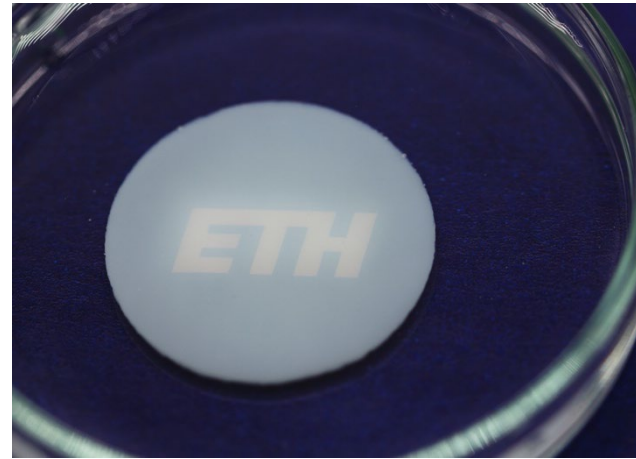
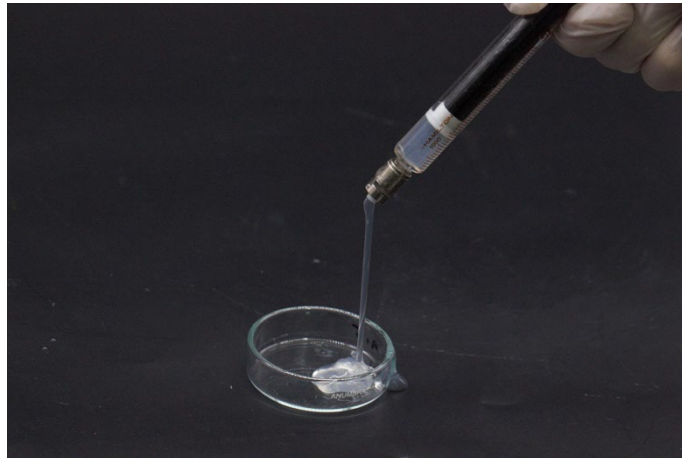
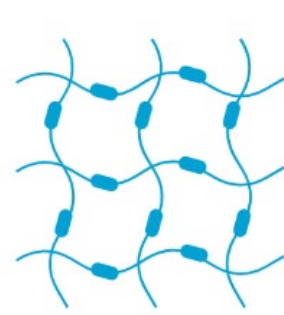


Thermo-responsive Nanocellulose Reinforced Hydrogels as a Universal Drug Release Platform

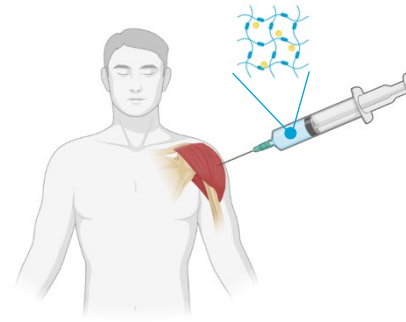


Qiyao Sun, Giovanni Bovone, Siyuan Tao, Garam Han, Pascal Bertsch, Dhananjay Deshmukh, Luca Müller, Mark Tibbitt, Qun Ren, Gilberto Siqueira and Peter Fischer*

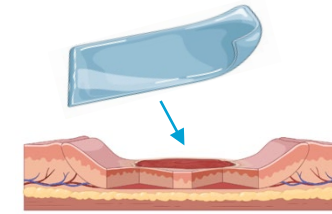
Motivation



- Tunable structures
- Tunable mechanical, physiochemical properties, stimuli-responsiveness

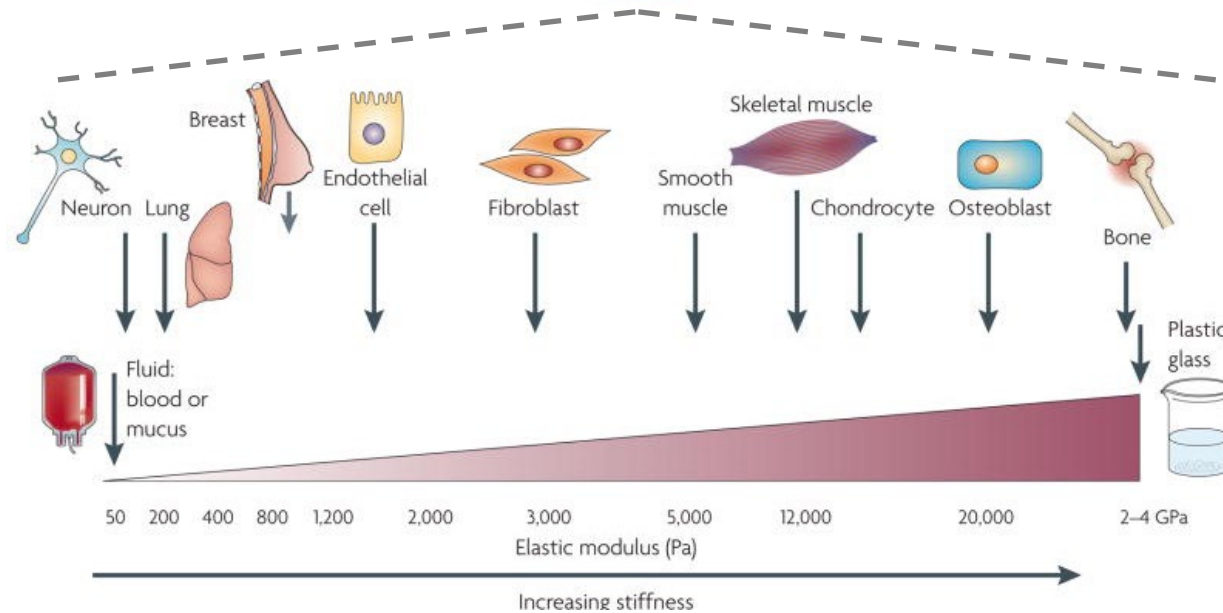
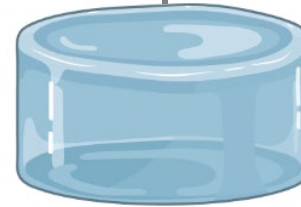


- Injectable hydrogel
- Therapeutic local administration



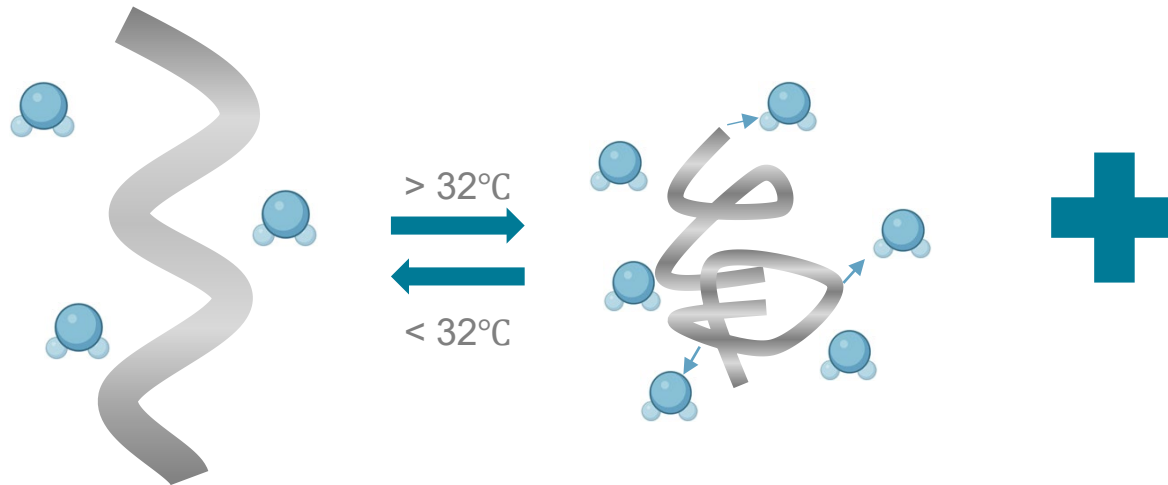
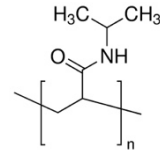
- Wound dressing

