Engineering Biomaterials to Address Neural Injury

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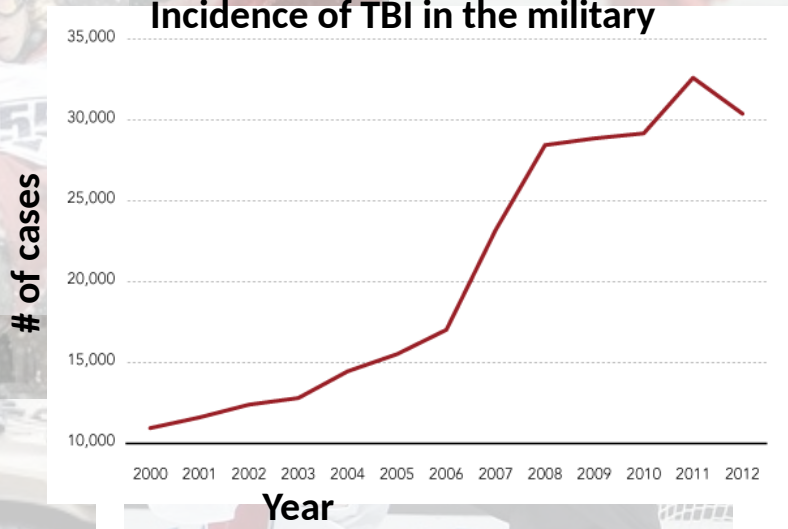
ARIZONA STATE UNIVERSITY

Motivation: Traumatic Brain Injury (TBI)

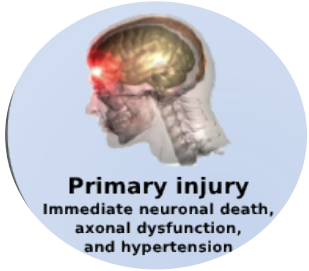
1.7 million TBIs reported annually in the U.S.

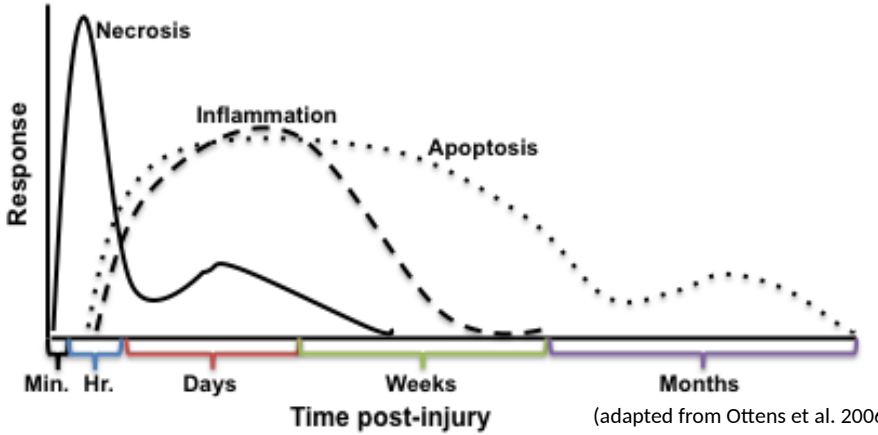
Indirect and direct costs **$77 billion** yearly in the US

TBI contributes to **1/3** of injury related deaths



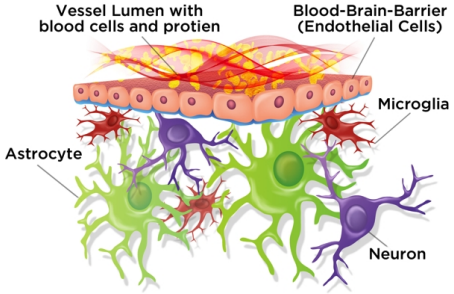
Faul et al. CDC, 2010, Huffington Post, Department of Defense


Primary injury
Immediate neuronal death,
axonal dysfunction,
and hypertension



(adapted from Ottens et al. 2006).

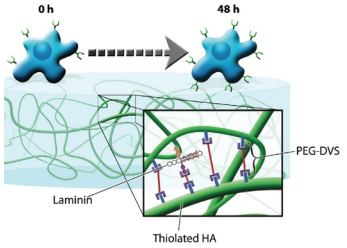
Healthy Brain Microenvironment



Temporally and spatially complex pathology may ensue

Limited clinical diagnostic and therapeutic approaches available

Traumatic Brain Injury (TBI)

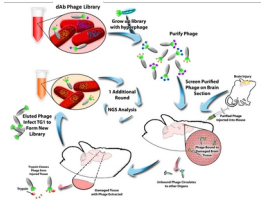


Addington et al. *Matrix Bio 2016*

Diagnostics

Biomarker Discovery: Phage display for novel TBI biomarkers

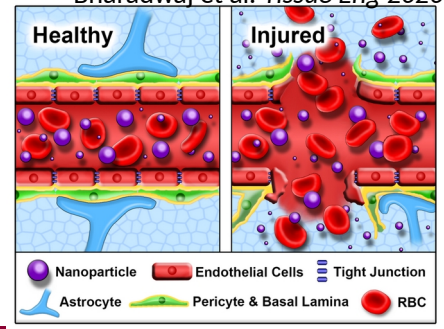
Witten et al. *J Neurotrauma* 2016
Marsh et al *Drug Delivery Methods* 2018
Martinez et al. *J Biological Eng* 2019
Martinez et al. *Protocols in Neurosci* 2021
Martinez et al. *Sci Adv* 2022



Therapeutics

Development and characterization of nanoparticle systems

Bharadwaj et al. *Scientific Reports* 2016
Bharadwaj et al. *Nanomedicine* 2018
Bharadwaj et al. *Tissue Eng 2020*



Endogenous neural regenerative signaling

Dutta et. al. *J Mater Chem B Mater Biol Med*. 2015.
Dutta et al. *JBMR-A* 2016
Dutta et al. *Biomaterials Science* 2017
Hickey et al. *Biomedical Materials*. 2018
Hickey et al. *Cellular and Molecular Bioengineering* 2021

Neural tissue engineering

Addington et al. *Biomaterials* 2014
Addington et al. *Biomaterials* 2015
Addington et al. *Matrix Biology* 2016
Bjorklund et al. *Int. J. Mol. Sci*. 2021

Bharadwaj et al. *Nanomedicine 2018*

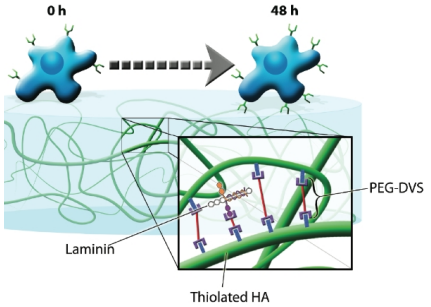
Goal of Regenerative Medicine

Restore

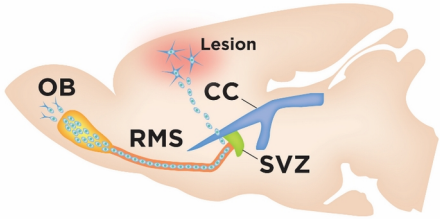
Maintain

Enhance

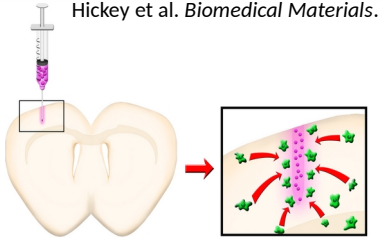
} ***Tissue Function***



Addington et al. *Matrix Bio 2016*



Hickey et al. *Biomedical Materials*. 2018



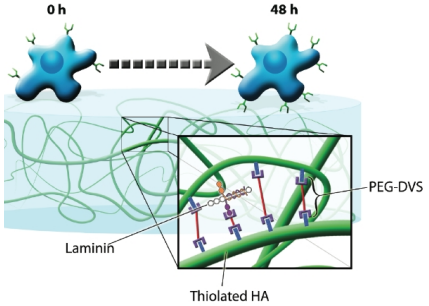
Dutta et al. *Biomat Sci 2017*

Regenerative Medicine Strategies

Approach 1: Neural transplantation

- Limitations: Low cell survival (~2-4%)
- Scaffold/delivery systems that work in concert with injury signals

Addington et al., *Biomaterials*, 2014
Addington et al., *Biomaterials*, 2015
Addington et al. *Matrix Biology* 2016
Bjorklund et al. *Int. J. Mol. Sci*. 2021

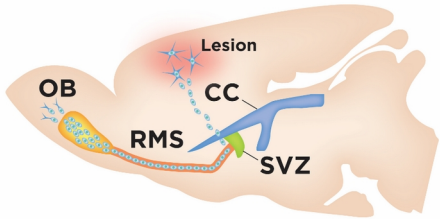


Addington et al. *Matrix Bio 2016*

Approach 2: Endogenous cell recruitment

- Harness endogenous regenerative signaling of the injured brain
  - Drug delivery approaches to tune regenerative profile after TBI

