BIOMIMETIC NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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DISSOLVING MICRONEEDLES TO ATTENUATE INFLAMMATION

Tomás Bauleth-Ramos[1,2]*, Mohammad-Ali Shahbazi[1,2], Christos Tapeinos[1], Raquel Bártolo[2], Maria Lobita[2], João Pedro Martins[1], Ermei Mäkilä[1,3], Ja... Salonen[3], Hélder A. Santos[1,2]

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[2]Department of Biomedical Engineering and W. J. Kolff Institute for Biomedical Engineering and Materials Science, University Medical Center Groningen/University of Groningen, 9713 AV Groningen, The Netherlands
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SANTOSLAB

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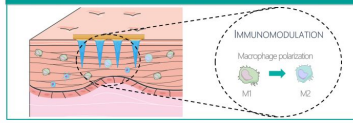
INTRODUCTION

Inflammation is a crucial healing process for many diseases, including cardiovascular diseases such as myocardial infarction (MI). However, excessive inflammatory re... detrimental effect, exacerbating the consequences and leading to a poor clinical outcome.[1] Therefore, there is the need to find ways to easily attenuate inflammation ... **Microneedles (MNs)** are remarkable drug delivery systems for immunomodulation due to their ability to deliver transdermally a broad array of molecules in a painless and m... Moreover, **nanoparticles (NPs)** due to their multiple advantages, such as targeting capacity, controlled delivery, and immunomodulatory properties, are also promis... immunotherapy.[4,5] Considering this, we hypothesized that by delivering D-Mannose modified NPs loaded with interleukin-4 (IL-4) through dissolving MNs, we are able to mod... from M1 (pro-inflammatory) to a M2 (anti-inflammatory) state, attenuating inflammation.

AIMS



1. To prepare fast dissolving MNs with intrinsic immunomodulatory properties and ability to transdermal deliver NPs and cytokines.
2. Physicochemical characterization of the developed NPs and MNs independently and combined.
3. *In vitro* evaluation of the cytocompatibility of the developed system, cellular uptake and macrophage polarization.

RESULTS

NANOPARTICLES

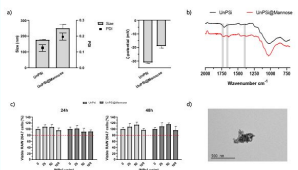


Figure 1: Characterization of the prepared NPs. (a) Average size, PdI and ζ-potential, (b) ATR-FTIR of the developed NPs. (c) Cytotoxicity. (d) TEM image of UnPSI NPs. Results are represented as mean ± s.d. (n ≥ 3).

Table 1: IL-4 Loading (AE: association efficiency; LD: loading degree

IL-4 loading	AE%	EE%
UnPSI	90.81±1.51	0.38±0.01

MICRONEEDLES

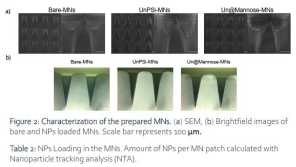


Figure 2: Characterization of the prepared MNs. (a) SEM, (b) Brightfield images of bare and NPs loaded MNs. Scale bar represents 100 µm.

Table 2: NPs Loading in the MNs. Amount of NPs per MN patch calculated with Nanoparticle tracking analysis (NTA).

NPs loading	250 µg/mL	500 µg/mL	1000 µg/mL
UnPSI	3.78±3.03	5.38±2.03	9.78±1.03
UnPSI@Mannose	2.62±2.03	5.18±1.03	7.18±2.03

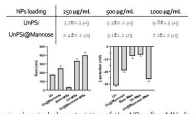


Figure 3: Physicochemical characteristics of the NPs after MN dissolution. DLS measurement.

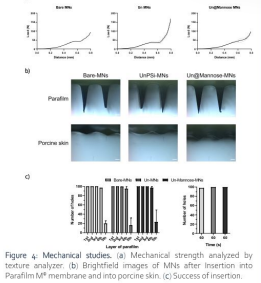


Figure 4: Mechanical studies. (a) Mechanical strength analyzed by texture analyzer. (b) Brightfield images of MNs after insertion into Parafilm M® membrane and into porcine skin. (c) Success of insertion.

CONCLUSIONS & FUTURE WORK

CONCLUSIONS

- We successfully produced NPs modified with mannose and MNs in which we were able to load NPs in a controlled manner.
- The produced system was able to be inserted into a depth *ca.* 500 µm, and completely dissolved within the skin.
- The system was also shown to be biocompatible in the tested conditions.

FUTURE WORK

In vitro and *in vivo studies* to assess the release profile and immunomodulatory properties of the developed system.

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4. T. Bauleth-Ramos, et al. Adv. Funct. Mater. 2021, 1703393
5. MA. Shahbazi, et al. Biomaterials. 2014, 9227-9235

ACKNOWLEDGEMENTS

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Poster #389

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TRAGACANTH-BASED INJECTABLE HYDROGEL LOADED WITH SPERMINE-ACETALATED DEXTRAN NANOPARTICLES FOR TARGETED MIRNA DELIVERY

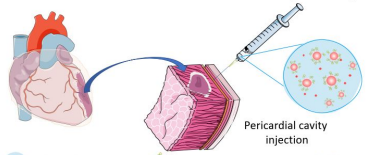
Raquel Bártolo[1], Tomás Bauleth-Ramos[1,2], Giulia Torrieri[2], Shiqi Wang[2], Karla Gomez[3], Mohammad-Ali Shahbazi[1], Peter v. d. Meer[3], Hélder A. Santos[1,2]

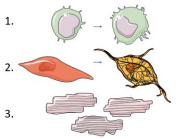
[1]Department of Biomedical Engineering and W.J. Kolff Institute for Biomedical Engineering and Materials Science, University Medical Center Groningen/University of Groningen; [2]Drug Research program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland; [3]Department of Cardiology, University Medical Center Groningen, Groningen, the Netherlands

1. Introduction and Objectives

Myocardial infarction (MI) is a life-threatening condition characterized by irreversible cell death [1]. During the past decades, several therapeutic strategies have held the promise of restoring the full functionality of a damaged heart. However, MI approved therapies, to date, only ameliorate the state of care of these patients [2]. Therefore, new therapeutic approaches need to be explored.

Herein, we have developed a nanoparticle (NP) composite injectable hydrogel for MI treatment. This system consists of a tragacanth-based injectable hydrogel (**Scheme 1**), containing cytokines and spermine-acetalated dextran-based (AcDXSp) [3] functional NPs loaded with miRNA. We hypothesized that this system can modulate the immunoenvironment present in the heart after MI and induce cardiac fibroblasts differentiation to myofibroblasts, while inducing a cardioprotective effect.





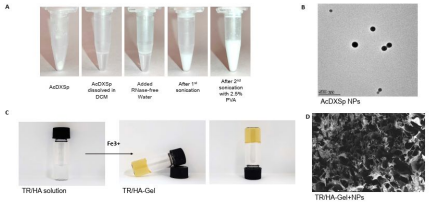
1. Macrophages reprogramming (Mo-M2)
2. Fibroblasts differentiation to myofibroblasts
3. Cardiomyocytes: ↓ CaMKII

TR/HA-Gel · IL-4 · miR@AcDxSp-PEG-ANP NPs · M0 and M1 macrophages · Fibroblast · Myofibroblast · Cardiomyocyte

Scheme 1. Mechanism of action of the injectable composite. AcDXSp-PEG-ANP NPs are released from the gel and taken up by cardiac cells, inducing fibroblasts differentiantion to myofibroblasts and cardioprotective effect, while the IL-4 induces macrophage polarization toward anti-inflamatory phenotype.

2. Methods and Results

- NPs fabrication by double emulsion technique
- NPs PEGylation and further conjugation with ANP through EDC/NHS reaction
- Metal coordination: Gel preparation by vigorous vortexing



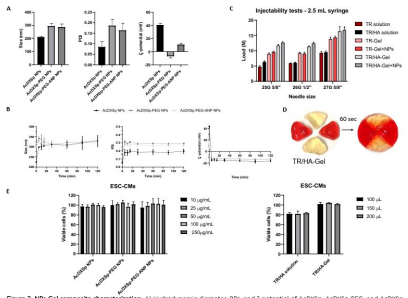


Figure 1. NPs and Gel fabrication. A) AcDXSp NPs fabrication by double emulsion; B) TEM images of the structural morphology of AcDXSp; C) Gel formation images; D) TEM image of freeze-dried Gel.

Figure 2. NPs-Gel composite characterization. A) Hydrodynamic diameter, PDI, and ζ-potential of AcDXSp, AcDXSp-PEG, and AcDXSp-PEG-ANP NPs; B) Evaluation of the impact of cell medium on the colloidal stability of AcDXSp, AcDXSp-PEG, and AcDXSp-PEG-ANP NPs upon incubation for 120 min at 37 °C; C) Gel injectability tests with different needle sizes; D) Self-healing properties of the Gel fabricated with different dyes; E) Cellular biocompatibility of embryonic STEM cell derived cardiomyocytes measured by Almar Blue upon 24h incubation with NPs and Gel . The results are represented as the mean ± s.d. (n ≥ 4).

3. Conclusions and Future work

- We successfully produced and modified NPs with PEG and ANP.
- The produced NPs were loaded into the injectable Gel for NPs' control release.
- The combined system showed biocompatibility in the tested conditions.
- In the future, studies for immunomodulation and cell differentiation *in vitro* and *in vivo* will be conducted.

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Poster #355

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CHALLENGES IN CANCER AND CVDS TREATMENTS

LIMITATIONS OF CURRENT TREATMENT

Surgery:

1. Cannot treat most of metastatic cancer, pancreas, as well as some brain tumors;
2. Associated with potential risks.

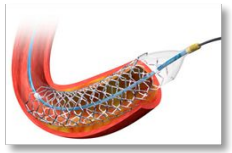
Radiotherapy:

1. Limited curative effect;
2. Body damage and development of other cancers

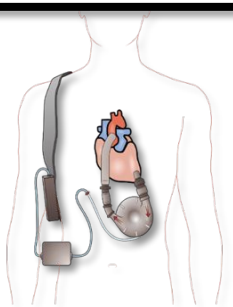
Chemotherapy:

1. Increase in the development cost;
2. Mortality rates increase continuously

Restore perfusion
Decrease blood presure, cardiac overload
Aid blood systemic distribution
Prevent arrhithmias



Stent Angioplasty

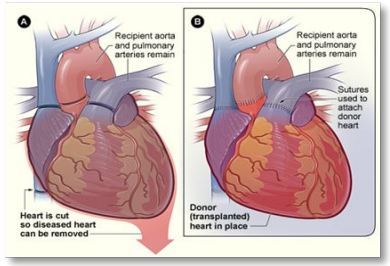


Ventricular assist devices

Pharmacological therapy

- ACE inhibitors
- Ang-II receptor blockers
- β-blockers
- Mineralocorticoid-receptor antagonists
- Diuretics
- Digoxin
- Thrombolytic therapy

Pacemaker



Heart transplant

New treatment solutions are urgently needed!

