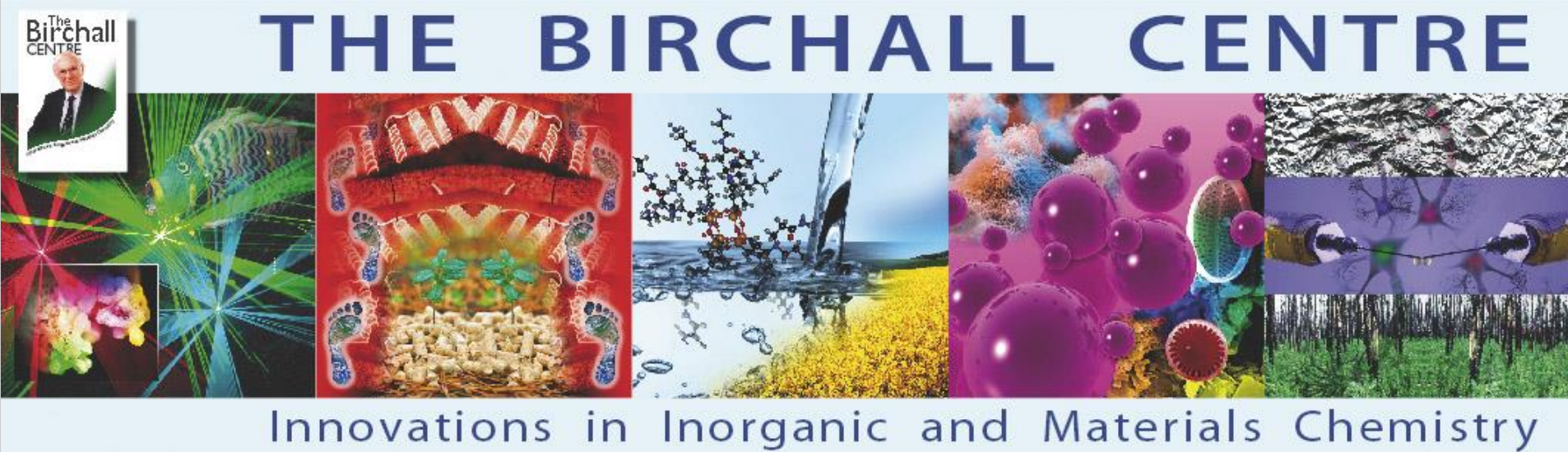




Systemic Toxicity of Aluminium Adjuvants

Christopher Exley, Emma Shardlow & Matthew Mold

The Birchall Centre, Lennard-Jones Laboratories, Keele University, Keele, Staffordshire, ST5 5BG, UK.

c.exley@keele.ac.uk e.shardlow@keele.ac.uk m.j.mold@keele.ac.uk

<http://www.keele.ac.uk/aluminium/>



<https://www.hippocraticpost.com/?s=Exley>

Warsaw, 2019



There are no clinically-approved
(aluminium) adjuvants!

There are only clinically-approved vaccines.

The safety of adjuvants is established
alongside the safety of vaccines.

So, why are aluminium adjuvants used as
placebos in vaccine safety trials?!

For example, in demonstrating the ‘safety’ of HPV vaccines

The NEW ENGLAND
JOURNAL *of* MEDICINE

ESTABLISHED IN 1812

MAY 10, 2007

VOL. 356 NO. 19

Quadrivalent Vaccine against Human Papillomavirus
to Prevent High-Grade Cervical Lesions

The FUTURE II Study Group*



Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

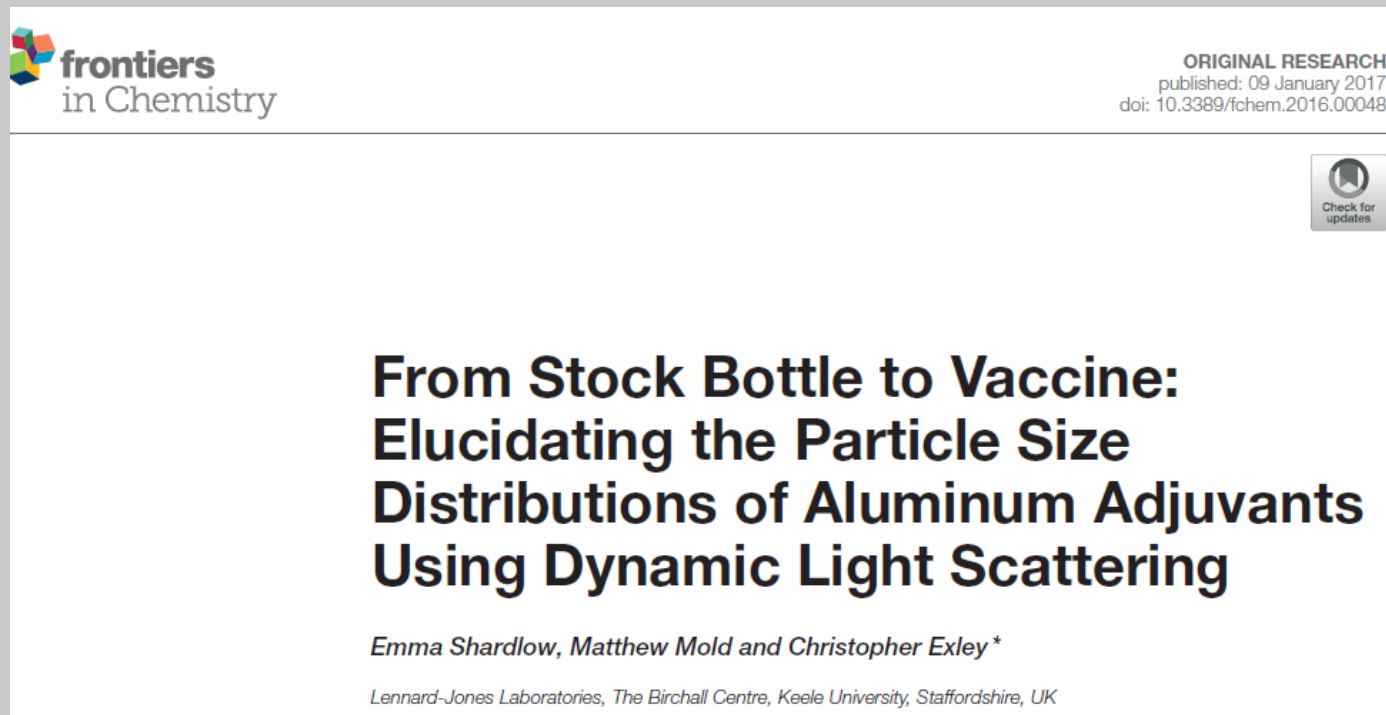


Letter to the Editor

Aluminium-based adjuvants should not be used as placebos in clinical trials

- [2] Hem SL, Johnston CT, HogenEsch H. Imject Alum is not aluminum hydroxide adjuvant or aluminum phosphate adjuvant. Vaccine 2007;25:4985–6.
- [3] Exley C, Siesjö P, Eriksson H. The immunobiology of aluminium adjuvants: how

So, what do we know about aluminium adjuvants that are used in clinically-approved vaccines?



<https://www.frontiersin.org/articles/10.3389/fchem.2016.00048/full>

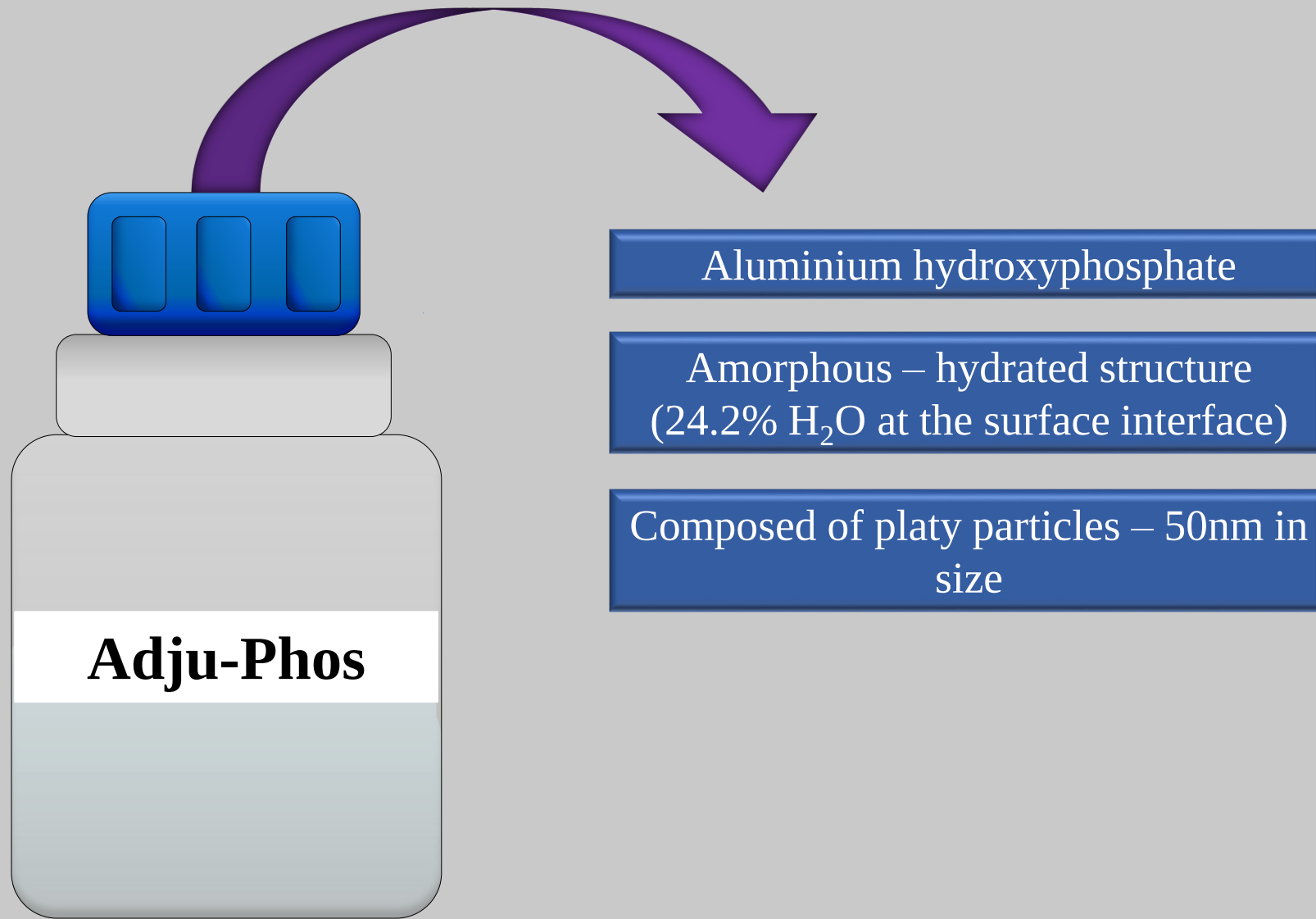


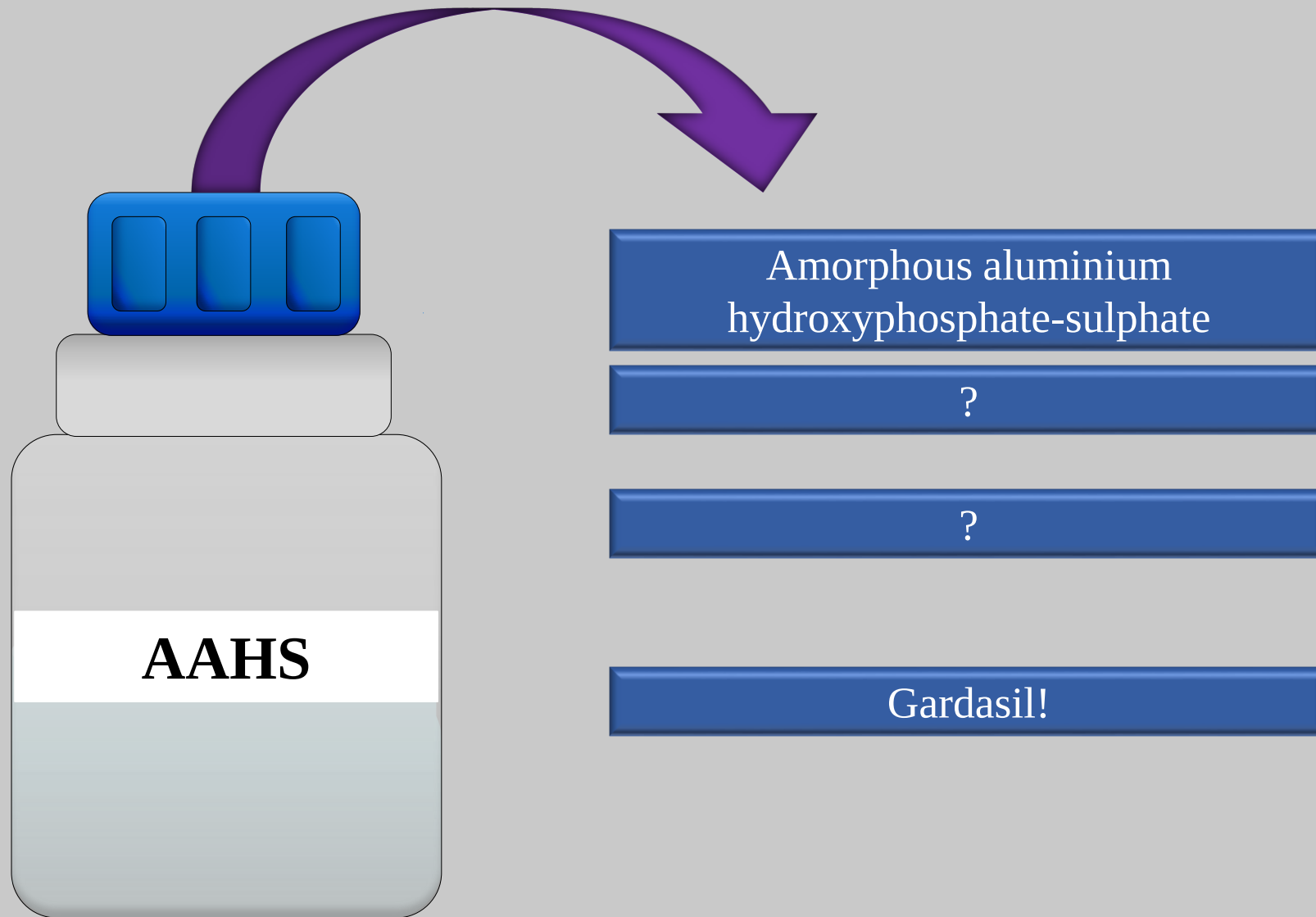
Aluminium oxyhydroxide (boehmite)

Poorly crystalline – hydrated structure
(14.1% H₂O at the surface interface)

Composed of nanoneedles - 4.5 nm ×
2.2 nm × 10 nm in size

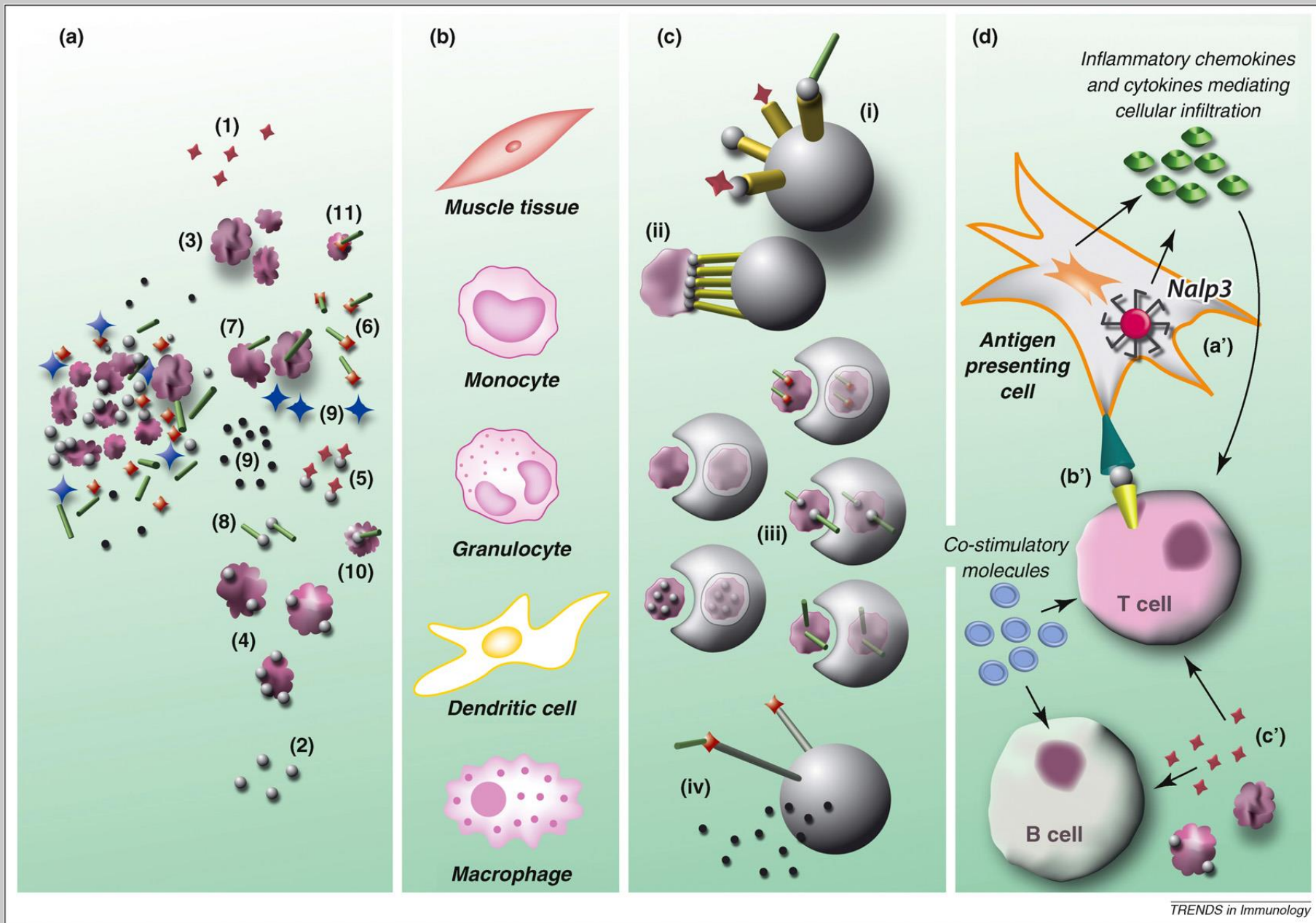
Most frequently used adjuvant in
commercial vaccines



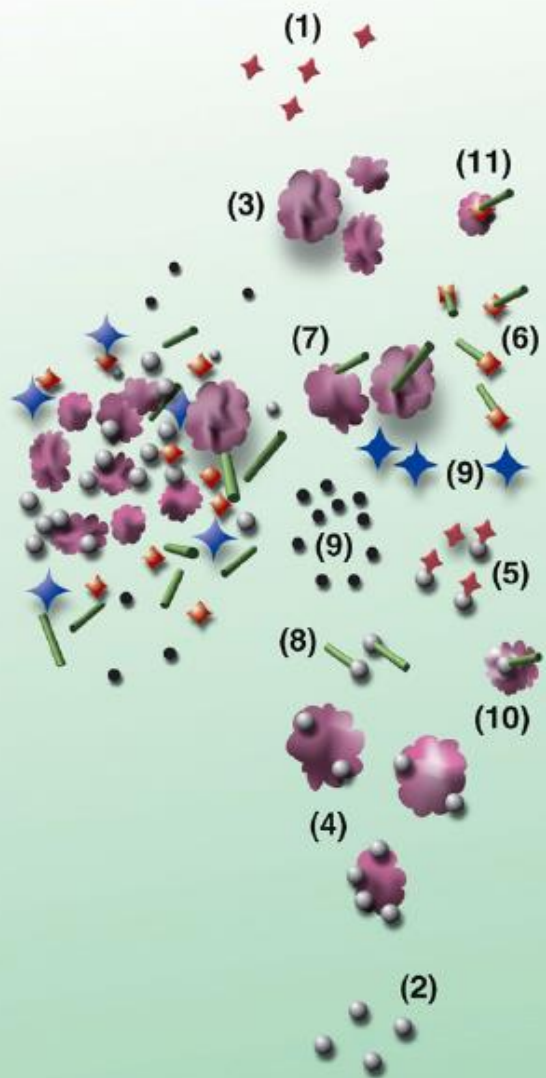


So, how do aluminium adjuvants work?

