Alleviation of aorta atherosclerosis by Antibody-coated Mesoporous silica nanoparticles

Jong Oh Kim, PhD

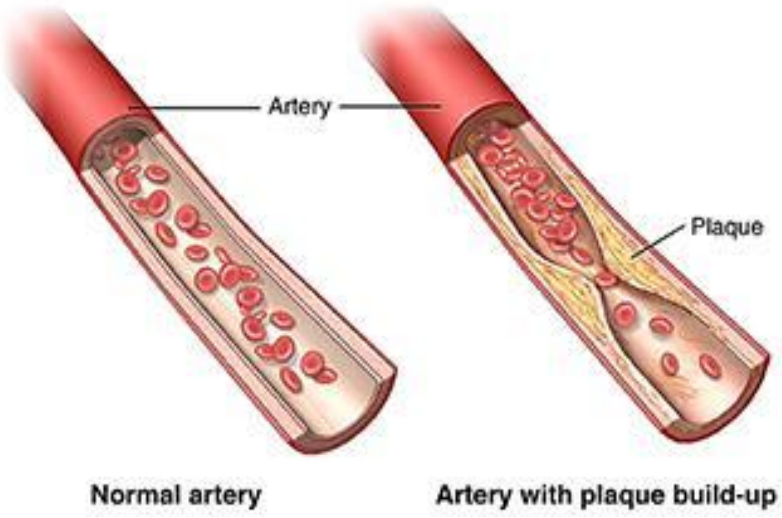
Professor, Dean
Lab of Applied Nanomedicine
Associate Editor, Journal of Controlled Release
Director, BK21 Four program Team
College of Pharmacy, Yeungnam University
Republic of Korea

CRS Controlled Release Society

영남대학교 YEUNGNAM UNIVERSITY 1947

Atherosclerosis

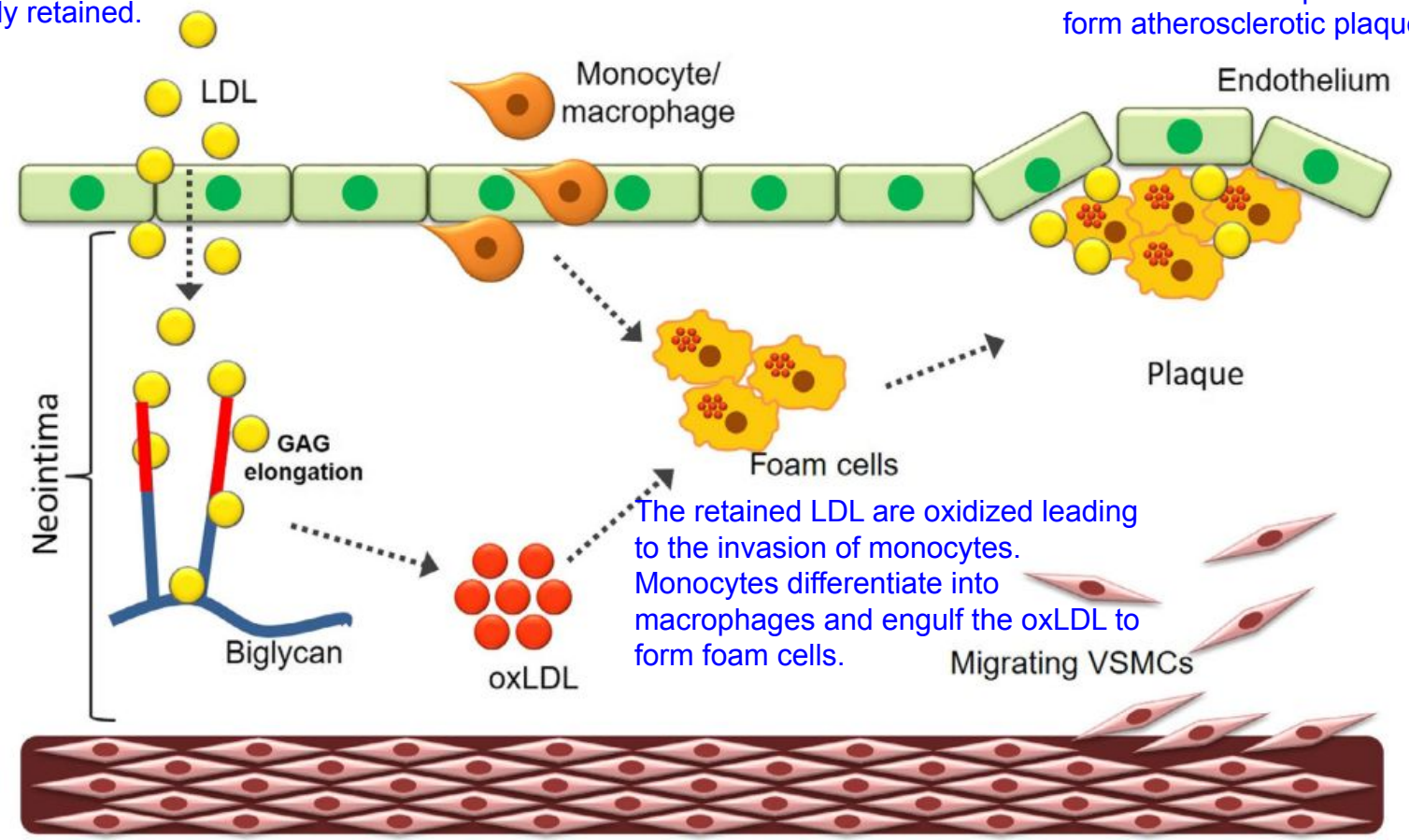
- Thickening or hardening of the arteries
- Caused by a build-up of plague due to the accumulation of low-density lipoprotein and fibrous substances in the inner lining of an artery
- The lisk factors: hypertension, diabetes, serum total and LDL cholesterol, smoking
- Also associated with premature or accelerated vascular aging, and cellular senescence



Schematic representation of the initiation and progression of atherosclerosis

LDL diffuse into the vessel wall where it is bound by the modified proteoglycans and subsequently retained.

Foams cells and lipids accumulate to form atherosclerotic plaque

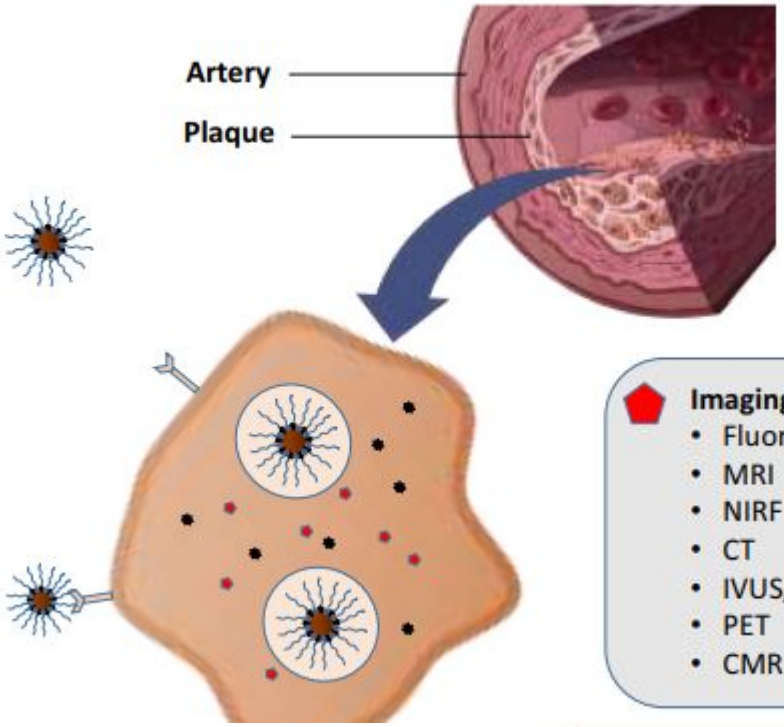


The retained LDL are oxidized leading to the invasion of monocytes. Monocytes differentiate into macrophages and engulf the oxLDL to form foam cells.

Vascular smooth muscle cell (VSMC) secrete a range of proteoglycans, such as biglycan.
When growth factors are released, the glycosaminoglycan chains on proteoglycans become enlongated.

Zhang et al J Pharmacy and Pharmacology 71 (2019), 1029-1043

Theranostic nanomedicine for atherosclerosis



Target cells or molecules in atherosclerosis:
- Macrophages
- Integrin $\alpha_v\beta_3$
- Annexin V
- Vascular cell adhesion molecule-1 (VCAM-1)

Nanocarrier:
- Lipid-based nanoparticles
- Micelles
- Polymeric nanoparticles
- Dendrimers
- Gel-like nanoparticles
- Magnetic nanoparticles
- Inorganic nanoparticles

Imaging mode:
- Fluorescence imaging
- MRI
- NIRF
- CT
- IVUS/IVPA imaging
- PET
- CMR molecular imaging

Therapy agent for atherosclerosis:
- Anti-inflammatory drugs
- Immunomodulation drugs
- Gene (DNA/RNA)
- Antibodies
- Proteins
- Photoabsorbers
- Photosensitisers

