Antimicrobial *N* -Carboxyanhydride-Derived Polypeptide Functionalized Surfaces

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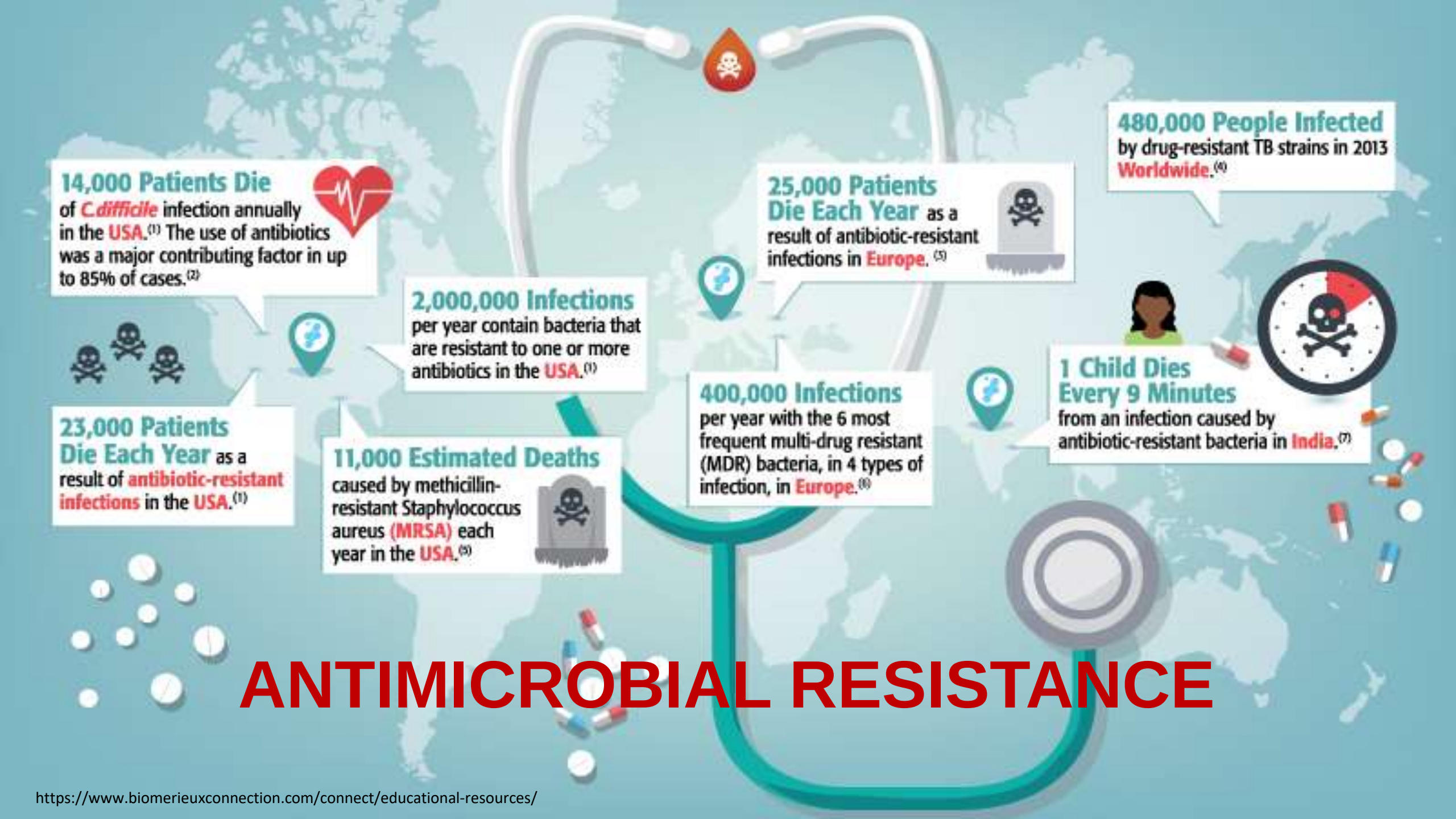

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ANTIMICROBIAL RESISTANCE

- *Antimicrobial resistance (AMR) is recognized as* ***one of the greatest threats to human health*** *worldwide.*

