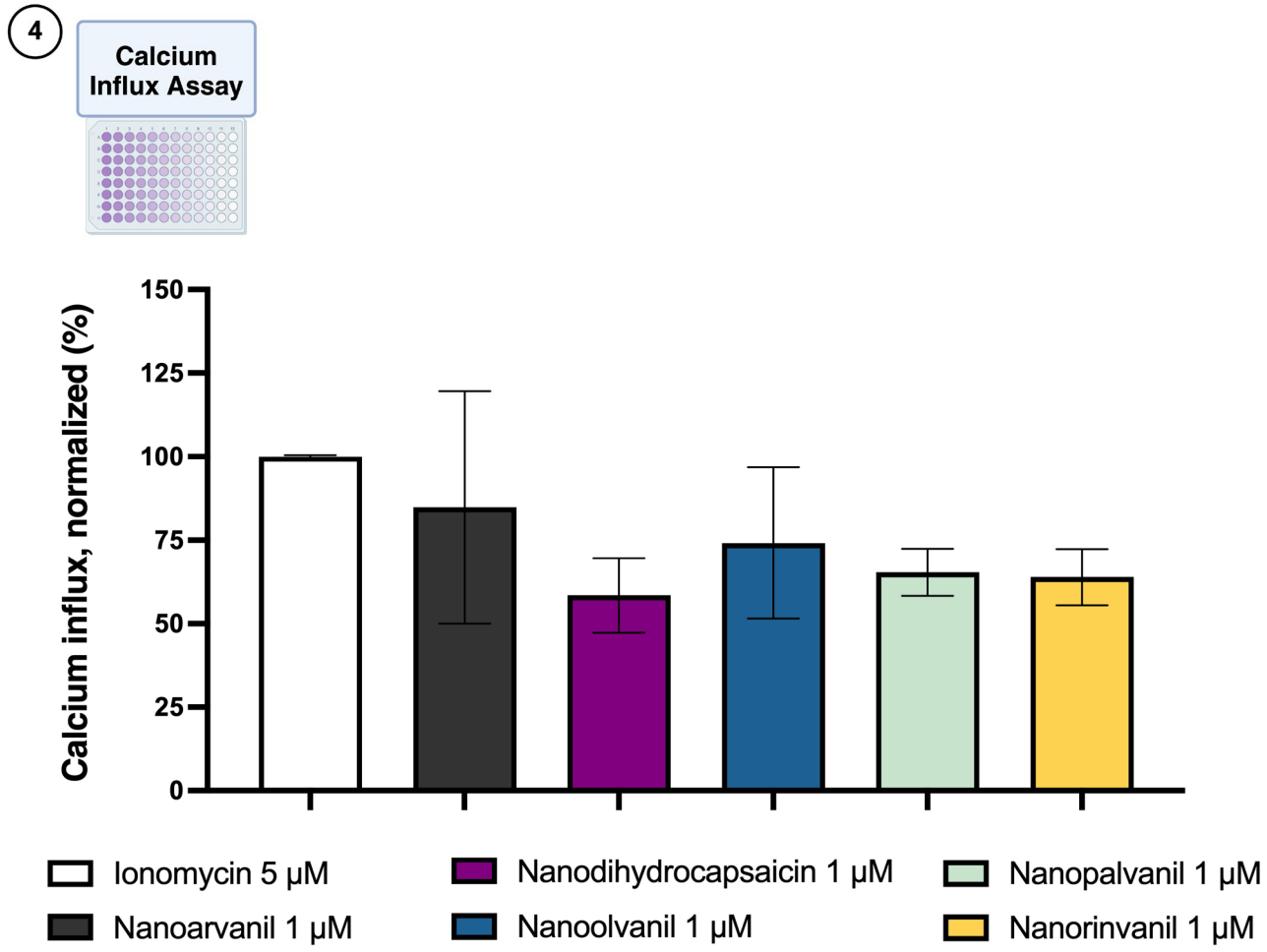
A two-way ANOVA was performed to analyze the data, with results reported as the **Relative Standard Deviation (RSD) of N = 3 replicates**. Results are non-significant unless noted otherwise.

