HIPS HELMHOLTZ Institut für Pharmazeutische Forschung Saarland

Acid-cleavable Tobramycin cross-linked nanogels for antibiotic delivery to *P. aeruginosa* biofilms

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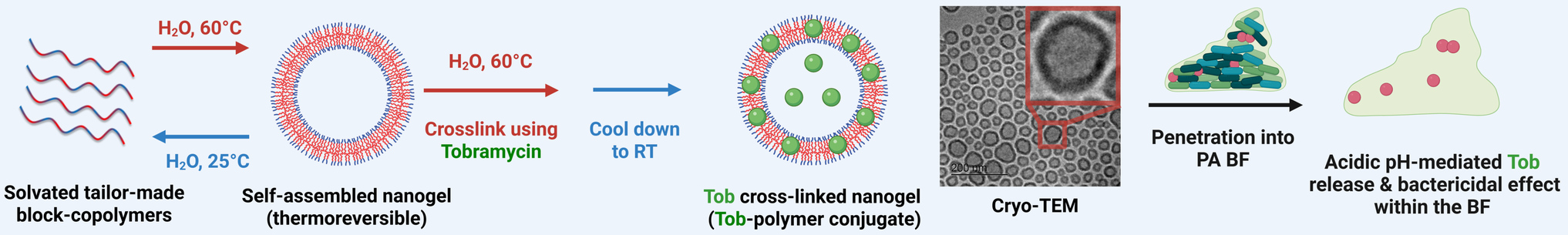
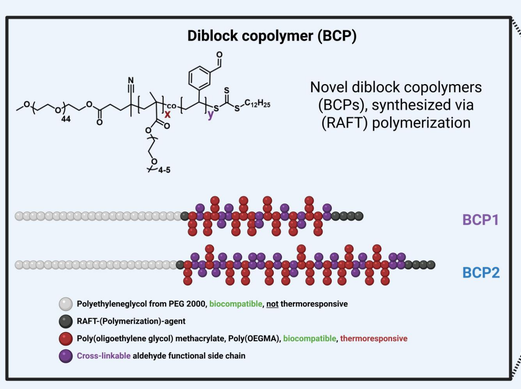
Synopsis

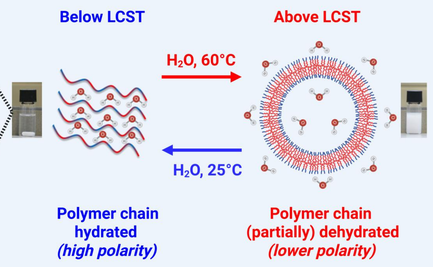
Biofilm (BF) formation significantly reduces the susceptibility of *Pseudomonas aeruginosa* (PA) to Tobramycin (Tob) and other aminoglycosides (AGs). This is mainly due to the electrostatic interactions between cationic AGs and anionic species within the BF (1), especially at acidic pH. We hypothesized that nanogels (NGs) (2) can shield the cationic charge of AGs and therefore enhance their BF penetration. To produce such NGs, a new diblock copolymer was synthesized. The first block of the copolymer comprises linear PEG. In the second block, a thermoresponsive poly(oligoethylene glycol) methacrylate (POEGMA) and an aldehyde functional monomer are randomly distributed.

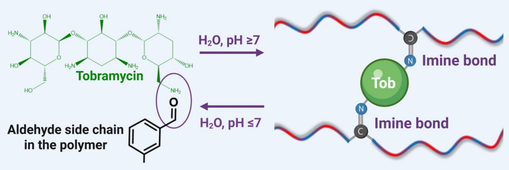
To create NGs, the polymer was cross-linked using Tob in water above its lower critical solution temperature (LCST) via imine bonds. The NGs were found to be biocompatible, rapidly released Tob at pH ≤ 6.0, as reported for PA BF (3), and killed planktonic PA. Targeted Tob release in infected or inflamed areas ensures bacterial killing primarily at those sites. This approach may reduce collateral damage to the microbiome and potentially slow the development of AMR. However, in biofilms the killing is insufficient, likely both due to PA having decreased susceptibility to Tob in acidic conditions, as well as the general susceptibility decrease observed in biofilms.