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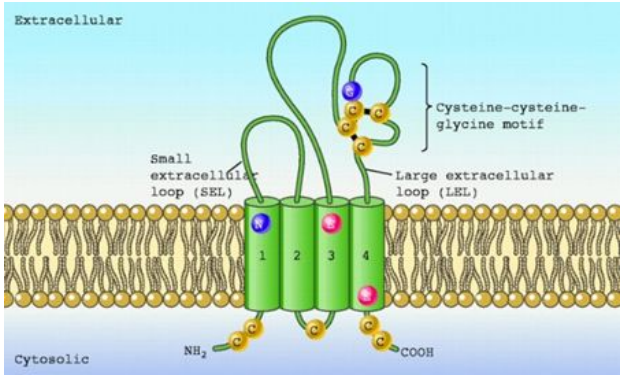
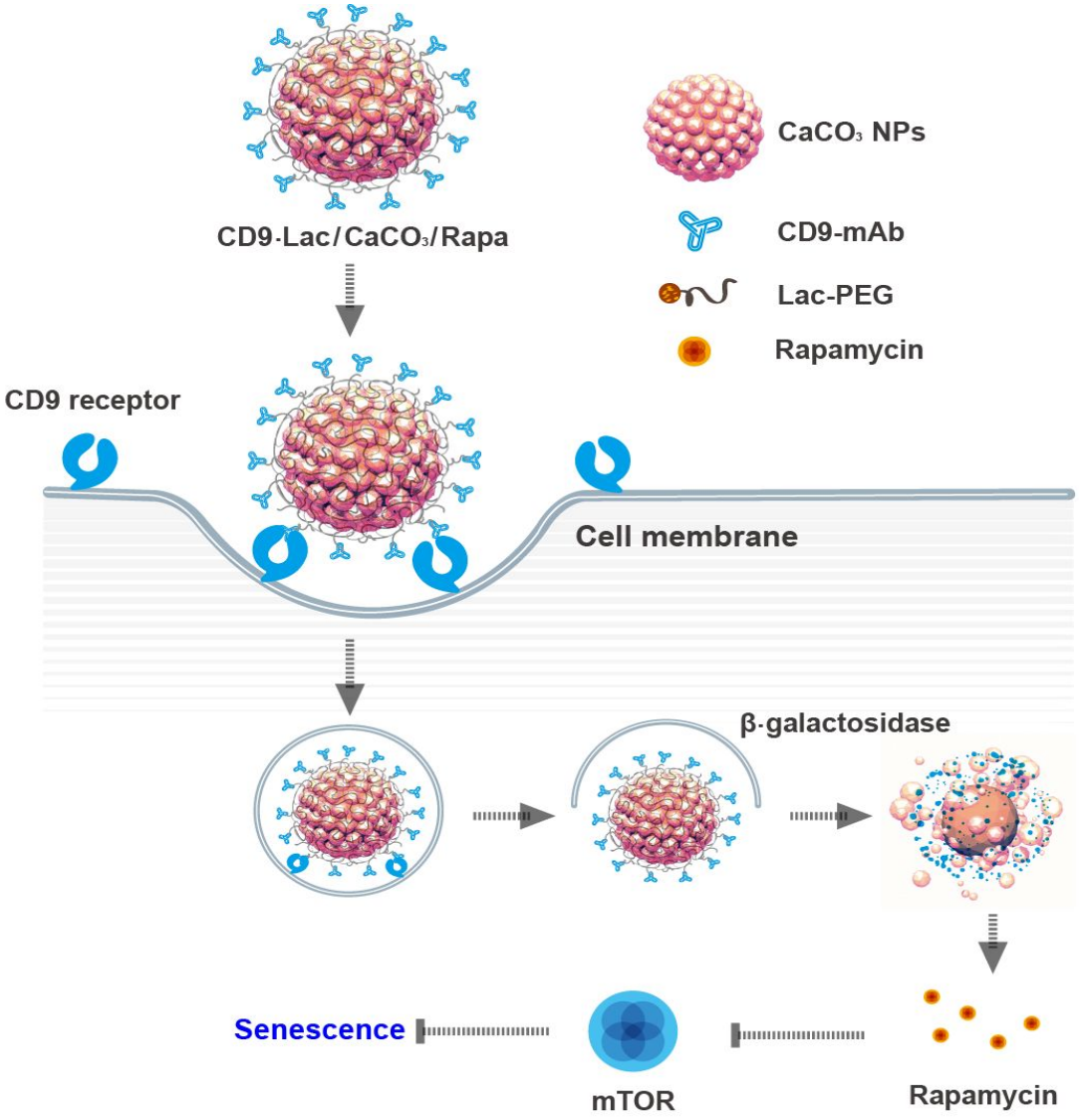
CD9 receptor as a target molecule of atherosclerosis

- **CD9 is a cell-surface glycoprotein and controls a variety of cellular activities such as migration, proliferation, and adhesion.**
- **It is highly expressed in the human aorta and coronary arteries, especially in areas with atherosclerotic lesions.**
- **In addition, some smooth muscle cells and most macrophages in macrophage-rich plaques exhibited a CD9 immunoreactivity.**
- **According to our recent finding, CD9 plays a crucial role in inducing cellular senescence through the phosphatidylinositide 3 kinase-AKT-mTOR-p53 signal pathway, advancing atherosclerotic plaque formation.**
- **Selective knockdown of CD9 in senescent cells significantly restricts senescence, whereas CD9 upregulation in young cells initiates cellular senescence.**



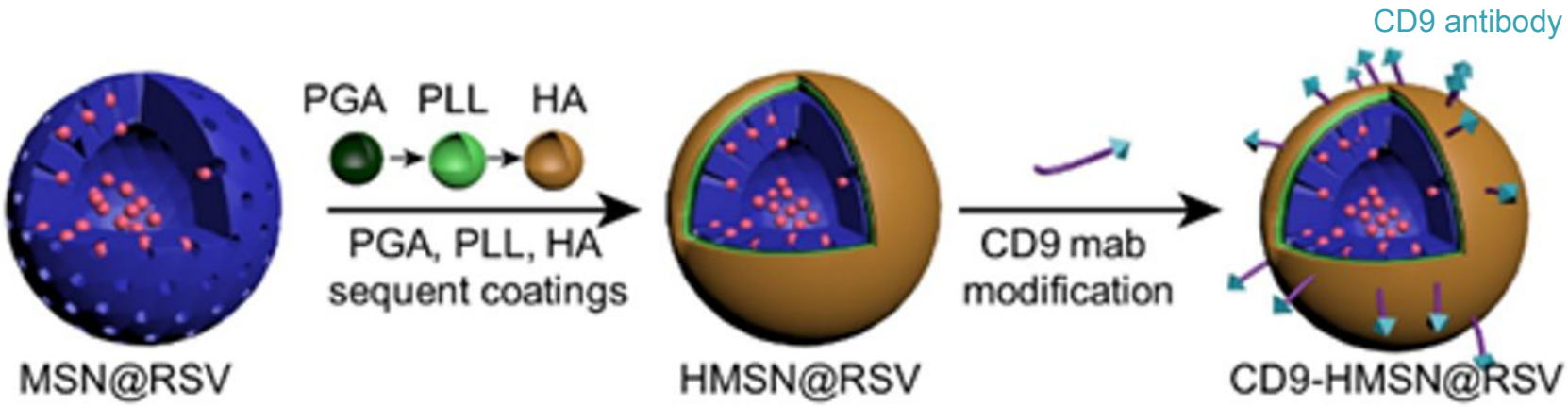
CD9 receptor-targeted delivery of rapamycin to aging cells



High uptake and **anti-senescence effects** (reduced β-galactosidase and reduced population doubling time, enhanced cell proliferation and migration, and prevention of cell cycle arrest) in old human dermal fibroblasts

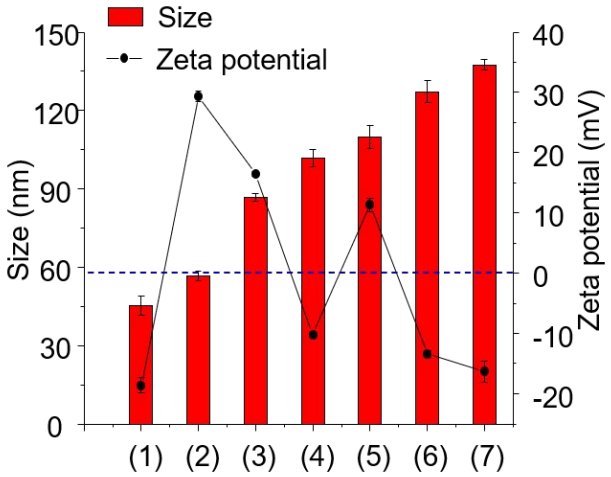
Thapa et al Scientific reports 7 (2017), Article number 43299

Preparation of CD9-HMSN@RSV



layer-by-layer coating

RSV: Rosuvastatin calcium
PGA: poly(*L*-glutamic acid)
PLL: Poly(*L*-lysine hydrochloride)

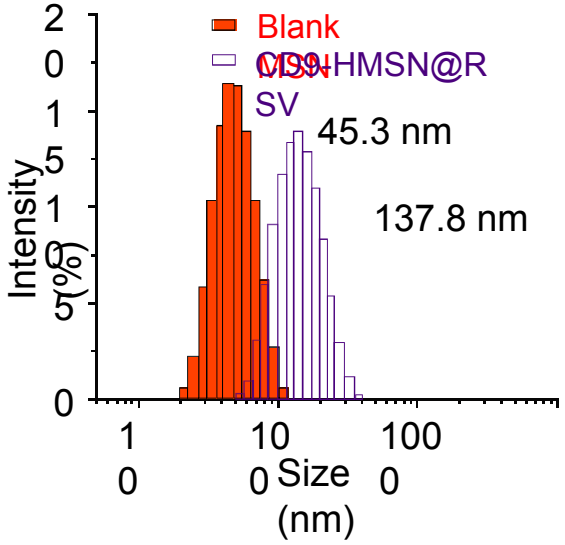


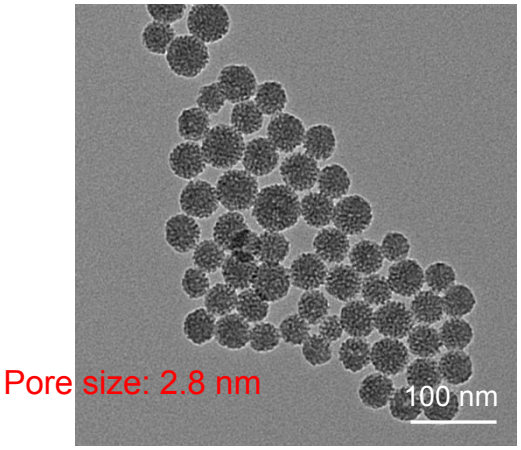
(1) MSN-OH
(2) MSN
(3) MSN@RSV
(4) PGA@MSN@RSV
(5) PLL@PGA@MSN@RSV
(6) HA@PLL@PGA@MSN@RSV
(7) CD9-HMSN@RSV

LC: 8.8 ± 0.2%
LE: 48.2 ±1.0%

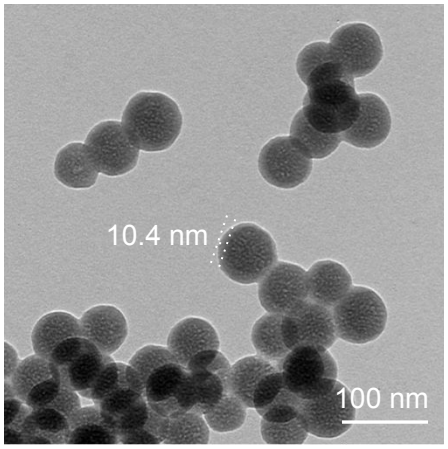
LM Pham et al Biomaterials 269 (2021), 120677