How might understanding this also begin to explain the known adverse events associated with their use in vaccines?



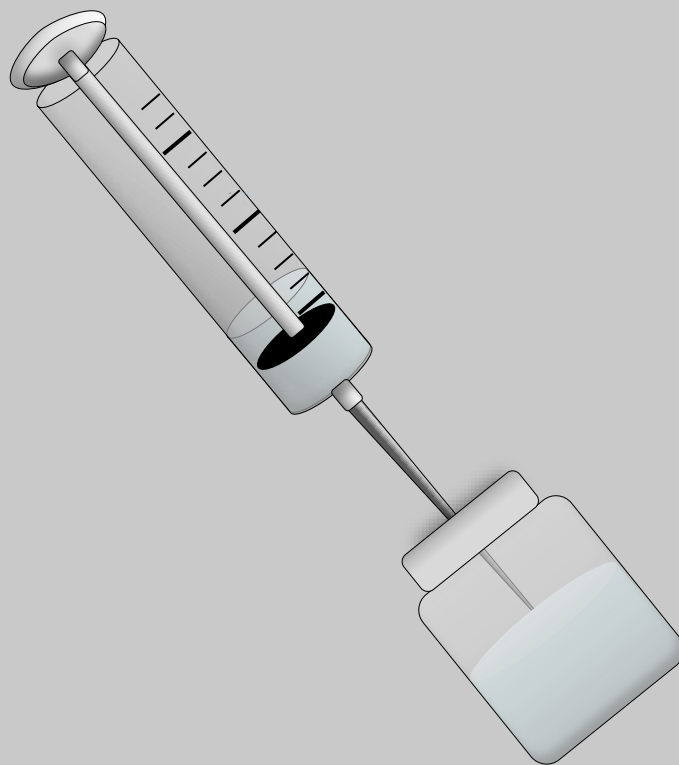
(a)



The Critical Environment of the Injection Site

A. Dilution of the vaccine preparation into the muscle interstitial fluid (MIF) results in an array of potential agonists of the immune cascade including; **(1)** $\text{Al}^{3+}_{(\text{aq})}$; **(2)** free antigen (AG); **(3)** particulate adjuvant (ADJ); **(4)** ADJ with associated AG; **(5)** AG-Al complex; **(6)** MIF ligand-Al complex; **(7)** ADJ with associated MIF ligand; **(8)** MIF ligand-AG complex; **(9)** particulate iron (as contaminant of adjuvant) either free or with adsorbed Al/AG and resultant reactive oxygen species (ROS); **(10)** ADJ with associated MIF ligand-AG complex; **(11)** ADJ with associated MIF ligand-Al complex. MIF ligands might include biomolecules such as; ATP, albumin, transferrin, citrate, fibrinogen.

What Happens to the Aluminium Adjuvant?



Vaccine preparations (adjuvants in 0.9% NaCl)

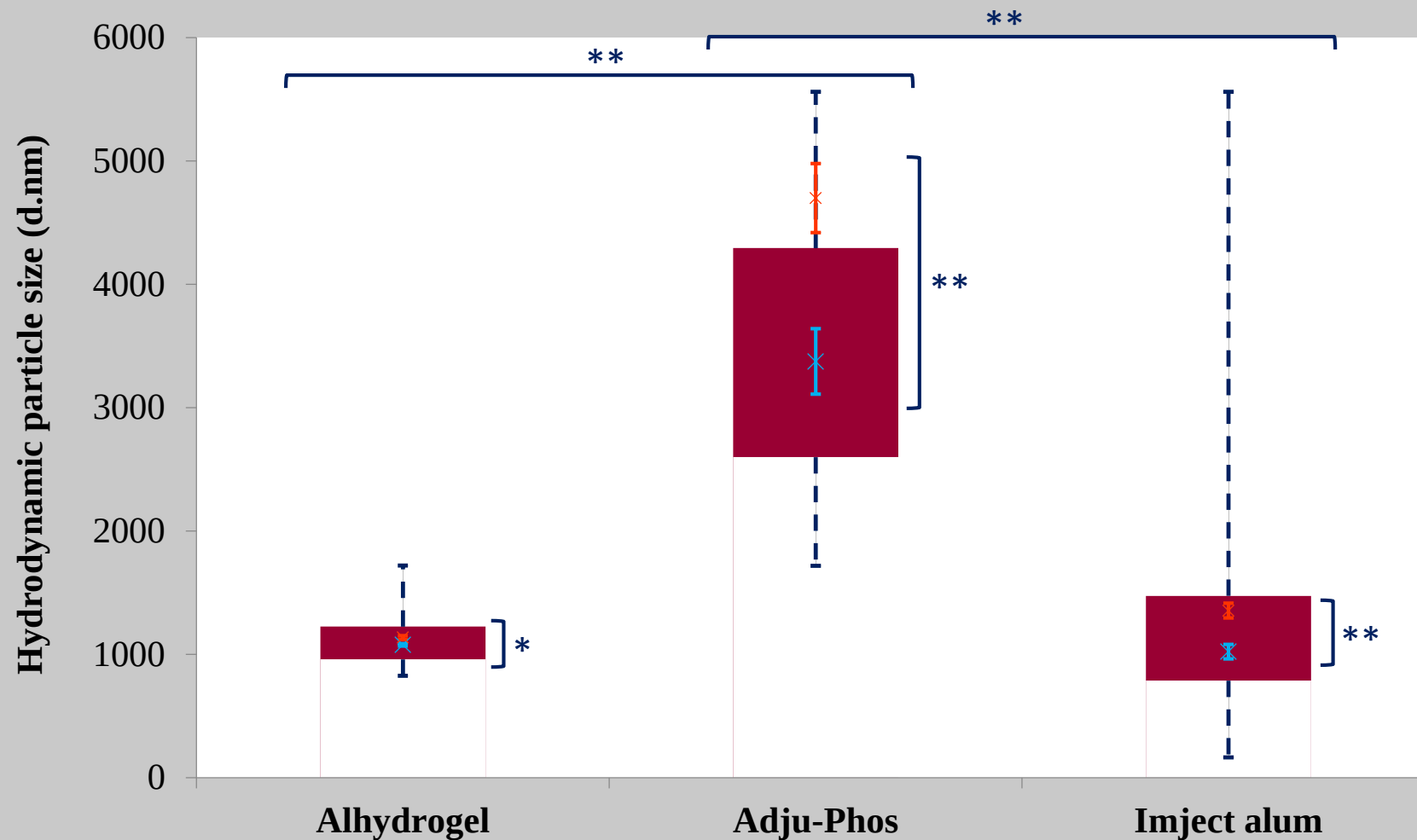


Fig 1: Size distributions of Alhydrogel, Adju-Phos & Imject alum in 0.9% NaCl following initial formulation (0hrs). Box plots are representative of the interquartile range of the data while blue dashed lines indicate the maxima and minima. Orange crosses indicate Z-average cumulant size values (nm) while light blue crosses represent the median peak size value (nm). Error bars represent the $\pm SE$ of the measurement where $n = 5$.

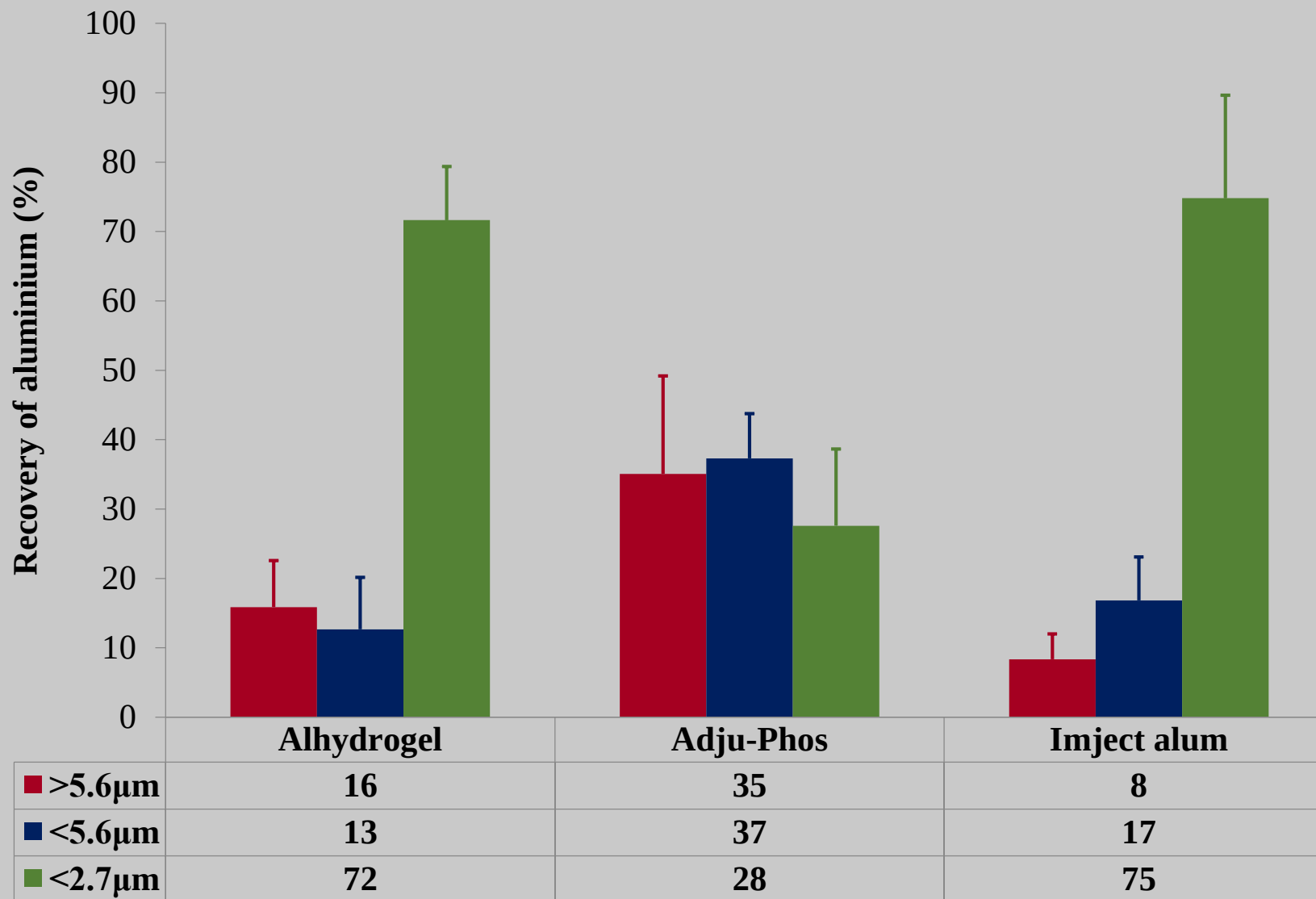


Fig 2: Recovery of Al (%) following selective filtration of Alhydrogel, Adju-Phos & Imject alum in 0.9% NaCl post initial formulation (0hrs). Error bars represent the %RSD of the measurement where $n = 5$.

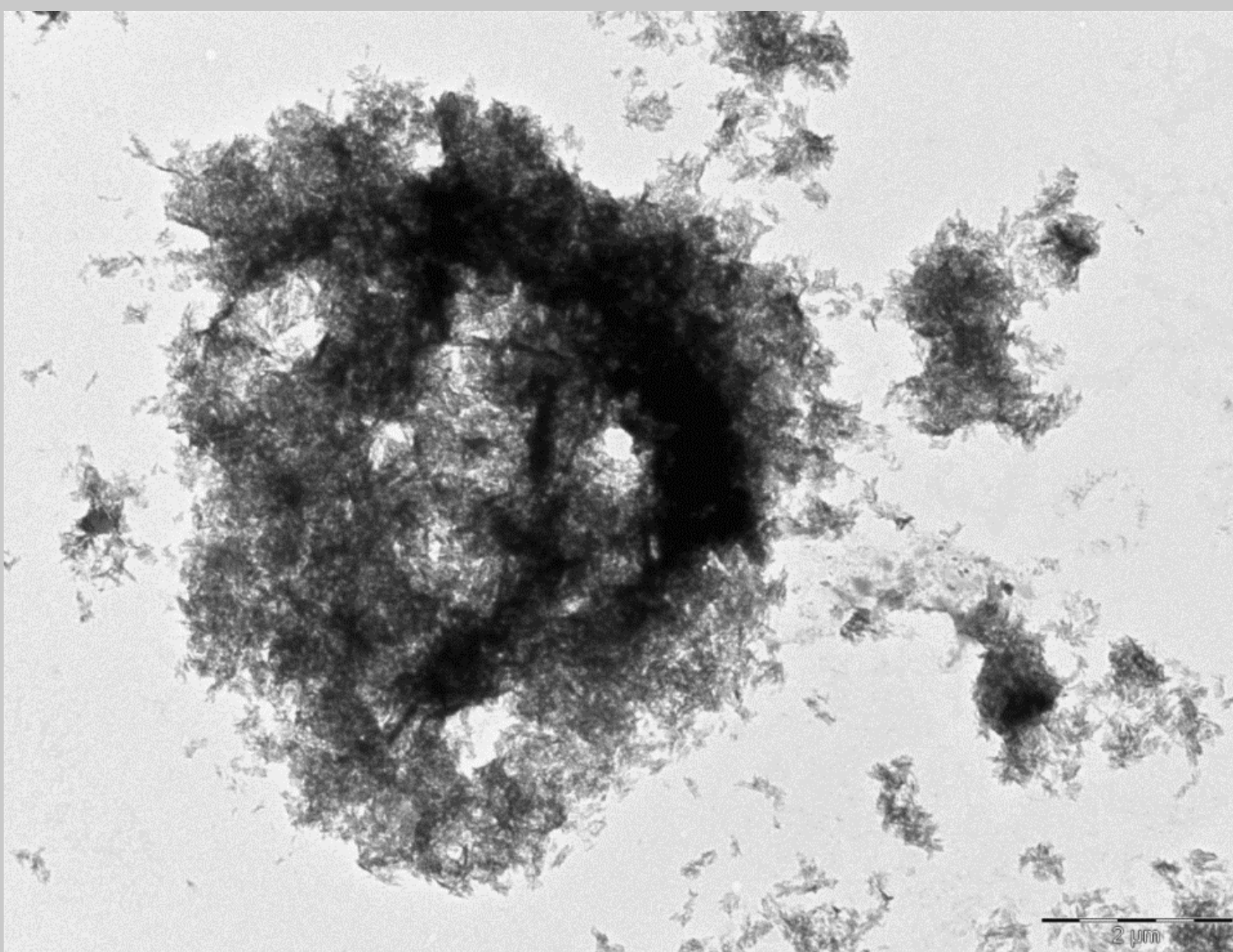


Fig 3: TEM image of Alhydrogel in 0.9% NaCl (0hrs). Mag. 10,000X, scale bar 2μm.

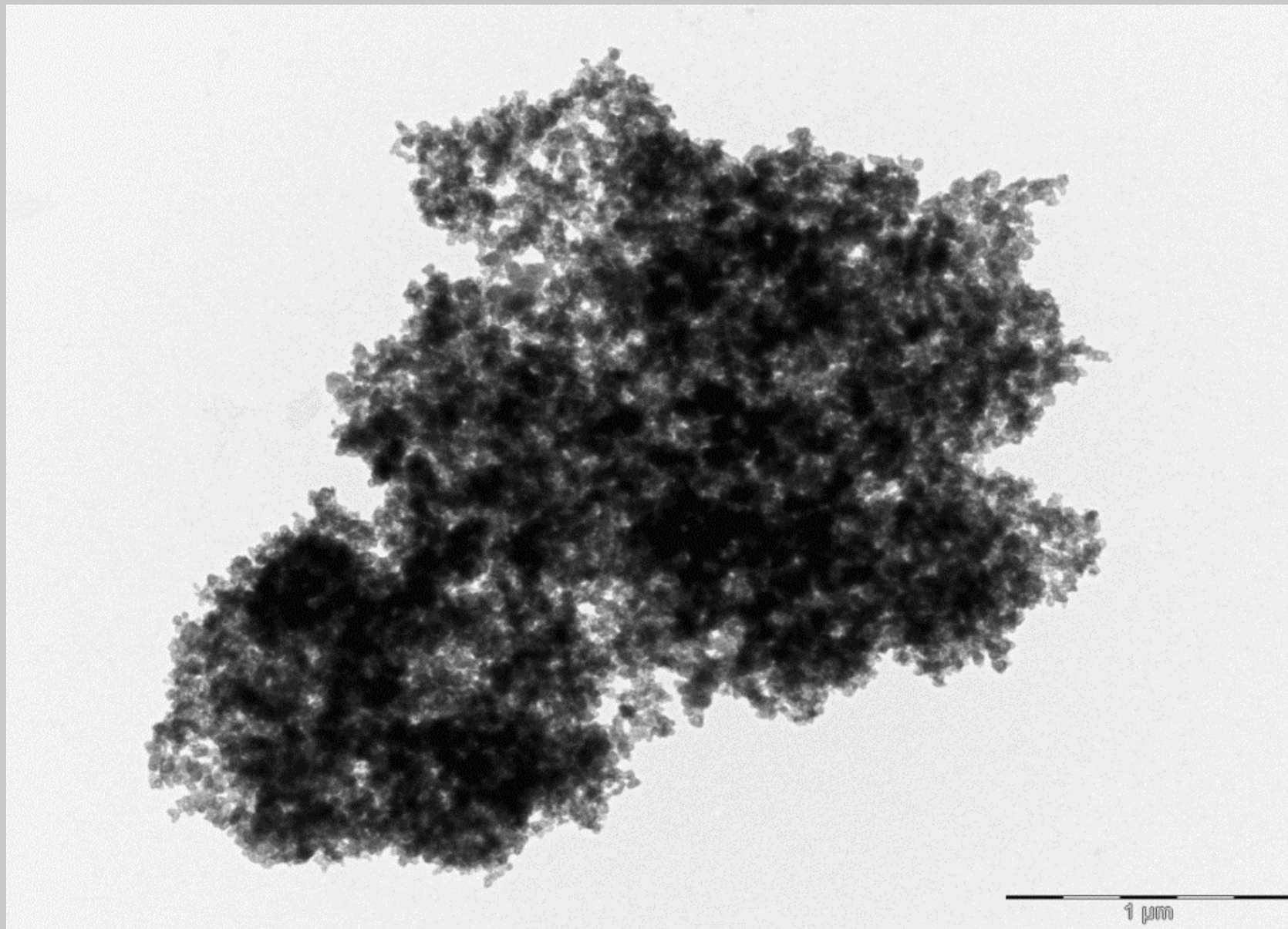


Fig 4: TEM image of Adju-Phos in 0.9% NaCl (0hrs). Mag. 30,000X, scale bar 1μm.

