Biomaterials for translational regenerative medicine

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Research interests

1. Materials design: synthesis of biomaterials with different properties

Small 11.34 (2015): 4284-91

ACS Appl Mater Inter, 2018, 10(43): 36615-21

Theranostics, 2017, 7(6), 1650.

Adv Healthc Mate, 2016, 5,108-118.

Biomaterials. 2020: 8:120378

2. Scaffolds fabrication: microspheres, nanofibers, injectable and 3D printable scaffolds

Adv Funct Mater,2017, 27(2): 1604617.

Adv Funct Mater, 2016, 26(17), 2809-2819

Biomaterials, 2016, 83, 169-181

Biomaterials, 2015,61, 61-74

NPG Asia Mater, 2019, 11(1): 3.

ACS Appl Mater Inter, 2019, 11(37): 33716-24

3. Biomaterial application: tissue regeneration (bone, tendon, skin) and cancer therapy

Adv Funct Mater, 2018, 29(4): 1807559

Angewandte Chemie, 2018, 57(26), 7878-82

Biomaterials, 2019, 178: 1-10

Biomaterials, 2019, 194: 117-129

Small, 2019: 1903939

Nanoscale, 2018;11(1):60-71.

Research interests

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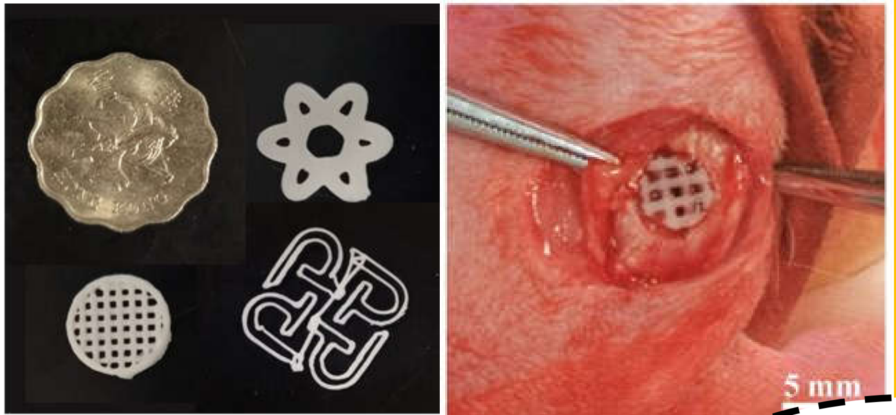
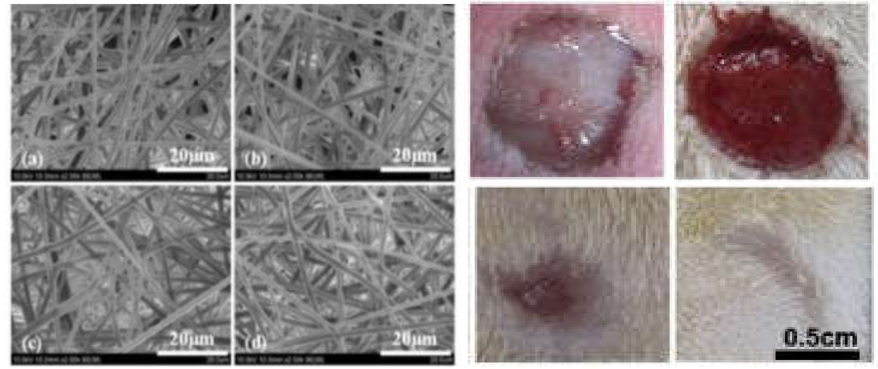
3D Printing



Electrospinning

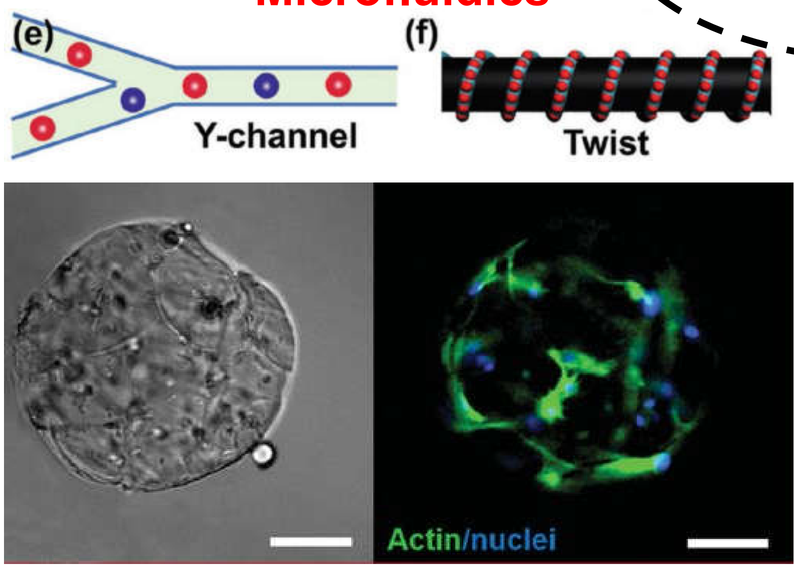


Materials design

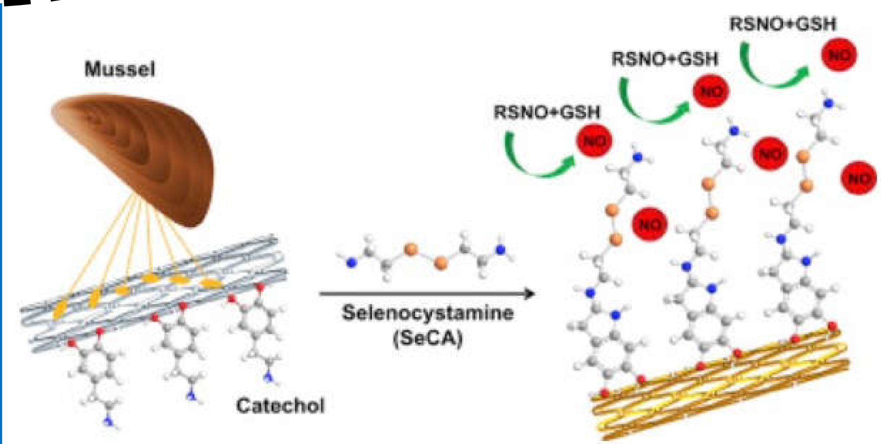
Scaffolds fabrication

Biomaterial application

Microfluidics

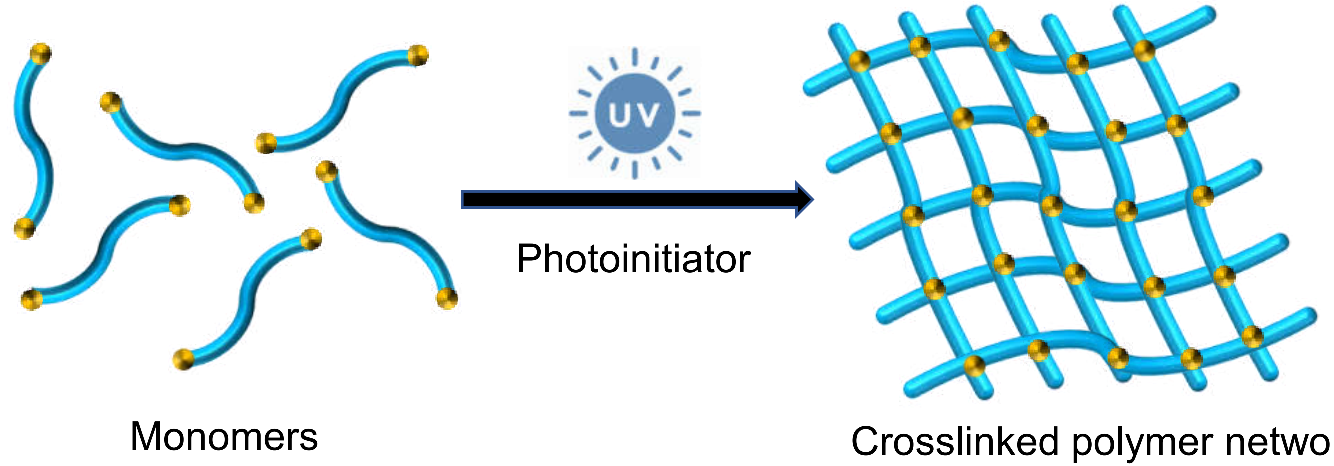


Biocoating



Photocrosslinkable polymers- Photopolymerization

- Chain-growth polymerization which is initiated by the absorption of visible or ultraviolet light.



Photocrosslinkable polymers

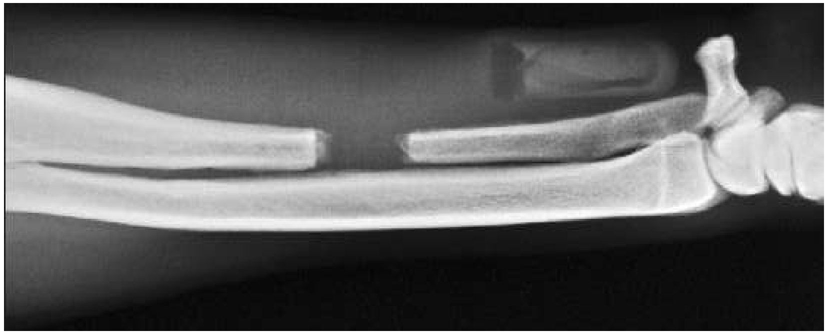
Synthetic polymers

- Poly(ethylene glycol) diacrylate
- Propylene fumarate
- Methyl methacrylate

Natural polymers

- Methacrylated alginate
- Acrylated hyaluronic acid
- Methacrylated gelatin

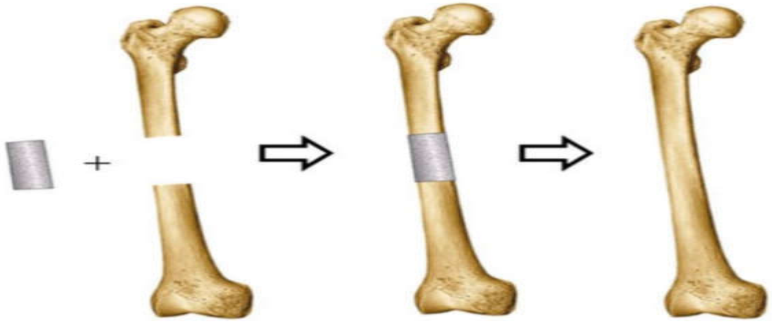
Critical bone defect



Theyse et al. JBMM, 1;24(4):266-73.

- ➢ Caused by injuries, diseases or trauma
- ➢ Unable to self repair or remodel

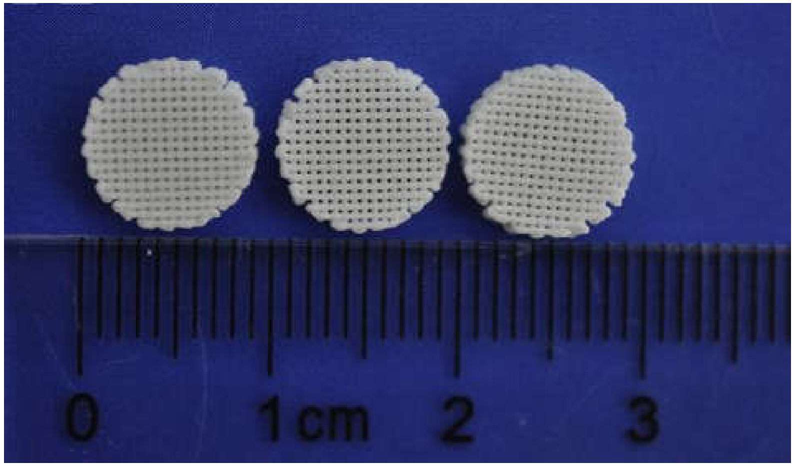
Grafting treatments for bone defect



Rossi et al. JTERM. 2015;9(10):1093-119.

- ➢ Autograft
  - ✓ **Gold standard**
- ➢ Allograft
  - ✓ **No donor morbidity**

✘ **Limited availability**

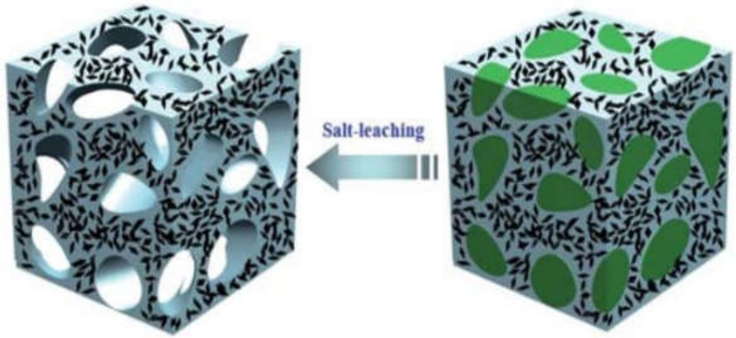


Chen et al. Biomaterials. 2019 1;196:138-50.

Bone tissue engineering (TE)

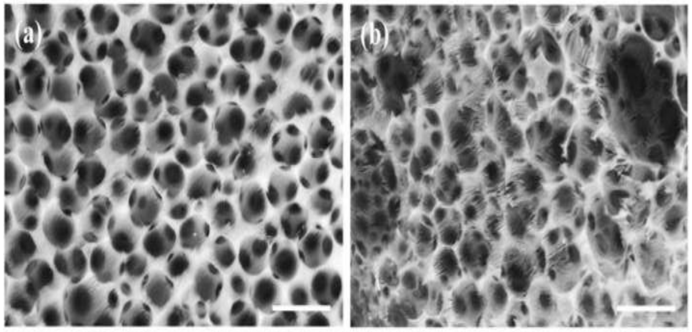
- ➢ Requirements of bone scaffolds
  - ✓ **High biocompatibility**
  - ✓ **Osteoconductivity**
  - ✓ **Sufficient mechanical property**
  - ✓ **Distributed, interconnected pores**
  - ✓ **Degradability**

Particle leaching
（颗粒浸出）



Yan et al. JMC. 2012;22(36):18772-4.

Gas foaming
（气体发泡）

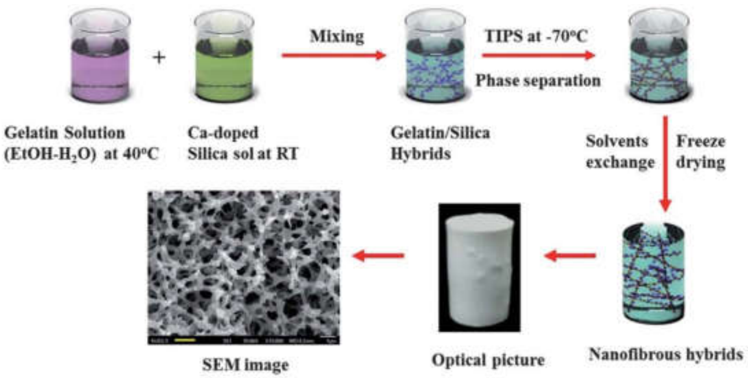


Costantini et al. MSE, 2016 1;62:668-77.

Freeze drying
（冷冻干燥）

Michael et al. Int J Nanomedicine. 2012; 7: 2087–2099.

Phase separation
（相分离）



Kim et al.J. Mater. Chem., 2012, 22, 14133–14140

✘ **Poor control over porosity and interconnectivity**

3D printing → ***Complex 3D structures with controlled architecture***

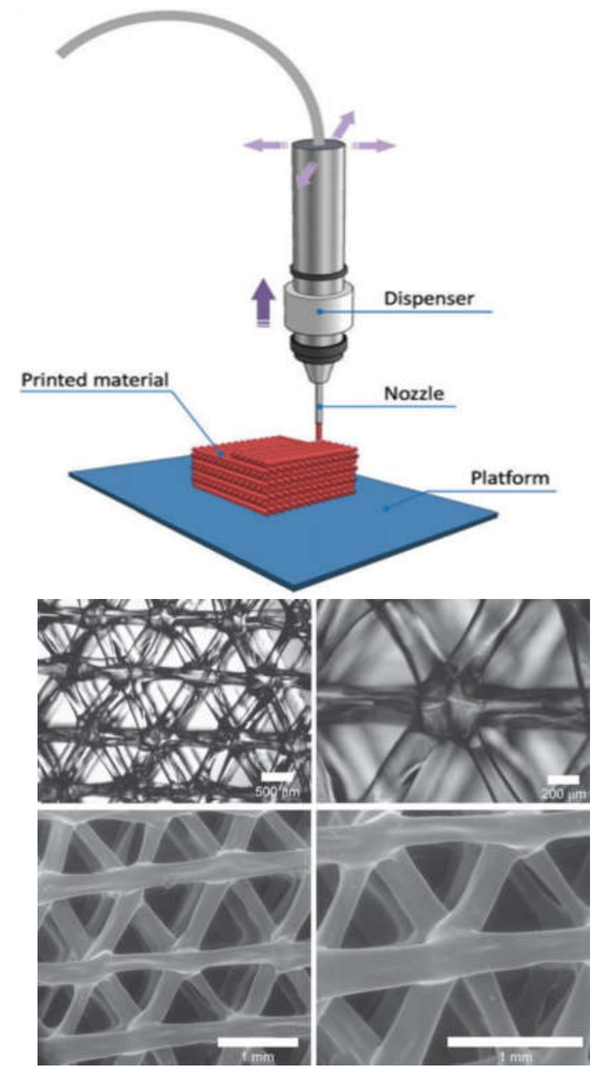
↓

Direct Ink Write (DIW)

- **Extrusion-based 3D printing**
  - ✓ Simple set-up, low operation cost;
  - ✓ High printing speed;
- **Strategies to tune the materials rheology**
  - ✓ High temperature melting;
  - ✓ Volatile solvent;
  - ✓ Photocrosslinkable monomers;

✘ **Cannot load bioactive molecules**

- **Photocrosslinking-assisted DIW**
  - ✓ Controllable curing kinetics;
  - ✓ Increased bioactivity



Ambrosi et al. Chemical Society Reviews. 2016;45(10):2740-55

Photocrosslinkable DIW inks

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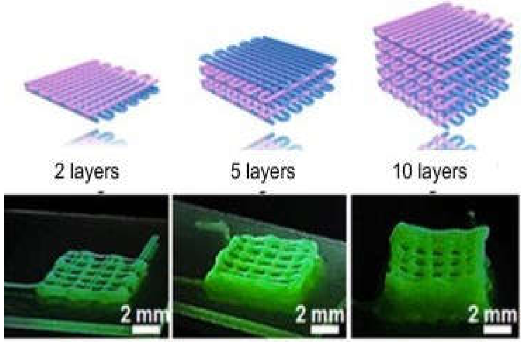
Natural polymer derivatives

- ***Methacrylated alginate, Gelatin methacryloyl (GelMA), etc.***

  ✓ Excellent biocompatibility

  ✘ **Low mechanical property**



J. Jang et al. Biomaterials 156 (2018) 88-106

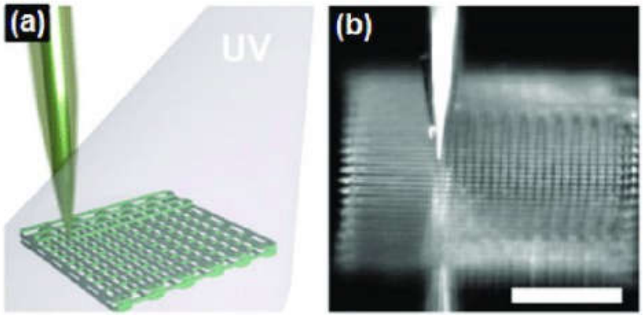
Synthetic polymers

- ***Poly (hydroxyethyl methacrylate) (pHEMA), polymethylmethacrylate (PMMA), etc.***

  ✓ High mechanical property

  ✘ **Non-degradability**

  ✘ **High exothermic heat during crosslinking**



Wentao Shi et al. Eur. J. Med. Res. 1.3 (2015): 3-8.

Composites

- ***Polymer + Ceramics (e.g., hydroxyapatite)***

  ✓ **Chemical similarity to natural bone**

  ✘ **Ceramics aggregation**

  ✘ **Weak polymer/ceramics interaction**

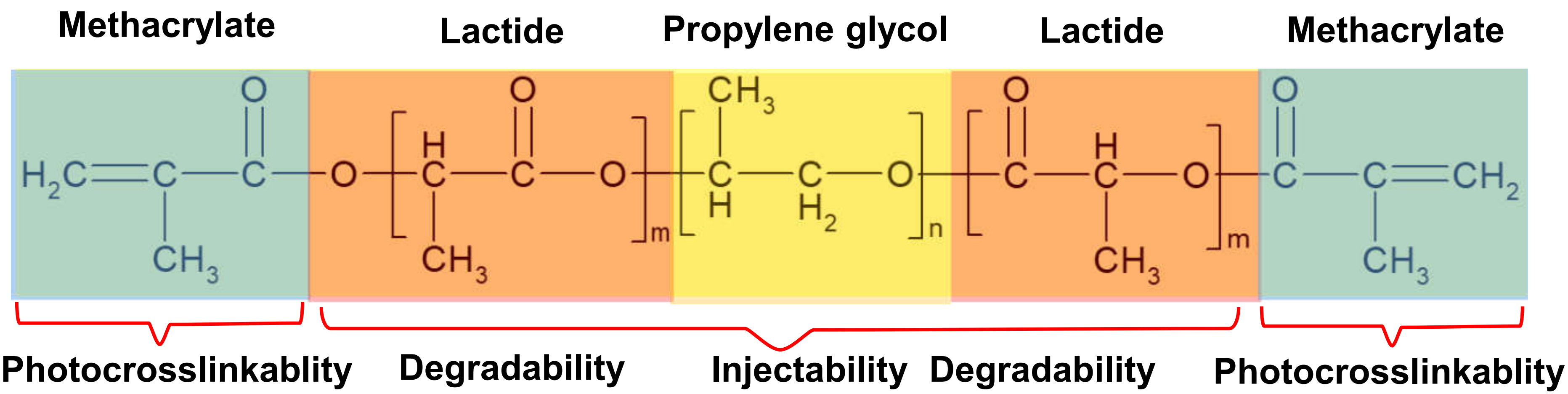
ACS AMI. 2014 29;6(15):13061-8.

Project Aim

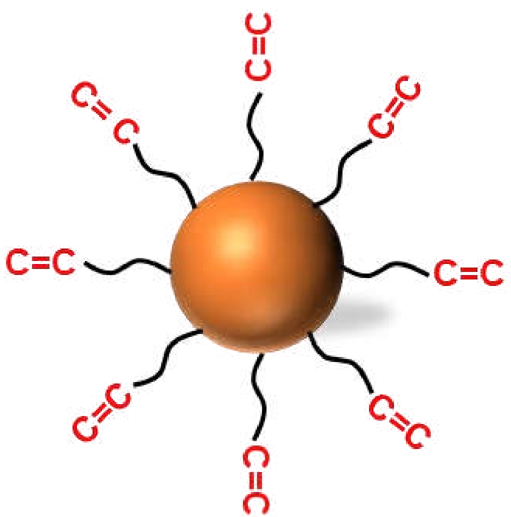
To develop a photocrosslinkable and 3D printable composite materials with excellent mechanical, biological and biomolecule releasing properties for bone TE.

Composite materials

Poly (lactide-co-propylene glycol-co-lactide) dimethacrylates (P_mL_nDMA)



Hydroxyethyl methacrylate-functionalized hydroxyapatite (nHAMA)



- ✓ **Crosslink with polymer**
- ✓ **Increase mechanical properties of polymer;**
- ✓ **Modulate the rheology behavior of the composites;**
- ✓ **Buffer the acidic product during polymer degradation;**

Biomaterials. 2020: 8:120378

Project hypothesis

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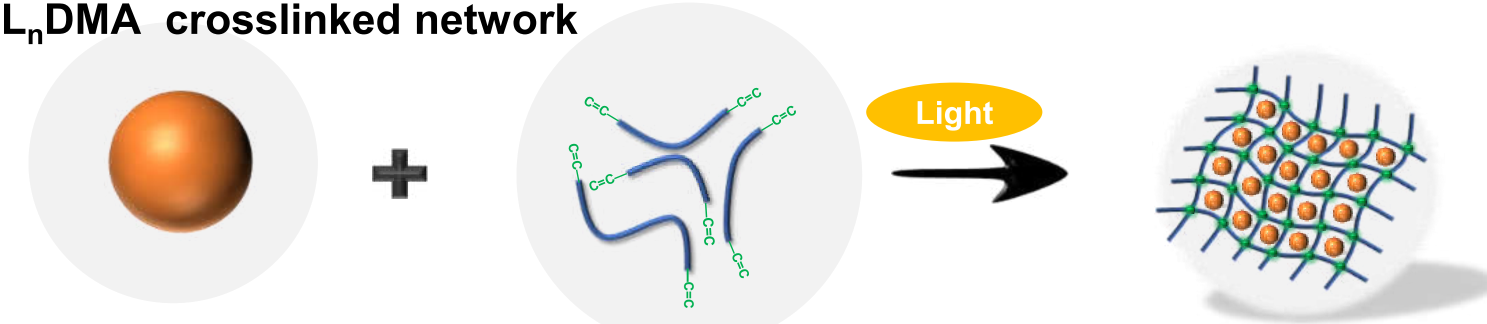
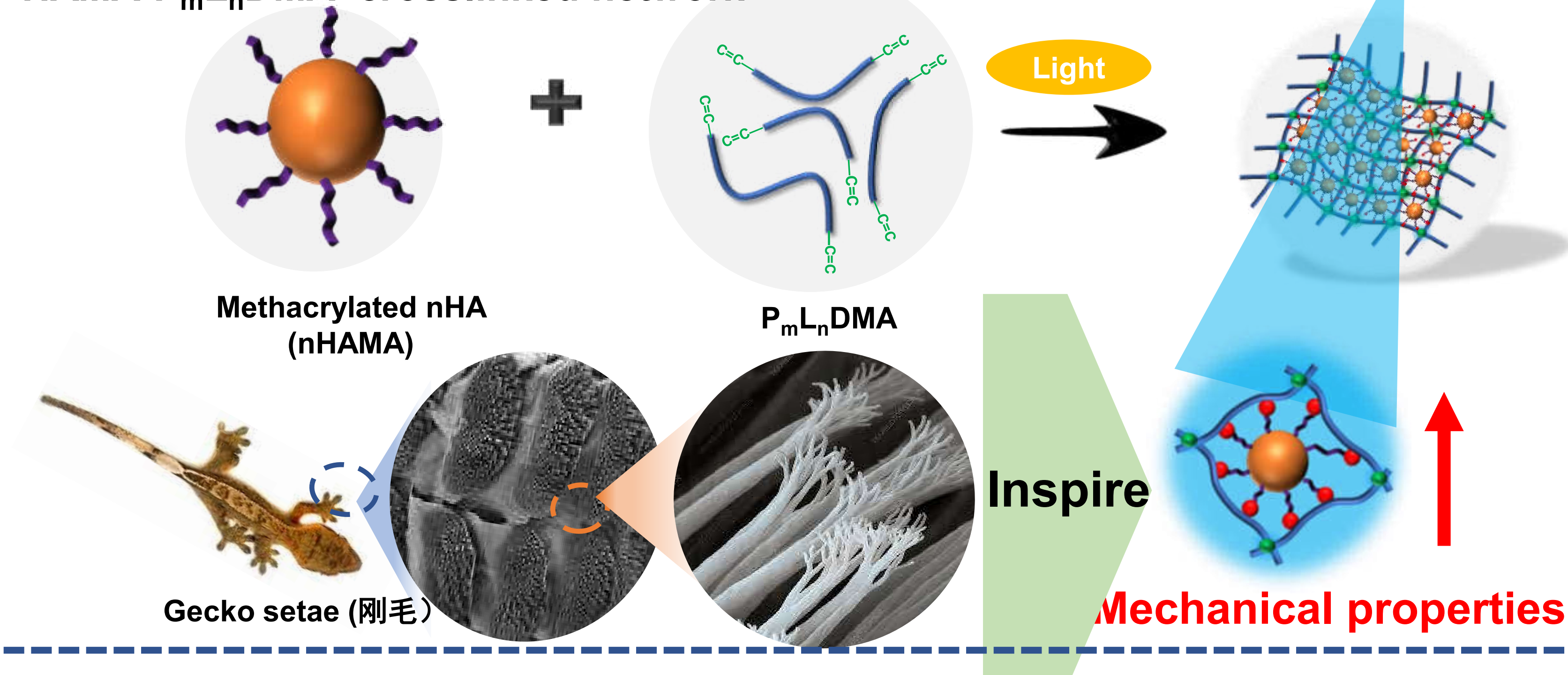
HA-P_mL_nDMA crosslinked network



nHA

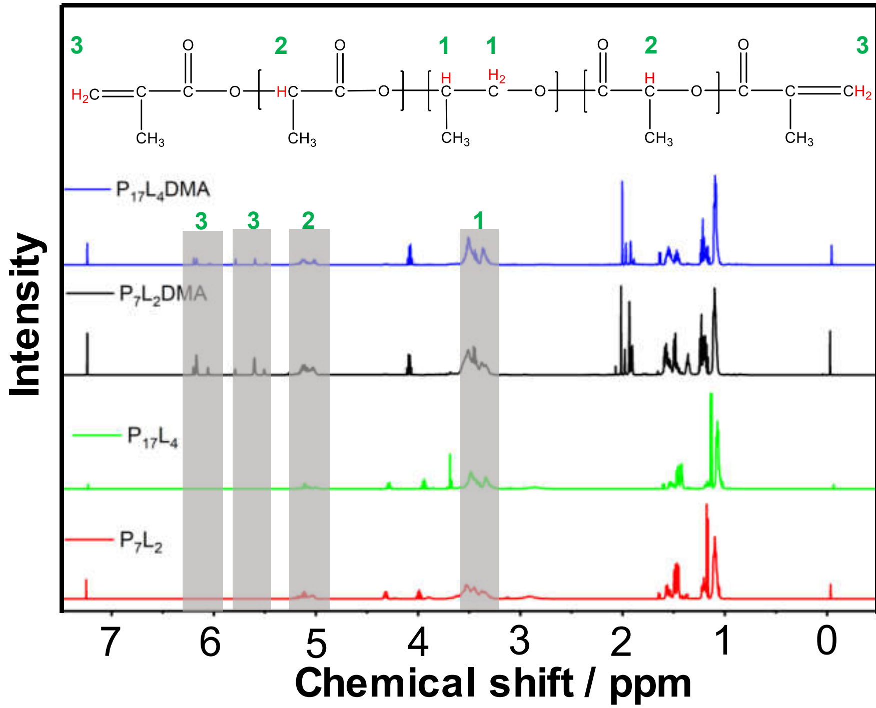
P_mL_nDMA

HAMA-P_mL_nDMA crosslinked network



Result 1: Characterizations of P_mL_nDMA using ^1H NMR

Nuclear Magnetic Resonance spectroscopy (NMR)