Characterization of CD9-HMSN@RSV

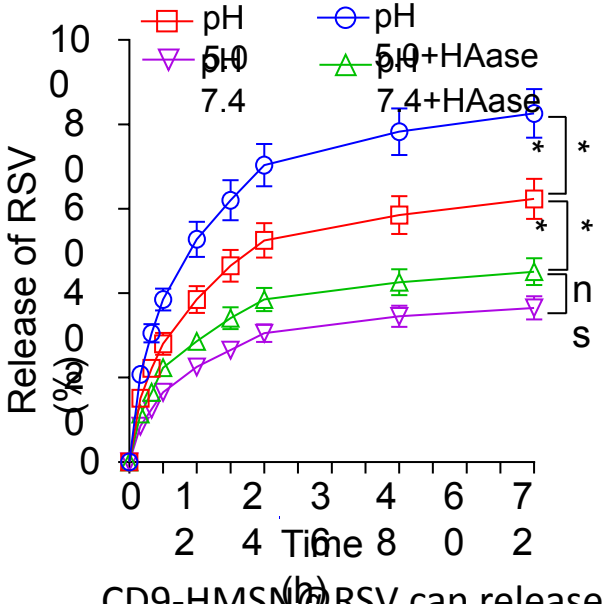


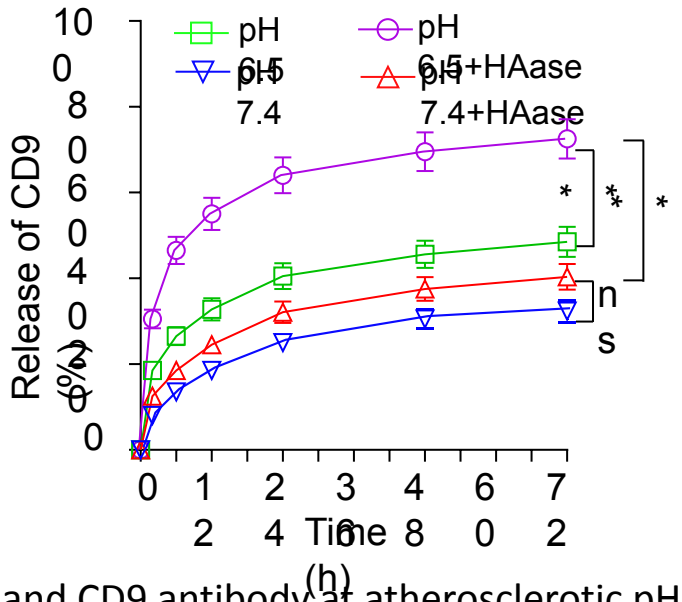


Blank MSN



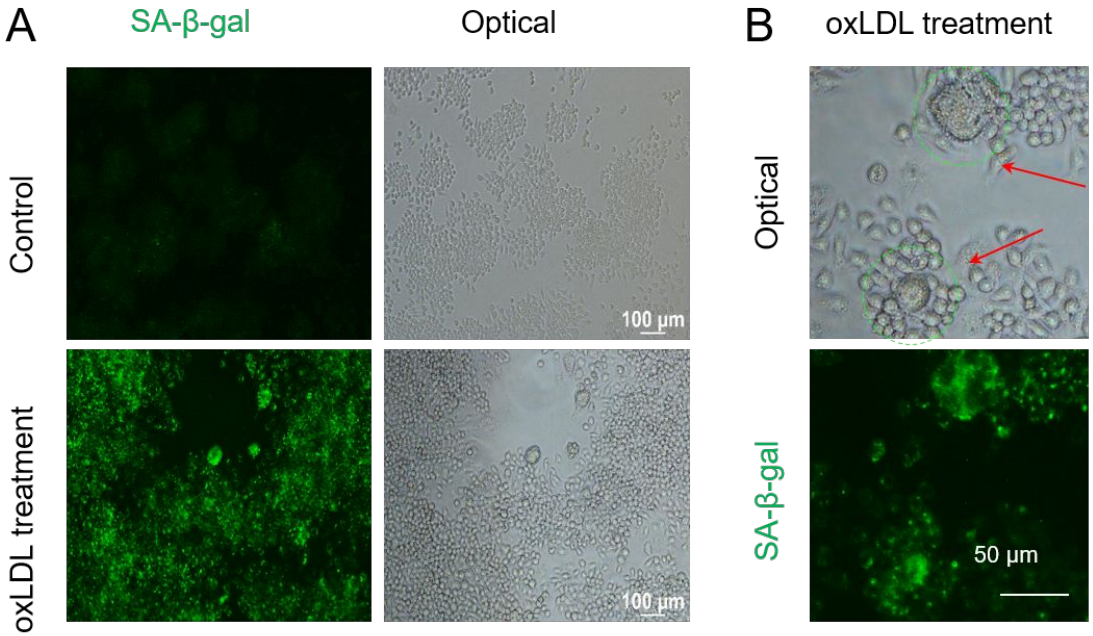
CD9-HMSN@RSV





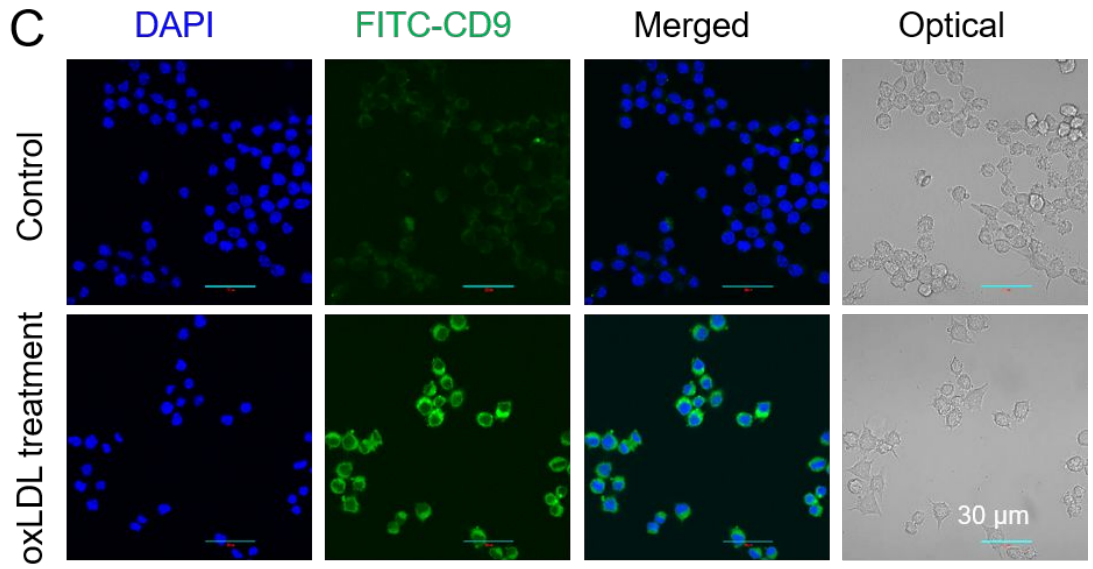
CD9-HMSN@RSV can release both RSV and CD9 antibody at atherosclerotic pH in an HAase-responsive manner

In vitro SA-β-gal staining of macrophages stimulated with oxLDL



In vitro SA-β-gal staining of macrophages stimulated with oxLDL for 24 h in RAW 264.7 cells.

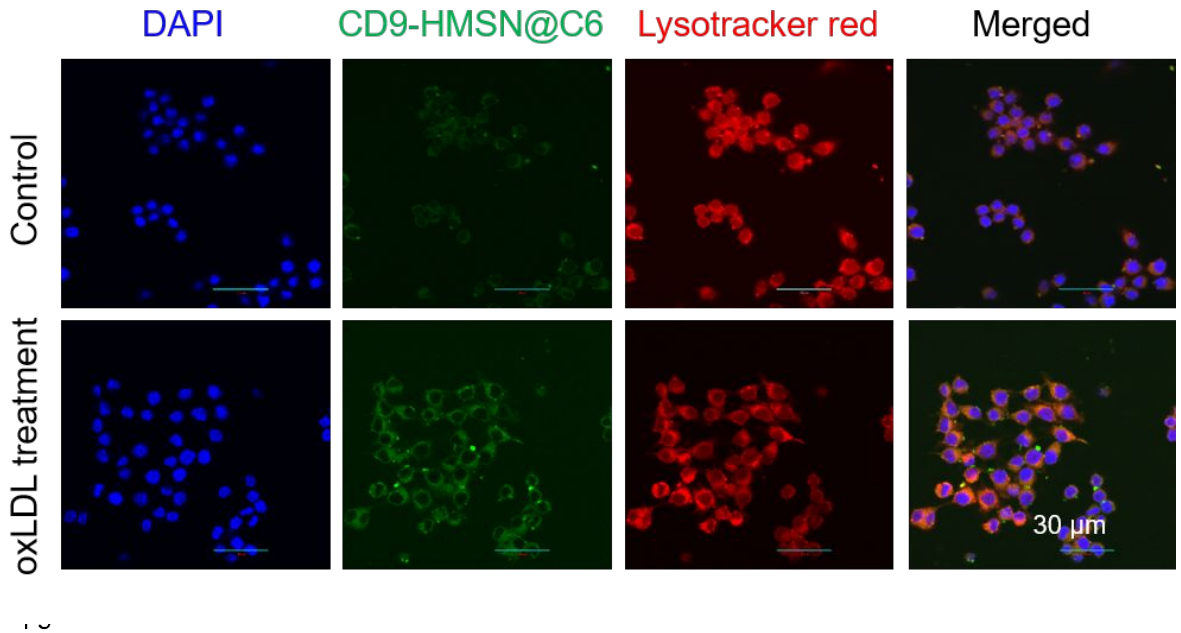
Magnified image of macrophages stimulated with oxLDL for 24 h, showing the formation of foamy macrophages with SA-β-gal-positive staining.