Dutta, et. al. *J Mater Chem B Mater Biol Med*. 2015
Dutta, et al. *JBMR-A* 2016
Dutta, Hickey et al. *Biomaterials Science* 2017
Hickey et al. *Biomedical Materials*. 2018
Hickey et al. *Cellular and Molecular Bioengin* 2021



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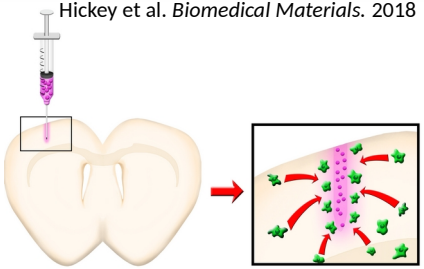

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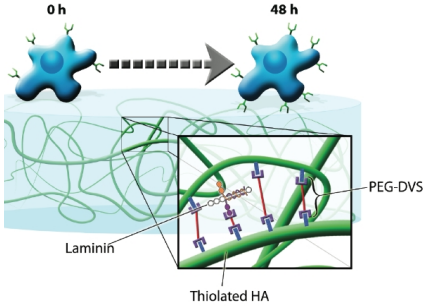

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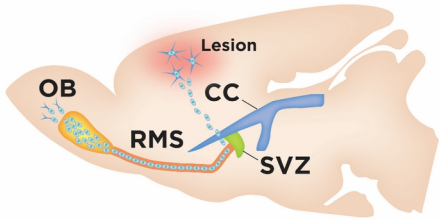

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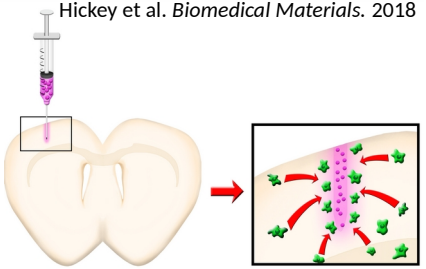
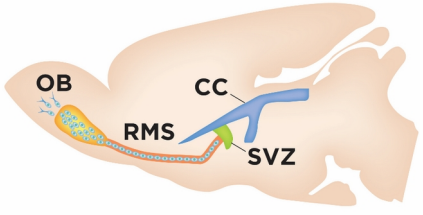


Dutta et al. *Biomat Sci 2017*

Stimulating Endogenous Neural Regeneration

Neural progenitor/stem cells (NPSC) niches:

- Hippocampus
- Subventricular zone (SVZ)

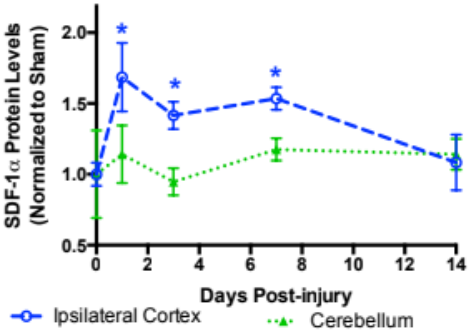


After brain injury, NPSCs migrate and selectively accumulate at the lesion site[1,2,3]

- Trophic support
- Angiogenesis
- Neurogenesis

Increased levels of chemokine stromal-cell derived factor-1α (SDF-1α) in TBI models

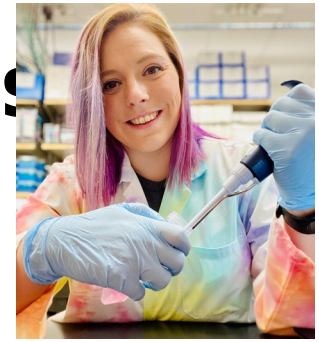
- Correlated with **chemotactic recruitment of NPSCs after TBI**[1]



Does prolonged presence of SDF-1 lead to enhanced NPSC recruitment?

1. Xin Y, et. al. PLoS ONE, 2013.
2. Itoh T, et. al., Neurol Res, 2009.
3. Xiong L, et. al., Brain Research, 2012

First things first – Key questions



How are chemotactic gradients formed in the brain?

Hickey, K, Grassi, S, Caplan, MR, Stabenfeldt, SE. Stromal Cell-Derived Factor-1a Autocrine/Paracrine Signaling Contributes to Spatiotemporal Gradients in the Brain. *Cellular and Molecular Bioengineering*. 2021; 14: 75-87.

Kassondra (Kassy) Hickey, PhD

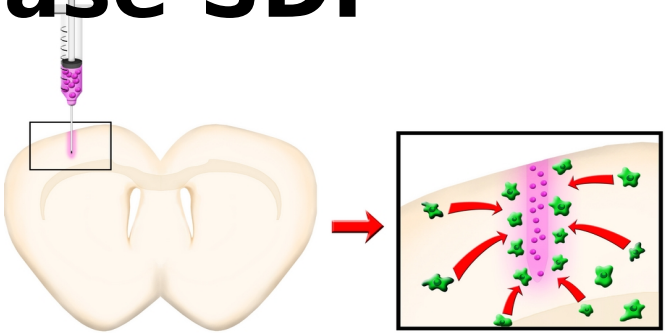
Can we design controlled release systems to modulate chemotactic profiles within the injured brain?

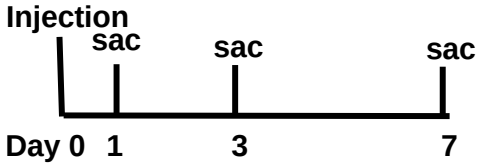
Dipankar Dutta, PhD

**Collaboration with Drs. Julianne Holloway and Mehdi Nikkhah at ASU

Head-to-Head Comparison of Bolus vs. Sustained Release SDF-1

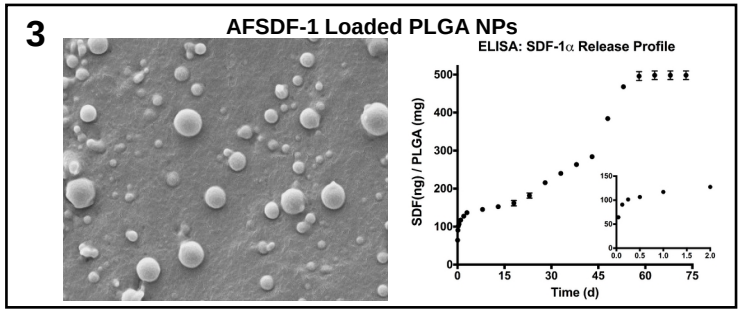
Determine the endogenous SDF-1/CXCR4 response to bolus vs. sustained release (Intracortical injections)