^1H NMR calculation

Theoretical formula	P_7L_2DMA	$P_{17}L_4DMA$
N_{LA}	1.9	3.7
E_{LA} (%)	95	92.5
N_{MA}	1.6	1.5
E_{MA} (%)	80	75
Observed formula	$P_7L_{1.9}MA_{1.6}$	$P_{17}L_{3.7}MA_{1.5}$

Successful synthesis of $P_{17}L_4DMA$ and P_7L_2DMA with high efficiency

Result 2. Characterizations of nHAMA using FTIR and TGA

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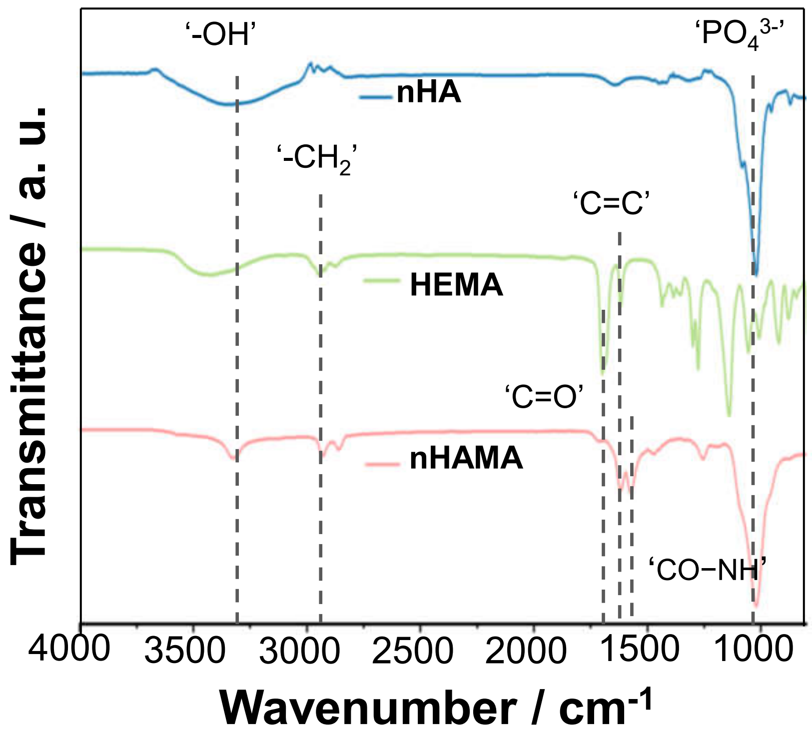
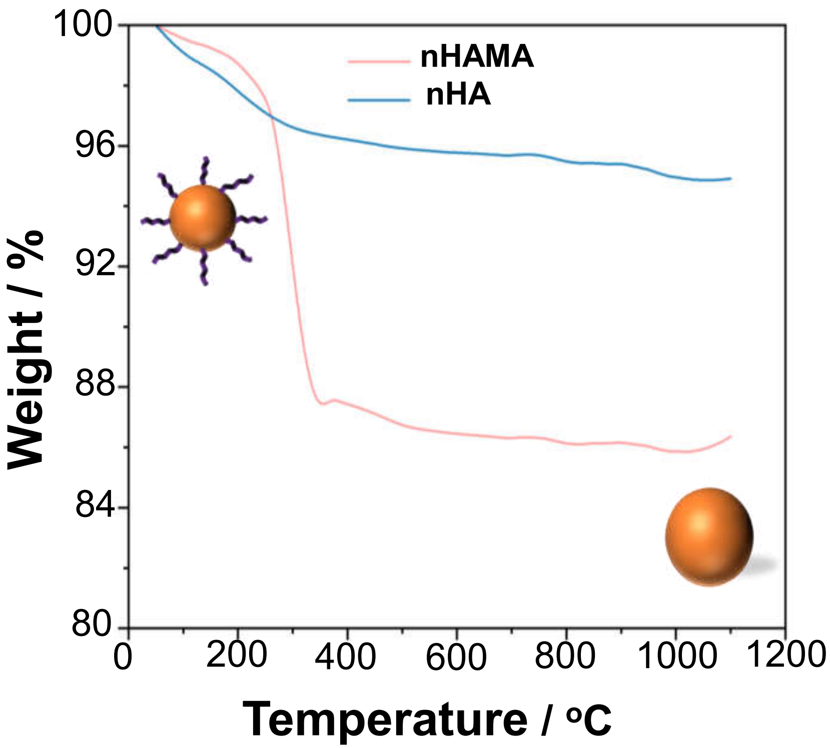
Fourier-transform infrared spectroscopy (FTIR)

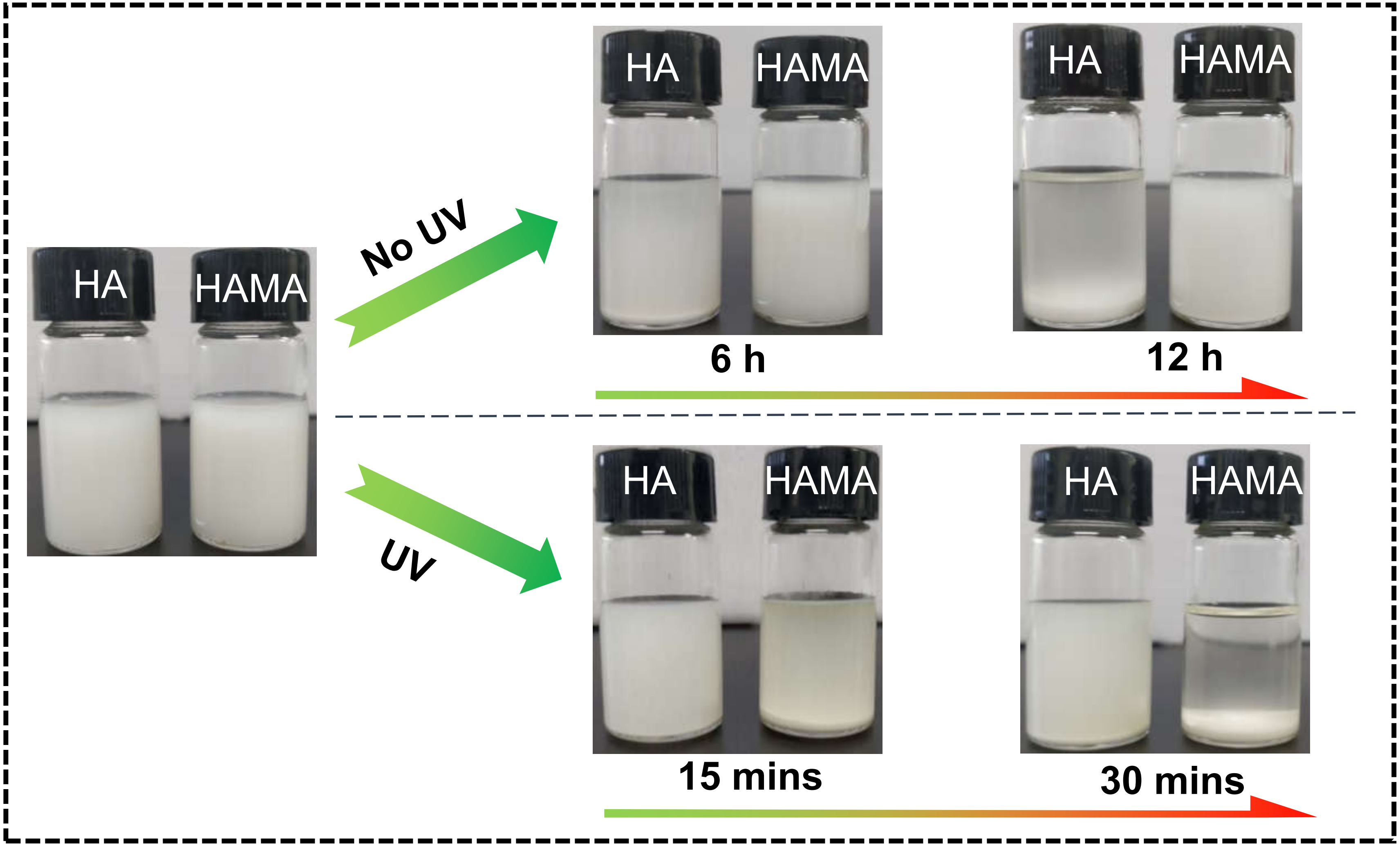


Thermogravimetric analysis (TGA)



Methacrylate brushes with 7.6 wt% has been coupled to nHA successfully;

Result 3. Colloid stability of HAMA in HFIP with photoinitiator



- ✓ The hydroxyethyl methacrylate groups can **enhance the colloid stability** of nHA;
- ✓ The nHAMA can crosslink with each other under UV radiation;

Result 4. Photocrosslinking kinetics using FTIR

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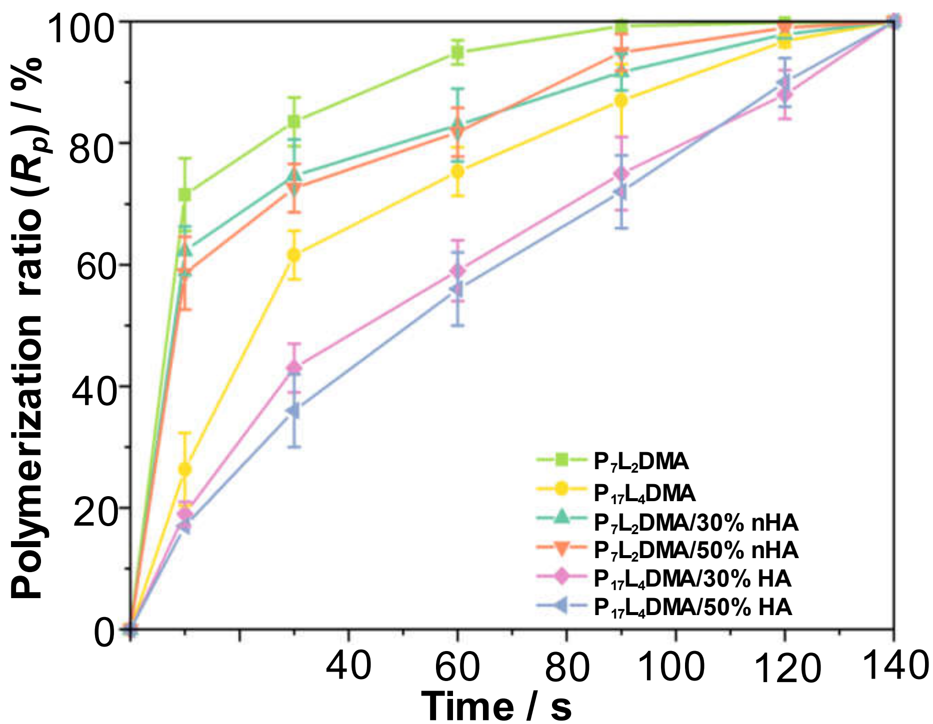
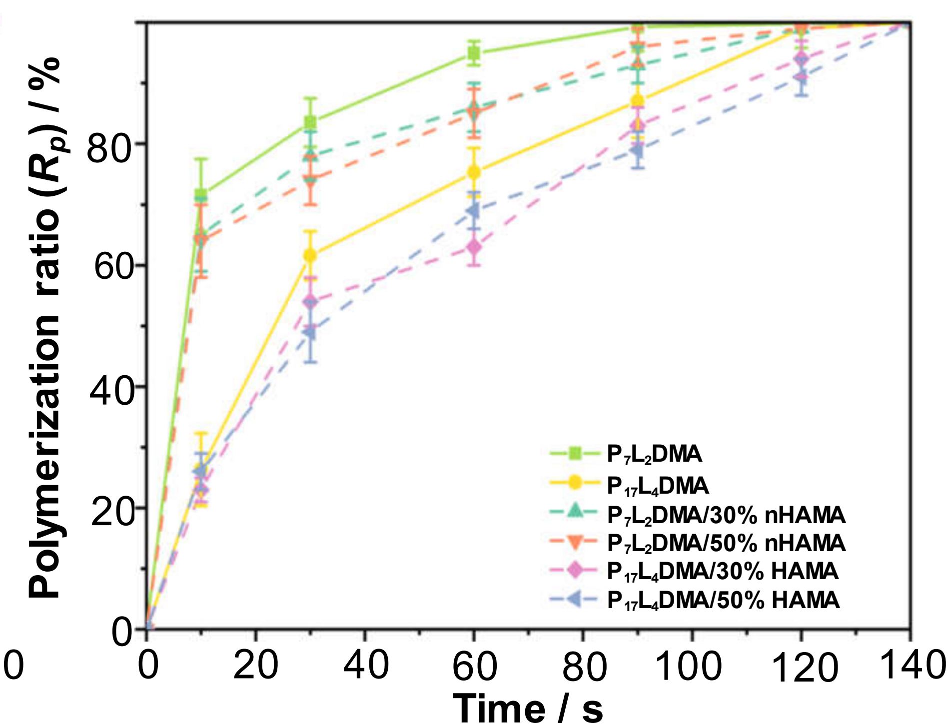
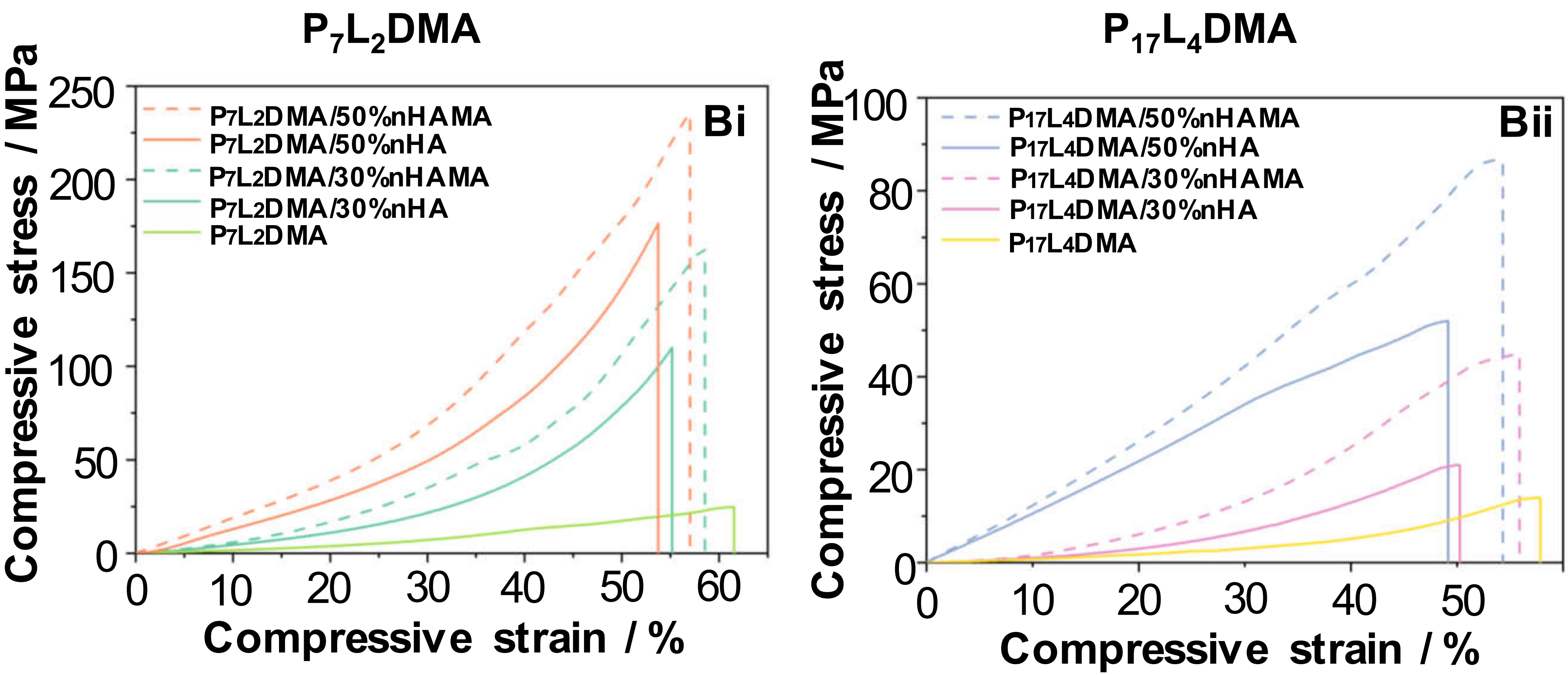




- ✓ P_mL_nDMA fully crosslinked **within 140 s**;
- ✓ P_7L_2DMA possessed higher polymerization rate;
- ✓ No detrimental effect of nHAMA on crosslinking rate;

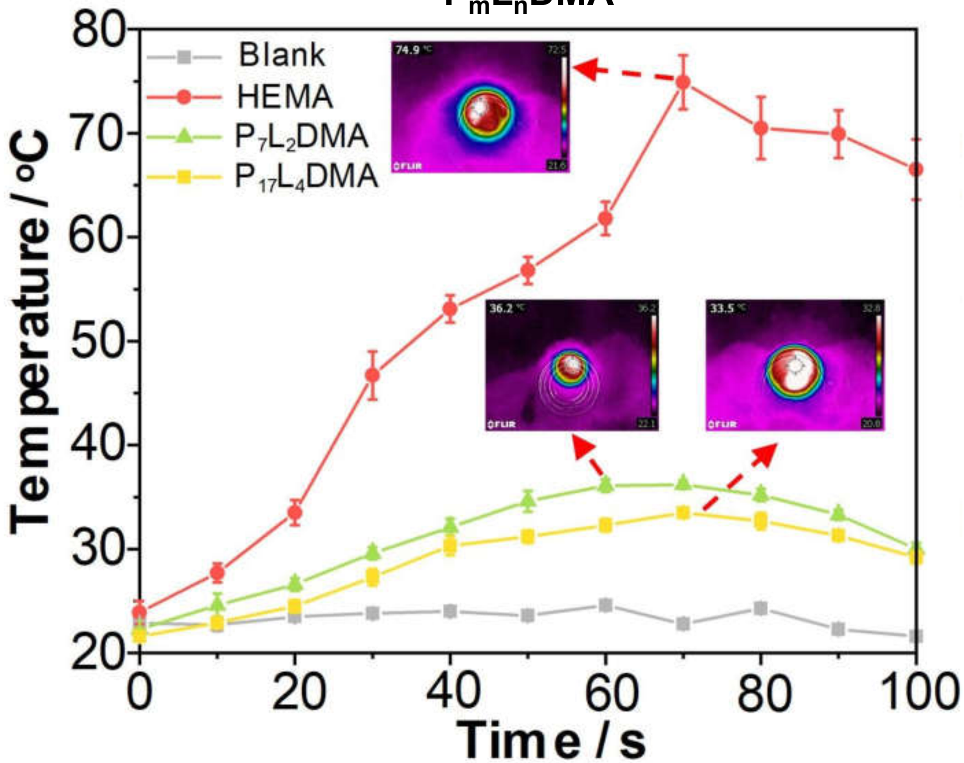
Result 5. Mechanical properties - compression test



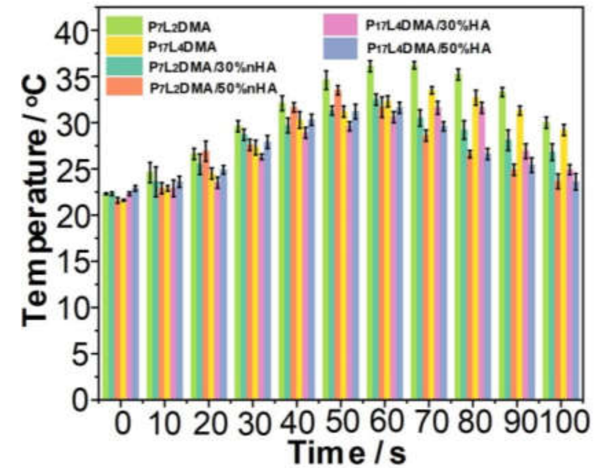
- ✓ **9- fold** increase in compressive modulus (P_7L_2DMA) after incorporating 50% nHAMA (**from 43 to 362 MPa**);
- ✓ Comparable to natural trabecular bones **(80 -1000 MPa in compressive modulus**);

- **C2 compact infrared thermal camera (FLIR, US)**

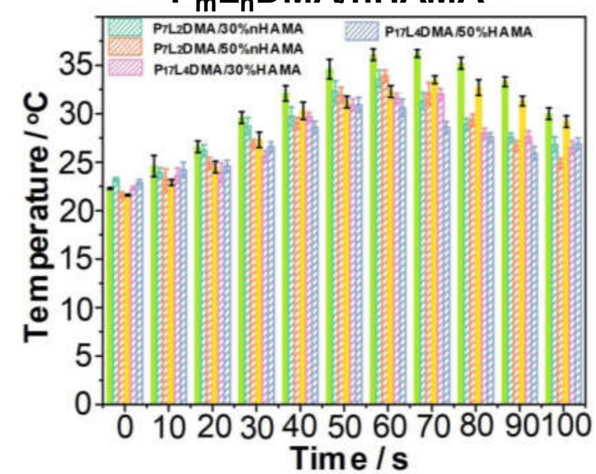
P_mL_nDMA



P_mL_nDMA/nHA



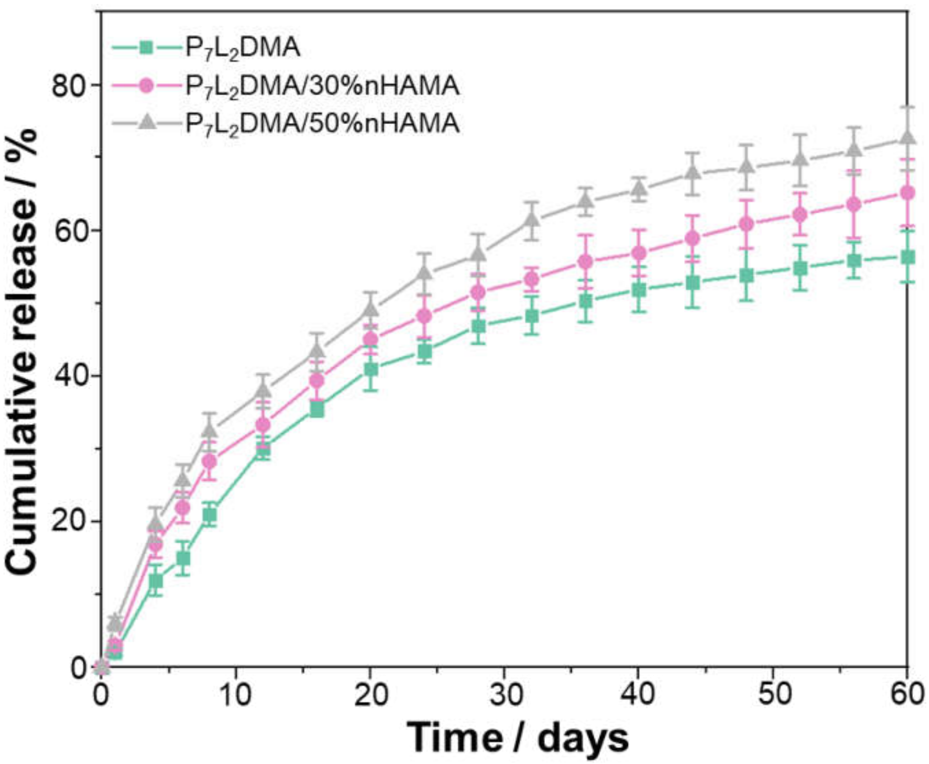
$P_mL_nDMA/nHAMA$

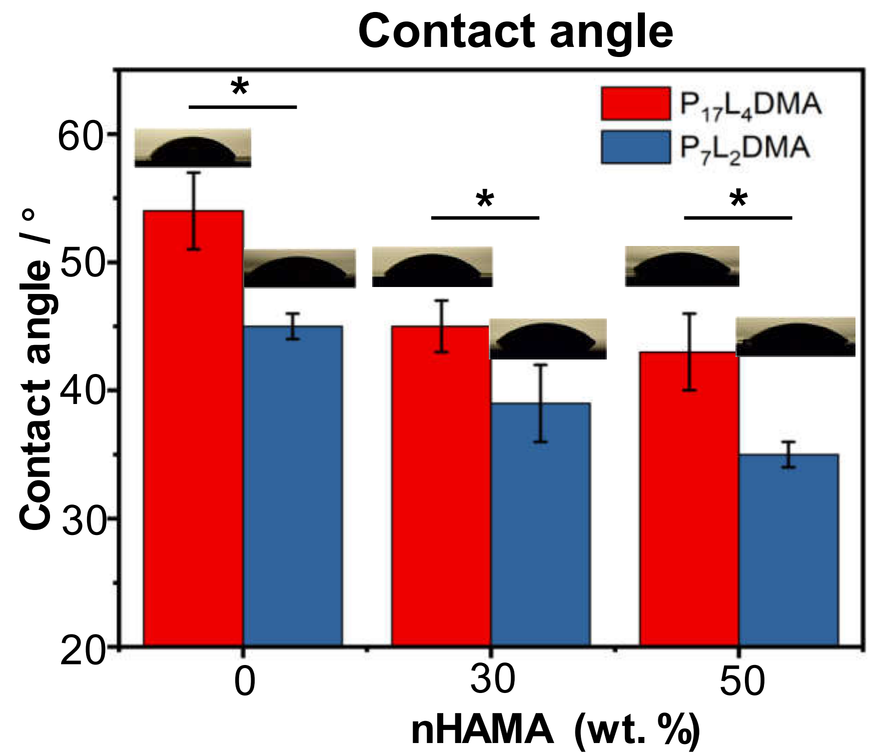


- ✓ P_7L_2DMA presented **slight temperature elevation** compared to (hydroxyethyl) methacrylate (HEMA), peaking at 36.2 ^oC;
- ✓ After incorporating nHAMA, the temperature rise was further reduced;

Result 7. BMP-2 release in $P_7L_2DMA/nHAMA$ composites

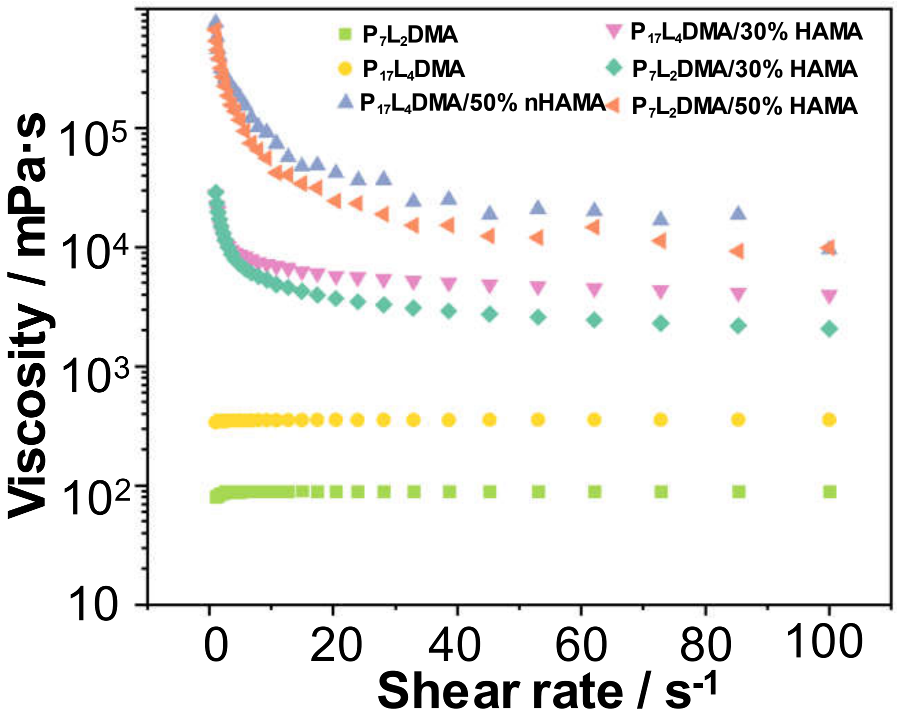
- 30 µg BMP-2 in 100 mg material

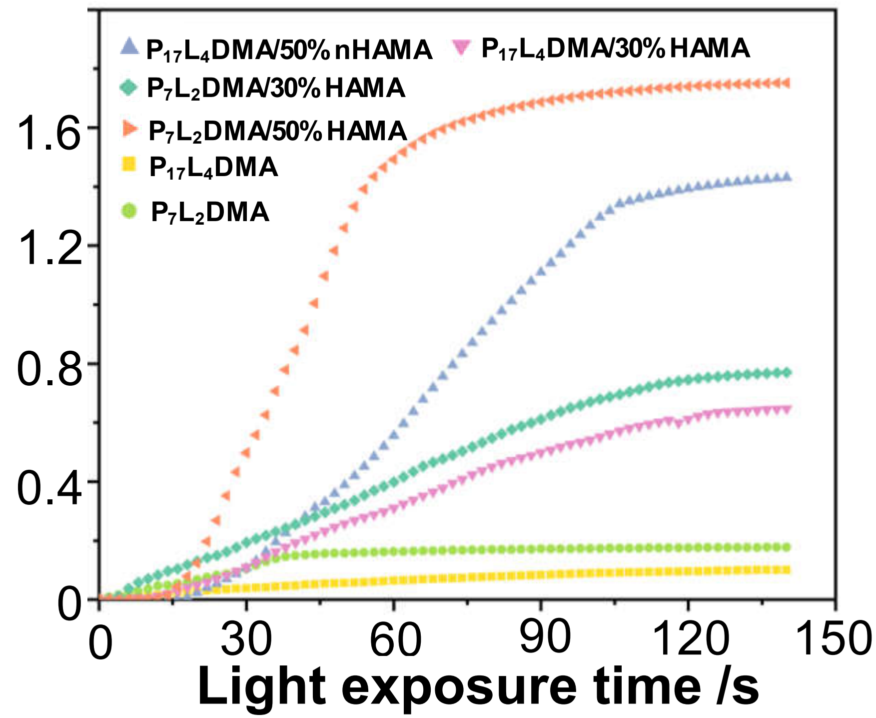




The materials could achieve over 60-day long-term release of BMP-2; Composites have higher BMP-2 release due to increased hydrophilicity.

