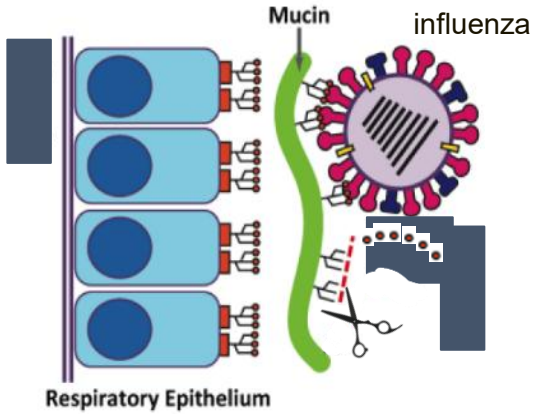
Integrated Fiber Microneedles in the Oral Mucosa Prime Antigen-Specific Immunity

Carmen Ioana Tobos

CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025
NEXT-GENERATION DELIVERY INNOVATIONS

Bean, R. et al. (2024). *mBio*.
Lapuente, D. et al. (2021). *Nat. Comm.*

Heterologous immunization can elicit both systemic and mucosal immunity



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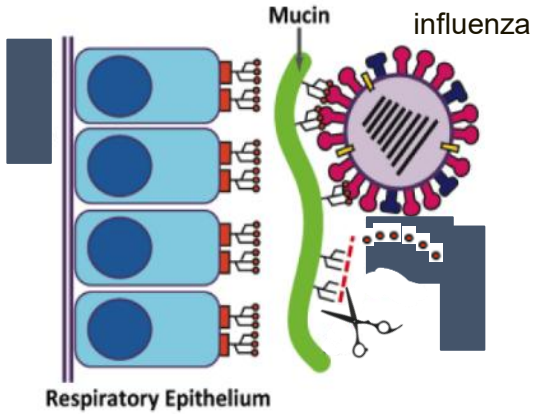

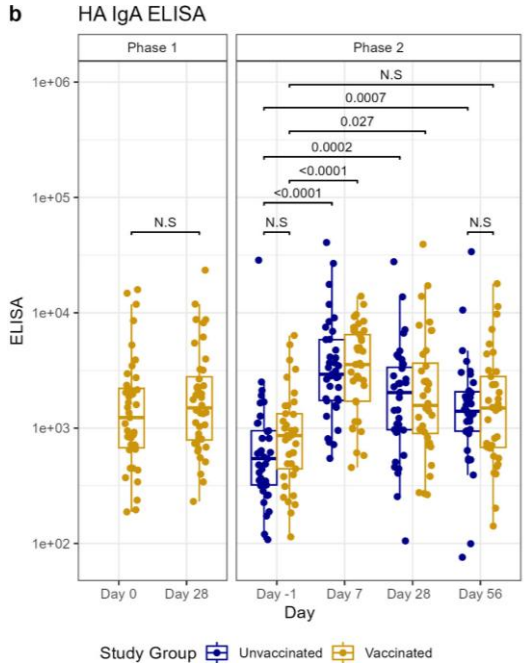

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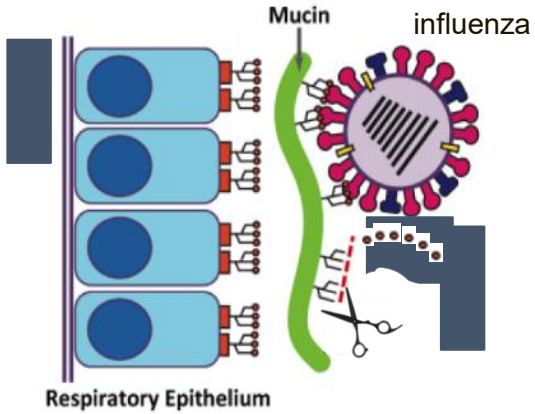

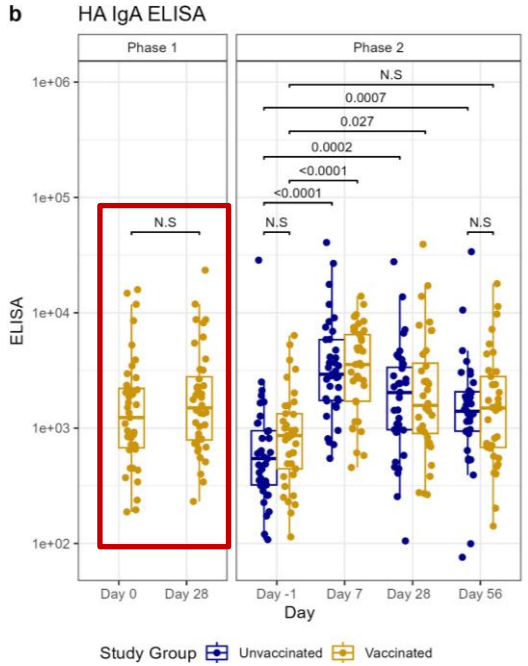

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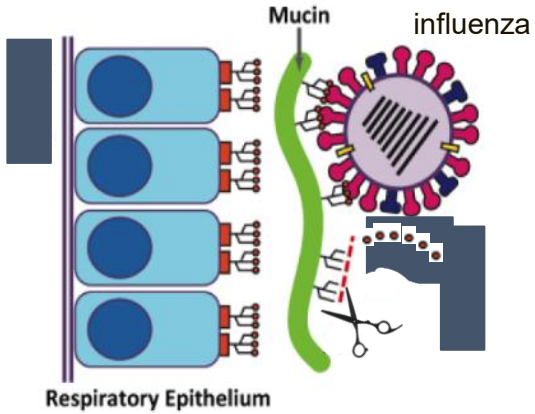

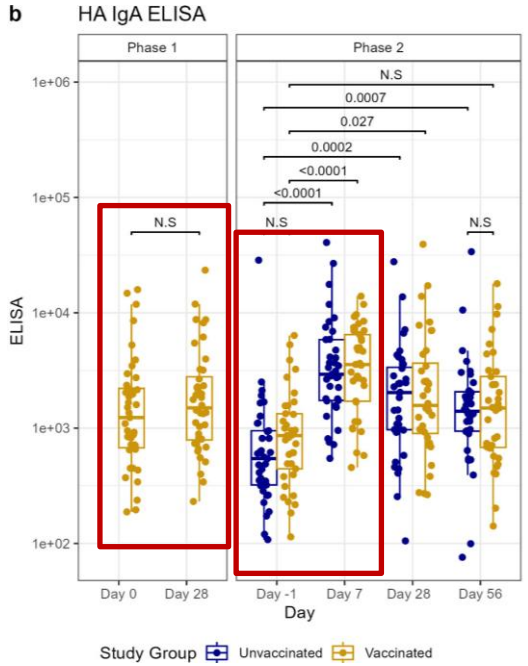

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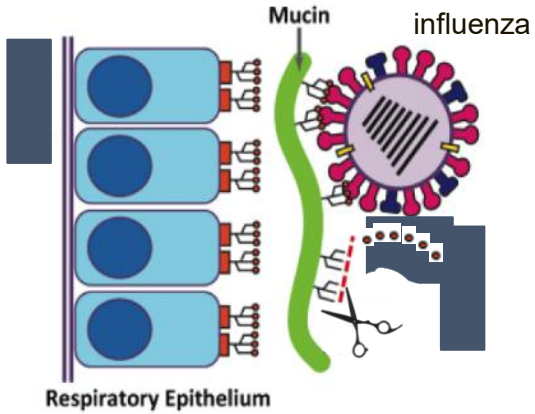

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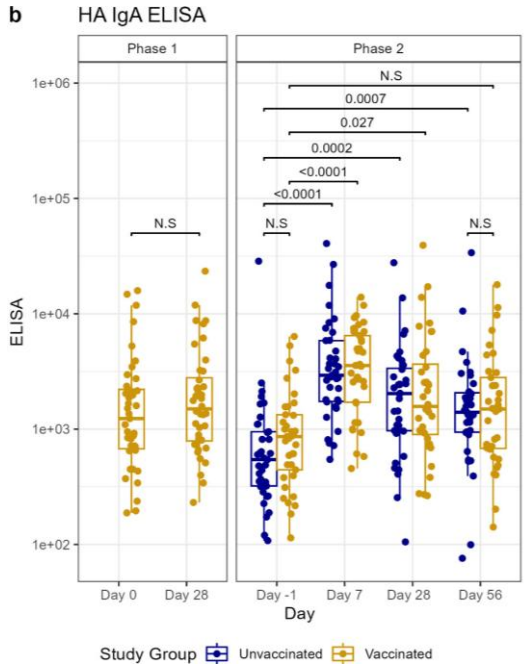
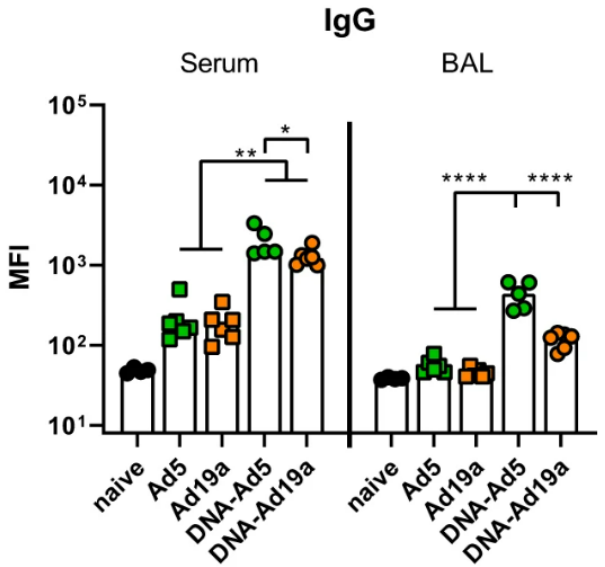
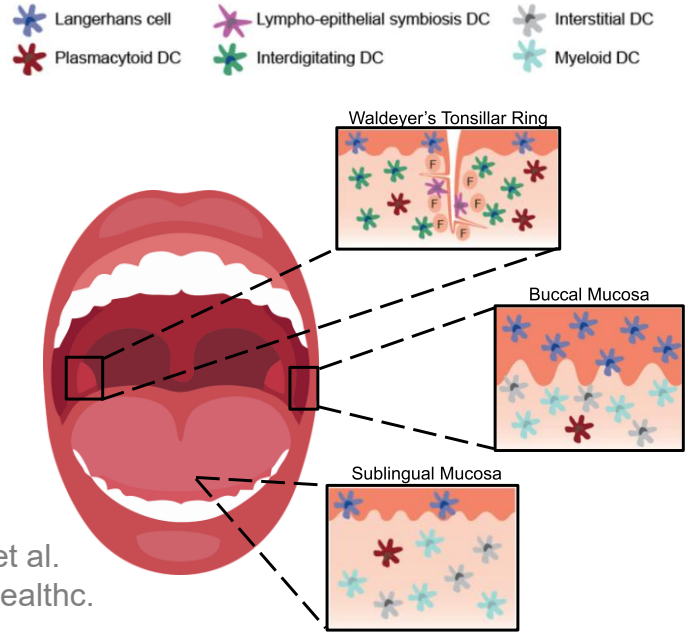


Systemically administered vaccines often elicit **poor mucosal immunity** compared to natural infection.



Heterologous immunizations that incorporate mucosal routes of vaccination elicit both systemic and mucosal immunity.

The oral mucosa is a promising site to induce mucosal immunity



Creighton, R. et al. (2019). Adv. Healthc. Mater.

The oral cavity is easily accessible, harbors diverse APC populations, and can induce effector responses in distal mucosae…

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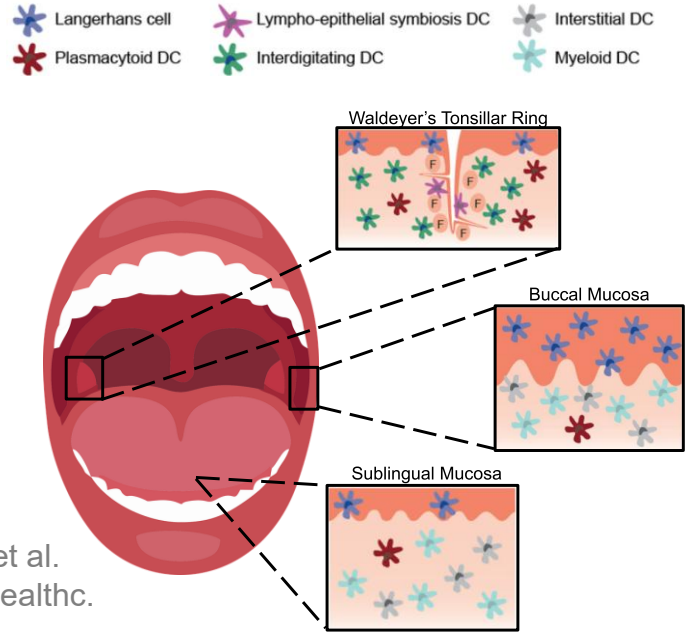

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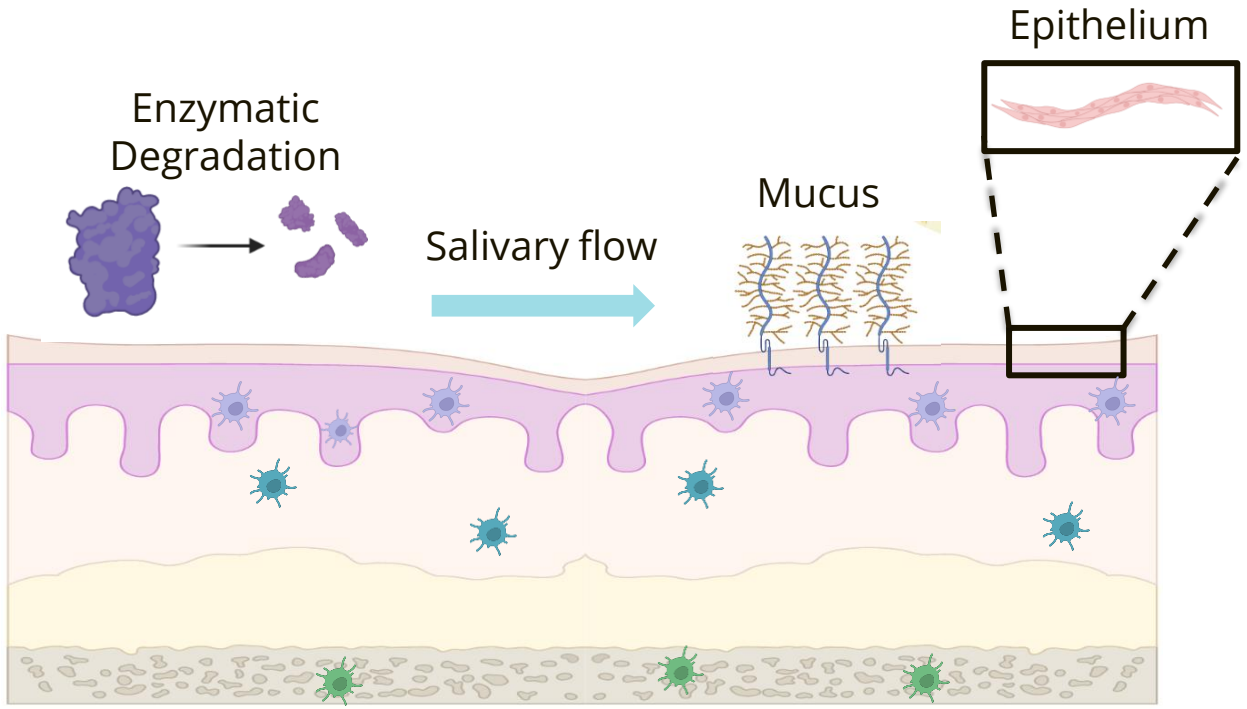
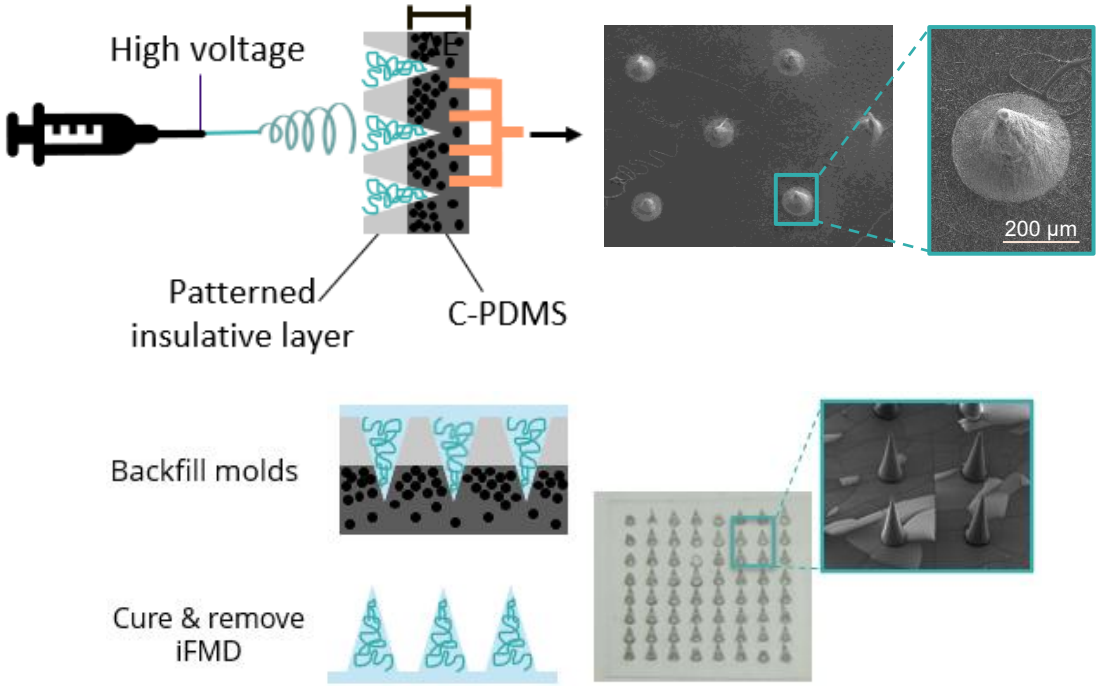