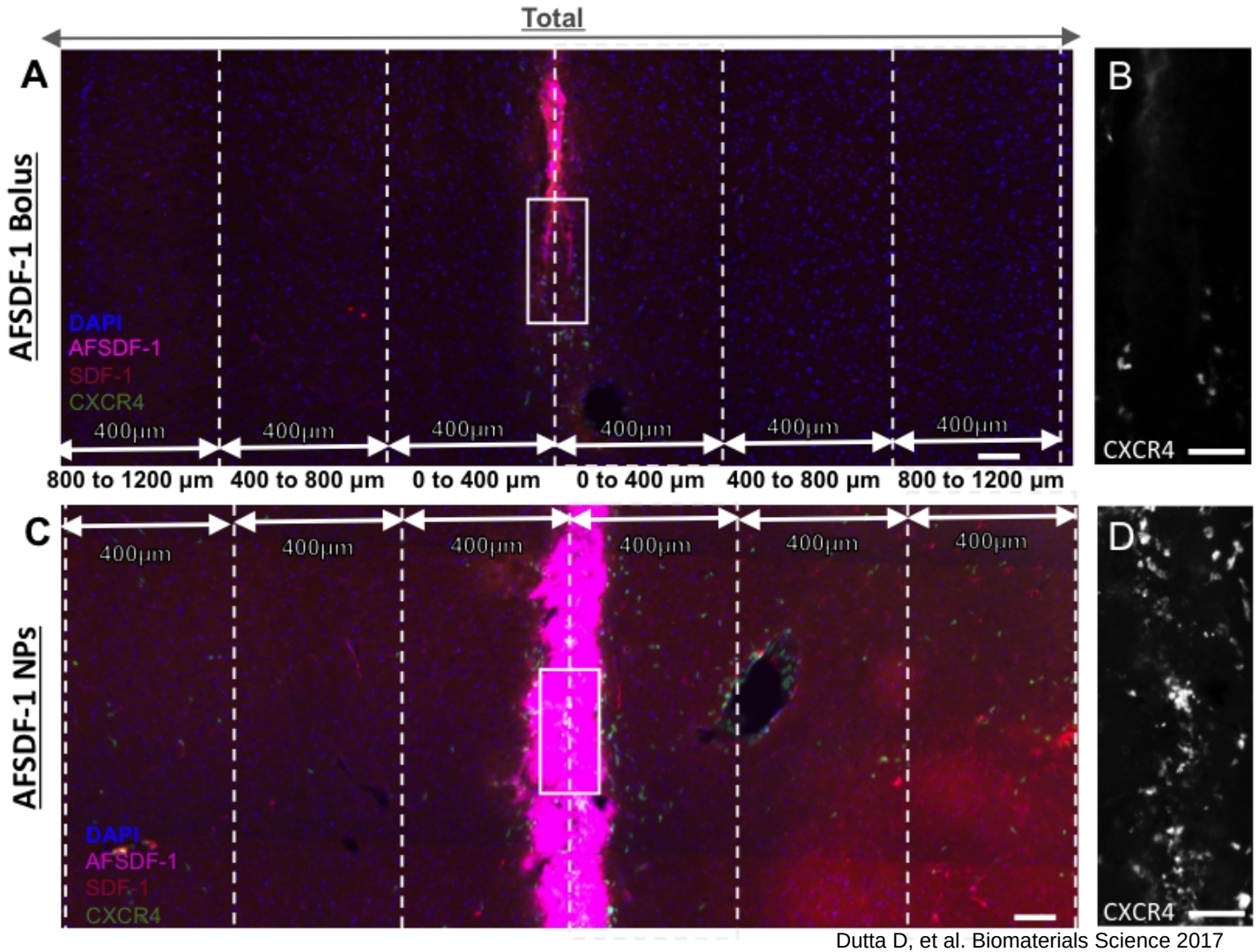


Available toolset:

1. Bioactive SDF-1α with fluorophore attached to C-terminus (**AFSDF-1**)
2. Transgenic (**EGFP-CXCR4**) mice
   - Intracellular enhanced green fluorescent protein (EGFP) transcription reporter for CXCR4
3. **AFSDF-1 loaded PLGA NPs**[1]
   - Sustained release of AFSDF-1
4. **SDF-1 immunostaining** ✉ total SDF-1

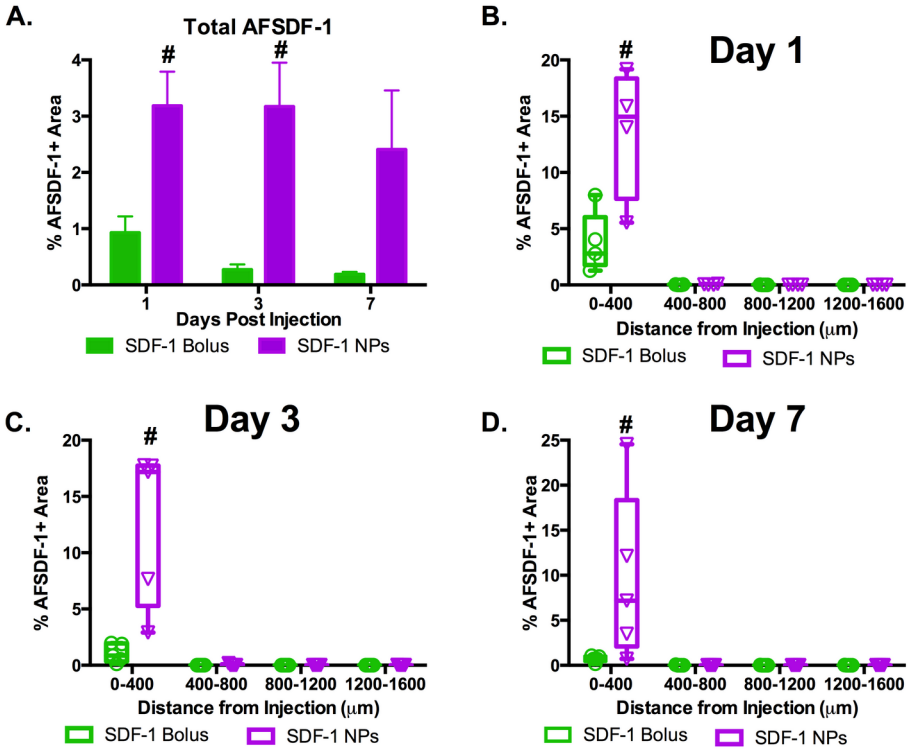


Dutta D, et. al. J Mater Chem B Mater Biol Med. 2015.
Dutta D, et al. Biomaterials Science 2017

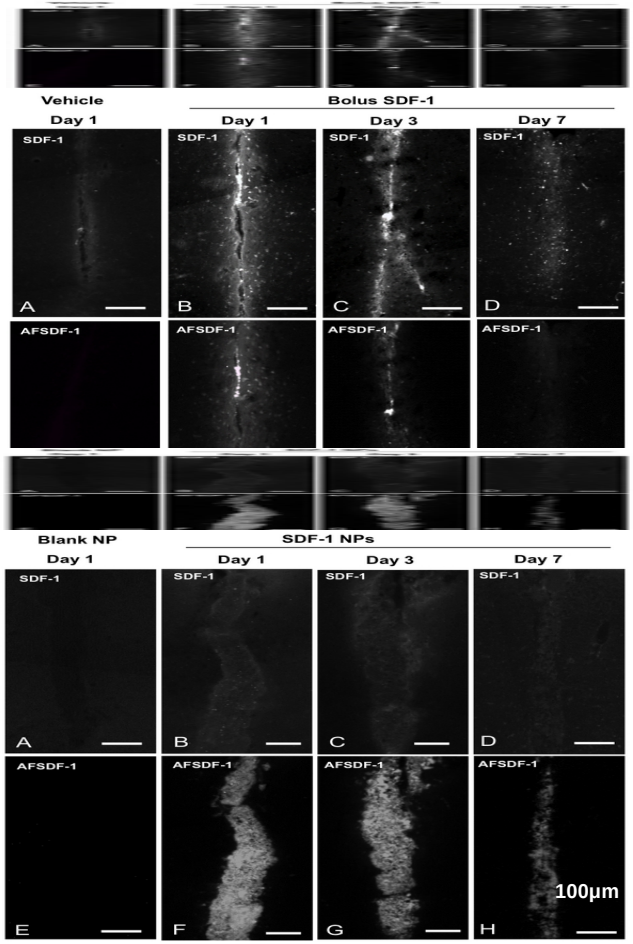


Dutta D, et al. Biomaterials Science 2017

Limited Penetration of Exogenous SDF-1



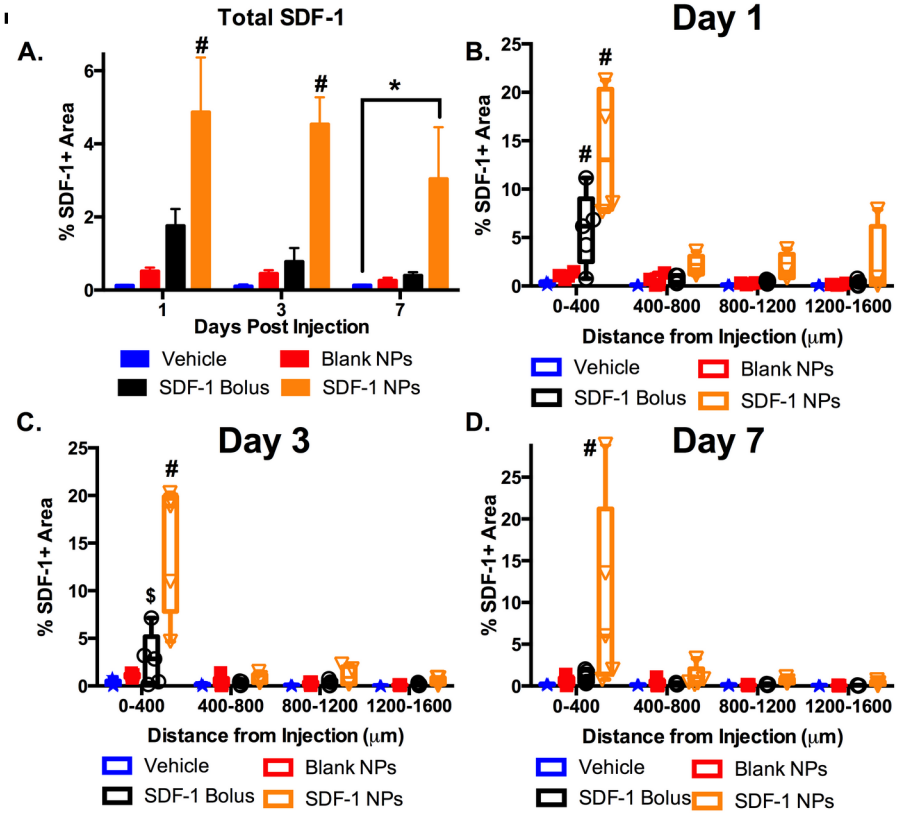
#p<0.01 compared SDF-1 bolus, n= 4-5 animals per group, 4-6 ROI per animal