PNAS
Proceedings of the National Academy of Sciences of the United States of America
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NEW RESEARCH IN Physical Sciences | Social Sciences

Photothermal-responsive nanosized hybrid polymersome as versatile therapeutics codelivery nanovehicle for effective tumor suppression

Hongbo Zhang, Wenguo Cui, Xiangmeng Qu, Huayin Wu, Liangliang Qu, Xu Zhang, Ermei Mäkilä, Jarno Salonen, Yueqi Zhu, Zhou Yang, Dong Chen, Hélder A. Santos, Mingtan Hai, and David A. Weitz

PNAS April 16, 2019 116 (16) 7744-7749; first published March 29, 2019 https://doi.org/10.1073/pnas.1817251116
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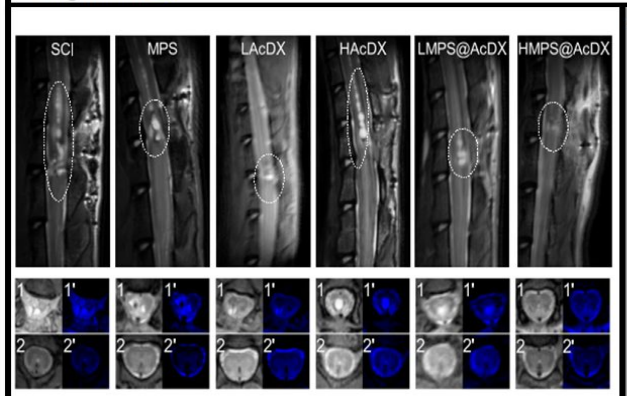
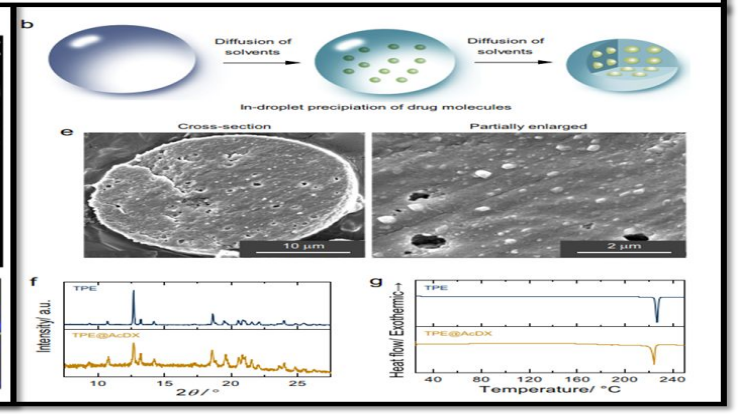
ARTICLE
https://doi.org/10.1038/s41467-022-28787-7 OPEN

High drug-loaded microspheres enabled by controlled in-droplet precipitation promote functional recovery after spinal cord injury

Wei Li[1,7], Jian Chen[2,7], Shujie Zhao[2], Tianhe Huang[3], Huiyan Ying[1], Claudia Trujillo[1], Giuseppina Molinaro[1], Zheng Zhou[2], Tao Jiang[2], Wei Liu[2], Linwei Li[2], Yuancheng Bai[3], Peng Quan[1,4], Yaping Ding[1], Jouni Hirvonen[1], Guoyong Yin[2✉], Hélder A. Santos[1,5,6✉], Jin Fan[2✉] & Dongfei Liu[3,1,5✉]

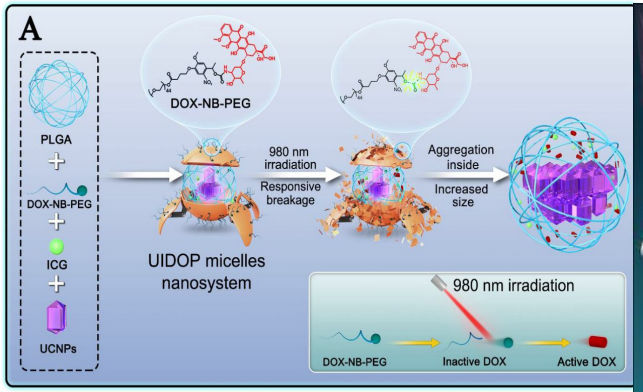


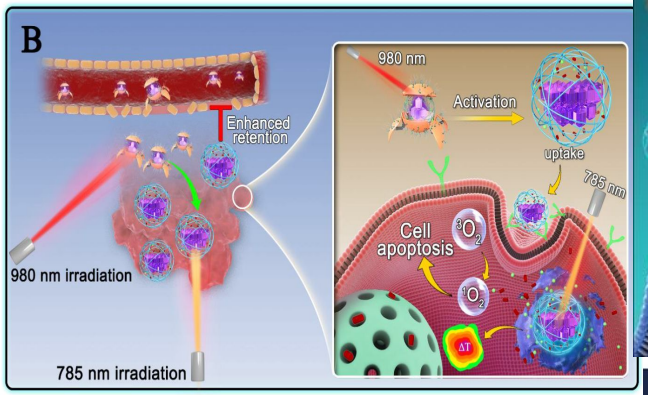


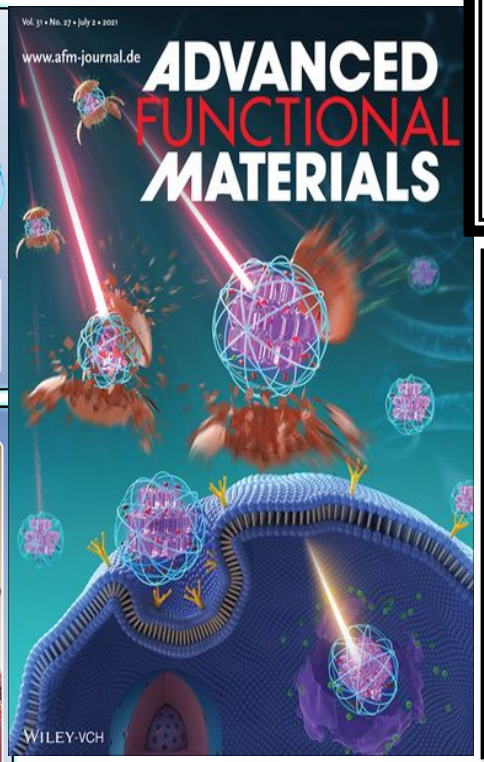
Microfluidics technology

Zhang, Santos *et al.*, *Cell Rep.* **2021**, *35*, 109131; *Adv. Funct. Mater.* **2021**, *31*, 2101262

LIGHT-CONTROLLED NANOSYSTEM WITH SIZE FLEXIBILITY IMPROVES TARGETED RETENTION FOR TUMOR SUPPRESSION







Cell Reports

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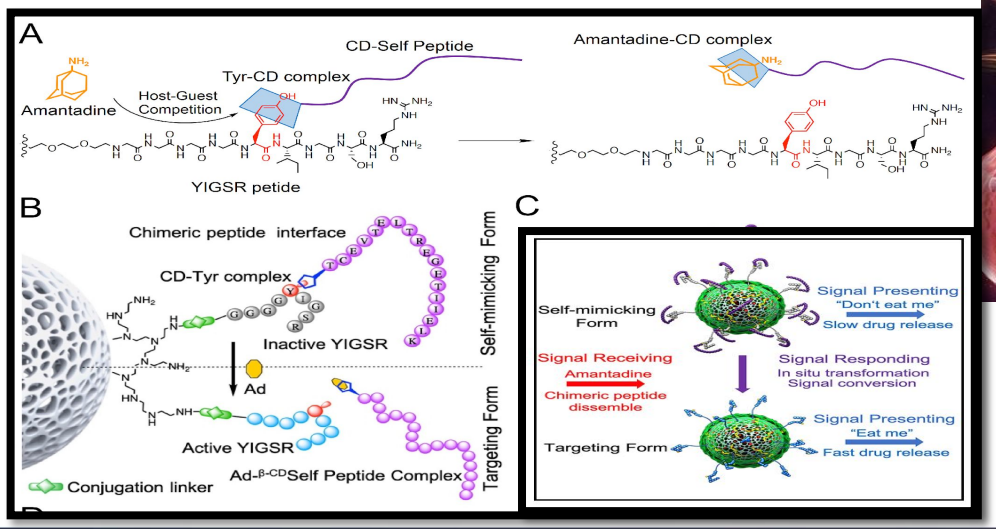
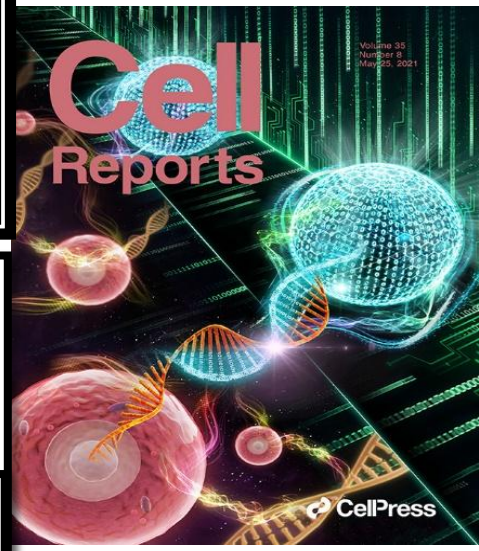
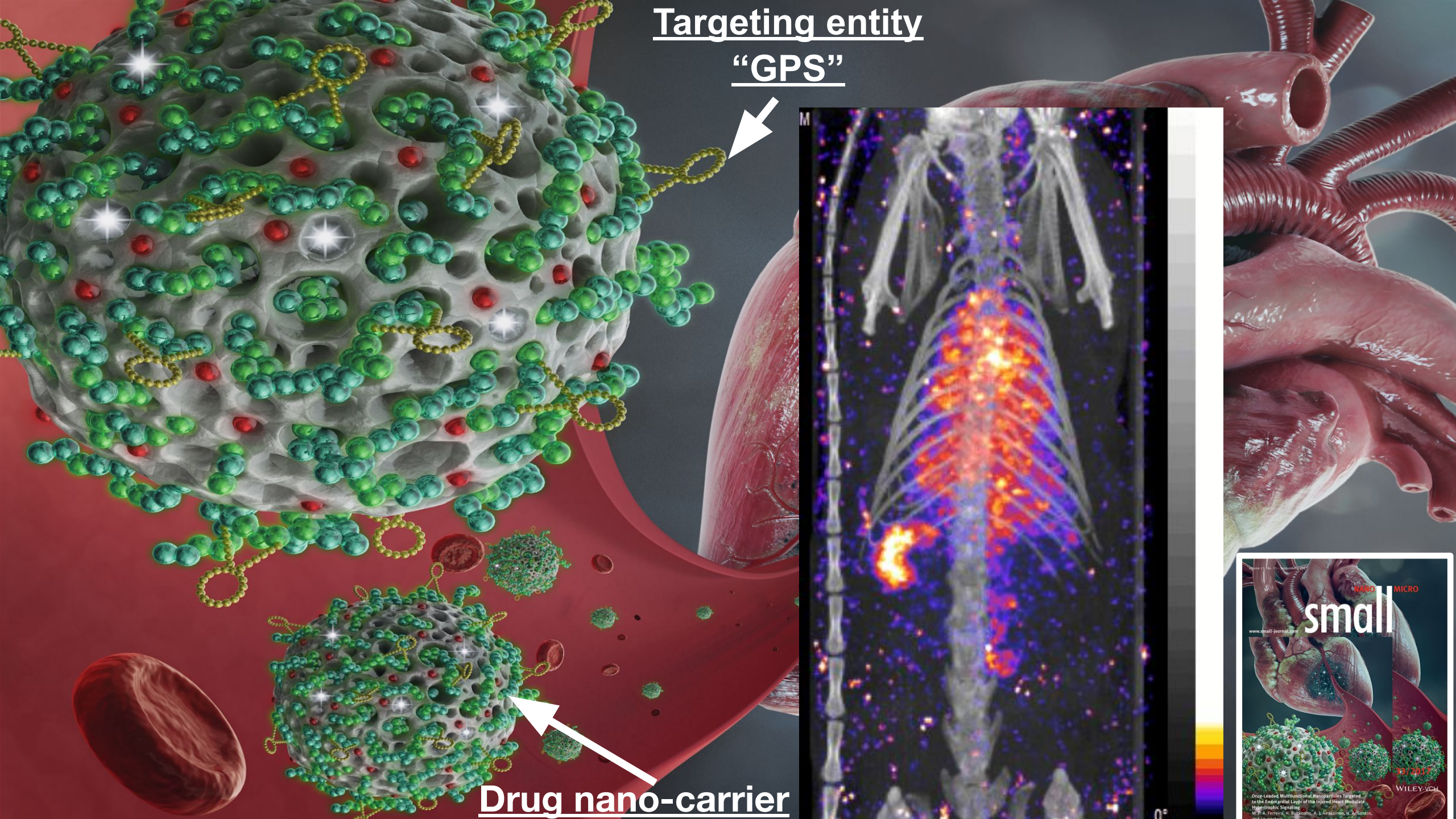
Report

Multistage signal-interactive nanoparticles improve tumor targeting through efficient nanoparticle-cell communications

Feng Zhang,[1,2,10] Yiran Zhang,[2,10] Li Kong,[3,4,10] Huanhuan Luo,[2] Yuezhou Zhang,[5] Ermei Mäkilä,[6] Jarno Salonen,[6] Jouni T. Hirvonen,[1] Yueqi Zhu,[7] Yingsheng Cheng,[7] Lianfu Deng,[2] Hongbo Zhang,[2,8,*] Alexander Kros,[4] Wenguo Cui,[2,*] and Hélder A. Santos[1,9,11,*]