Figure created with BioRender

The oral cavity is easily accessible, harbors diverse APC populations, and can induce effector responses in distal mucosae...

... but approaches must overcome physical and immunological barriers

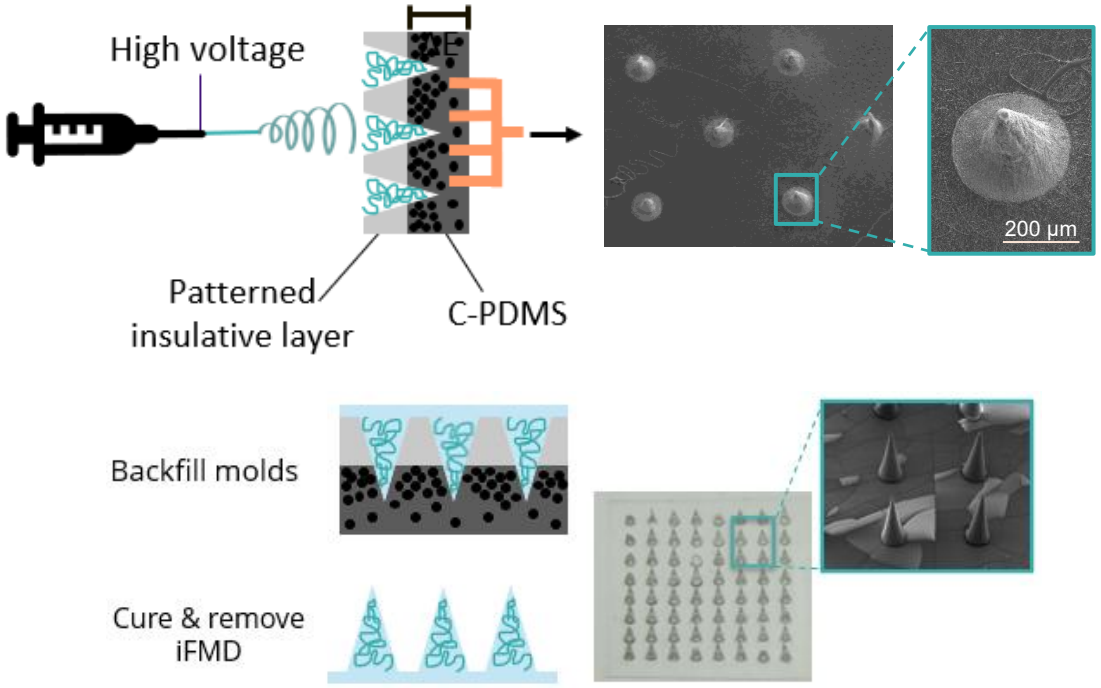
iFMDs deliver a wide range of reagents with tunable release kinetics



(iFMD) combine an electrospun fiber core with a polymeric backfill…

iFMDs deliver a wide range of reagents with tunable release kinetics

Rachel Creighton, PhD



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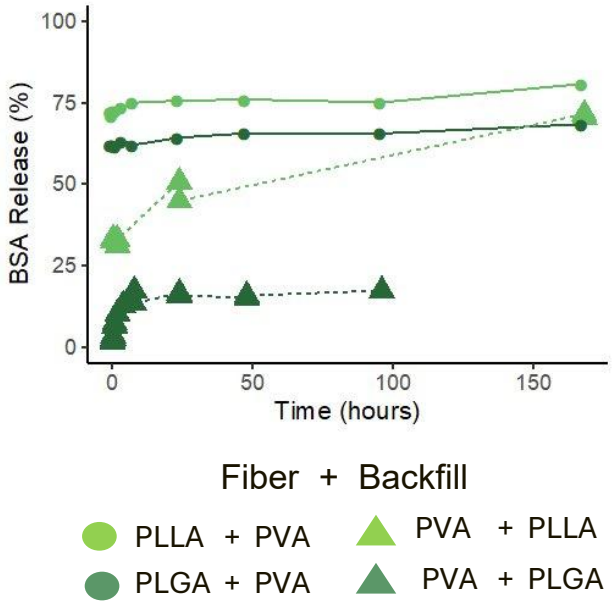

Creighton, R. et al. (2024). *JCR*.

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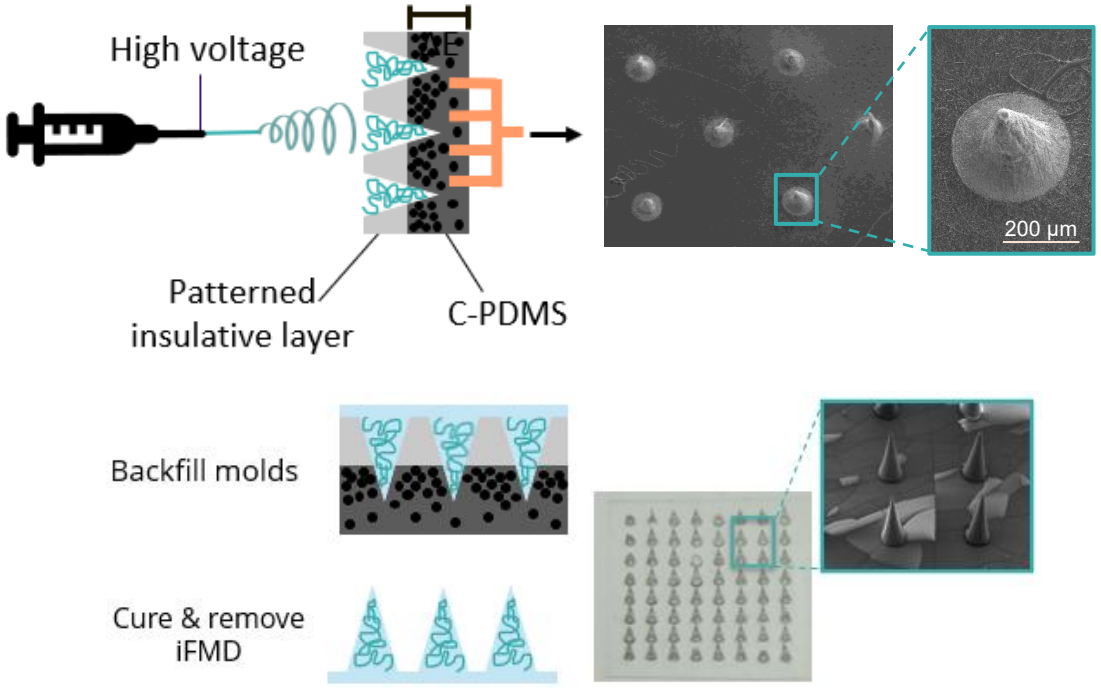

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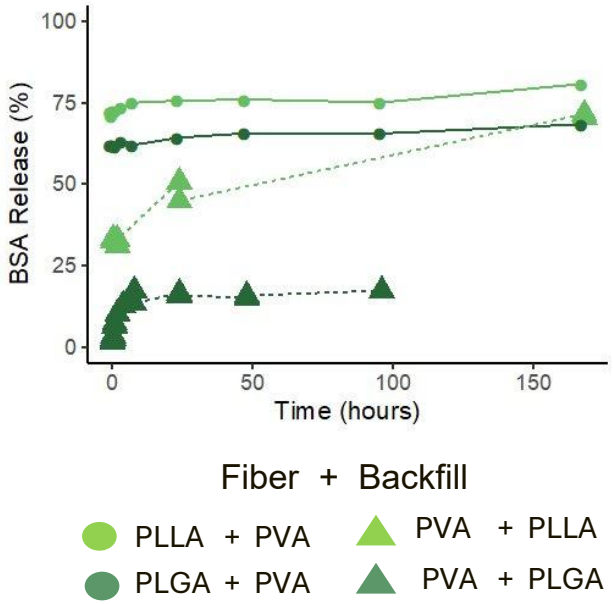
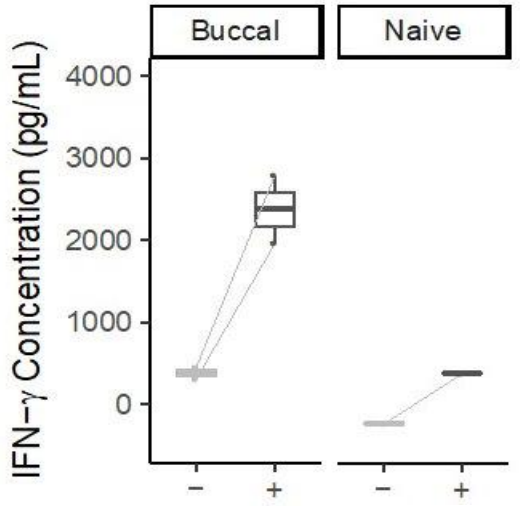


… and are immunogenic in the buccal mucosa of mice



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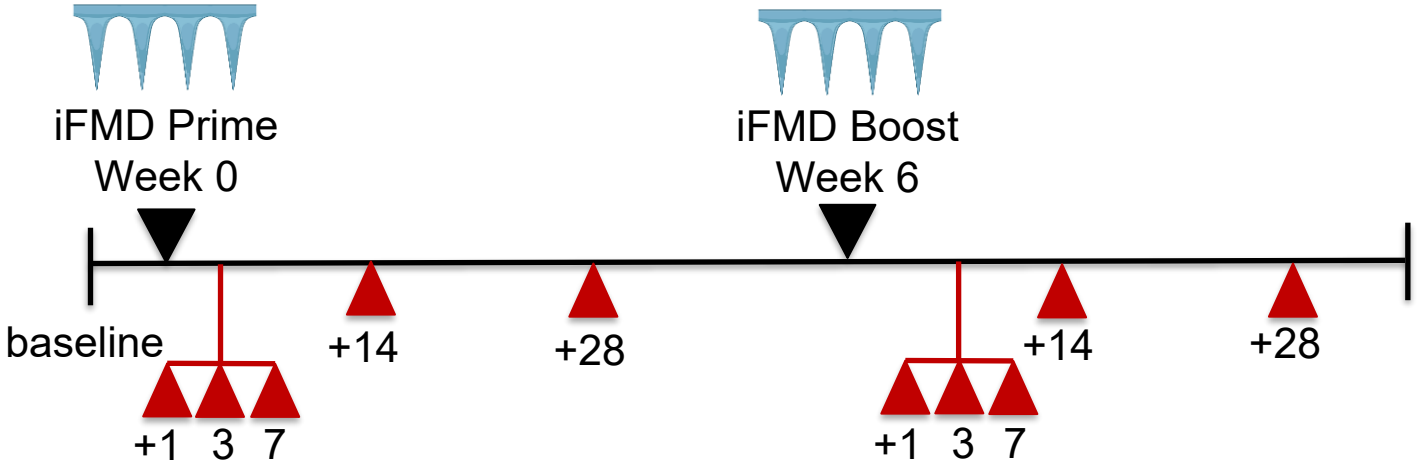
iFMDs in a Homologous Immunization Scheme

Cohort 1: With iFMD Priming



Antigen: NP plasmid
Adjuvant: IL-12 plasmid
Animal Model: Rhesus Macaques
Dose: 24 µg NP + 2.4 µg IL-12

iFMDs in a Heterologous Immunization Scheme

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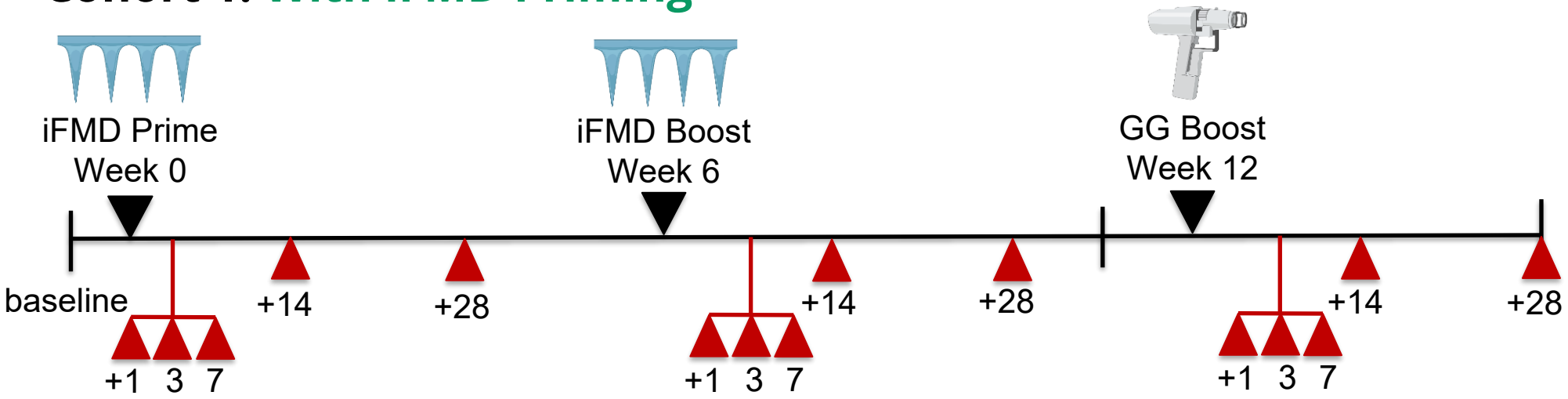