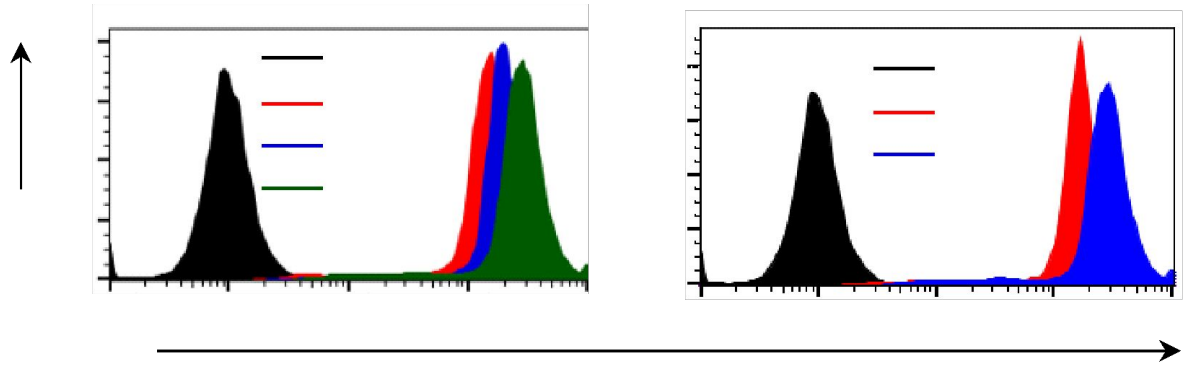


High CD9 level in macrophages stimulated with oxLDL for 24 h

SA-β-gal (Senescence-associated beta-galactosidase): a marker of senescence

In vitro cellular uptake in macrophages stimulated with oxLDL

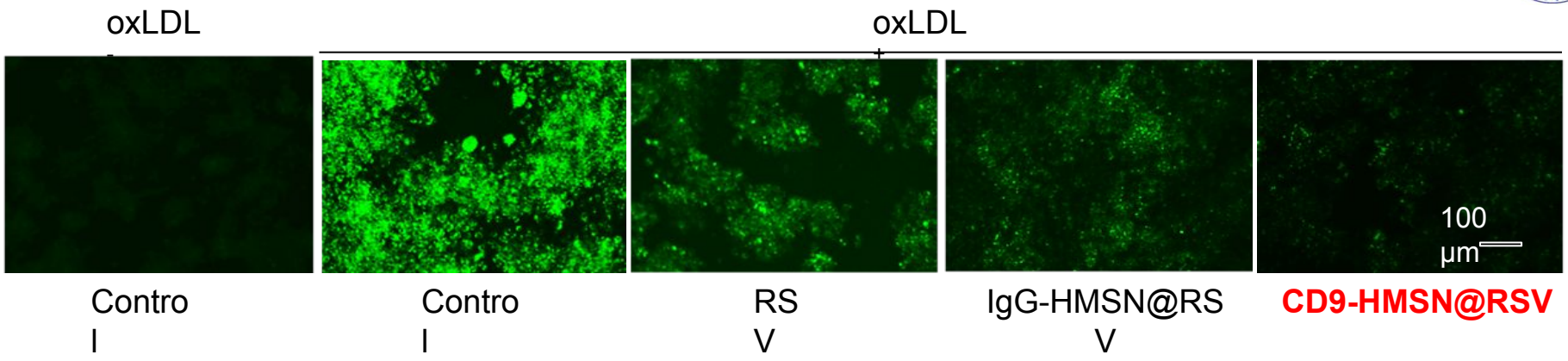


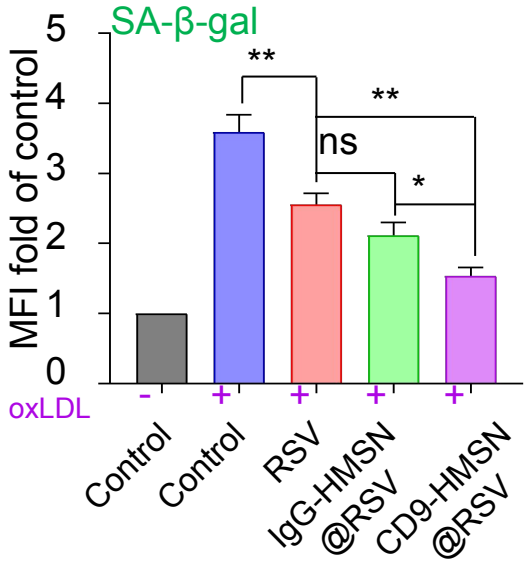


Time-dependent and dose-dependent uptake

oxLDL-stimulated senescent foamy macrophages exhibited markedly strong coumarin 6 fluorescence in the cytoplasm that merged well with lysotracker-red-labeled lysosomes, indicating good cellular internalization of CD9-HMSN@C6 into the senescent foamy macrophages

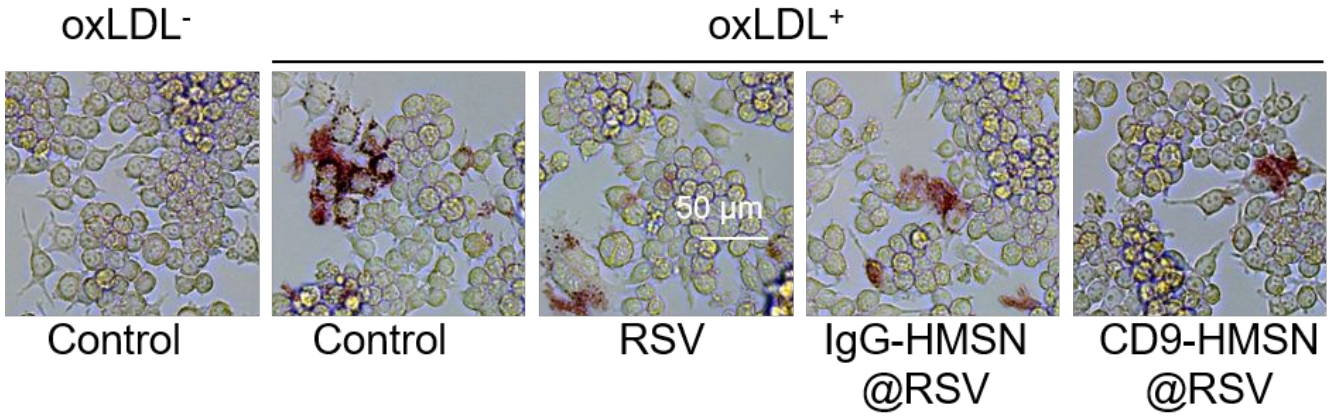
Secretion of intracellular SA-β-gal in macrophages

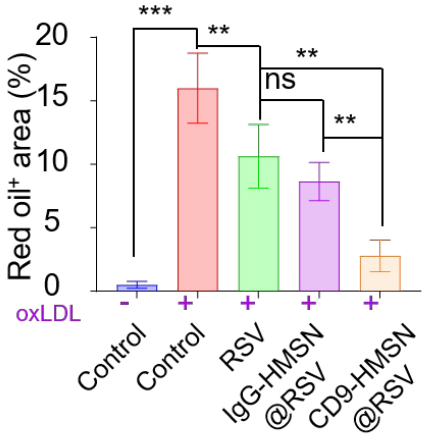




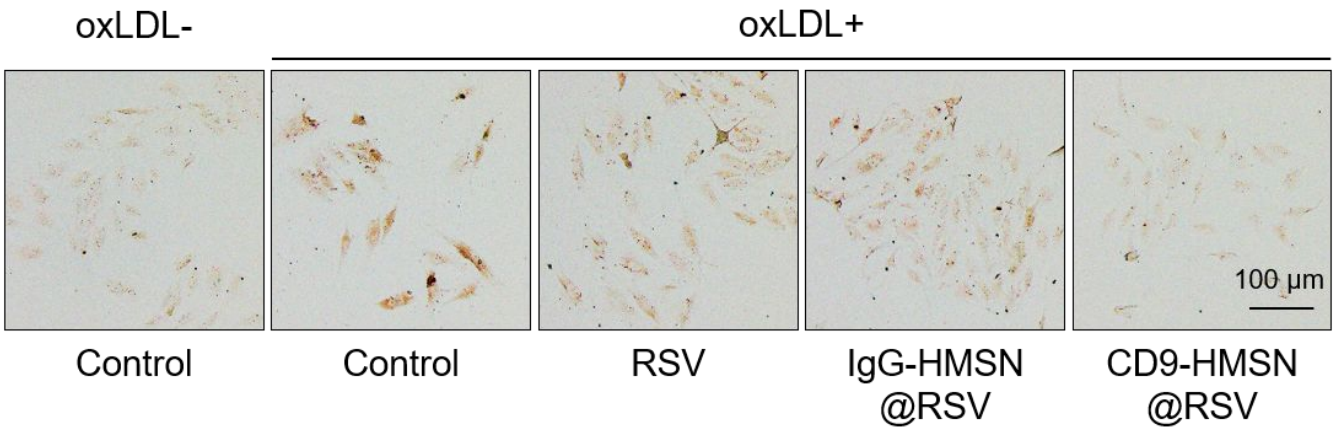
- **RAW 264.7 cells treated with oxLDL significantly enhanced intracellular SA-β-gal approximately 3.6 folds higher than non-oxLDL group**
- **Following treatment with CD9-HMSN@RSV, the level of intracellular SA-β-gal decreased significantly in blank oxLDL-stimulated macrophages.**

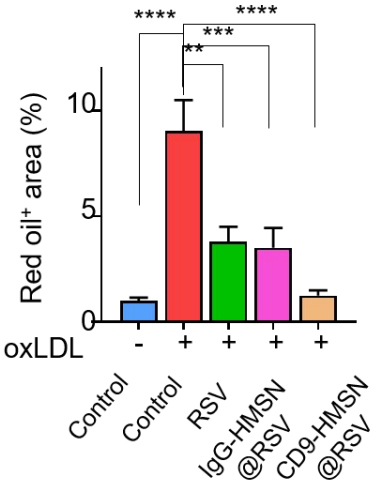
In vitro representative Oil red O staining images of macrophages treated with CD9-HMSN@RSV + oxLDL for 24 h.