Targeting entity
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Drug nano-carrier
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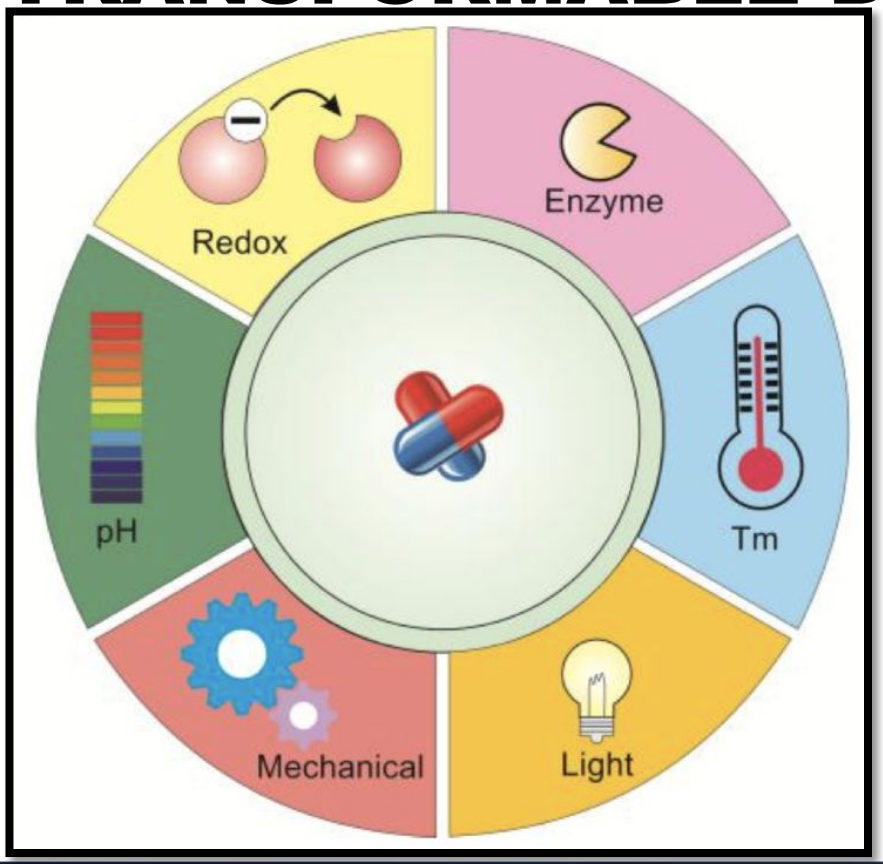
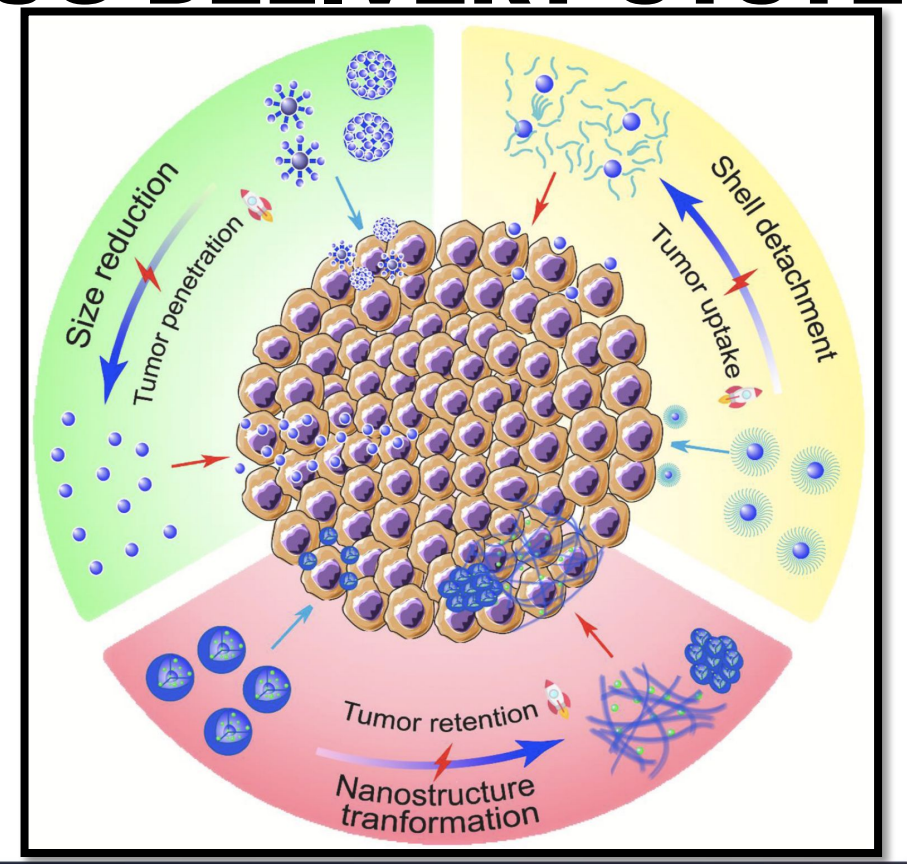
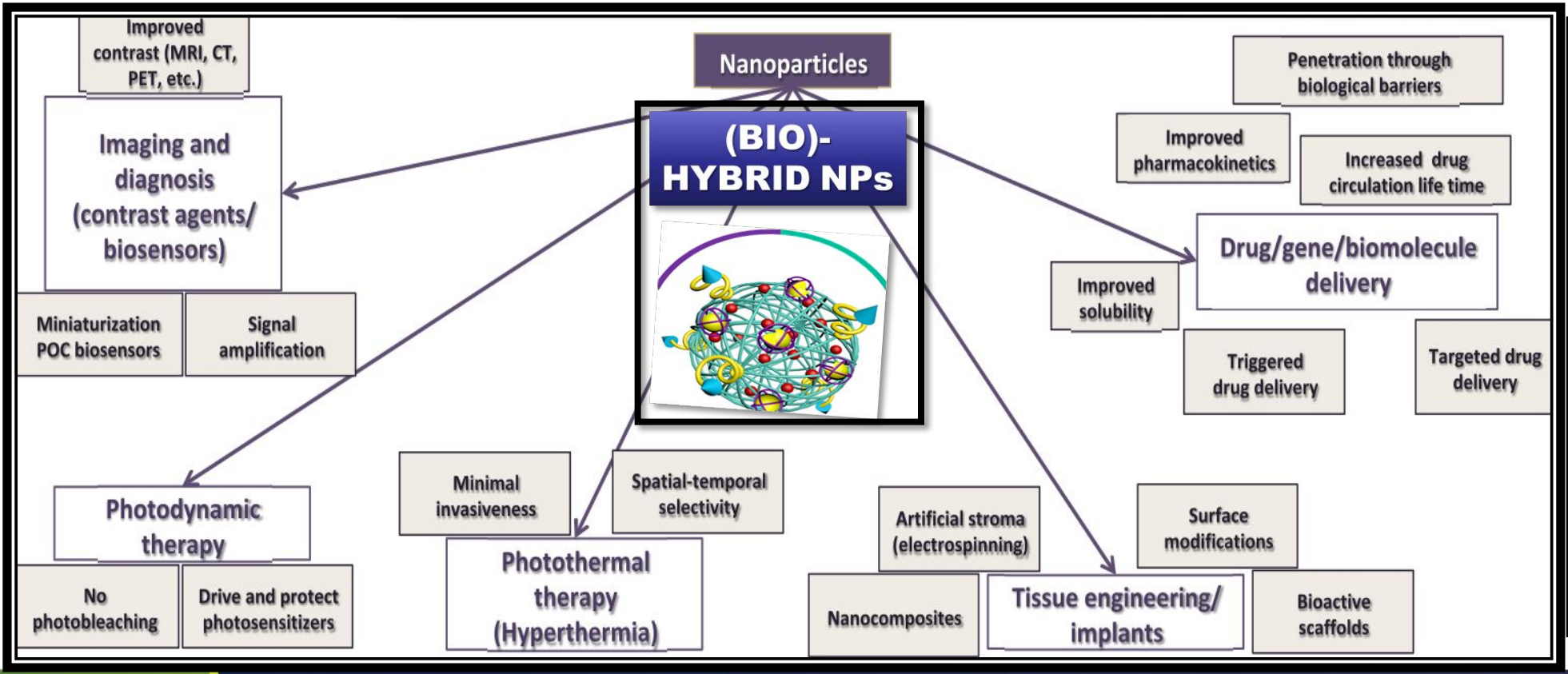
TRANSFORMABLE DRUG DELIVERY SYSTEMS



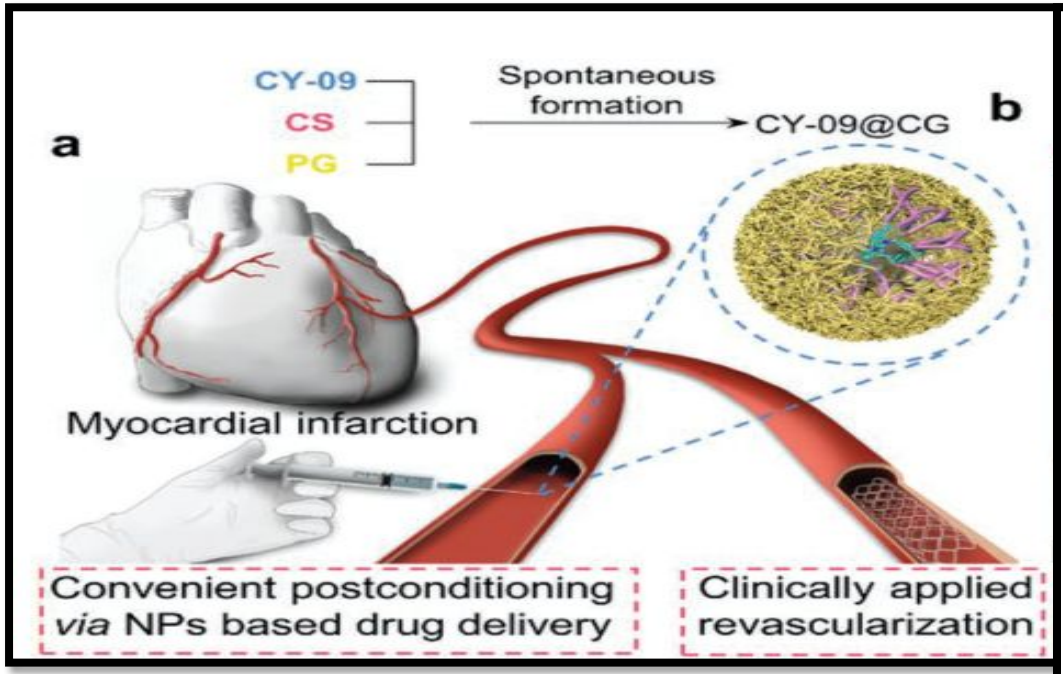


NANOPARTICLES IN MEDICINE

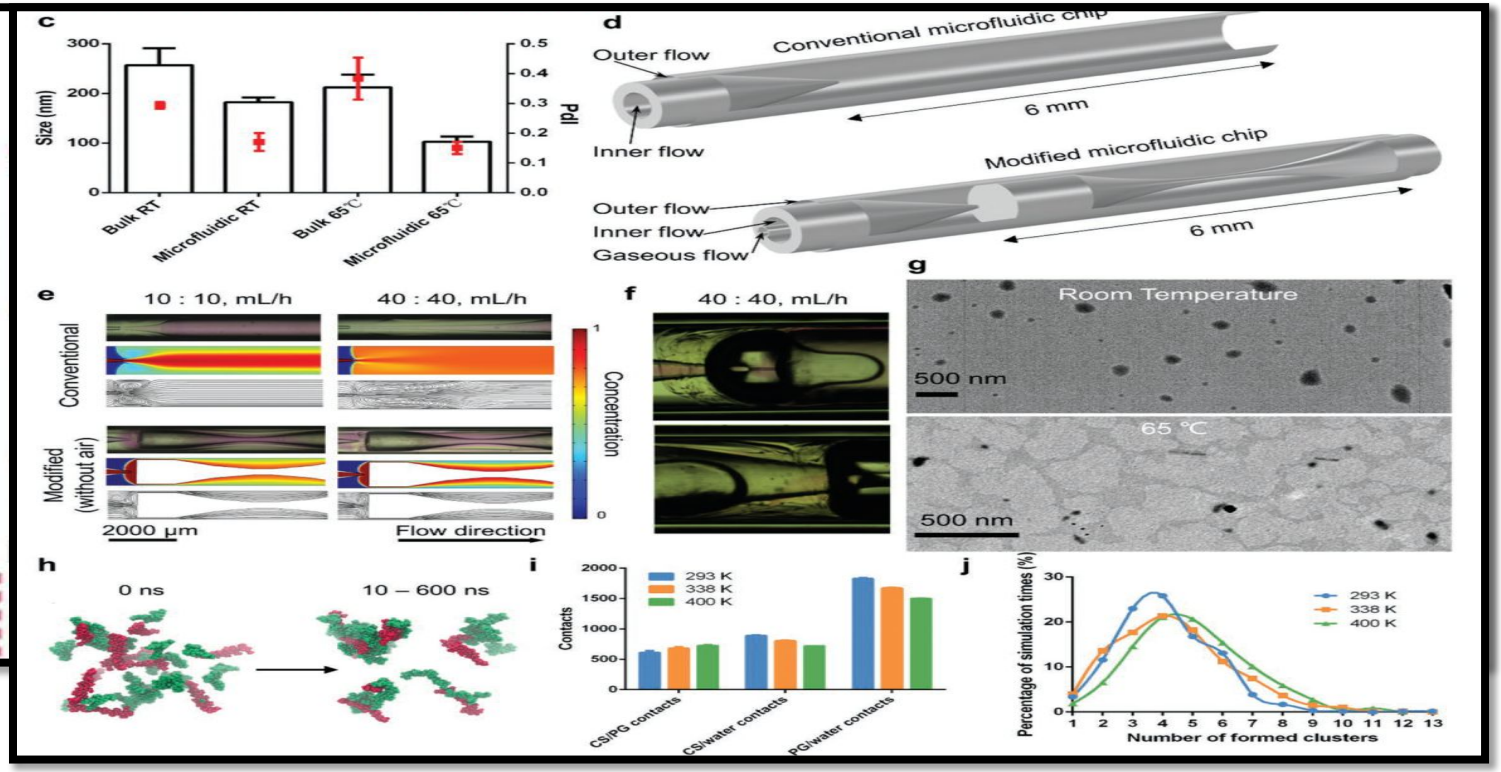


Liu, Santos *et al.*, Adv. Funct. Mater., **2022**, *10.1002/adfm.202204666*

PROMOTING CARDIAC REPAIR VIA NANOPARTICLES WITH EXCLUSIVE TARGETING CAPABILITY TOWARD MYOCARDIAL REPERFUSION INJURY USING MICROFLUIDICS