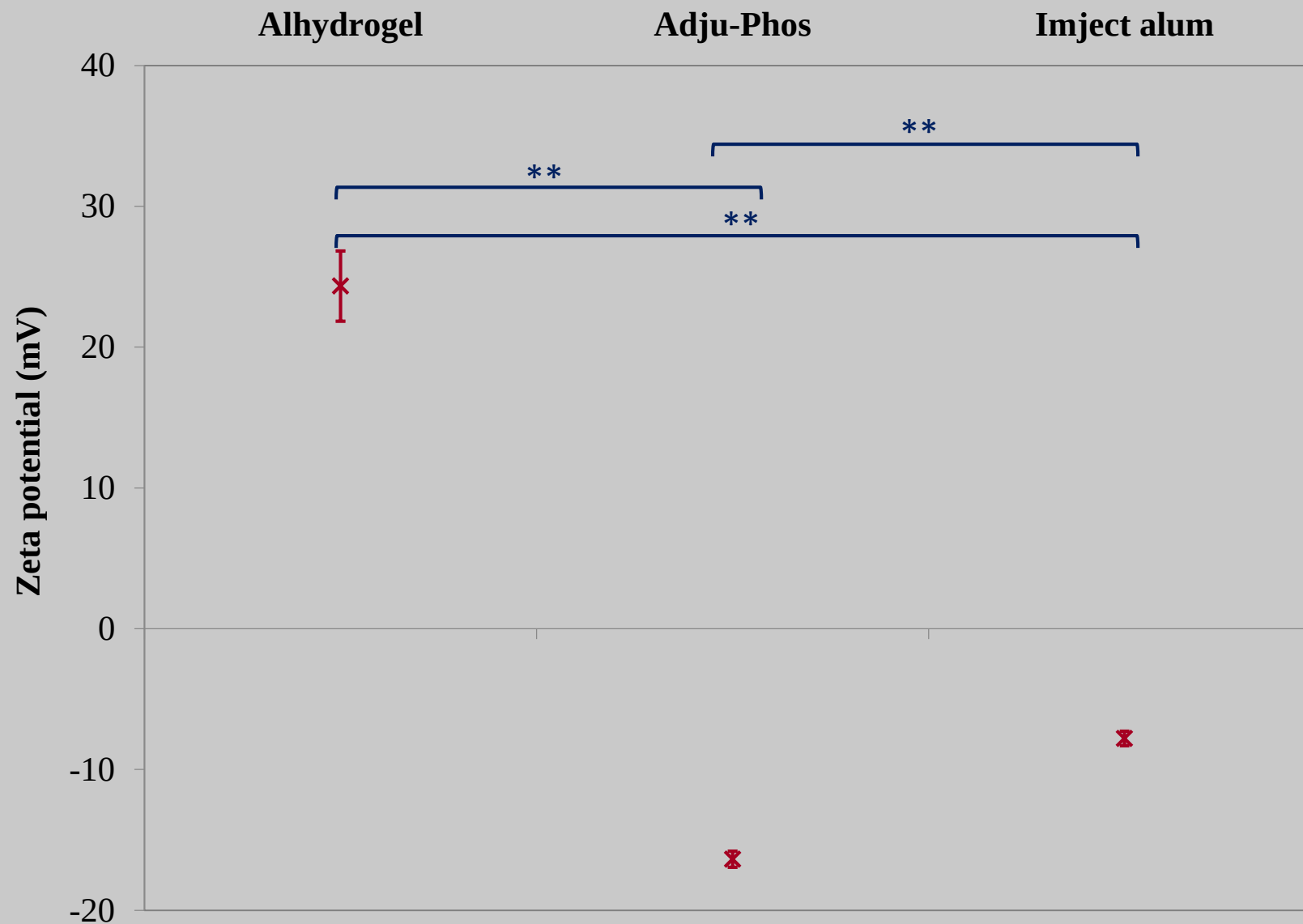
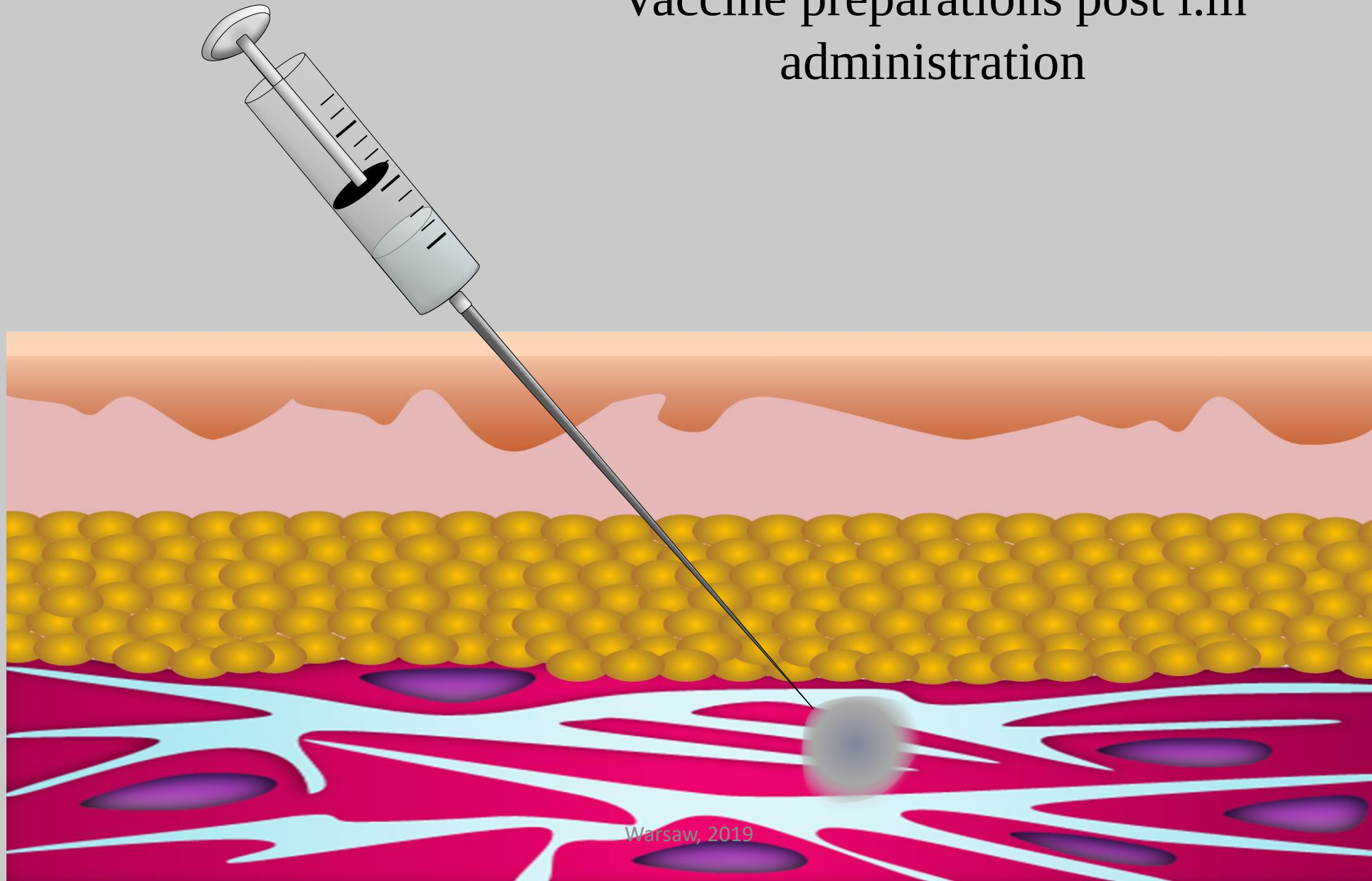


Fig 6: Zeta potential measurements of Alhydrogel, Adju-Phos & Imject alum in 0.9% NaCl following initial formulation (0hrs). Error bars represent the $\pm$ SD where $n = 5$.

Vaccine preparations post i.m administration



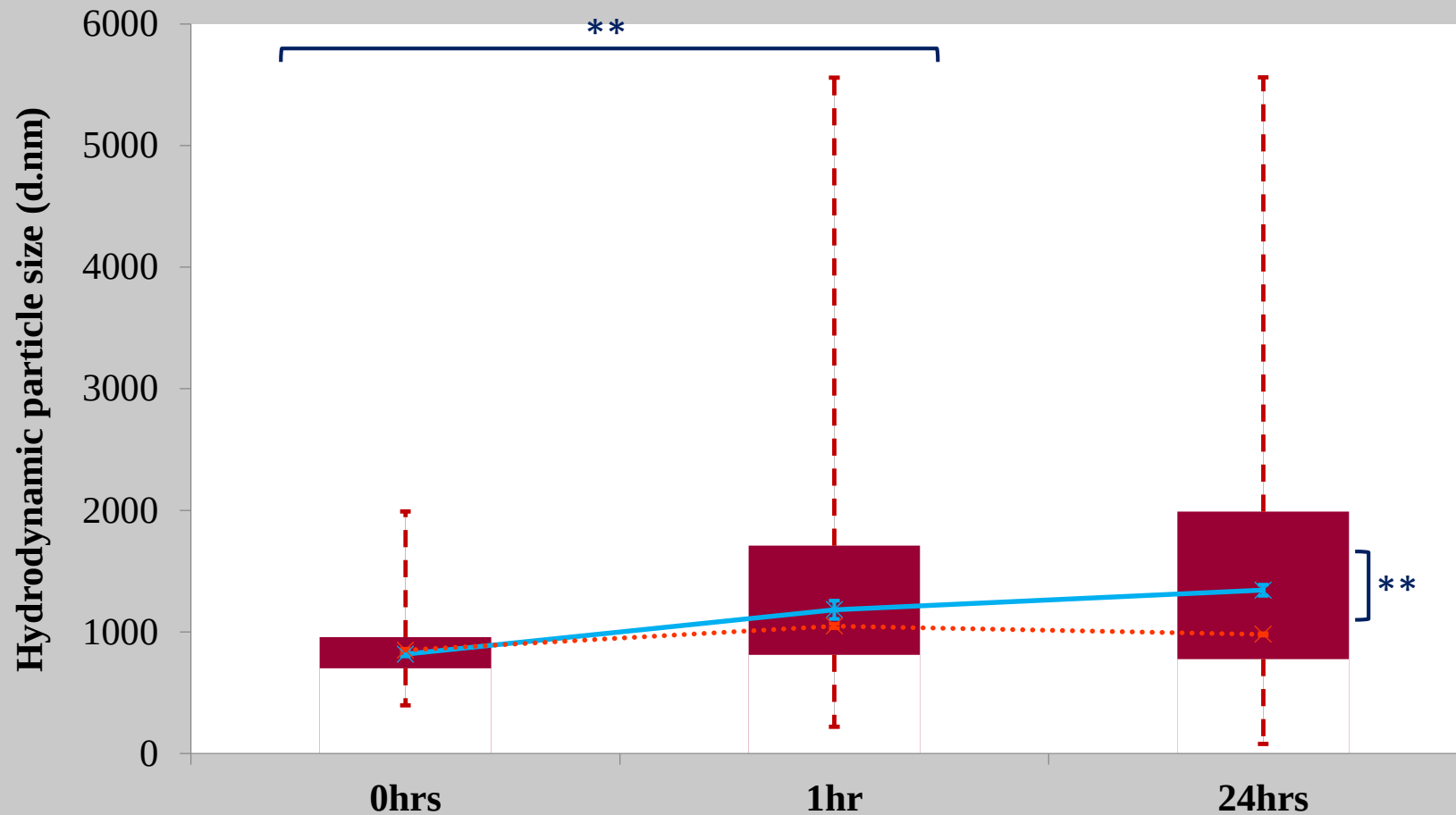


Fig 7: Size distributions of Alhydrogel in R10 medium following 0, 1 & 24hrs incubation (37°C). Box plots are representative of the interquartile range of the data while blue dashed lines indicate the maxima and minima. Orange crosses indicate Z-average cumulant size values (nm) while light blue crosses represent the median peak size value (nm). Error bars represent the $\pm$ SE of the measurement where $n = 5$.

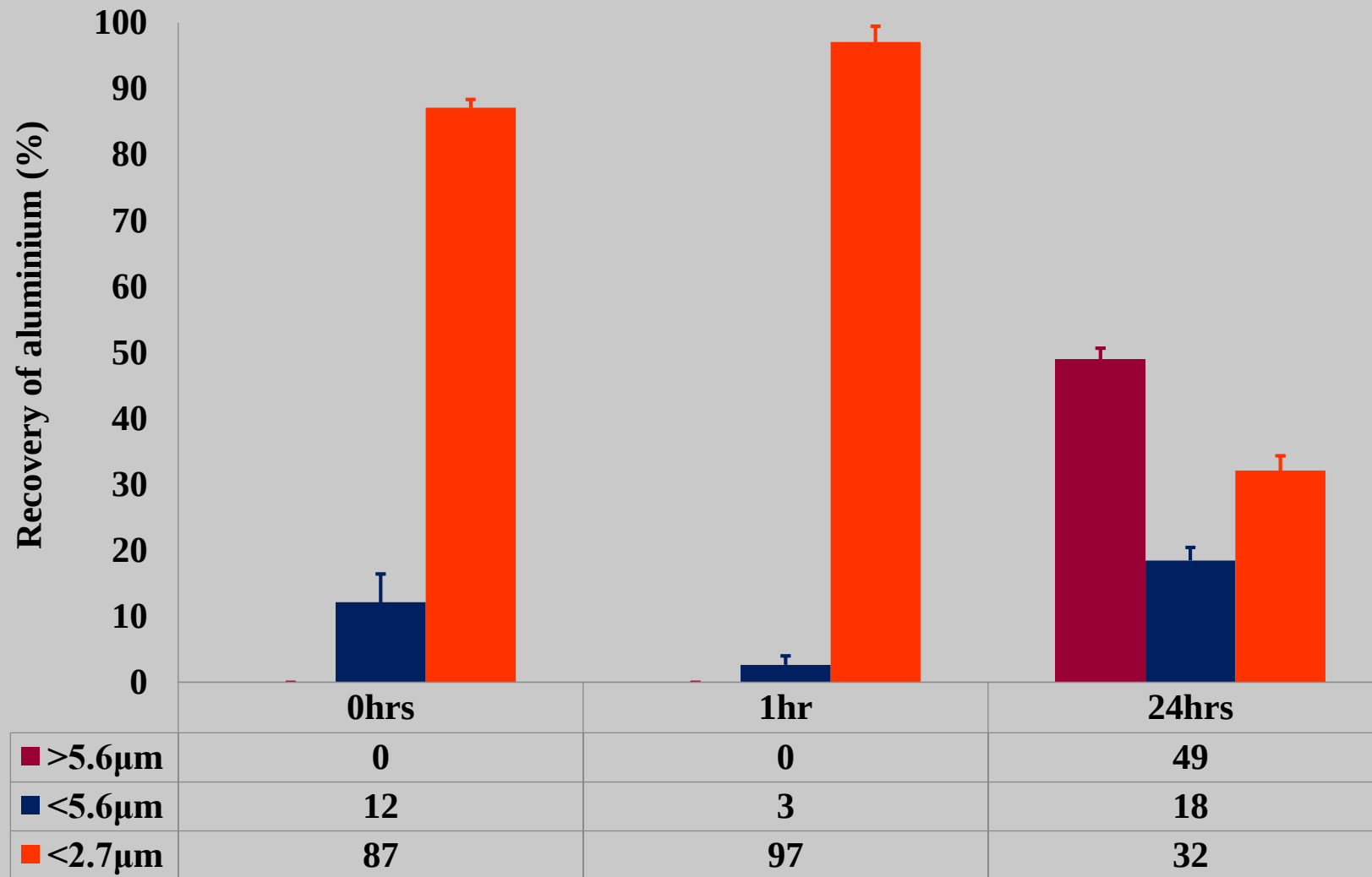


Fig 8: Recovery of Al (%) following selective filtration of Alhydrogel in R10 medium following 0, 1 & 24hrs incubation (37°C). Error bars represent the %RSD of the measurement where n = 5.

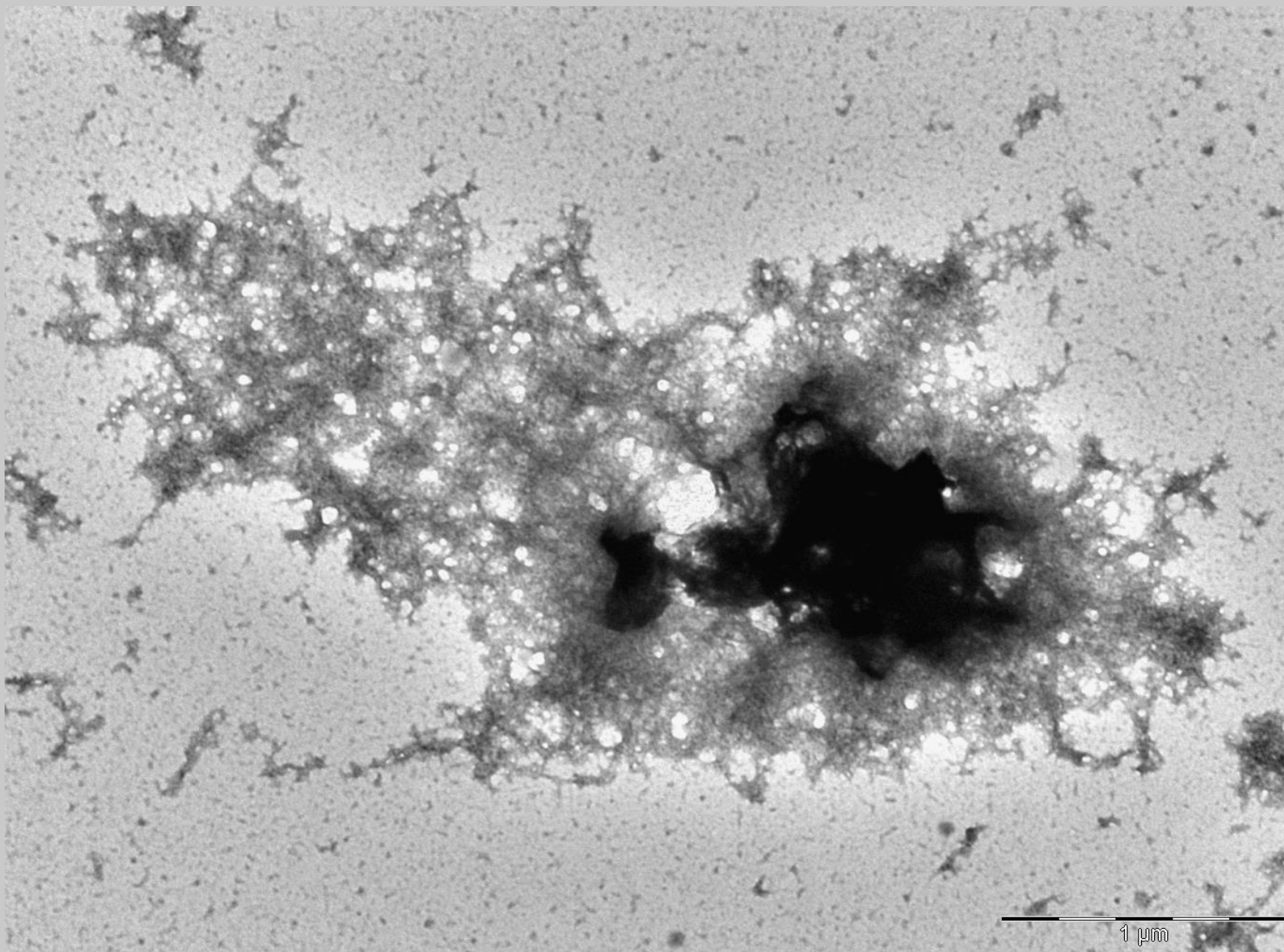


Fig 9: TEM image of Alhydrogel in R10 medium (0hrs). Mag. 30,000X, scale bar 1μm.