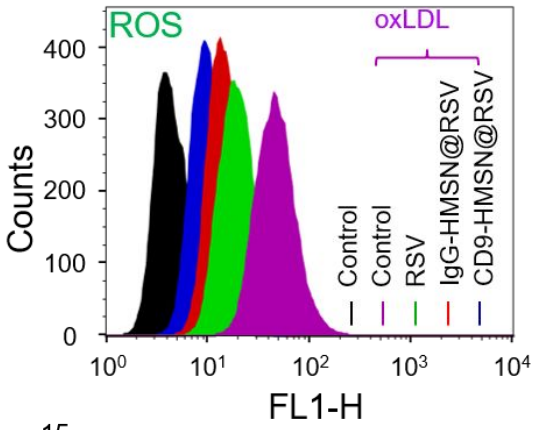
after incubation with endothelial cells

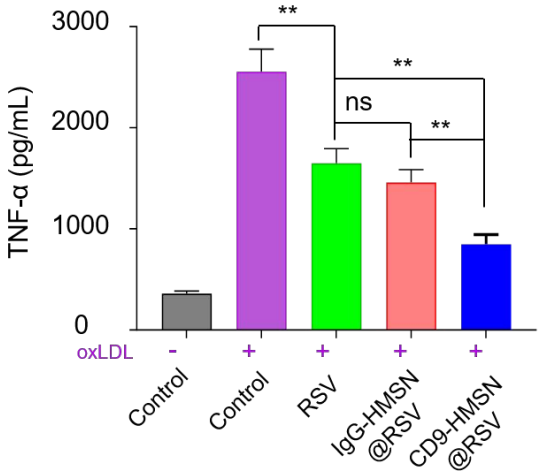


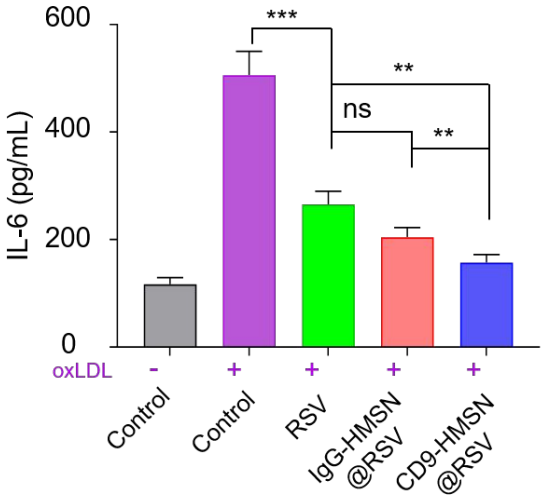


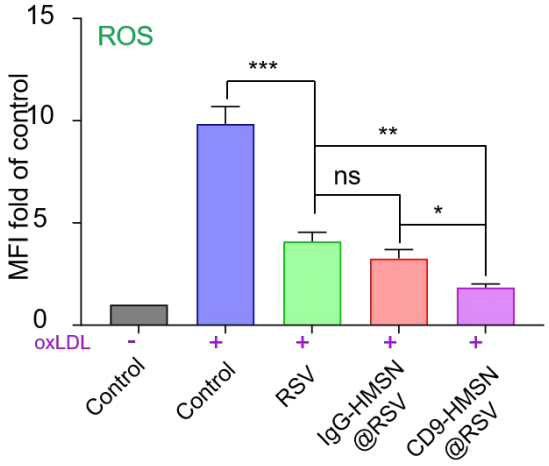
CD9-HMSN@RSV exhibited the lowest LDL oxidation than other groups

ROS generation

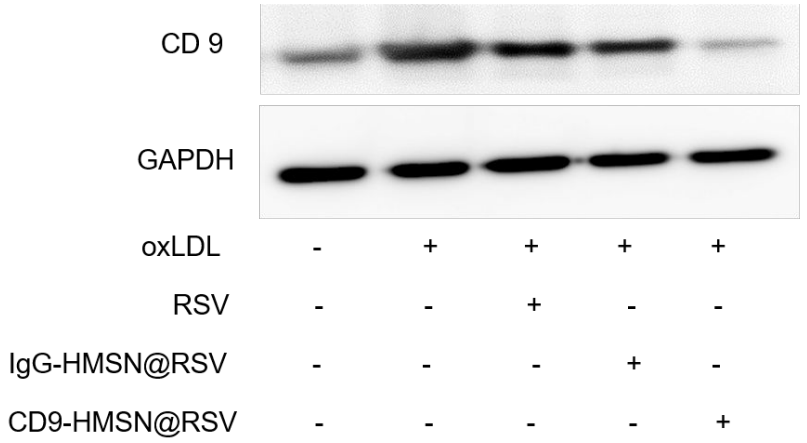








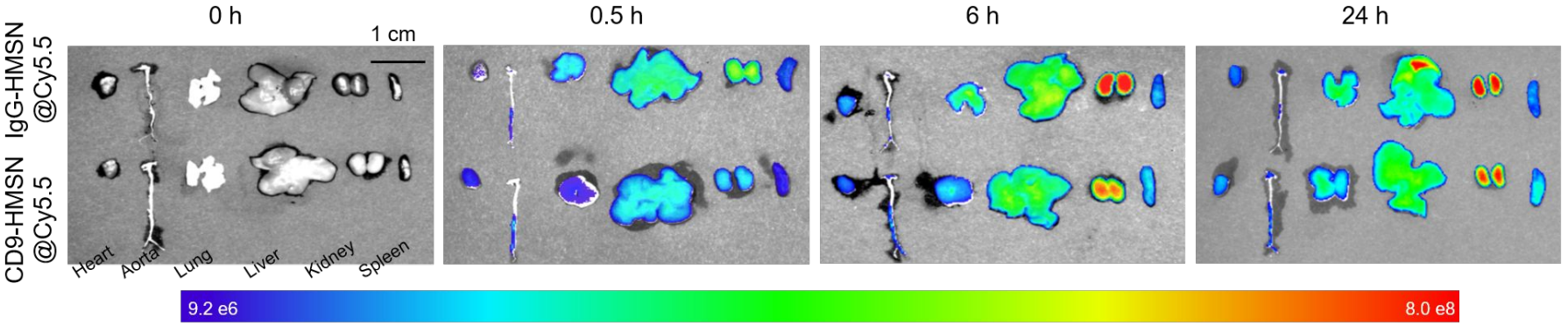
CD9-HMSN@RSV induced a substantial decrease in pro-inflammatory cytokines such as the TNF-α and IL-6 levels

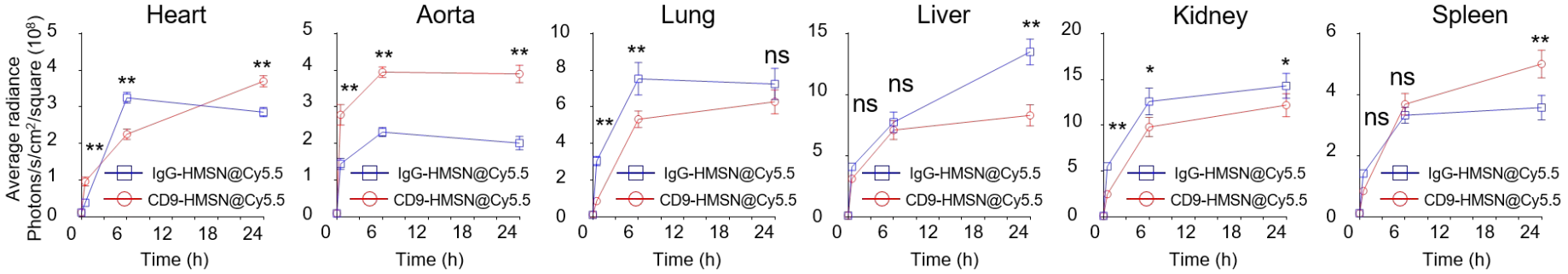


A high level of ROS production was detected in the oxLDL-stimulated macrophages through flow cytometry. However, this level was significantly reduced after CD9-HMSN@RSV treatment

CD9 expression of RAW 264.7 cells

In vivo targeting efficacy and pharmacokinetic of CD9-HMSN@RSV to the atherosclerotic lesions

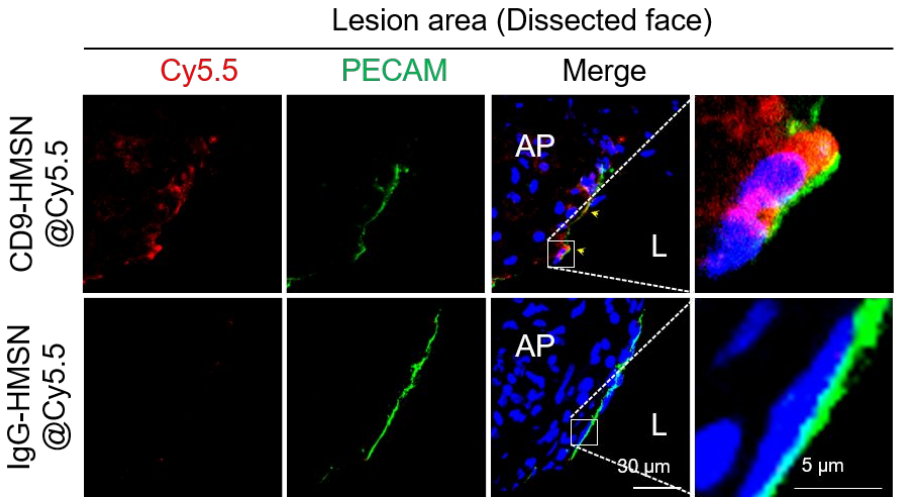




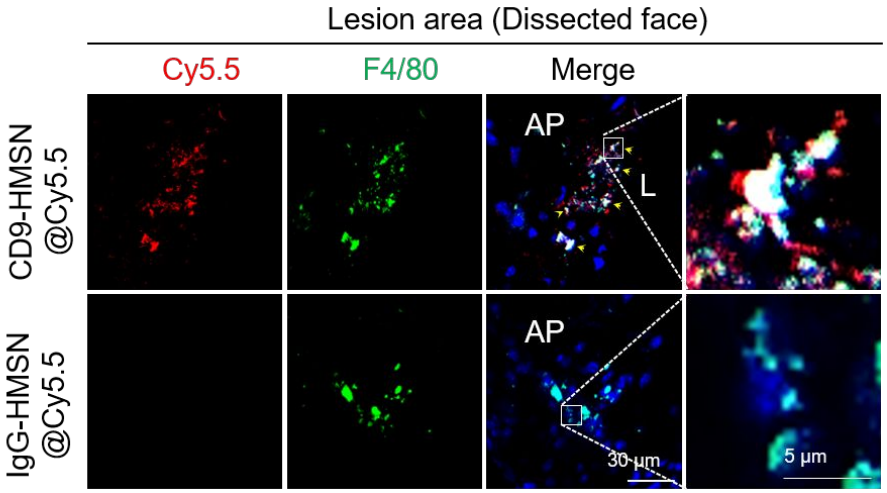
IV injection

9-week-old apolipoprotein E knockout (*ApoE*$^{-/-}$) mice with a high fat diet for 5 weeks

: Well established model for the study of human atherosclerosis.