Hydrogel

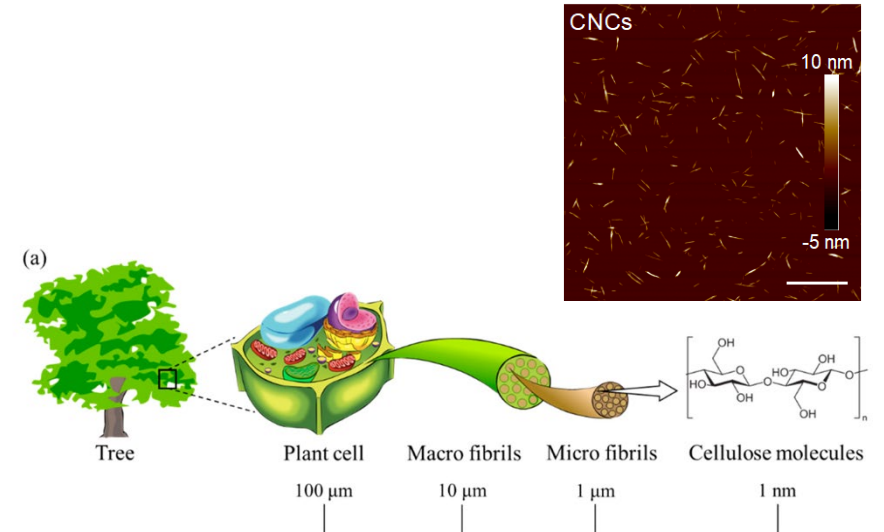


Synthesis

PNIPAM



Nanocellulose (CNC, CNF)

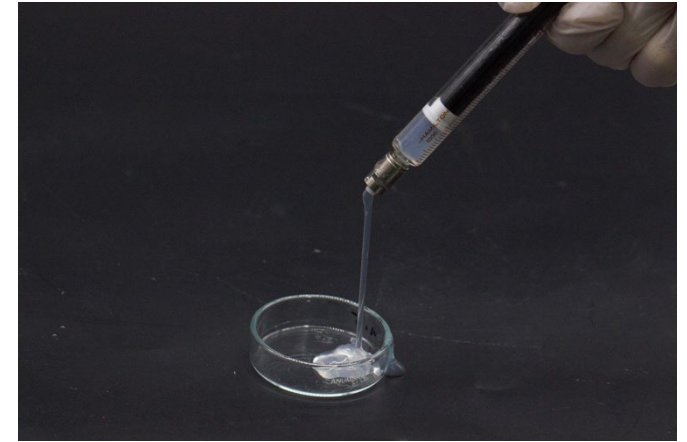
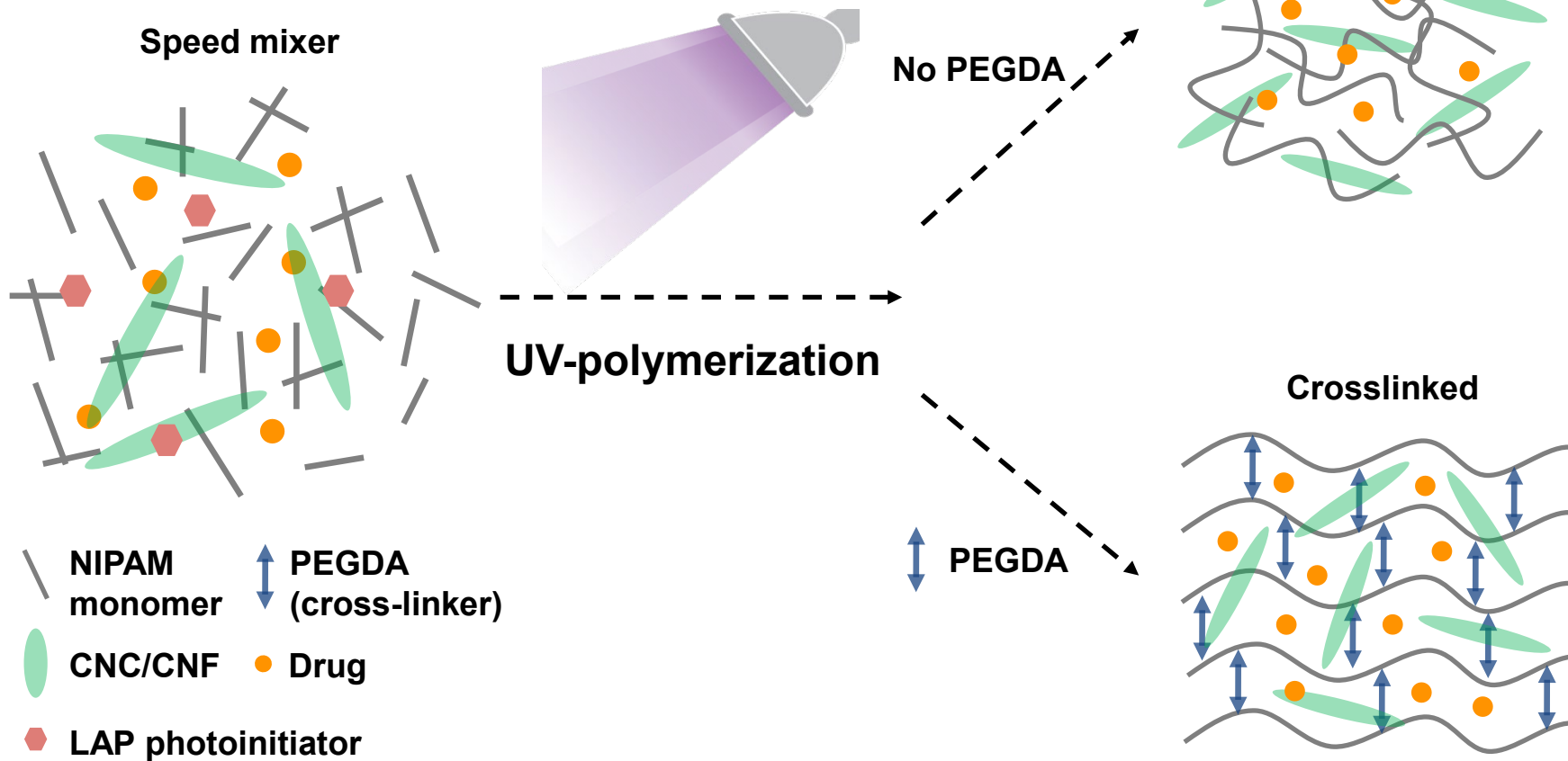


Miyashiro et al. *Nanomaterials* 10.2 (2020).
Bertsch et al. *Langmuir* 34.50 (2018).