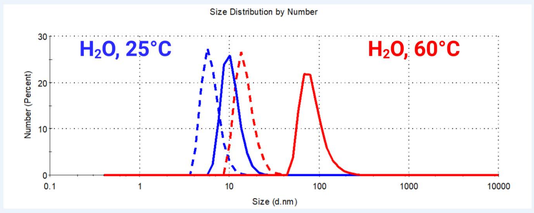
Polymer design and characterization







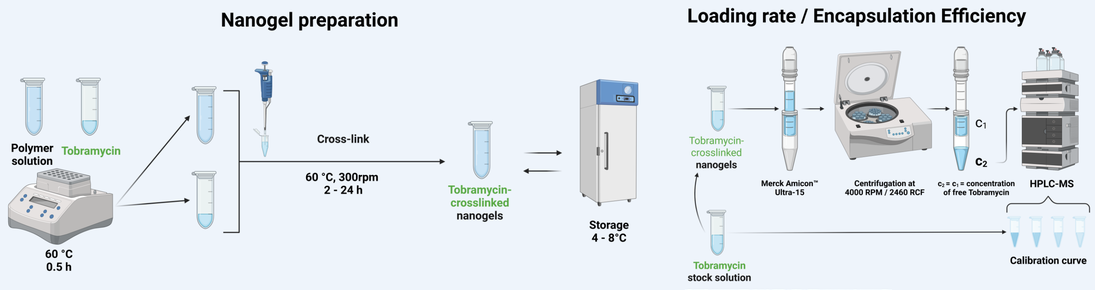
Cross-linker = the antibiotic **Tobramycin**
5 cross-linkable primary amino groups to form acid-cleavable **imine bonds** (Schiff base)

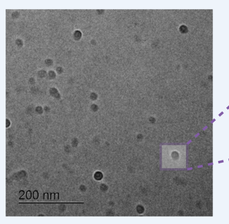


BCP1 ---------- BCP2

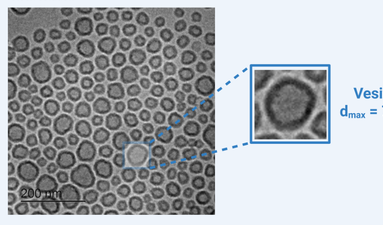
Thermoreversible **LCST**-driven self-assembly behaviour observed via Dynamic Light Scattering (DLS)
(**LCST** = **L**ower **C**ritical **S**olution **T**emperature)

Nanogel preparation and characterization



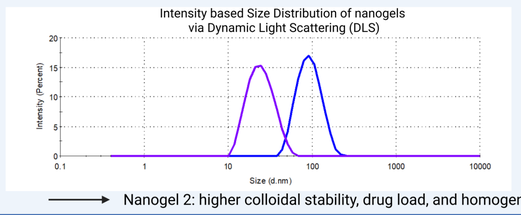


Cryo-TEM



Nanogel 1 resulting from BCP1 shows micelles

Nanogel 2 resulting from BCP2 shows homogenous vesicles



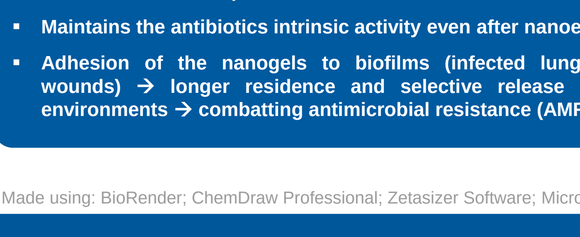
	Nanogel 1 from BCP1	Nanogel 2 from BCP2
Encapsul. Efficiency	52%	85%
Loading rate	11%	11%
Conc. of loaded Tob [µg/mL]	1209	1141
Z-Average	23.4 ± 0.3nm	85.7 ± 2.8 nm
PDI	0.155 ± 0.009	0.085 ± 0.016
Zeta potential	0.19 ± 0.18 mV	-0.52 ± 1.29 mV

→ Nanogel 2: higher colloidal stability, drug load, and homogenous morphology, thus subsequent studies were mostly performed using it

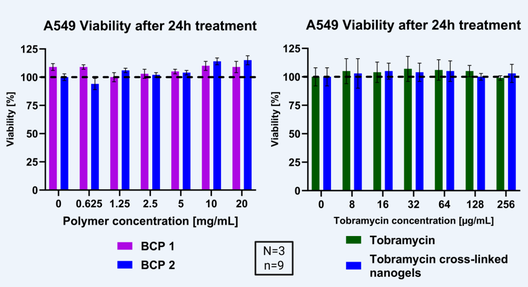
Cytocompatibility and bactericidal effect

A549 cells → Nanogel

Optical image analysis of alive and dead cell count using *TECAN Image Analyzer Software*



A549 cells treated for 24h with highest nanogel 2 concentration (256µg Tob/mL ; 1,5mg BCP2/mL) stained with LIVE/DEAD™ Kit (Calcein-AM / ethidium homodimer-1)



→ The nanogel 2 is cytocompatible

Pseudomonas aeruginosa → Nanogel

MIC/MBC/MBEC of Tobramycin and Nanogel 2 for *Pseudomonas aeruginosa* (PAO1)