Fig 10: Zeta potential measurements of Alhydrogel in R10 medium following 0, 1 & 24hrs incubation (37°C). Error bars represent the $\pm$ SD where $n = 5$.

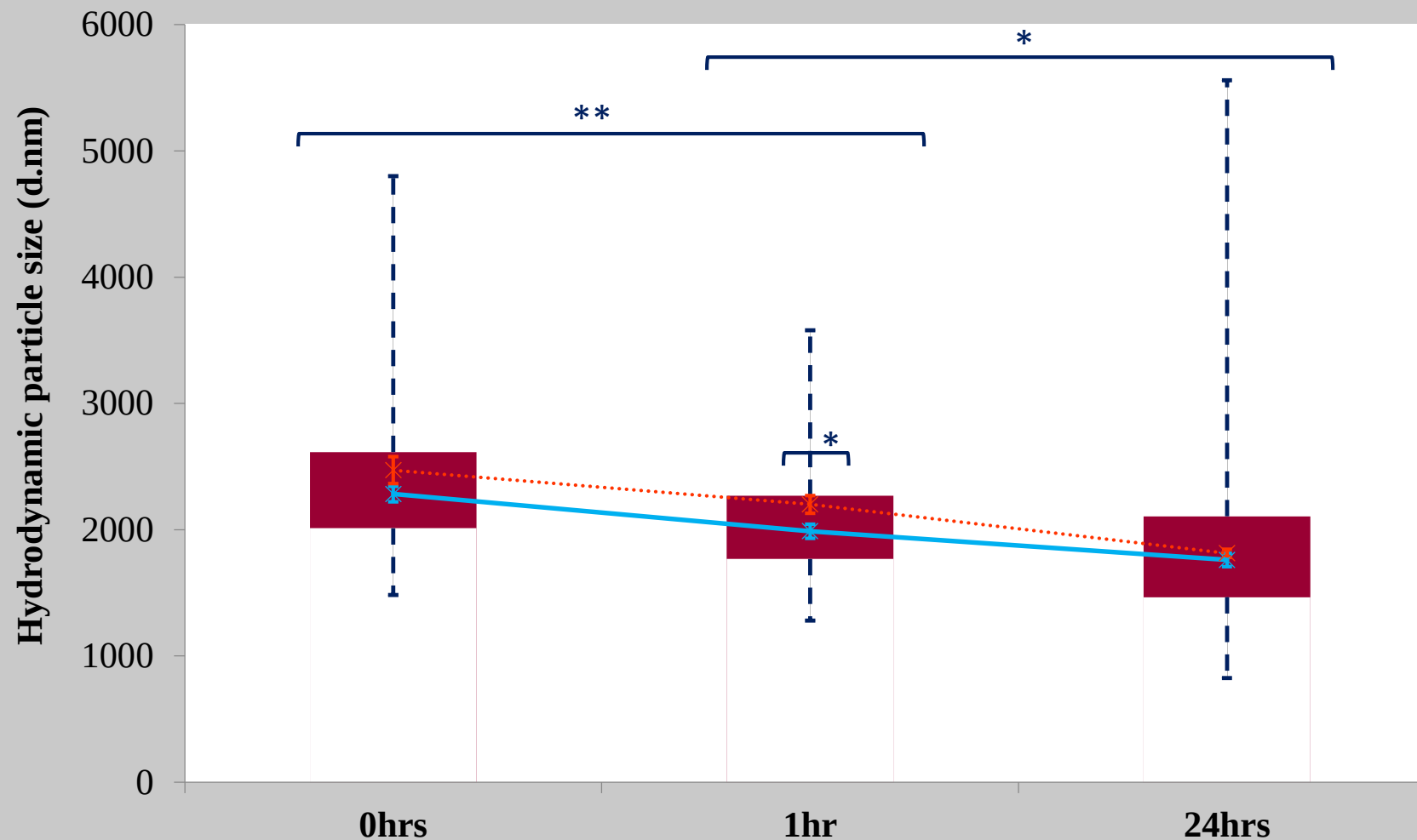


Fig 11: Size distributions of Adju-Phos in R10 medium following 0, 1 & 24hrs incubation (37°C). Box plots are representative of the interquartile range of the data while blue dashed lines indicate the maxima and minima. Orange crosses indicate Z-average cumulant size values (nm) while light blue crosses represent the median peak size value (nm). Error bars represent the $\pm$ SE of the measurement where $n = 5$.

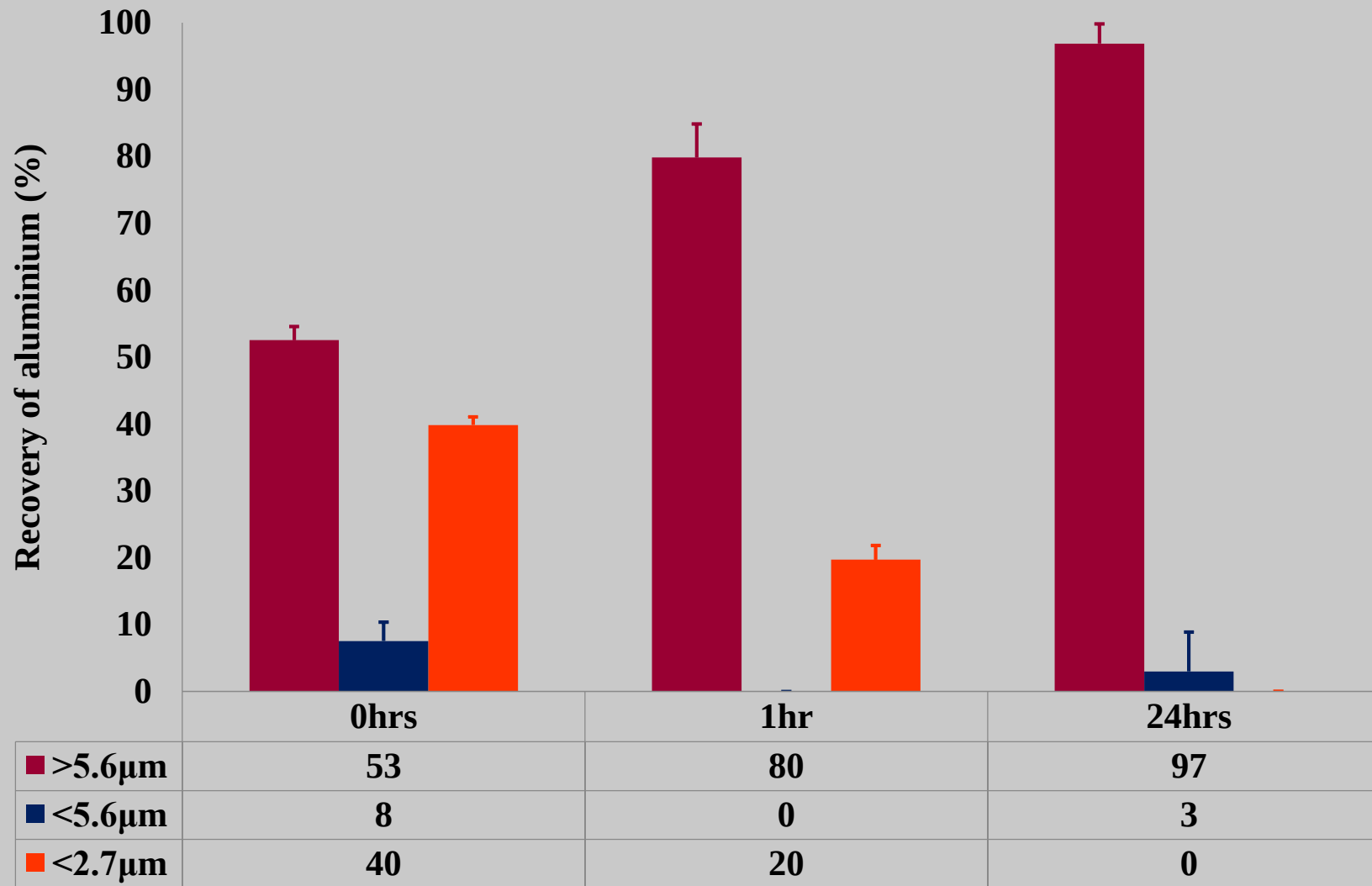


Fig 12: Recovery of Al (%) following selective filtration of Adju-Phos in R10 medium following 0, 1 & 24hrs incubation (37°C). Error bars represent the %RSD of the measurement where n = 5.

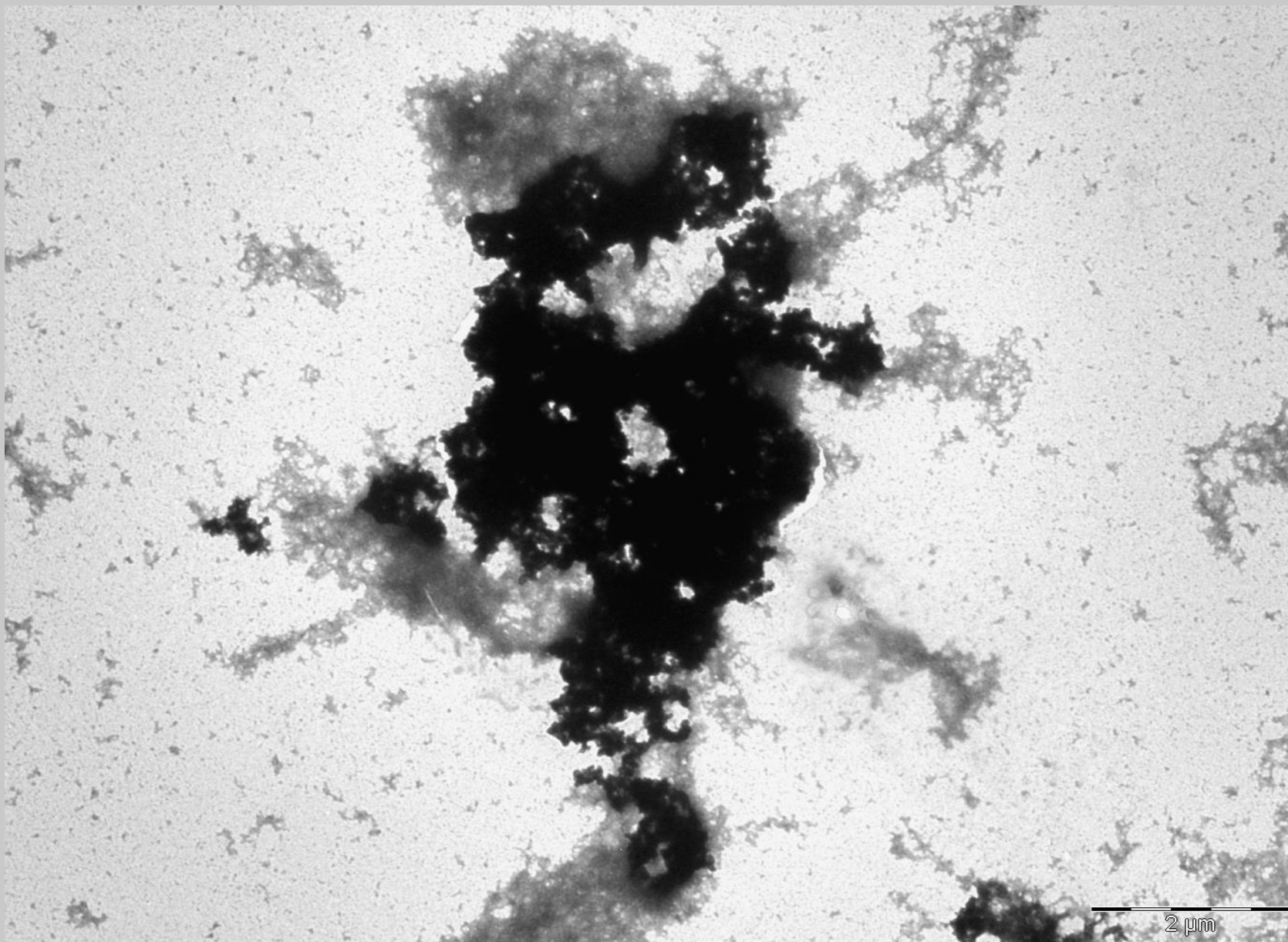


Fig 13: TEM image of Adju-Phos in R10 medium (0hrs). Mag. 30,000X, scale bar 2μm.



Fig 14: Zeta potential measurements of Adju-Phos in R10 medium following 0, 1 & 24hrs incubation (37°C). Error bars represent the $\pm SD$ where $n = 5$.

Conclusions

➤ In 0.9% NaCl, negatively charged Adju-Phos has a larger overall particle size than positively charged Alhydrogel

❖ Alh - $\sim 72\% \leq 2.7\mu\text{m}$

❖ Adj - $\sim 28\% \leq 2.7\mu\text{m}$

➤ At the site of injection both adjuvants become negatively charged upon administration

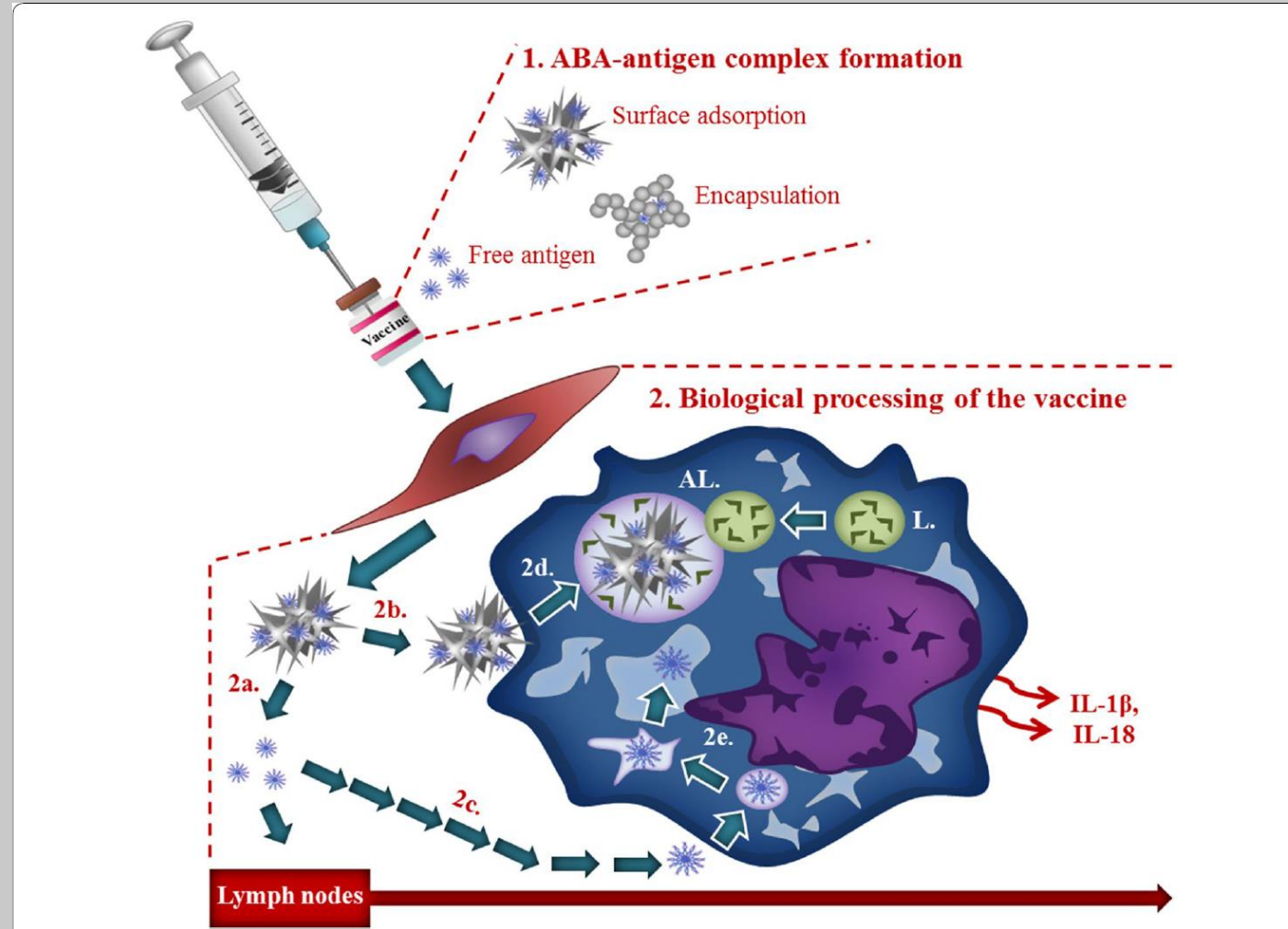
➤ Following administration Alhydrogel has a larger abundance of particles available for phagocytosis.

❖ Alh - $\sim 97\% \leq 2.7\mu\text{m}$

❖ Adj - $\sim 20\% \leq 2.7\mu\text{m}$

What About the Cellular Response to Aluminium Adjuvants?