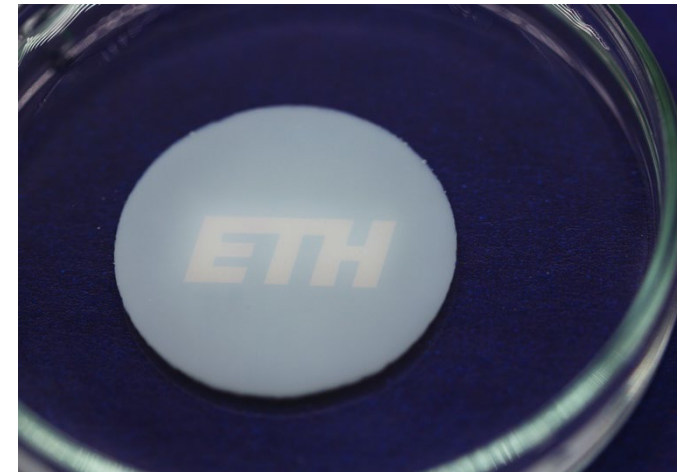
- PNIPAM: Poly(N-isopropylacrylamide)
- **Thermo-responsive:** Lower critical solution temperature at **32°C**
- Biocompatible
- **Disadvantage: Low mechanical strength**

- Natural abundancy, biocompatible, biodegradable
- Obtained from sulfuric acid hydrolysis of **wood** pulp
- Rigid, rod-like, anisotropic, negatively charged
- **High mechanical strength** → **reinforcement agent**

Synthesis

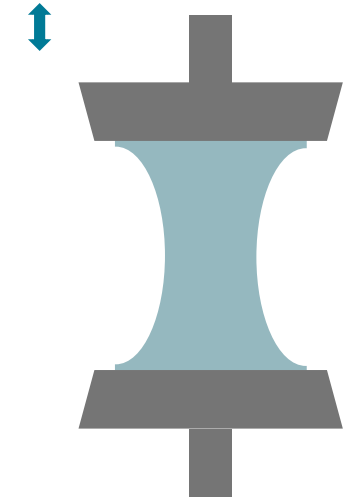
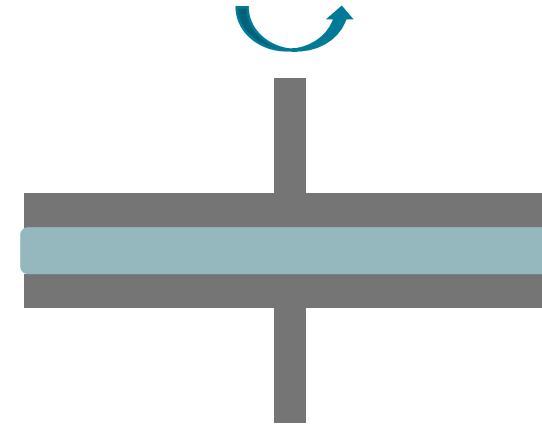
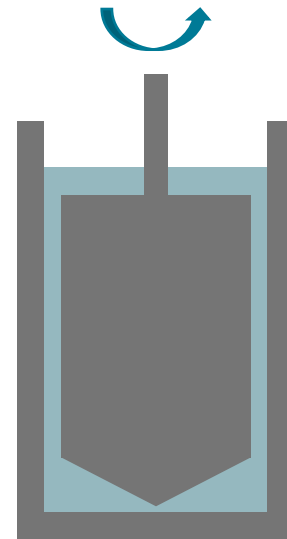
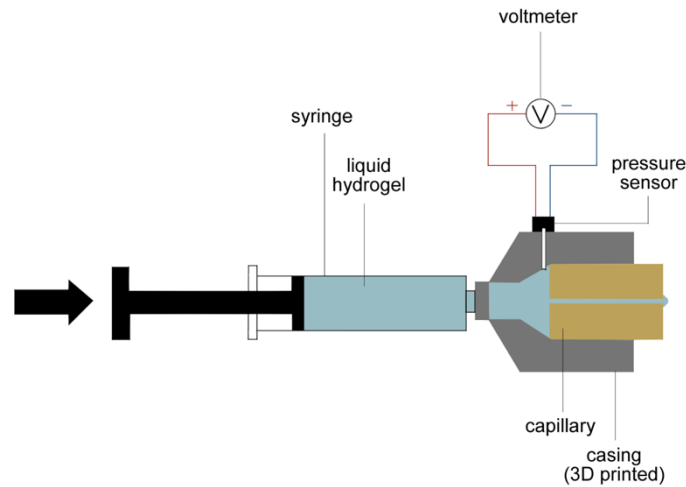
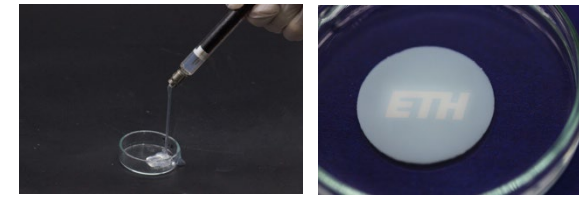


Liquid: Injectable hydrogel



Solid: Wound Dressing

Rheological Characterization Toolbox



Liquid: Capillary Rheology

- Mimic injection
- In situ viscosity measurement

Liquid: Shear rheology

- Temperature sweep
- Oscillatory test
- Steady shear rheology

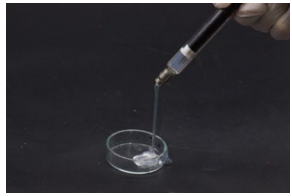
Solid: Plate-Plate

- Temperature sweep

Solid: Tensile

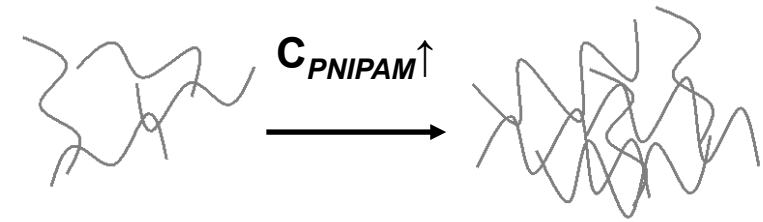
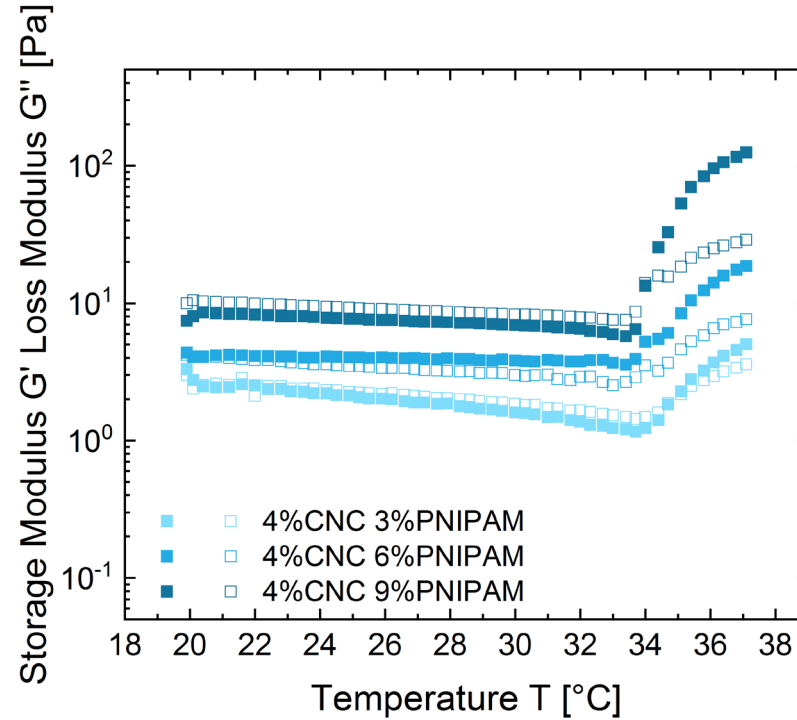
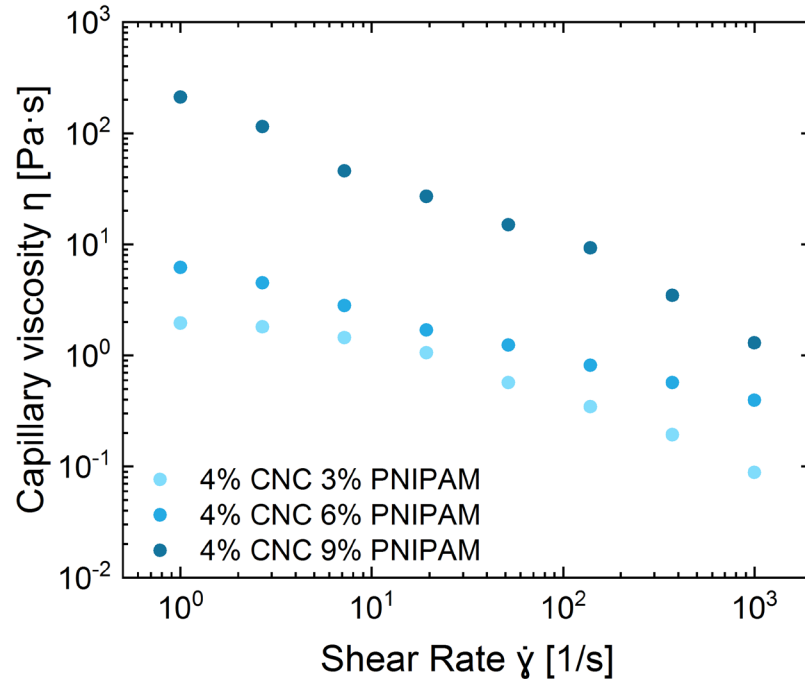
- Force amplitude sweep