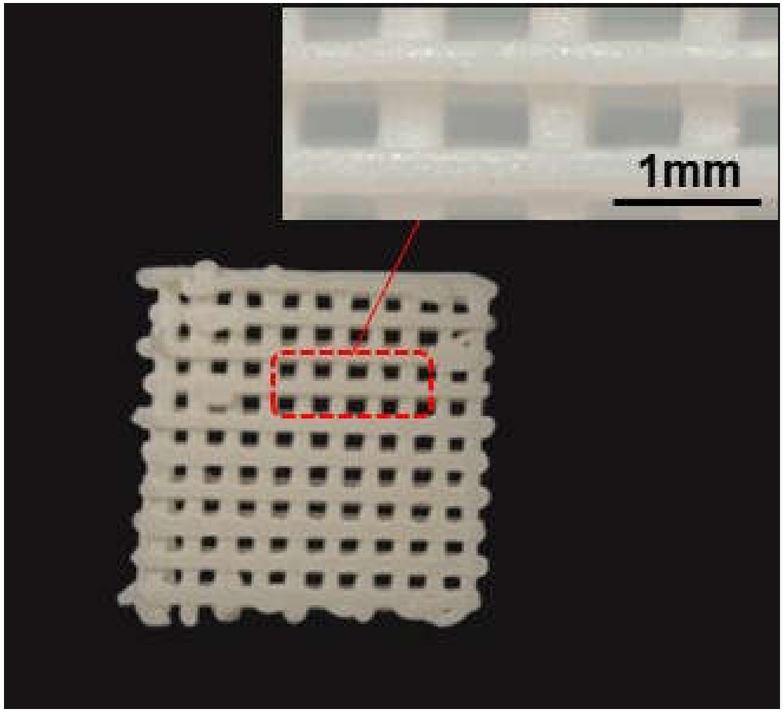
Result 8. Viscosity and rheology using rheometer

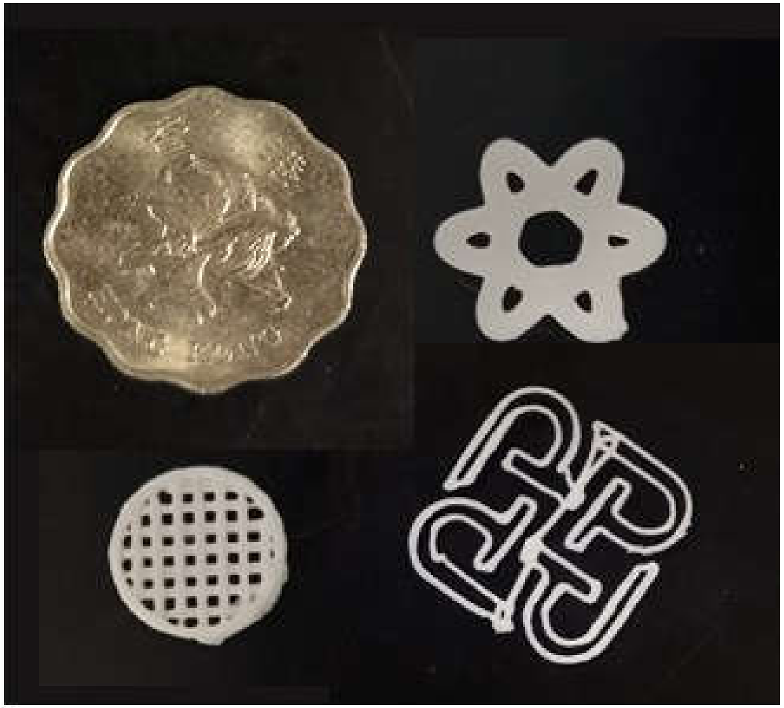




✓ **Tunable rheology behaviors** and **high printability** of the composites;

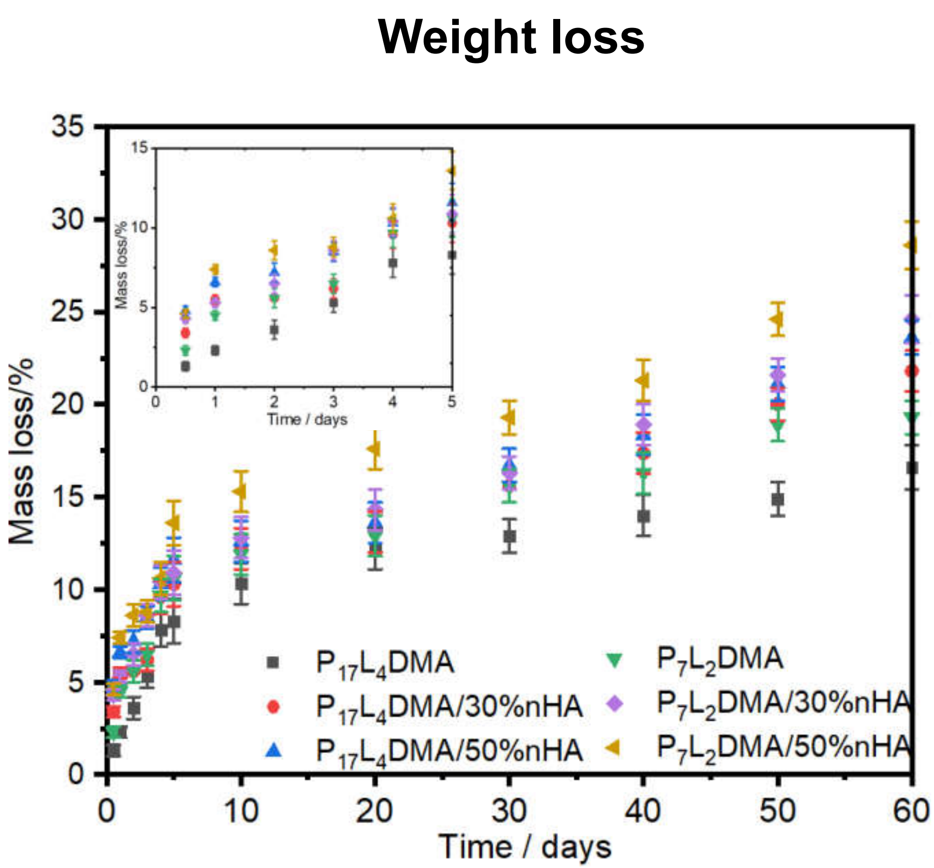
Multi-layer scaffolds and structures

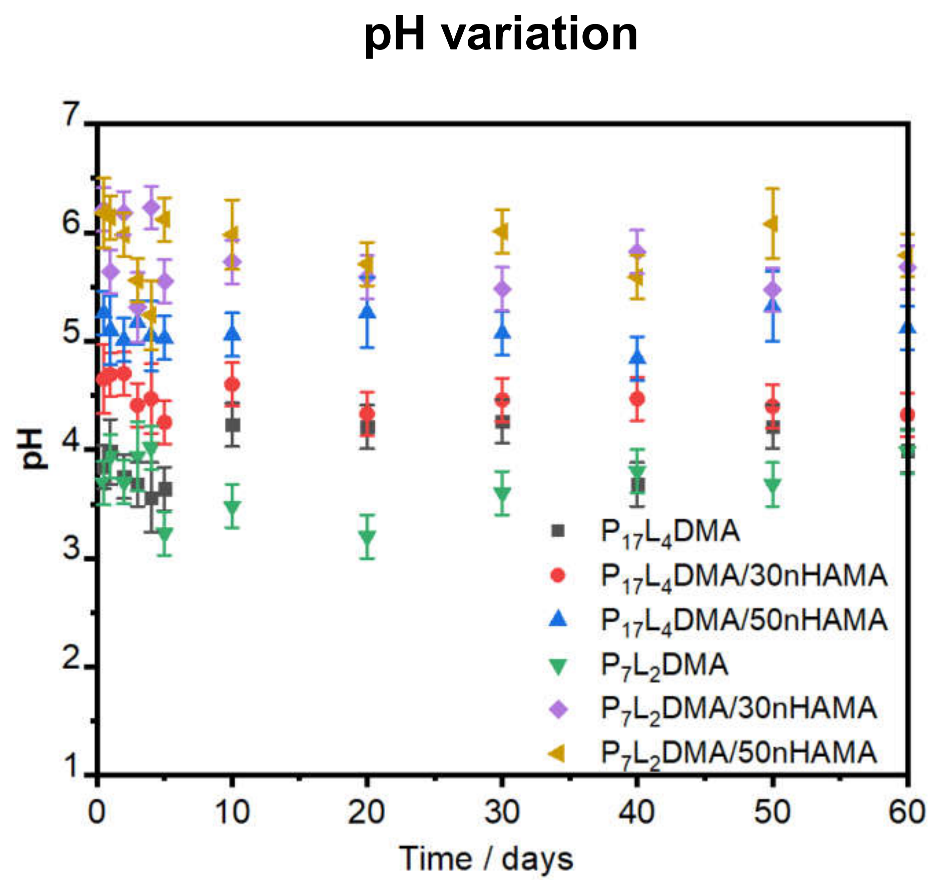




Printed scaffolds with fiber diameter of 350 μm by P_7L_2DMA/50%nHAMA.

Result 10. Degradation kinetics

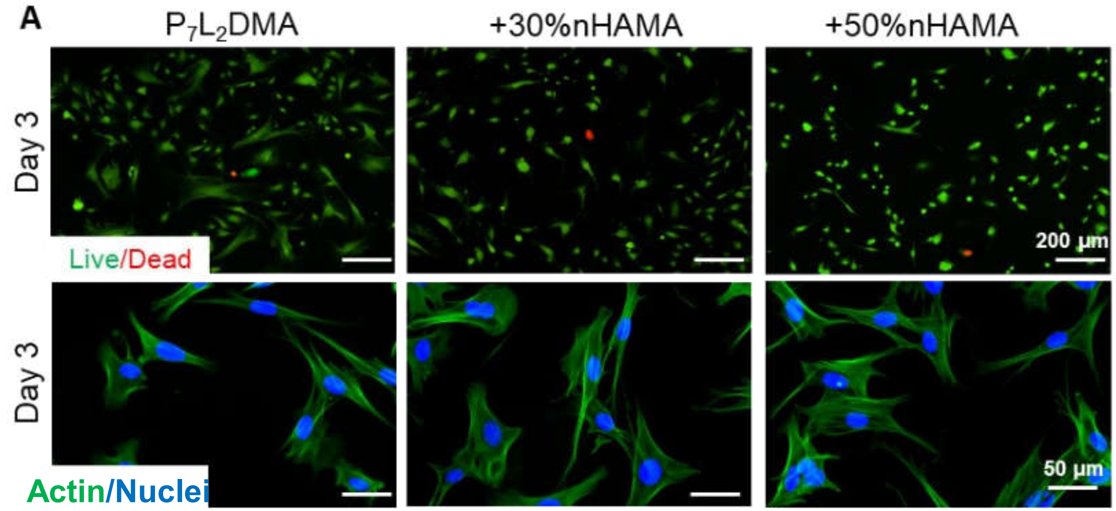




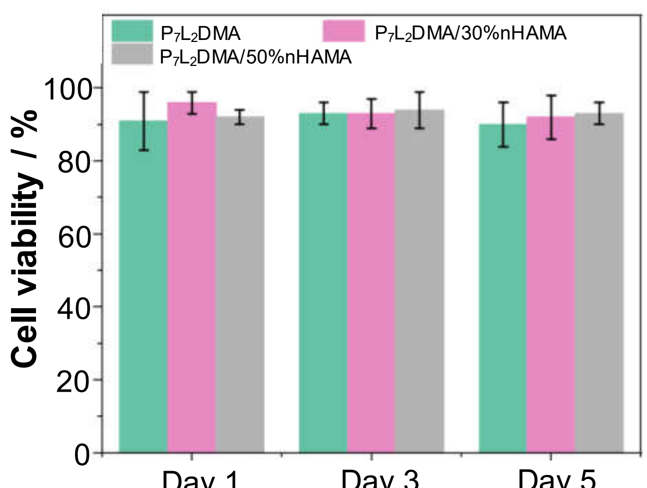
- ✓ Higher concentrations of nHAMA in the polymer were correlated with higher degradation rate;
- ✓ No obvious fluctuations in the pH due to the **buffering effect of HA**;

Result 11. *In vitro* biocompatibility

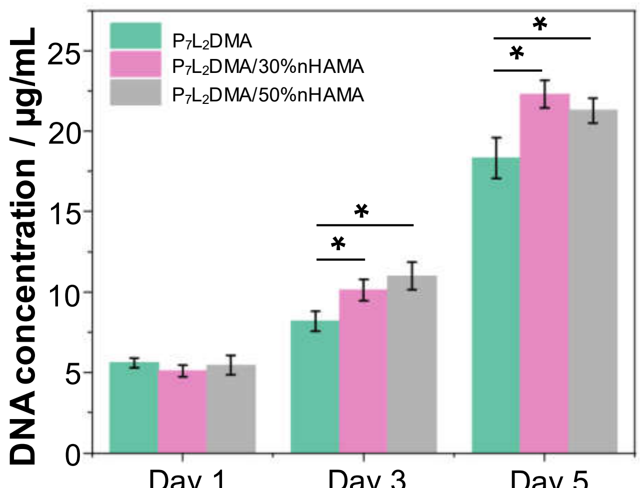
➢ **Rabbit mesenchymal stem cell (rMSC)**



Cell viability



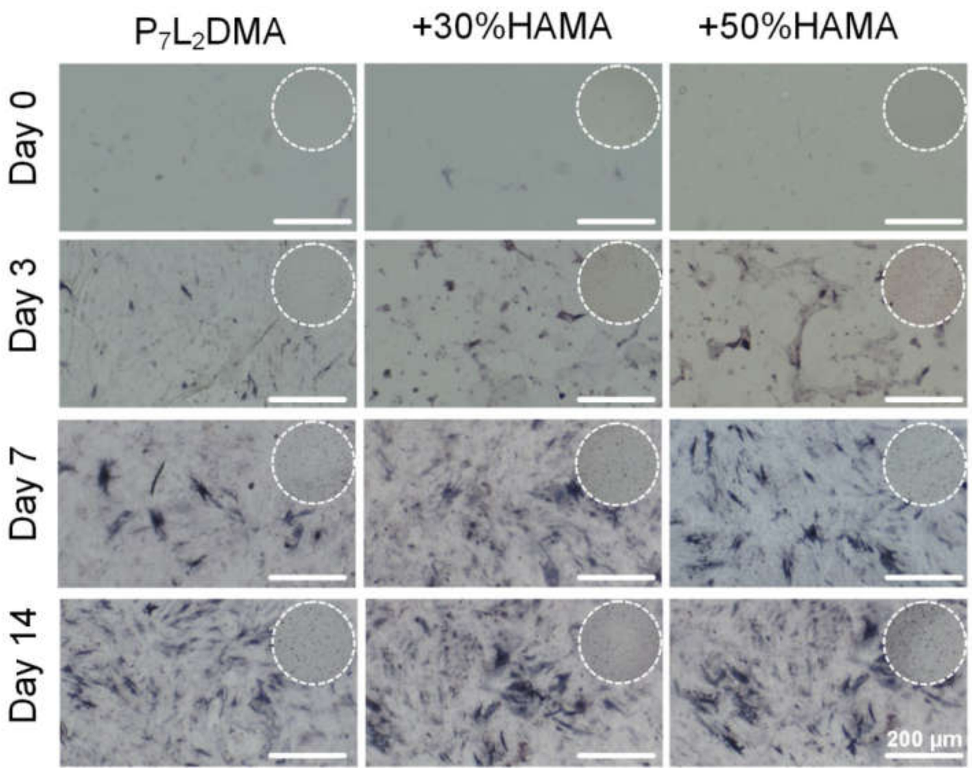
Cell proliferation



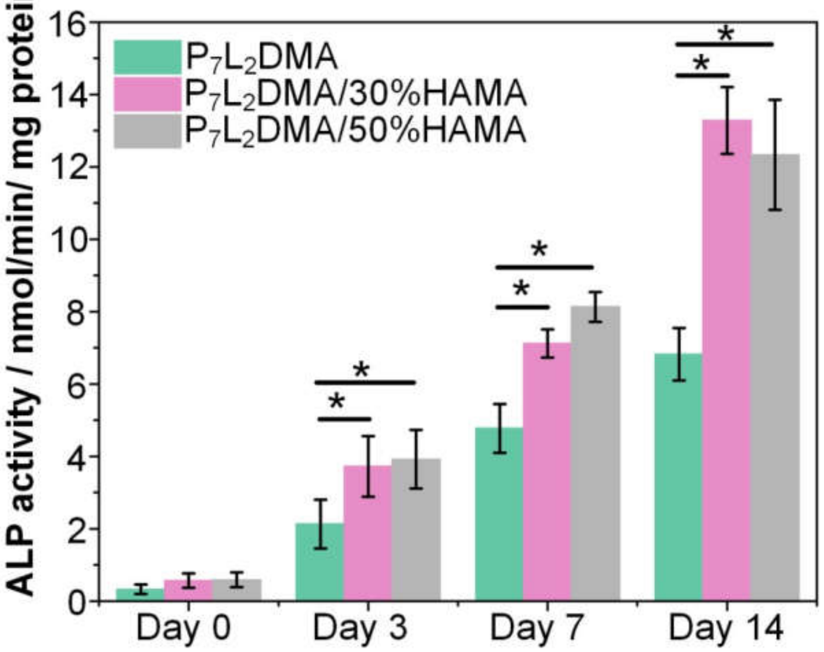
✓ Both polymer and composites can support cell survival, adhesion and proliferation.

Result 12a. *In vitro* osteogenesis-ALP activity

ALP staining



ALP activity



nHAMA-containing materials exhibited remarkably higher ALP activities compared to the nHAMA-free groups

Result 12b. *In vitro* osteogenesis-alizarin red (ARS) staining

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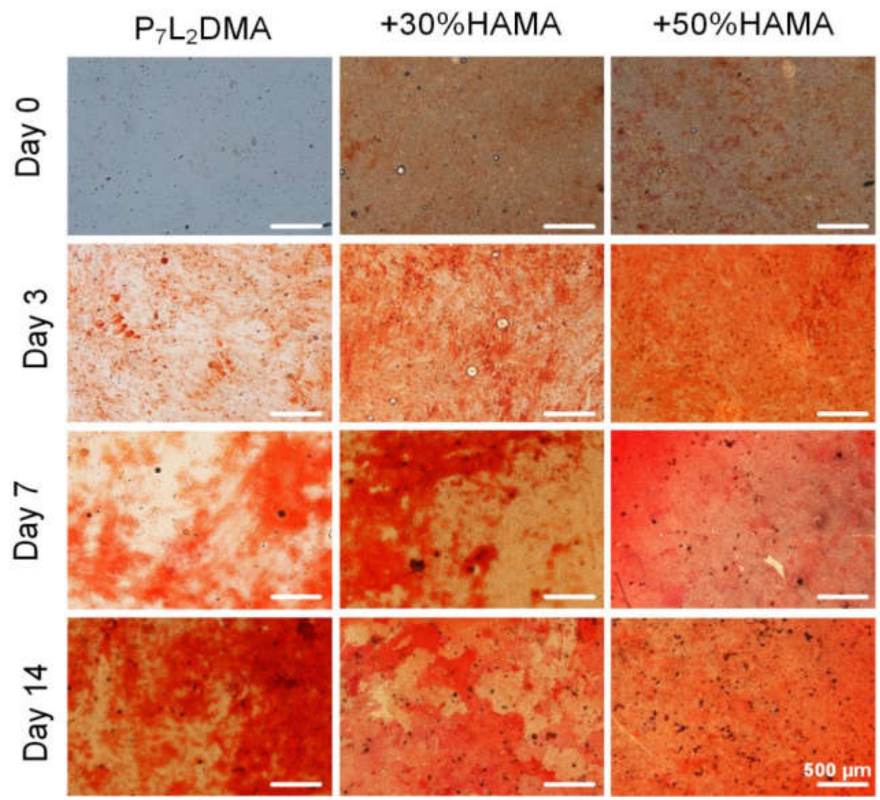
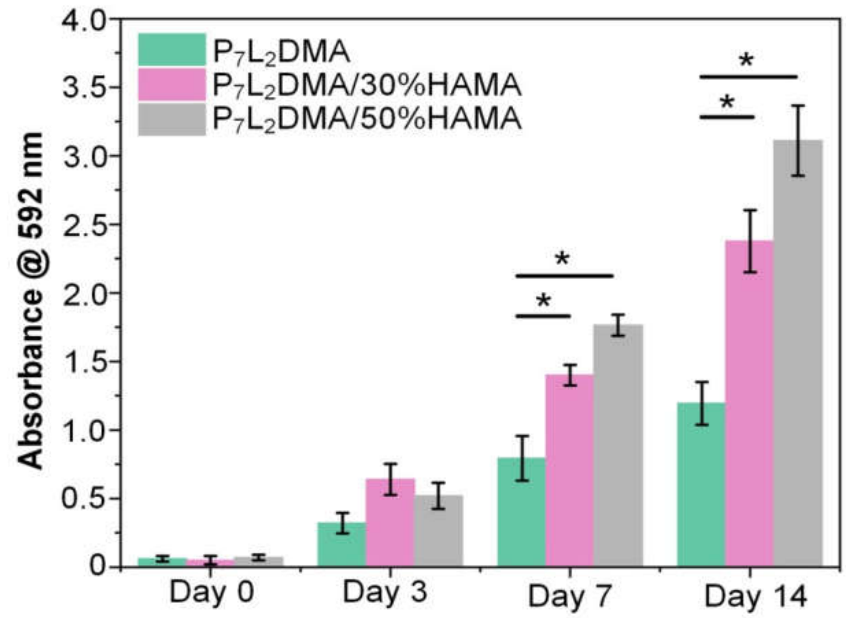
ARS staining



Mineralization concentration



The nHAMA could facilitate the extracellular matrix mineralization of the rMSC

Result 13. In vivo evaluation - Micro-CT analysis

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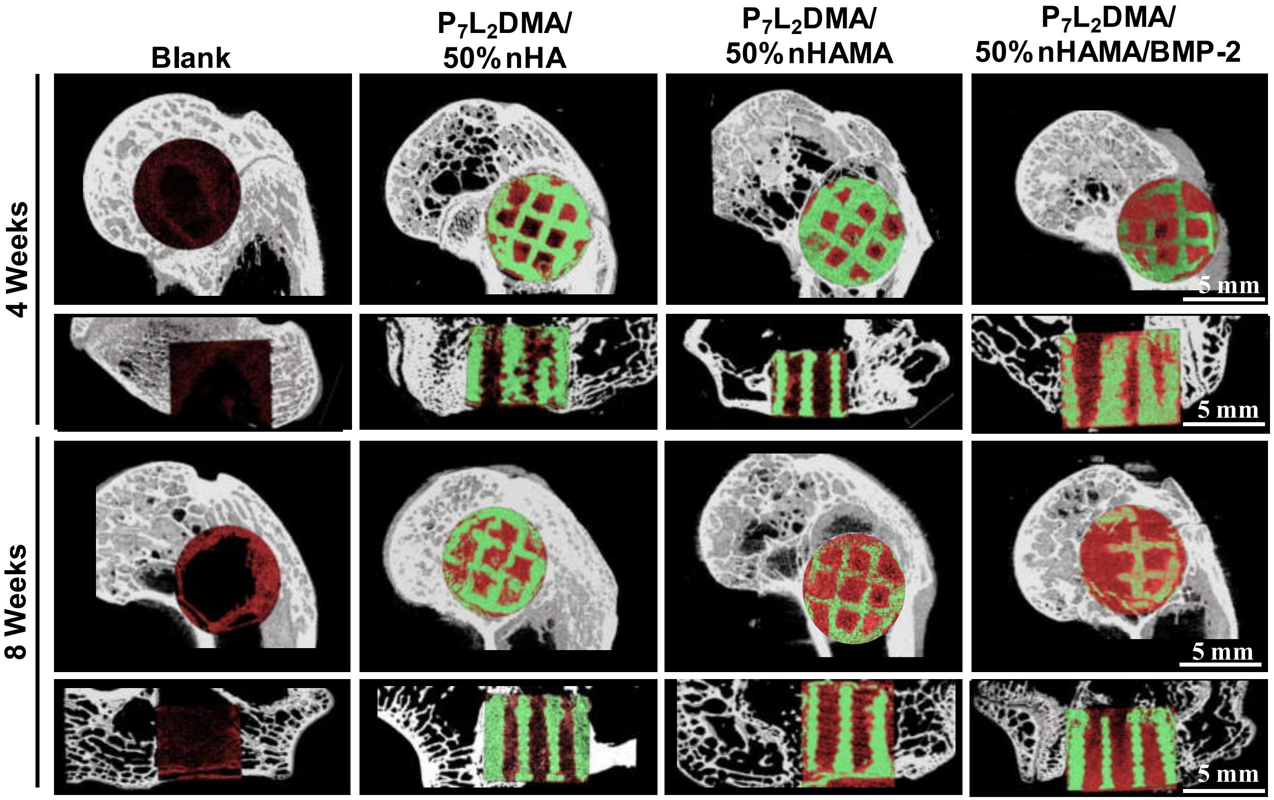
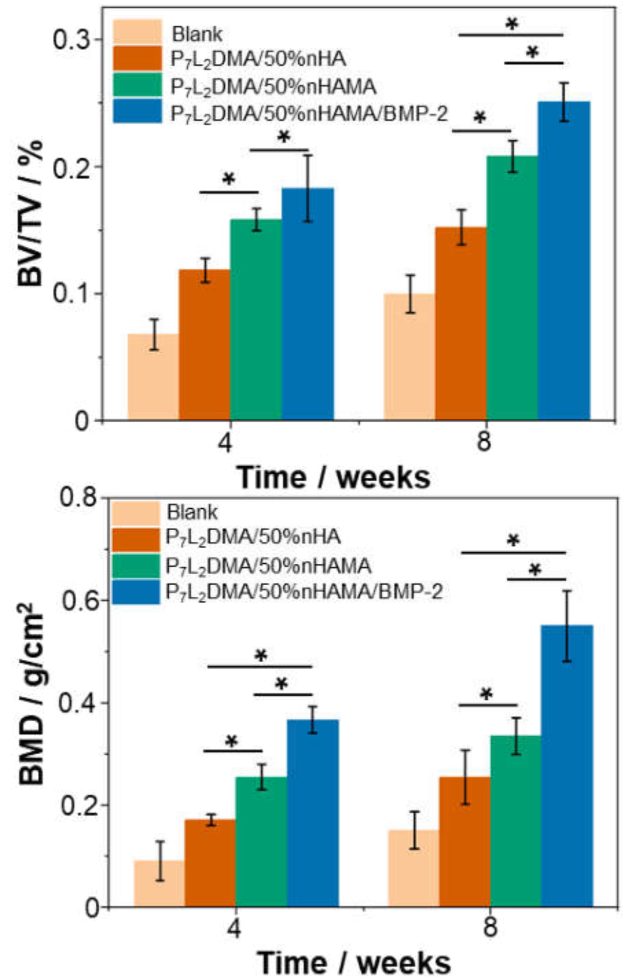
Rabbit Femoral Condyle Defect Model





Green: Scaffolds **Red**: New bone

- ✓ The P_7L_2DMA/50%HAMA scaffolds can support bone regeneration;
- ✓ The released BMP-2 can promote osteogenesis.