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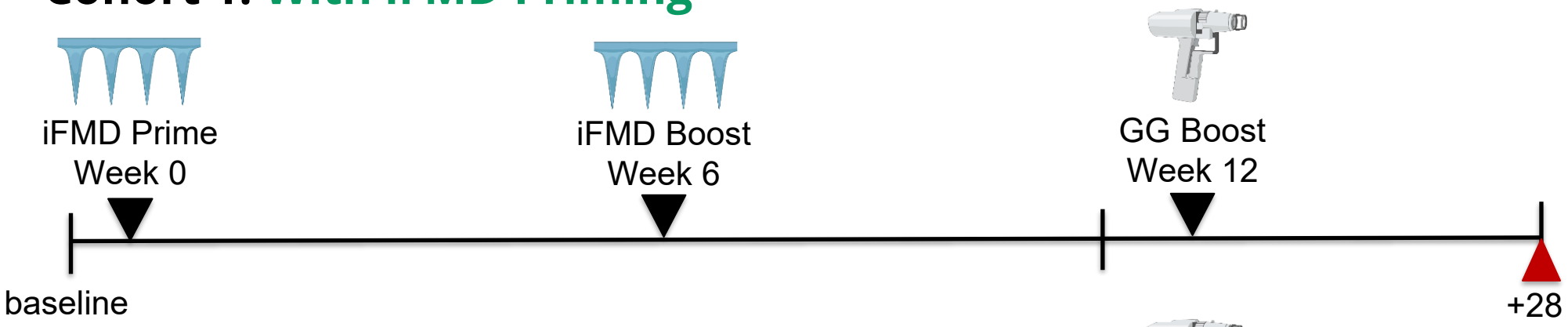

Cohort 2: Without iFMD Priming

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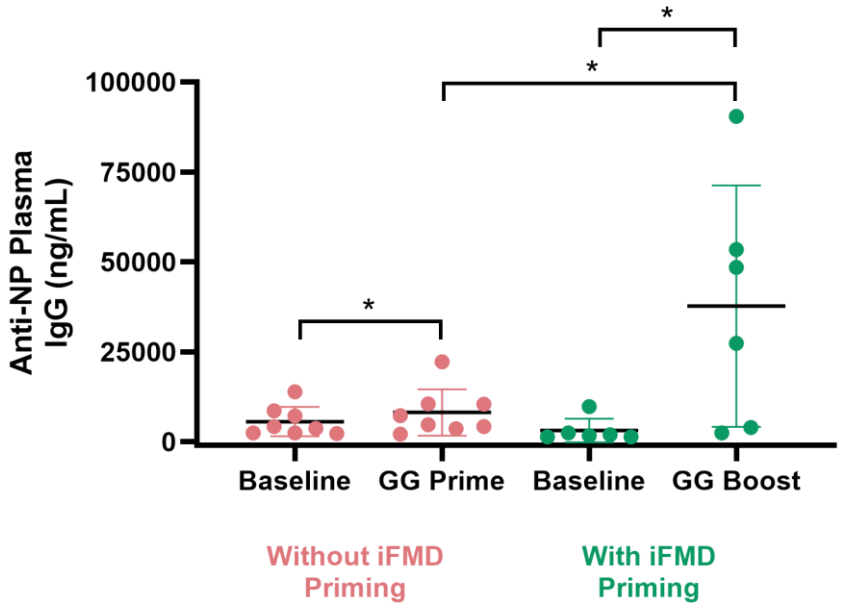
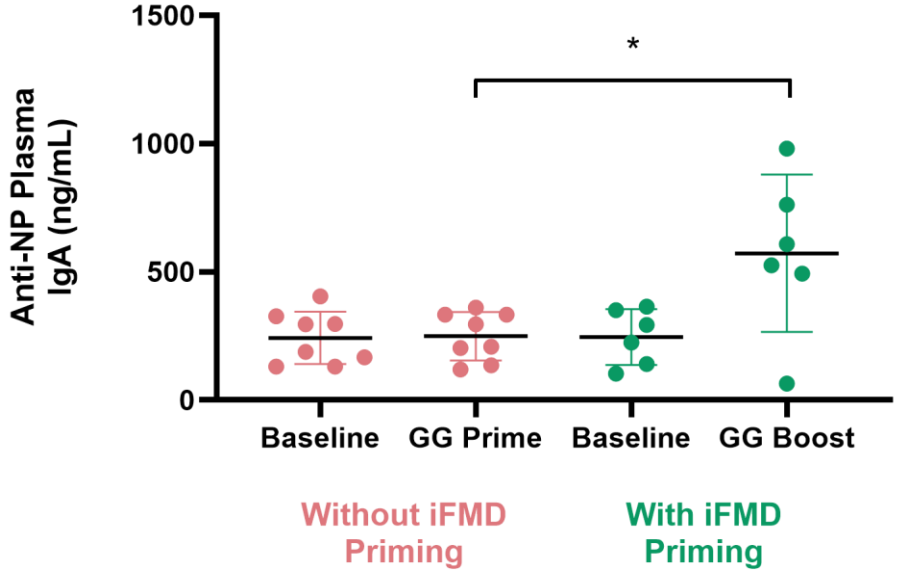
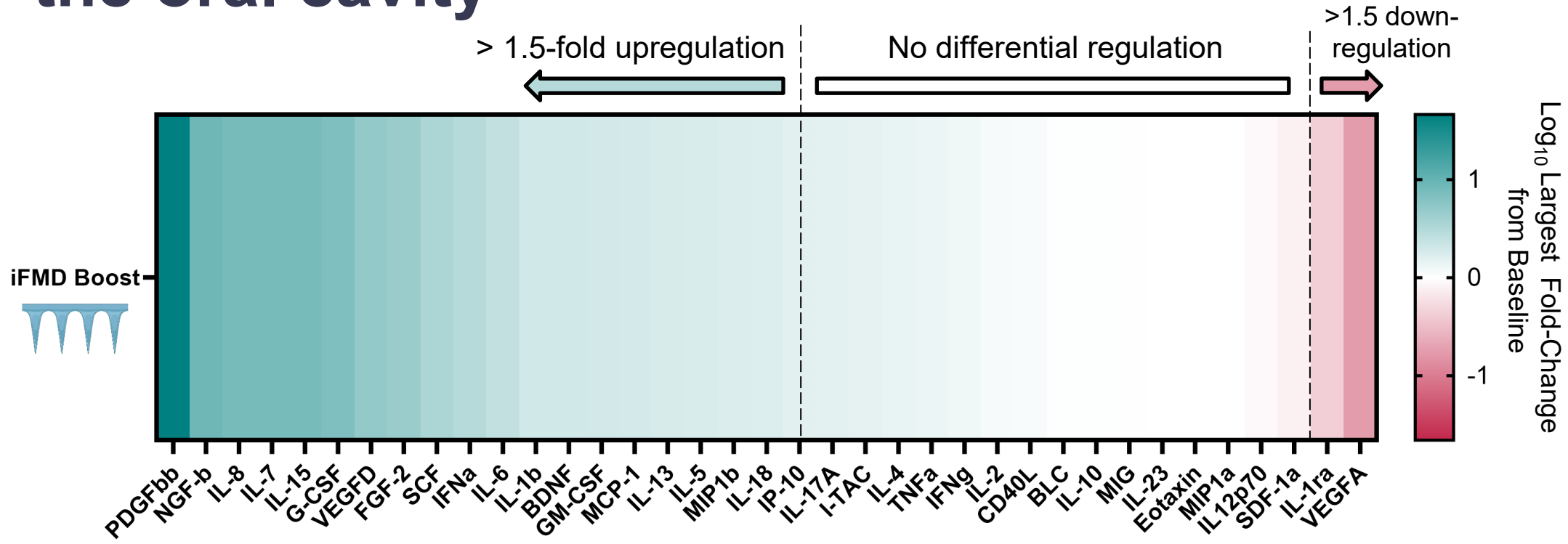
iFMDs prime antigen-specific immune responses in plasma





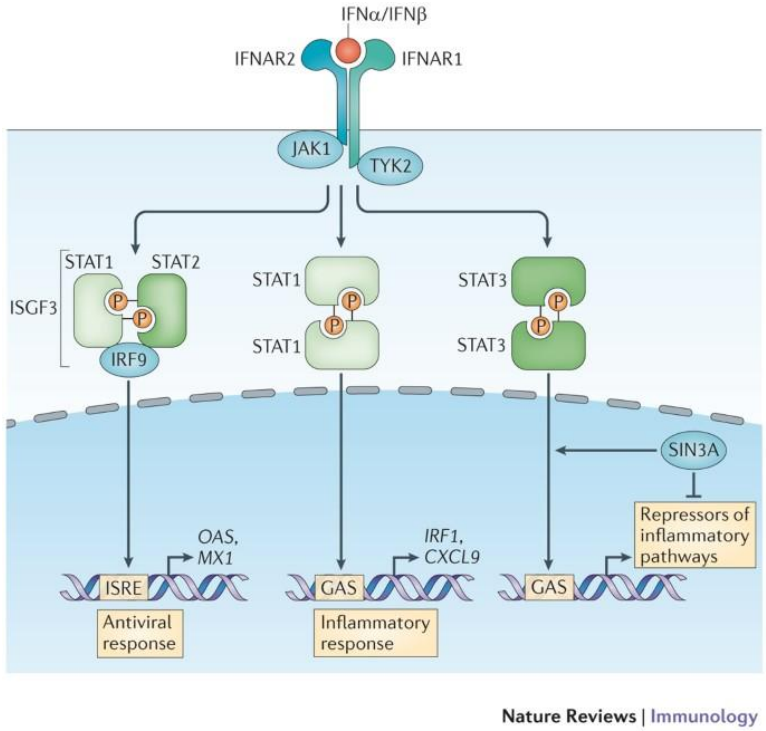
Microneedle and gene gun administration in tandem yield significantly higher anti-NP plasma IgG and IgA than gene gun administration alone.

iFMD administration activates innate immunity in the oral cavity

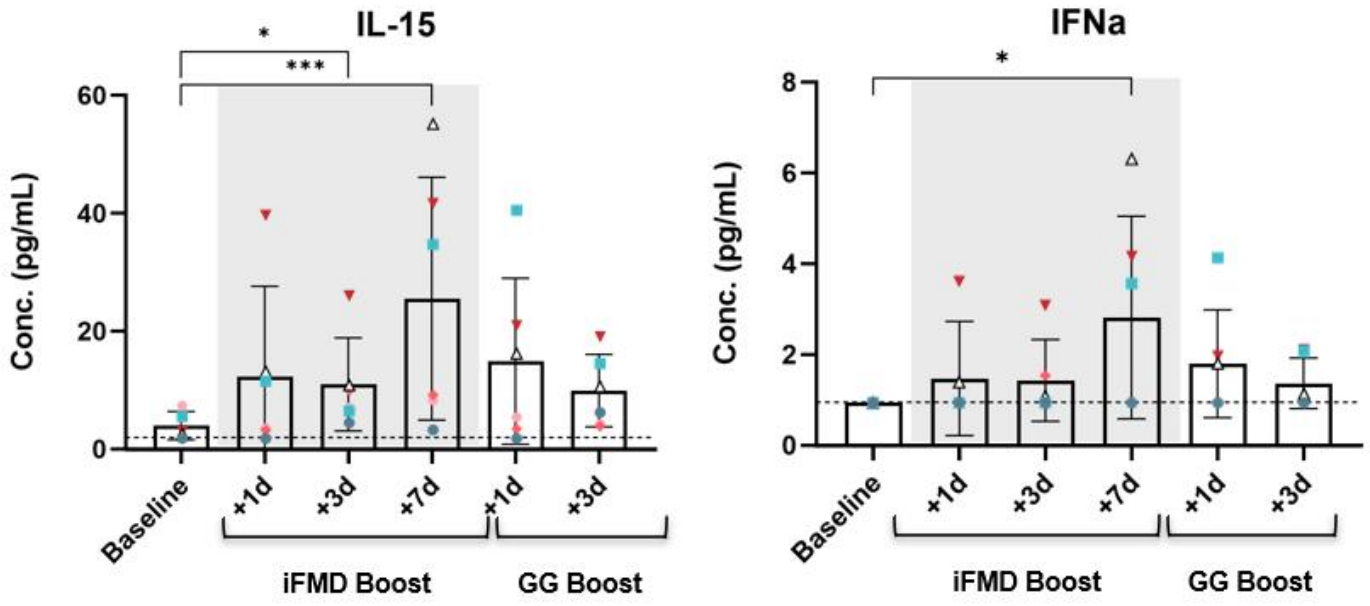


Upregulation of **20 salivary pro-inflammatory markers** (ranging from 1.5-fold to 46.0 fold) indicated innate immune activation in the local mucosa.

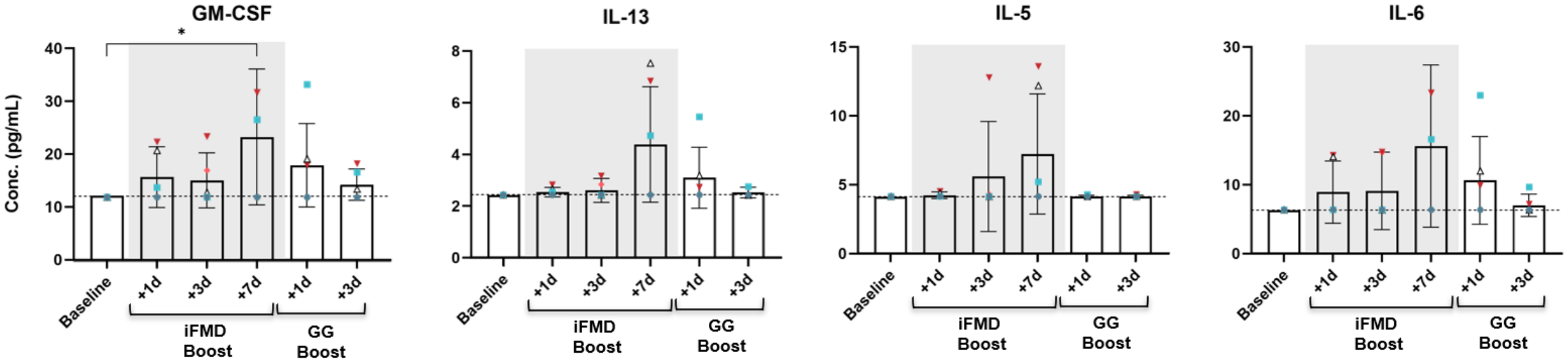
iFMD administration activates markers of... lymphocyte activation & antiviral functions