AP: atherosclerotic plaque area
L: lumen area
PECAM: endothelial cell marker

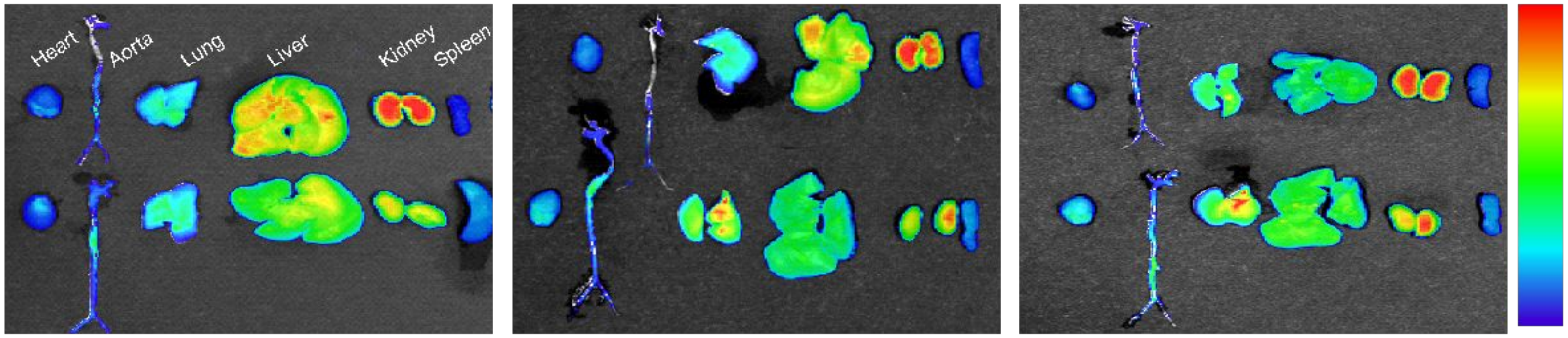


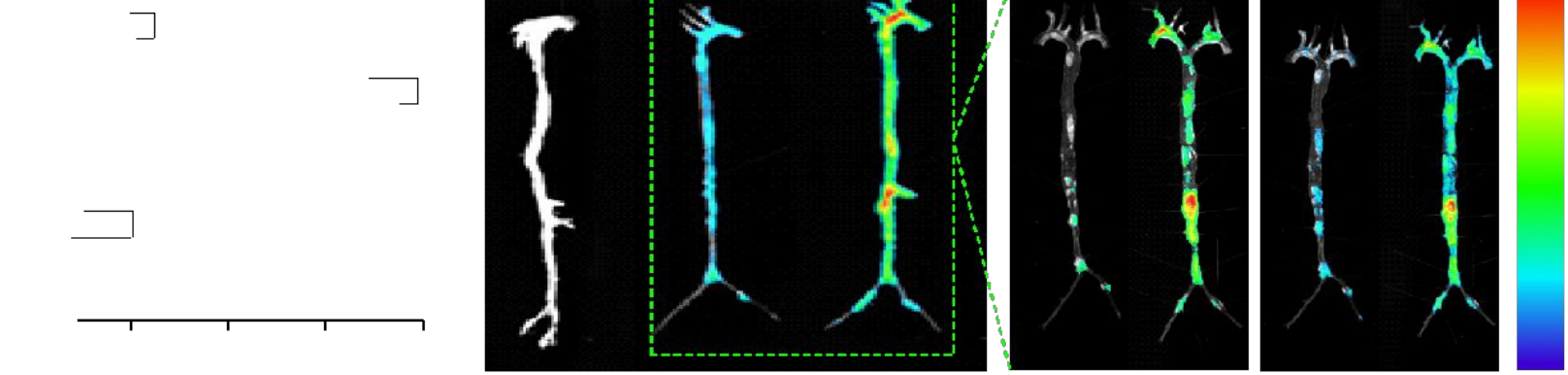
AP: atherosclerotic plaque area
F4/80: macrophage marker

Compared to IgG-HMSN@Cy5.5, more specific targeting of CD9-HMSN@Cy5.5 was observed to both endothelial cells and macrophages in the atherosclerotic plaques

in vivo biodistribution

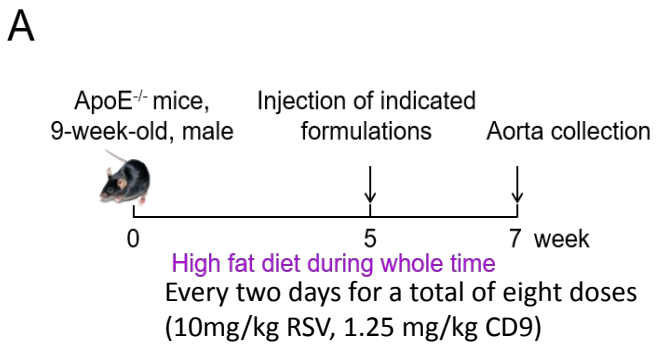
After 24h IV injection
9-week-old *ApoE*$^{-/-}$ mice with a high fat diet for 5 weeks

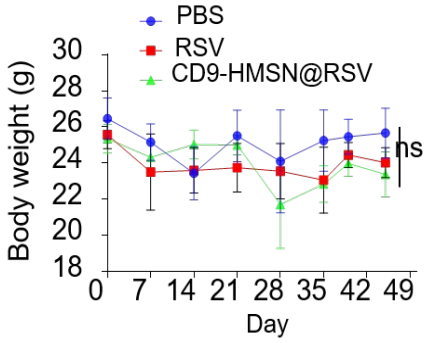


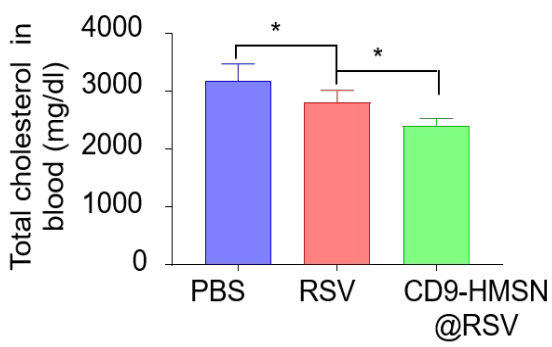


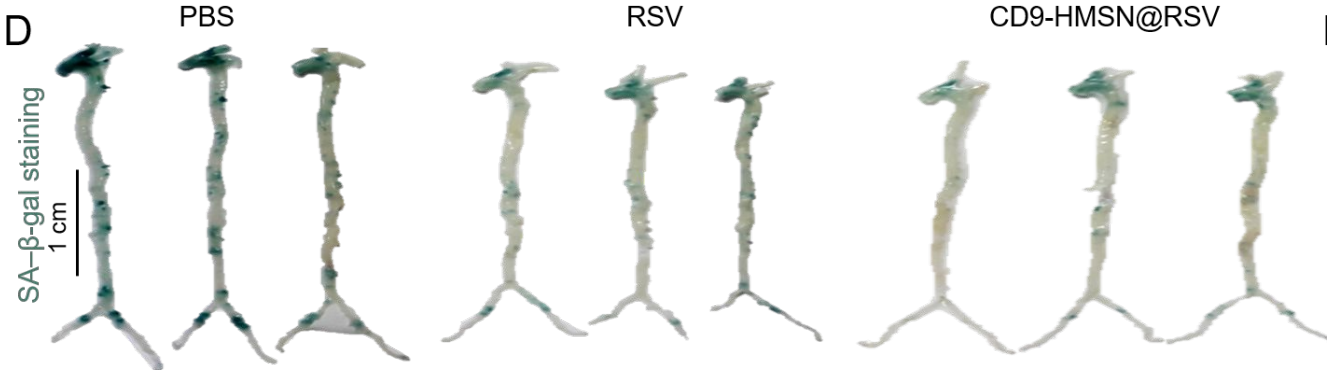
Both the *in vivo* and *ex vivo* distribution and circulation results indicate that the CD9-HMSN@RSV can target atherosclerotic sites and firmly bind to senescent plaques.