Designing Liquid Injectable Hydrogel for *in vivo* Drug Delivery



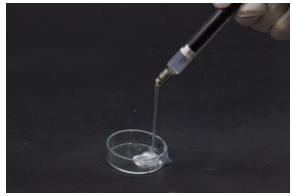
➤ Rheological characterization: Fixed CNC%, varying NIPAM%

Capillary rheology



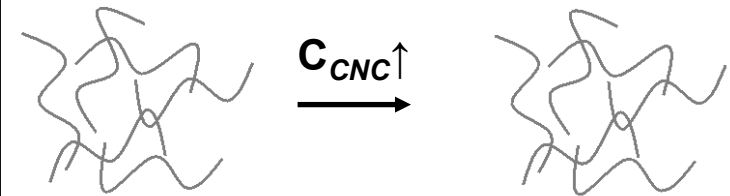
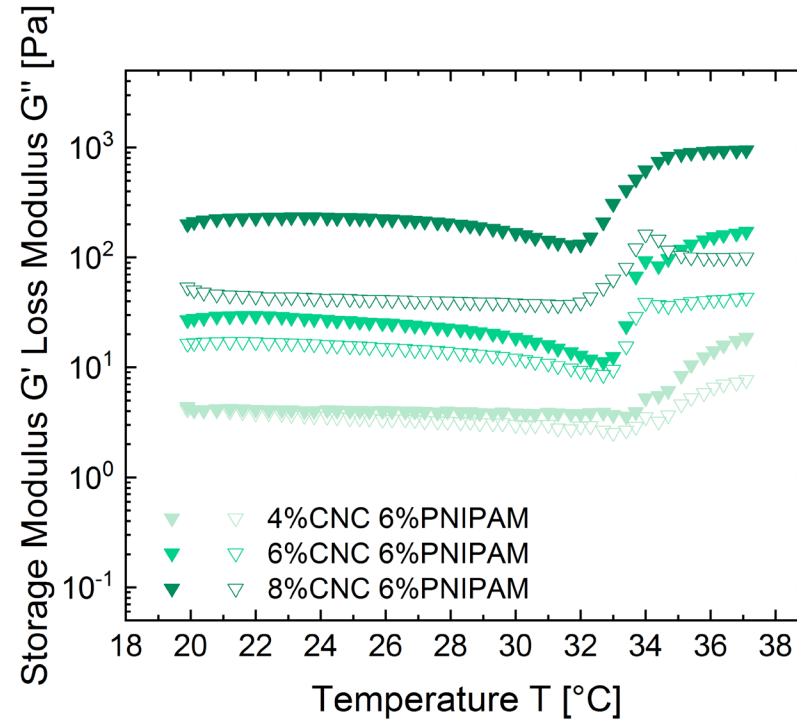
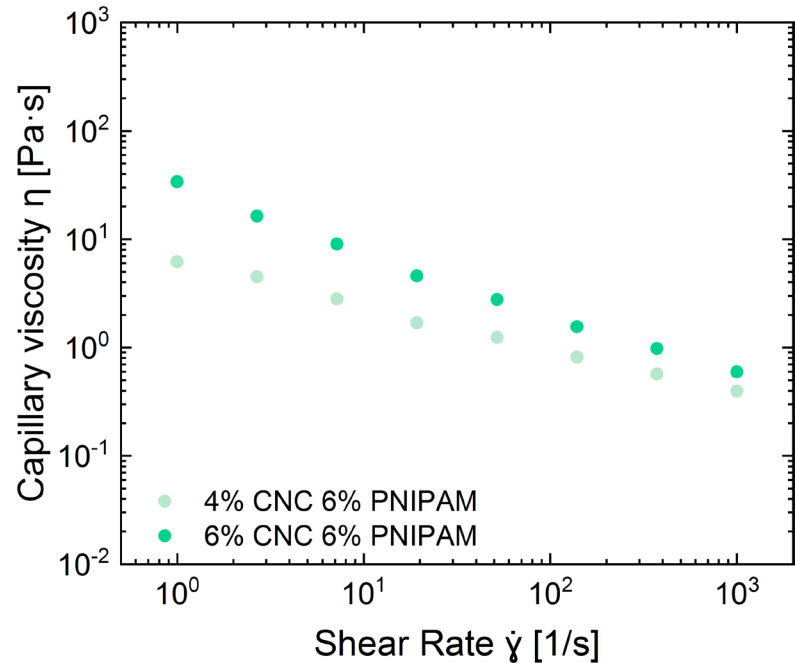
- Injectability: **Shear-thinning behavior** under capillary flow
- $G' > G''$ increases significantly at 33°C, $G' > G''$ indicating PNIPAM's **thermo-gelation**
- **Higher PNIPAM% leads to higher $\Delta G'$**

Designing Liquid Injectable Hydrogel for *in vivo* Drug Delivery



➤ Rheological characterization: Fixed NIPAM%, varying CNC%

Capillary rheology

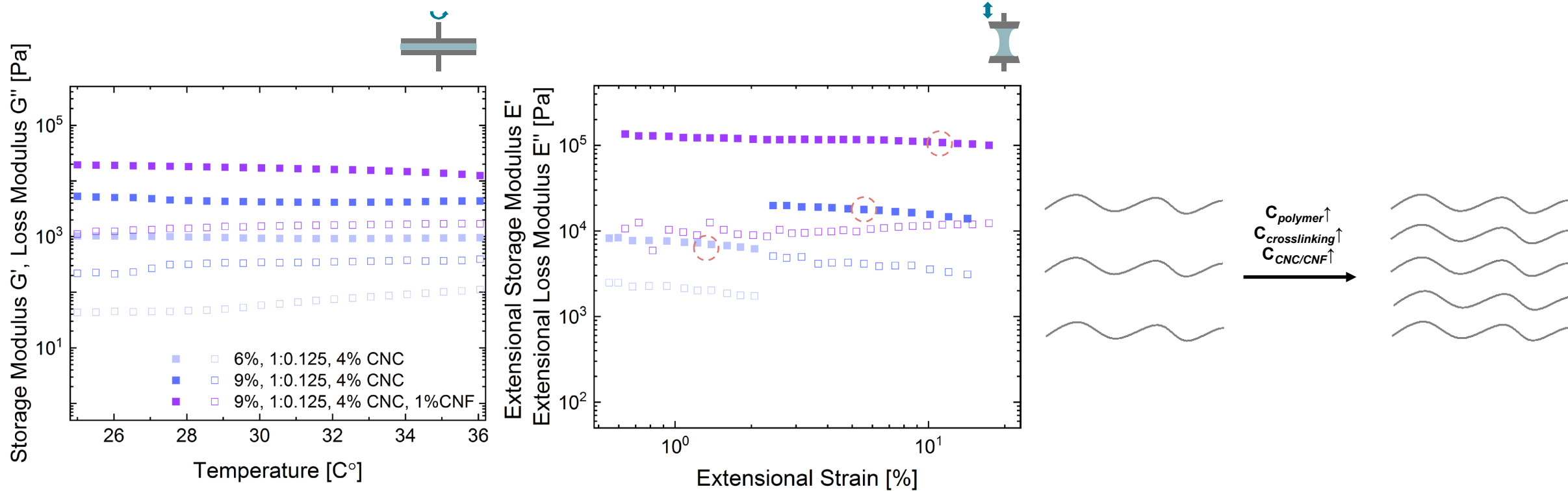


- Injectability: **Shear-thinning** behavior under capillary flow up to 6% CNC addition
- **Higher CNC% lifts up G' G''**, no effect on the thermo-responsiveness