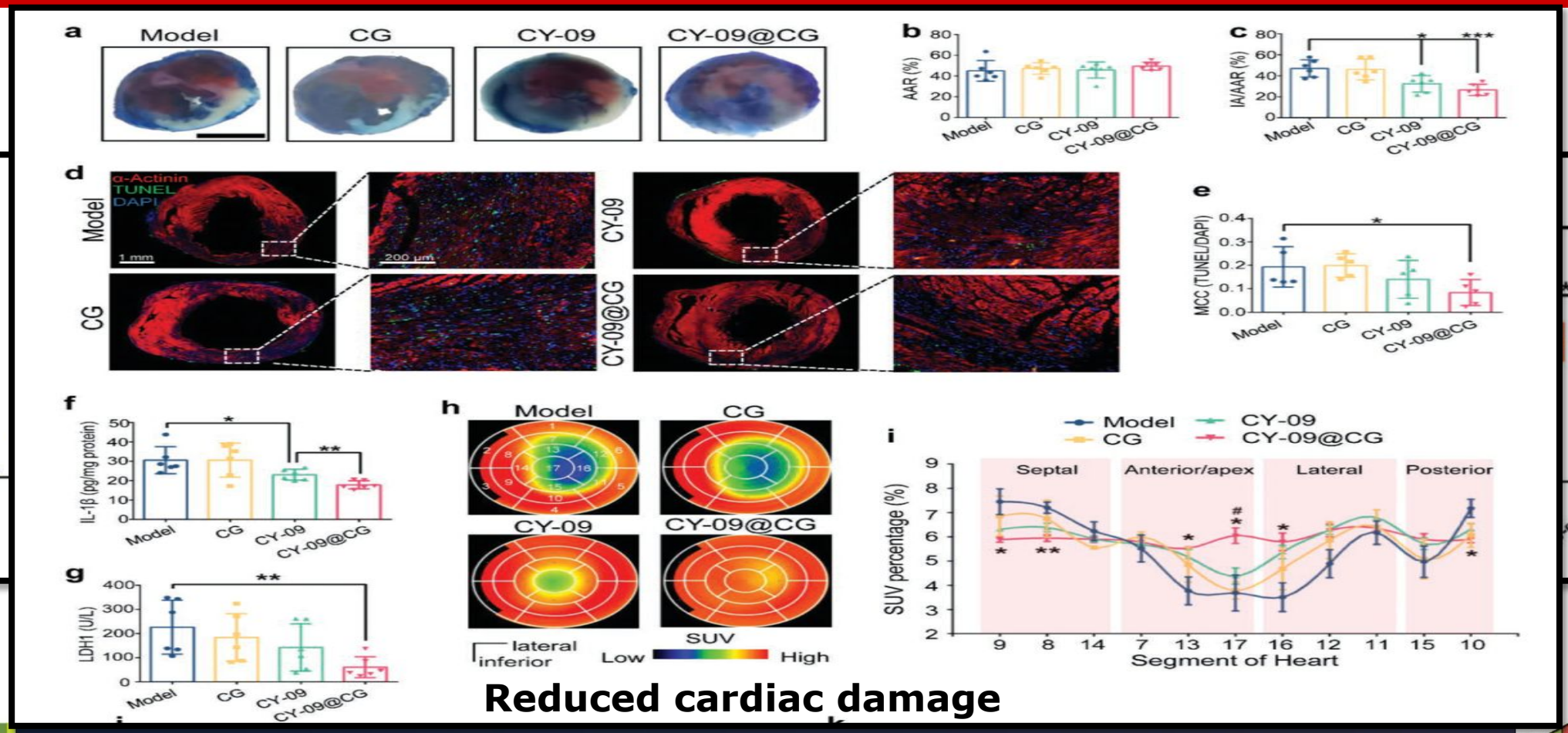


β-glucan NPs loaded with NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome inhibitor (CY-09)



Thermal resistant microfluidics assisted NPs production

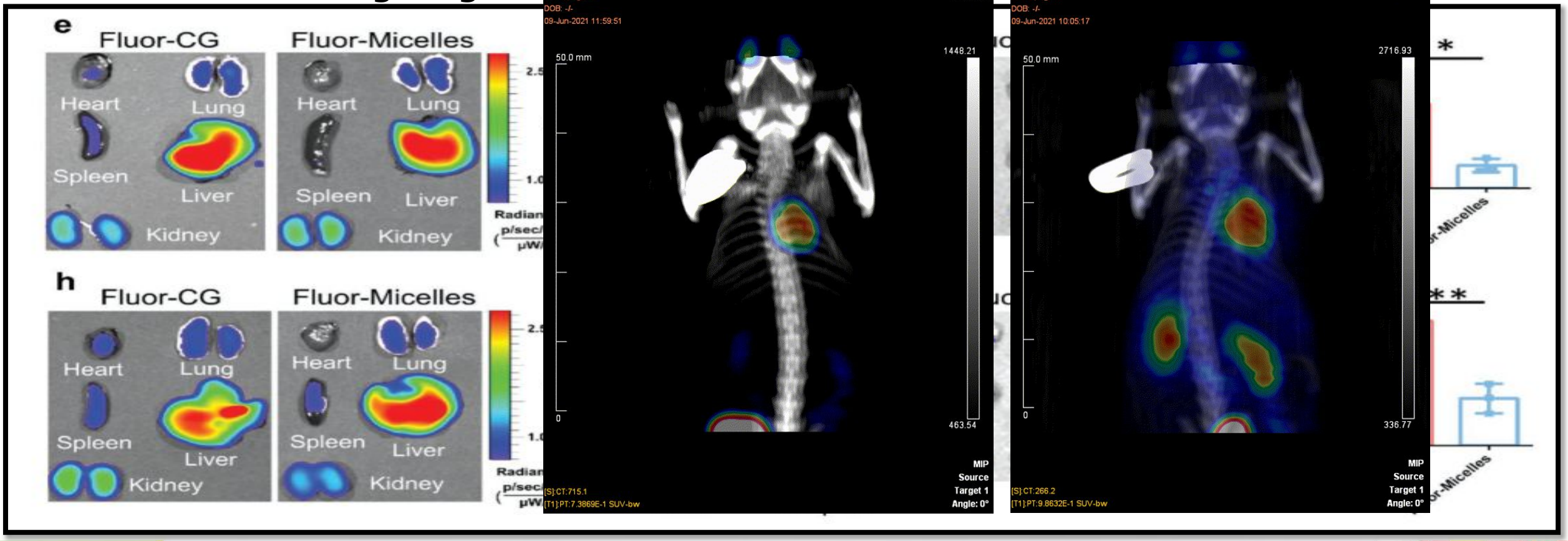
Liu, Santos *et al.*, Adv. Funct. Mater., **2022**, *10.1002/adfm.202204666*



Reduced cardiac damage

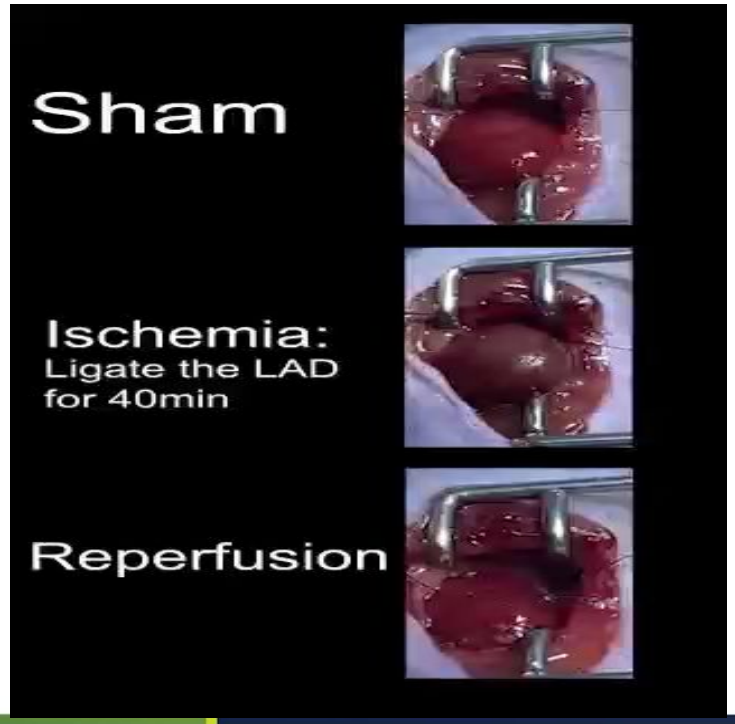
Liu, Santos *et al.*, Adv. Funct. Mater., **2022**, *10.1002/adfm.202204666*

Enhanced cardiac targeting

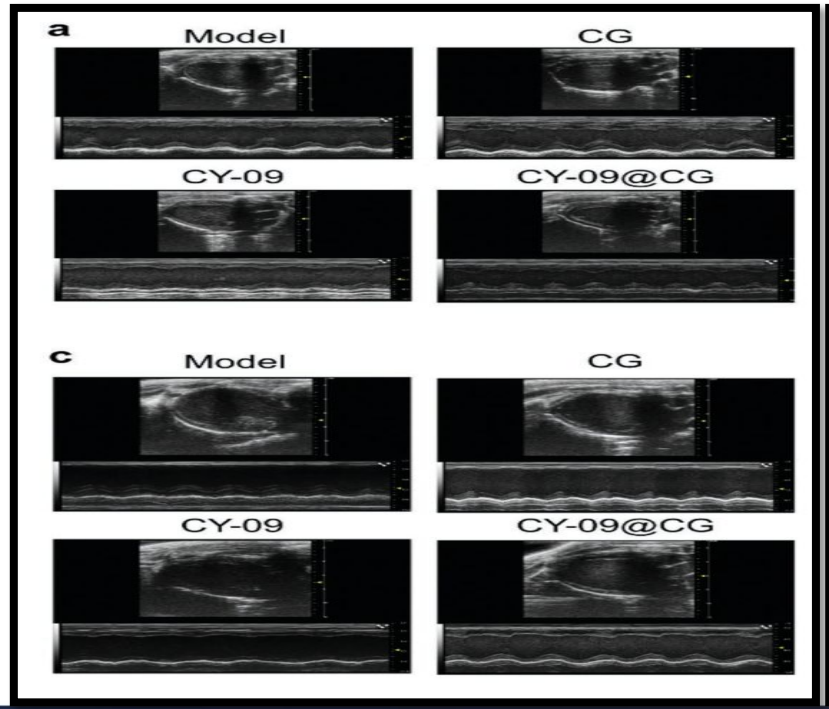


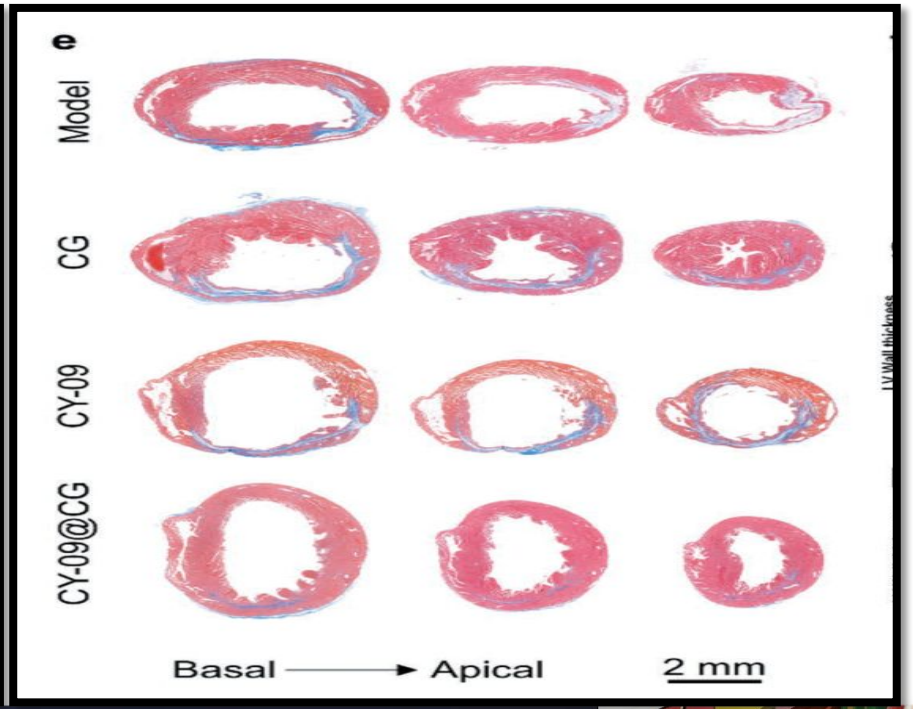
Liu, Santos *et al.*, Adv. Funct. Mater., **2022**, *10.1002/adfm.202204666*