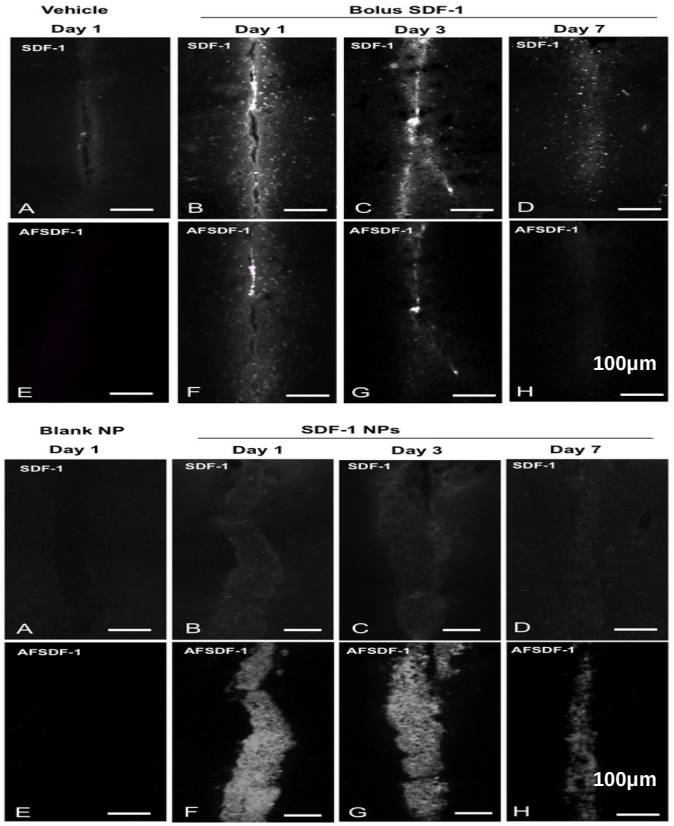
Limited penetration of exogenous SDF-1, yet, persistent presence with SDF-1 NP

Dutta D, et al. Biomaterials Science 2017

Total (Endogenous+Exogenous) SDF-1 Localization



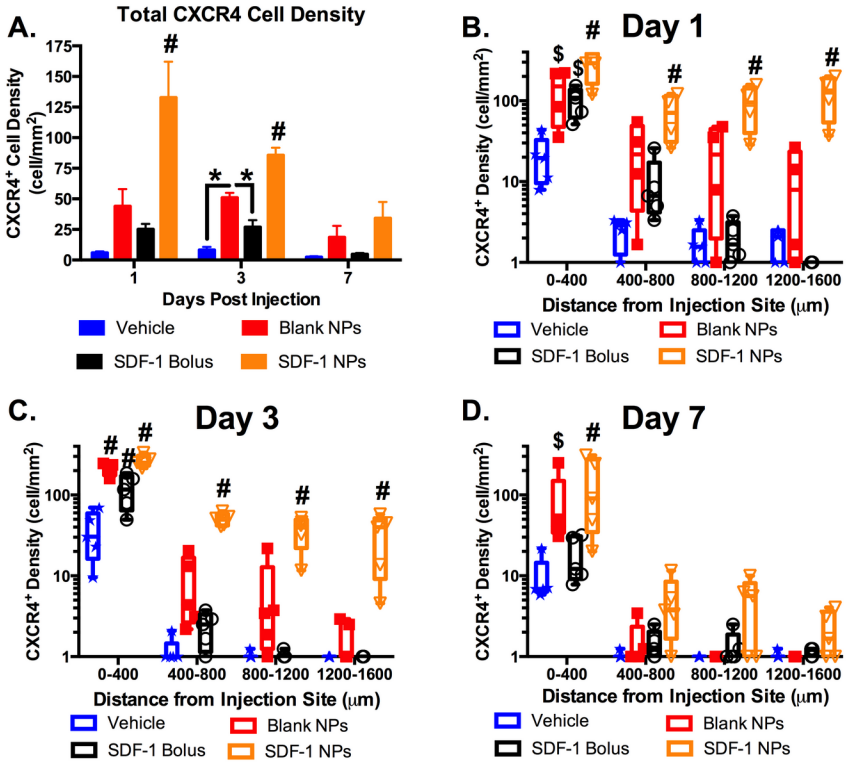
#p<0.01 compared to all groups, $p<0.05 compared vehicle,*p<0.05; n= 4-5 animals per group, 4-6 ROI per animal



SDF-1 NP increased temporal bioavailability of exogenous SDF-1 and transiently induce endogenous SDF-1 expression

Dutta D, et al. Biomaterials Science 2017

Spatiotemporal Localization of CXCR4+ Cells



#p<0.01 compared to all groups, $p<0.05 compared vehicle, *p<0.05; n= 4-5 animals per group, 4-6 ROI per animal

CXCR4 Activation for SDF-1 Bolus = Local & Transient

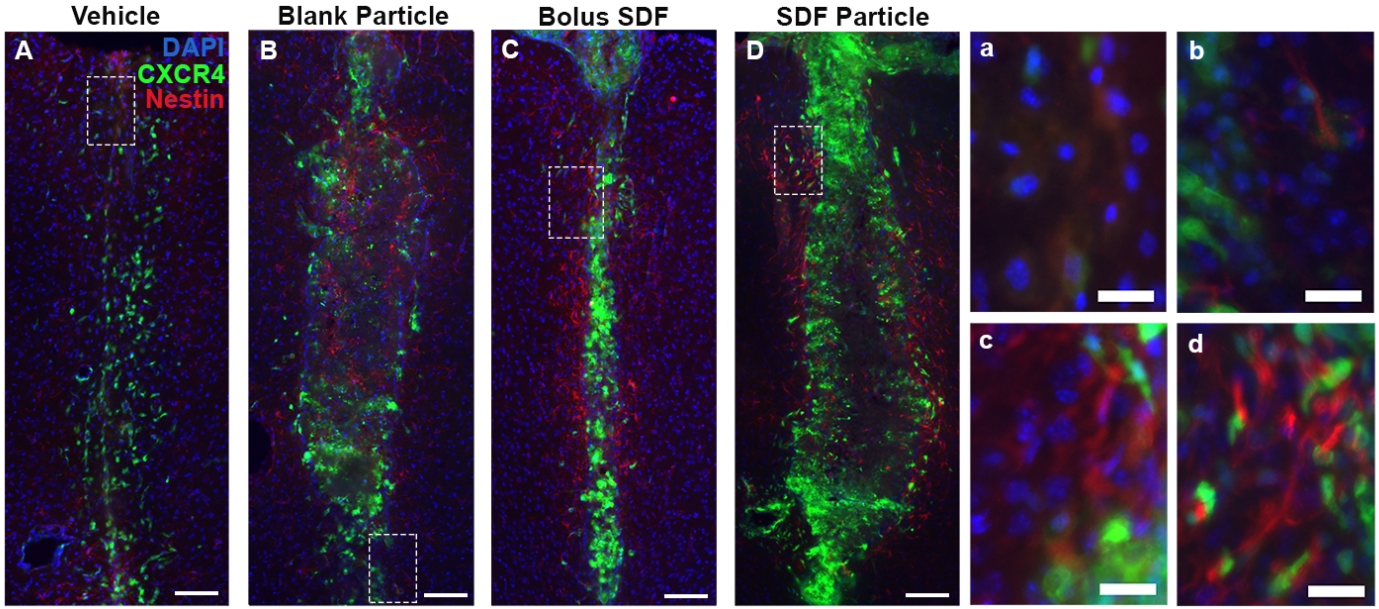
SDP-1 NP Sustained Release = Spatially Dispersed & Transient

Dutta D, et al. Biomaterials Science 2017

What cells are activated/recruited?