Designing Solid Hydrogel Targeting Wound Dressing



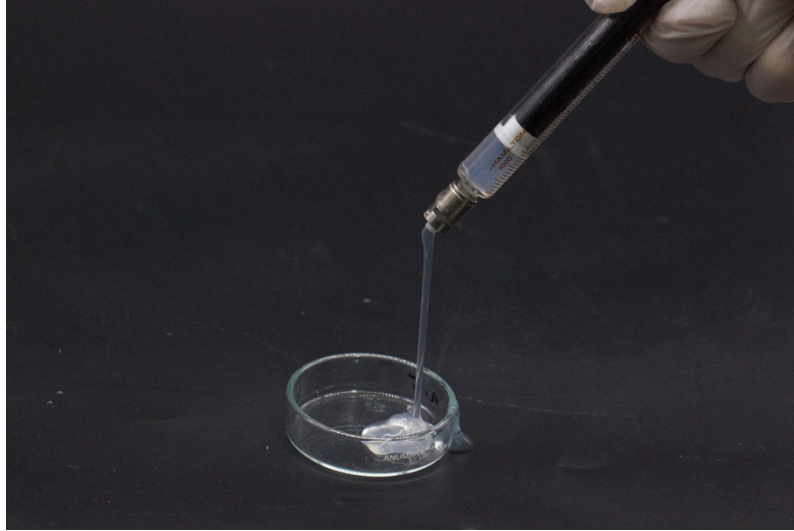
➤ Rheological characterization: Varying NIPAM+PEGDA%, CNF addition



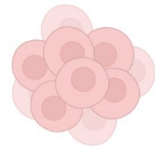
- **Loss of thermo-responsiveness**, possibly due to cross-linking
- **Significant higher mechanical strength** compared to liquid hydrogels
- **Higher NIPAM+PEGDA%, CNF addition** → $G' G''$, $E' E''$ ↑, tensile yield strength ↑

Hydrogel as a universal drug delivery platform

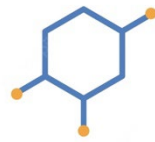
Liquid injectable hydrogel:
in vitro drug delivery



Release kinetics



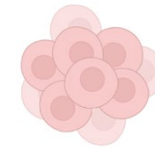
Biocompatibility



Bioactivity



Antimicrobial



Biocompatibility

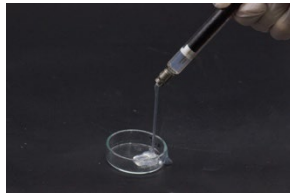


Ex vivo

Solid hydrogel:
Antimicrobial wound dressing



Liquid Injectable Hydrogel *in vitro* Drug Delivery

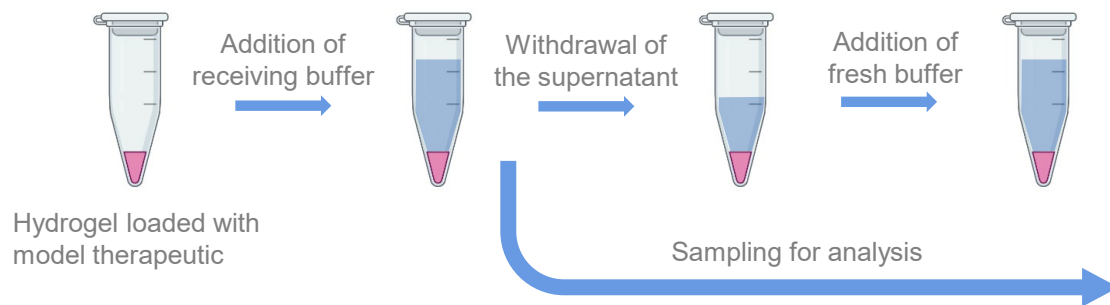


➤ Drug release kinetics

Model drug selection

- Small molecule drugs
 - Rhodamine (hydrophilic)
 - Doxorubicin (hydrophobic)
- Macromolecule drugs
 - FITC-Albumin (model protein, negatively charged)
 - IGG-Atto (antibody, weak negatively charged)

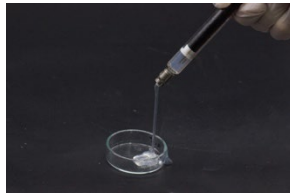
Method: UV-Vis spectroscopy



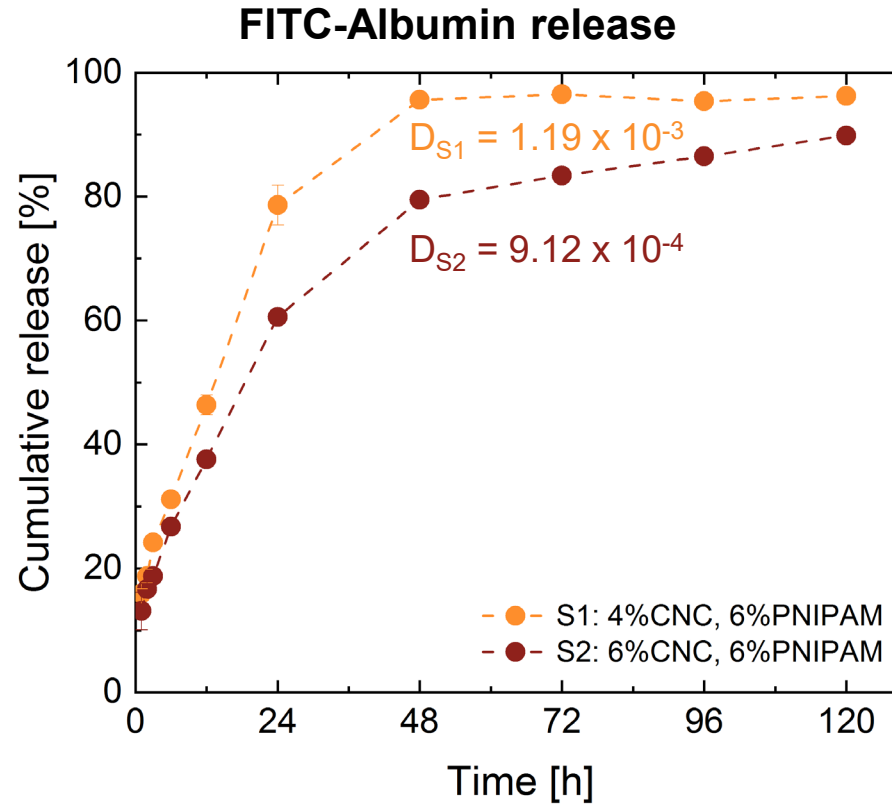
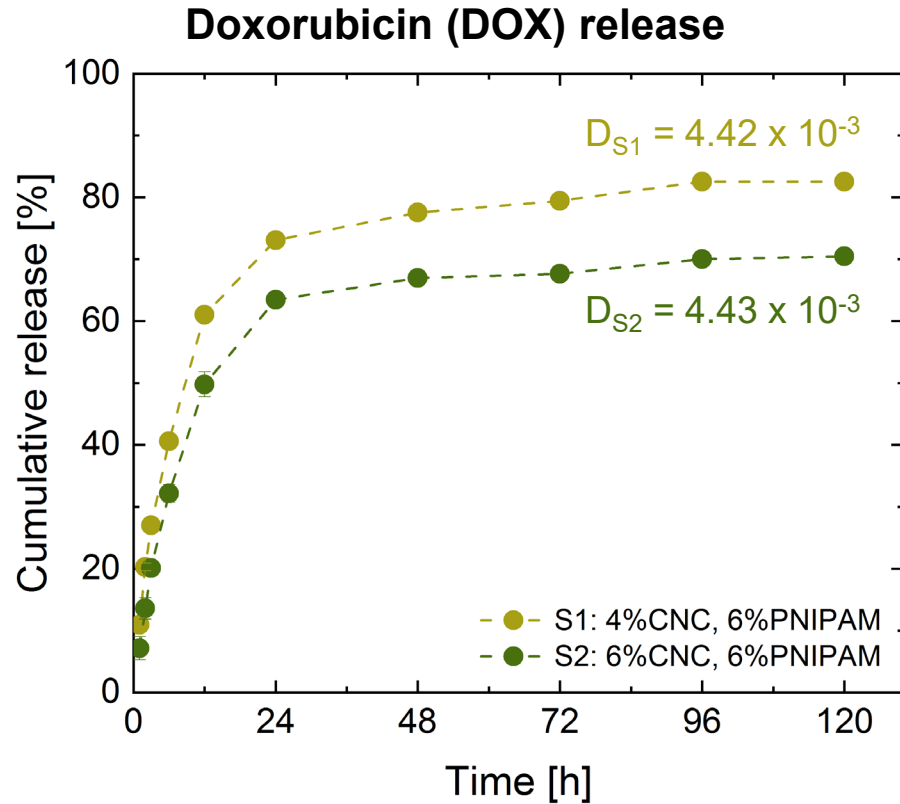
Kim, Yejin, et al. *Pharmaceutics* 13.8 (2021).