CY-09@CG showed better efficiency in ameliorating long-term prognosis after I/R cardiac injury



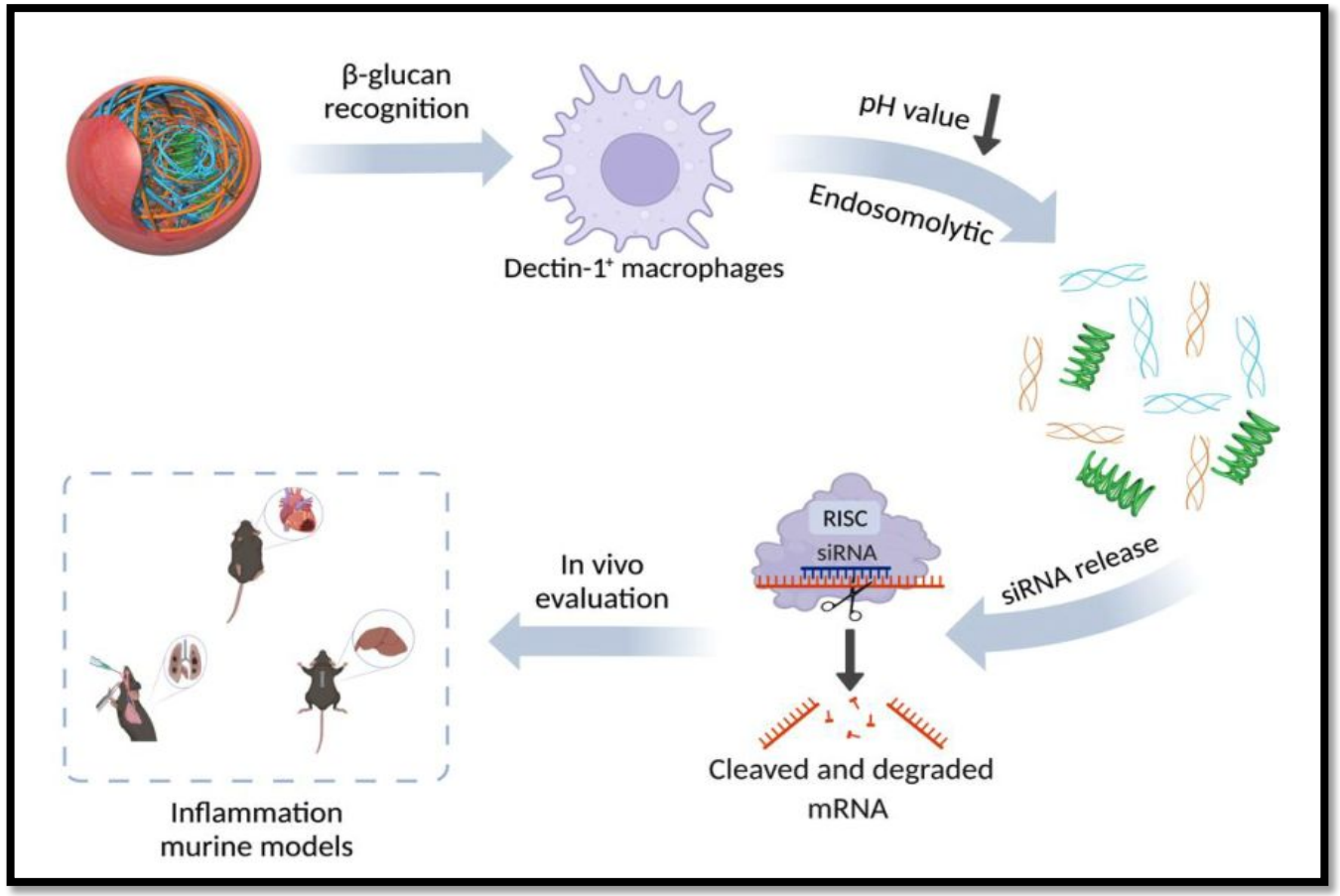
Enhanced cardiac function



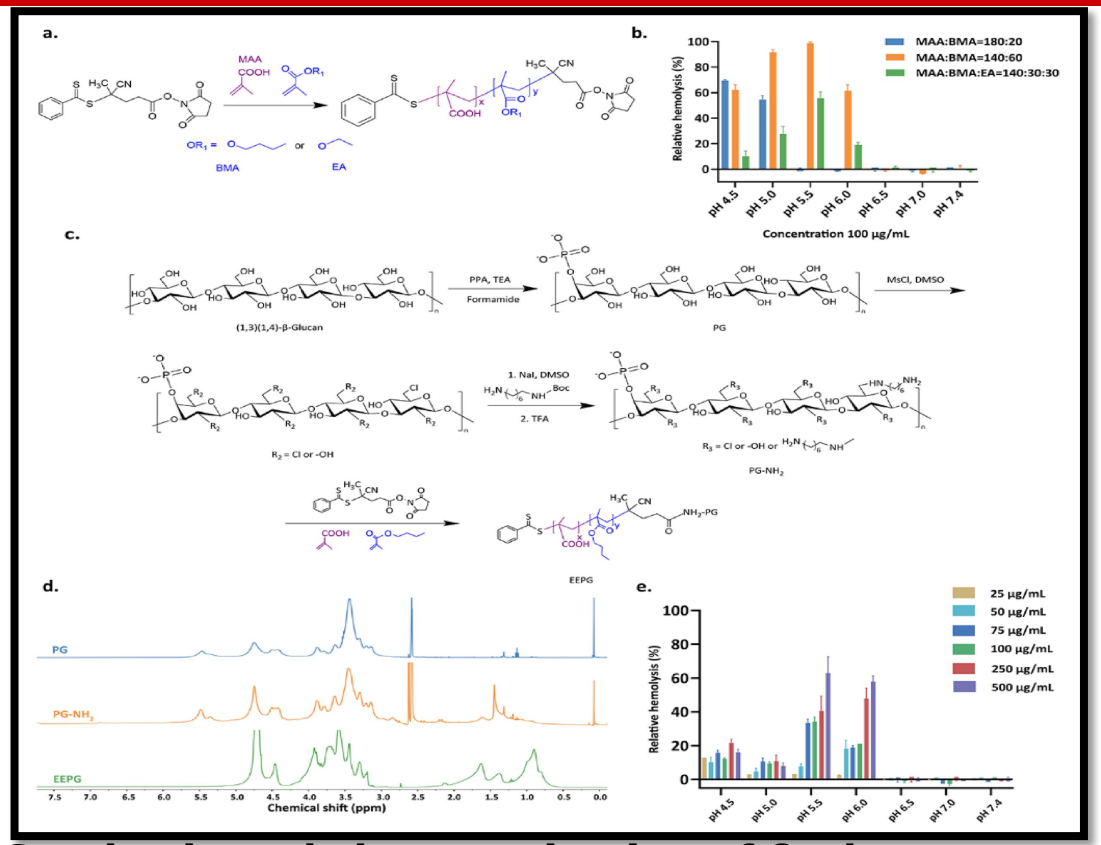


Gao, Santos *et al.*, *under review*

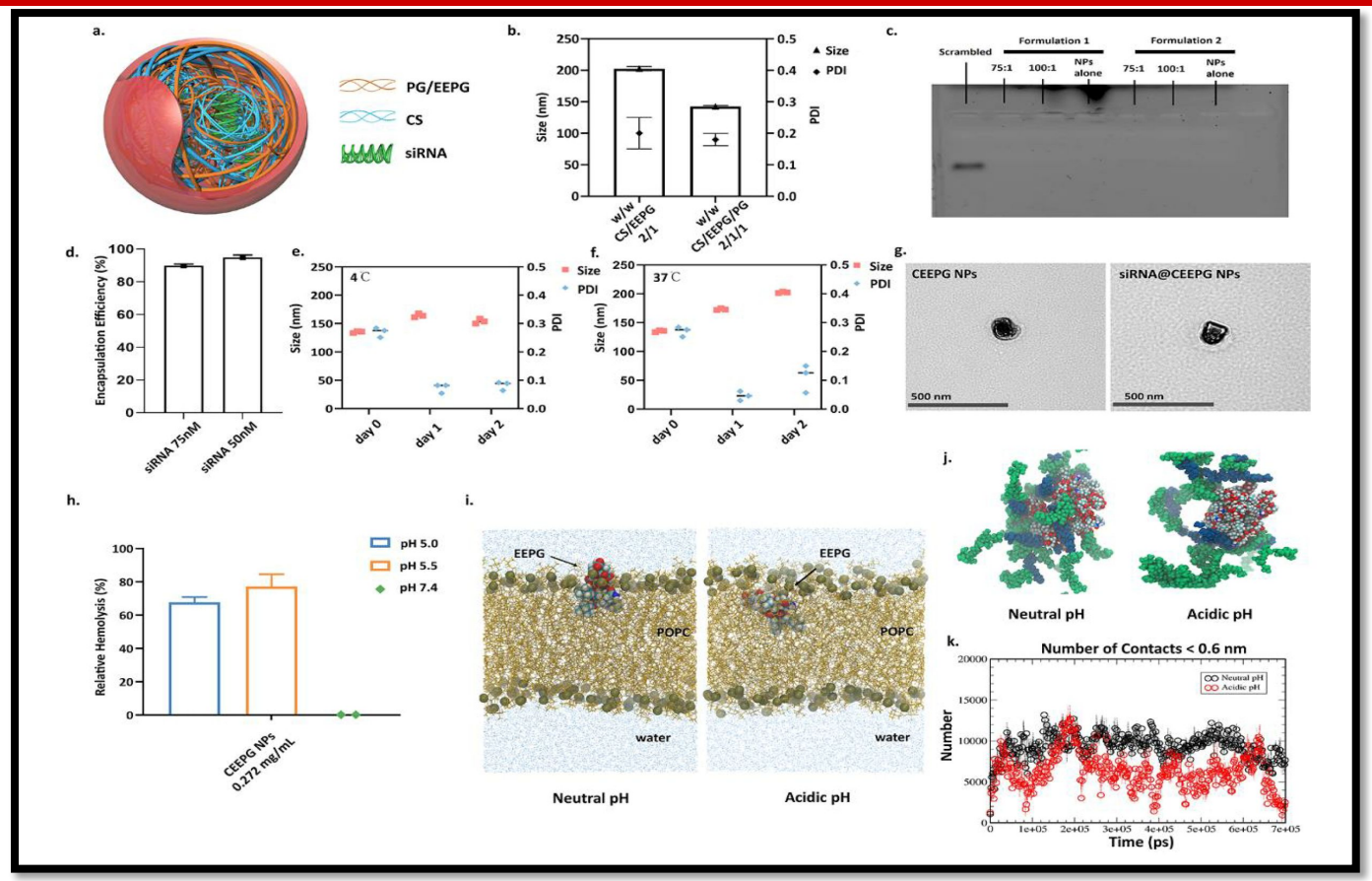
Polysaccharide-based viral mimicry nanocomplex with pH-responsive membrane destabilization and macrophage-targeting capability for gene silencing in inflammatory tissue