Ivashkiv, L. B. and Donlin, L. T., (2014) *Nat. Rev. Immun.*



iFMD administration activates markers of... mucosal immunity



Recruitment of mucosal DCs
Potent mucosal adjuvant

△ 11C050 ● 11C157 ▼ 15R516 ◆ A10N101 ■ A10N112 ● A11N010

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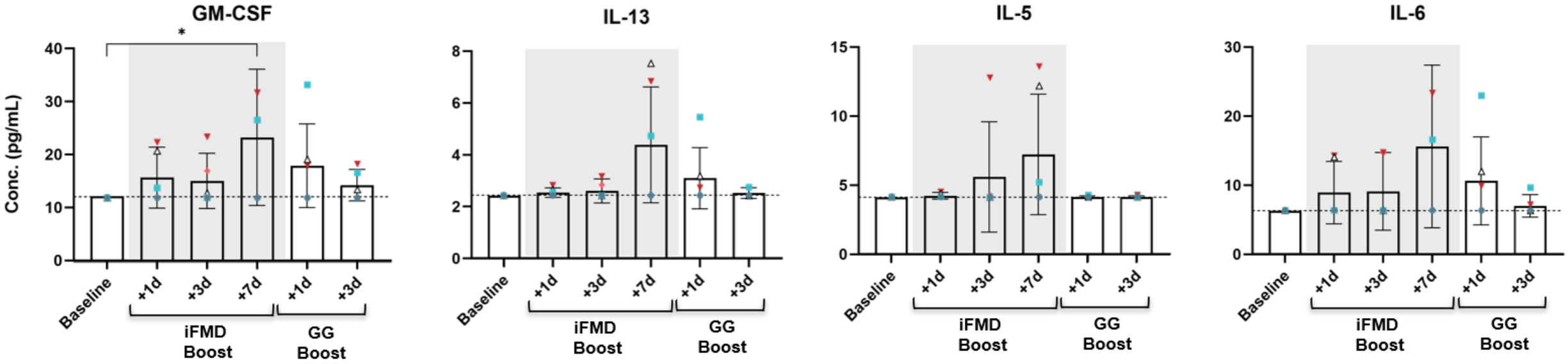

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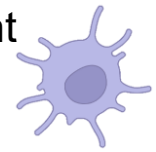
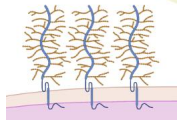


Mucosal homeostasis & mucus production



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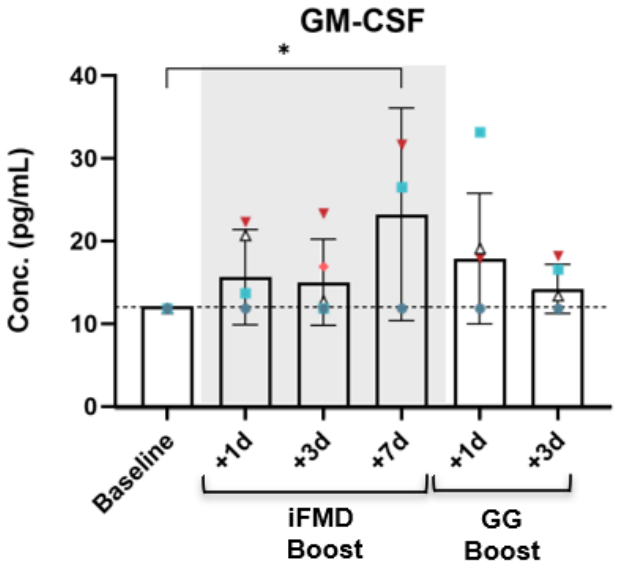

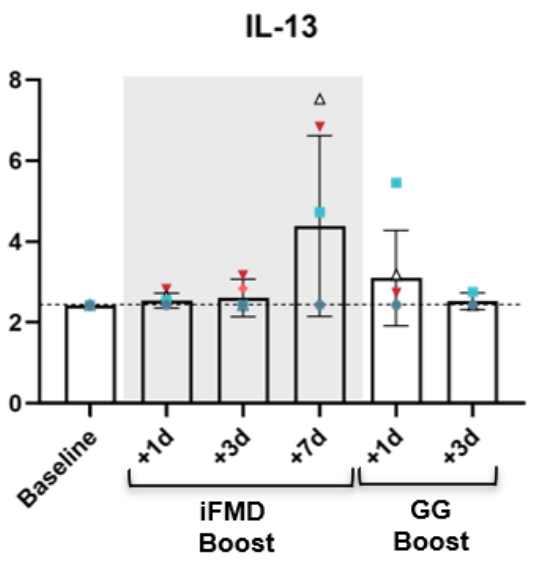

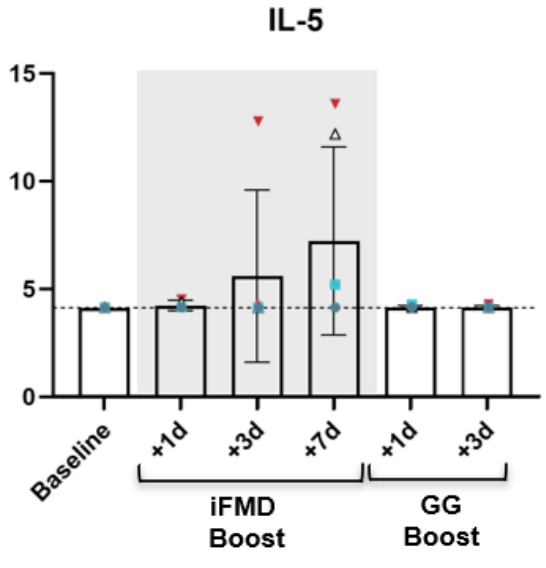

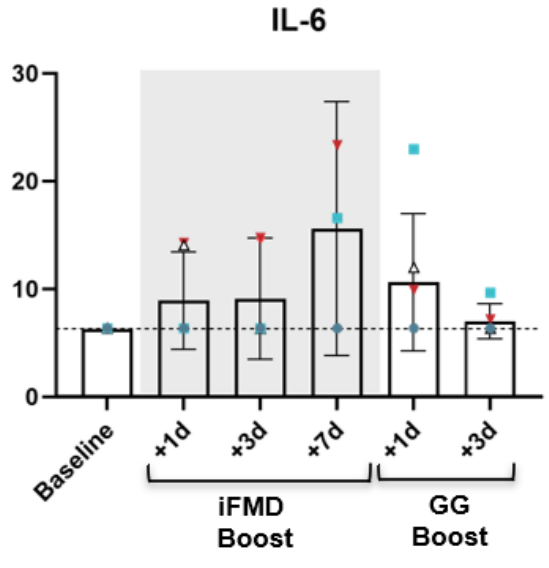

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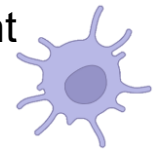

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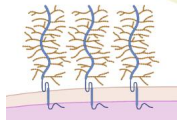
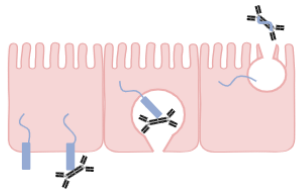
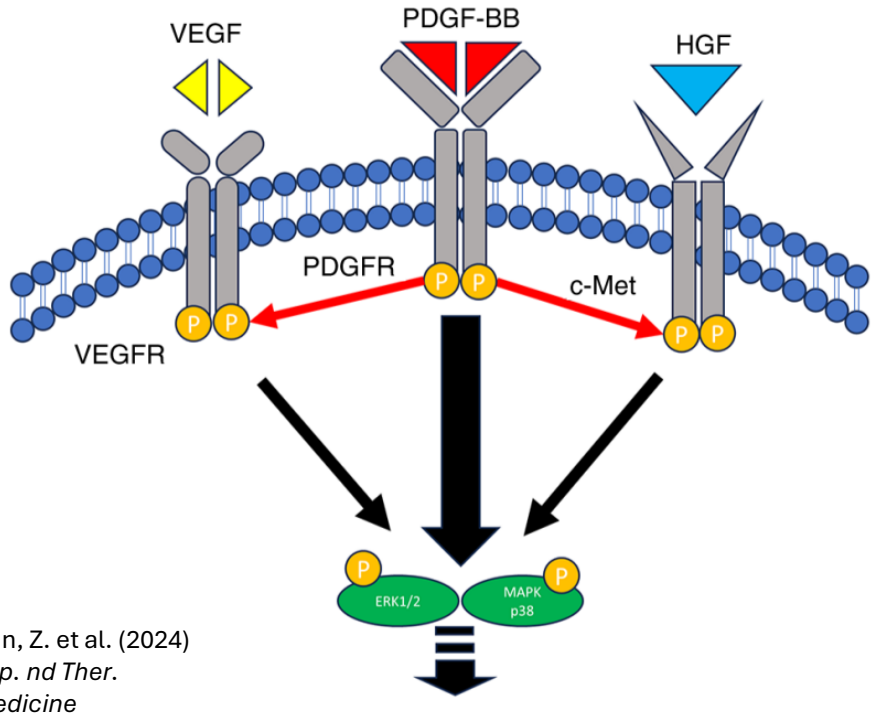


Poly Ig receptor (pIgR) regulators



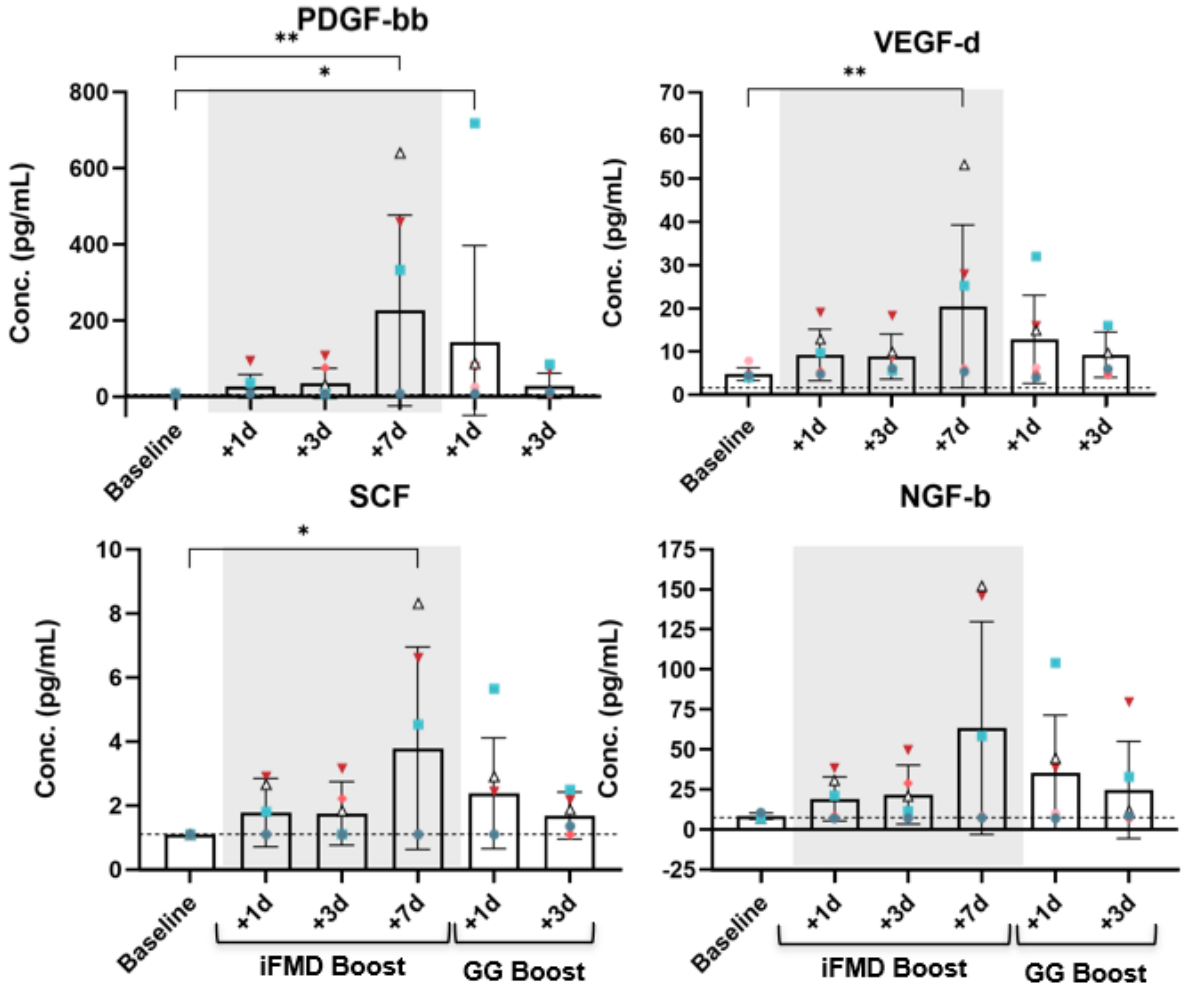
△ 11C050 ● 11C157 ▼ 15R516 ◆ A10N101 ■ A10N112 ● A11N010

iFMD administration activates growth factors



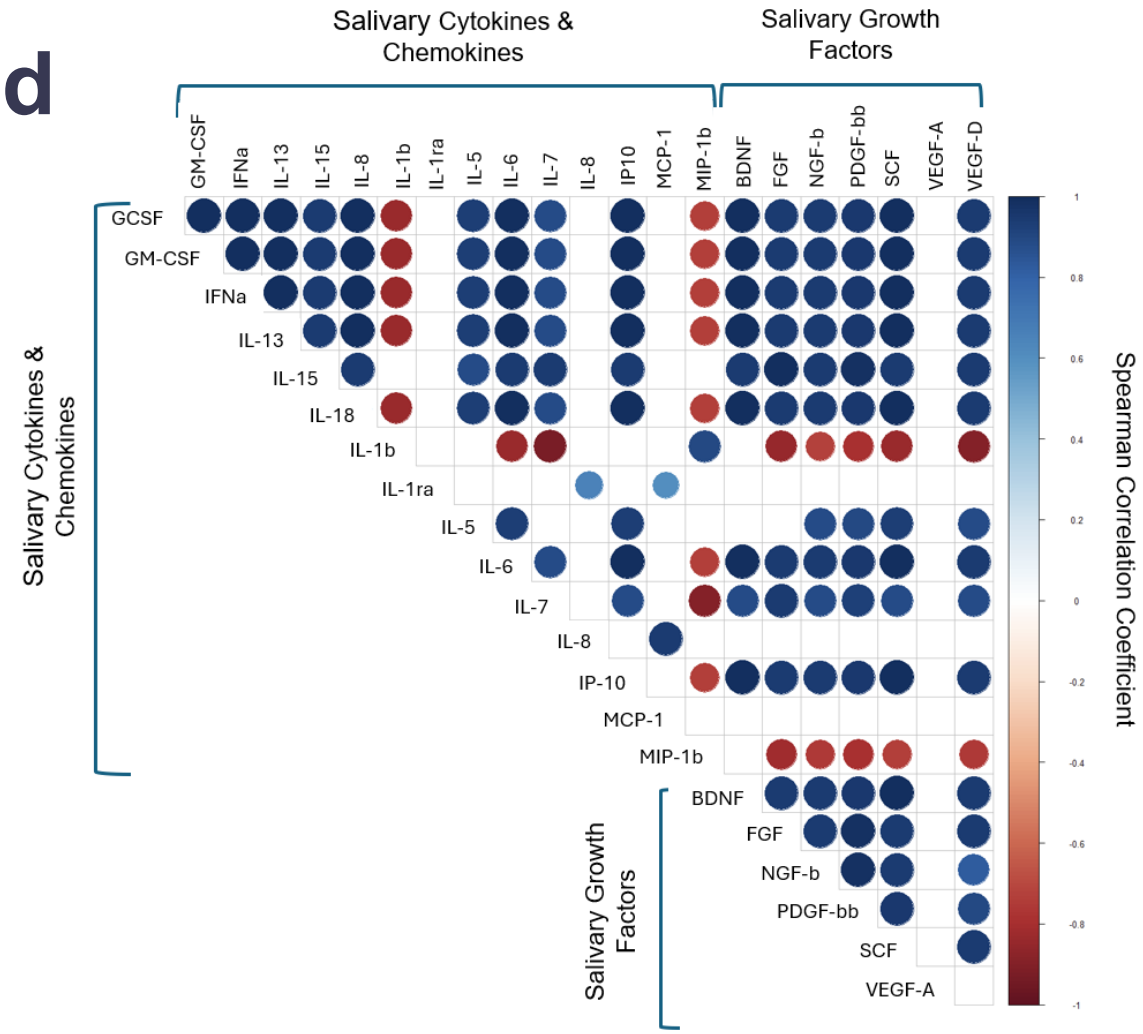
Sun, Z. et al. (2024)
Exp. nd Ther. Medicine