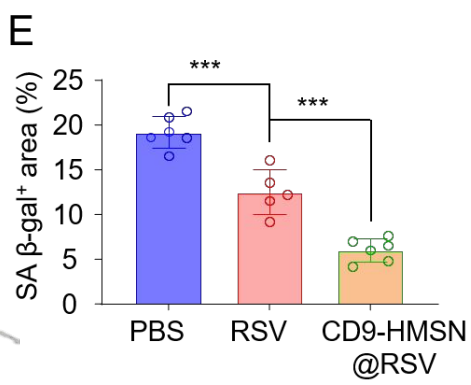
In vivo effect of CD9-HMSN@RSV

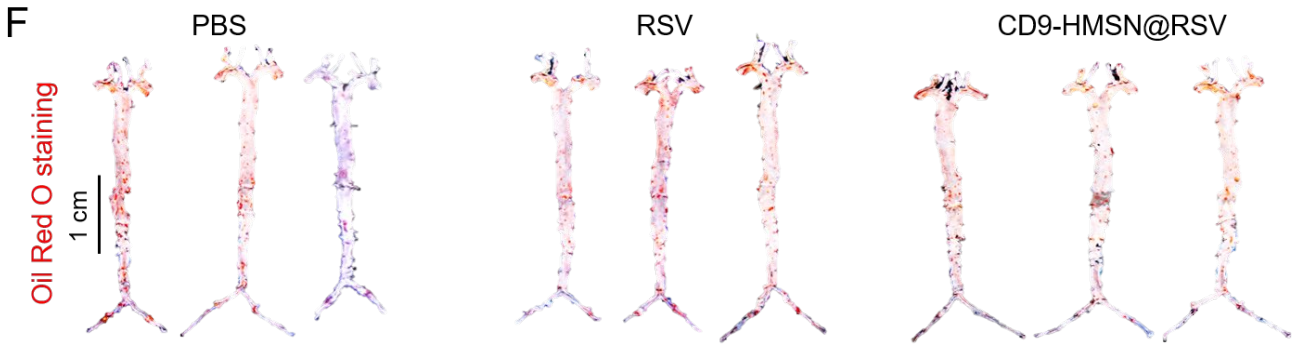


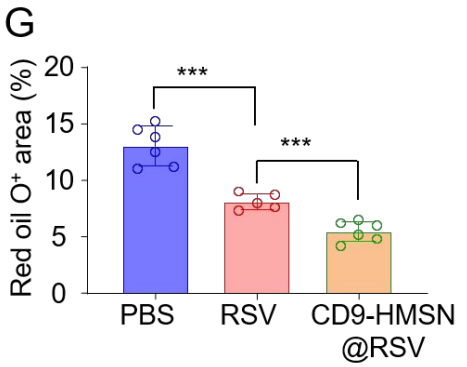






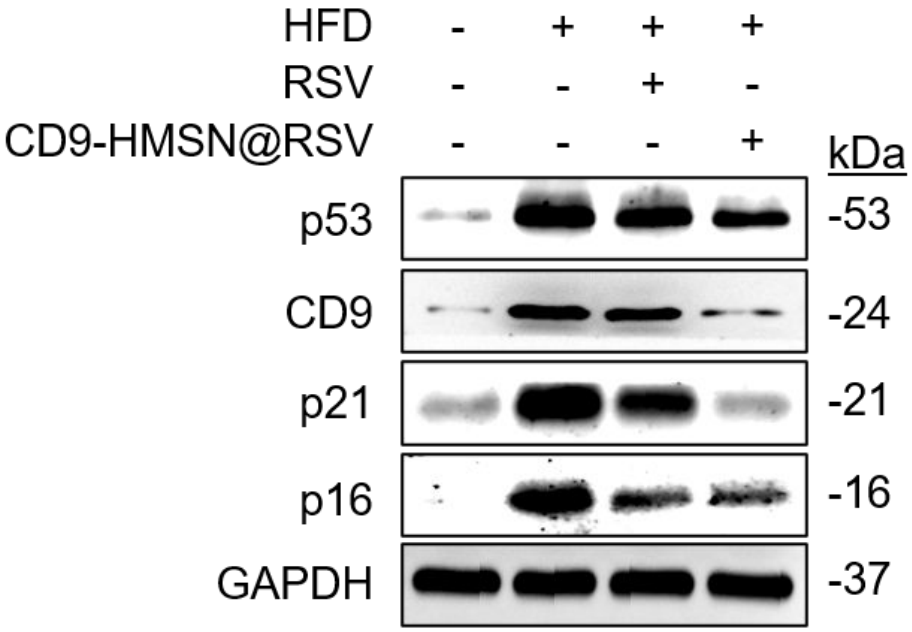


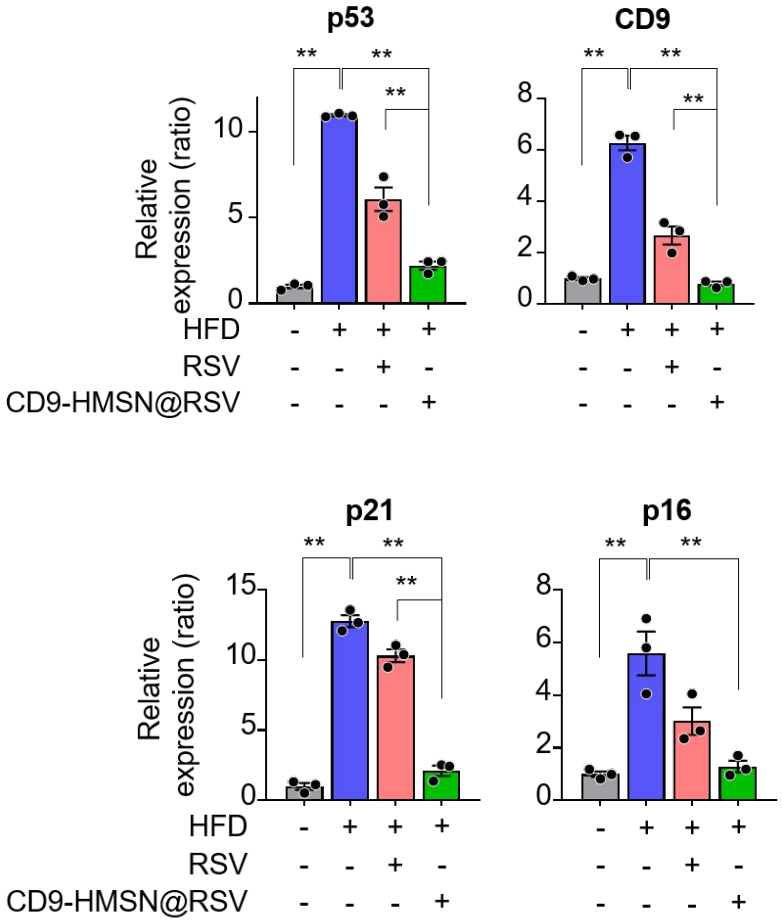




CD9-HMSN@RSV treatment attenuated the number of plaques in the atherosclerotic lesion

In vivo expression of senescence markers in aortas of *ApoE*$^{-/-}$ mice





HFD: high fat diet

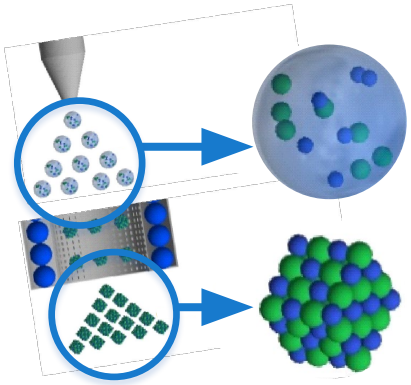
Senescence markers: p53, p21, p16

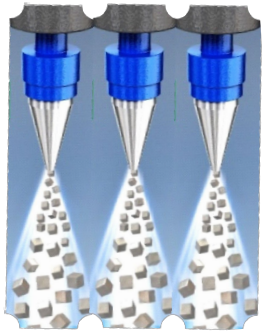
Conclusion

- **We successfully established CD9-antibody-modified nanoparticles, CD9-HMSN@RSV.**
- **It can not only carry a high amount of the anti-senescence drug RSV and release them in an HAase-responsive manner, but also enable precise targeting of senescent plaques and attenuation of senescent cells associated with atherosclerosis in *ApoE*$^{-/-}$ mice.**
- **Treatment of these senescent foamy macrophages with CD9-HMSN@RSV reduced ROS production, decreased LDL oxidation, alleviated the senescence process, and downregulated the secretion of pro-inflammatory cytokines such as TNF-α and IL-6.**
- **This study provides a closer look into the cell markers expressed by senescent cells in aortic plaques, and may signify a more precise and effective strategy for targeting and attenuating atherosclerosis.**

Aerosol-based Synthesis

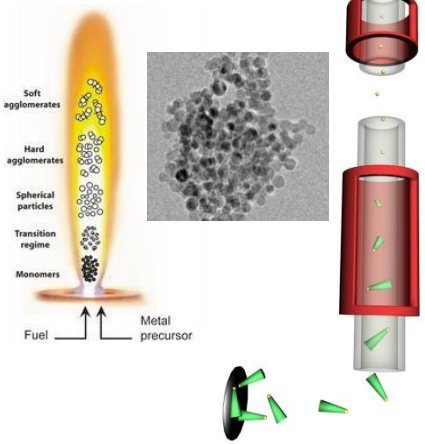
The word **AEROSOL** refers to matter 'floating' in the air (a mixture in which solid or liquid or combined solid-liquid particles are suspended in a fluid).



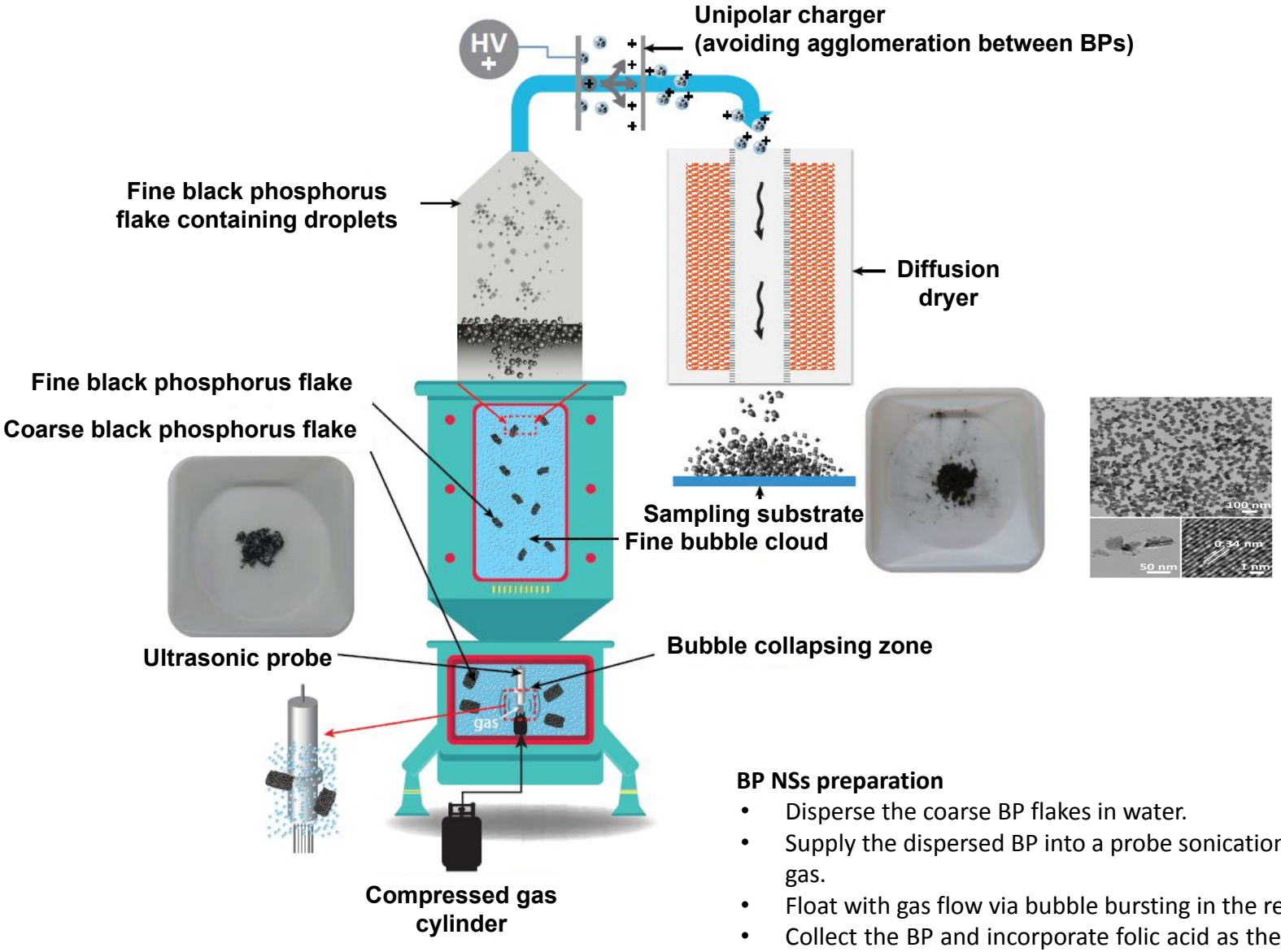


Why? The usual routes to fabricate novel nanoscale materials and patterns are wet chemical and lithographical methods; however, the synthesis method involves multiple chemical steps and high vacuum. In contrast to these classical methods, **aerosol processing involves far fewer preparation steps.**

The combination of aerosol processing and more conventional chemical routes has the potential to bring a **"wind of change" to the preparation of advanced nanoscale materials** and patterns.