7 Day Post Injection

Nestin: Neural progenitor and Glial Cell Marker



Low level Nestin+ cells within the injection track

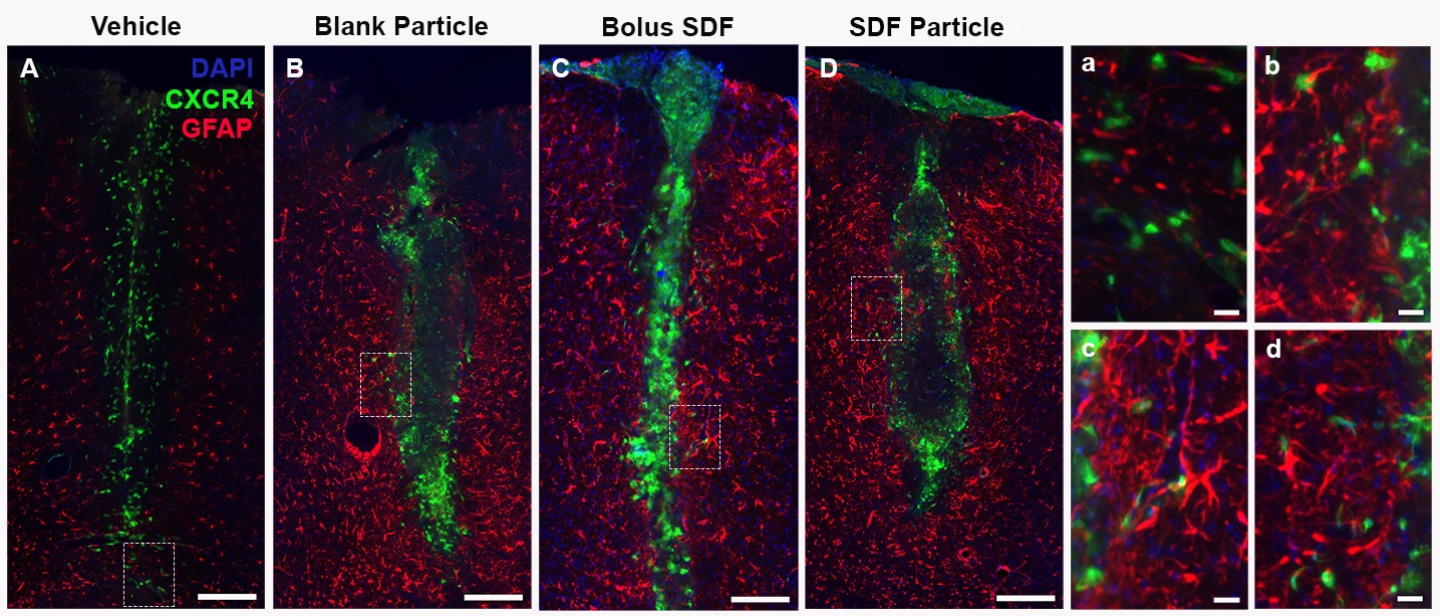
Modest neurogenesis OR related to astrogliosis

Dutta D, et al. Biomaterials Science 2017

What cells are activated/recruited?

7 Day Post Injection

GFAP: Astroglial Marker



Robust GFAP+ in response to nanoparticles and/or SDF-1

Yet no co-localized with CXCR4

Dutta D, et al. Biomaterials Science 2017

PLGA NP SDF-1 System: Conclusions

SDF-1 NPs extended bioavailability of exogenous SDF-1

SDF-1 NPs resulted in prolonged immunopositive staining of total SDF-1 locally nearly the injection tract as compared to bolus SDF-1 and control groups.

Bolus SDF-1 delivery resulted in transient and localized CXCR4 response

CXCR4 positive cells were activated acutely following bolus SDF-1 injection. This response was confined to within 400µm of the center of injection tract.

SDF-1 NPs delivery resulted in dispersed, yet, transient CXCR4 response

In contrast to the bolus SDF-1 administration, the SDF-1 NPs elicited a pronounced CXCR4 response that was dispersed across the cortex on day 1 and day 3. Yet, this response was confined to a localized response (0-400µm) by day 7.

Modest evidence of neurogenesis/immature neuronal recruitment, namely robust astroglial response

Continuing to explore activated CXCR4 cell phenotype. **Shifting to hyaluronic acid hydrogel based system with faster degradation rates and low levels of inflammation.**

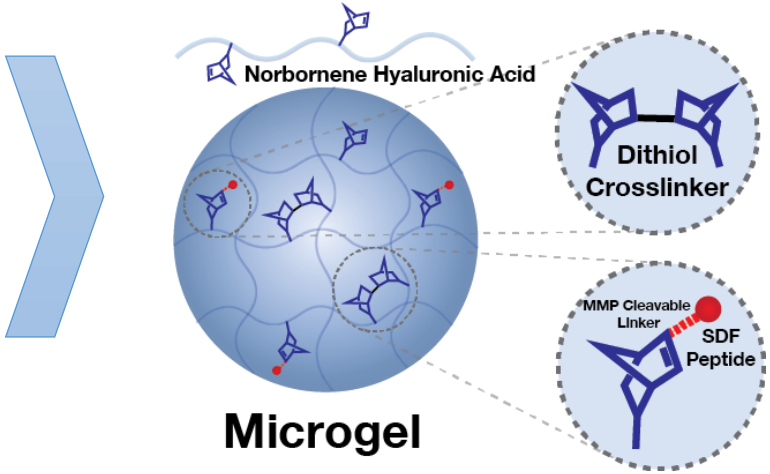
Hyaluronic Acid (HA) Particle Delivery Platform

Design criteria:

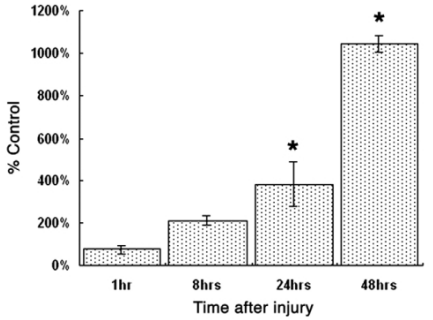
- High **biocompatibility**
- Injectable via needle (<400um diameter)
- Mechanical properties matching brain tissue
- Tunable release of SDF-1a

Norbornene HA Microgels:

- Native ECM material
- Precise chemical modification
- Ability to prolong SDF-1a release through matrix metalloproteinase cleavage sites



MMP-9 Expression after TBI; Hayashi et al.



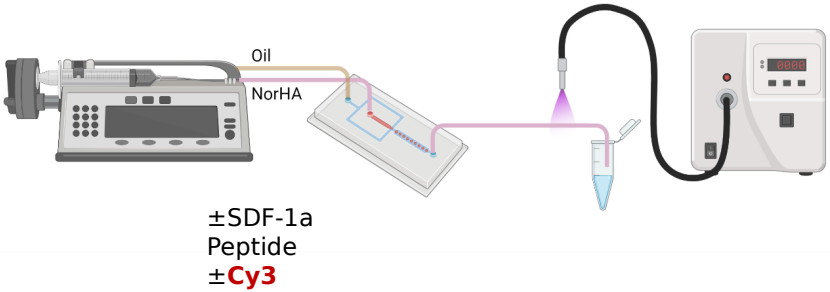
Microgel Characterization: Size Distribution

Microgel Production:

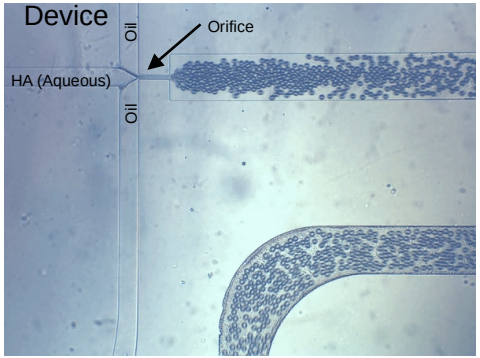
- Flow focusing microfluidics

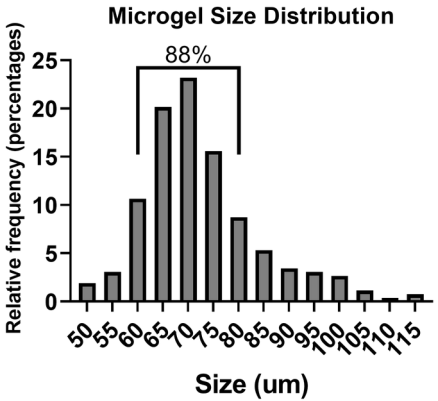
Results:

- 73 ± 12 µm
- Over 88% between 60-80µm

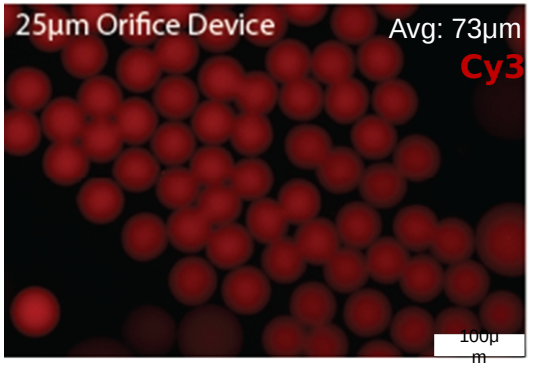


Flow Focusing Microfluidics Device



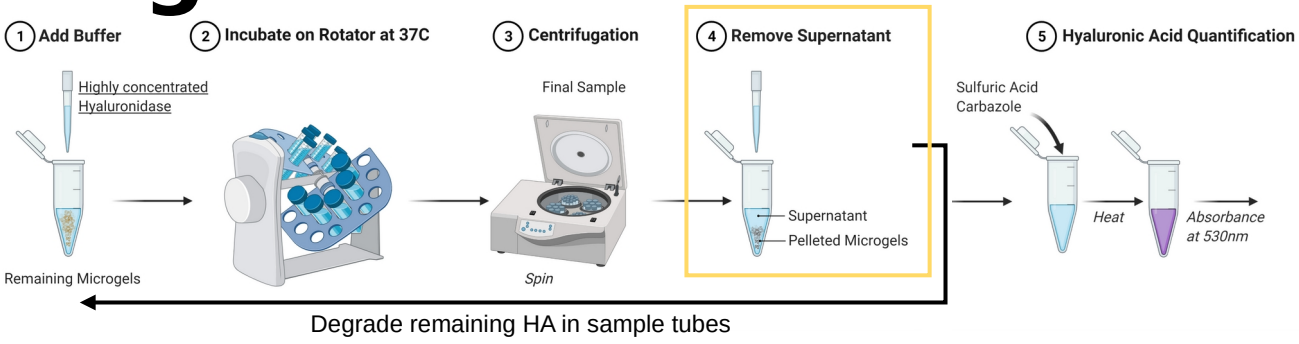


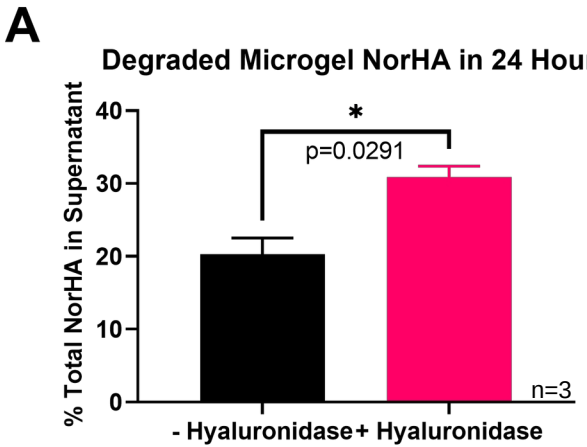
4 Devices, n=263

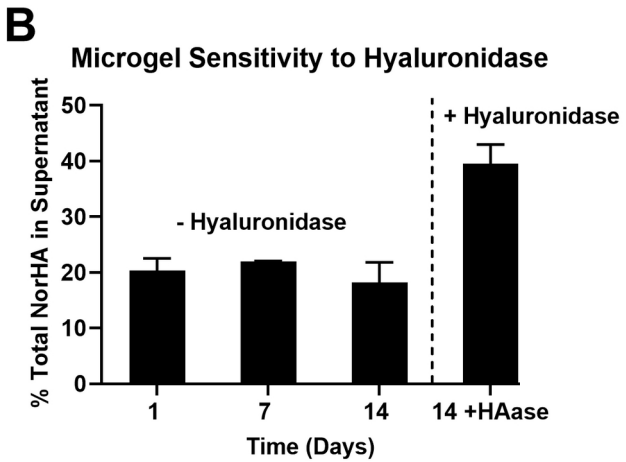


Monodisperse, injectable microgels were generated via microfluidics

Microgel Characterization: Degradation

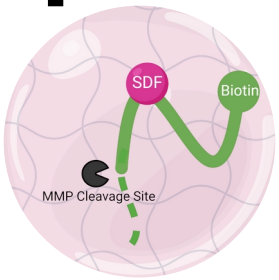




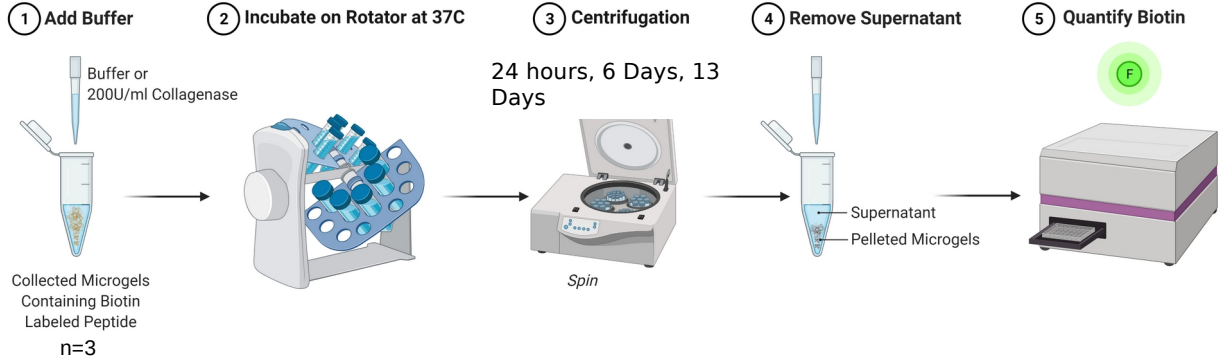


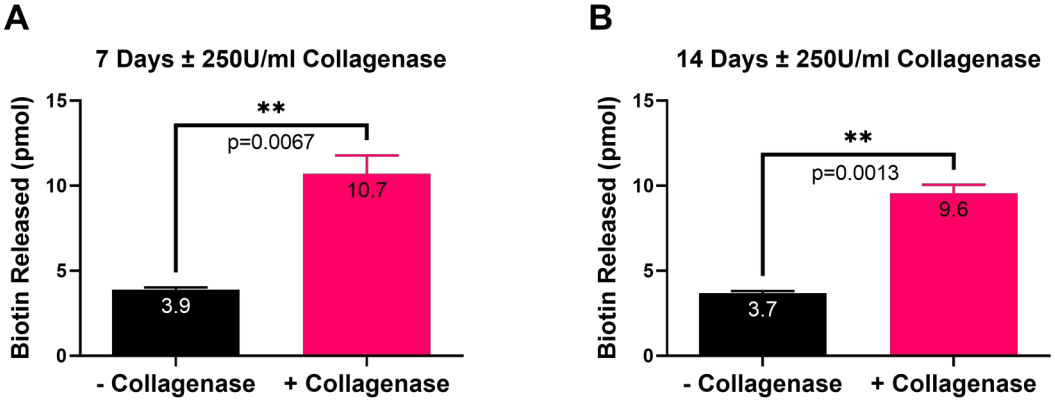
Microgels are sensitive to hyaluronidase degradation over 14days in vitro

Microgel Characterization: SDF-1a Peptide Release



Peptide Schematic within Microgel

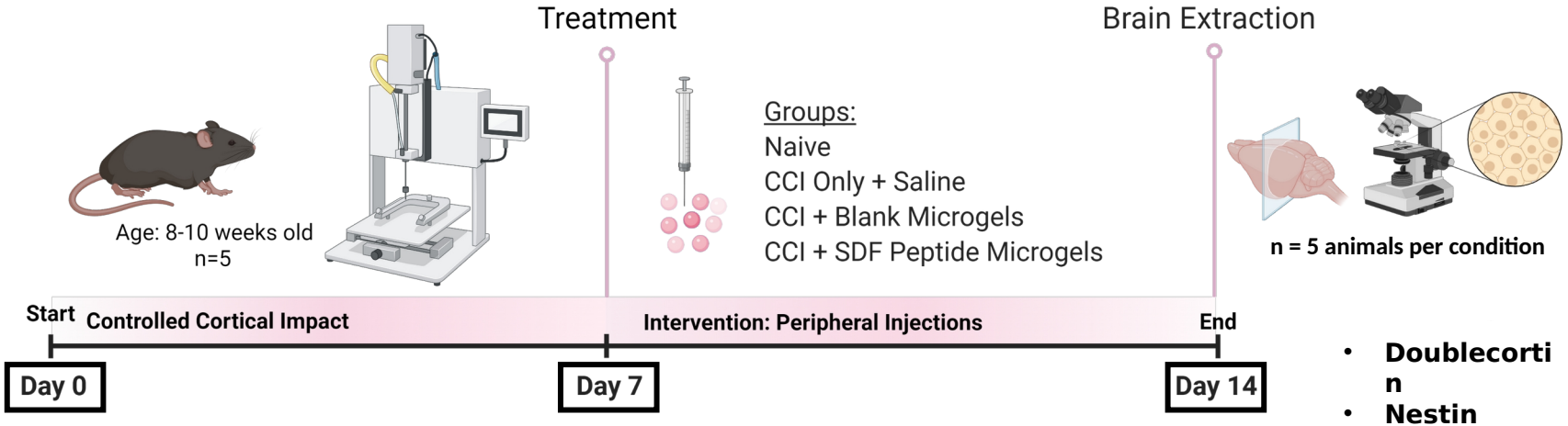




Microgels demonstrate retention and MMP-mediated SDF-1a peptide release for 14 days

In Vivo Experimental Design

In Vivo Experimental Timeline

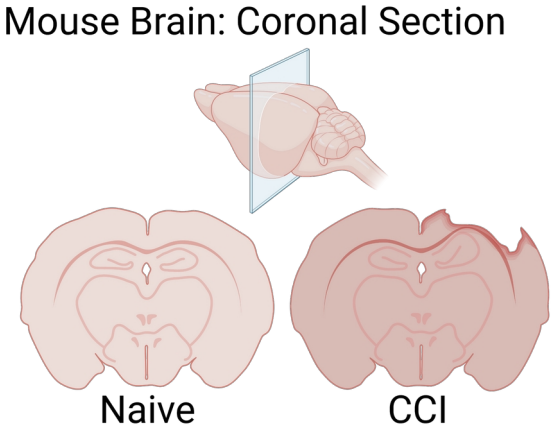


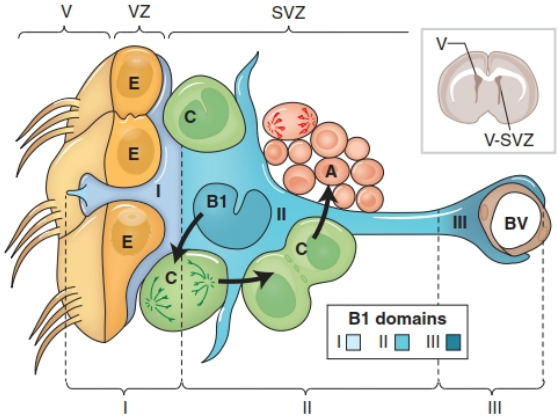
- **Doublecortin**
- **Nestin**
- **GFAP**