Migration
Proliferation
Angiogenesis
Lymphangiogenesis

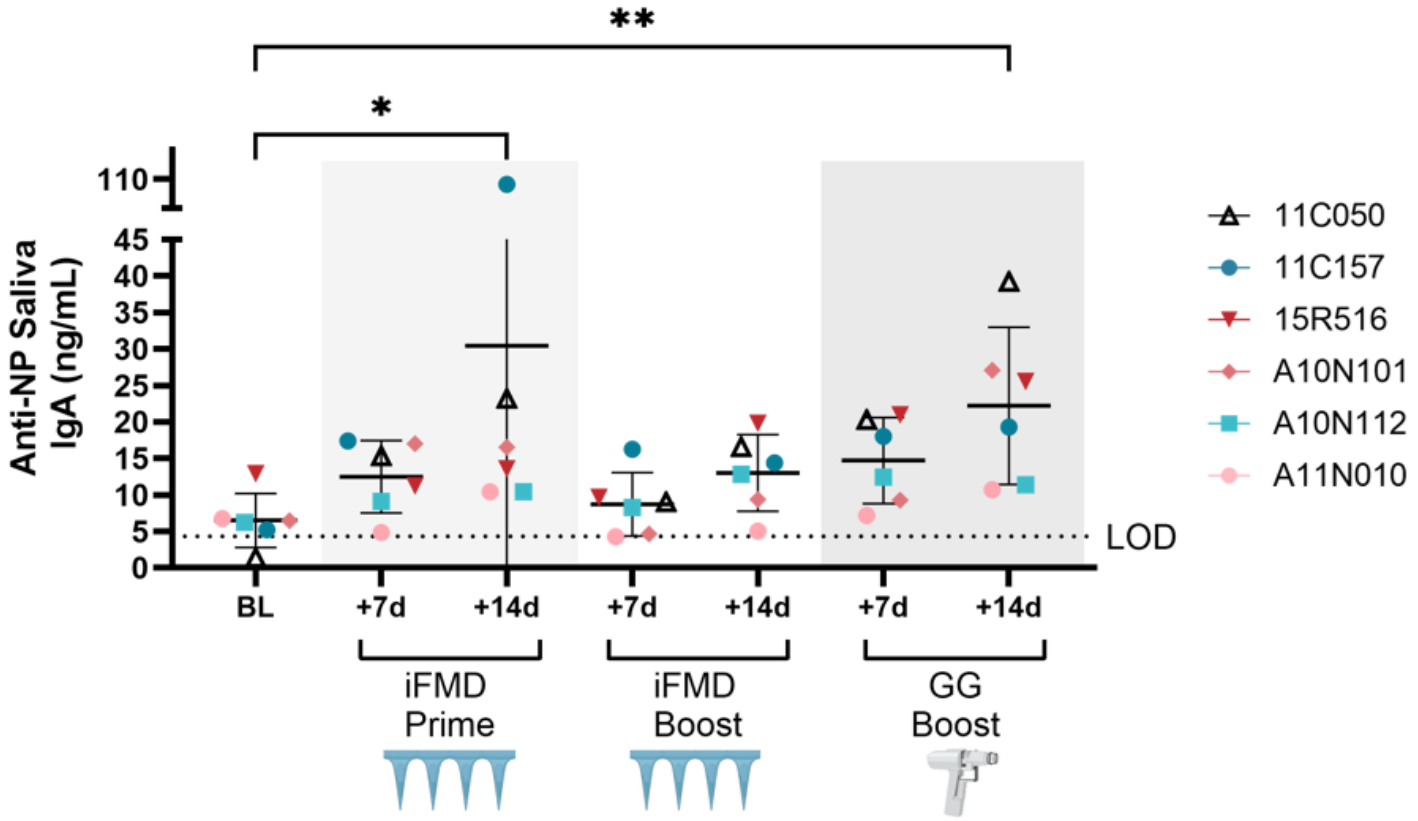


Salivary Growth Factors and Cytokines Correlate

- There was a **strong, positive correlation** between upregulation of salivary growth factors and cytokines.
- This suggests that microneedles invoked a wound healing response that serves as an adjuvant to augment immunogenicity.



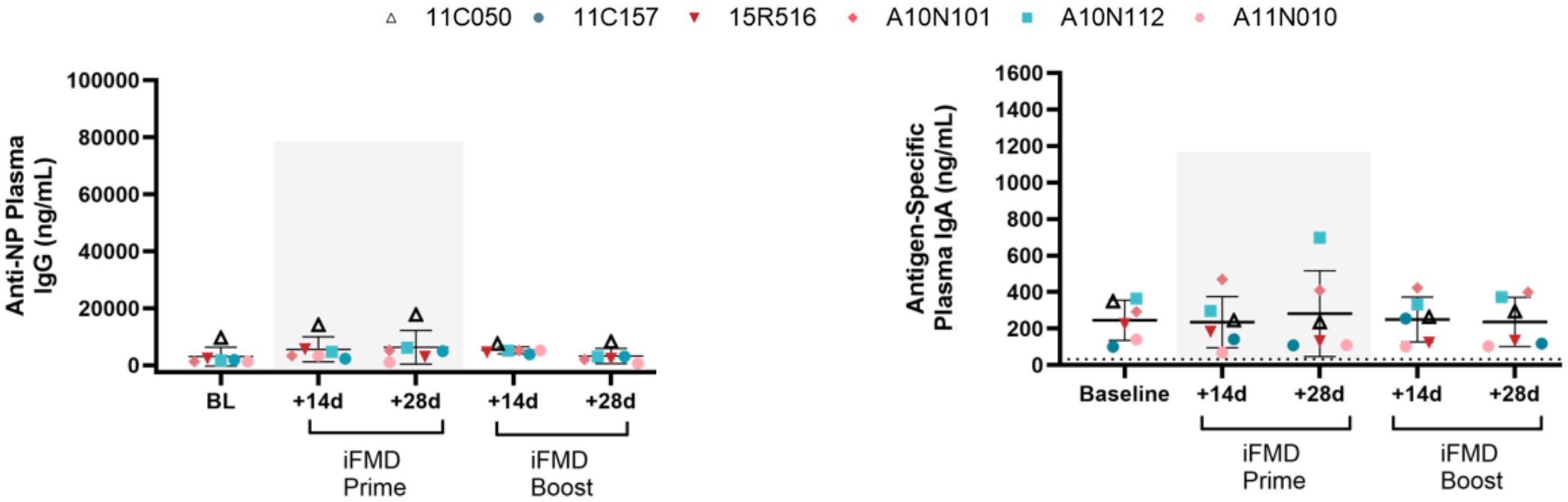
iFMDs induce antigen-specific immune responses in the local mucosa



- Significant **increases in salivary IgA** were measured after the iFMD prime and gene gun boost.
- Salivary IgA responses after gene gun administration **strongly correlated** with earlier responses after iFMD prime and boost, suggesting the systemic heterologous **systemic boost recalls a pre-existing mucosal immune response.**

Homologous iFMD prime boost does not elicit systemic immunity...

Om Sahaym,
Undergraduate Student



But heterologous iFMD + GG yields robust plasma titers

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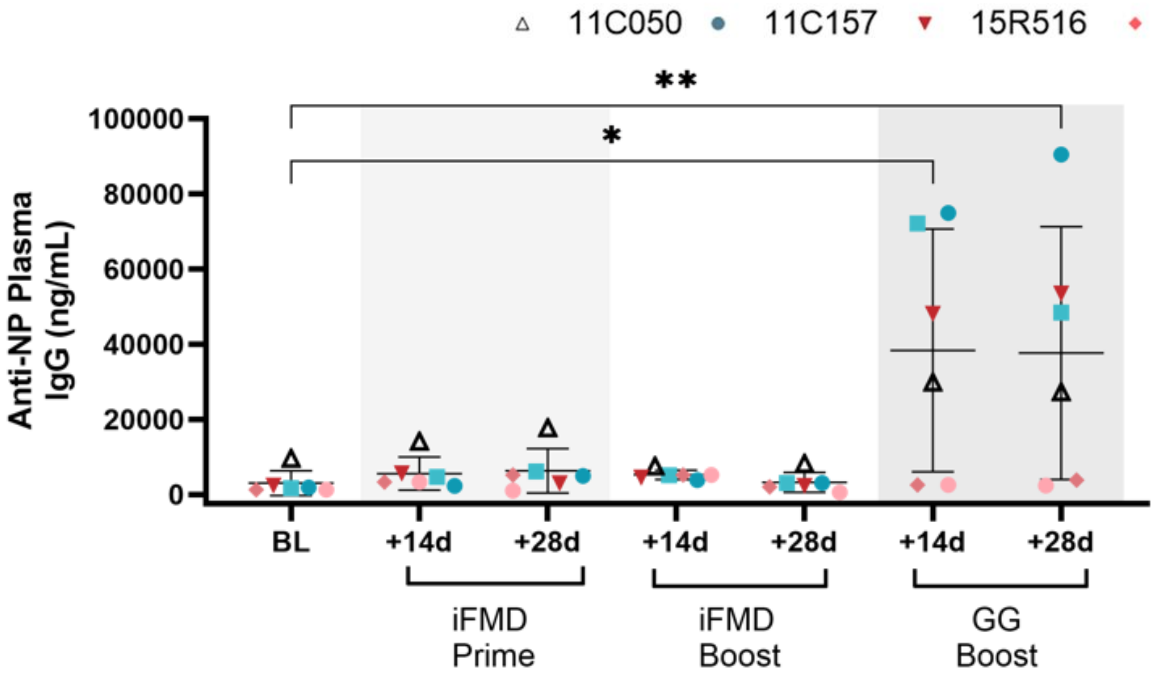
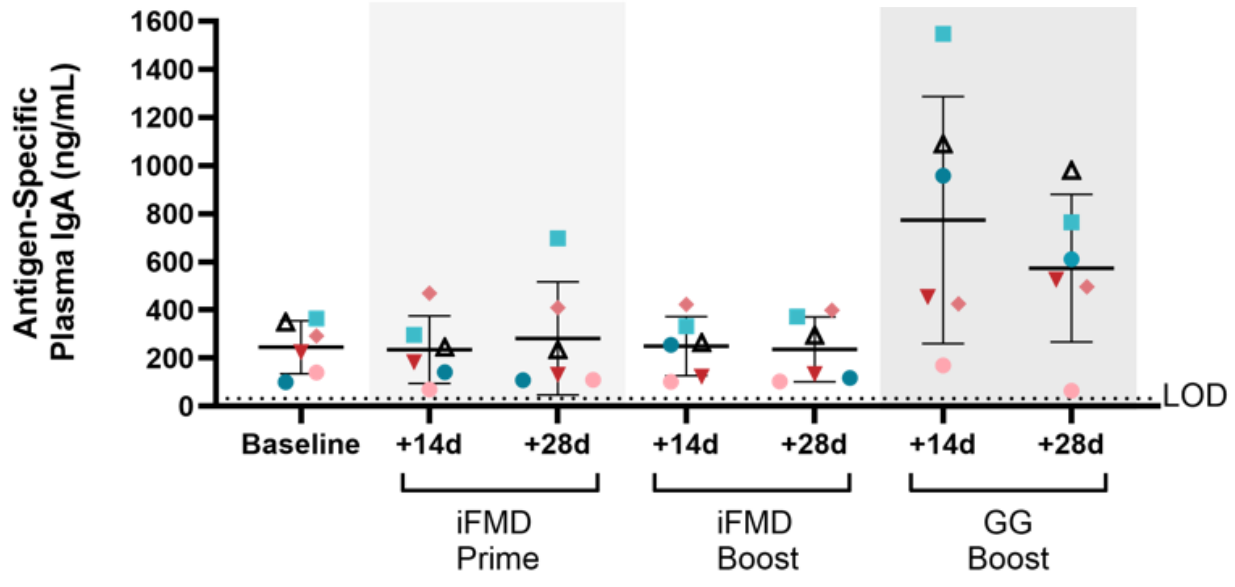
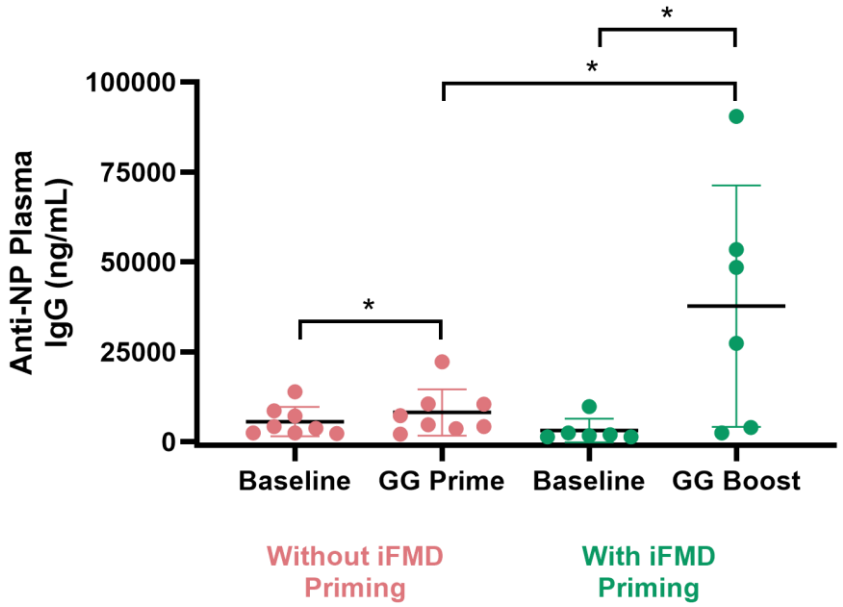
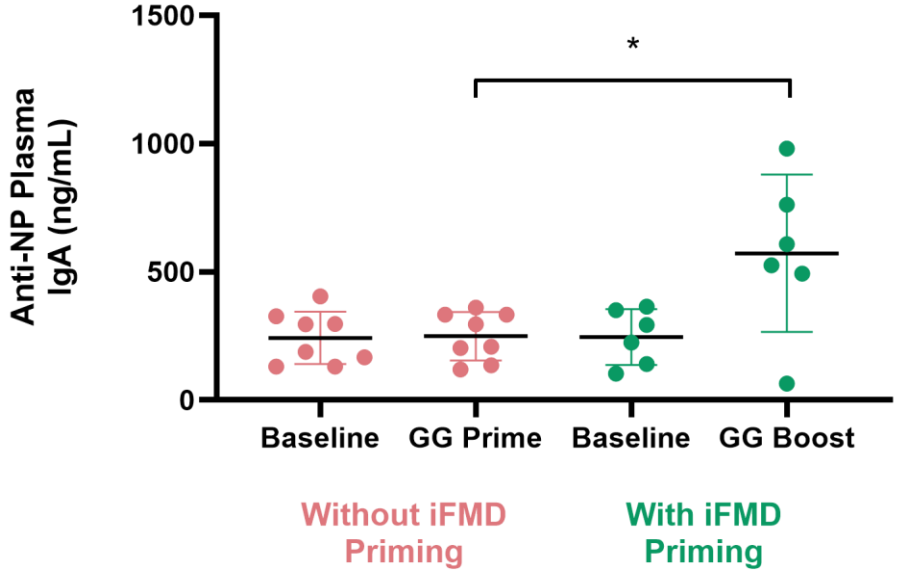




- Anti-NP plasma IgG concentrations increased significantly after the heterologous gene gun boost, with 4/6 animals seroconverting.
- Anti-NP plasma IgA mirrored these trends, with two animals sero-converting.

iFMDs prime antigen-specific immune responses in plasma





> Microneedle and gene gun administration in tandem yield significantly higher anti-NP plasma IgG and IgA than gene gun administration alone.