Conditions	MIC90 in LB Broth [µg/mL]		MBC in LB Broth [µg/mL]	
	Standard pH 7	Acidified pH 5	Standard pH 7	Acidified pH 5
Free Tobramycin	1	4	2	8
Tob nanogel 2	4	4	4	8

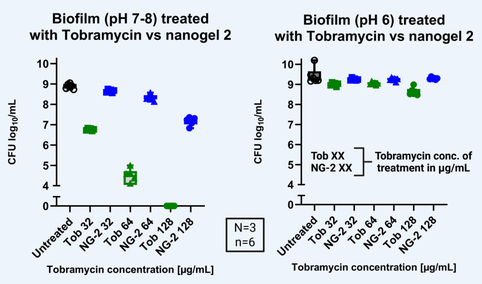
MIC = Minimum Inhibitory Concentration, MBC = Minimal bactericidal concentration, MBEC = Minimal biofilm eradication concentration

N=2 n=4

PAO1 was incubated in for 72h to form biofilms → treated for 48h with Tob or NG

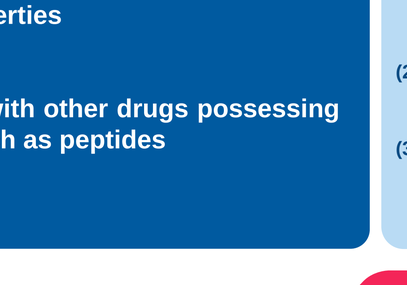
Release kinetic issue in bacterial killing? → pre-release experiment: Tob and nanogel 2 pre-incubated at pH 6 37°C for 72h before used in treatment

→ same negligeable decrease in CFU like in the pH 6 assay

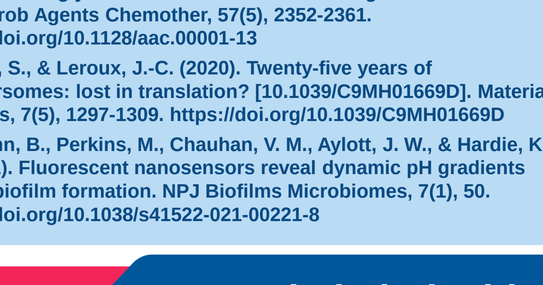


→ Lower effectiveness of Tobramycin in acidic pH

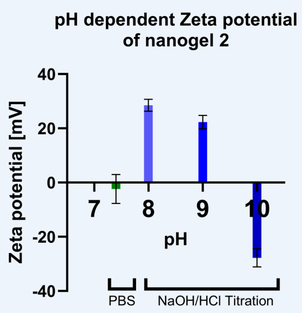
pH-responsive behaviour of nanogel 2

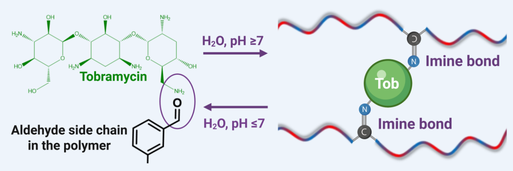


→ Fast release/degradation in acidic conditions



→ Stable/minimal drug release in neutral/basic conditions





→ Without buffering positive Zeta Potential, due to the positively charged primary amino groups in **Tobramycin**

Conclusion

- Using a novel, thermoresponsive diblock copolymer with aldehyde functionality, we formulated acid-cleavable Tob-crosslinked nanogels
- Drug loading contents of 1300 µg/mL while a PEG-shell effectively shields the positive charge of Tobramycin
- Promising new pH-controlled release system for prolonged release of Tob in antibacterial therapies
- Maintains the antibiotics intrinsic activity even after nanoencapsulation
- Adhesion of the nanogels to biofilms (infected lung or topical/burn wounds) → longer residence and selective release only in infected environments → combatting antimicrobial resistance (AMR)

Outlook

- Co-delivery by loading/conjugation of another antibiotic or adjuvant with antimicrobial properties
- Use outside of infection research with other drugs possessing multiple primary amino groups, such as peptides

References

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(2) Matoori, S., & Leroux, J.-C. (2020). Twenty-five years of polymersomes: lost in translation? [10.1039/C9MH01669D]. Materials Horizons, 7(5), 1297-1309. https://doi.org/10.1039/C9MH01669D

(3) Hollmann, B., Perkins, M., Chauhan, V. M., Aylott, J. W., & Hardie, K. R. (2021). Fluorescent nanosensors reveal dynamic pH gradients during biofilm formation. NPJ Biofilms Microbiomes, 7(1), 50. https://doi.org/10.1038/s41522-021-00221-8

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