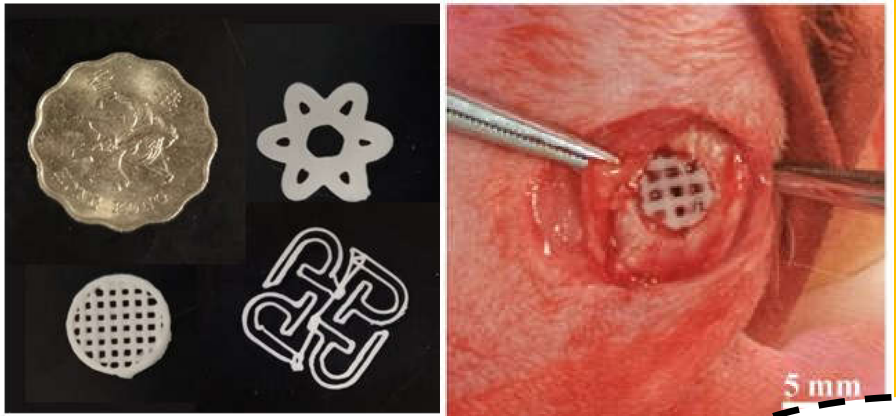
Conclusion

Photocrosslinkable composite materials consisting of P_mL_nDMA and methacrylate-functionalized hydroxyapatite presented:

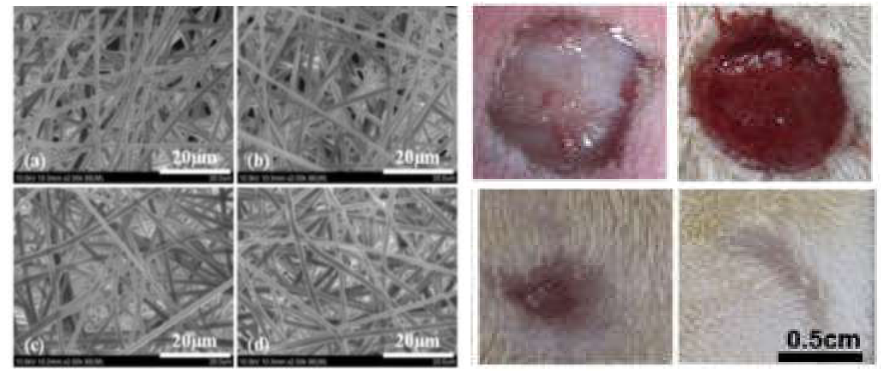
1. **9-fold increase in compressive modulus (362 MPa), comparable to natural trabecular bone (80 – 1000 MPa)**
2. **Enables loading and release of biomolecules**
3. **Tunable wettability, rheological behaviors and degradation, as well as printability**
4. **The composites can support the *in vitro* and *in vivo* osteogenesis**

Research interests

3D Printing



Electrospinning

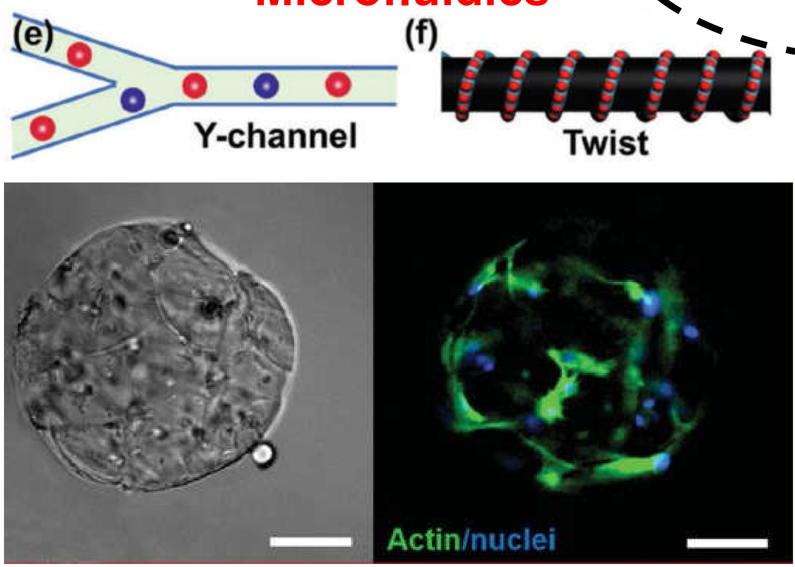


Materials design

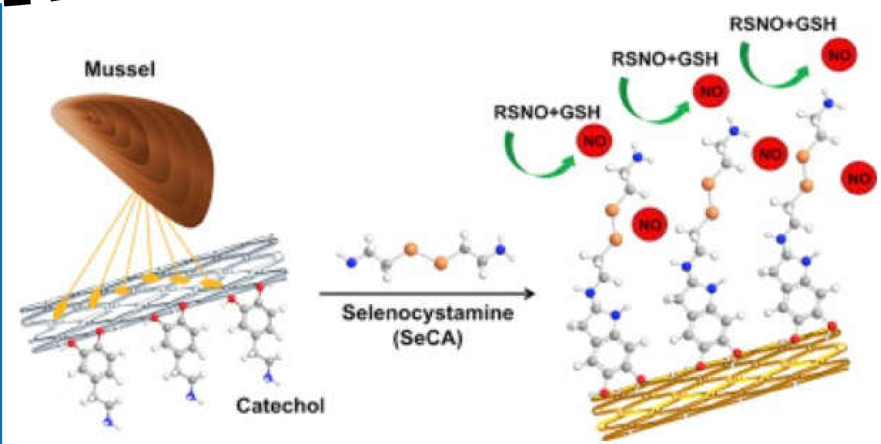
Scaffolds fabrication

Biomaterial application

Microfluidics



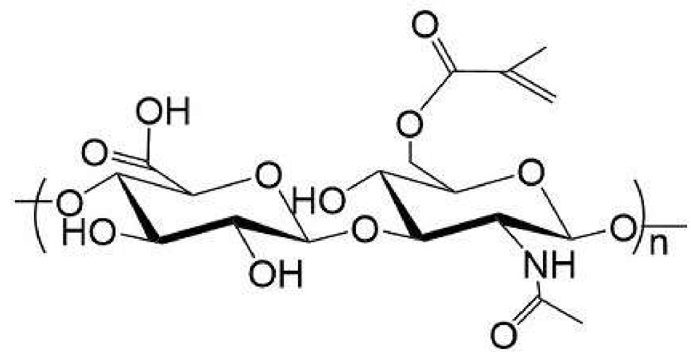
Biocoating



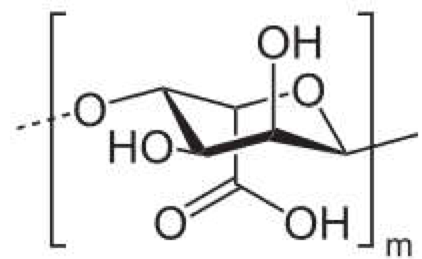
Natural photocrosslinkable polymer- background

Current methacrylated/acrylated natural polymer

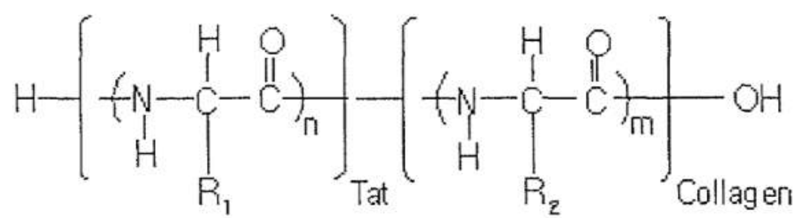
- **Hyaluronic acid**
  - ✓ Biocompatible
  - ✘ Need arginylglycylaspartic acid (RGD) for cell spreading



- **Alginate**
  - ✓ Biocompatible
  - ✓ Mild crosslinking
  - ✘ Need RGD modification for cell spreading
  - ✘ Non-degradable

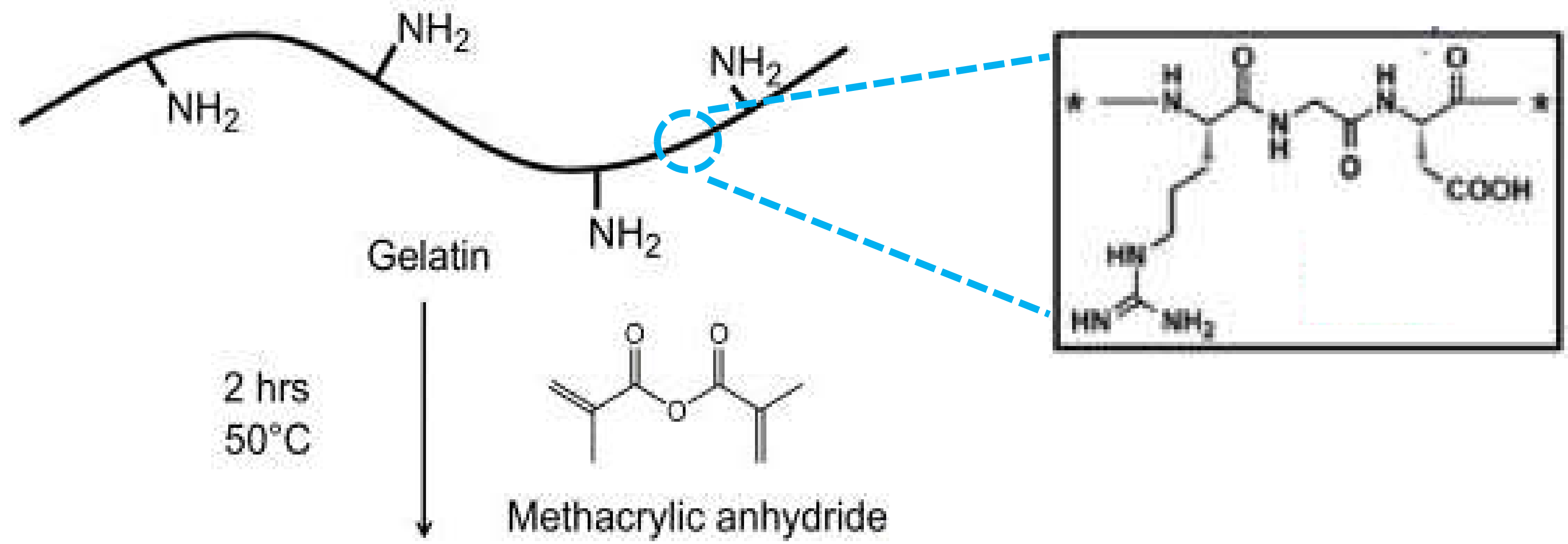


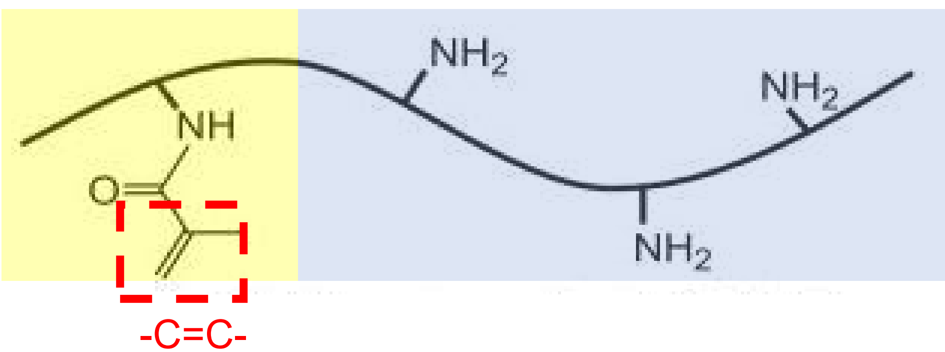
- **Collagen**
  - ✓ Biocompatible
  - ✓ Support cell spreading
  - ✓ Biodegradable



✘ Long UV crosslinking time (few mins, not good for cell encapsulation)

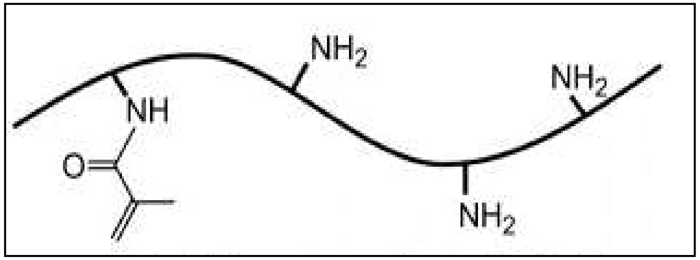
Photocrosslinkable gelatin (GelMA)





- ✓ **Cell adhesive**
- ✓ **Biodegradable**
- ✓ **Photocrosslinkable (rapid, non-toxic)**

Tunable physical properties



Change the substitution ratio of -NH_2 and GelMA concentration → **Change the crosslinking density** → **Change the physical properties(e.g., mechanical property)**

A. In situ forming hydrogels

B. Electrospun fibrous membrane for skin regeneration

C. Injectable cell-laden hydrogel microspheres

Zhao et al., *Adv. Healthcare Mater.*, 2015

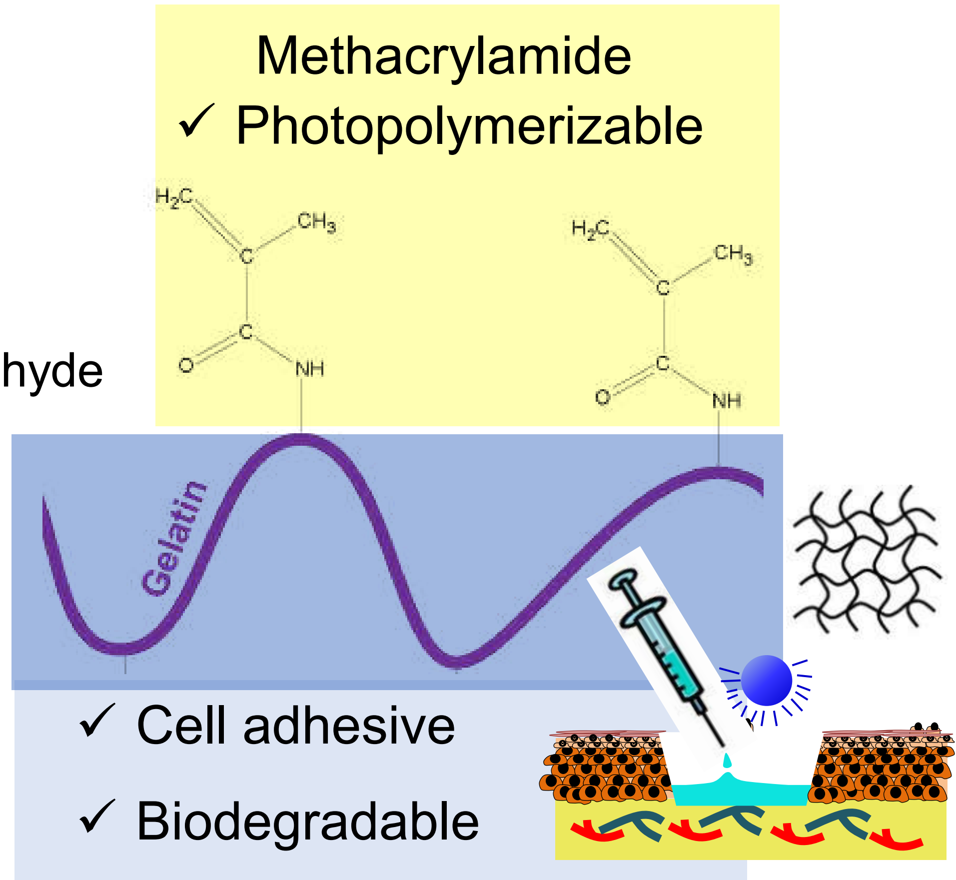
➢ Current natural hydrogels for skin regeneration

- Collagen
- Gelatin

✓ Biocompatible
✗ Mechanical property
✗ Degradation
✗ Toxic when crosslinking with glutaraldehyde

➢ Strategy

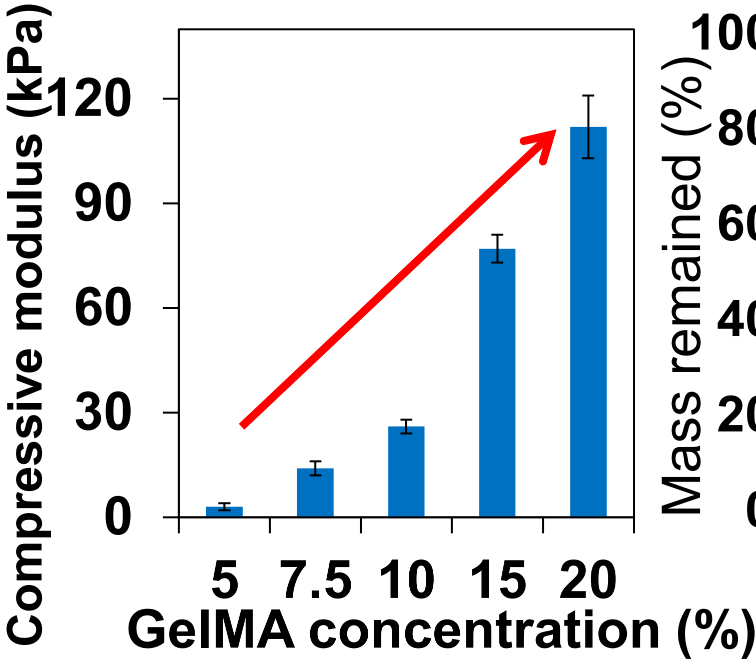
Gelatin methacryloyl (GelMA)

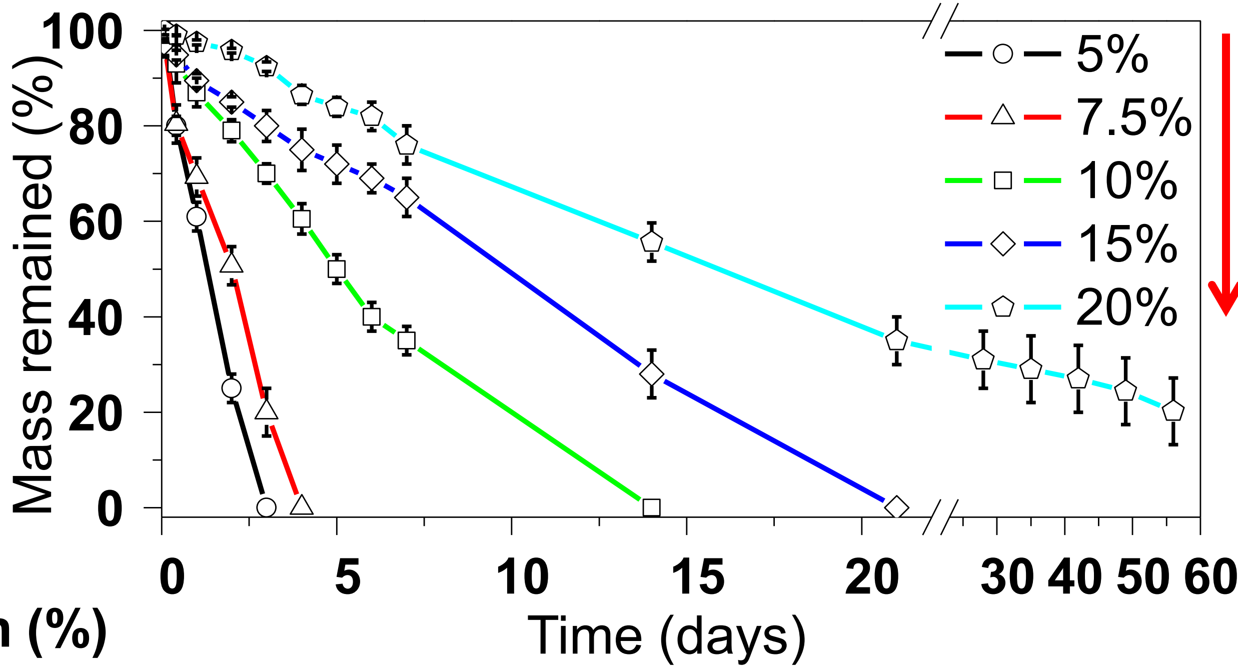


Aim:

To develop natural hydrogels with tunable mechanical and degradation properties

Zhao et al., *Adv. Healthcare Mater.*, 2015





Tunable modulus few kPa to few hundred kPa

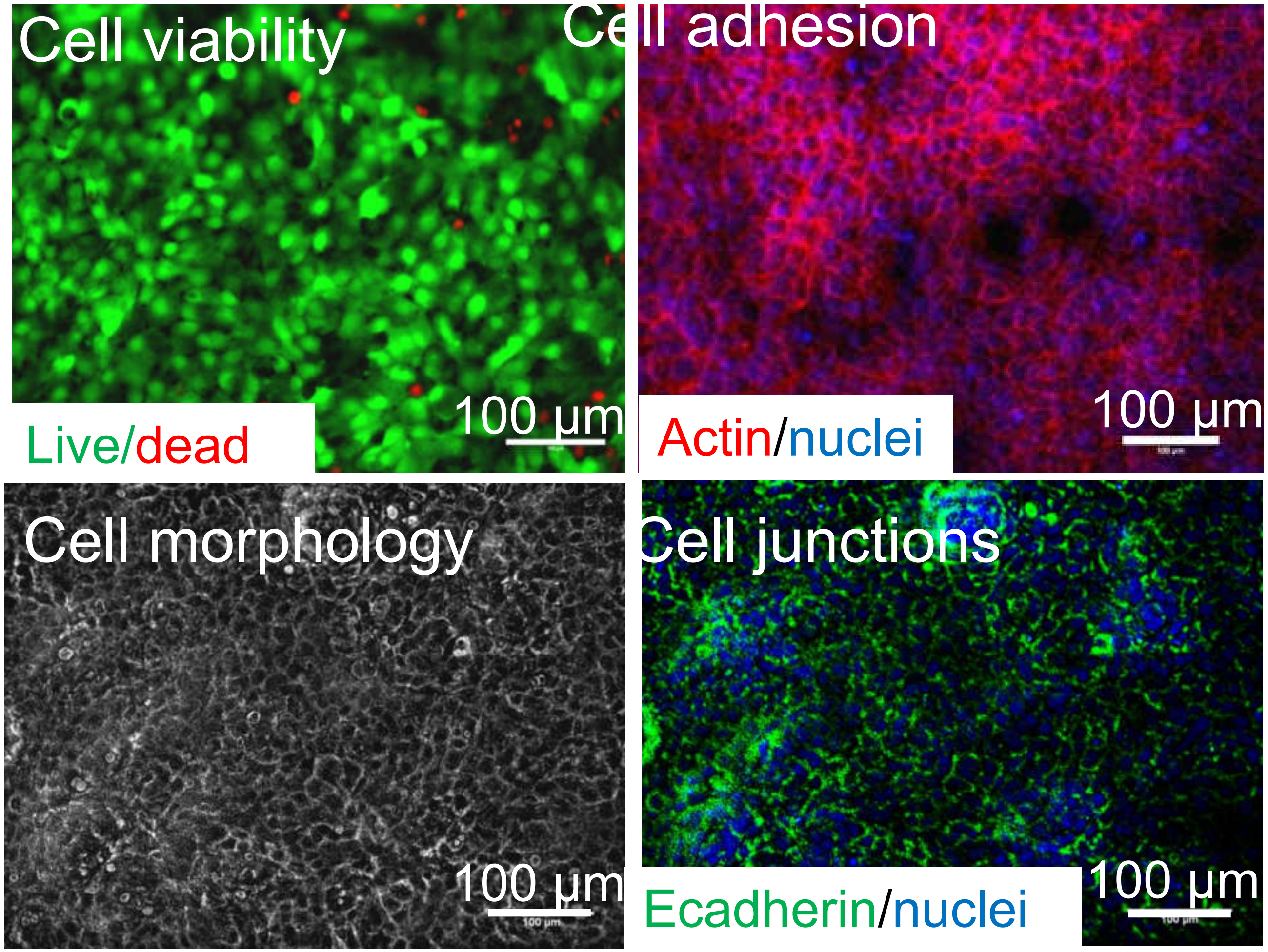
Tunable degradation from few days to few months

➢ Increase in GelMA concentration resulted in increased compressive modulus and decreased degradation due to increased crosslinking density.

➢ Result 2. Biological characterization

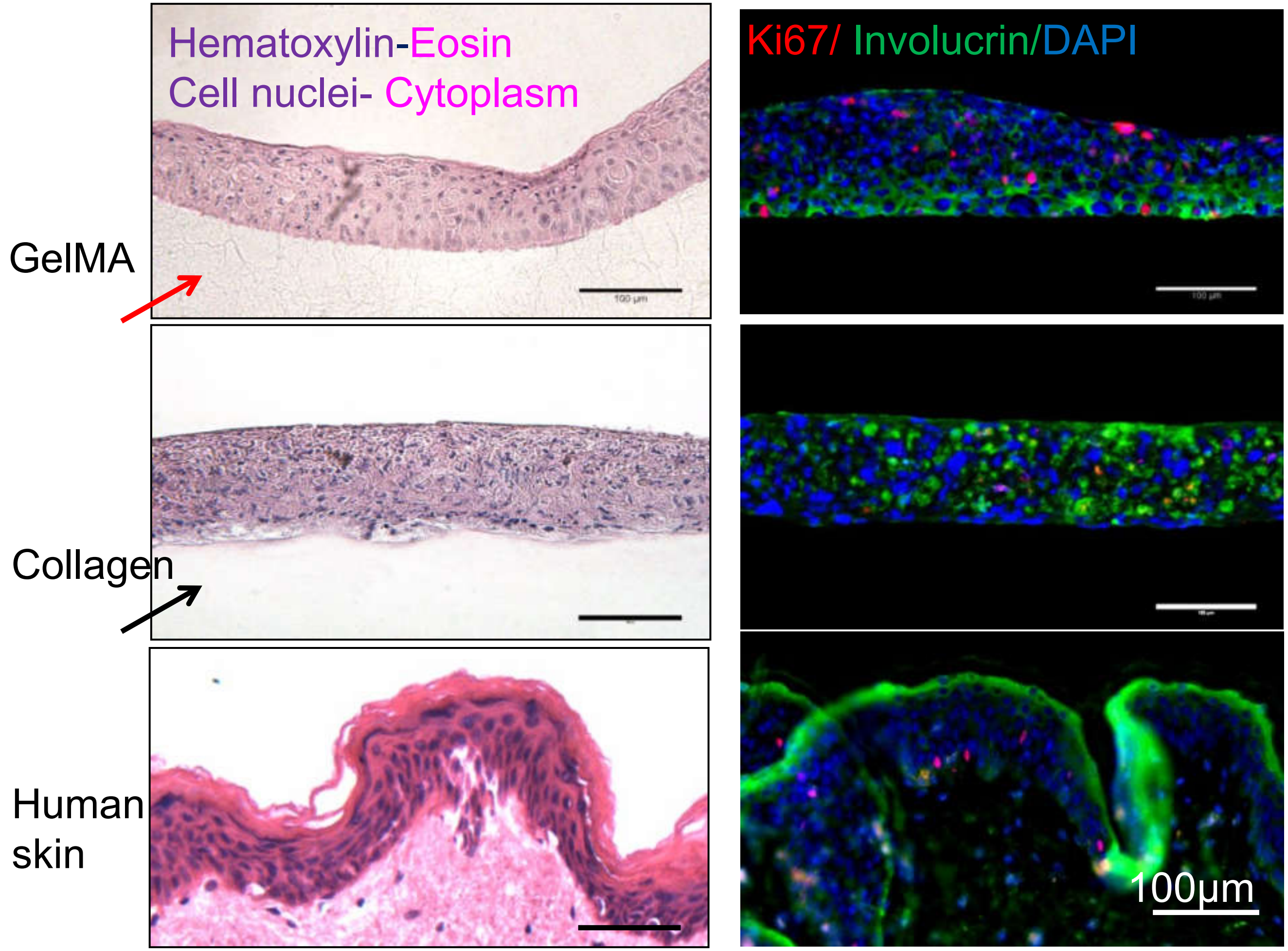
Cells:
Human keratinocyte cell line (HaCaT)

1 week culture



- High cell viability
- Good cell adhesion
- Tight cell junctions

➢ Result 3. Characterization of reconstructed epidermis (6 weeks)



Epidermis developed on GelMA showed comparable thickness (~ 100 µm) to that on control collagen and human skin.