Conclusions

- Integrated fiber microneedles (iFMD) can deliver **multiple classes** of biologics (proteins, nucleic acids) with **tunable release kinetics**.

- iFMD administered to the buccal mucosa provoke a **wound healing response** which **augments immunogenicity.**

- Heterologous iFMD and GG immunization induced both **systemic and mucosal antigen-specific immune responses.**

Acknowledgements

PI: Dr. Kim A. Woodrow, PhD

Woodrow Lab
University of Washington Bioengineering
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Maya Nathani-Sim

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Dr. Deborah H. Fuller
UW Microbiology
Thomas B. Lewis
Om Sahaym

Dr. Francois Villinger
New Iberia Research Center

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- NSF Graduate Research Fellowship

Appendix

Carmen Ioana Tobos

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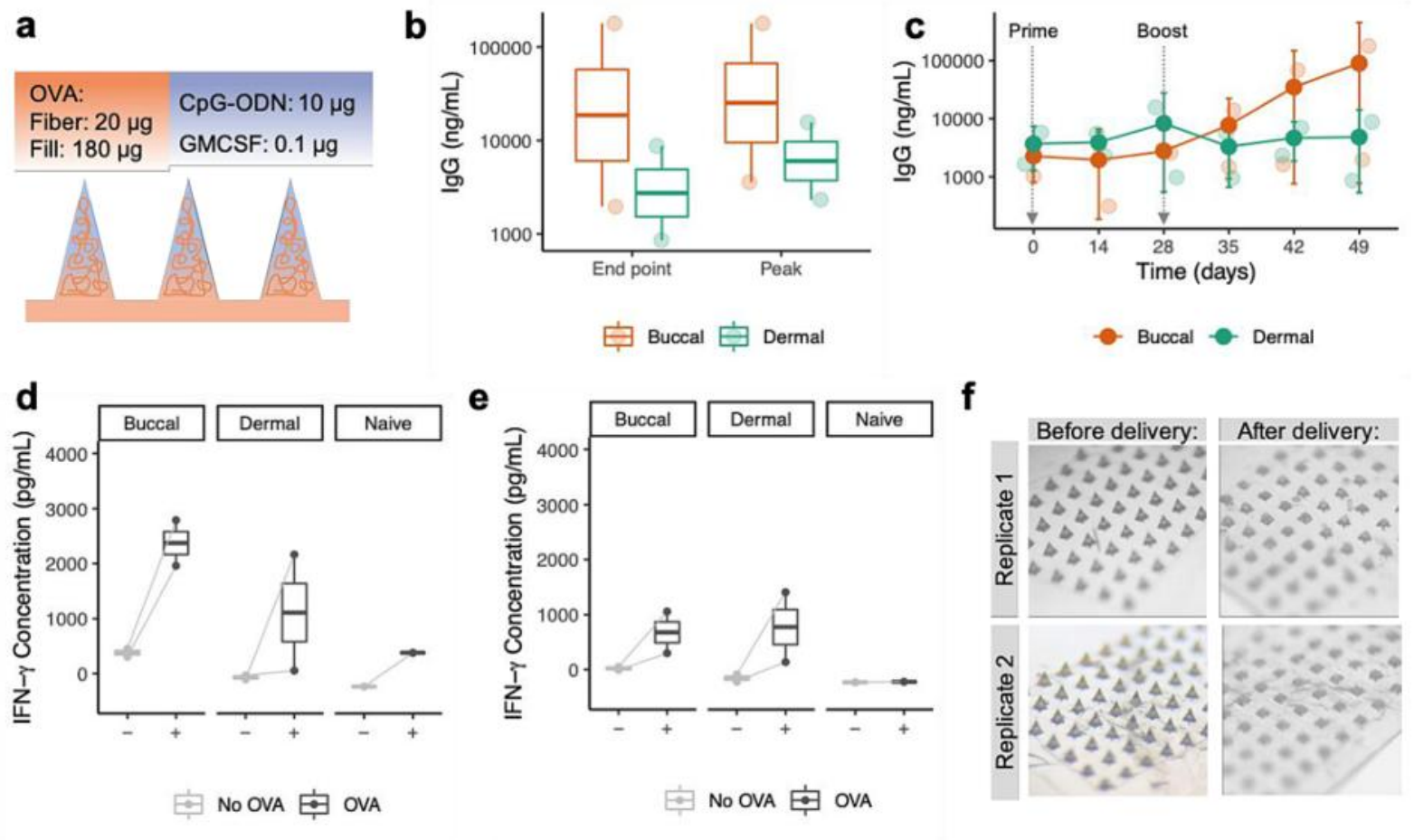
NEXT-GENERATION DELIVERY INNOVATIONS

iFMDs are immunogenic in the buccal mucosa

Rachel Creighton, PhD



Ovalbumin iFMD are modestly immunogenic in the buccal mucosa, leading to a 19-fold increase in OVA-specific serum IgG and antigen-specific IFNγ secretion from submandibular splenocytes and lymphocytes.

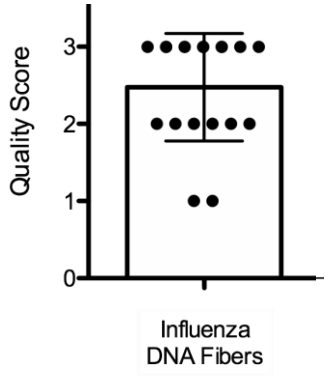
Creighton, R. et al. (2024). *JCR*.

Nucleic Acid iFMDs can be fabricated with high quality

Electrospinning Quality Score

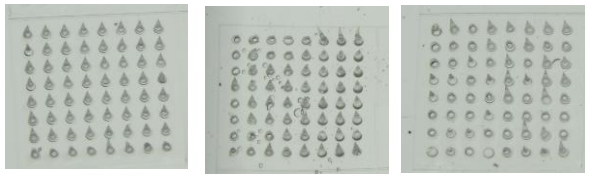
3 2 1 0

N/A

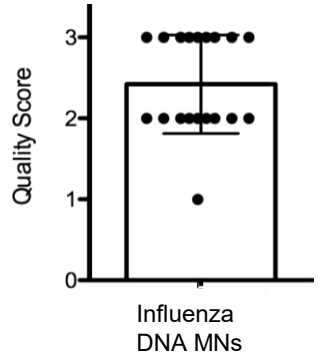


Backfill Quality Score

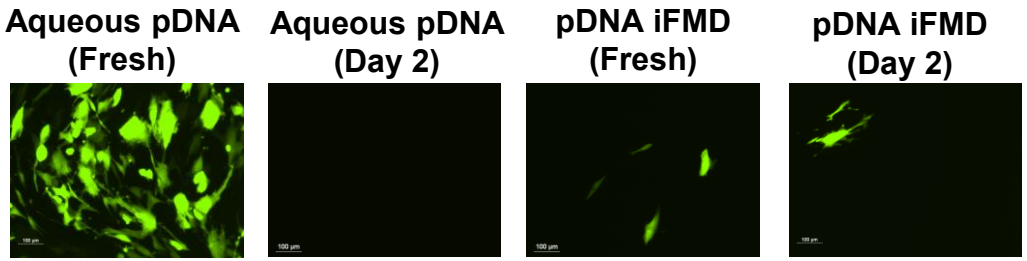
3 2 1 0

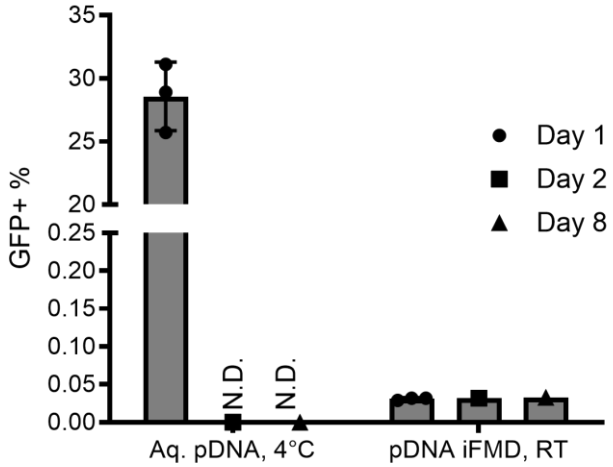


N/A



iFMDs enable pDNA storage for up to 8 days at RT with low but non-negligible transfection

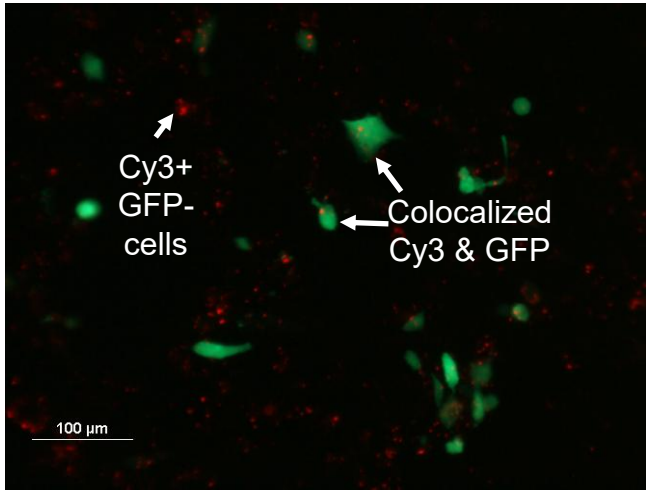




- iFMDs fabricated with pGFP transfect cells in vitro with low but non-negligible efficiency (0.033% as compared to 0.000% in the negative control).
- Aqueous pDNA complexes stored for 48 hours at 4°C lost all transfection efficiency, while pDNA iFMDs stored up to 8 days at room temperature yielded GFP positive cells (0.033% on day 1 vs 0.032% by day 8).

The barrier to higher GFP expression is not cellular entry

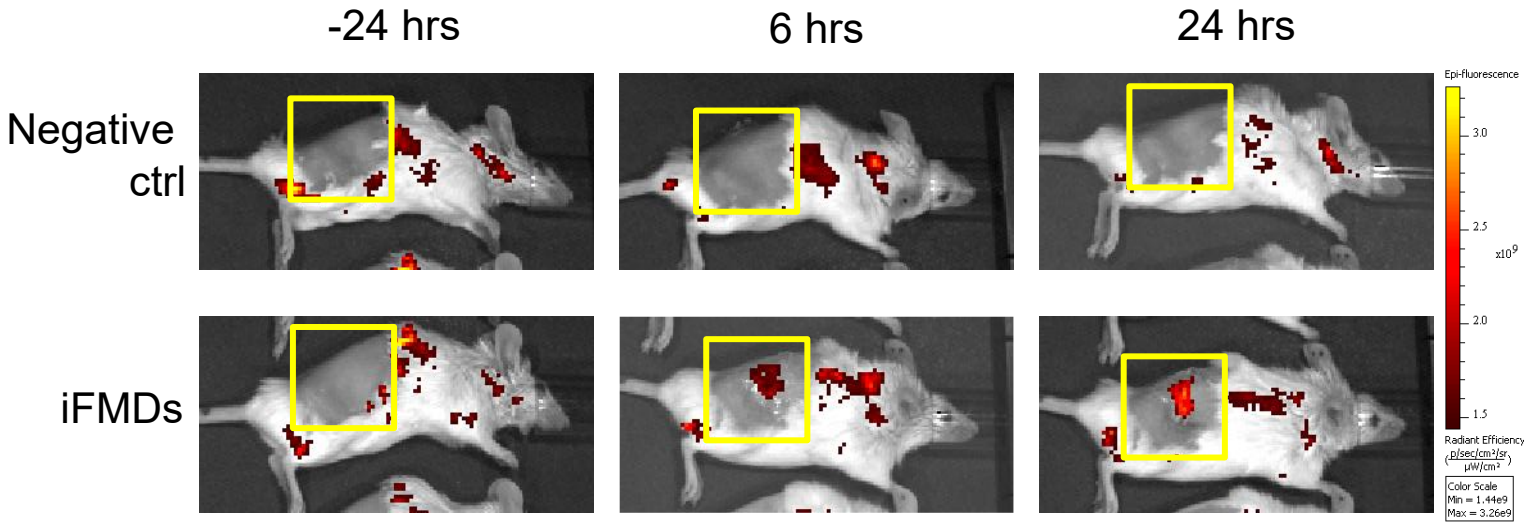
- **>50%** of cells were Cy3+ after transfection with Cy3-tagged pDNA in aqueous or iFMD format.
- Of these, 5.57% (aqueous pDNA) or 0.01% (iFMD pDNA) were Cy3+ GFP+.
- This suggests that iFMDs **deliver pDNA into the cell with high efficiency,** and that the **barrier to GFP expression is a subsequent step** (nuclear entry, translation, transcription).

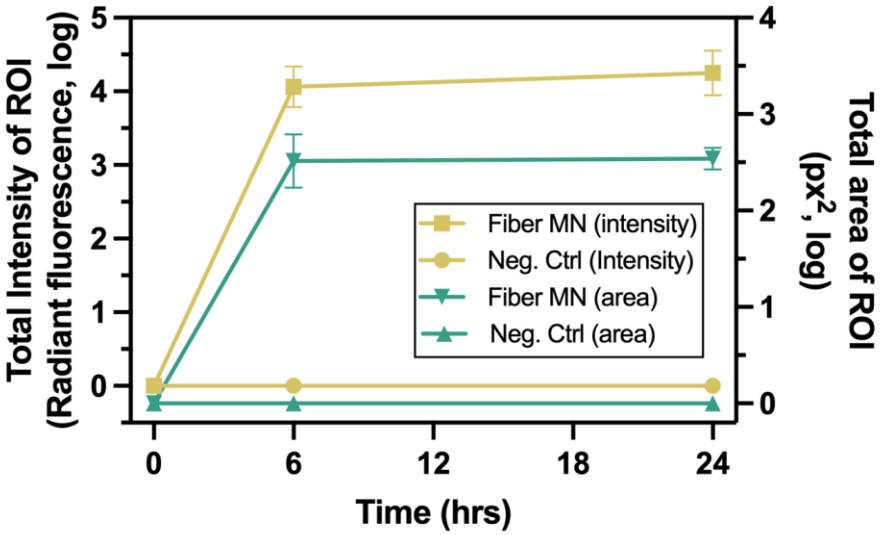


Sample	% Cy3+	%Cy3+, GFP+
Aqueous pDNA	44.7 ± 2.39 %	5.56 ± 0.57 %
pDNA iFMD	53.7 ± 1.98 %	0.0074 ± 0.005 %

iFMDs lead to quantifiable GFP expression in vivo

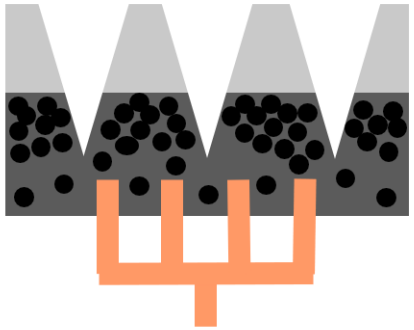
Vivi Yu,
Research Assistant



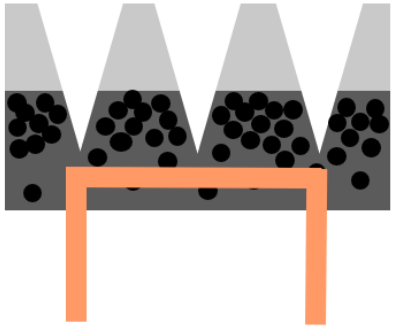


Doan, M.A, **Tobos, C.I.**, *et al.* (2023). Adv. Eng. Mat.