Gao, Santos *et al.*, *under review*

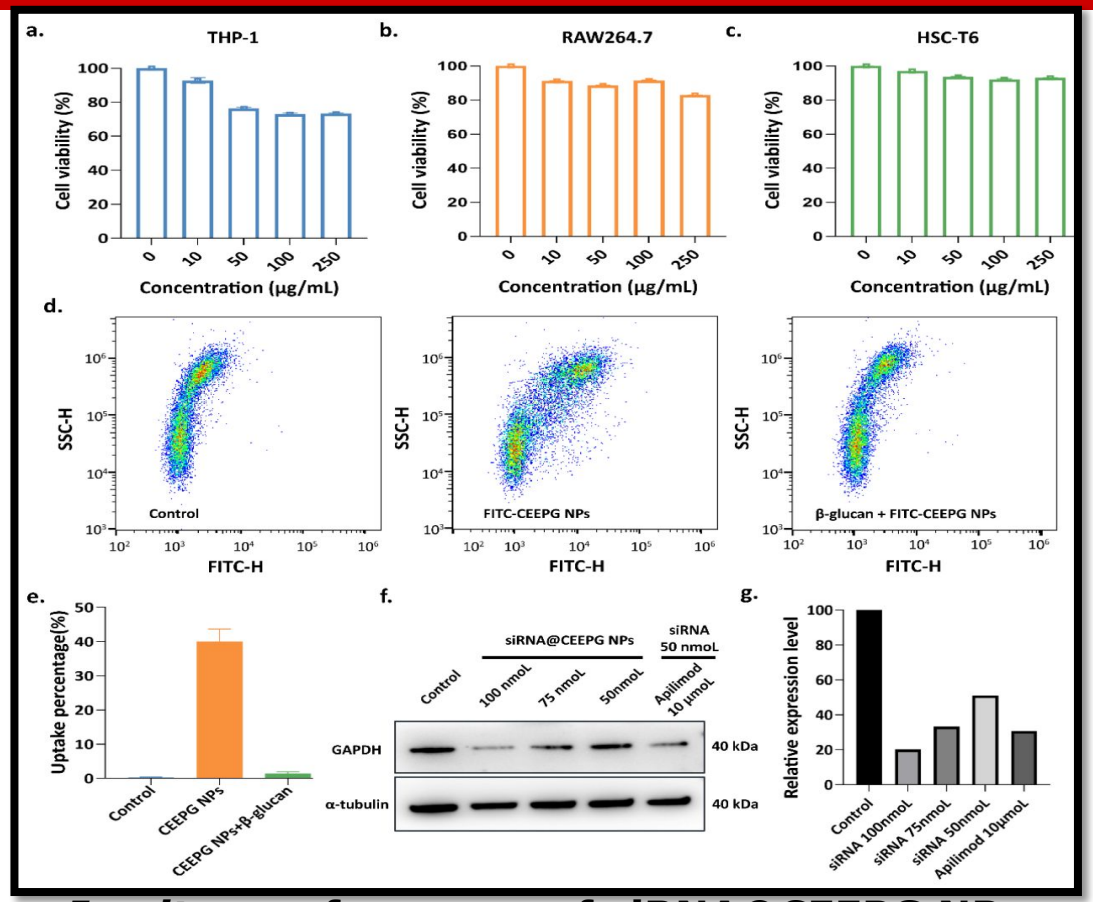


Synthesis and characterization of β-glucan based membrane-active polymer (EEPG)

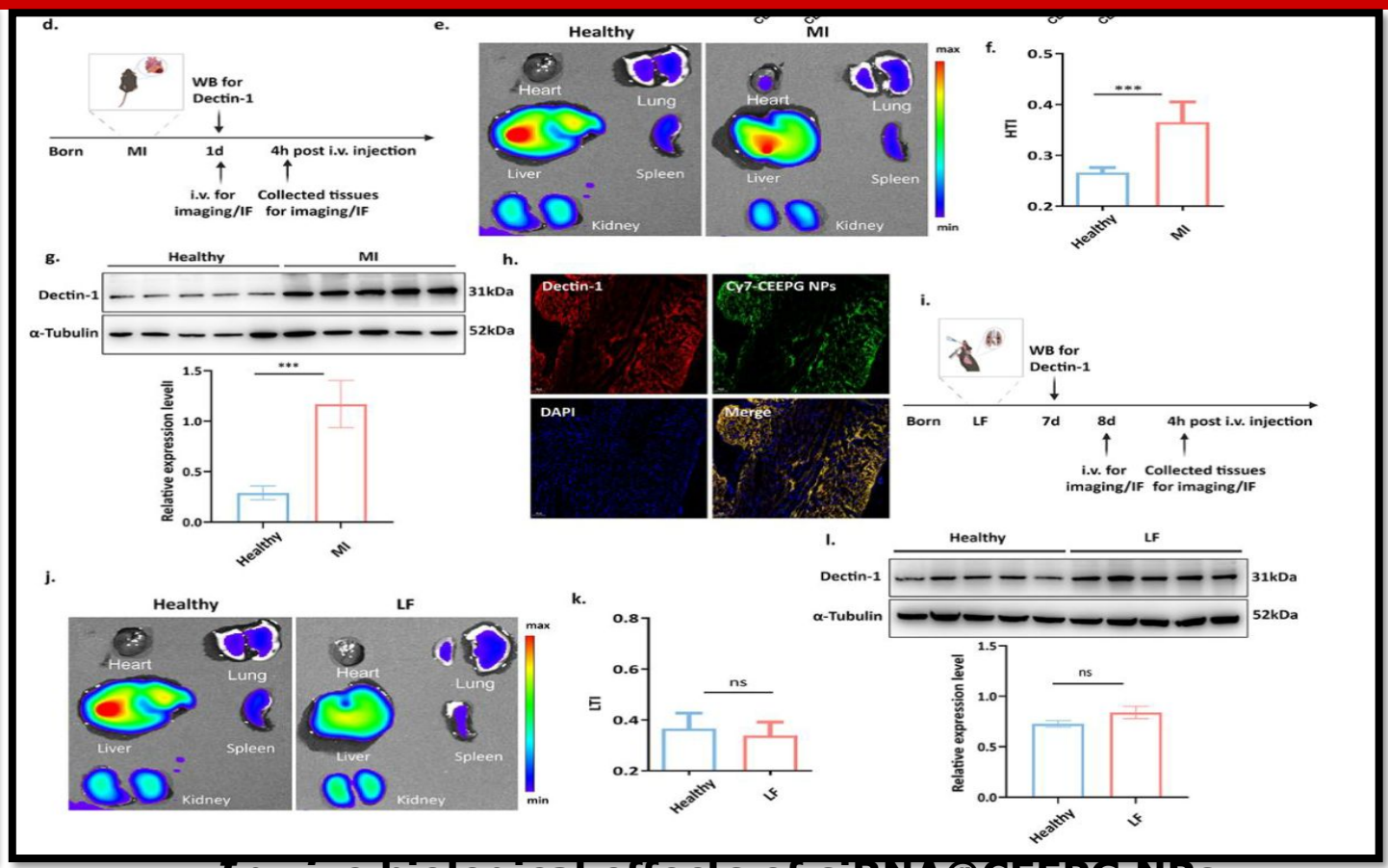


Preparation and characterization of polymeric NPs

Gao, Santos *et al.*, *under review*



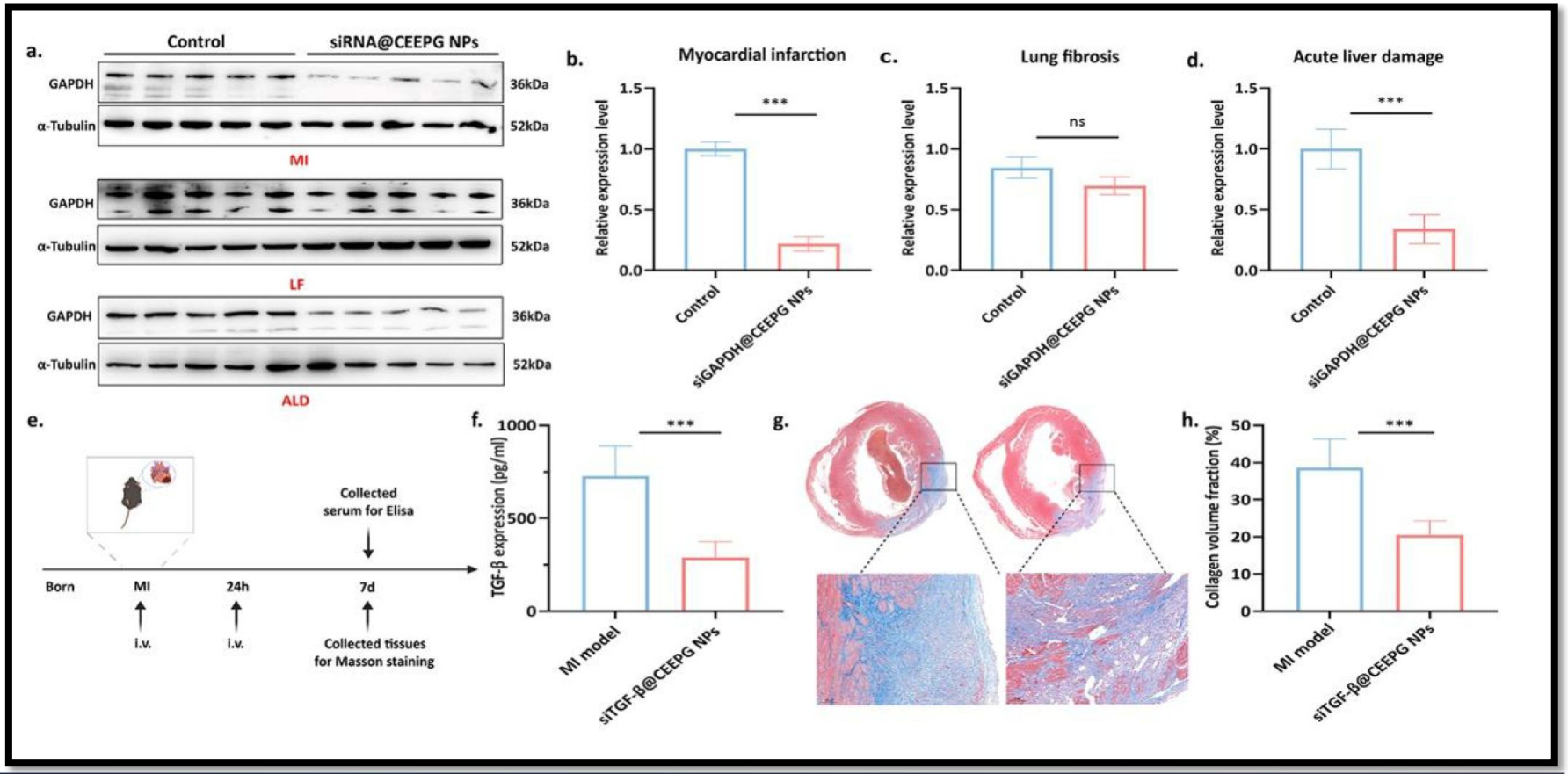
***In vitro* performance of siRNA@CEEPG NPs**



***In vivo* biological effects of siRNA@CEEPG NPs**

Gao, Santos *et al.*, *under review*

Functional evaluation of RNAi nanoformulation



- β-glucan-based NPs show precise and efficient targeting capability toward Dectin-1$^+$ monocytes/macrophages, which are main pathogenesis mediators for cardiac ischemic/reperfusion (I/R) injury.
- β-glucan NPs loaded with NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome inhibitor (CY-09) show better efficiency in ameliorating myocardial injury and heart failure induced by surgically induced I/R.
- A virus-inspired poly (alkyl methacrylate-co-methacrylic acid) fragment with pH-responsive membrane destabilization properties conjugated on barley β-glucans (EEPG) endow the nanocomplex with endosomal escape capabilities.
- EEPG nanocomplex with efficient siRNA loading can achieve potent *in vivo* gene silencing in a selective subset of leukocytes due to the inherent affinity of EEPG towards an innate phagocytic receptor, Dectin-1.