Treatment for 5 minutes



A two-way ANOVA was performed to analyze the data, with results reported as the **Relative Standard Deviation (RSD) of N = 3 replicates**. Results are significant P<0.0001 for all excipients

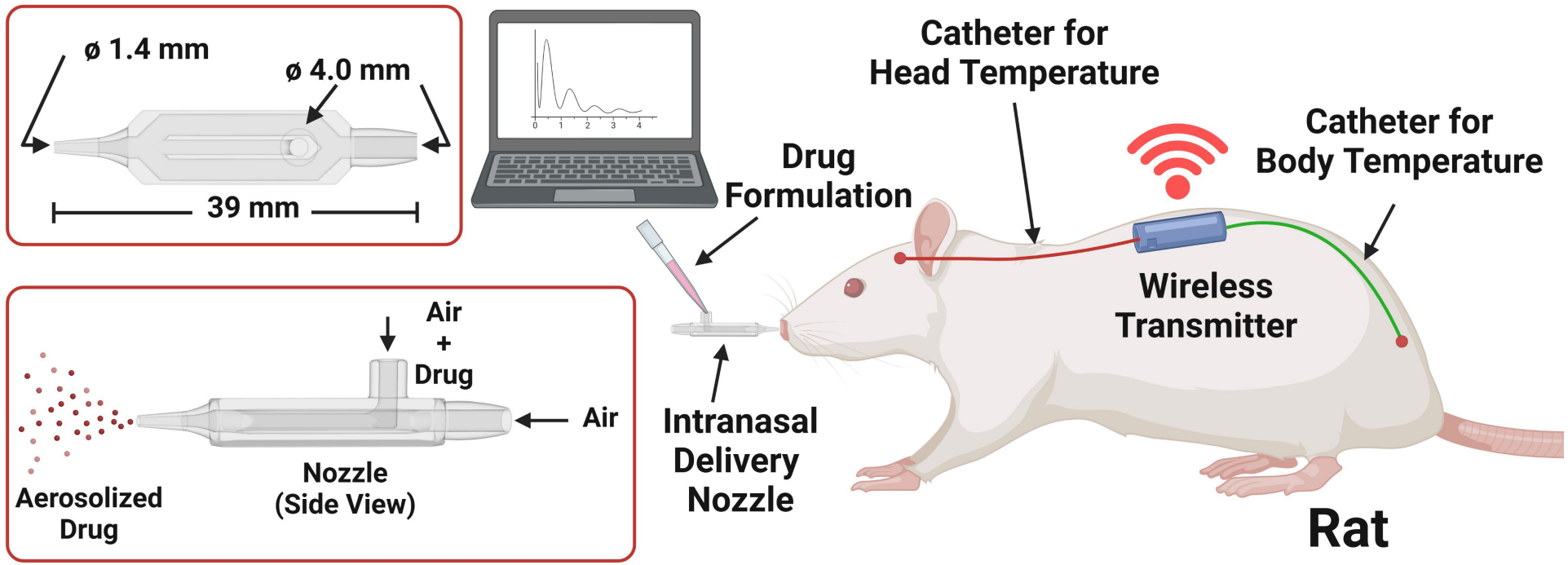
TRPV1 Activation Studies



- **Fluo-4 Dye**: Fluoresces upon binding to intracellular calcium.
- **Ionomycin**: Positive control to induce calcium influx.
- **Vanilloid Nanodrugs**: Tested for TRPV1 activation by measuring calcium entry.
- **Result**: Higher fluorescence = increased calcium influx, indicating TRPV1 activation.

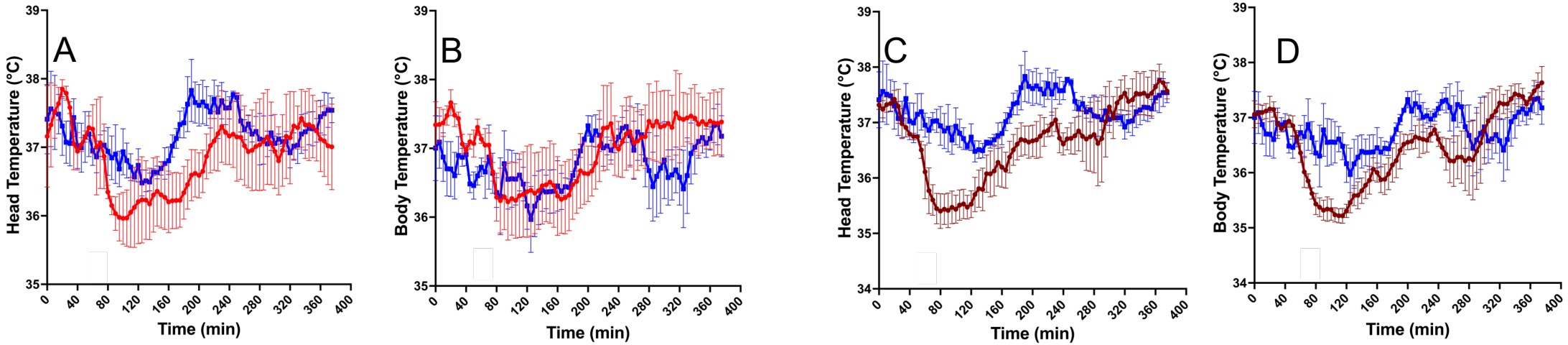
A two-way ANOVA was performed to analyze the data, with results reported as the **Relative Standard Deviation (RSD) of N = 3 replicates**. Results are non-significant unless noted otherwise.