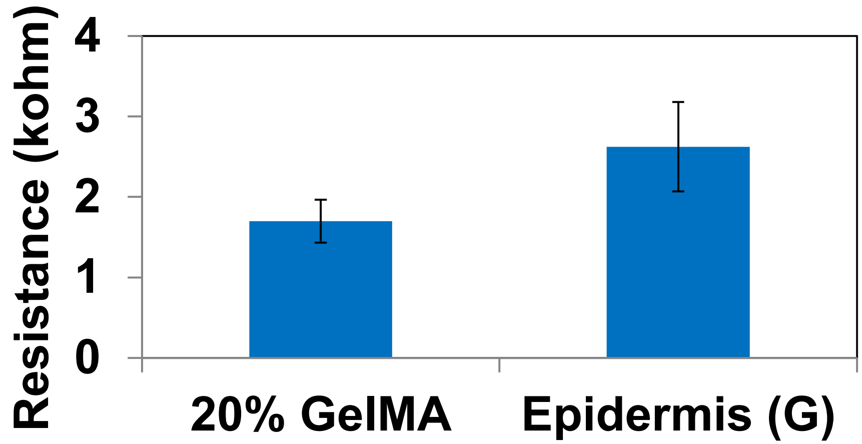
Collagen scaffolds lost their integrity after 6 weeks of culture whereas GelMA was still intact.

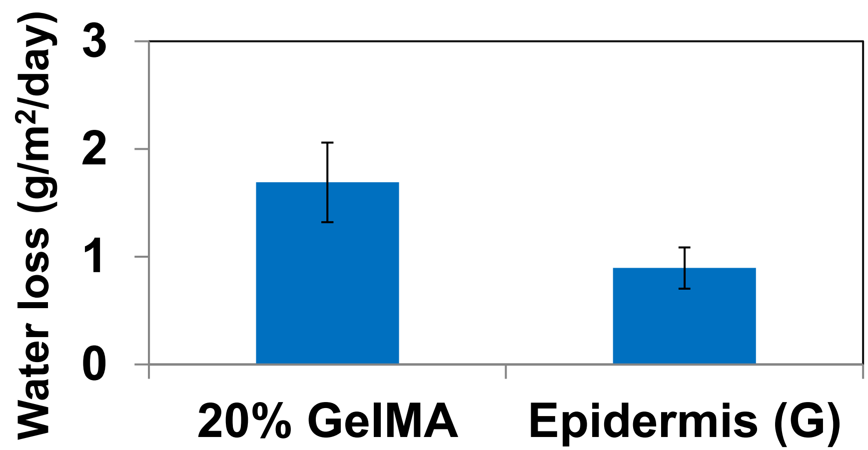
Expressions of proliferation marker (Ki 67) and differentiation marker (Involucrin) can be readily seen in all samples.

Reconstructed epidermis on hydrogel

Epidermis developed on top

Hydrogel scaffolds





- The reconstructed epidermis on GelMA exhibited increased resistance and reduced water vapor transmission, indicating improved barrier formation.

A. In situ forming hydrogels

B. Electrospun fibrous membrane for skin regeneration

C. Injectable cell-laden hydrogel microspheres

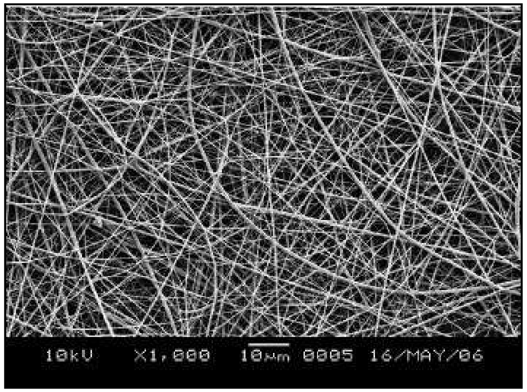
Zhao et al., *Acta Biomater.*, 2016; Sun et al., *Adv. Funct.* Mater. 2016

Electrospun fibrous scaffolds for skin regeneration

- **Hydrogel**

- ✗ Low surface-to-volume ratio for cell-material interaction
- ✗ Low nutrient diffusion

- **Electrospun mats**

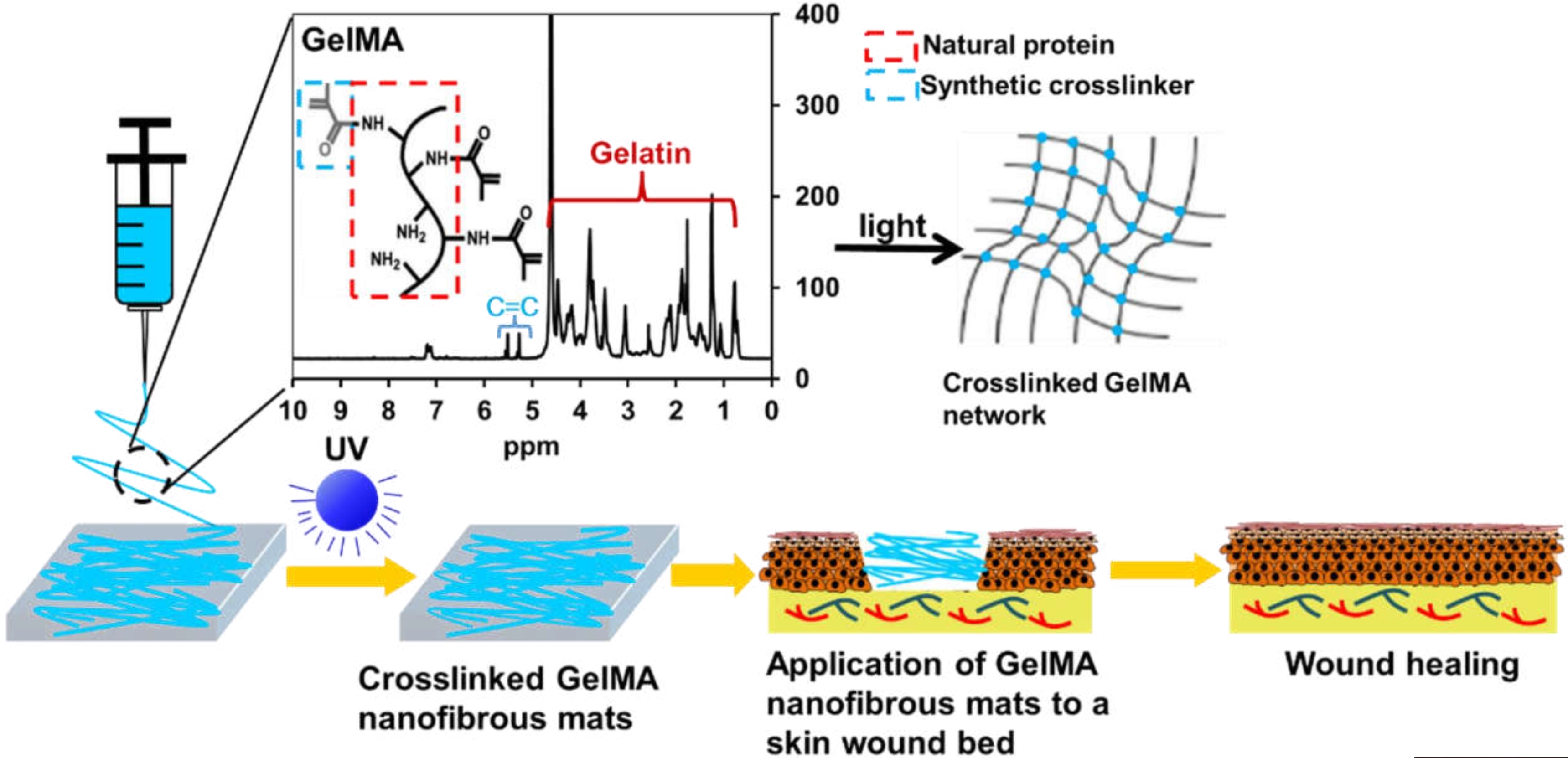


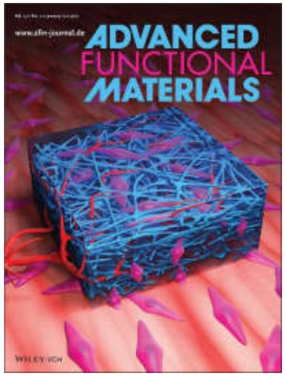
- ✓ Nutrition and waste diffusion
- ✓ Large surface-to-volume ratio
- ✓ Have three-dimensional (3D) micro/nanofibrous structure, mimicking the architecture of natural ECM
- ✗ Small pore size limit three-dimensional (3D) cellular infiltration

Project Aim:

To fabricate electrospun fibrous scaffolds with soft adjustable mechanical and controllable degradation properties.

Electrospun fibrous scaffolds for skin regeneration

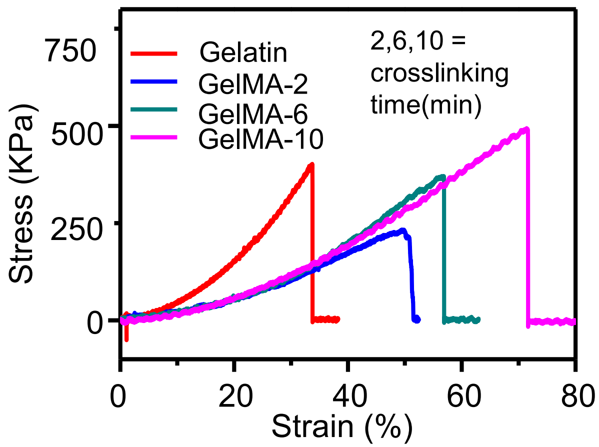


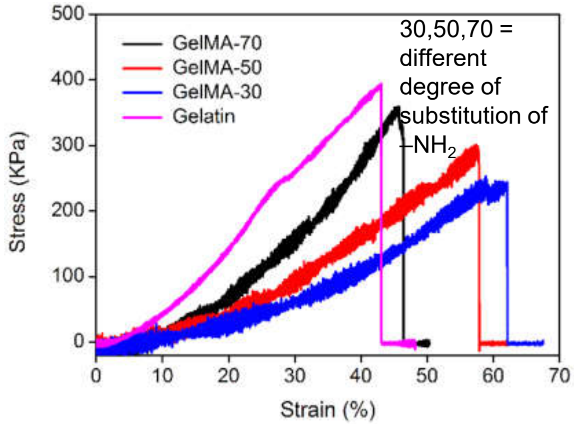


- **Advantages**
  - ✓ High surface area to volume ratio for cell–material interactions
  - ✓ Nutrient and waste diffusion
  - ✓ Tunable physical properties for cell infiltration

Zhao et al., *Acta Biomater*., 2016; Sun et al., *Adv. Funct*. Mater. 2016

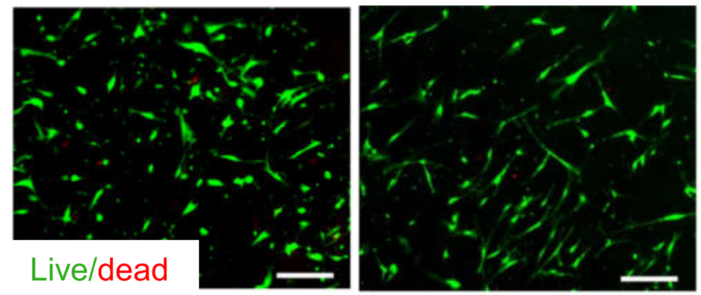
➢ Elasticity





➢ Cell viability

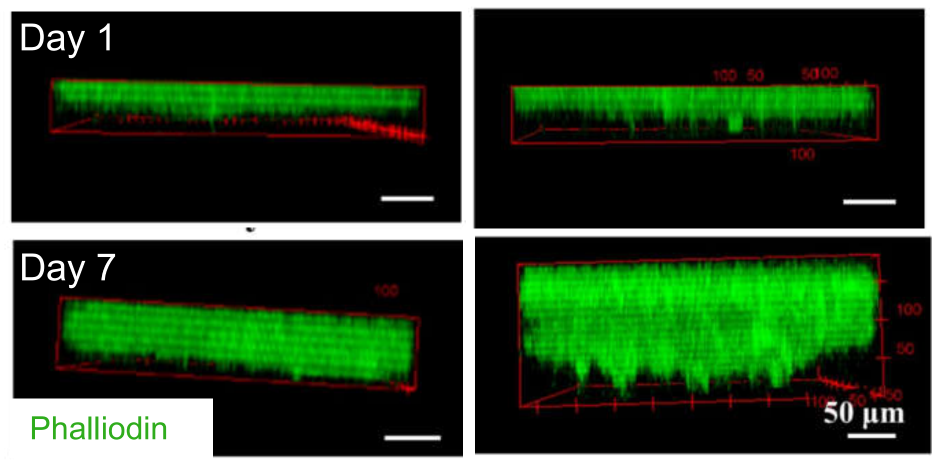
Gelatin GelMA



Fibroblasts Day 4

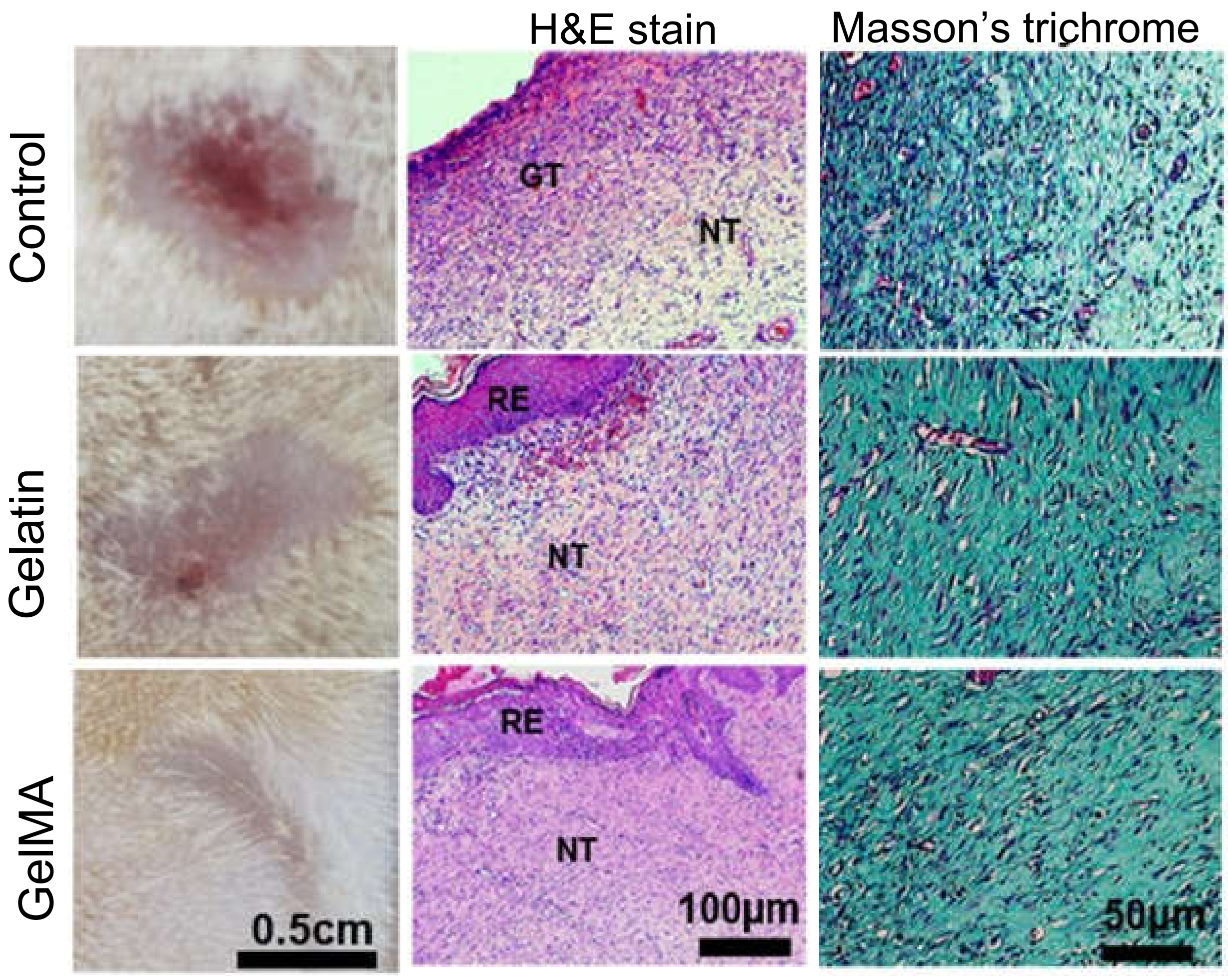
➢ Cell migration

Gelatin GelMA



- ➢ Via changing the crosslinking time and degree of substitution, the GelMA fibers exhibited tunable mechanical properties;
- ➢ GelMA scaffolds can support cell survival and migration.

➢ Result 3. *In vivo* wound healing



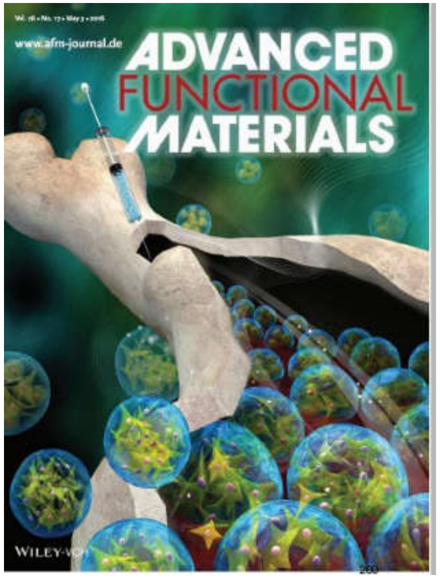
Cell nuclei/Cytoplasm Collagen/cytoplasm/nuclei

➢ Compared with control and gelatin, GelMA scaffolds obtained re-epithelialization and showed better wound closure and collagen organization.

A. In situ forming hydrogels

B. Electrospun fibrous membrane for skin regeneration

C. Injectable cell-laden hydrogel microspheres



Zhao et al., *Adv. Funct. Mater*, 2016

Cell-laden microspheres

Cell-laden hydrogel

Advantages

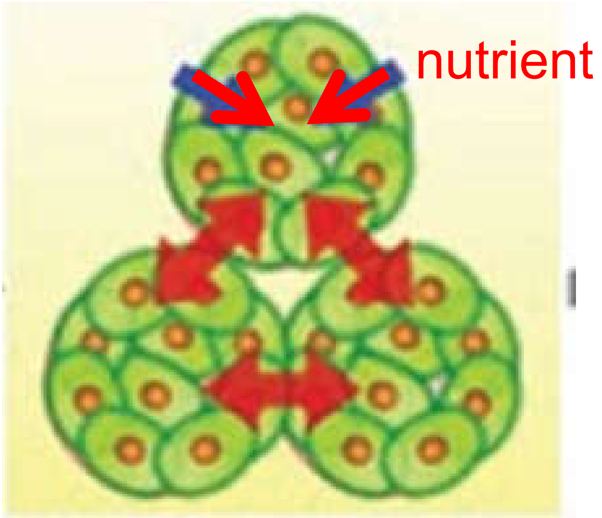
- ✓ Cell encapsulation capability
- ✓ Tunable physical properties
- ✓ Biocompatibility
- ✓ Biodegradability

Disadvantages

- ✘ Can not sustain cell viability due to lack of nutrient diffusion

Microstructured cell delivery system

- ✓ Allow ready nutrition diffusion between the microspheres



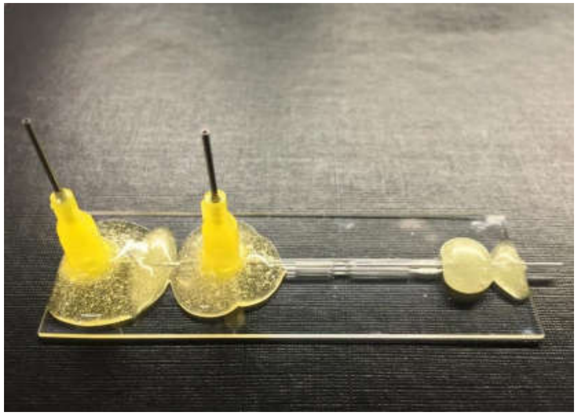
Microfluidics

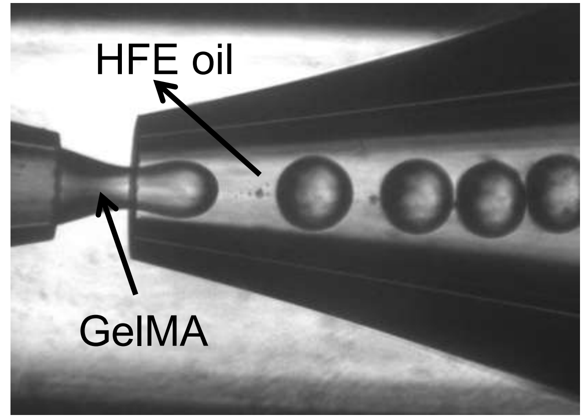
- ✓ One step fabrication of large quantity of cell-laden microspheres

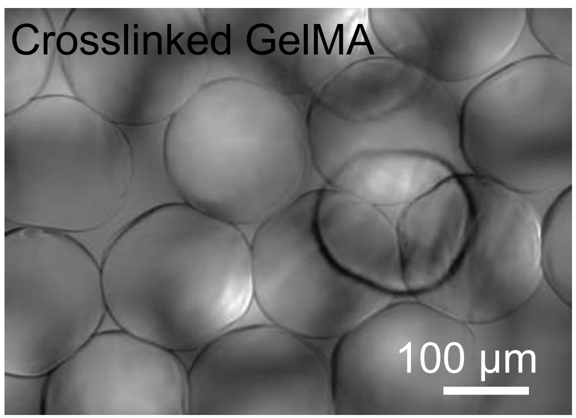
Project aim: Develop cell-laden microspheres for bone regeneration

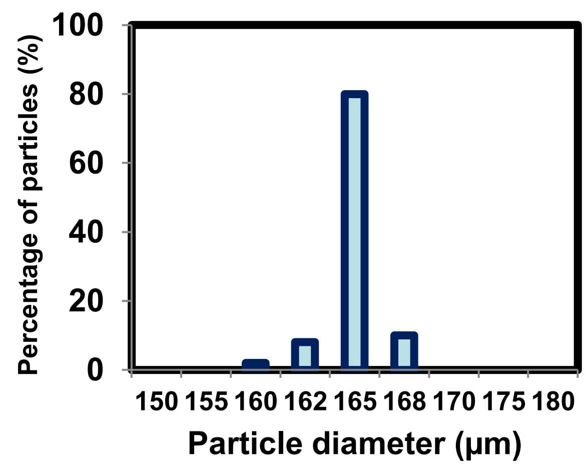
- **Fast setting**
- **Biodegradable**
- **Biocompatible**
- **Osteogenic *in vitro* and *in vivo***

Result 1. Morphology of GelMA microspheres



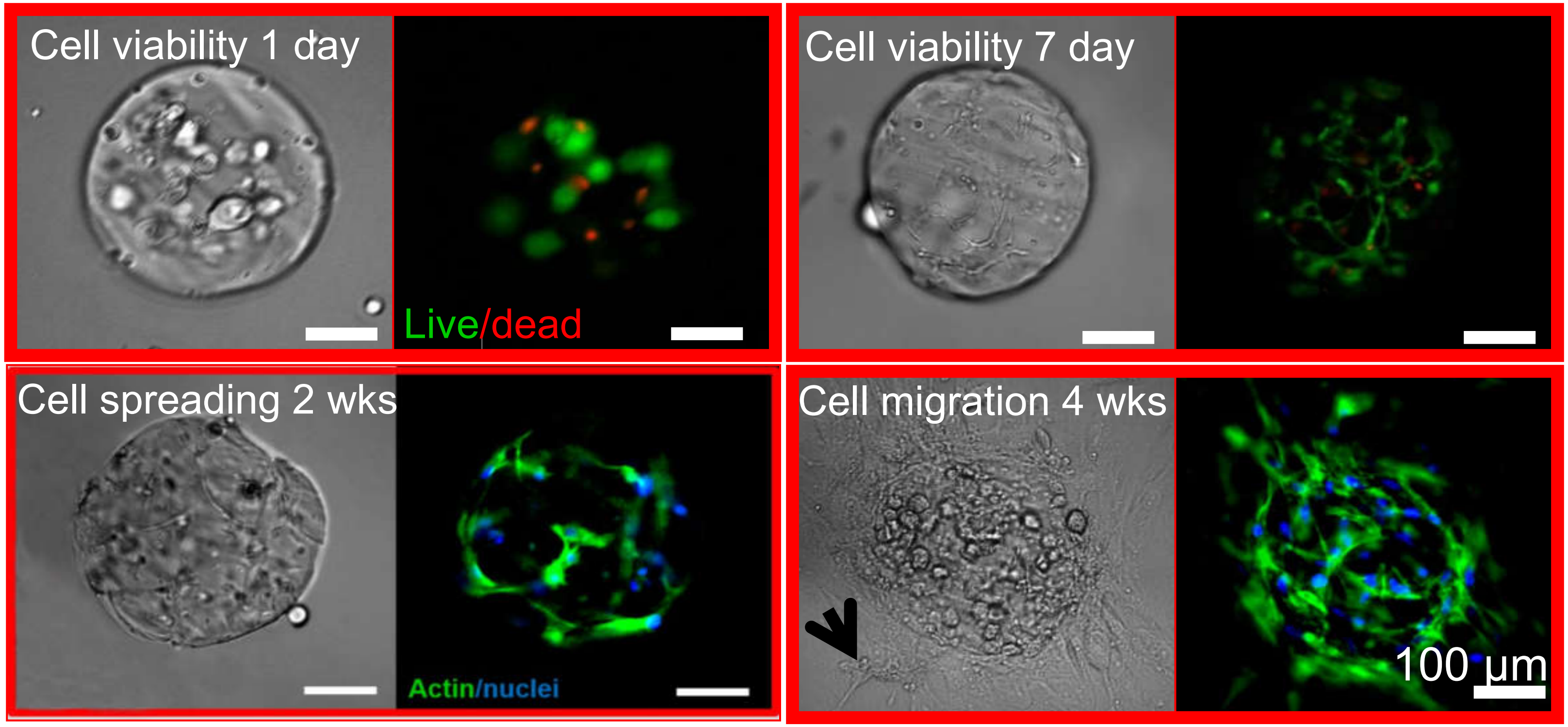




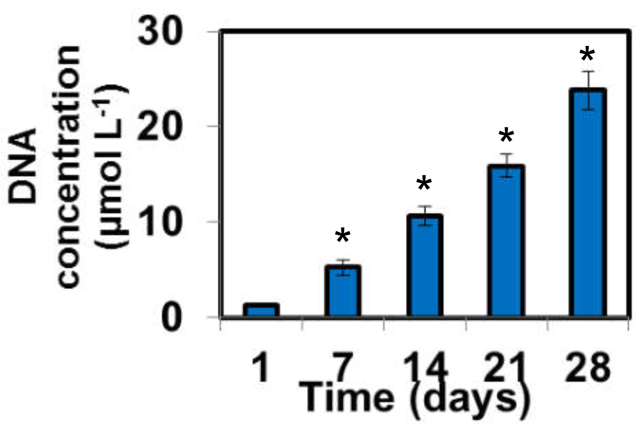


The resultant GelMA microspheres have uniform diameter around 160 μm

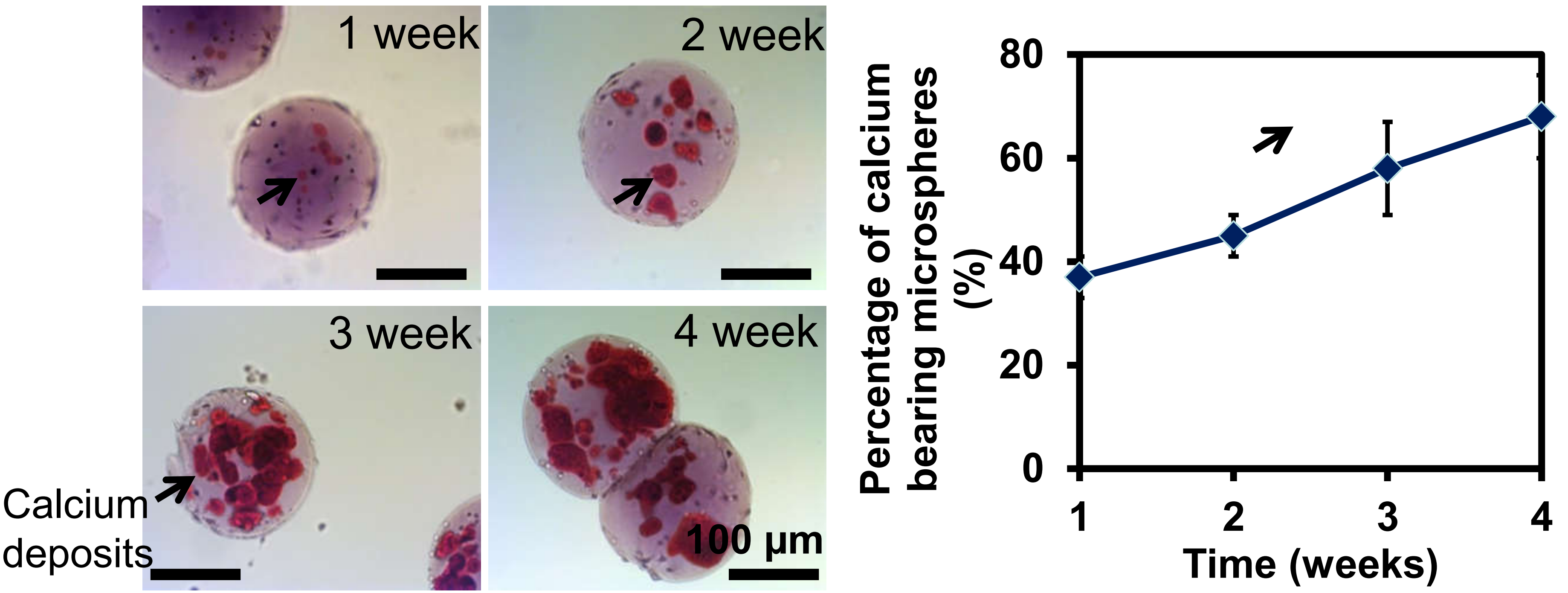
Result 2. *In vitro* biocompatibility of GelMA microspheres



GelMA microspheres can support bone mesenchymal stem cell (BMSC) survival, spreading, migration and proliferation.