Alternative Grounding Strategies & Smaller Carbon Black Particle Diameters Improve Electrospinning Quality

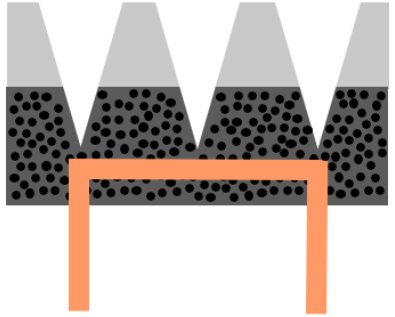


Shortcoming #1: The removable ground results in variable placement and physical degradation of the elastomer network



Approach: Use higher surface area implanted electrical grounds.

Shortcoming #2: Non-homogenous carbon black distribution and insufficient inter-particle contacts lead to inconsistent electrical potential.



Approach: Use carbon black particles with reduced diameter (50 nm → 29 nm) to lower percolation threshold

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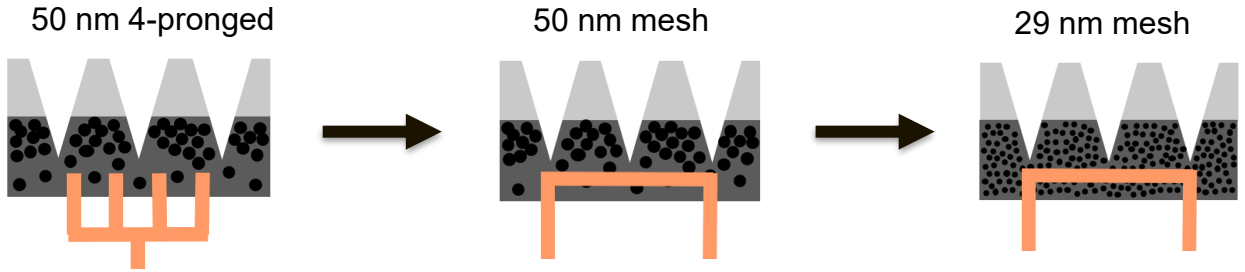

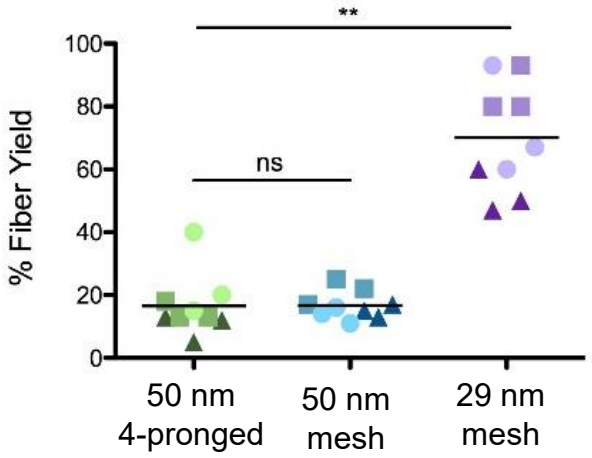

Fiber yield improves 4-fold

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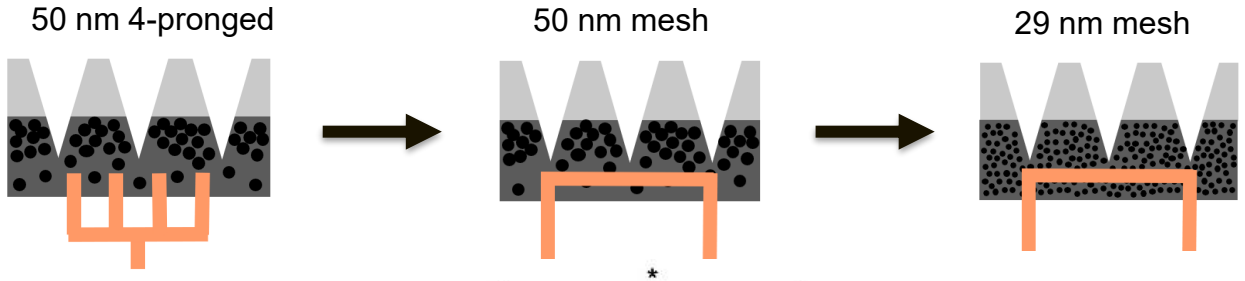
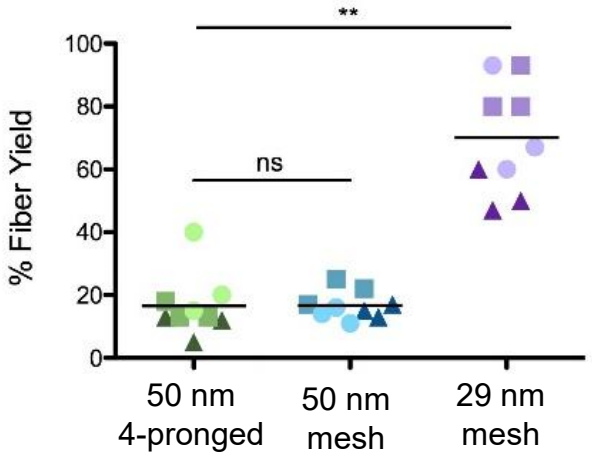
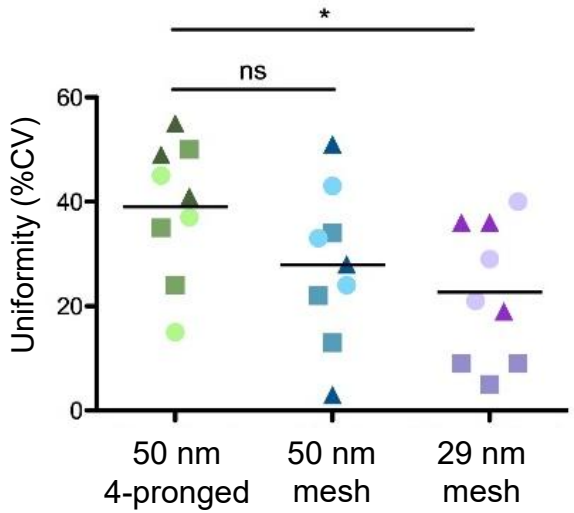




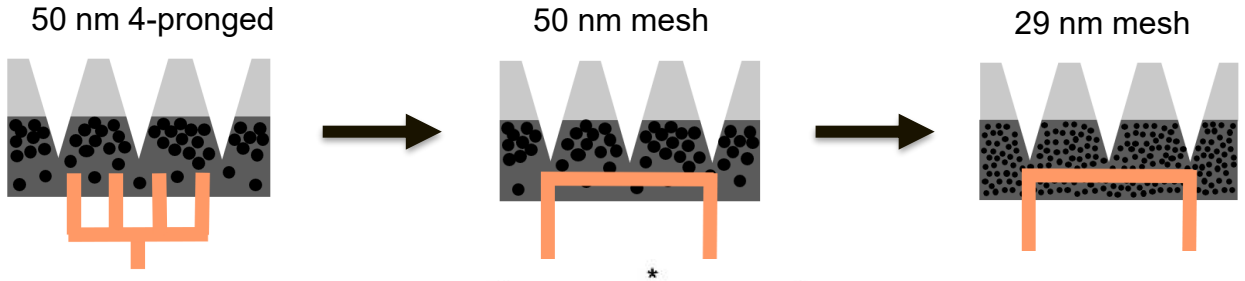
Fiber yield improves 4-fold

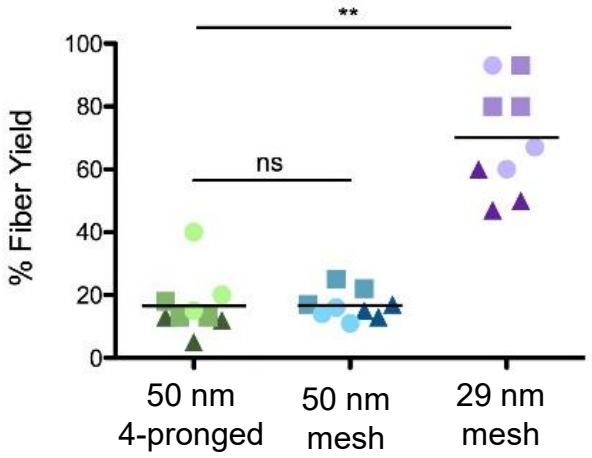


Uniformity: Variation within an array decreased from 39% to 23%

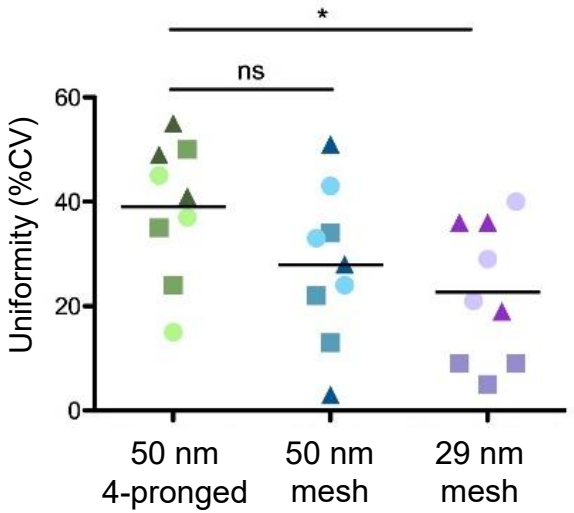
Doan, M.A, **Tobos, C.I.**, *et al.* (2023). Adv. Eng. Mat.

Alternative Grounding Strategies & Smaller Carbon Black Particle Diameters Improve Electrospinning Quality

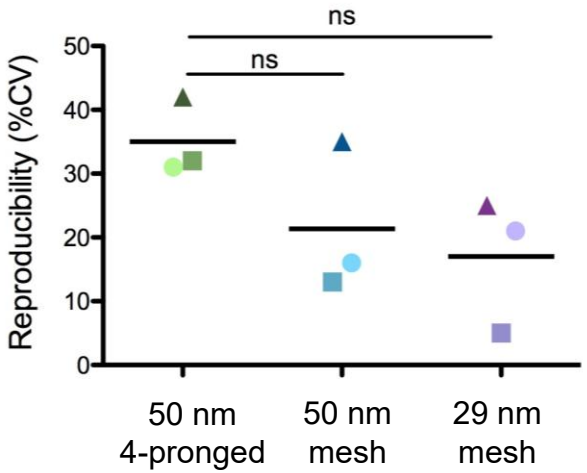




Fiber yield improves 4-fold



Uniformity: Variation within an array decreased from 39% to 23%



Reproducibility: Variation between arrays decreased from 35% to 17%

Doan, M.A, **Tobos, C.I.**, *et al.* (2023). Adv. Eng. Mat.

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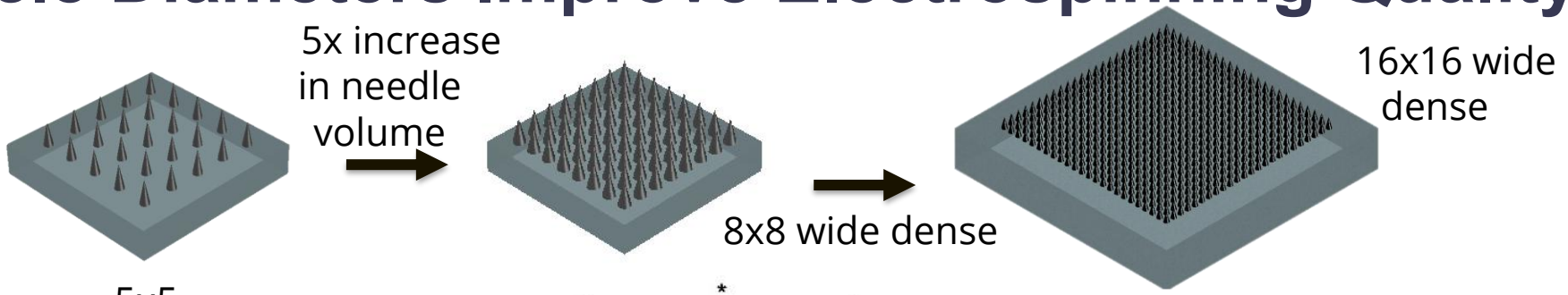
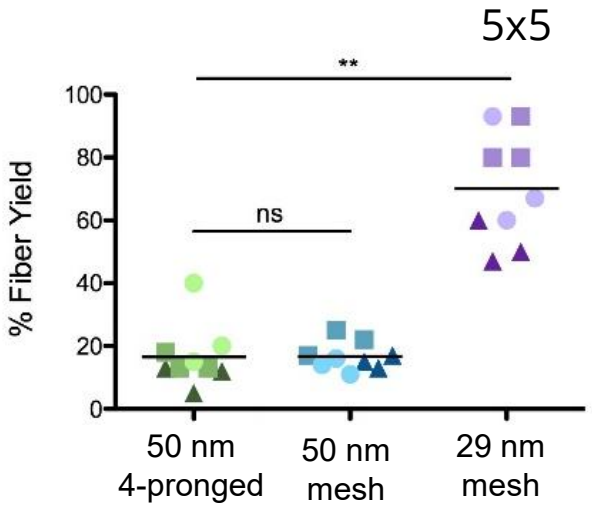
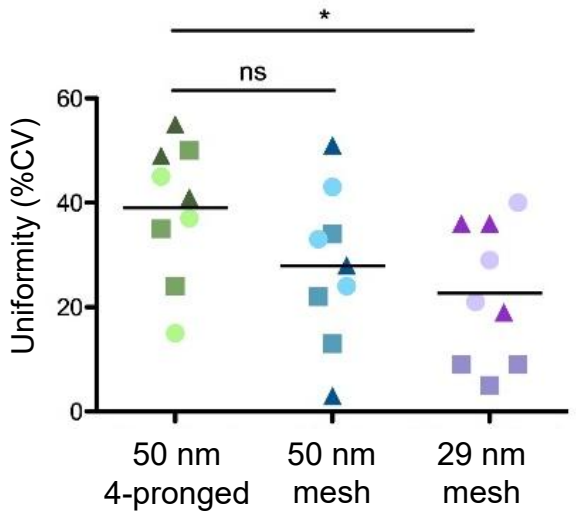