Liquid Injectable Hydrogel *in vitro* Drug Delivery

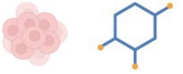
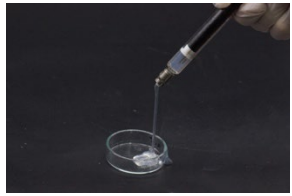


➤ Drug release kinetics preliminary results

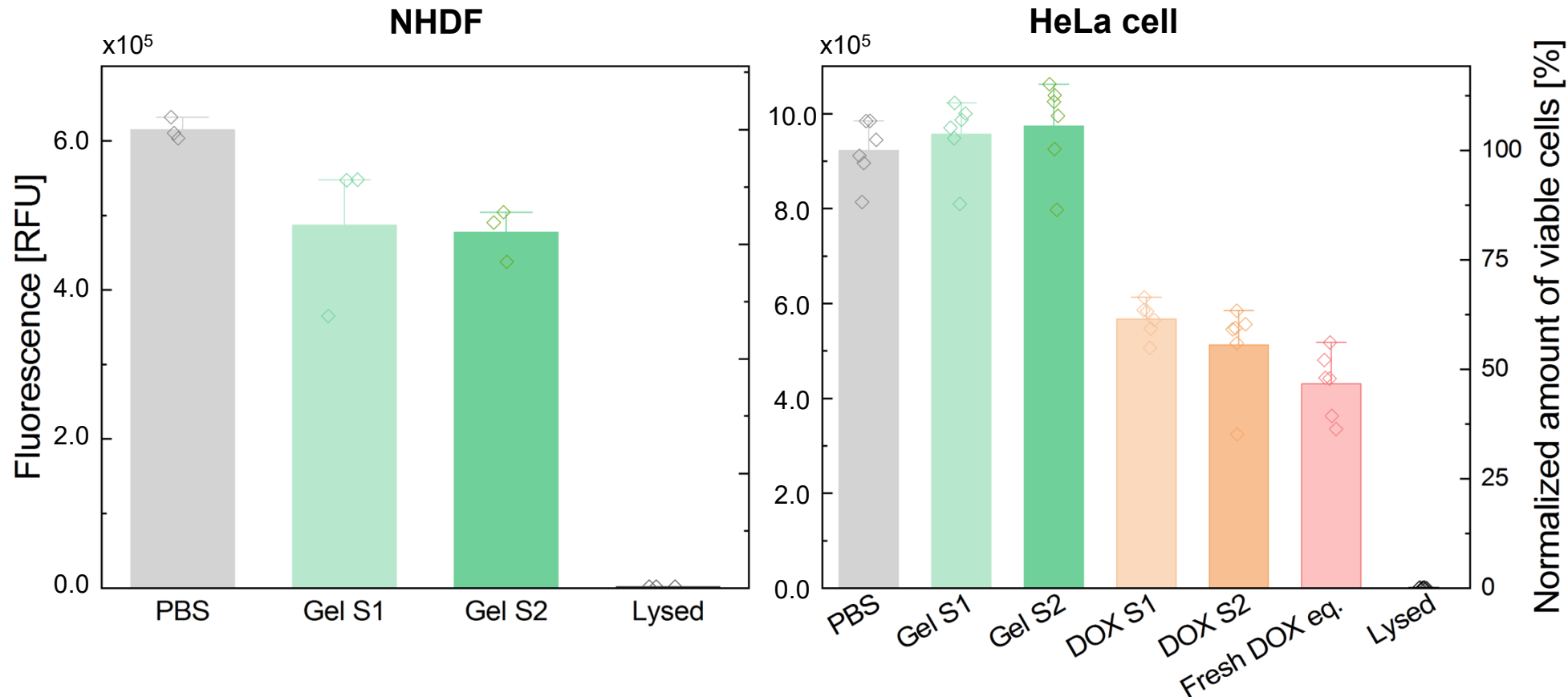


- **Burst release** in the first hours (~15%), followed by **sustained release over days** (DOX: 2-3 days, FITC-Albumin: 3-5 days)
- **60-80% of DOX** released cumulatively, whereas almost **100% of FITC-Albumin** released
- **CNC** concentration has **no significant impact on DOX** release, while **Albumin release was extended**

Liquid Injectable Hydrogel *in vitro* Drug Delivery



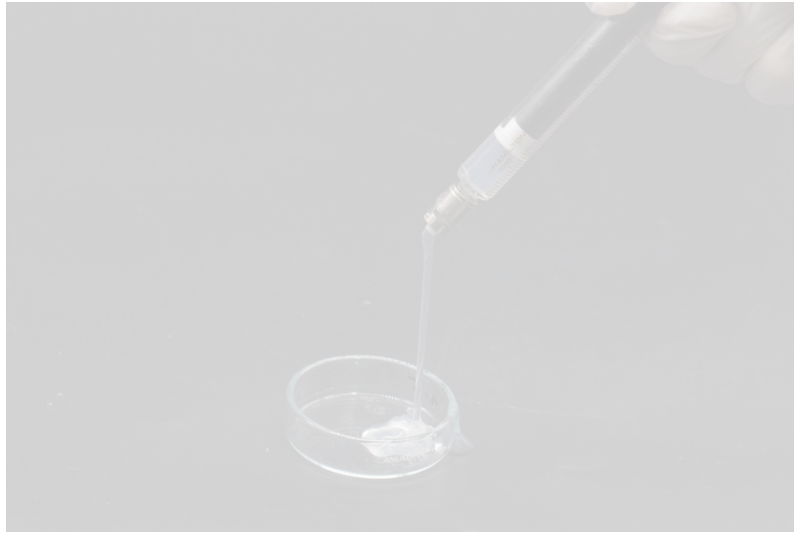
➤ Drug release kinetics preliminary results



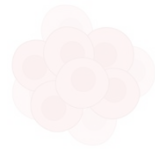
- Good **biocompatibility** confirmed by a normal human dermal fibroblast (NHDF) cell assay
- Released DOX exhibits equivalent **bioactivity** to fresh free DOX against model cancer HeLa cell

Hydrogel as a universal drug delivery platform

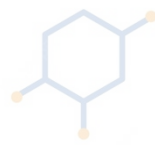
Liquid injectable hydrogel:
in vitro drug delivery