Preparation of BP Nanosheets: Ultrasonic bubble bursting method



BP NSs preparation

- Disperse the coarse BP flakes in water.
- Supply the dispersed BP into a probe sonication reactor with Ar gas.
- Float with gas flow via bubble bursting in the reactor.
- Collect the BP and incorporate folic acid as the target, Dox and PD-L1 siRNA as the drugs.

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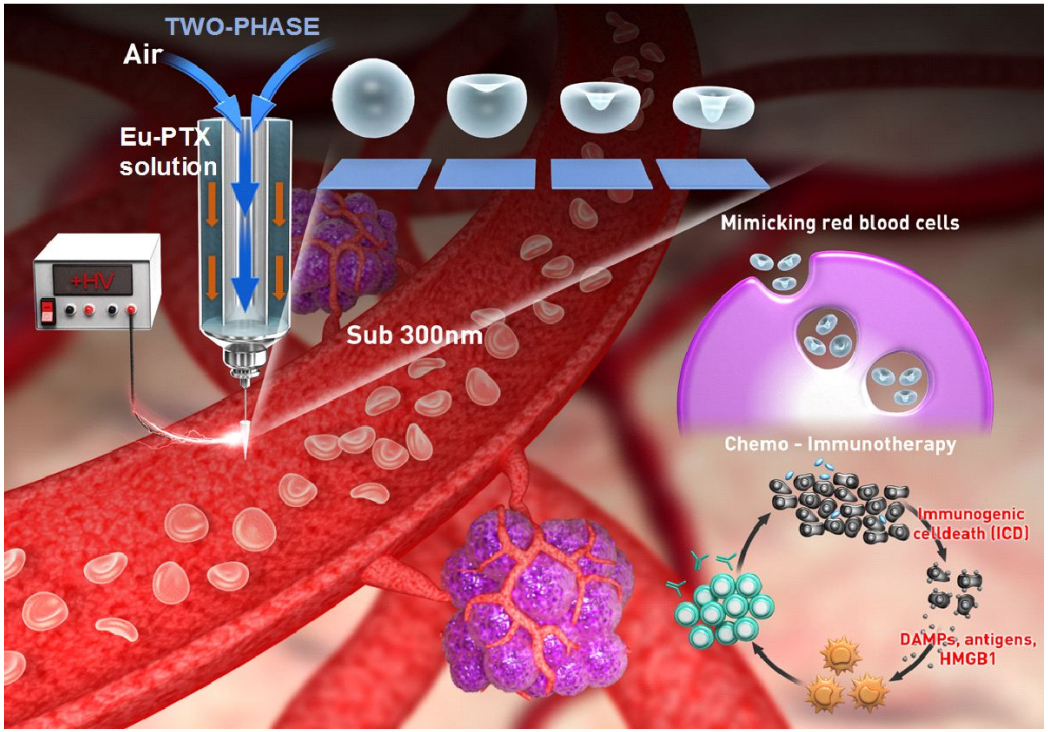
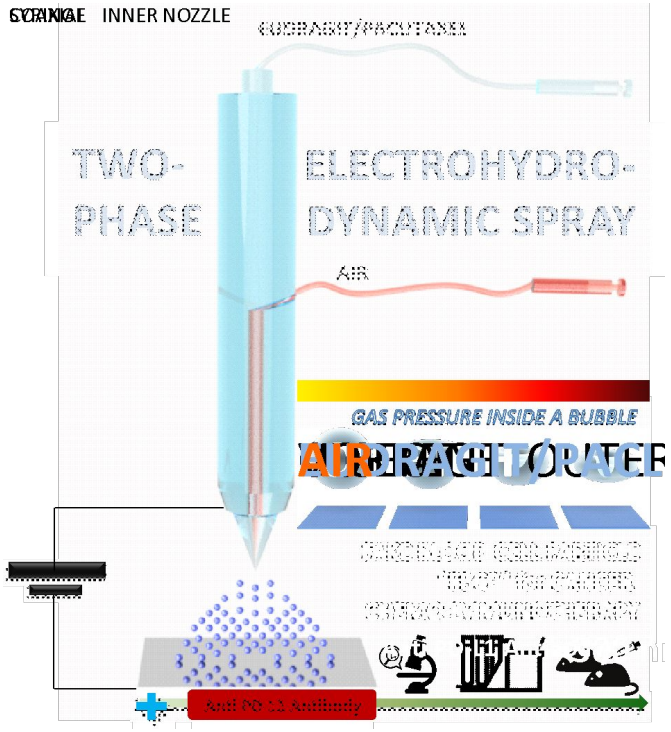
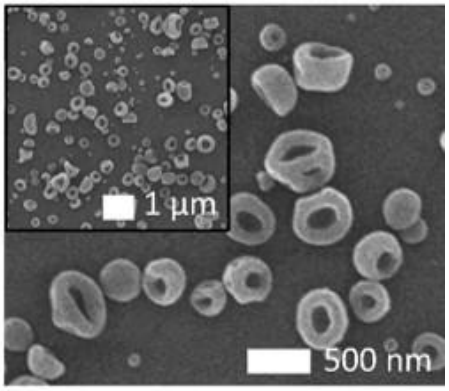
Artificial Nanoscale Erythrocytes for Enhancing Cancer Immunotherapy







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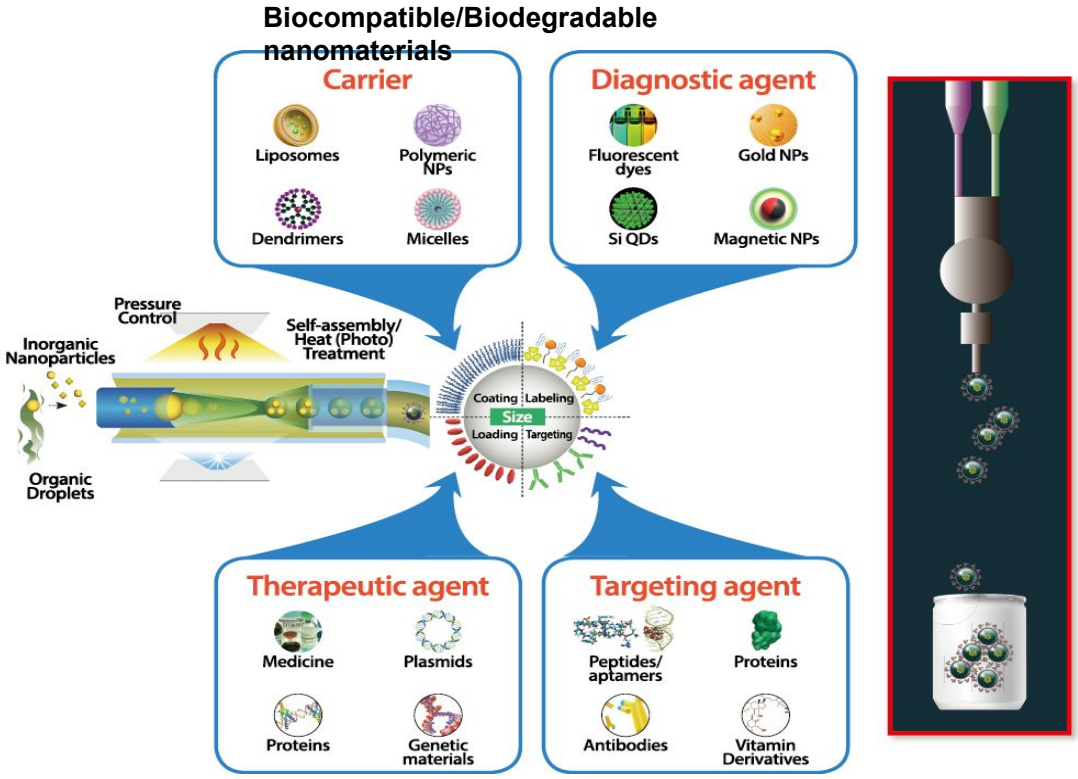

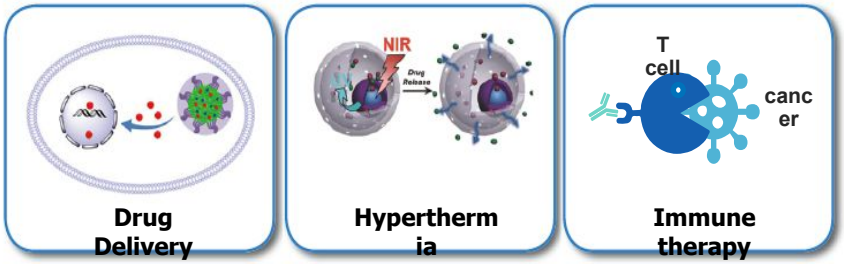

Collaborators

Dr. Han-Gon Choi, Hanyang University
Dr. Jeong Hoon Byeon, Yeungnam University
Dr. Jee-Heon Jeong, Yeungnam University
Dr. Sae Kwang Ku, Dague Hanny University

Postdoc

Dr. Basavaraj Patil

PhD Students

Kishwor Poudel
Srijan Maharjan
Prageena Karmacharya
Amit Shrestha

MS Students

Bao Loc Nguyen
Heesang Chun
Ngoc Duy Le
Jong Sze Ying

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