Fiber yield improves 4-fold



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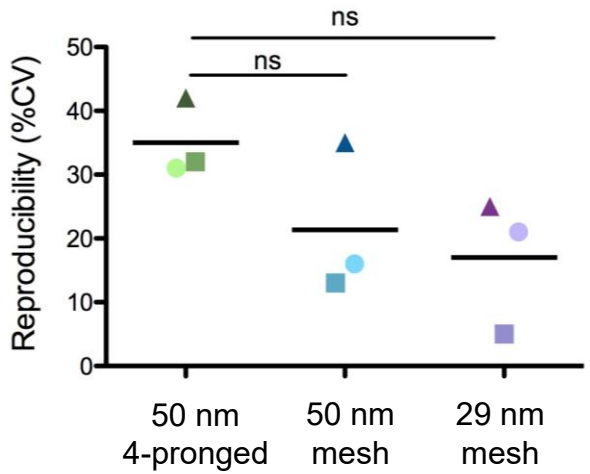
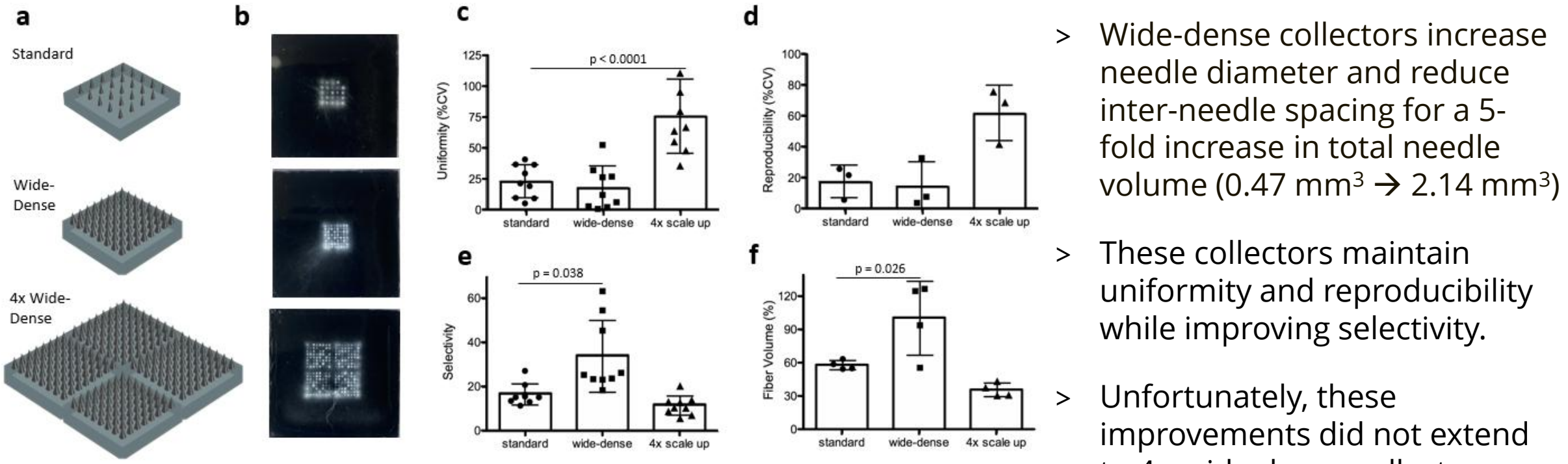


Reproducibility: Variation between arrays decreased from 35% to 17%

Collector optimization supports scaled up array geometries



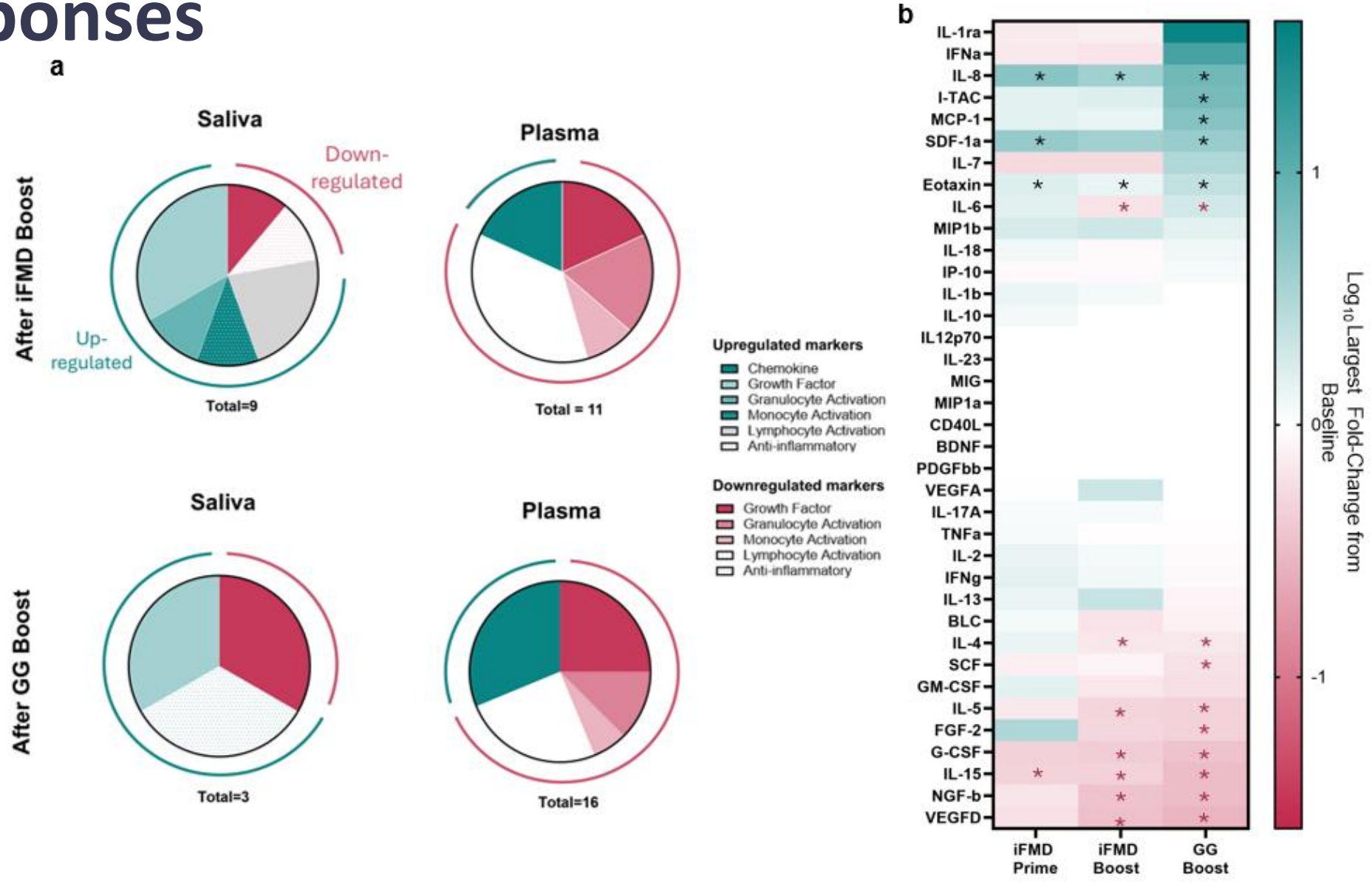
> Wide-dense collectors increase needle diameter and reduce inter-needle spacing for a 5-fold increase in total needle volume (0.47 mm^3 → 2.14 mm^3)

> These collectors maintain uniformity and reproducibility while improving selectivity.

> Unfortunately, these improvements did not extend to 4x-wide dense collectors,

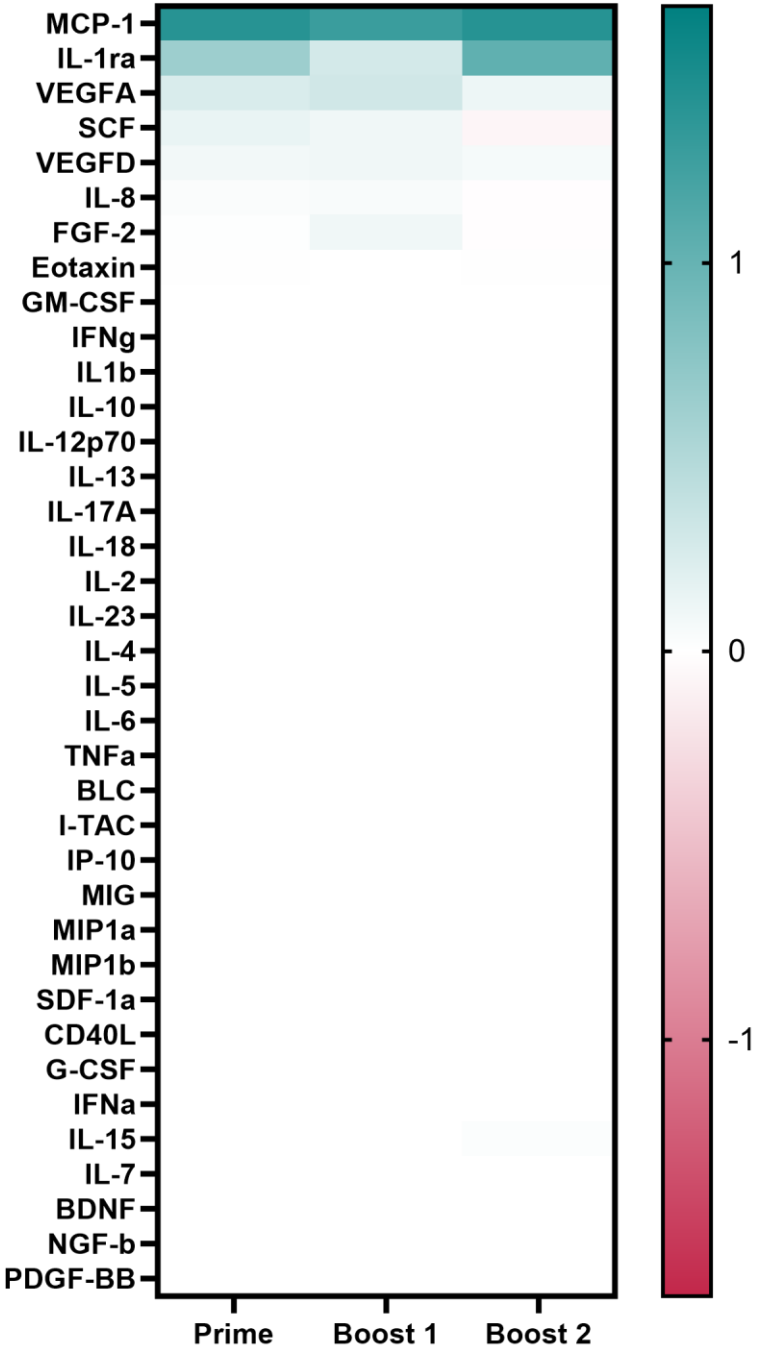
Doan, M.A, **Tobos, C.I.**, *et al.* (2023). Adv. Eng. Mat.

Plasma Cytokine, Chemokine, and Growth Factor Responses

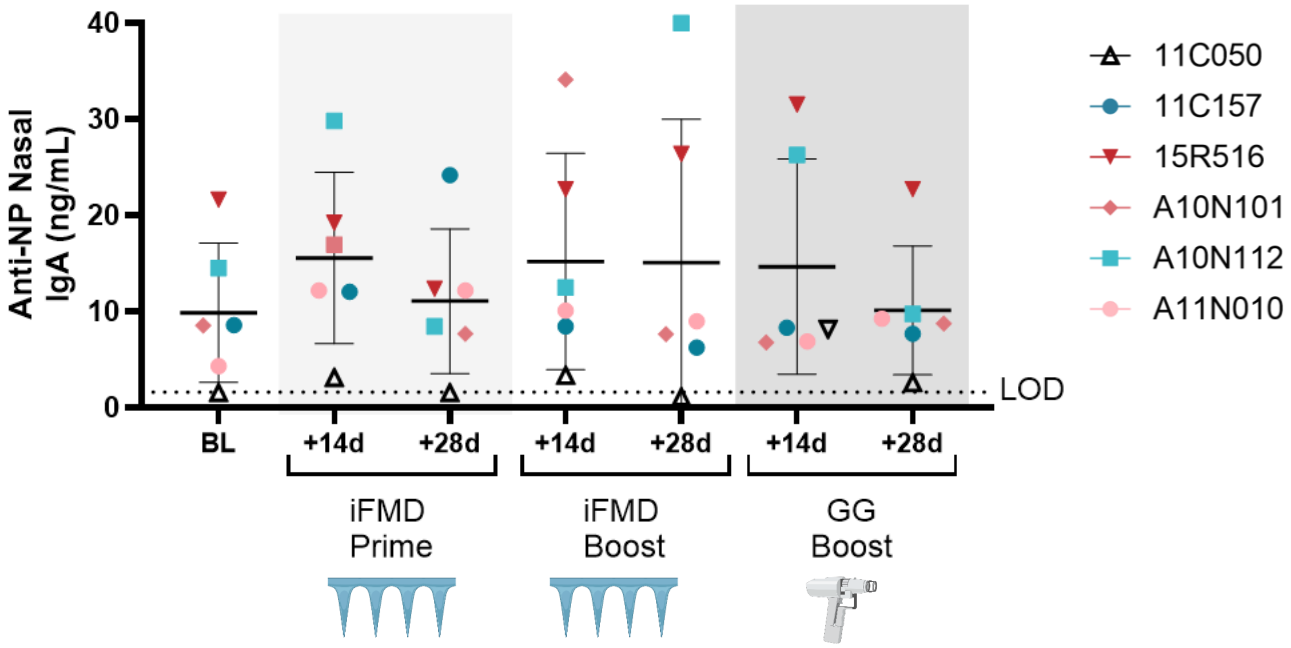


BAL Cytokine, Chemokine, and Growth Factor Responses

- Activation of innate immunity is restricted to the local mucosa, and protein concentrations in BAL after iFMD administration remained largely unmeasurable, with no statistically significant changes in protein expression compared to baseline.

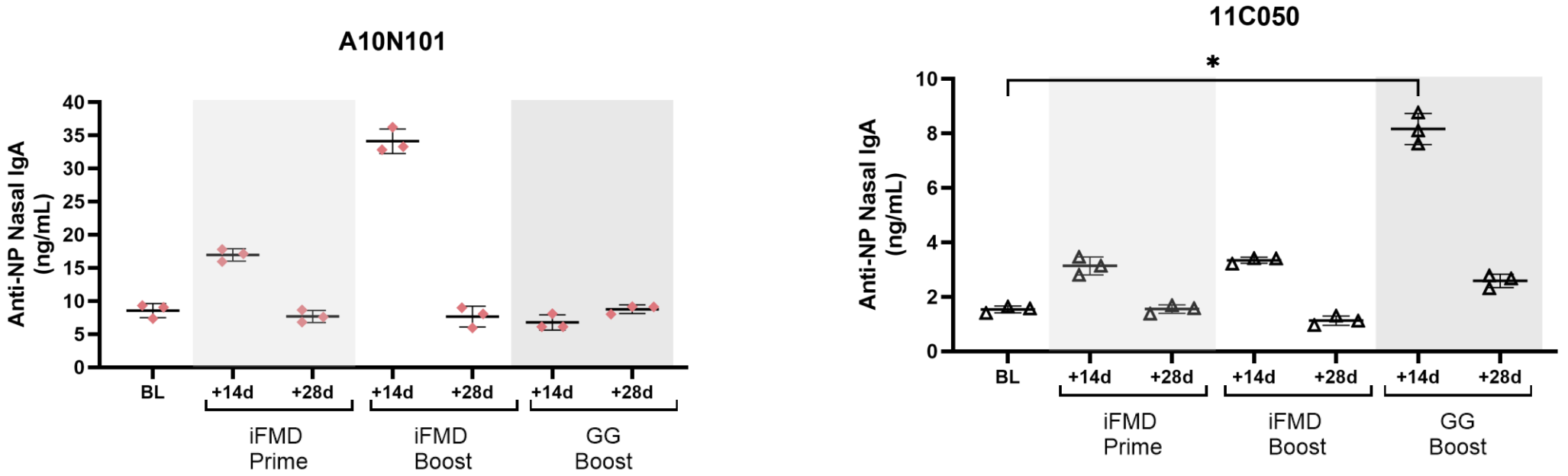


Evaluation of immunogenicity at distal mucosal sites



- Nasal IgA concentrations at baseline varied widely
- None of the immunization events induced a significant change Nasal IgA concentrations compared to baseline.

Evaluation of immunogenicity at distal mucosal sites



- Anti-NP IgA binding concentrations increased 3-fold over baseline or more in two animals.
- This suggests that iFMD have the **potential to induce distal mucosal immunity**, although they elicited suboptimal nasal humoral responses in this study.