Release kinetics



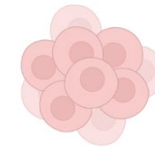
Biocompatibility



Bioactivity



Antimicrobial

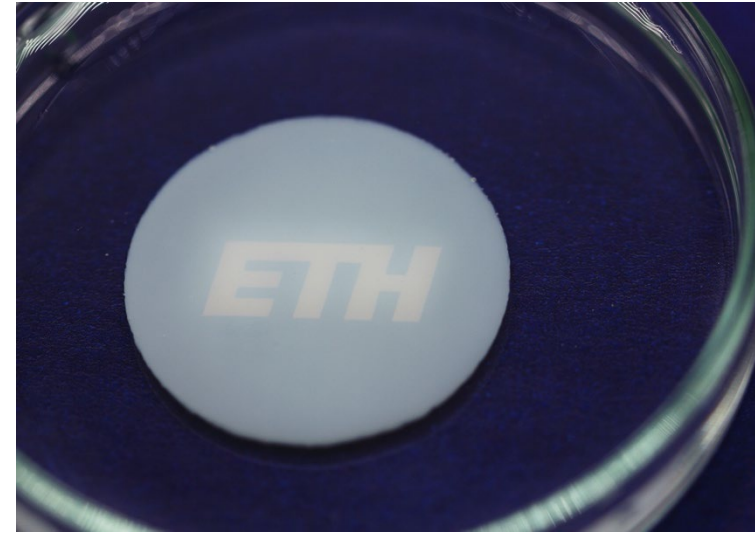


Biocompatibility



Ex vivo

Solid hydrogel:
Antimicrobial wound dressing



Solid Wound Dressing Hydrogel: Antimicrobial Trials



➤ Antimicrobial test



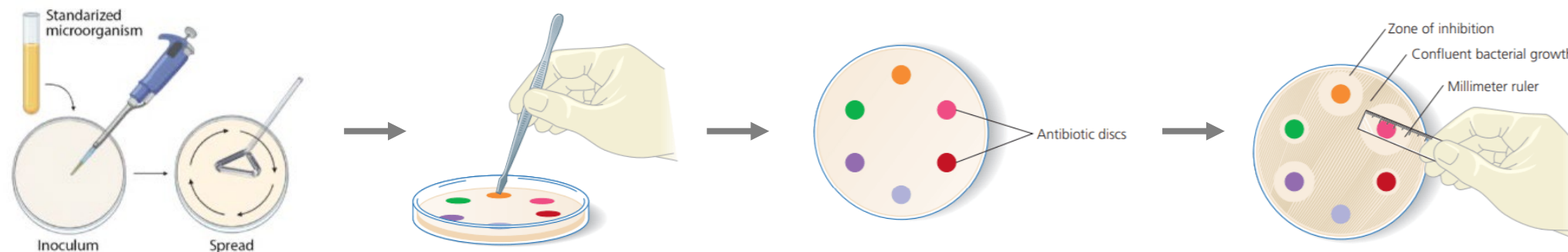
Antimicrobial agent

- Chlorohexidine digluconate (CHX): antimicrobial polymer, typically used in wound cleaning, safe in pregnancy

Typical wound pathogen

- Gram positive: *Staphylococcus aureus* (S.A.)
- Gram negative: *Pseudomonas aeruginosa* (P.A.)

Method: Agar diffusion assay + Quantitative bacteria suspension assay + ex vivo skin test



Correa, Matías Guerrero, et al. *Beilstein journal of nanotechnology* 11.1 (2020).

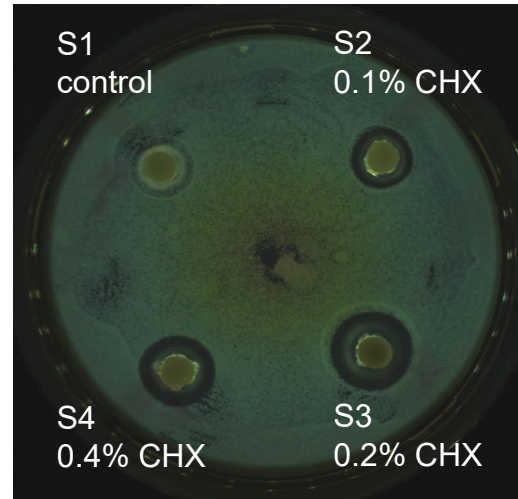
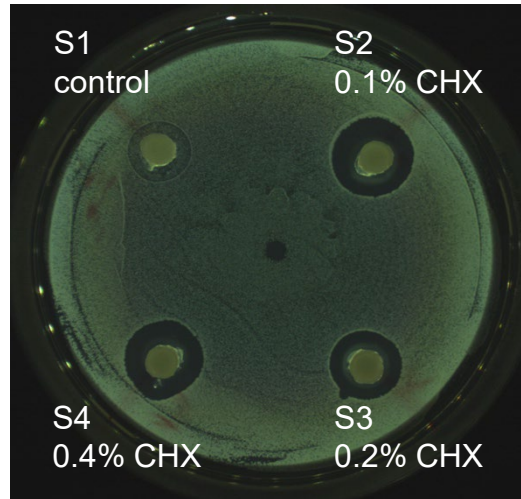
Solid Wound Dressing Hydrogel: Antimicrobial Trials



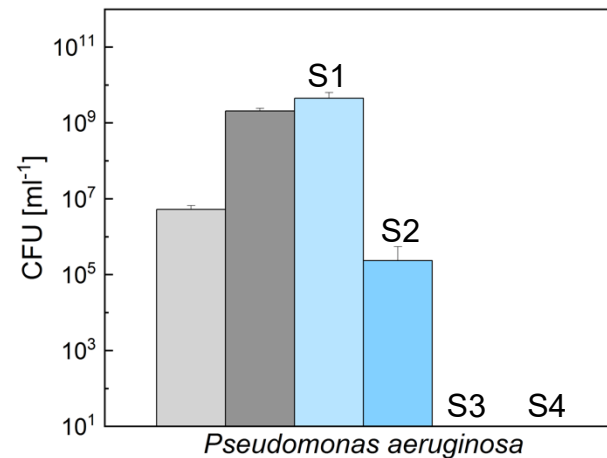
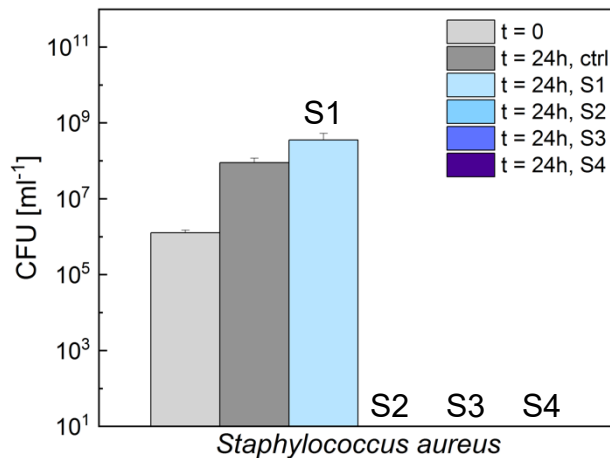
➤ Antimicrobial test: Agar diffusion assay & Quantitative bacteria suspension assay

Staphylococcus aureus
(Gram-positive)

Pseudomonas aeruginosa
(Gram-negative)



- Chlorohexidine digluconate (CHX) is **effective against both pathogens**, the effect enhanced by concentration
- **0.1% CHX** is detected to be critical effective concentration against *Pseudomonas aeruginosa* (**killing rate 99.99%**)
- Solid hydrogels exhibit **excellent biocompatibility** (results not shown)