ARTIFICIALLY CLOAKED VIRAL NANOVACCINE FOR CANCER IMMUNOTHERAPY

nature COMMUNICATIONS

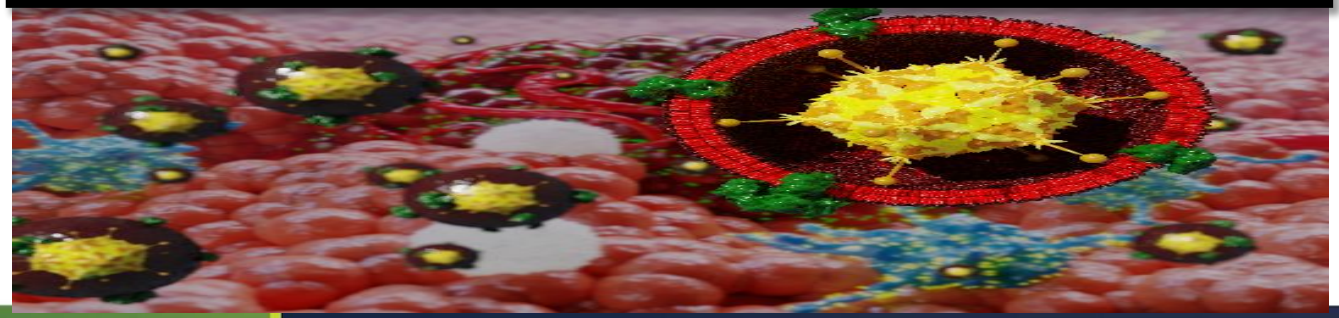
Nature Commun. **2019**, *10*, 5747

ARTICLE

https://doi.org/10.1038/s41467-019-13744-8 OPEN

Artificially cloaked viral nanovaccine for cancer immunotherapy

Manlio Fusciello[1,8], Flavia Fontana[2,8], Siri Tähtinen[1], Cristian Capasso[1], Sara Feola[1], Beatriz Martins[1], Jacopo Chiaro[1], Karita Peltonen[1], Leena Ylösmäki[1], Erkko Ylösmäki[1], Firas Hamdan[1], Otto K. Kari[1], Joseph Ndika[3], Harri Alenius[3,4], Arto Urtti[1,5], Jouni T. Hirvonen[2], Hélder A. Santos[2,6*] & Vincenzo Cerullo[1,6,7*]



TUMOR EXOSOME-BASED NANOPARTICLES FOR TARGETED CANCER CHEMOTHERAPY

nature COMMUNICATIONS

Nature Commun. **2019**, *10(1)*, 3838

Article | Open Access | Published: 23 August 2019

Tumor exosome-based nanoparticles are efficient drug carriers for chemotherapy

Tuying Yong, Xiaoqiong Zhang, Nana Bie, Hongbo Zhang, Xuting Zhang, Fuying Li, Abdul Hakeem, Jun Hu, Lu Gan, Hélder A. Santos & Xiangliang Yang

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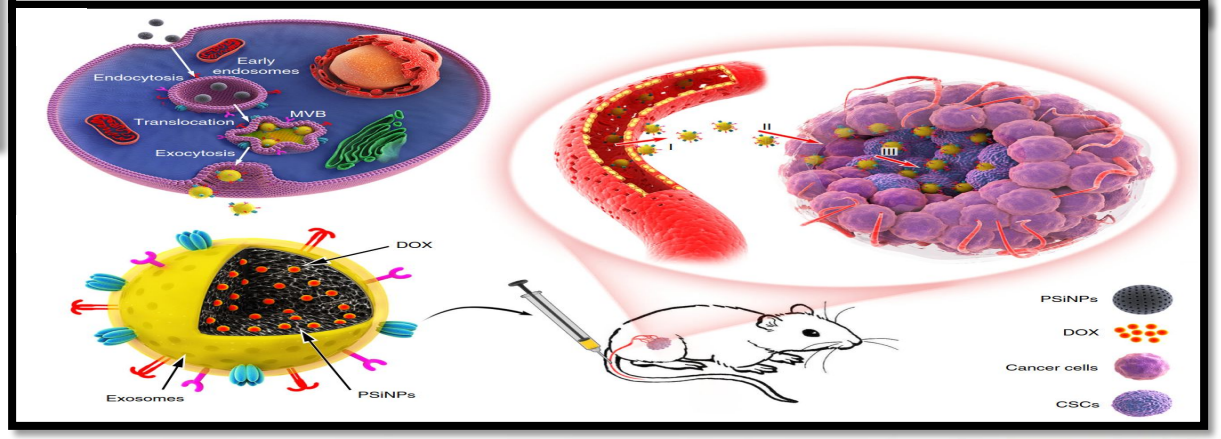
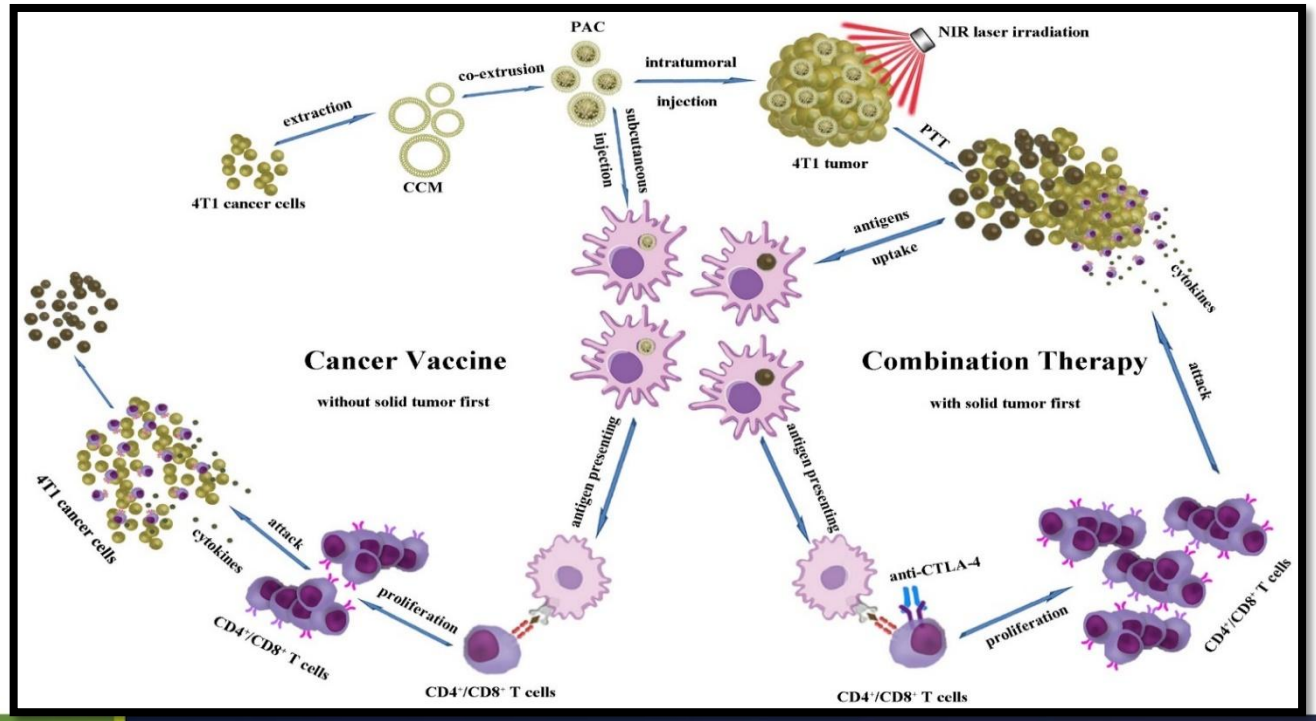
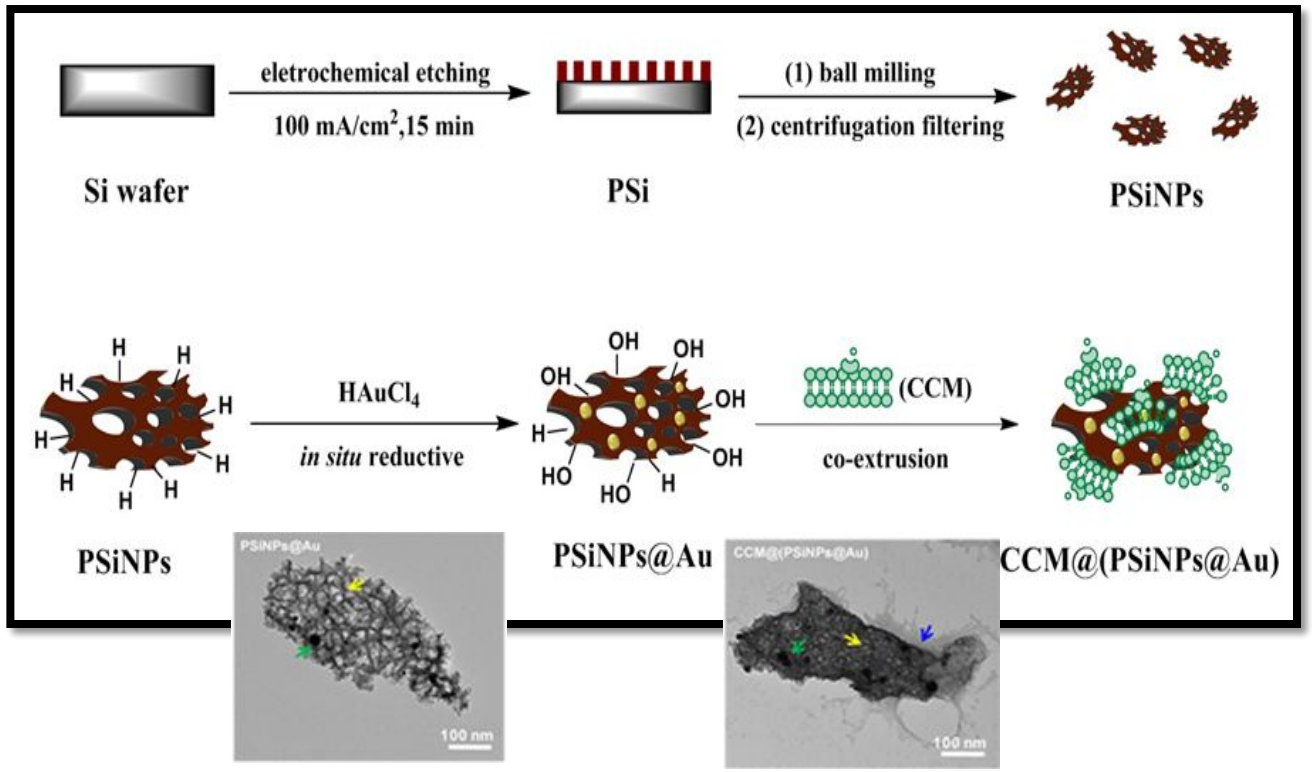


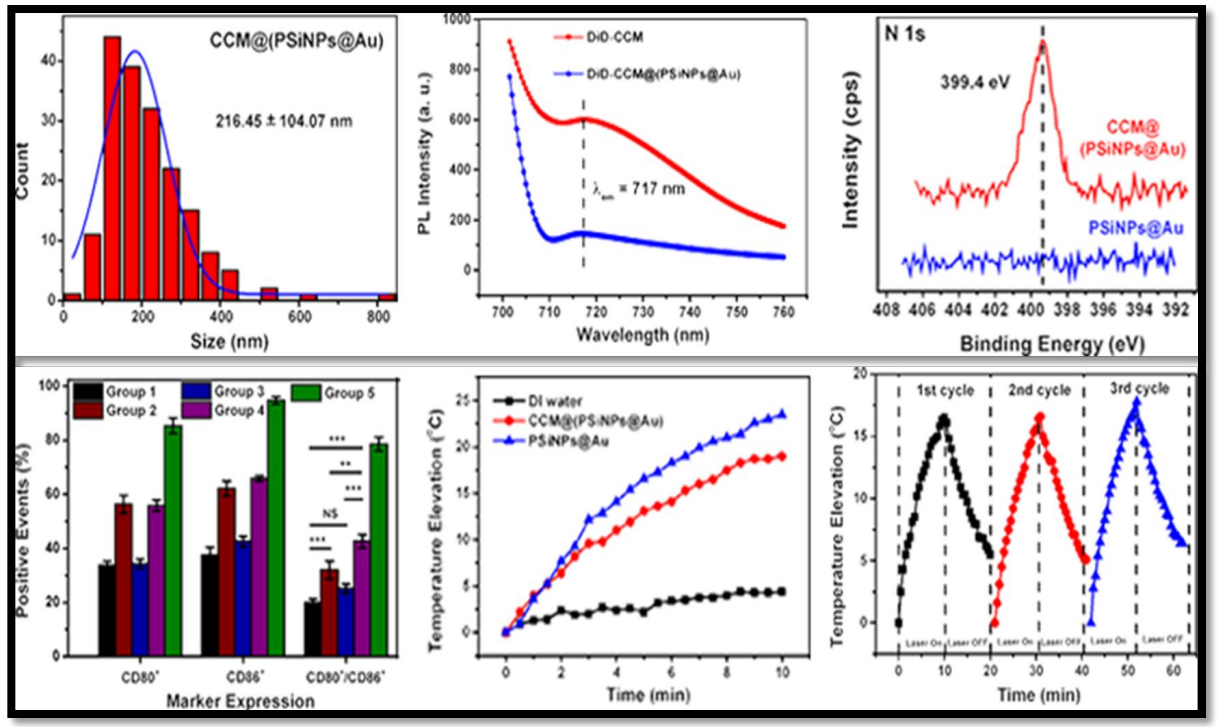
MULTIFUNCTIONAL NANOVACCINES BASED ON PHOTOTHERMAL AND IMMUNOSTIMULATORY NANOPARTICULATE CORES FOR IMMUNOTHERAPY OF SOLID TUMORS