<https://aacijournal.biomedcentral.com/articles/10.1186/s13223-018-0305-2>



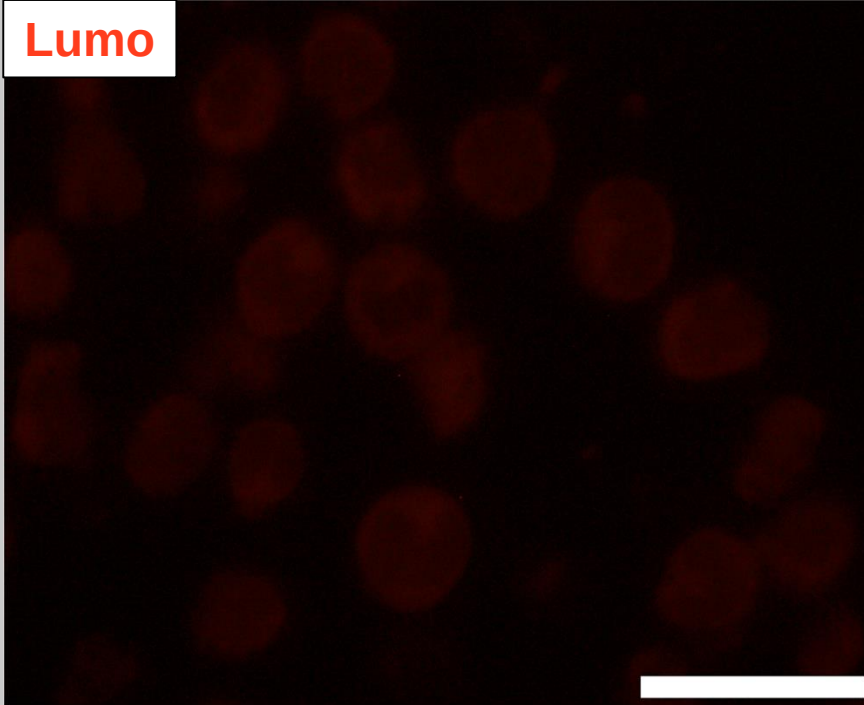
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Warsaw, 2019

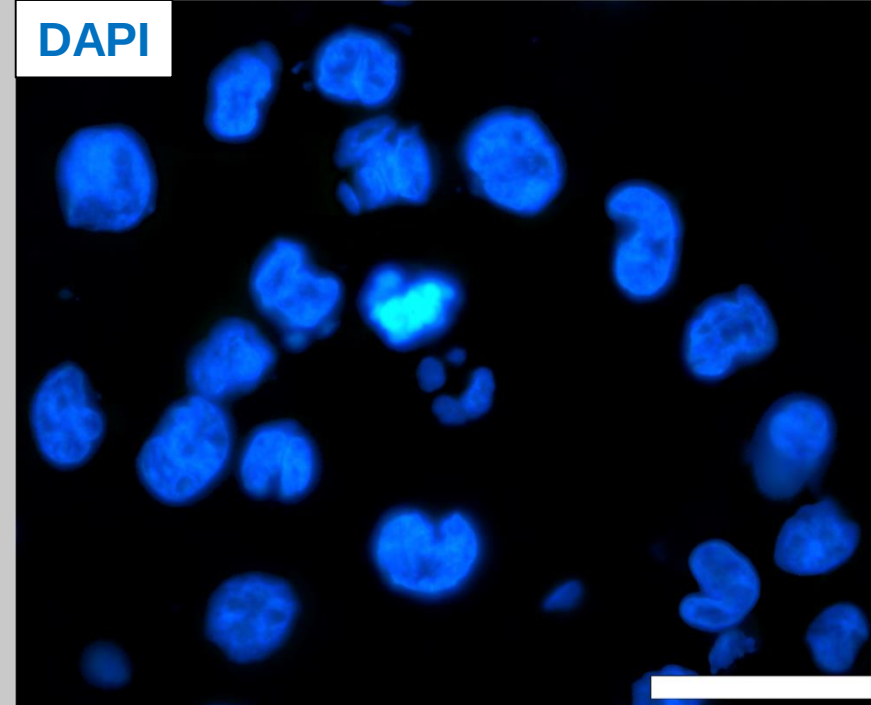
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Native THP-1 cells (R10)

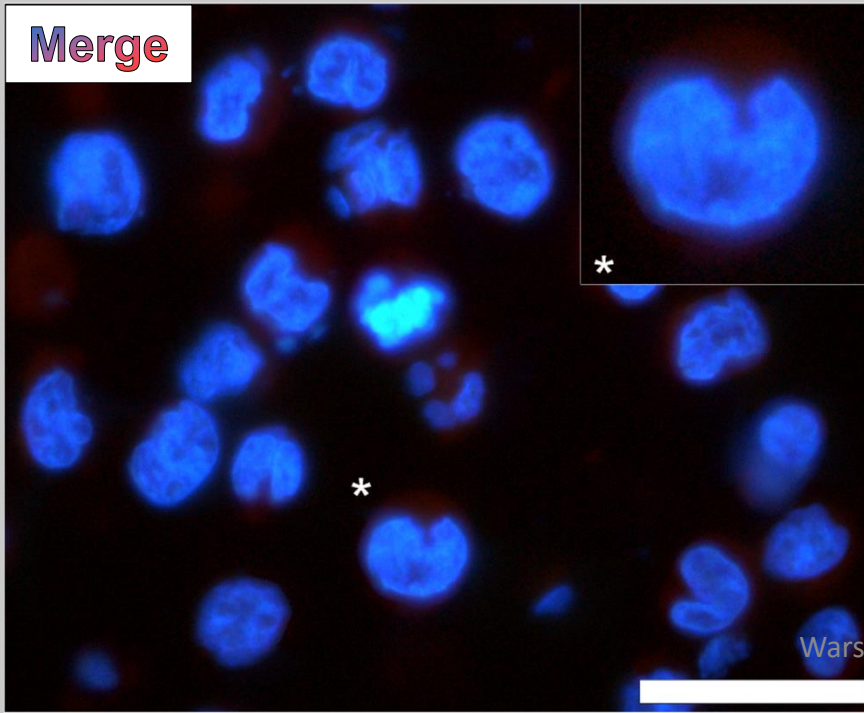
Lumo



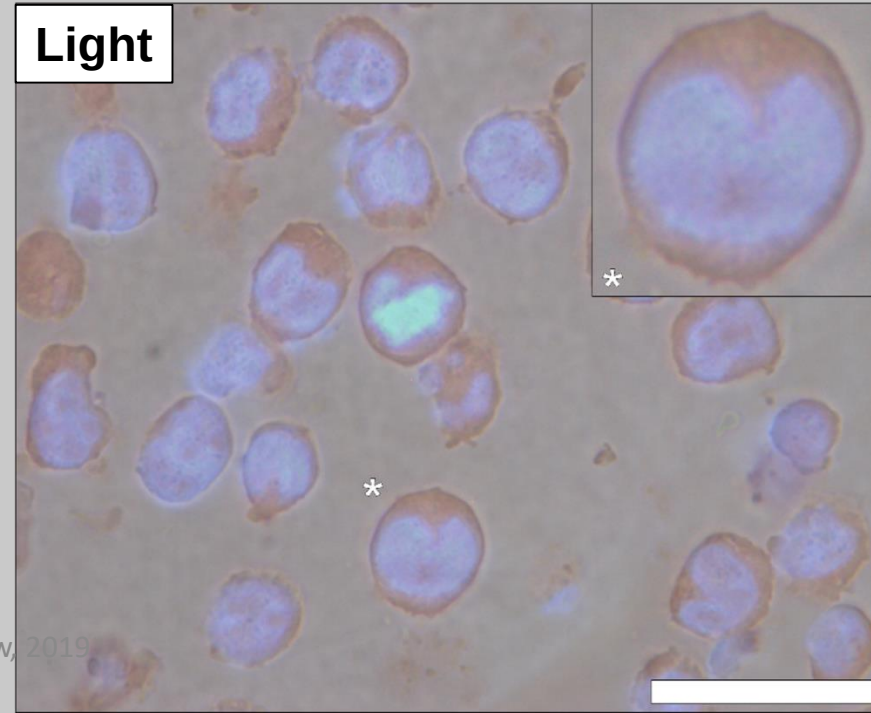
DAPI



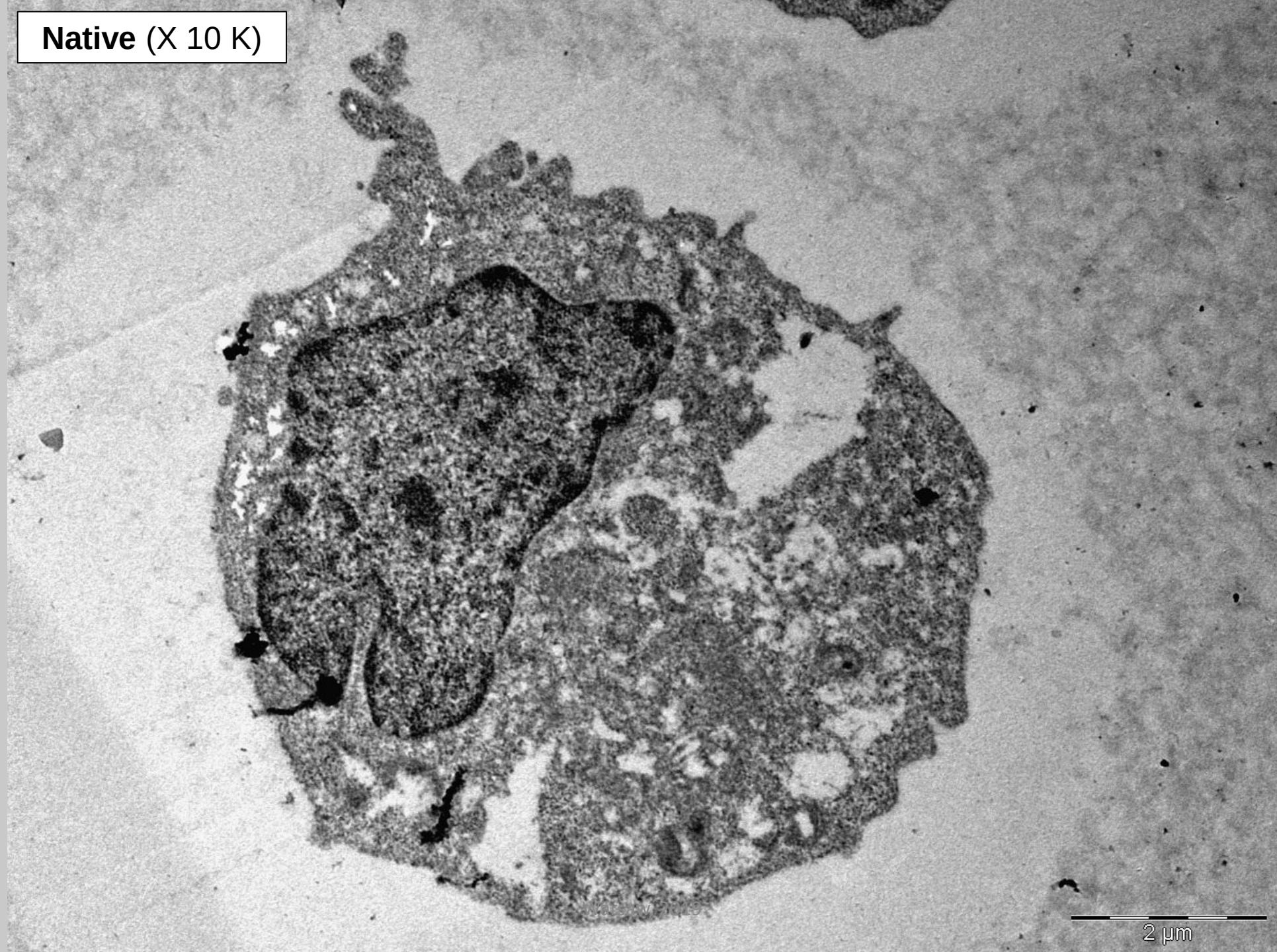
Merge



Light



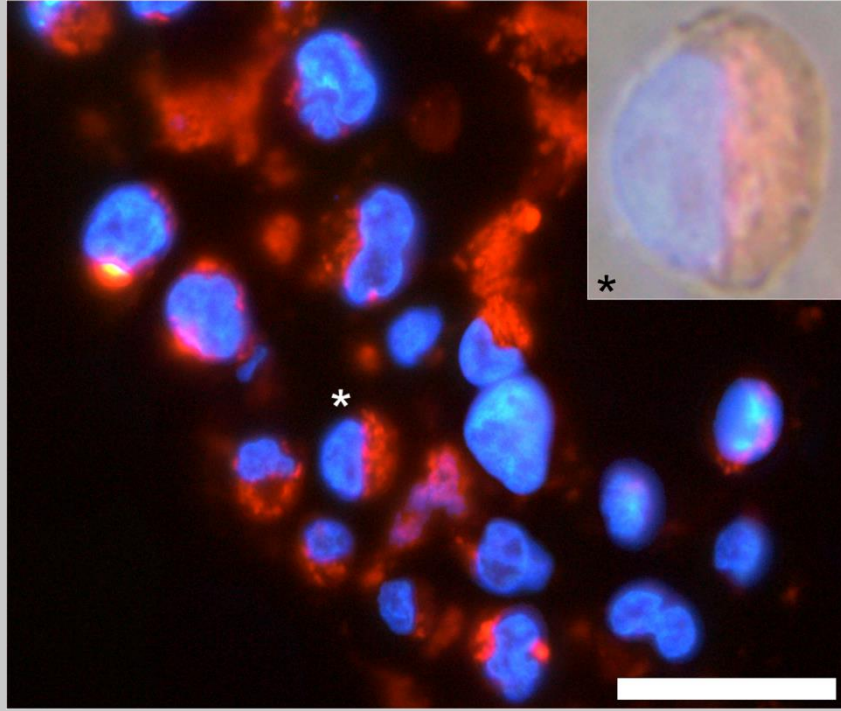
Native (X 10 K)



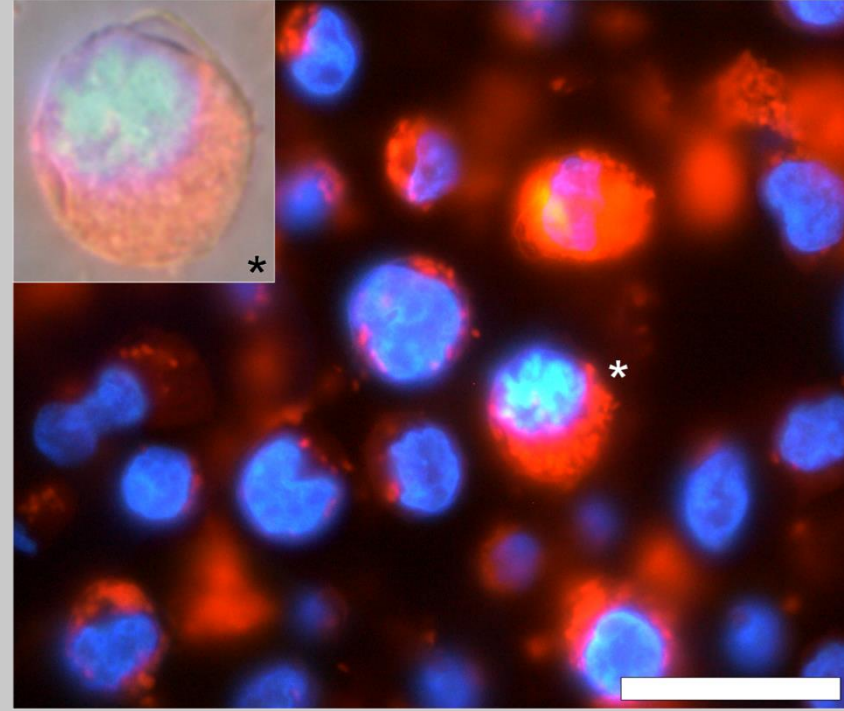
Alhydrogel®

2.5 - 100 $\mu\text{g/mL}$

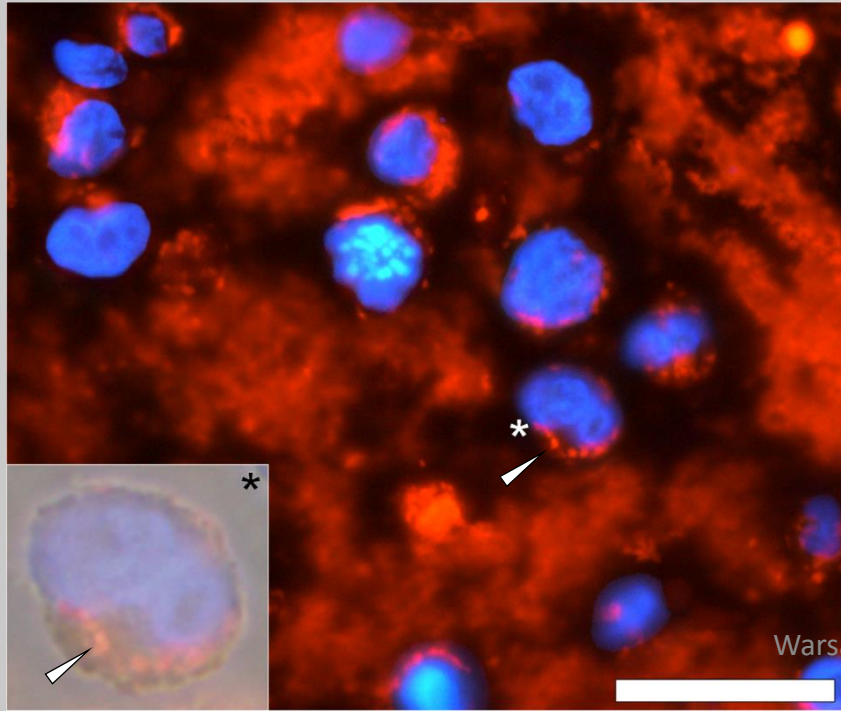
2.5 μ g/mL



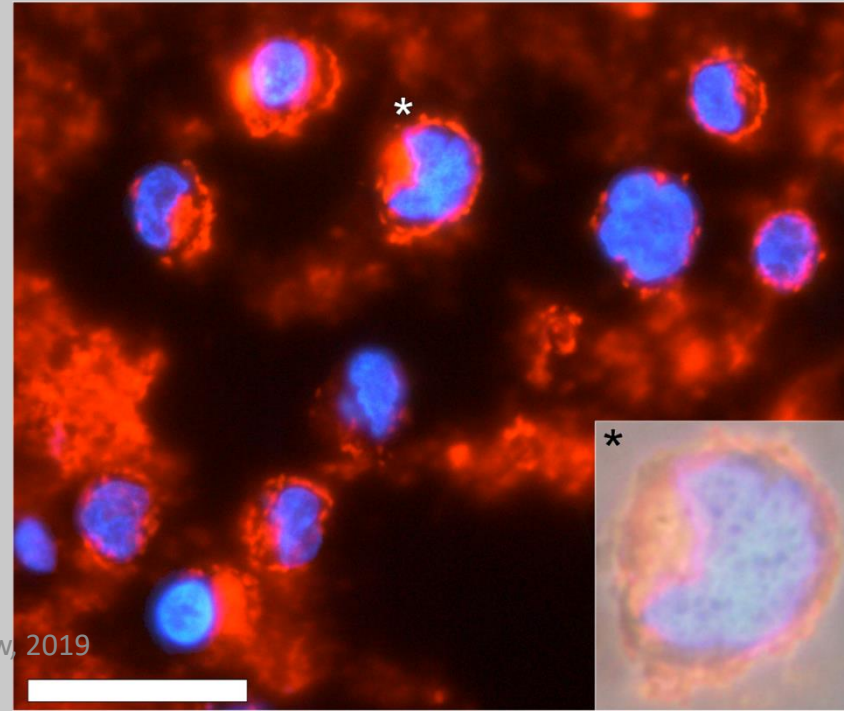
25 μ g/mL



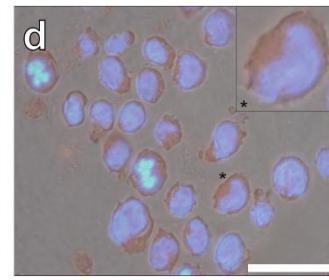
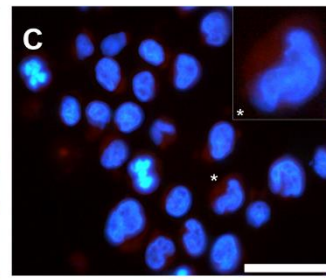
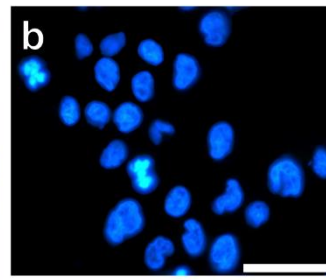
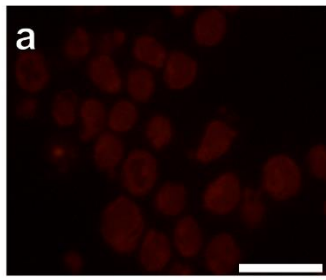
50 μ g/mL