In Vivo Set-up



Preliminary Data in Sham Animals

Preliminary data for **successful cooling induced by nanoolvanil**

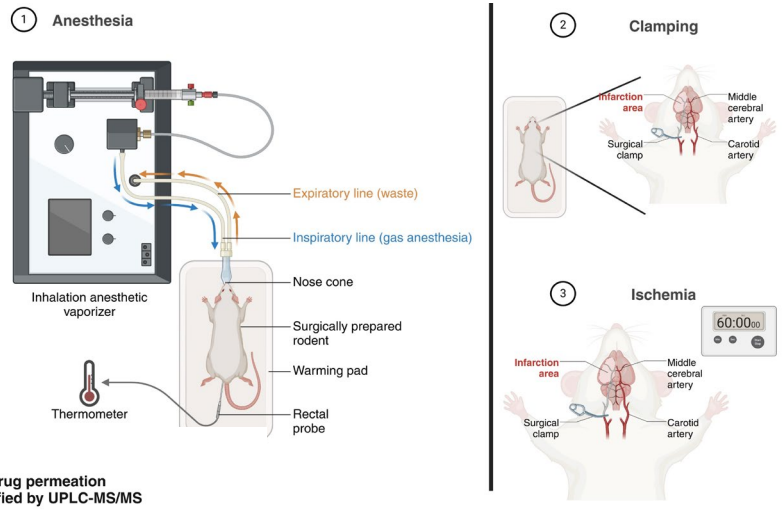


Mean head (A, C) and body (B, D) temperatures of 6 rats following nanoolvanil (red/brown; N = 3) or vehicle control (blue; N = 3) administration via the device (5 mg/kg (A, C) or 10 mg/kg (B, D) per nostril).

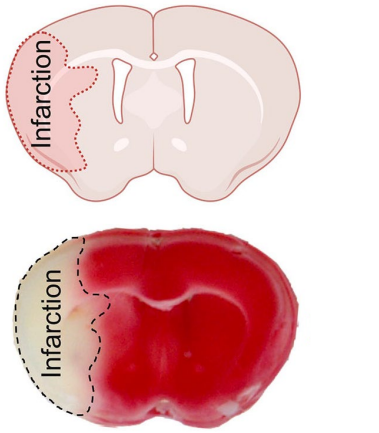
5-10 mg/kg intranasal nanoolvanil induced rapid cooling withing 20 min and sustained for 100 min. Vehicle had no significant decrease in temperature overall ($p < 0.0001$)

Future Work

Middle Cerebral Artery Occlusion (MCAO)



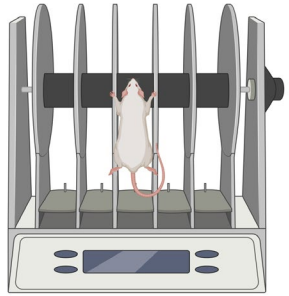
2,3,5-Triphenyltetrazolium chloride (TTC)



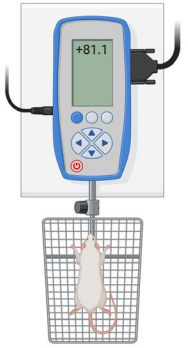
TTC staining at 48h

Yang, C., et al. (2023). "Therapeutic Benefits of Adropin in Aged Mice After Transient Ischemic Stroke via Reduction of Blood-Brain Barrier Damage." Stroke **54**(1): 234-244.