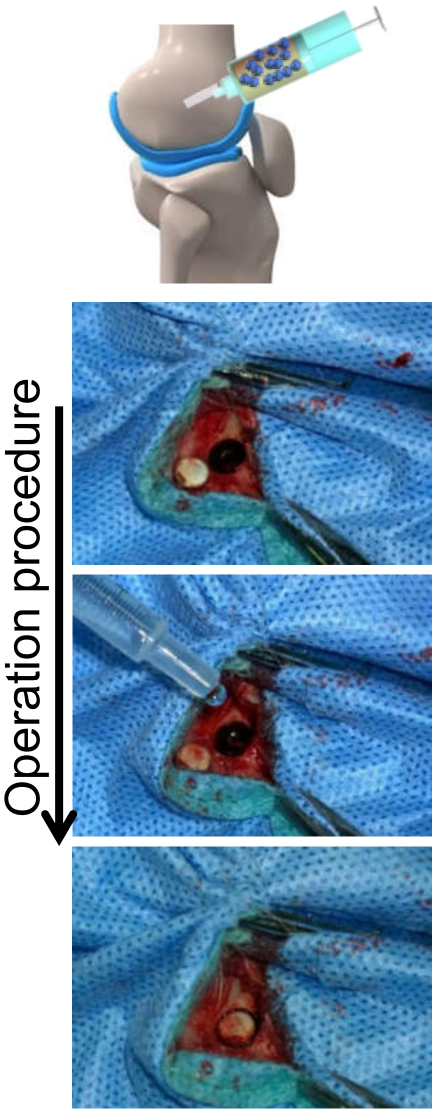


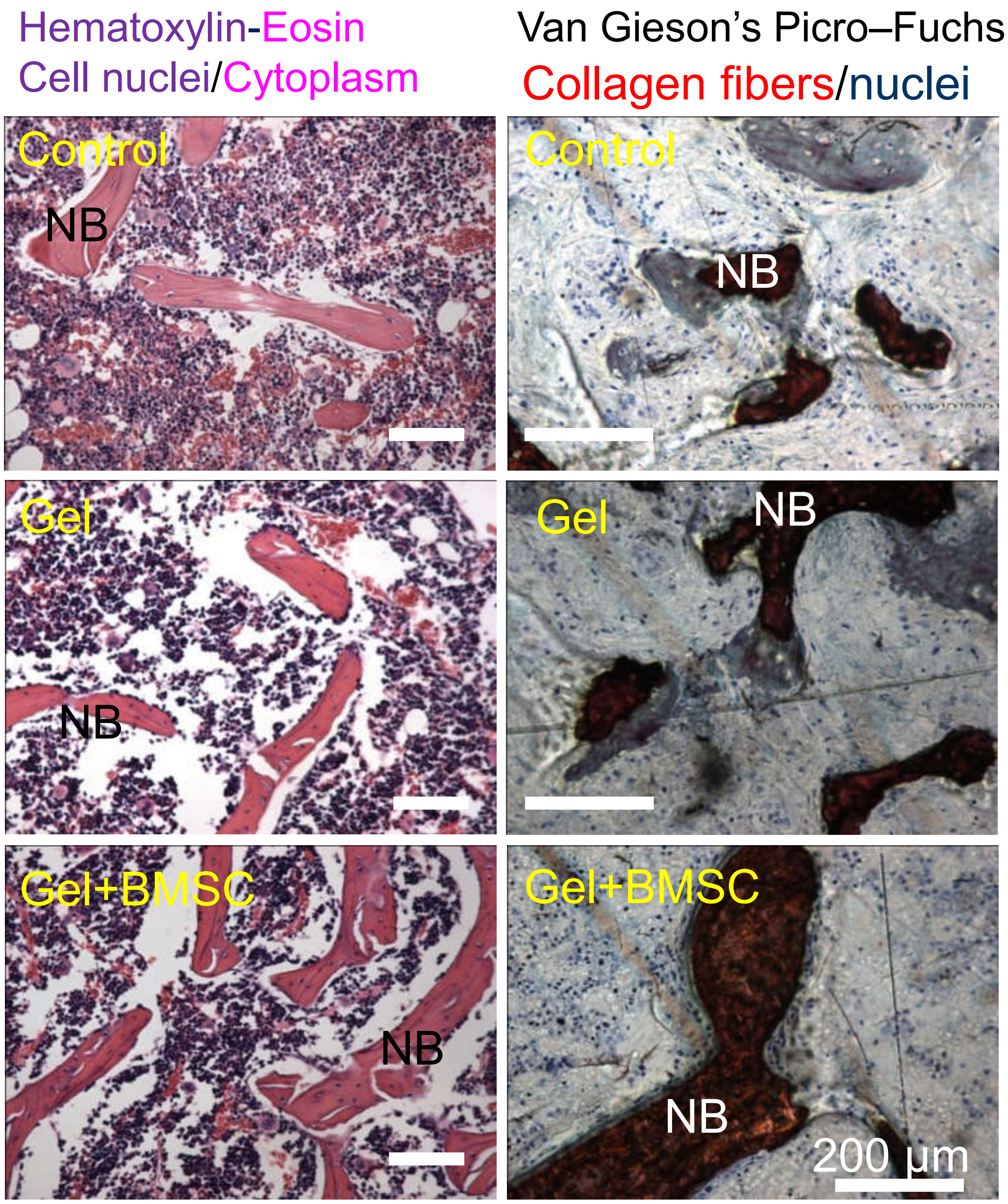
Result 3. *In vitro* osteogenesis potential



GelMA microspheres can support BMSC differentiation.

Result 4. *In vivo* osteogenesis potential





4 weeks implantation
NB = New bone

GelMA microspheres can support osteogenesis *in vivo*.

Conclusion

1. The photocrosslinkable gelatin (GelMA) have **tunable physical and biological properties.**

2. It can be used as in situ forming hydrogels for **wound healing.**

3. Electrospun GelMA-HAMA fibers have highly **tunable mechanical properties, and could support skin regeneration, osteogenesis and angiogenesis** *in vitro* and *in vivo*.

4. Cell-laden GelMA microspheres can support **osteogenesis** *in vitro* and *in vivo.*

Electrospun Fibers for Tendon regeneration

Sports injury leads to tendon rupture

Tendon repair generates adhesion

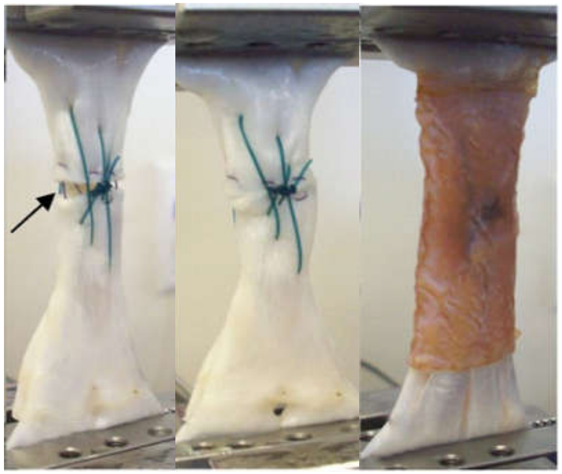
Tendon repair failure

How to regenerate tendon with no adhesion formation?

Zhao et al., *Biomaterials*, 2015

➢ Anti-adhesion tendon regeneration membrane

Tendon repair



Lee et al, *Biomed. Mater.*, 2011

➢ Current anti-adhesion membrane

- ✗ Naked physical anti-adhesion membrane
- ✗ Anti-adhesion effect is undesirable

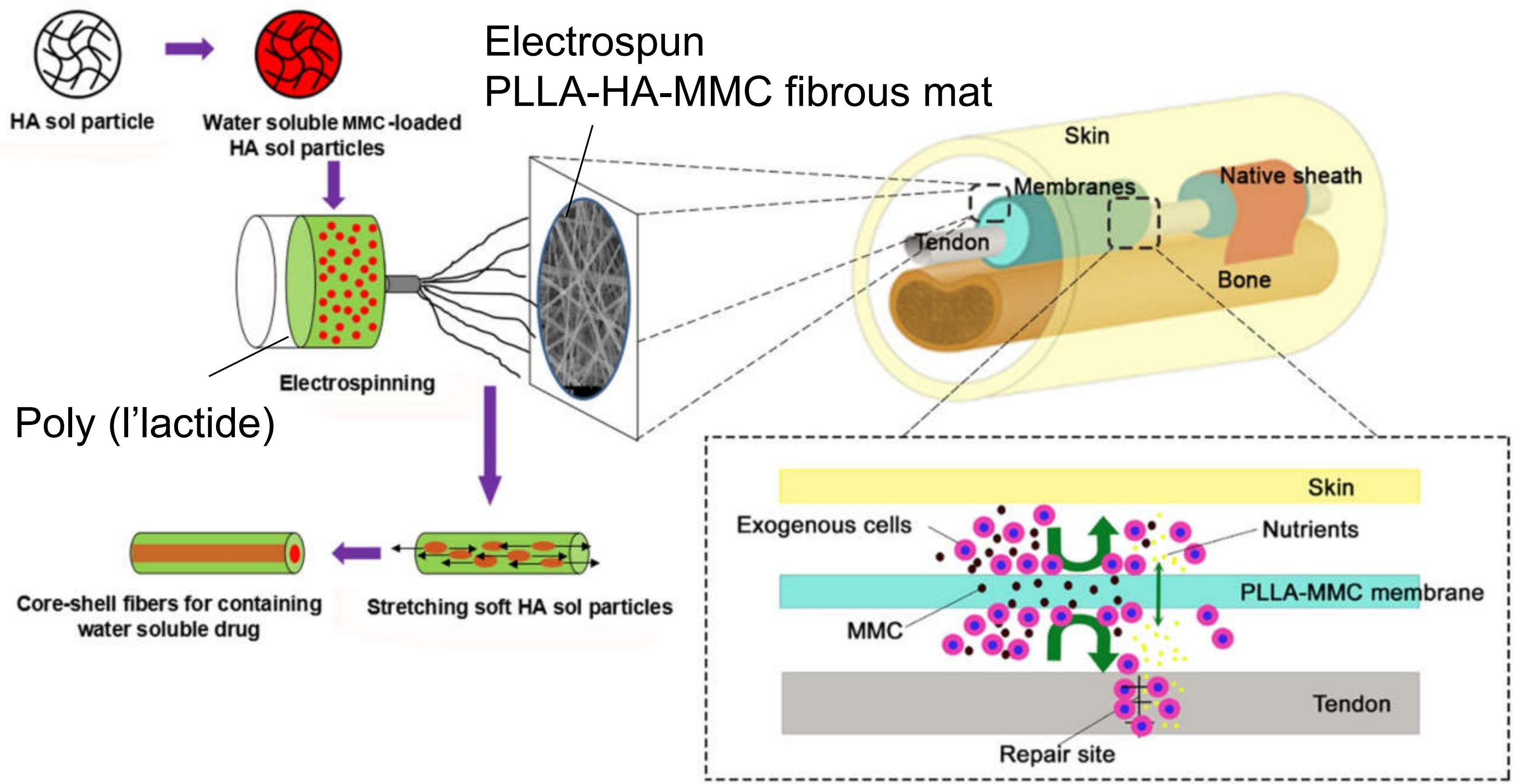
➢ Drug-loaded anti-adhesion membrane

- ✓ Effective anti-adhesion
- ✗ Drug toxicity inhibits tendon regeneration

Project aim

Develop an anti-adhesion membrane which can inhibit adhesion while facilitating tendon regeneration

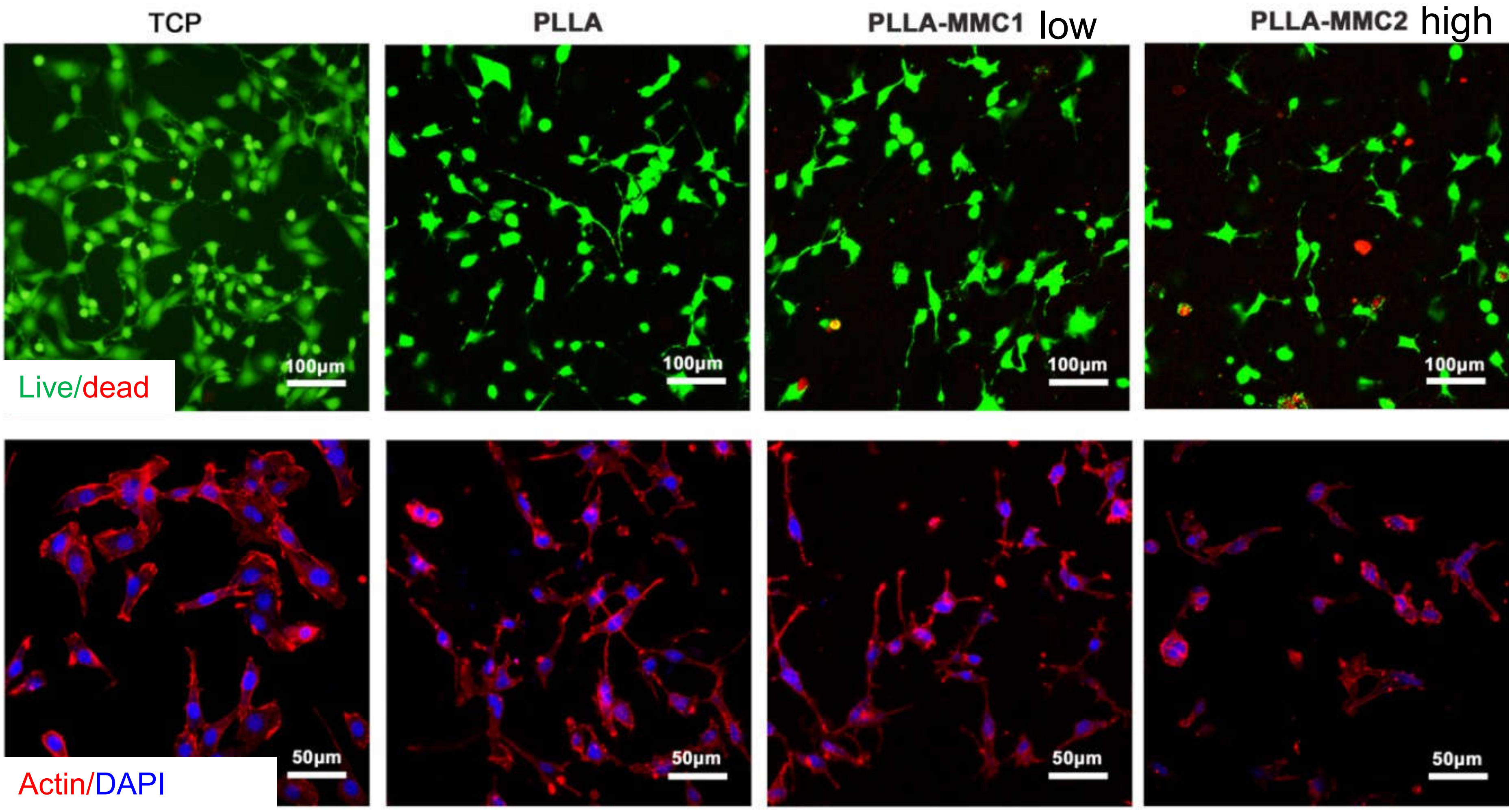
➢ Strategy



Hyaluronic acid (HA): Facilitate tendon regeneration
Mitomycin-C (MMC): Prevent adhesion formation

➢ Result 1. *In vitro* anti-adhesion

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學



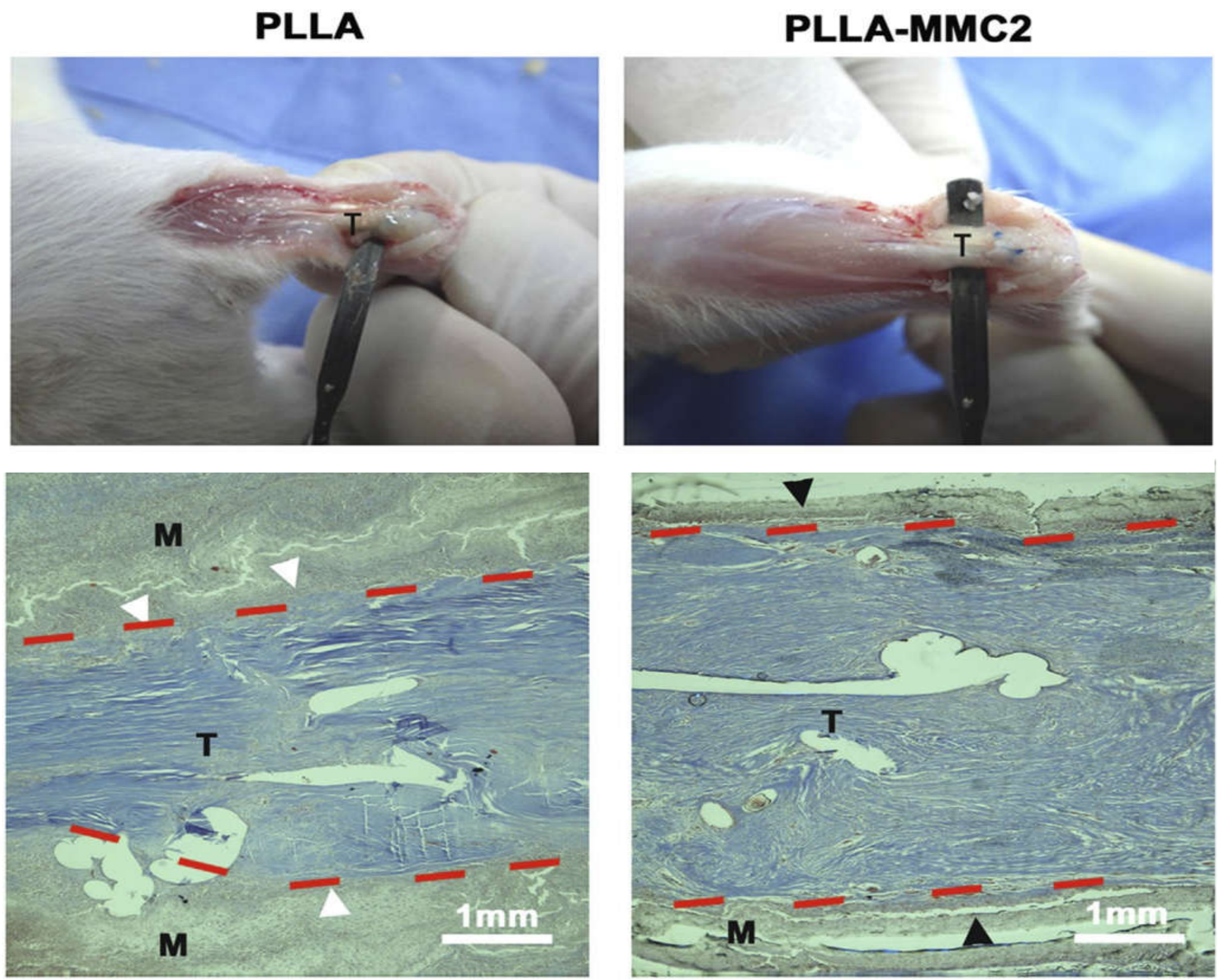
➢ PLLA with higher concentration of MMC can inhibit survival and adhesion of fibroblasts more efficiently.

➢ Result 2. *In vivo* anti-adhesion

T = tendon
M = membrane
△ Adhesion
▲ Anti-adhesion

3 weeks of implantation

- PLLA-MMC exhibited optimal adhesion-free tendon regeneration.



Masson's trichrome staining Collagen/cytoplasm/nuclei

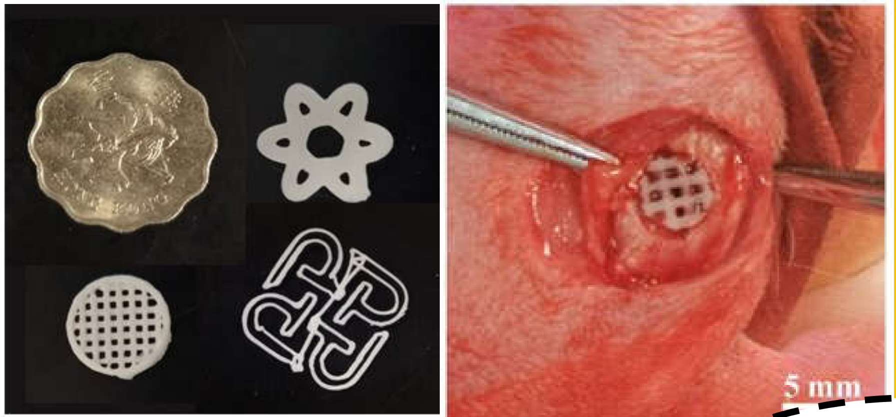
Conclusion

1. The developed drug loaded electrospun PLLA-HA-MMC fibrous mat can release drugs to prevent adhesion formation

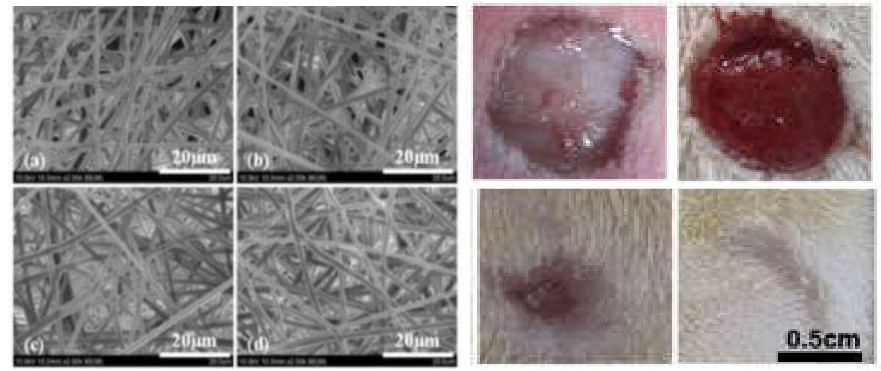
2. The developed drug loaded electrospun PLLA-HA-MMC fibrous mat can support tendon regeneration

Research interests

3D Printing



Electrospinning

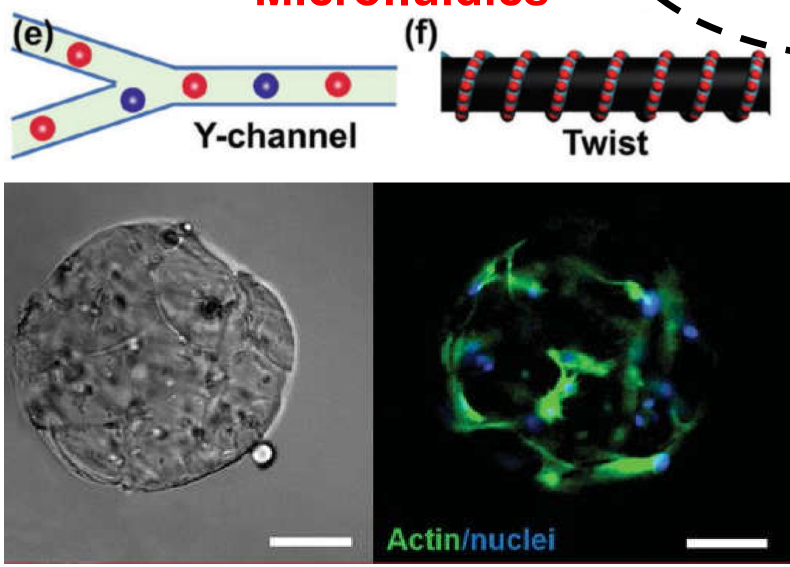


Materials design

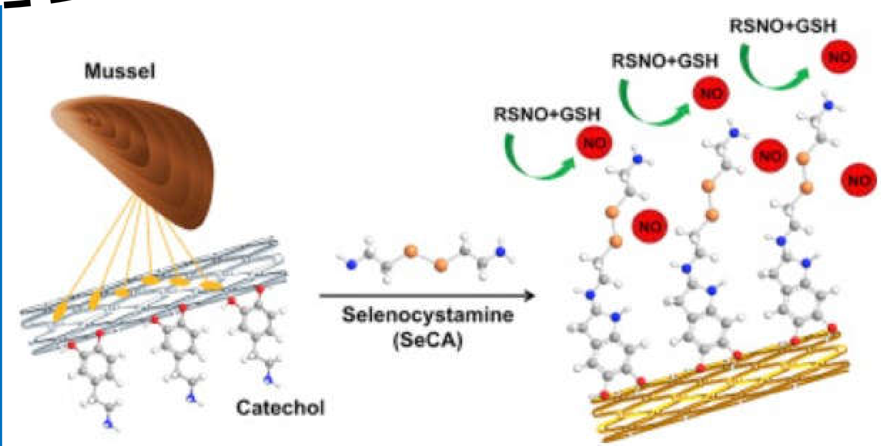
Scaffolds fabrication

Biomaterial application

Microfluidics



Biocoating

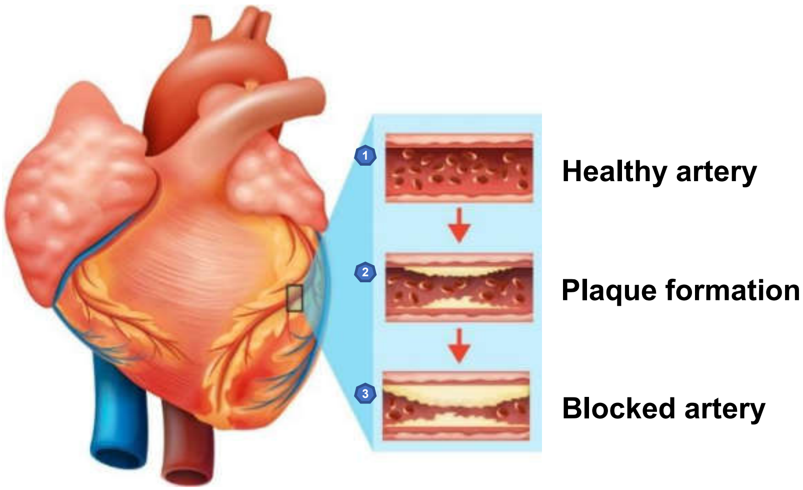


Mussel-inspired cardiovascular stent coatings for therapeutic gas generation to prevent thrombosis （血栓形成） and restenosis （支架内再狭窄）

Background

➢ Cardiovascular diseases

E.g., coronary artery diseases due to lipid deposit of plaque



Formation of atherosclerotic plaque

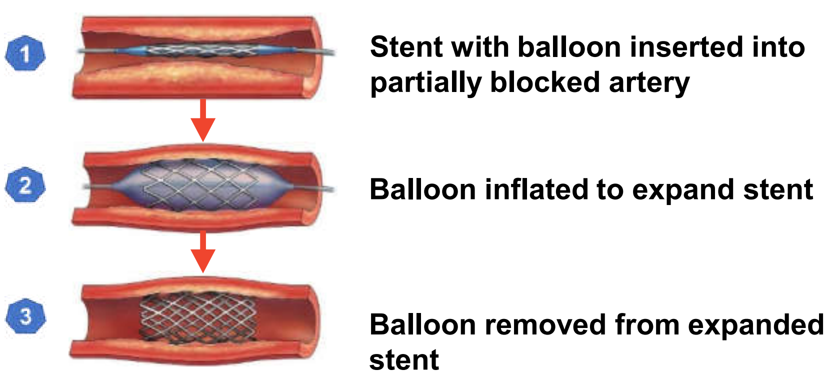
- ➢ Lead to > 18 million deaths and > 350 billion USD every year worldwide
- ➢ > 4 million deaths and > 10 billion USD every year in China
- ➢ 20% of registered deaths in Hong Kong

➢ Treatments for cardiovascular diseases

- ➢ Early-stage atherosclerosis
  - ✓ Pharmaceutical therapy (e.g. Aspirin)
- ➢ Late-stage atherosclerosis (blockage)
  - ✓ Bypass surgery
  - X *Open-heart surgery*
  - X *Long recovery period*

➢ Surgical interventions with stents

- ✓ The most widely performed procedures
- ✓ Minimally invasive surgery and fewer risks
- ✓ Reduce the narrowing and restore blood flow



Stent with balloon angioplasty

Current cardiovascular stents in clinic or lab

➢ Bare metal stent (BMS)

- ✓ Uncoated metal (e.g., 316L stainless steel) stent
- ✓ Reduce stenosis
- ✓ Improve symptoms
- × May cause in-stent restenosis and thrombosis

BMS

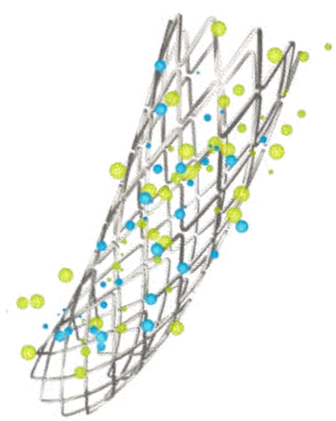
➢ Drug eluting stent (DES)

- ✓ Stent coated with anti-restenosis drug-loaded polymer
- × Uncontrollable drug release
- × Cause delayed re-endothelialisation or in-stent restenosis

DES

➢ Biomolecule-modified stent

- ✓ Surface coated with biomolecules such as <u>adhesive peptides</u>, <u>vascular endothelial growth factors (VEGFs)</u> and <u>nitric oxide (NO)-releasing/generating molecules</u>
- ✓ Preserve adequate mechanical properties of the stent
- ✓ Regulate the behaviors of blood cells including platelet, endothelial cell (ECs), smooth muscle cell (SMCs) to improve stent hemocompatibility
- × Burst or insufficient release of therapeutic molecules
- × Fabrication complexity with involvement of toxic organic solvent



Biomolecule-modified stent

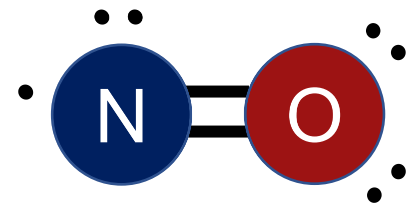
Project Aim

Project aim is to develop a blood-compatible stent coating that releases therapeutic agents in a long-term and controllable manner to prevent major post-surgery complications such as thrombosis （血栓形成） and restenosis （支架内再狭窄）.