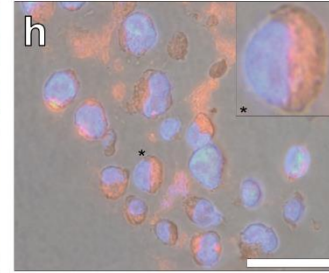
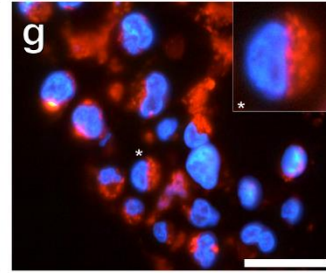
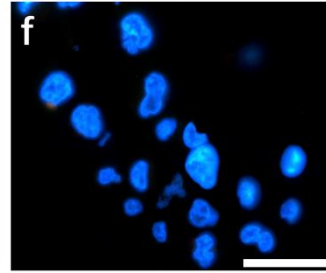
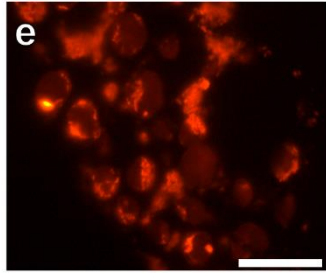
100 μ g/mL



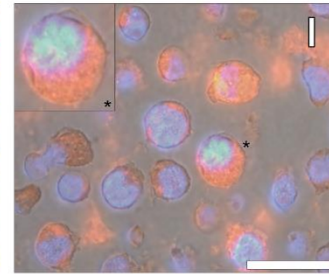
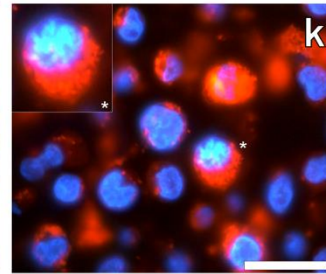
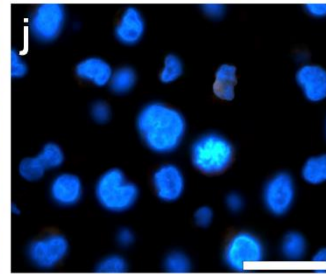
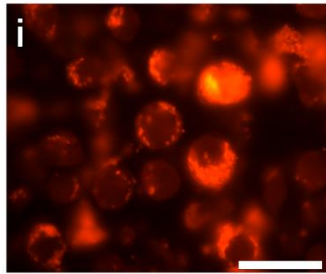
R10



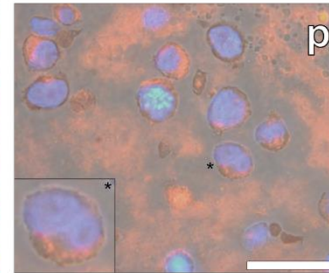
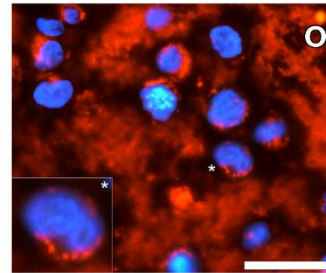
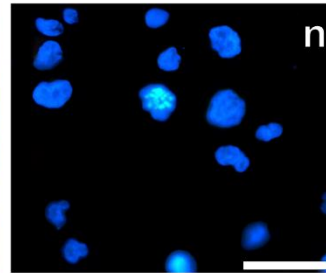
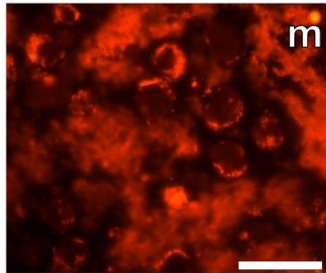
2.5µg/mL



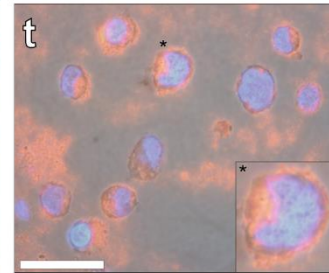
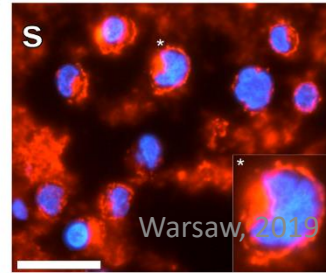
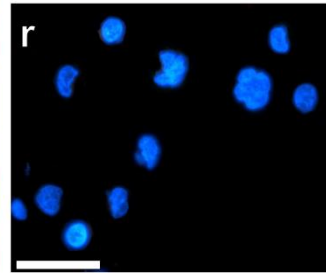
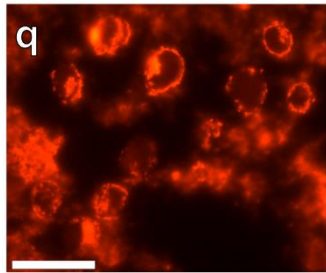
25µg/mL



50µg/mL



100µg/mL



- Alhydrogel® found localised in cell cytoplasm.
- ABA particles were found internalised in THP-1 cells (ca 1.0µm).
- Alhydrogel was found readily internalised at all [ABA]s.
- ABA particulates were also found **associated with plasma membranes** at 100µg/mL of the adjuvant.

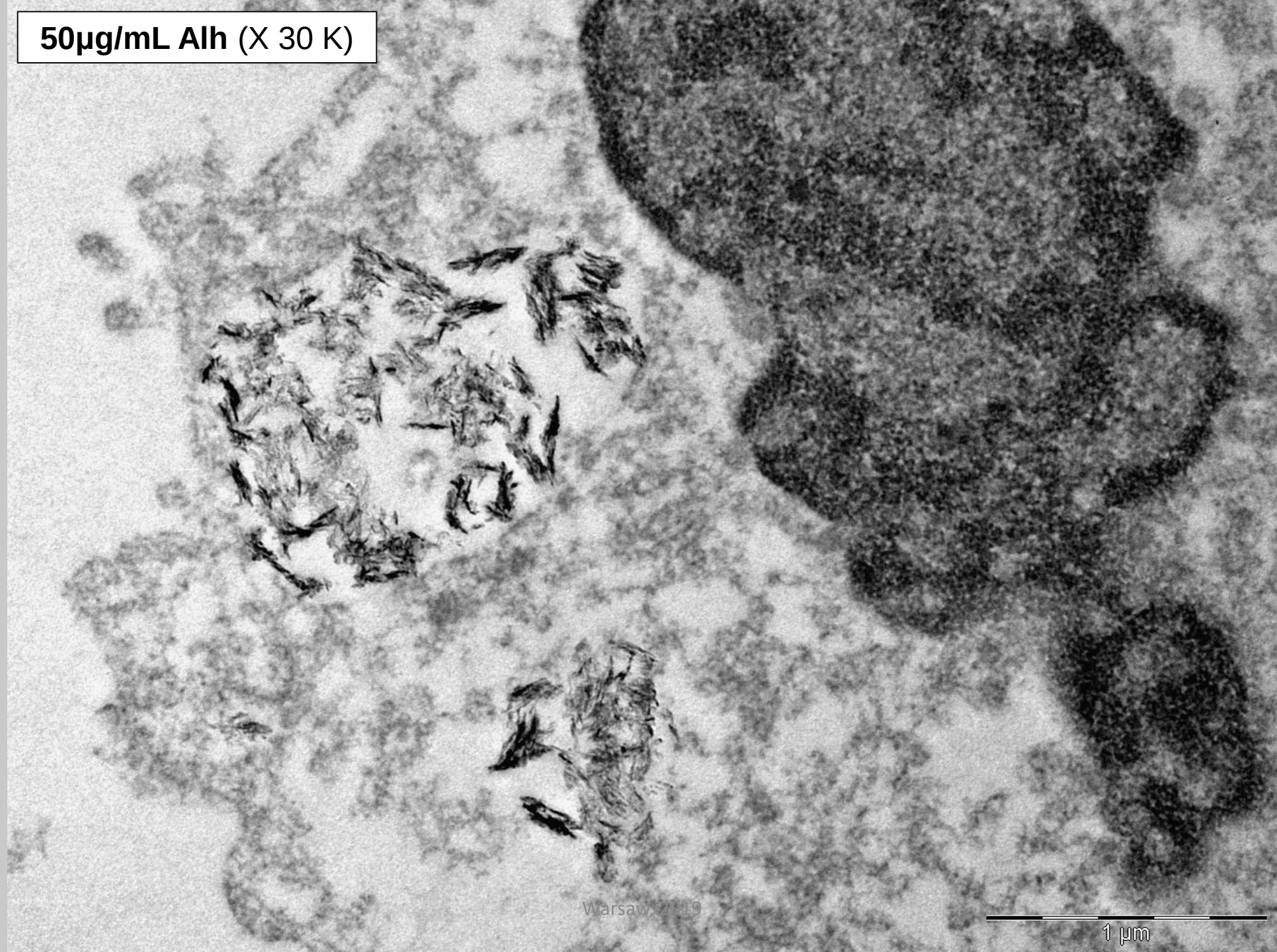
50 μ g/mL Alh (X 8 K)



Warsaw, 2019

5 μ m

50 μ g/mL Alh (X 30 K)



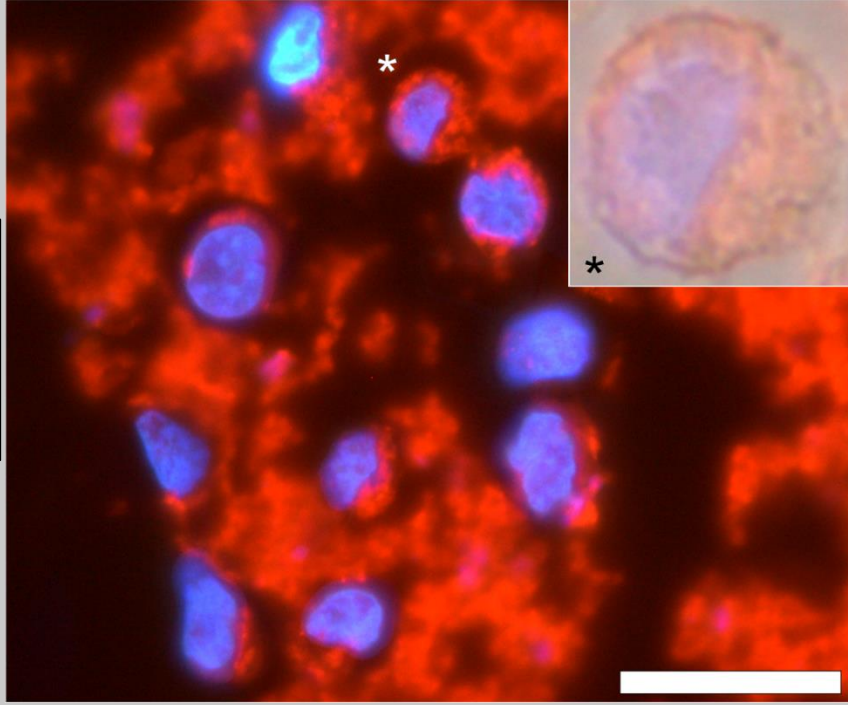
Warsaw, 2019

1 μ m

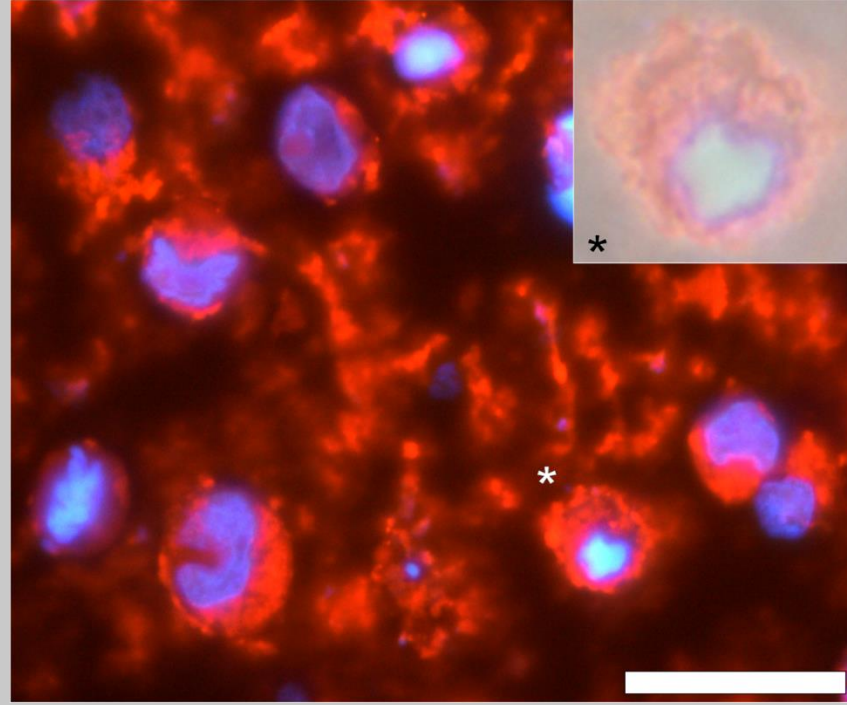
Adju-Phos[®]