Li, Santos, Bing *et al.*, *Adv. Mater.* ***2022***, *2108012*

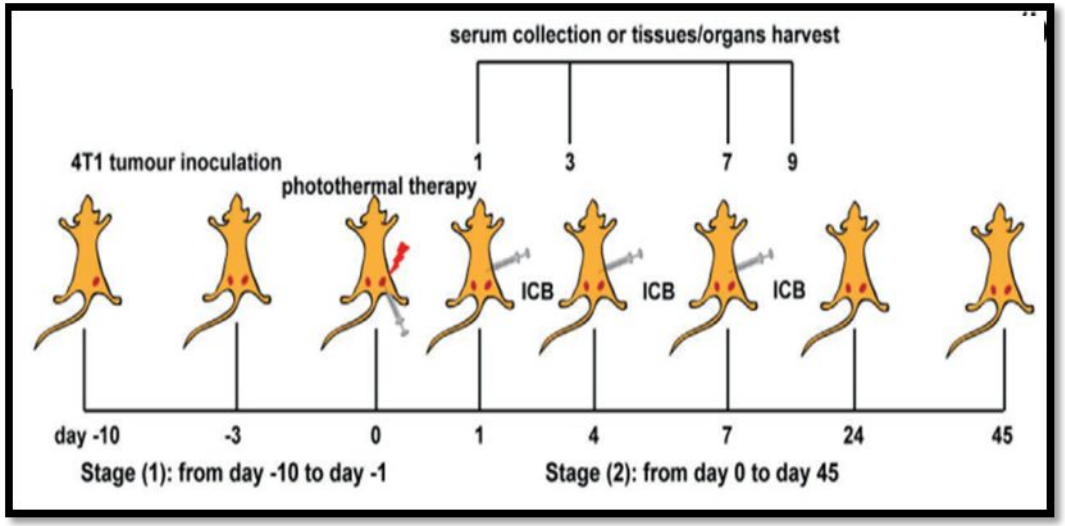
Nanovaccine preparation and characterization





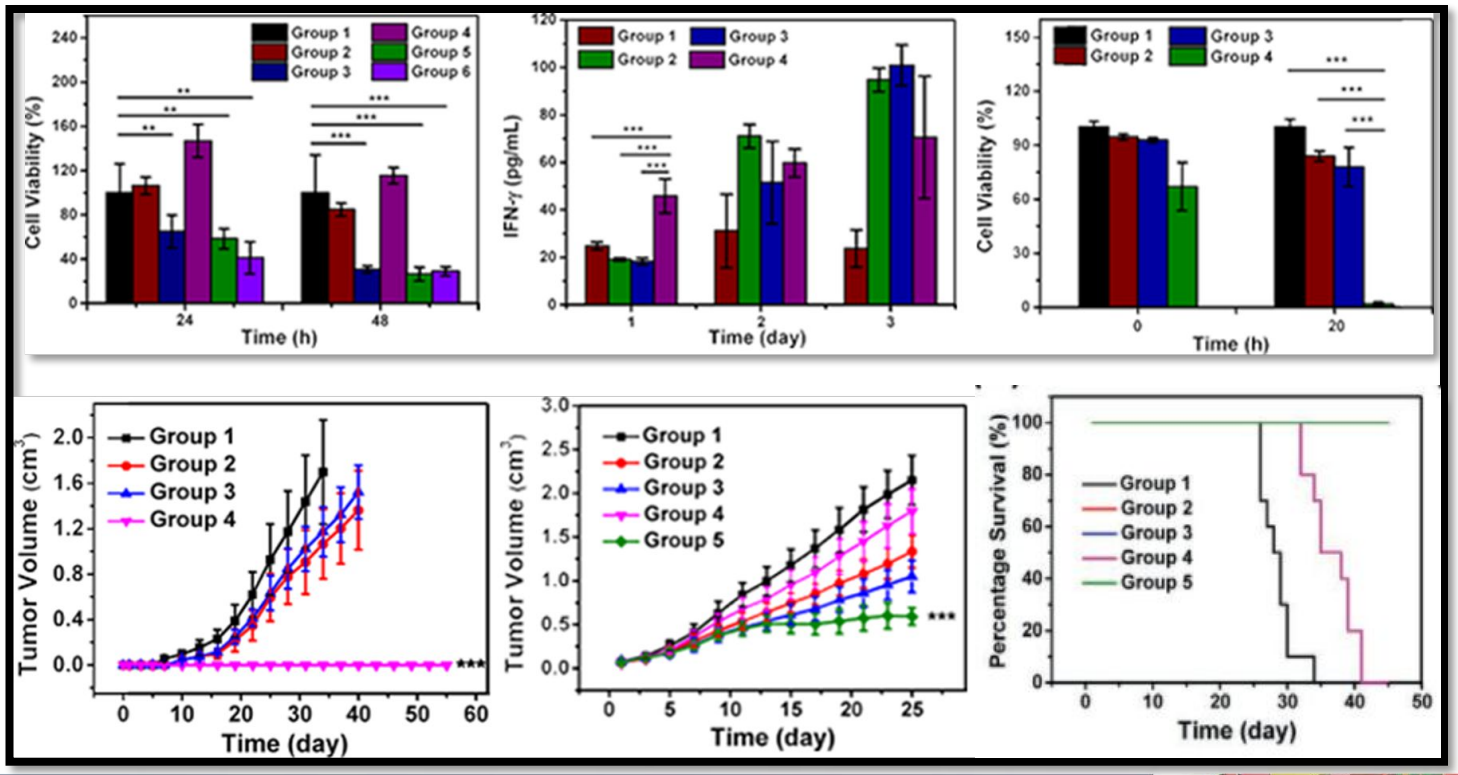
Li, Santos, Bing *et al.*, *Adv. Mater.* ***2022***, *2108012*

Nanovaccine *in vitro* and *in vivo* anticancer efficacy studies



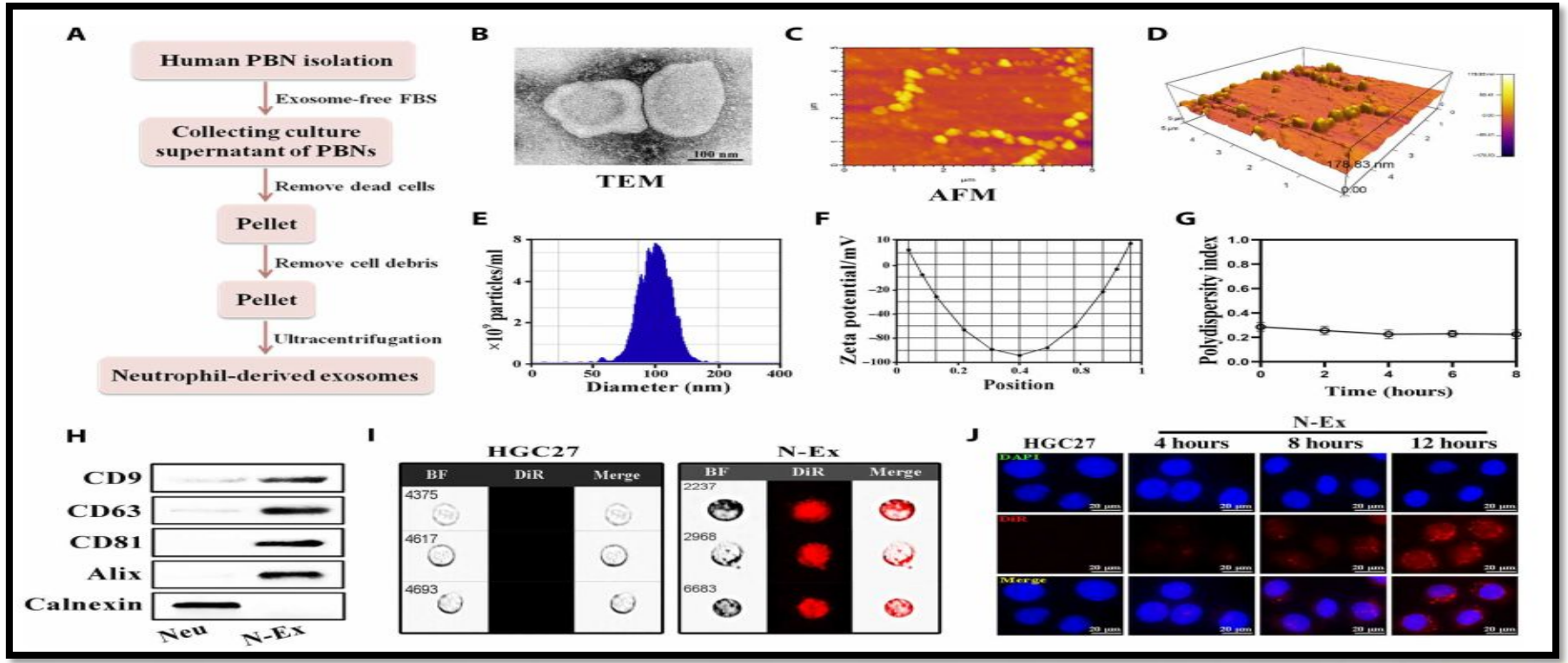
Group 1: Control
Group 2: NIR + ICB
Group 3: NIR + vaccine
Group 4: Vaccine + ICB
Group 5: NIR + vaccine + ICB

(Anti-CTLA-4 antibody)

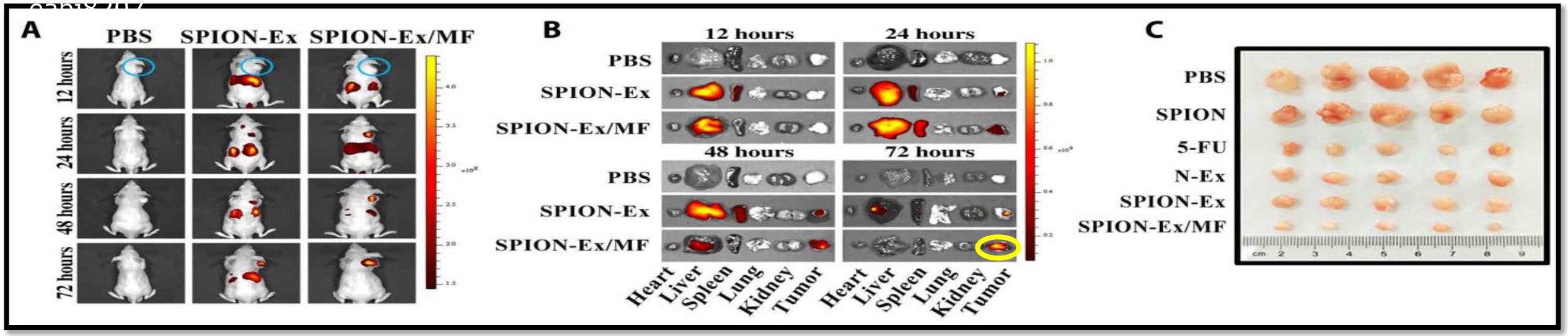


Zhang, Santos, Zhang *et al.*, *Sci. Adv.* **2022**, *8*, eabj8207

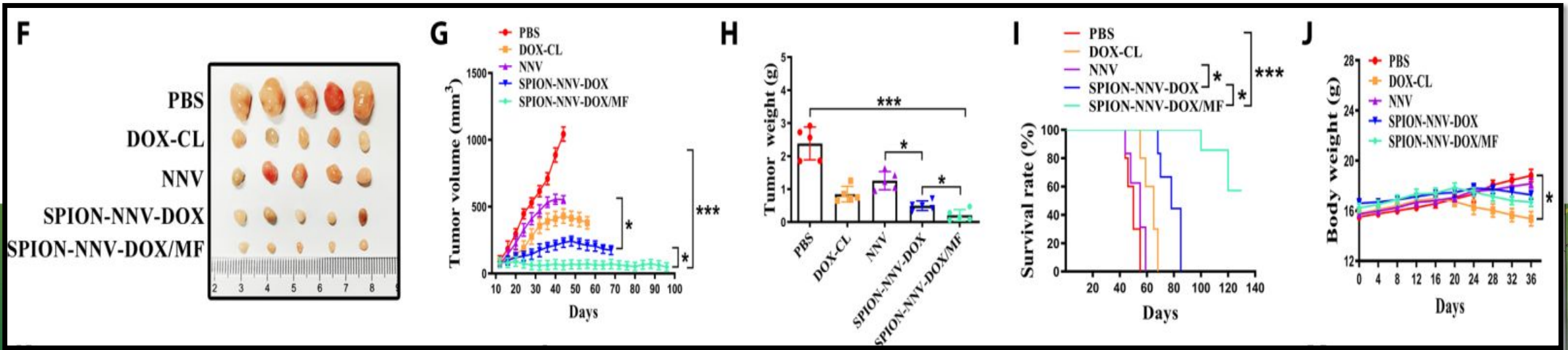
ENGINEERED NEUTROPHIL-DERIVED EXOSOME-LIKE VESICLES FOR TARGETED CANCER THERAPY



Zhang, Santos, Zhang *et al.*, *Sci. Adv.* **2022**, *8*, eabm7012



SPION-modified, N-Ex–like NVs deliver DOX to inhibit tumor growth



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- ICB immunotherapy + CCM@(PSiNPs@Au) nanovaccine and photothermal therapy, eliminate solid tumors and suppress their metastasis.
- Due to inhibition of the antitumor immune responses and the reversion of their immunosuppressive microenvironments.

- **Exosomes** from neutrophils (N-Ex) induced tumor cell apoptosis.
- Decorated N-Ex achieved higher tumor-targeting therapeutic effect.
- N-Ex with DOX-loaded improved inhibition of tumor cell proliferation and selectively accumulate at the tumor sites under an external magnetic field, preventing tumor growth and prolonging the survival rate in mice.

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