Which therapeutic agent we should use?

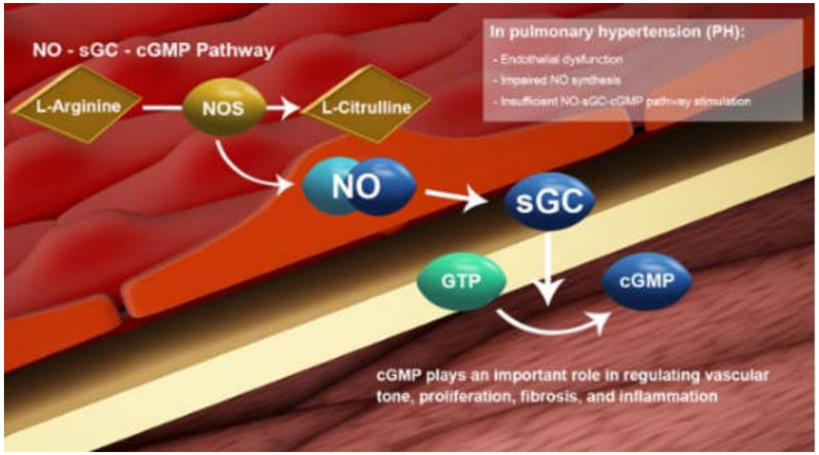
Nitric Oxide (NO)

- Signaling molecule
- Endogenously synthesized and secreted by ECs



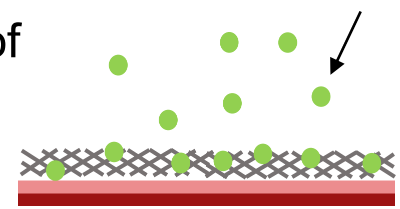
Significance of NO in maintaining cardiovascular system health

- Antithrombotic mediator
  - Up-regulate the expression of cyclic-guanylate monophosphate (cGMP, 环鸟苷酸)
  - Inhibit platelet adhesion and activation
- Stimulate ECs growth
- Inhibit SMC proliferation



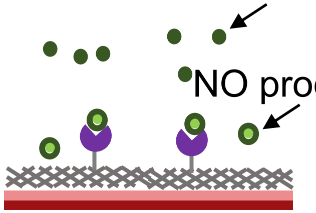
Pre-existing attempts:

NO-releasing coatings



- ➢ Incorporate NO donors (i.e., RSNOs) into the coating layer of vascular stents
- × NO donors are vulnerable to heat, moisture, and light
- × Insufficient NO release for a short time period

Enzyme-functionalized coatings



- ➢ Immobilizes catalytic enzymes onto the stent as coating
- × Require injection of exogenous NO prodrug as a source of NO

NO-generating coatings

- ➢ Conjugate catalytic molecules (i.e., glutathione peroxidase (GPx, 谷胱甘肽过氧化物酶)-like catalytic mimics onto stent surface
- ✓ Take advantage of the endogenous NO donors (i.e., RSNOs) for NO generation

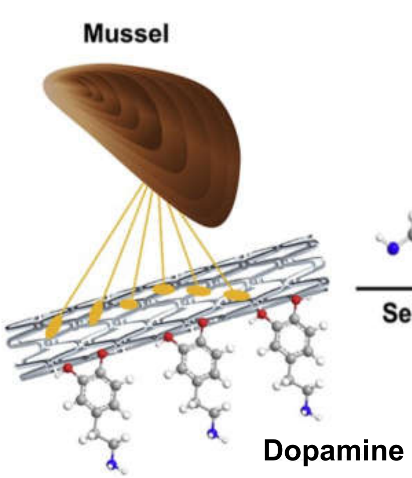
✓ Immobilizes catalytic selenocystamine (SeCA) onto stent surface

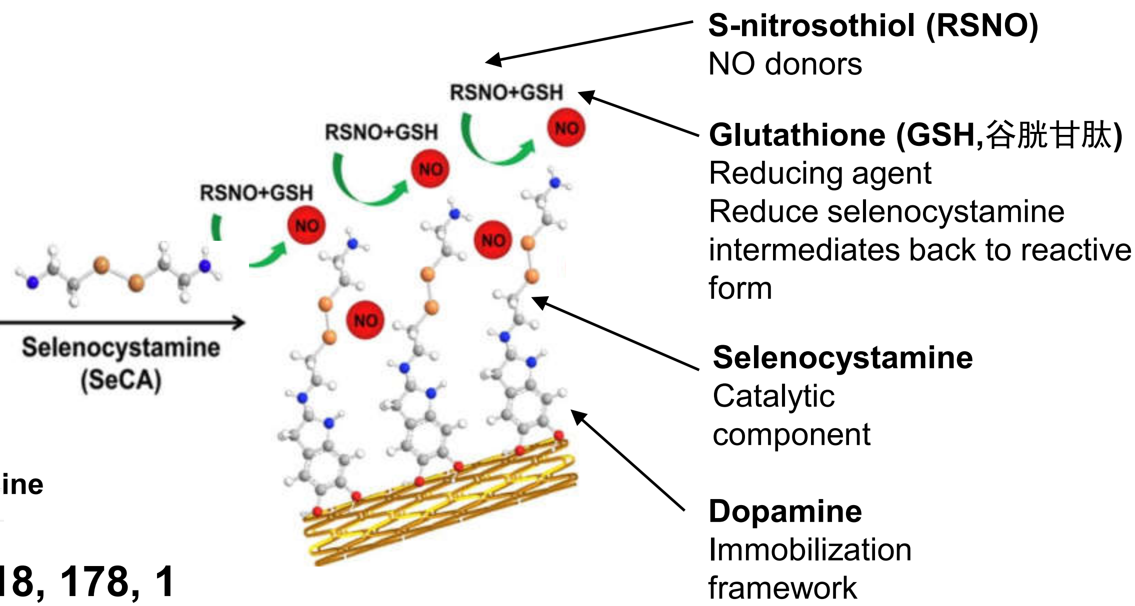
- Inspired by adhesion protein L-DOPA and lysine found in mussel byssus
- **SeCA** provides glutathione peroxidase (GPx,谷胱甘肽过氧化物酶)-like **NO catalytic activity**
- Dopamine are used as framework for immobilizing SeCA proteins

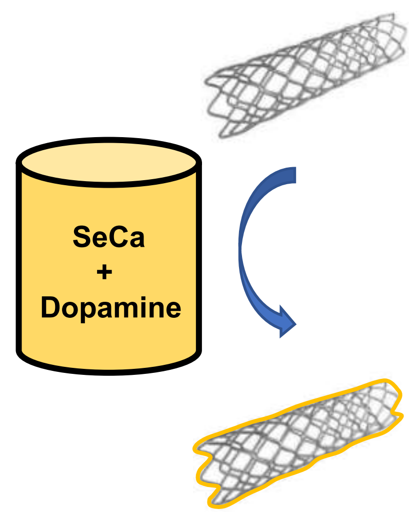
✓ Utilizes NO donors in blood as a source of NO gas

- **Maintain stable generation of NO in blood stream through catalysis of RSNO**
- **Avoid the depletion of NO donors**

Stents are coated through simple “one-pot” method by dipping into prepared solution.



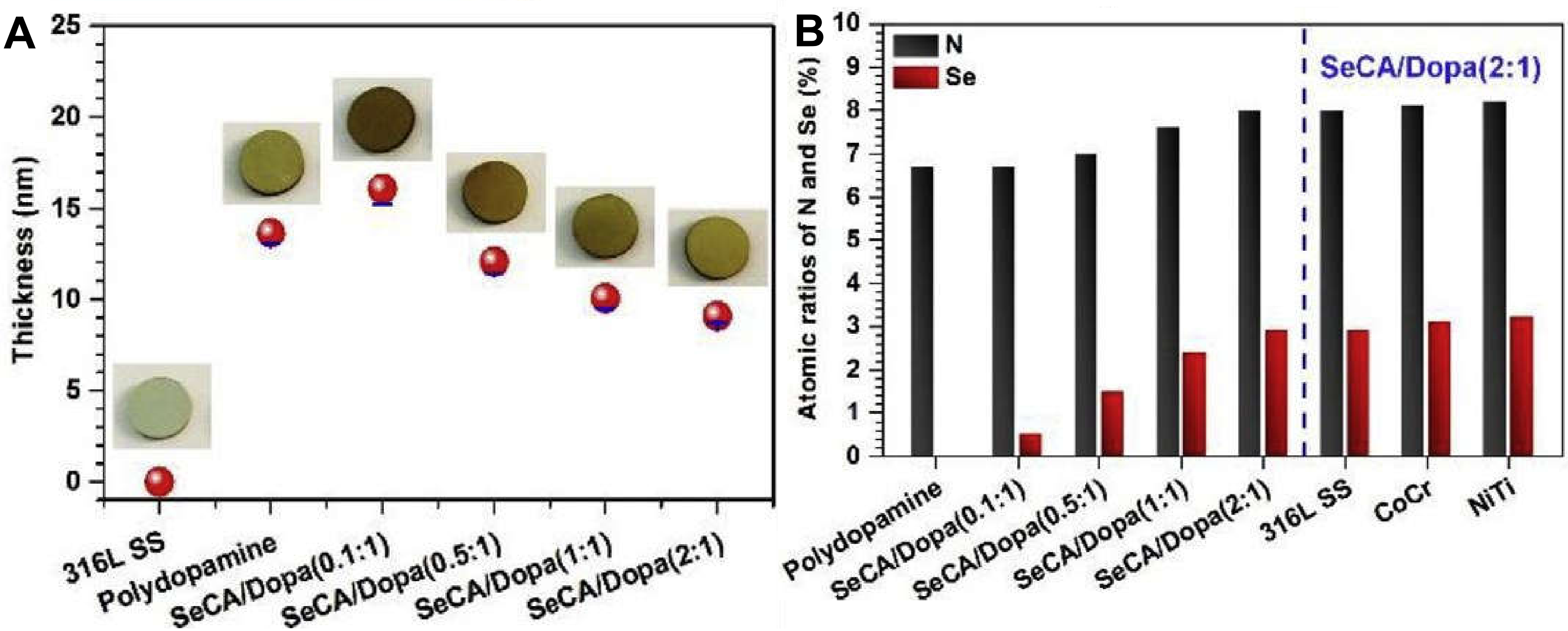




12 h reaction

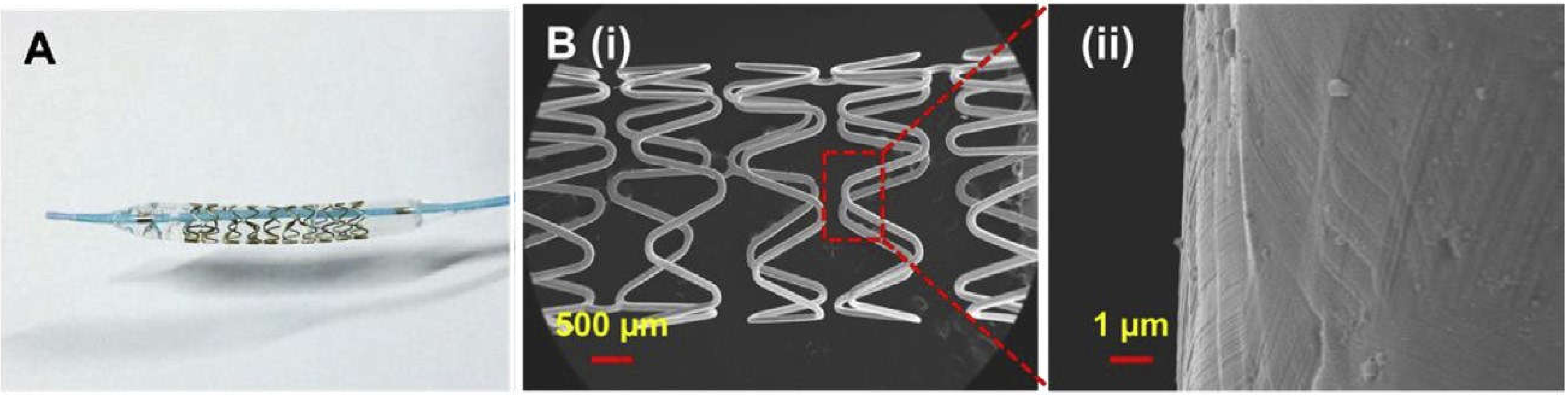
Biomaterials, 2018, 178, 1

Result 1: Formation of coating characterized by ellipsometer (偏振光椭圆率测量仪， A) and XPS (X射线光电子谱仪， B)



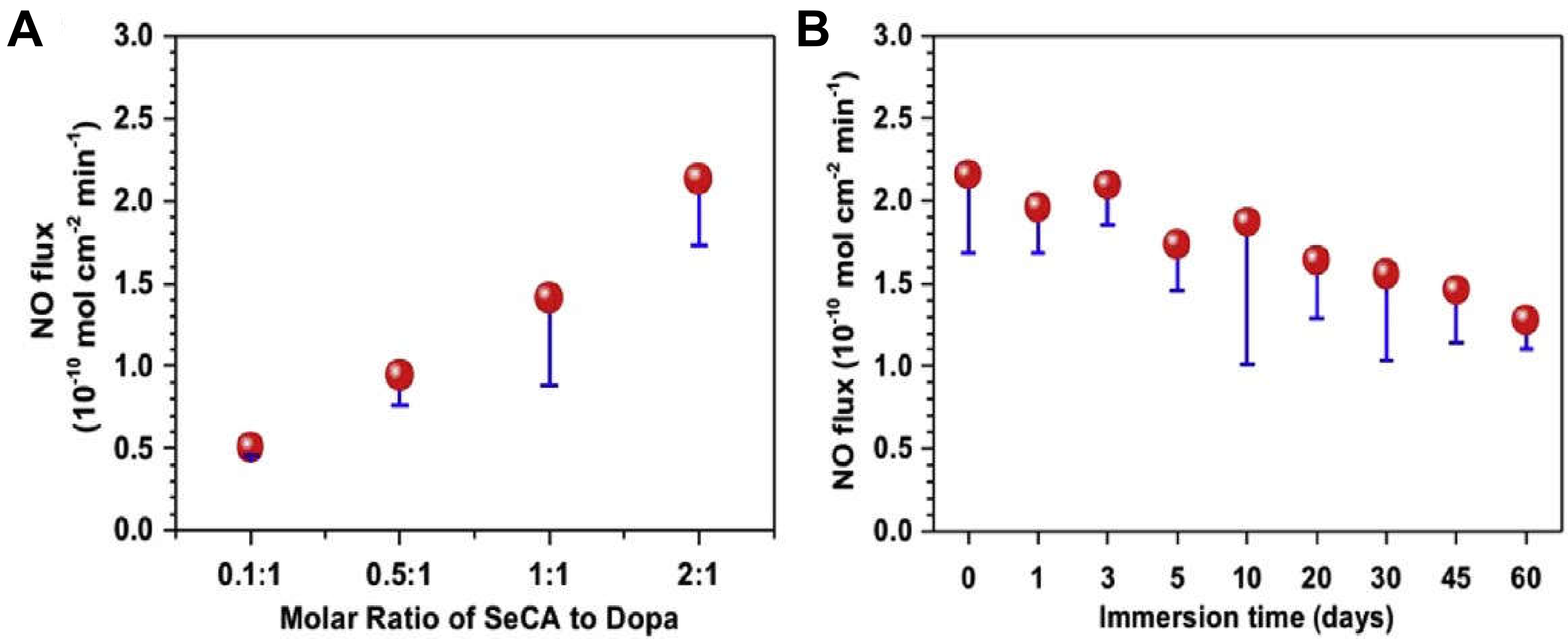
✓ Successful formation of catalytic SeCA/Dopa coatings on the stent.

Result 2: Stent endurance during balloon angioplasty （血管成形术）



✓ Catalytic SeCA/Dopa coatings can endure stresses during dilation without experiencing any coating damages.

Result 3: Nitric Oxide Generation using Chemiluminescence NO analyzer (化学发光NO分析仪）



- ✓ Increase in SeCA/Dopa ratios results in increasing NO generation.
- ✓ Catalytic SeCA/Dopa coatings can stably and continuously generate nitric oxide for **60 days.**
- ✓ Experimental rate: $0.5 - 2.2 \times 10^{-10} mol \cdot cm^{-2} \cdot min^{-1}$ (in the range of the NO physiological value: $0.5 - 4.0 \times 10^{-10} mol \cdot cm^{-2} \cdot min^{-1}$)

Coating's Advantages

✓ Simple manufacturing procedures

✓ Controlled **catalytic generation of nitric oxide**

- Long-term stability for 60 days
- Optimal rate of nitric oxide generation compared to physiological value
- **Experimental Rate:** $\mathbf{0.5 - 2.2 \times 10^{-10} mol \cdot cm^{-2} \cdot min^{-1}}$

Major Disadvantages

× still low and unsatisfactory generation rate of NO

physiological value: $0.5 - 4.0 \times 10^{-10} mol \cdot cm^{-2} \cdot min^{-1}$

Therefore, because of excellent GPx-like NO catalytic activity of Cu^{II} ions, we are inspired to design a coating using Cu^{II} to replace SeCA

Approach 2: Dopamine – Cu^{II} Stent Coating

➢ Copper II ions (Cu^{II}) ■ NO catalytic component

✓ Excellent **glutathione peroxidase** (GPx)-like NO catalytic activity

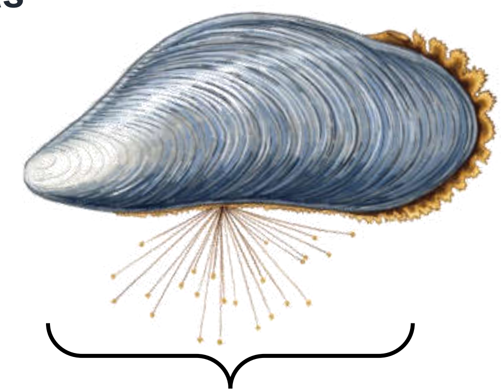
✓ Decomposition of S-nitrosothiols (RSNOs) existing in blood into NO gas

✓ Angiogenesis stimulus beneficial for wound healing

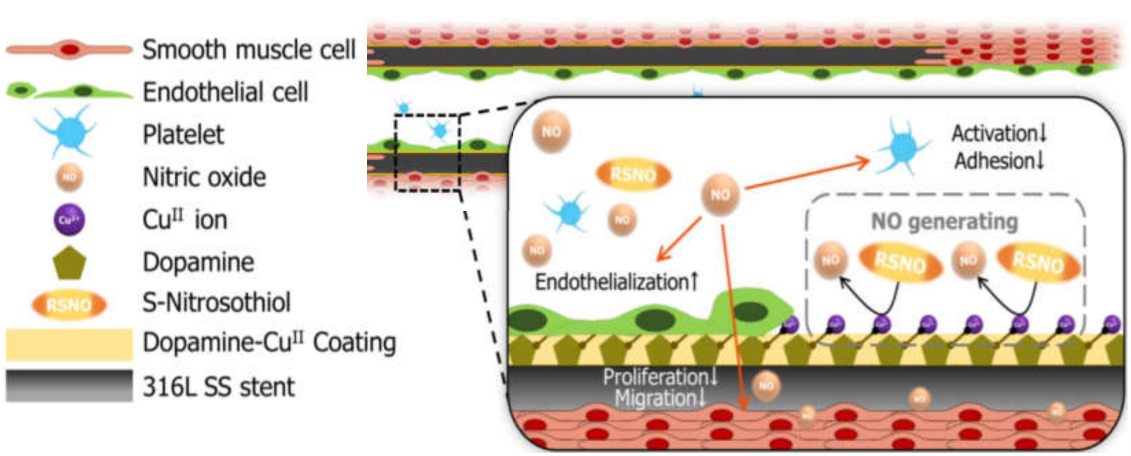
➢ Dopamine (DA) ■ A framework to immobilize the Cu icons

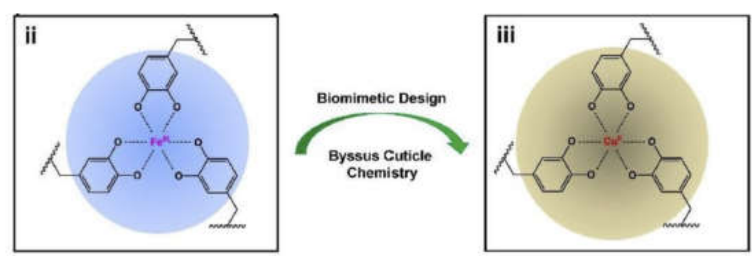
✓ Metal-catecholamine assembling strategy

✓ Inspired by the adhesion and protein cross-linking chemistry of $[Fe(DA)_3]$ complexes found in mussel byssus

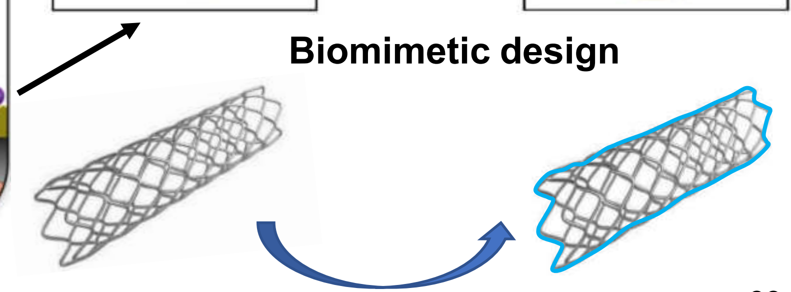


Inspired by mussel byssus



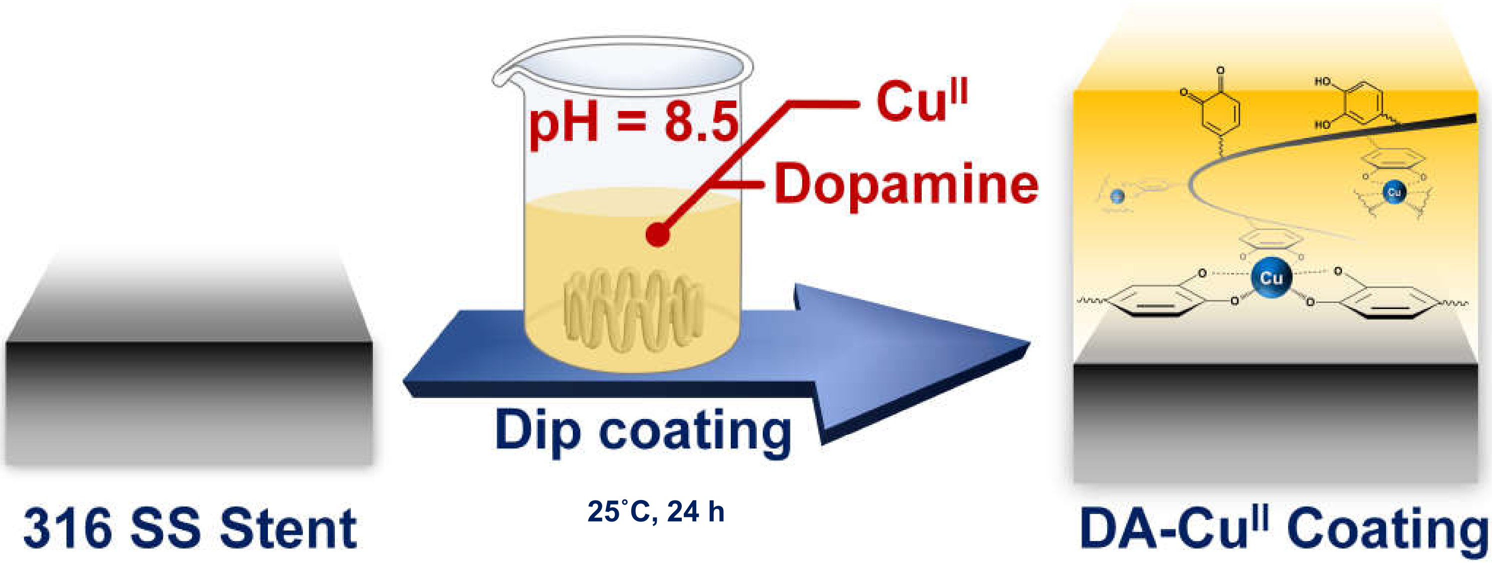


Biomimetic design



Biomaterials, 194, 117, 2019

➢ Fabrication of Dopamine-Cu^{II} coating



Cu: sustainable and local NO generation by decomposition of endogenous S-nitrosothiols (RSNOs) from fresh blood