Behavioral Tests for Rats

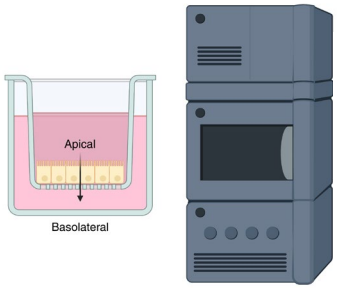


Rotarod (motor coordination)

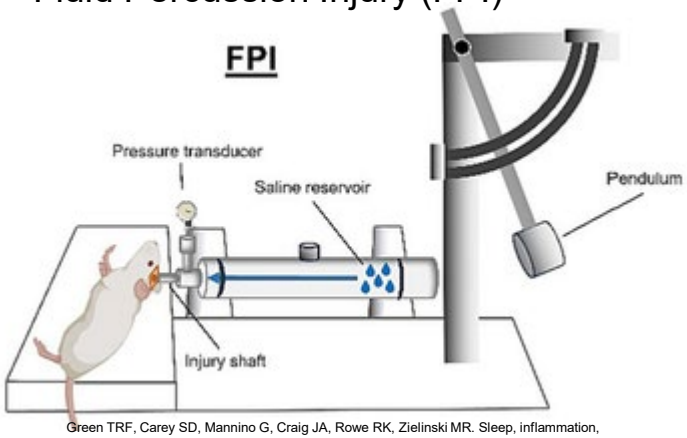


Grip strength (behavioral test)

Nanodrug permeation quantified by UPLC-MS/MS



Fluid Percussion Injury (FPI)



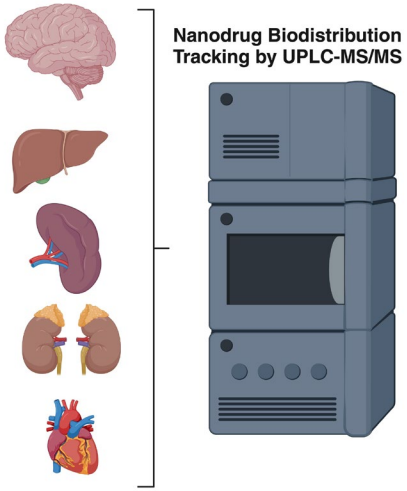
Green TRF, Carey SD, Mannino G, Craig JA, Rowe RK, Zielinski MR. Sleep, inflammation, and hemodynamics in rodent models of traumatic brain injury. Front Neurosci. 2024 Feb 15;18:1361014. doi: 10.3389/fnins.2024.1361014. PMID: 38426017; PMCID: PMC10903352.

Blood Sampling: After administration, samples will be collected at specific time points (1h, 6h, 24h, 48h, 72h).

Liver Function Tests: Measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin.

Kidney Function Tests: Measure blood urea nitrogen (BUN) and creatinine levels.

Histopathology: Organs will be stained (H&E) and examined under a microscope for signs of toxicity (e.g., inflammation, necrosis).



Nanodrug Biodistribution Tracking by UPLC-MS/MS

Acknowledgements

- Daunert/Deo Lab
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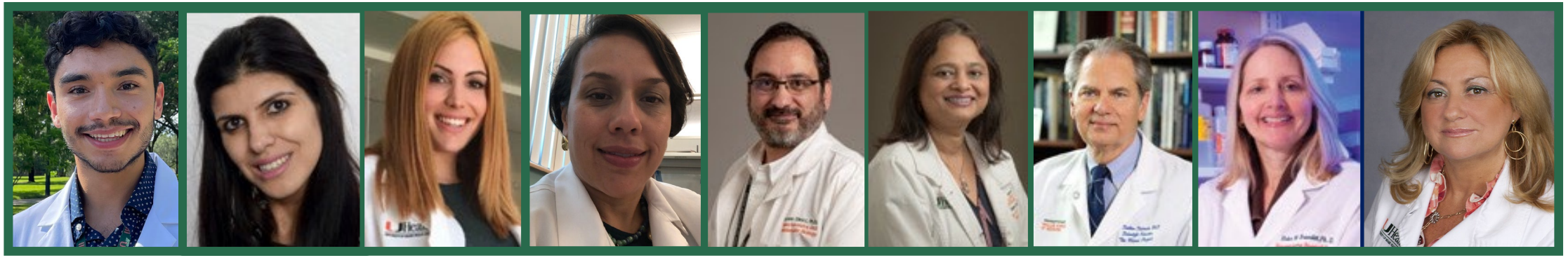

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Not pictured: Jake Borgia