2.5 - 100 $\mu\text{g/mL}$

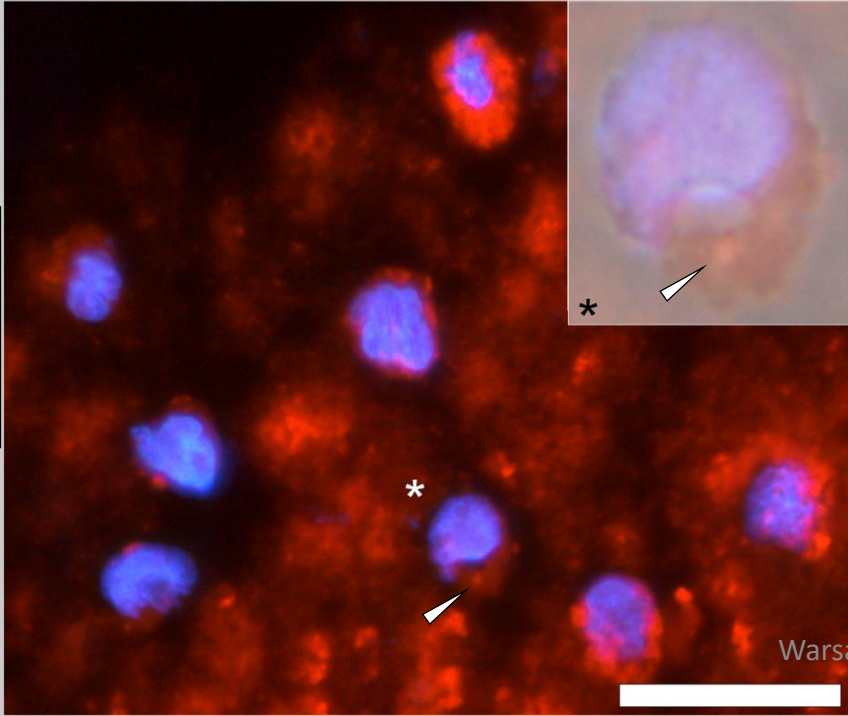
2.5µg/mL



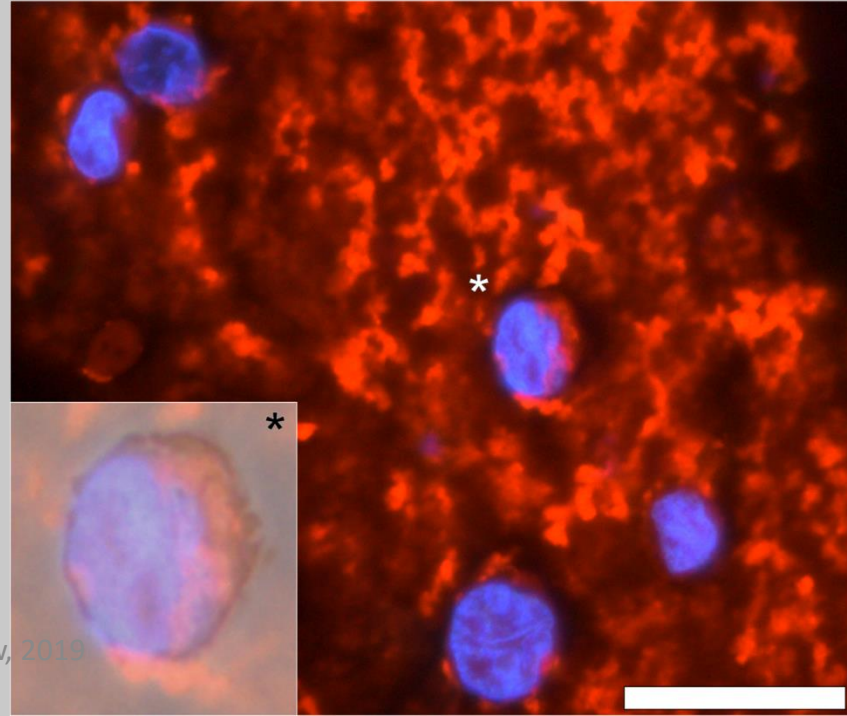
25µg/mL



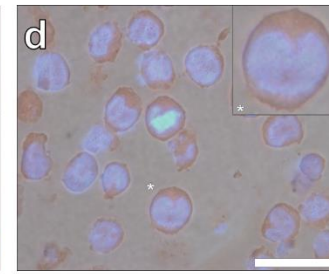
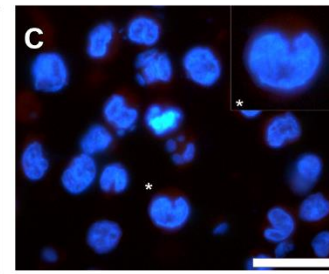
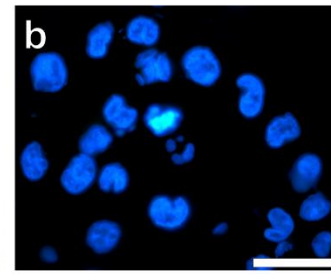
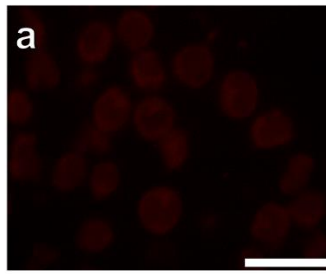
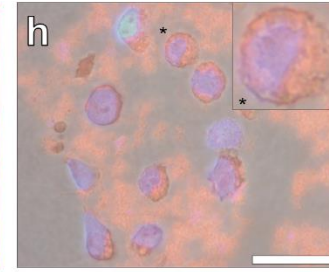
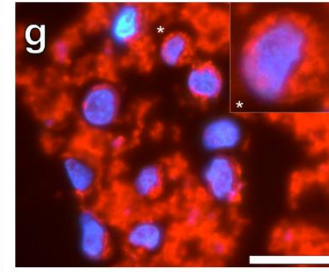
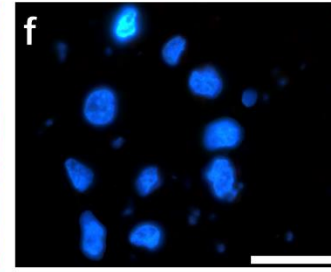
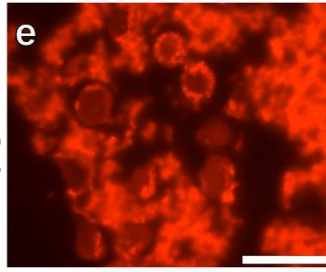
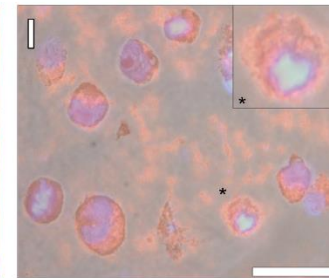
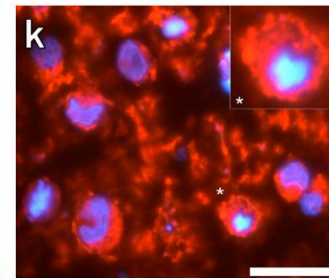
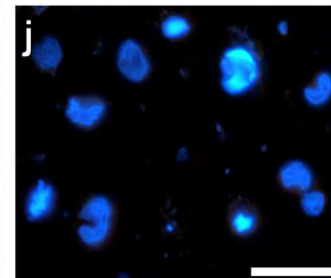
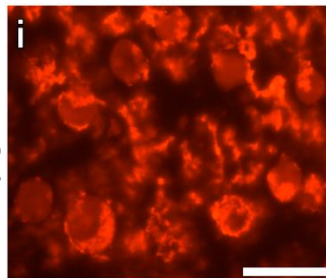
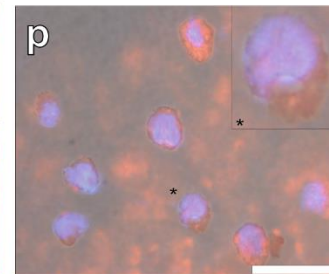
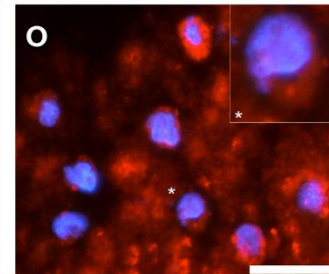
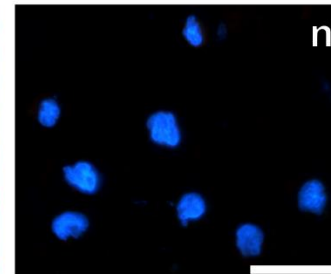
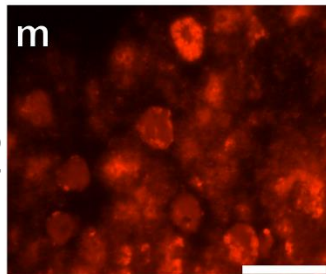
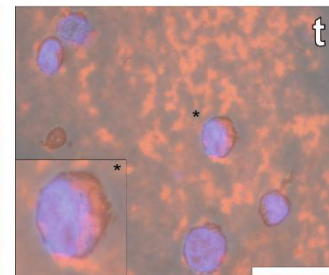
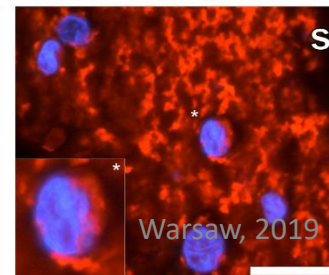
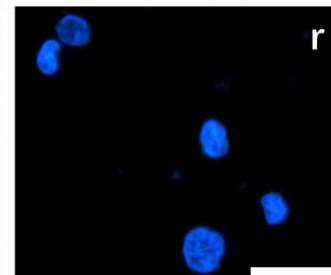
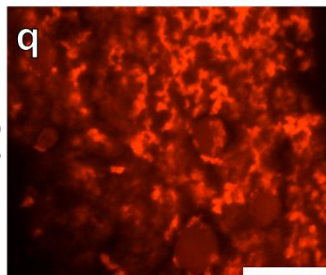
50µg/mL



100µg/mL

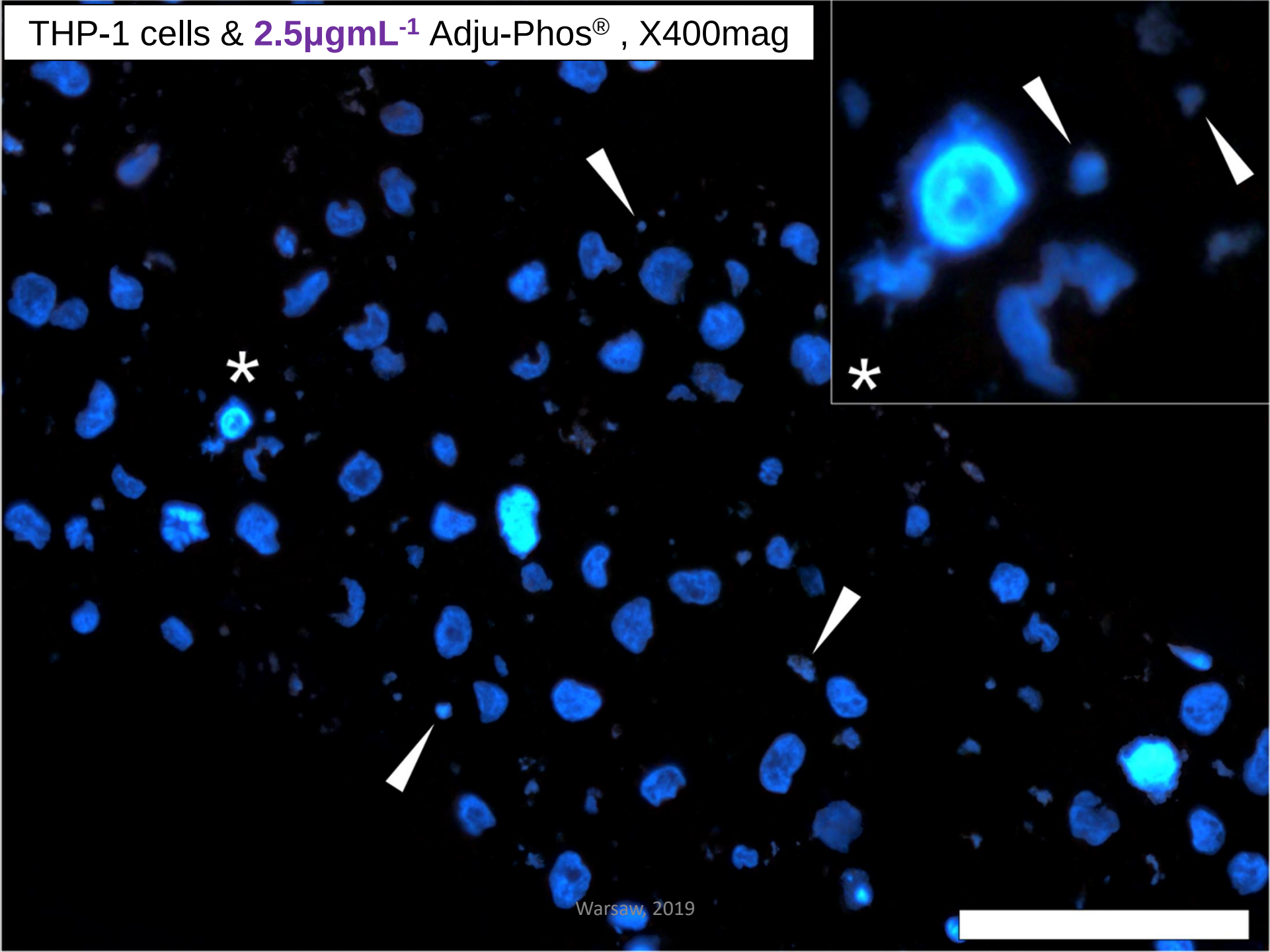


R10

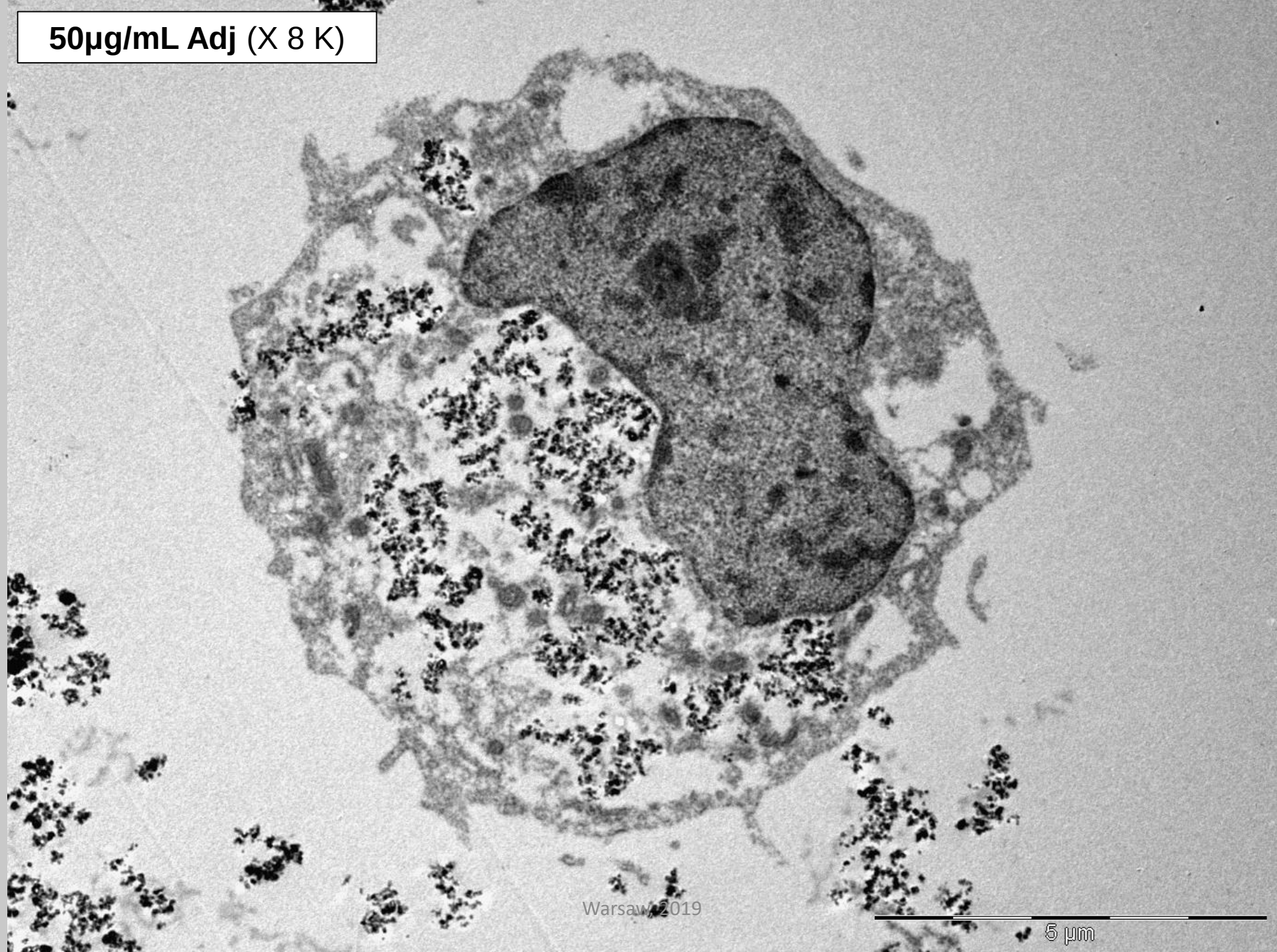
2.5 $\mu\text{g/mL}$ 25 $\mu\text{g/mL}$ 50 $\mu\text{g/mL}$ 100 $\mu\text{g/mL}$ 

- Adju-Phos[®] found localised in cell cytoplasm only.
- Discreet ABA particles were found internalised in THP-1 cells, however their identification were sometimes difficult.
- Adju-Phos was readily internalised at 2.5 and 25 $\mu\text{g/mL}$ of the ABA.
- Uptake less pronounced at 50 and 100 $\mu\text{g/mL}$ of the adjuvant.

THP-1 cells & $2.5\mu\text{gmL}^{-1}$ Adju-Phos[®] , X400mag



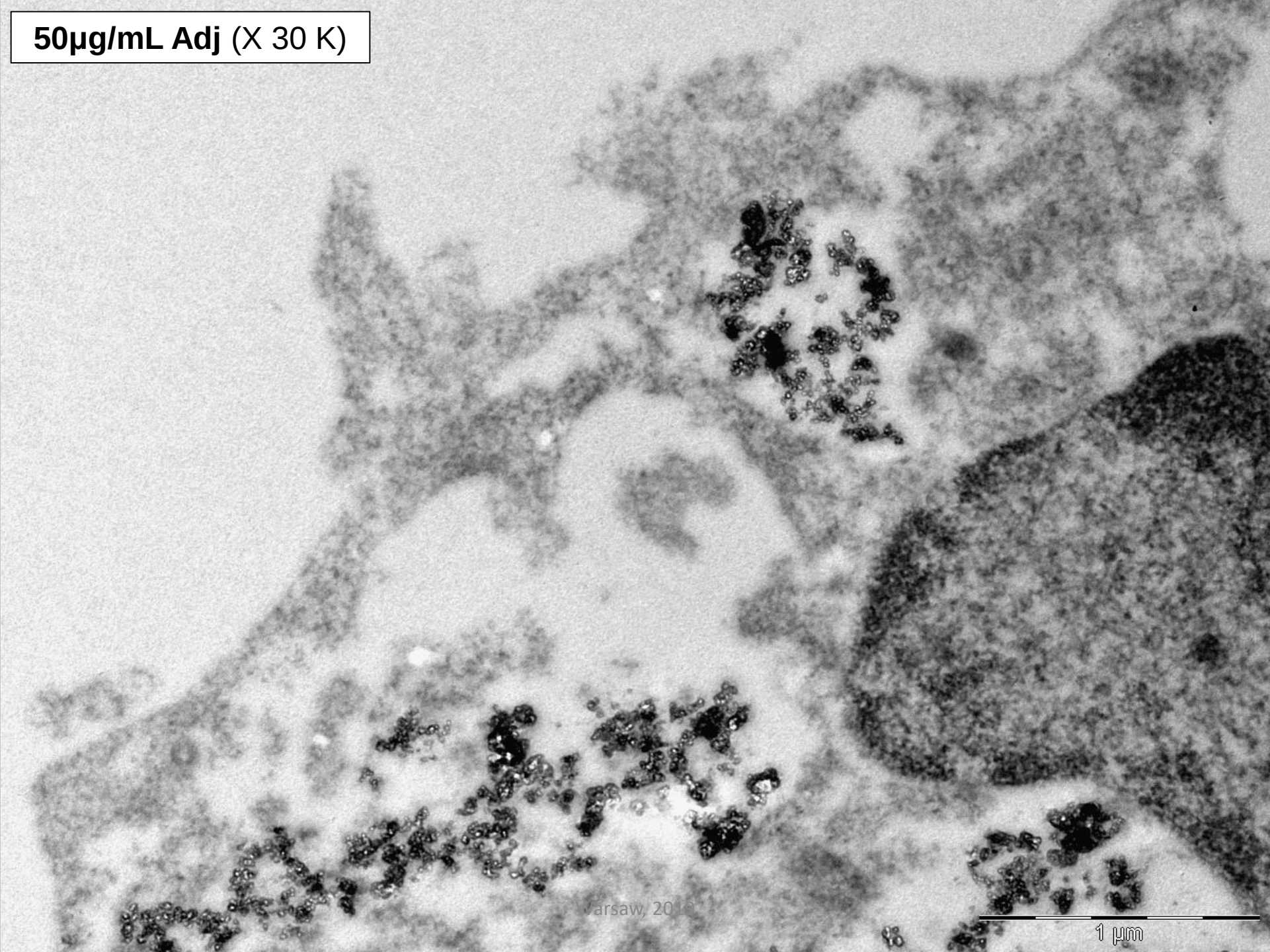
50 μ g/mL Adj (X 8 K)



Warsaw 2019

5 μ m

50µg/mL Adj (X 30 K)

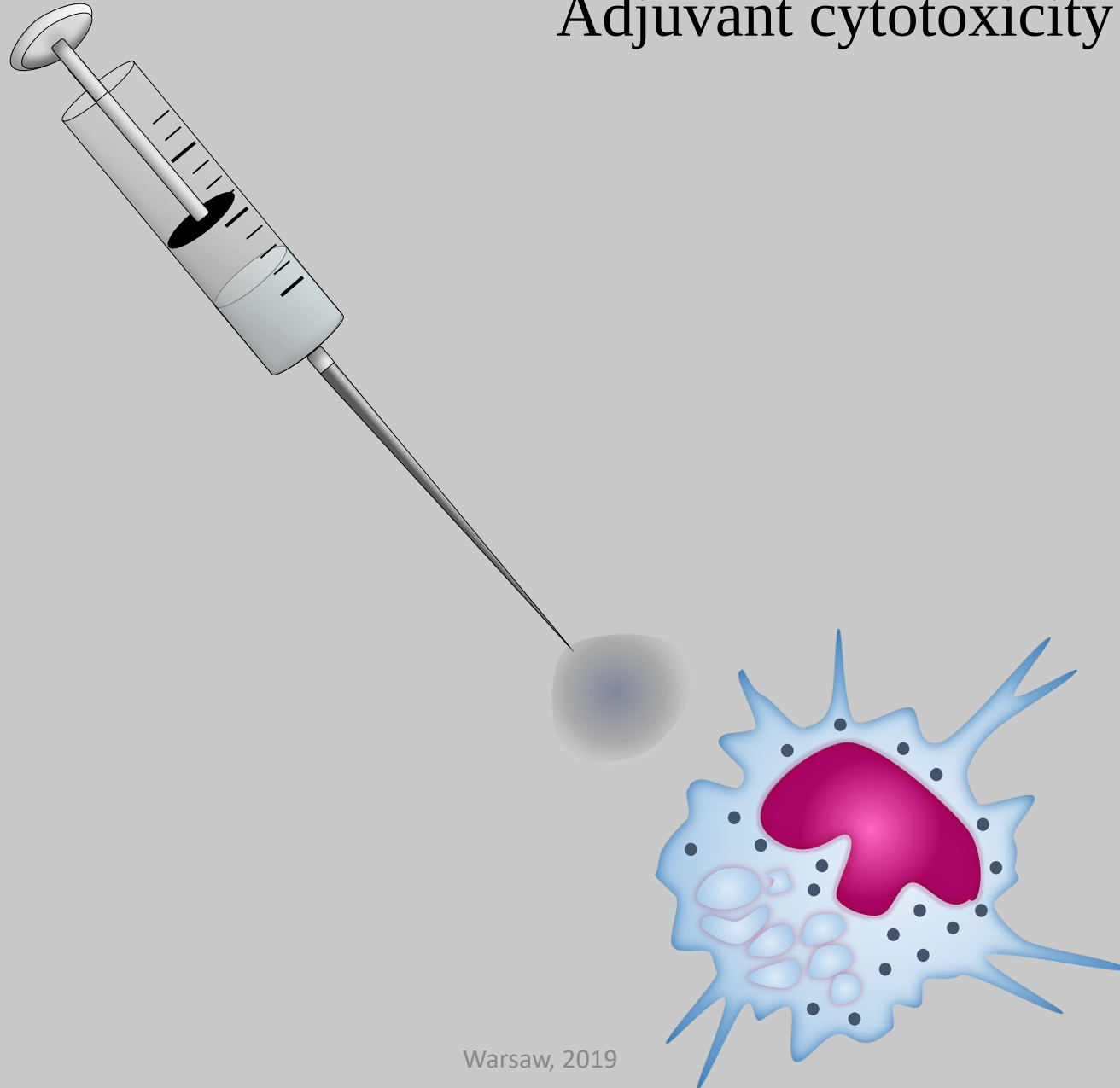


Warsaw, 2010

1 µm

So, What About the Toxicity of Aluminium Adjuvants?