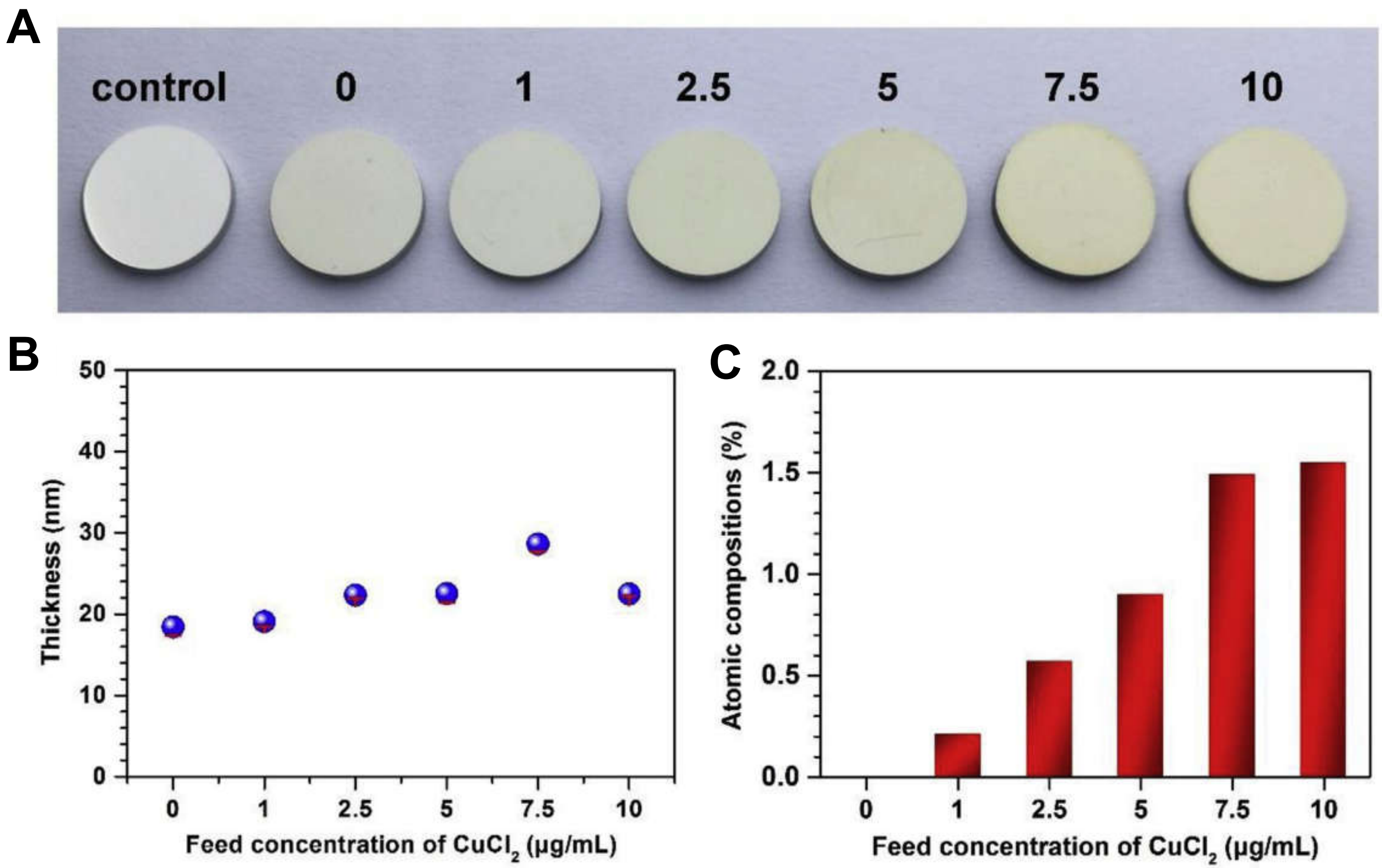
Polydopamine: robust adhesion and mechanical strength to withstand stent deformation and maintain surface performance

Advantages:

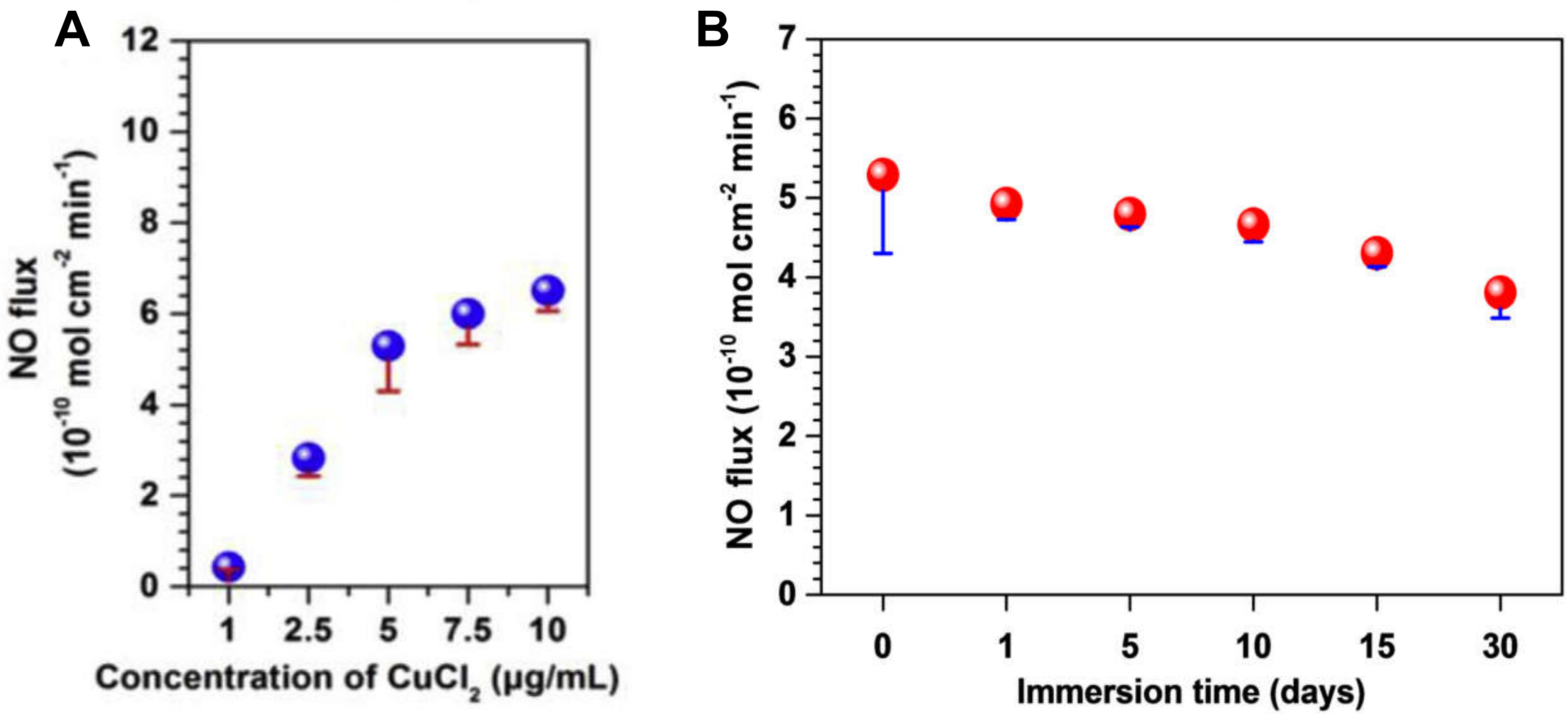
1. **Simple manufacture procedures**
2. **Environmentally friendly nature**
3. **High bioconjugation efficiency**
4. **High reproducibility**

Stents are coated through simple "one-pot" method by dipping into prepared solution

Result 1: Physical characteristics by ellipsometer (B) and XPS (C)

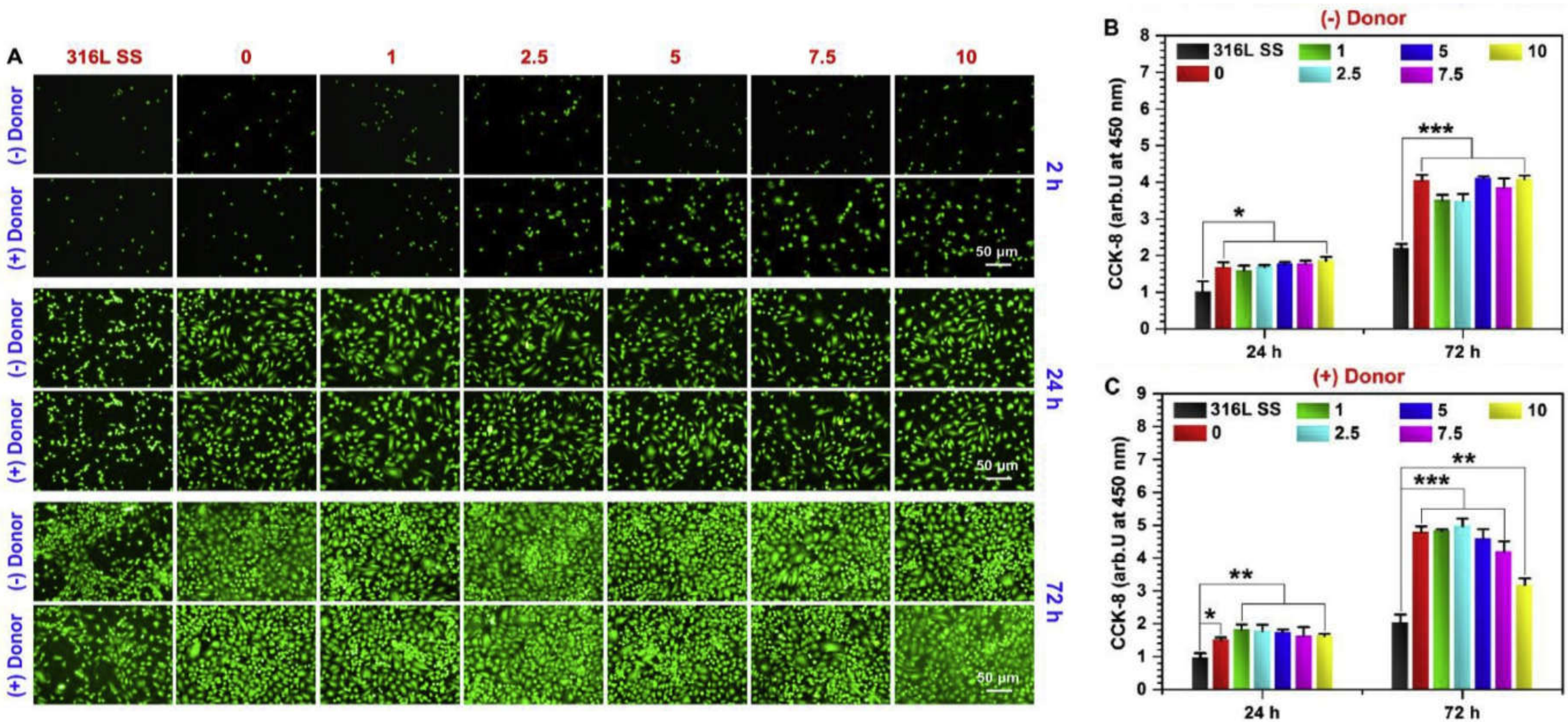


- ✓ Successful formation of catalytic DA-Cu^{II} coatings on the stent.
- ✓ Content of Copper II ions in the thin DA-Cu^{II} coating is adjustable.



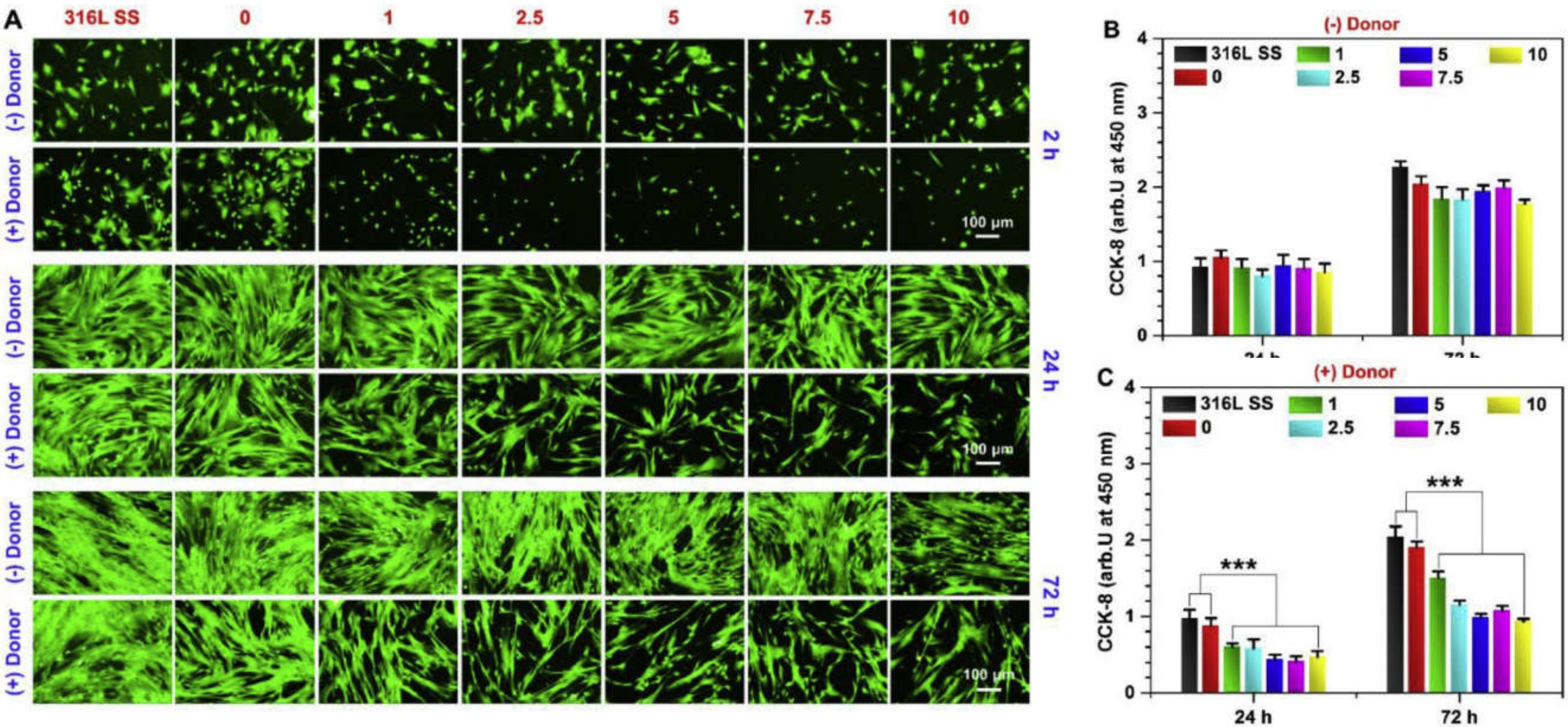
- ✓ Increase in Cu content resulted in increasing NO gas.
- ✓ Catalytic DA- Cu^{II} coatings can produce a controllable, stable, and durable release of NO flux for **30 days.** Experimental rate: $0.5 - 6.5 \times 10^{-10} mol \cdot cm^{-2} \cdot min^{-1}$
- ➢ (More comparable to the physiological value: $0.5 - 4.0 \times 10^{-10} mol \cdot cm^{-2} \cdot min^{-1}$)

Result 3: Endothelial cell growth behavior



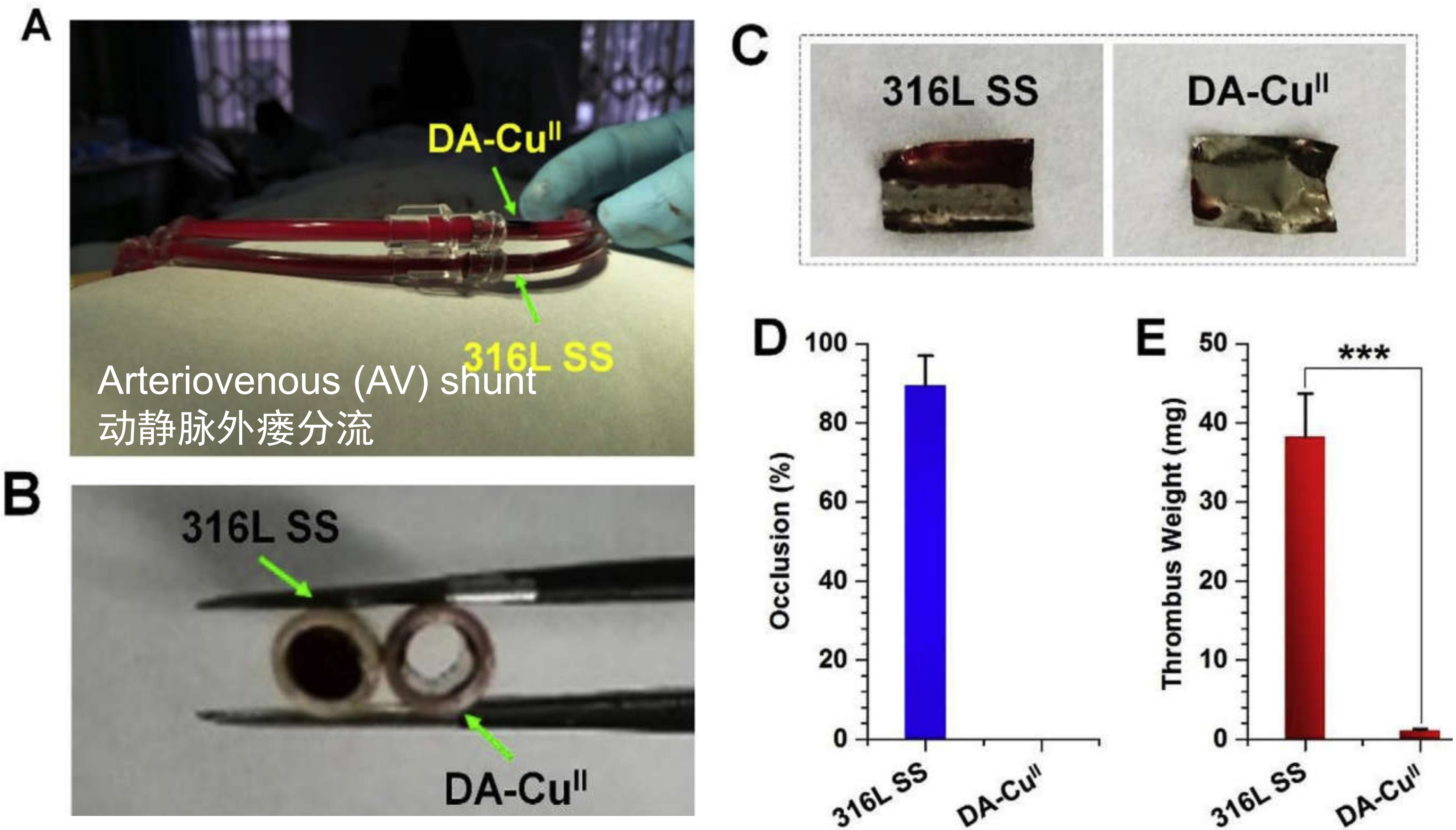
- ✓ DA-Cu^{II} coated surfaces can offer a better microenvironment and enhance the growth and attachment of endothelial cells in presence of NO donor.
- ✓ Inhibition of EC growth observed > 7.5 $\mu g/mL$.

Result 4: Smooth muscle cell behavior by phalloidin staining

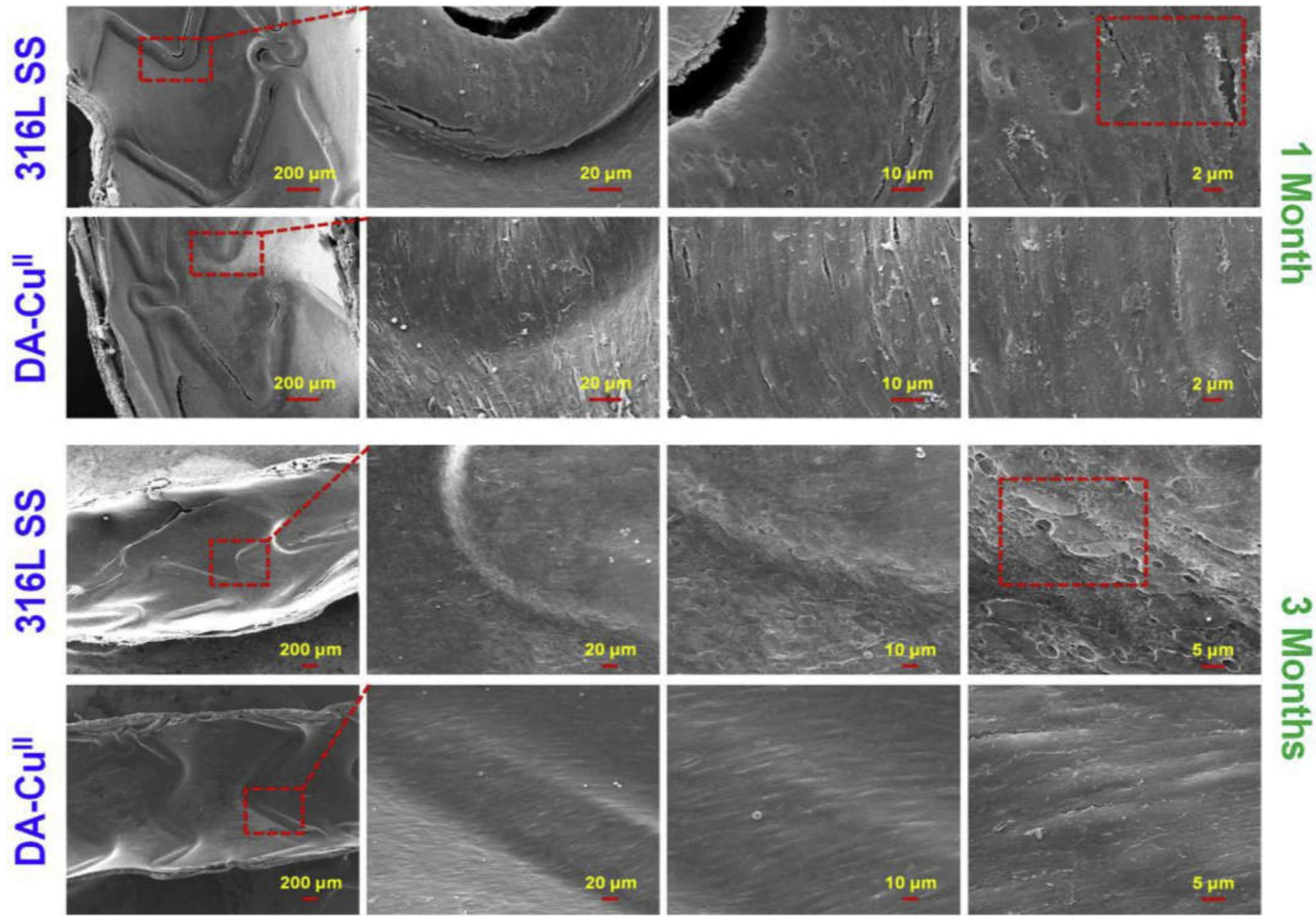


- ✓ DA-Cu^{II} coated surfaces can effectively discourage attachment of SMCs.
- ✓ Higher concentration of Cu further enhance the inhibition of SMC attachment.
- ✓ Optimal Cu^{II} concentration determined is $5\ \mu g/mL$ for best EC promotion and SMC inhibition.

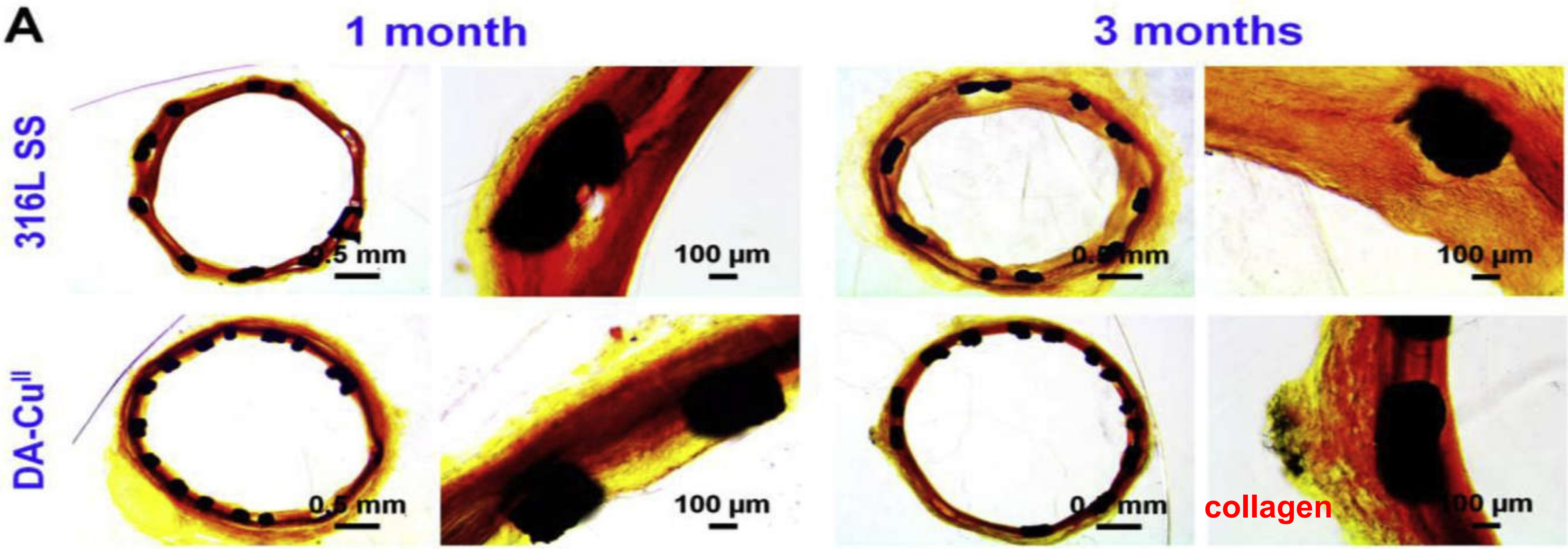
Result 5: *Ex vivo* thrombosis prevention using rabbit model



✓ *Ex vivo* circulation model shows that DA-Cu^{II} coated surfaces possess the ability to reduce the size of thrombus formed after 2 h circulation.



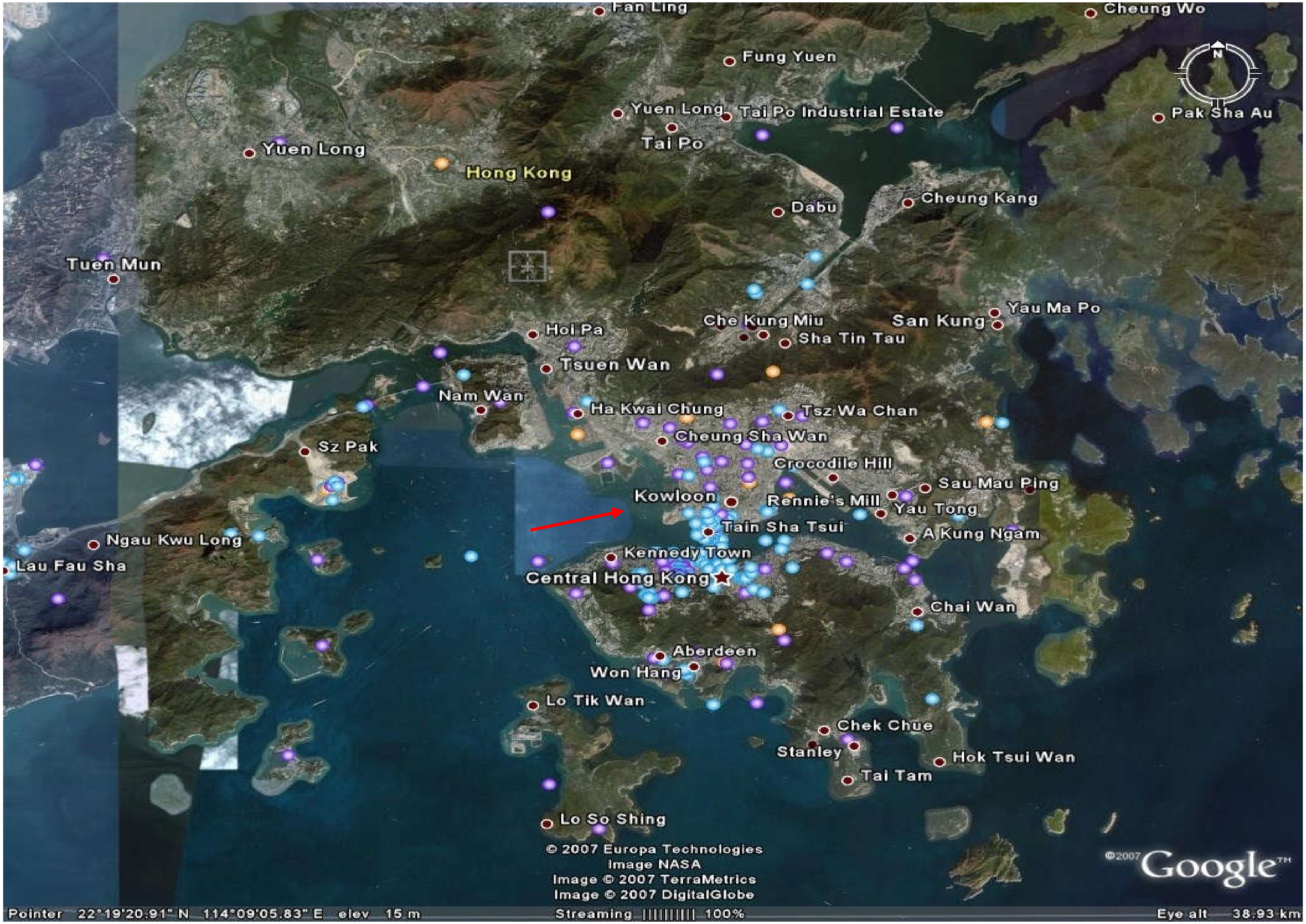
✓ DA-Cu^{II} coated surfaces can enhance healthy re-endothelialization after stent implantation by promoting complete coverage of ECs.



✓ DA-Cu^{II} coated surfaces can reduce neointimal stenosis (内膜狭窄）.

Advantages of our dopamine-Cu^{II} coatings

- ✓ **Simple manufacturing procedures** for stent modification
- ✓ **Long-term stability** and **highly controllable NO catalytic efficiency**
- ✓ **Rapid re-endothelialization**
- ✓ **Successful prevention** of thrombosis, restenosis, and neointimal stenosis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Thanks for your attention!