Created with BioRender.com

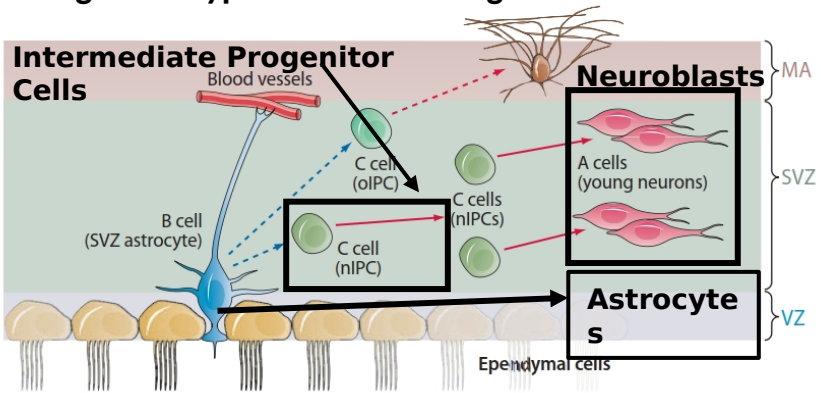
The Heterogenous SVZ

Ventricular-SVZ





Progenitor-type Cells and Lineages



Key Markers of Interest

- Doublecortin+ = Neuroblasts
- Nestin+/GFAP- = Neural Progenitor Cell
- Nestin+/GFAP+ = Astrocyte

Figu pect Biol. & Alavarez-Buylla, A. and Kriegstein, A. 2009. Annu. Rev. Neurosci.

Assessing Regional Nestin and GFAP Expression

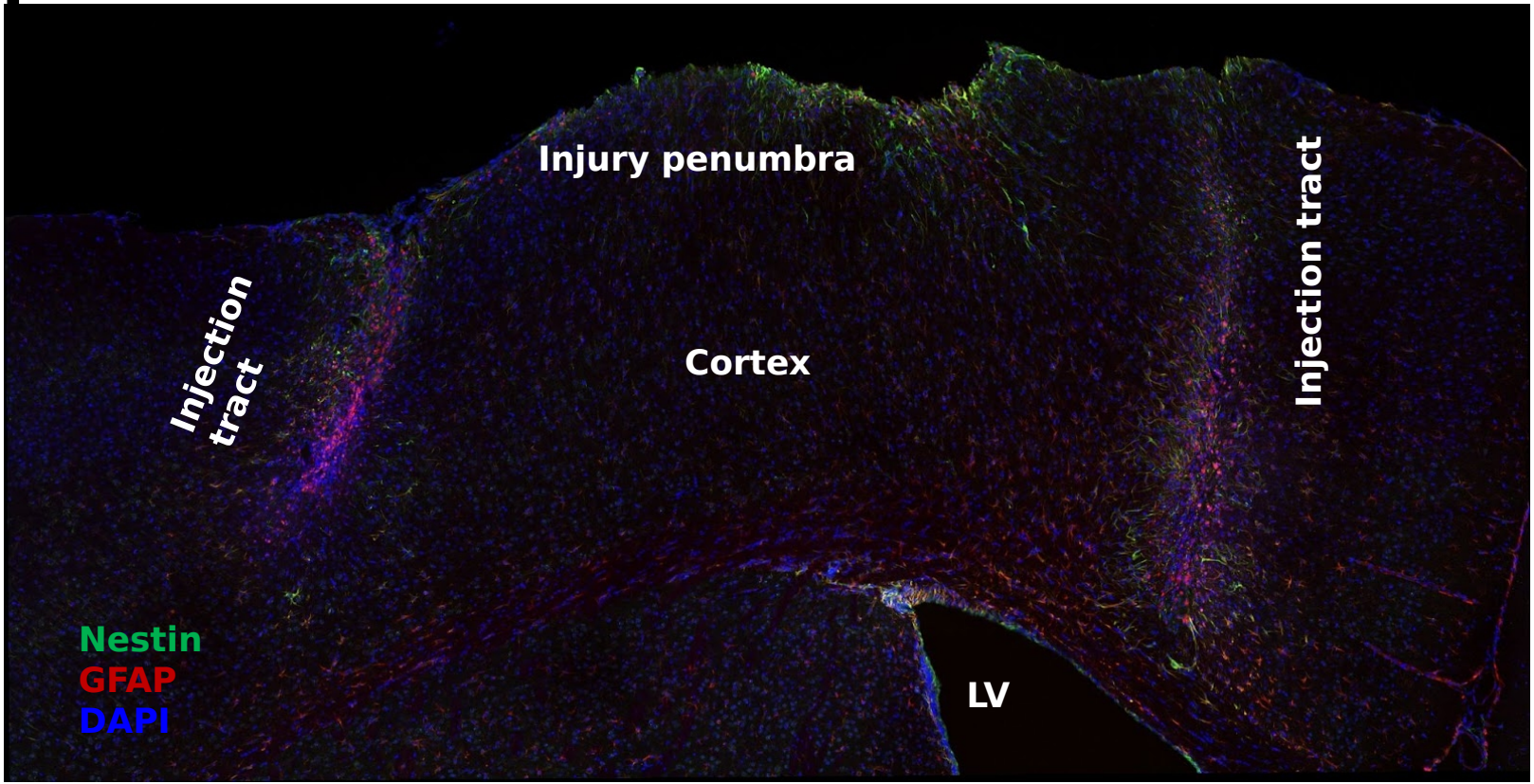
Questions and Regions of Analysis

- Is astrocyte activation increased due to the presence of microgels?
  - Assess astrocyte area in injection track and injury penumbra
    - Nestin+/GFAP+ AND Nestin-/GFAP+ = Astrocyte

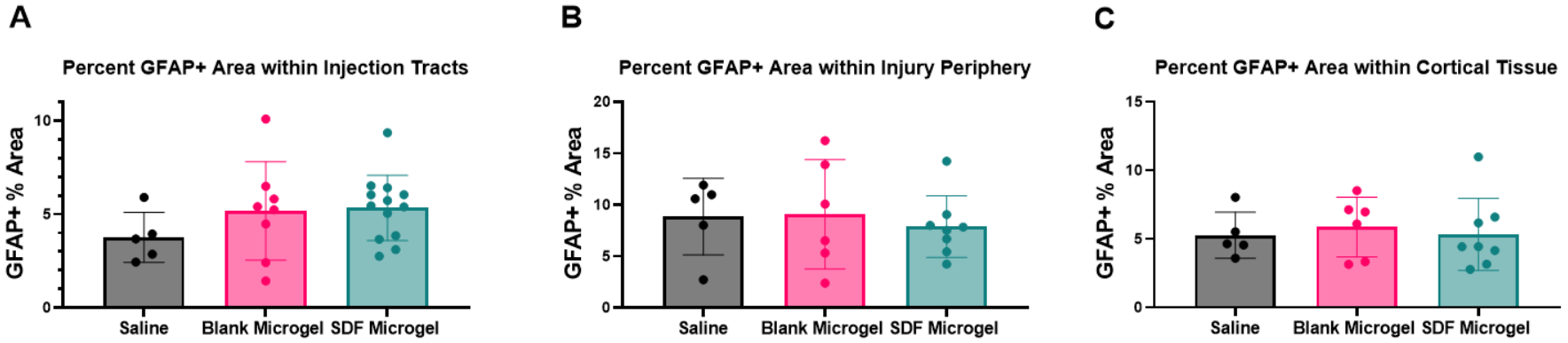
- Are neural progenitor cells prominent in injection site and/or injury penumbra?
  - Nestin+/GFAP- = Neural Progenitor Cell
  - Within injection tracks, cortical area between injections, and injury penumbra

- Are neuroblasts recruited to the microgel injection site and/or injury penumbra?
  - Doublecortin+ within injection tracks, cortical area between injections, and injury penumbra.