Adjuvant cytotoxicity



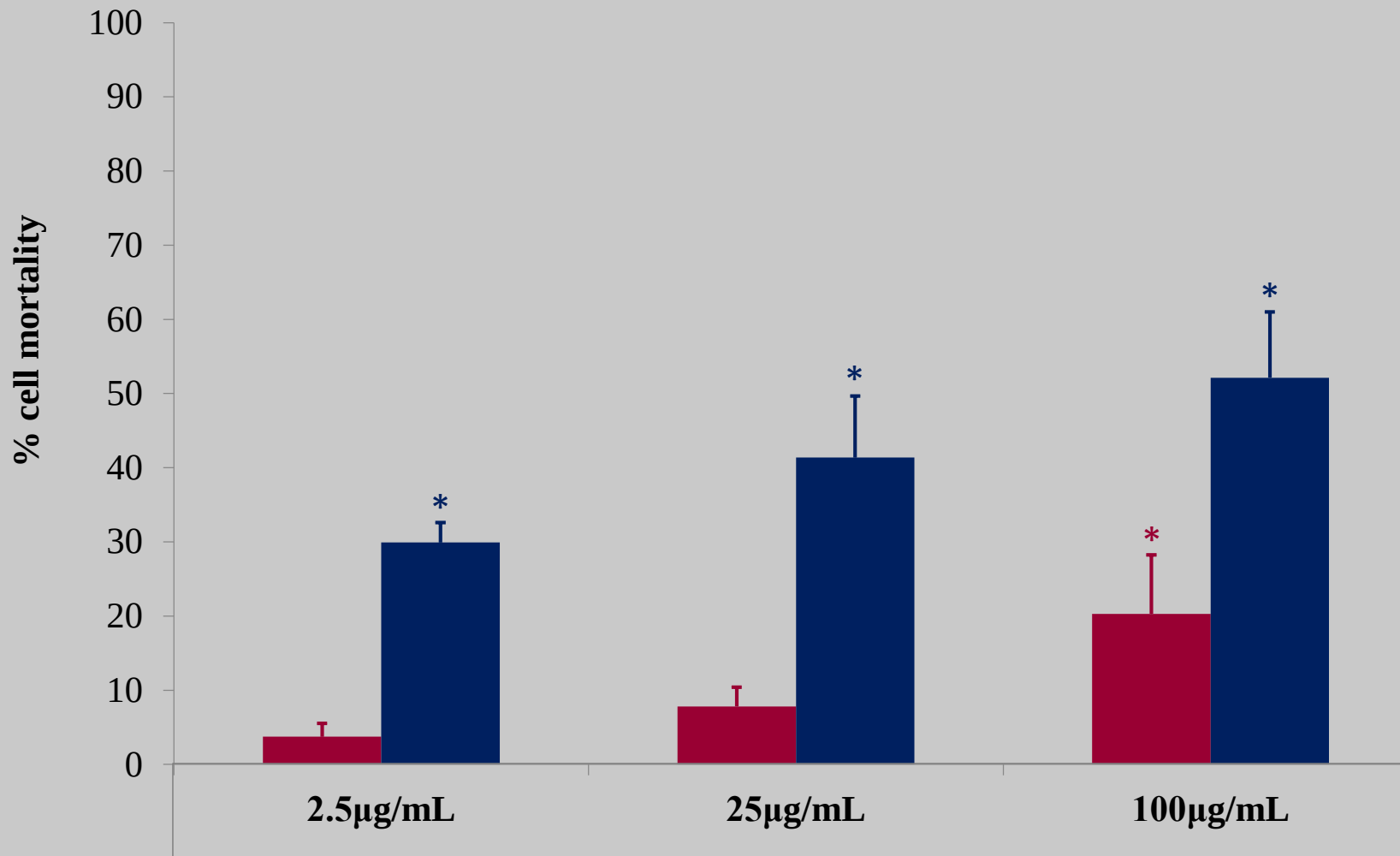


Fig 9: The % mortality experienced in THP-1 cell populations upon exposure to various concentrations of aluminium adjuvants relative to the control group, as elucidated using the live/dead cytotoxicity assay. Plum and blue bars represent Alhydrogel and Adju-Phos respectively. Error bars are representative of $\pm$ SD of 3 individual replicates and statistical significance is shown between treatments and respective control groups

Conclusions

For the two aluminium adjuvants used in clinically approved vaccines, intracellular particulates of Alhydrogel[®] and Adju-Phos[®], were observed localised in cell cytoplasm only.

Only co-culture with Adju-Phos[®] resulted in the release of extracellular genetic material.

Higher concentrations of aluminium adjuvants co-cultured with THP-1 cells were observed to result in their reduced cellular uptake (50 & 100µg/mL Adju-Phos[®]).

Conclusions cont.

The cytotoxicities of the two aluminium adjuvants used in clinically-approved vaccines are significantly different with Adju-Phos[®] expected to induce greater toxicity at the injection site.

The observed lower toxicity of Alhydrogel[®] despite its high intracellular burden may predispose this adjuvant to its translocation to (potentially) target tissues/organs away from the injection site.

Serious Adverse Events?

Khan *et al. BMC Medicine* 2013, **11**:99
<http://www.biomedcentral.com/1741-7015/11/99>



RESEARCH ARTICLE

Open Access

Slow CCL2-dependent translocation of biopersistent particles from muscle to brain

Zakir Khan^{1,2}, Christophe Combadière^{3,4,5}, François-Jérôme Authier^{1,2,6}, Valérie Itier^{1,2,11}, François Lux^{7,8}, Christopher Exley⁹, Meriem Mahrouf-Yorgov^{1,2,11}, Xavier Decrouy^{1,2}, Philippe Moretto¹⁰, Olivier Tillement^{7,8}, Romain K Gherardi^{1,2,6,12*†} and Josette Cadusseau^{1,2,11,12*†}

Serious Adverse Events?

Original Article

Granulomas Following Subcutaneous Injection With Aluminum Adjuvant-Containing Products in Sheep

Veterinary Pathology
1-11

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Javier Asín¹, Jéssica Molín¹, Marta Pérez², Pedro Pinczowski¹,
Marina Gimeno¹, Nuria Navascués³, Ana Muniesa¹,
Ignacio de Blas¹, Delia Lacasta¹, Antonio Fernández¹,
Lorena de Pablo⁴, Matthew Mold⁵ , Christopher Exley⁵,
Damián de Andrés⁴, Ramsés Reina⁴, and Lluís Luján¹ 

<http://journals.sagepub.com/doi/10.1177/0300985818809142>

New and important research on sheep and recently published in the journal Veterinary Pathology now provides direct evidence of the fate of aluminium adjuvants following sub-cutaneous injection. The research confirms the **accumulation of aluminium adjuvant in lymph glands**. However, it also shows that while lymph glands are a target destination for aluminium adjuvant for the whole vaccine this is not the case when only the aluminium adjuvant is injected. Essentially the handling of aluminium adjuvant is different between whole vaccine and that which is mainly used as the control or placebo in vaccine safety trials. These seminal data for sheep raise new and important questions about how vaccine safety trials are conducted in humans and offer further insight into the role of aluminium adjuvants in serious adverse events following vaccination.

Serious Adverse Events?

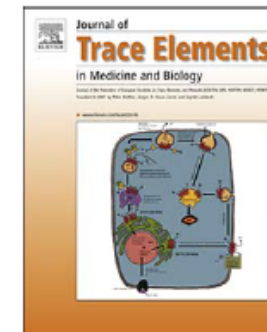
Journal of Trace Elements in Medicine and Biology 46 (2018) 76–82



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Journal of Trace Elements in Medicine and Biology

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Aluminium in brain tissue in autism

Matthew Mold^a, Dorcas Umar^b, Andrew King^c, Christopher Exley^{a,*}

^a The Birchall Centre, Lennard-Jones Laboratories, Keele University, Staffordshire, ST5 5BG, United Kingdom

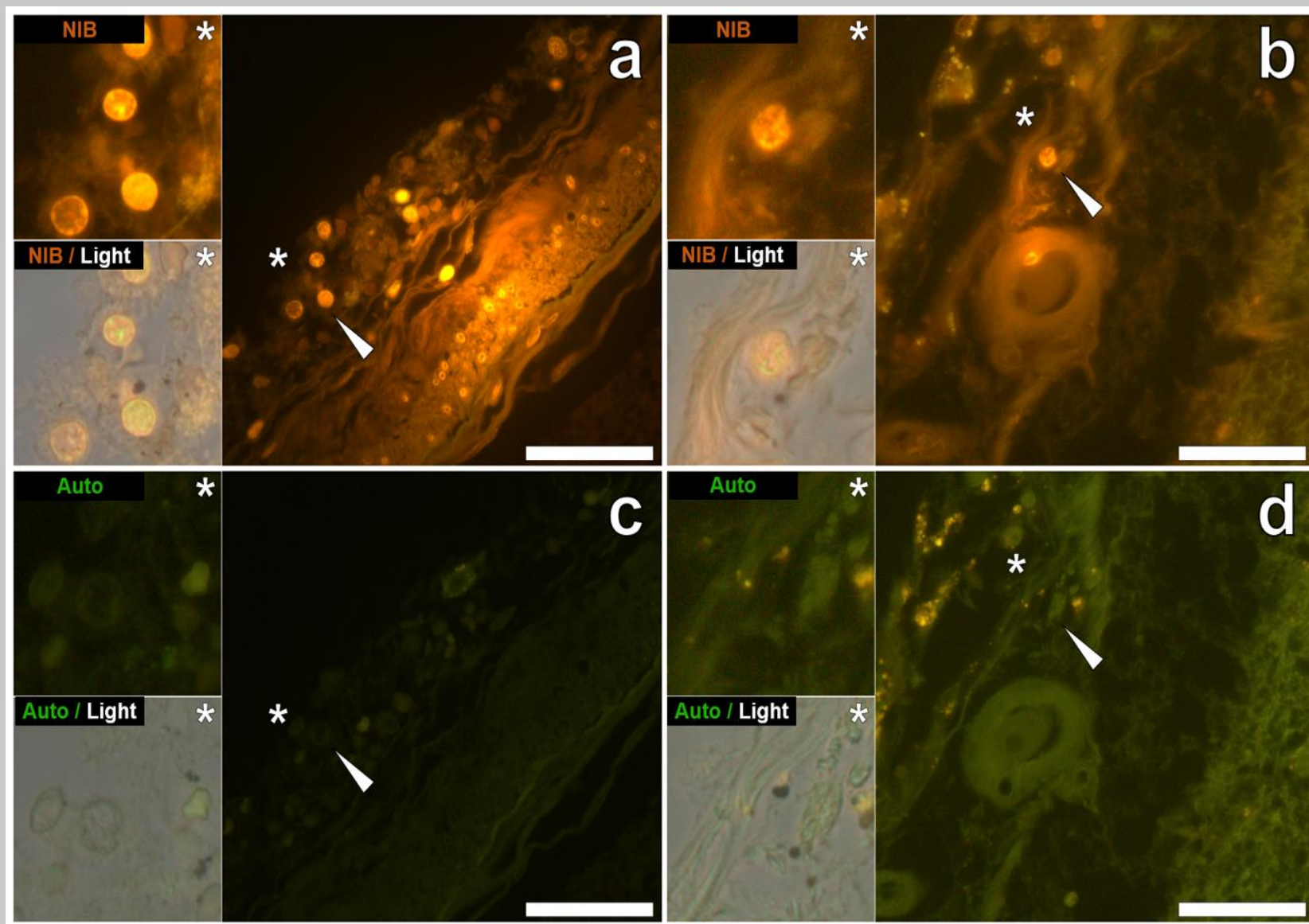
^b Life Sciences, Keele University, Staffordshire, ST5 5BG, United Kingdom

^c Department of Clinical Neuropathology, Kings College Hospital, London, SE5 9RS, United Kingdom



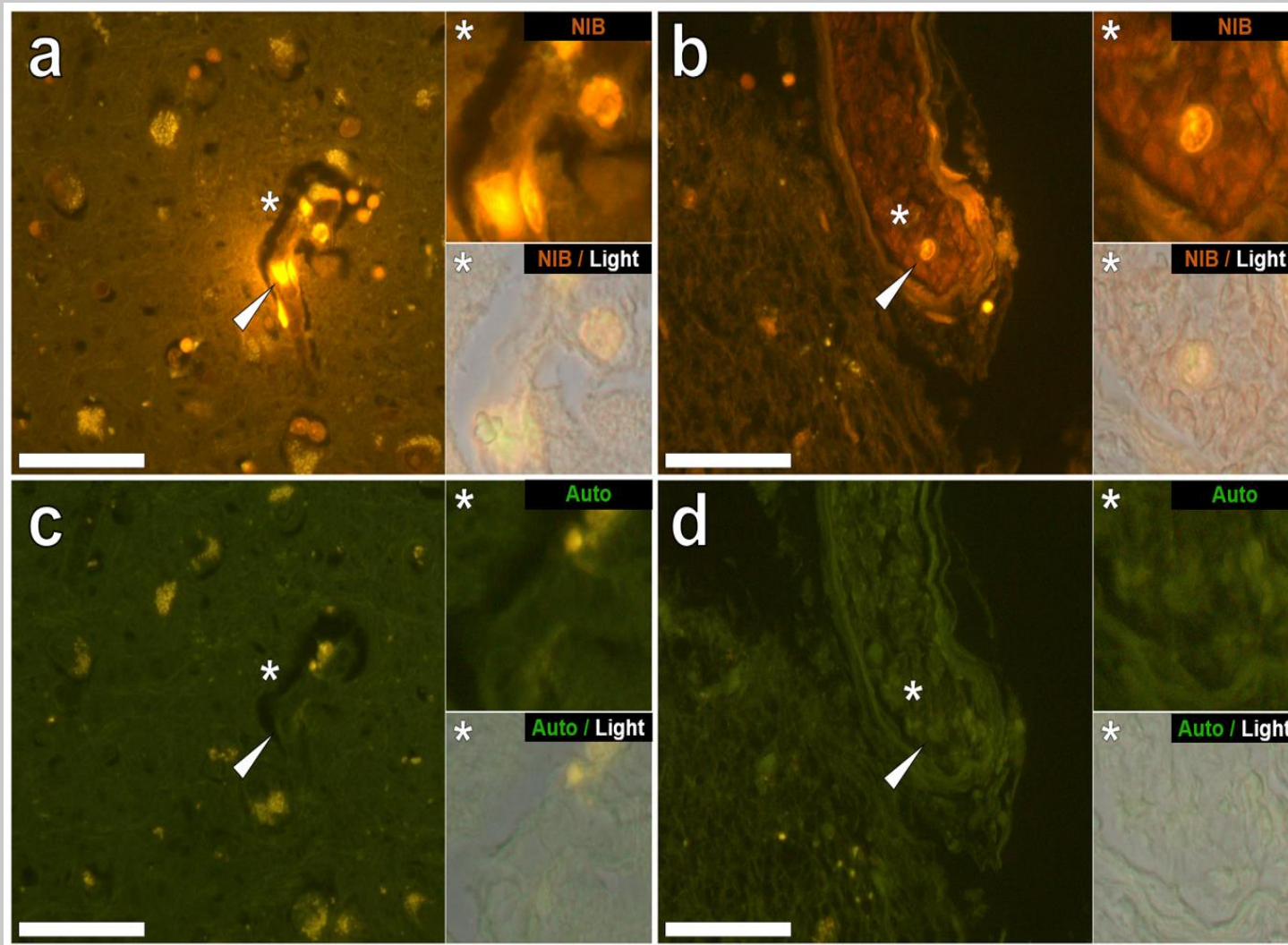
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Intrameningeal lumogallion-reactive aluminium identified in the hippocampus (**a & c**) and frontal lobe (**b & d**) of a 50-year-old male donor diagnosed with autism.

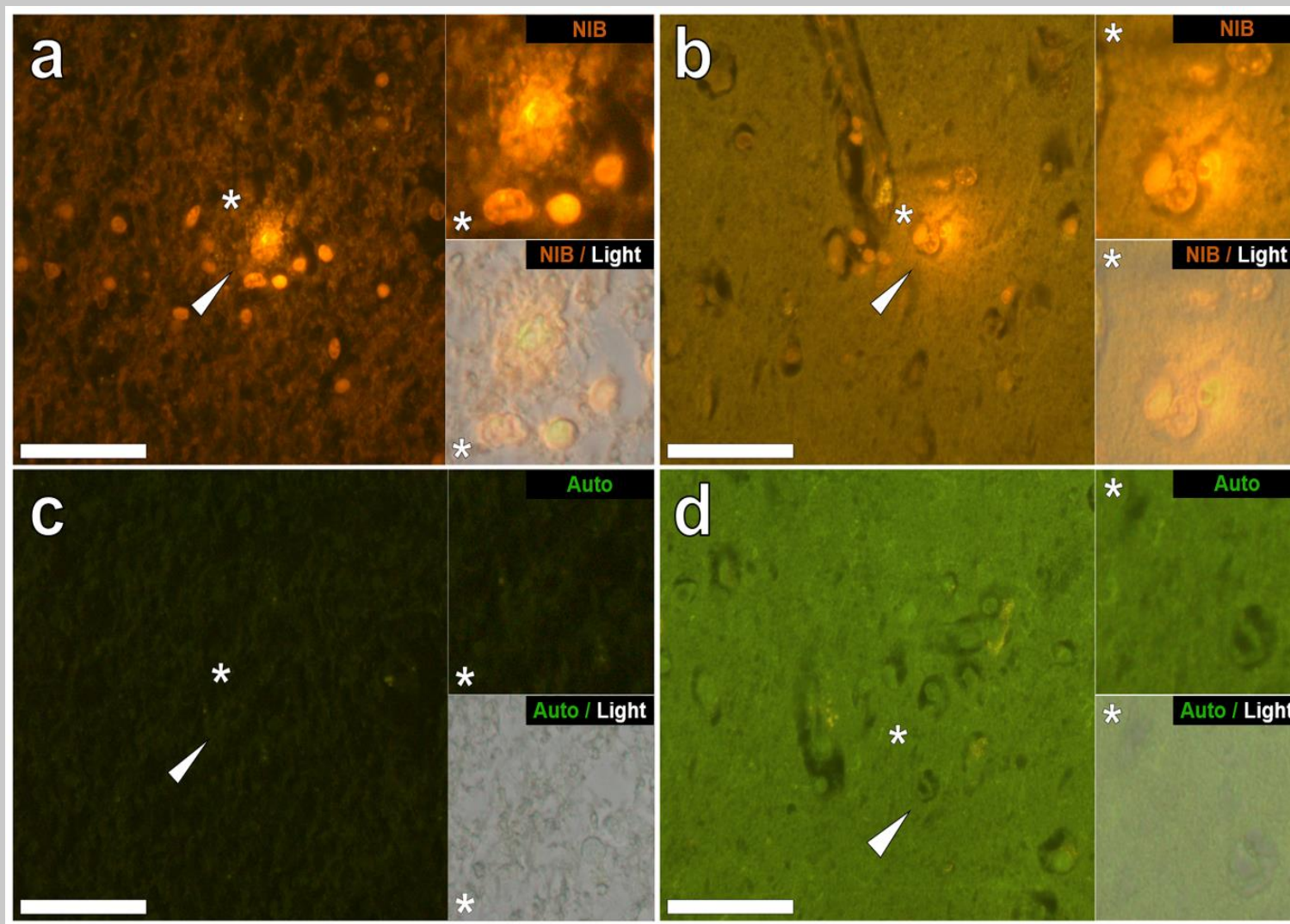
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Intravascular lumogallion-reactive aluminium identified in the hippocampus (**a – d**) of a 50-year-old male donor diagnosed with autism.

<https://www.sciencedirect.com/science/article/pii/S0946672X17308763>

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Lumogallion-reactive aluminium identified in the hippocampus (**a & c**) and parietal (**b & d**) lobe of a 15-year-old male donor diagnosed with autism.

<https://www.sciencedirect.com/science/article/pii/S0946672X17308763>

Warsaw, 2019

The Birchall Centre, Keele University and
Centro de Investigación Científica de Yucatán



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