Solid Wound Dressing Hydrogel: Antimicrobial Trials

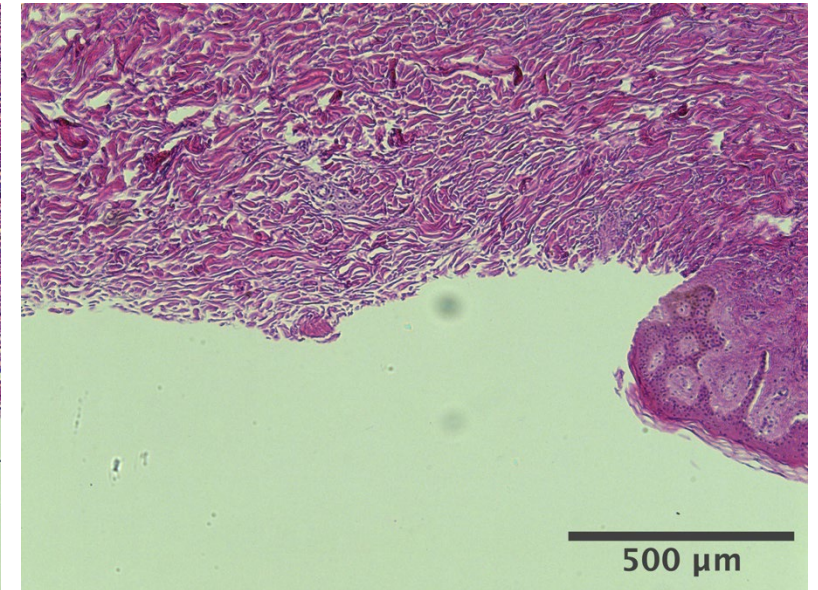
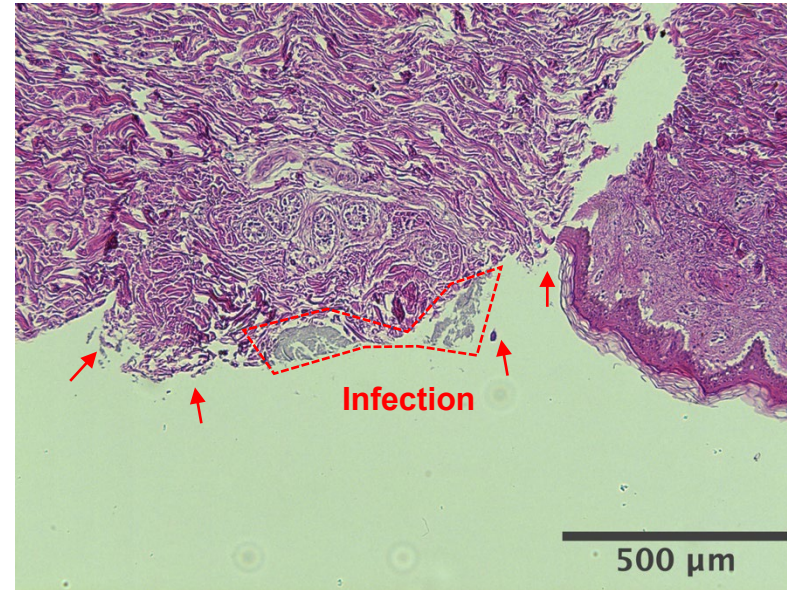
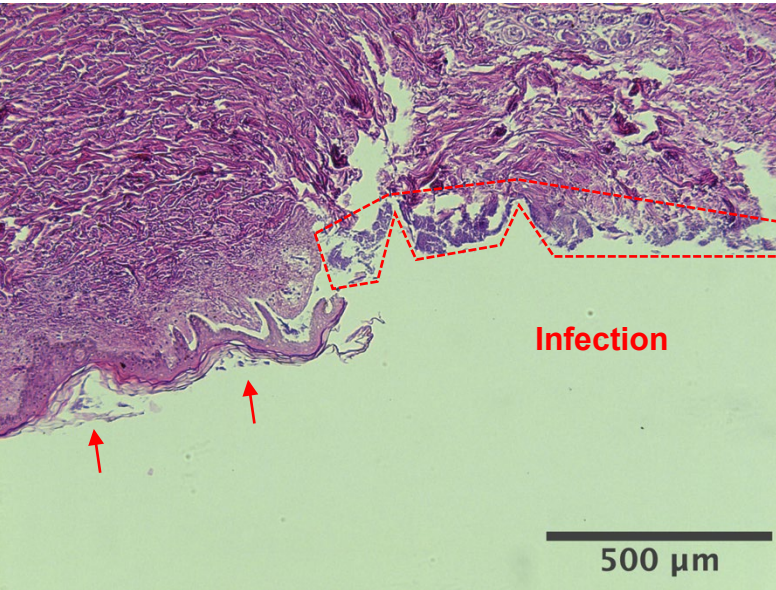


➤ *Ex Vivo* artificial wound test: Hematoxylin and eosin stain (H&E stain)

P. aeruginosa infected wound

Hydrogel treated wound

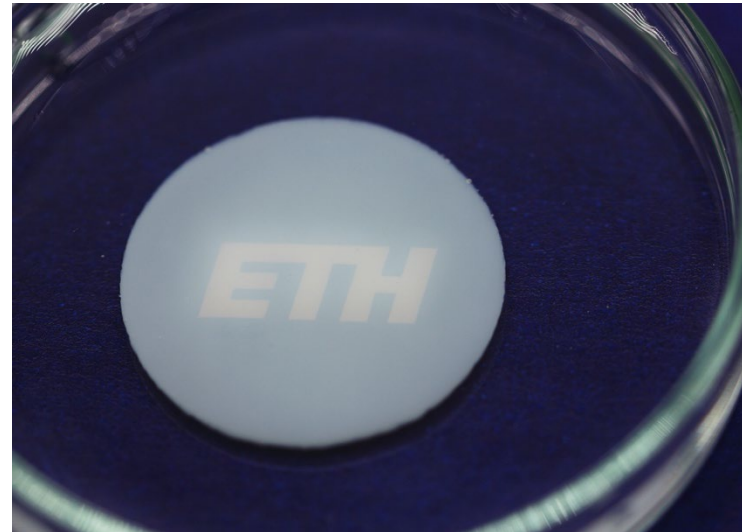
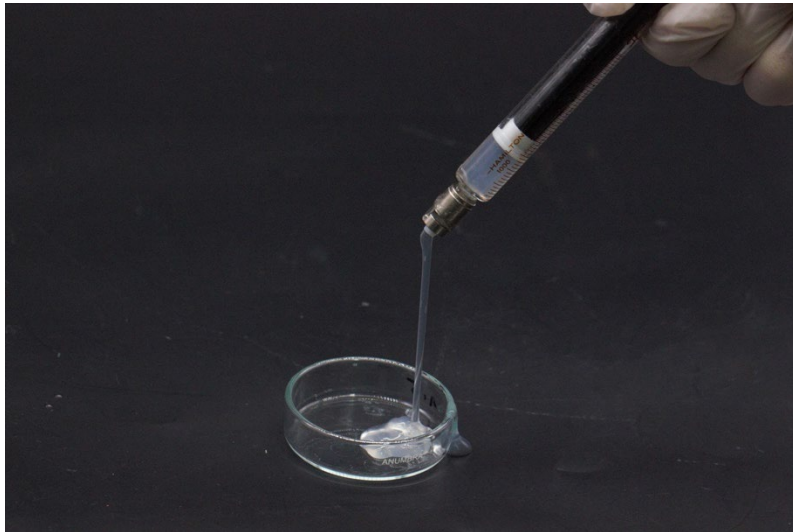
0.1% CHX loaded hydrogel treated wound



- **0.1% CHX** loaded hydrogel totally prohibits the infection (no p.a. colonies/biofilm)
- The hydrogel itself slightly prohibits the infection (p.a. colonies still found on the wound)

Conclusion & Outlook

- A “toolbox” of biocompatible nanocellulose reinforced hydrogel with **tunable mechanical strength** was established
- **Rheology** has been proven to be powerful tool for hydrogel **mechanical strength characterization**
- **Liquid hydrogel** shows potential in application of **in vivo drug delivery via injection**
- **Solid hydrogel** can be used as antimicrobial agent carrier for **wound dressing application**



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Siyuan Tao

Garam Han

Thank you!!!