Assessing Regional Nestin and GFAP Expression



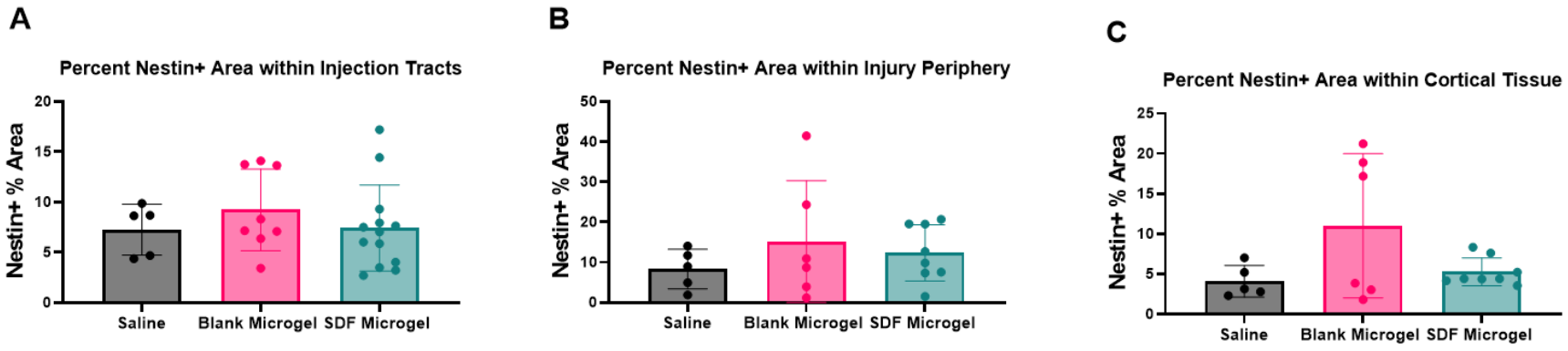
Assessing Regional GFAP Expression



Is astrocyte activation increased due to the presence of microgels?

- No evidence of heightened astrocyte activation locally or within in the injury penumbra.

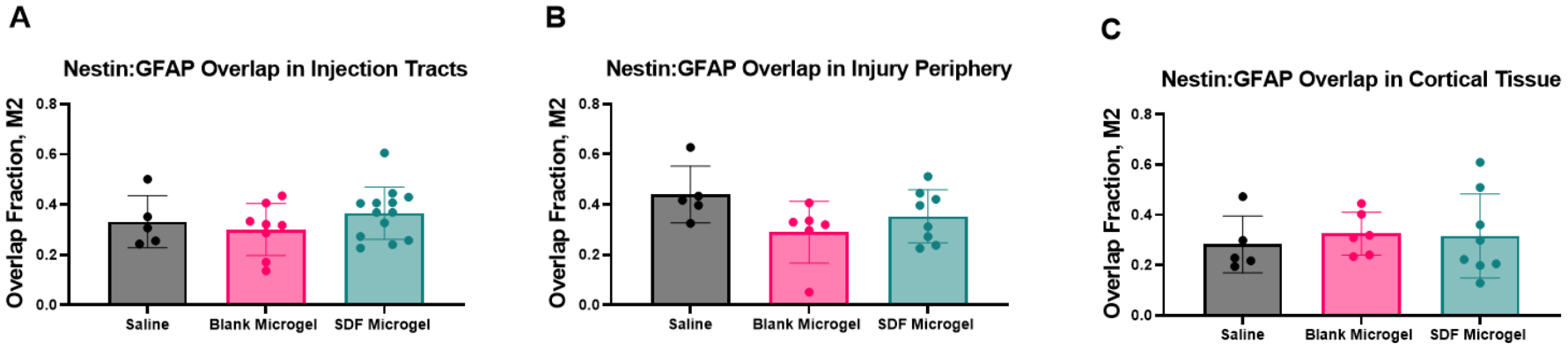
Assessing Regional Nestin Expression



Are neural progenitor cells prominent in injection site and/or injury penumbra?

- Nestin+ cells were prominent throughout the injection site, cortical tissue, and injury penumbra
- No difference among experimental groups

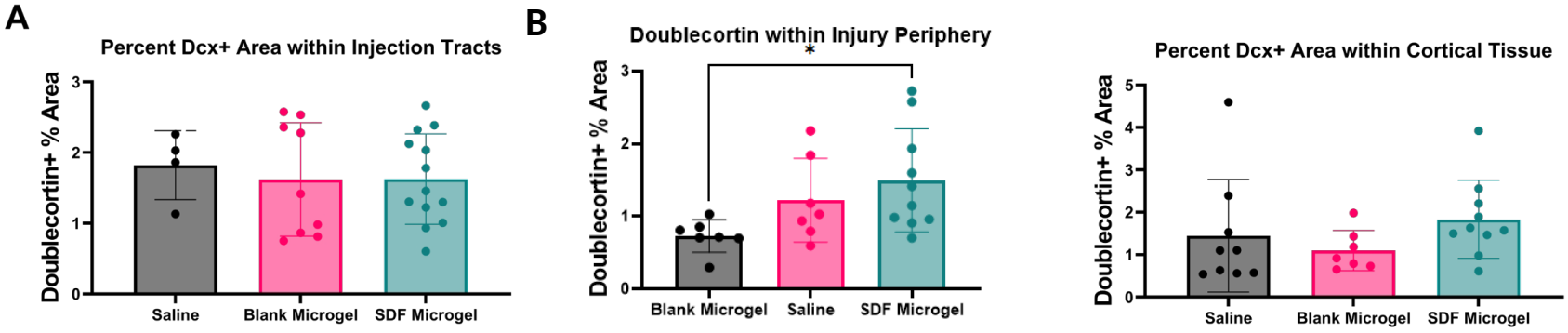
Assessing Regional Nestin/GFAP Expression



Are neural progenitor cells prominent in injection site and/or injury penumbra?

- Nestin+/GFAP+ accounted for ~25-40% of cells -> Nestin+ only accounted for 60-75% of cells = prominent NPC response due to injury
- No difference among experimental groups

Doublecortin Labeling for Neuroblasts

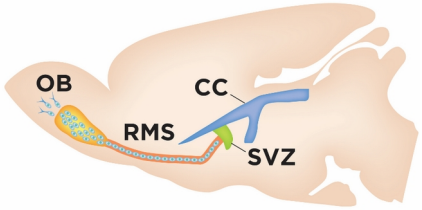


Are neuroblasts recruited to the microgel injection site and/or injury penumbra?

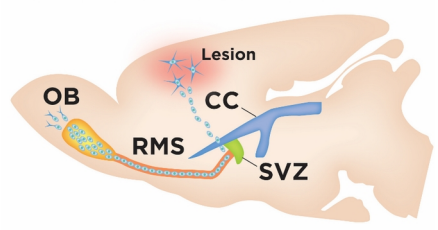
- DCX+ increased significantly within the injury penumbra with SDF microgels
- No difference among experimental groups in other regions

Approach 2: Endogenous cell recruitment - Conclusions

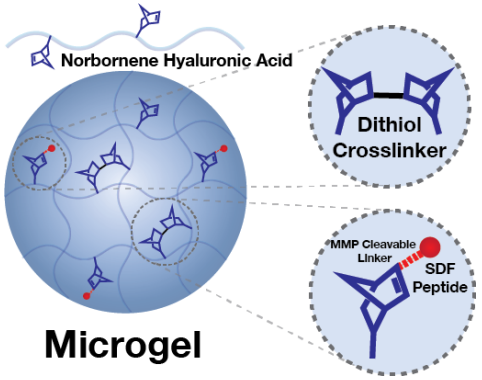
- **How are chemotactic gradients formed in the brain?**
  - ✓ Autocrine/paracrine signaling contributes to SDF-1a gradient Hickey et al. *Cellular and Molecular Bioengin* 2021



- **Can we design controlled release systems to modulate chemotactic profiles?**
  - ✓ HA microgel system with MMP-stimulated release of SDF-1a peptide



- **So what?**
  - Modest effect on neuroblast recruitment
  - No evidence of heightened astrocyte activation
  - Keep eye out for publication in 2024



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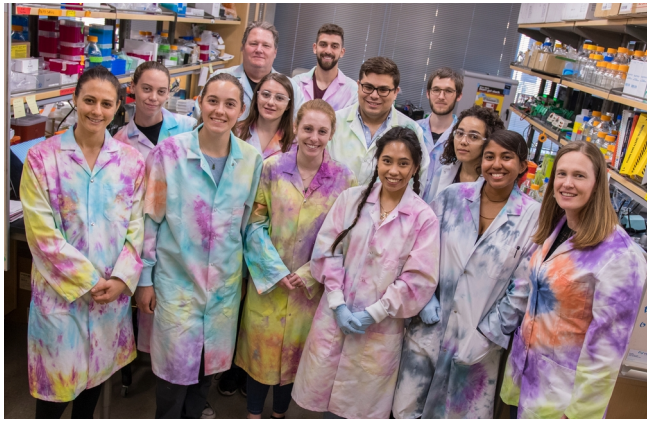

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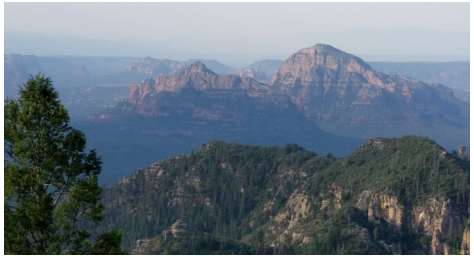

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