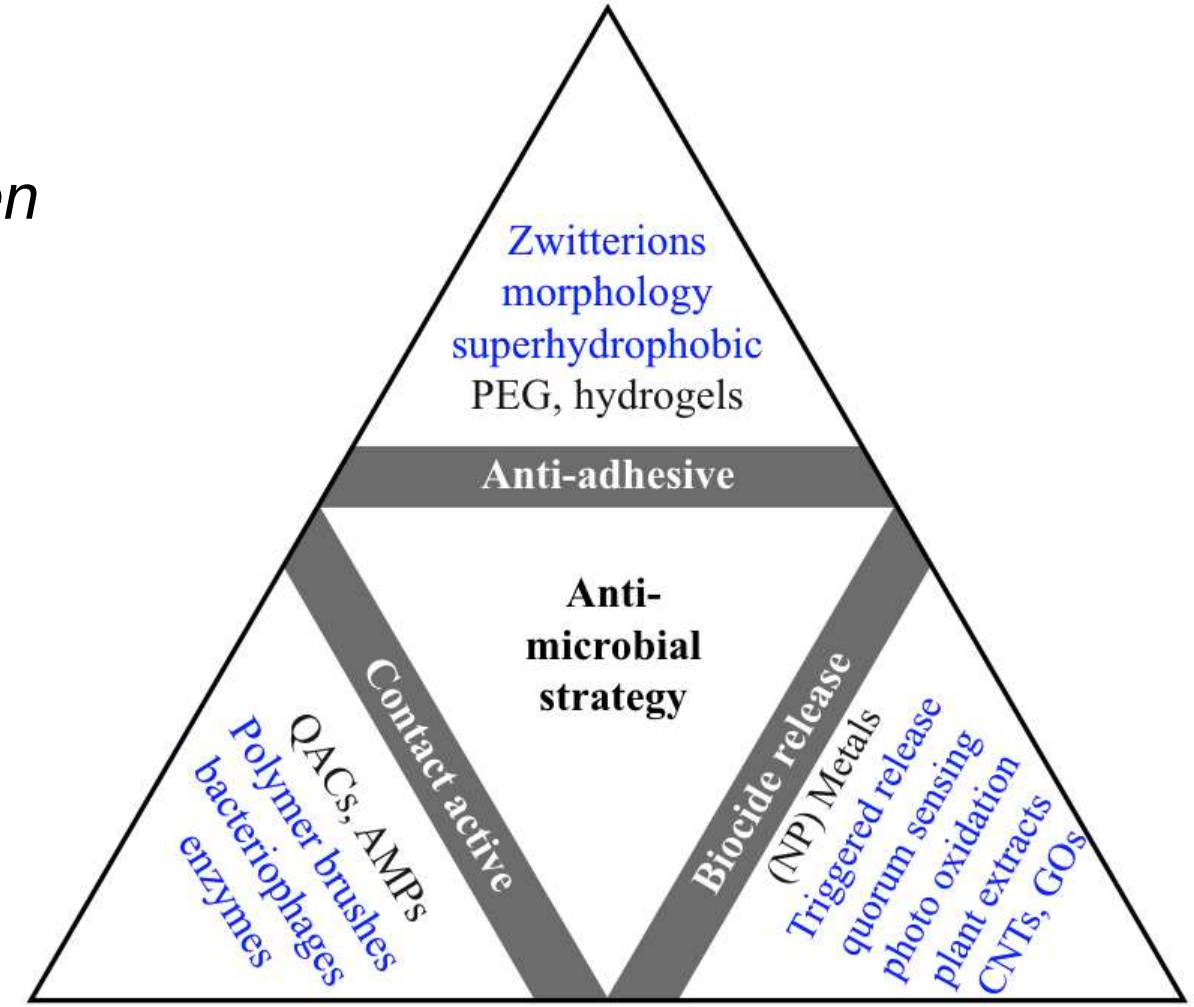
- *Antibiotic "one-size-fits-all" approach augments antimicrobial resistance.*

- *By 2050, it has been predicted that resistant infections will be the number one cause of death globally, with an estimated* ***10 million*** *attributable deaths per year.*

(1) WHO Antimicrobial Resistance Fact sheet N°194. Updated April 2015.
(2) O'Neill J. London: Review on Antimicrobial Resistance; 2014.

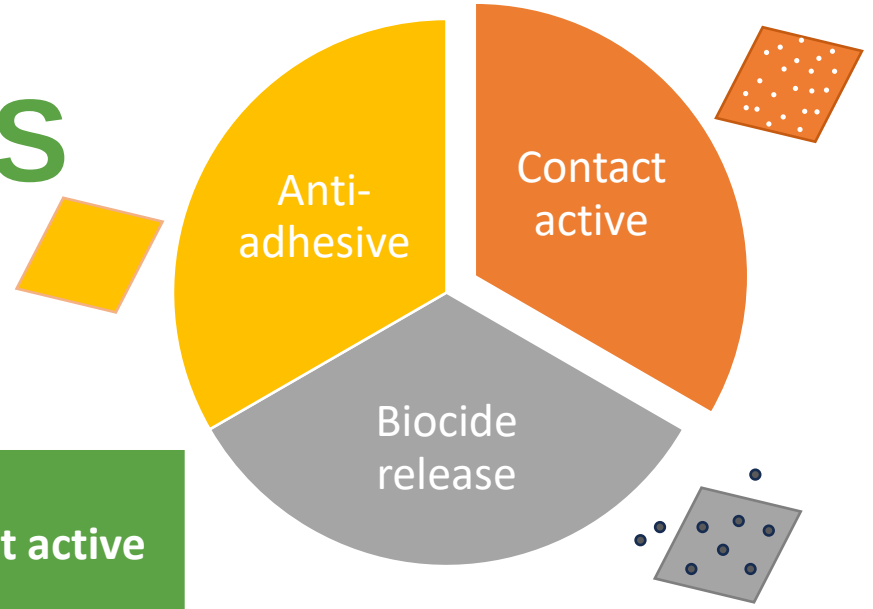
ANTIMICROBIAL SURFACES

- ***Anti-adhesive***
  - *Reduce the adhesion force between microbes and a solid surface*
- ***Biocide release***
  - *Release biocidal substances*
- ***Contact active***
  - *Biocidal substances immobilized on surface*



Adlhart, C. et al. (2018) Journal of Hospital Infection, pp 239–249.

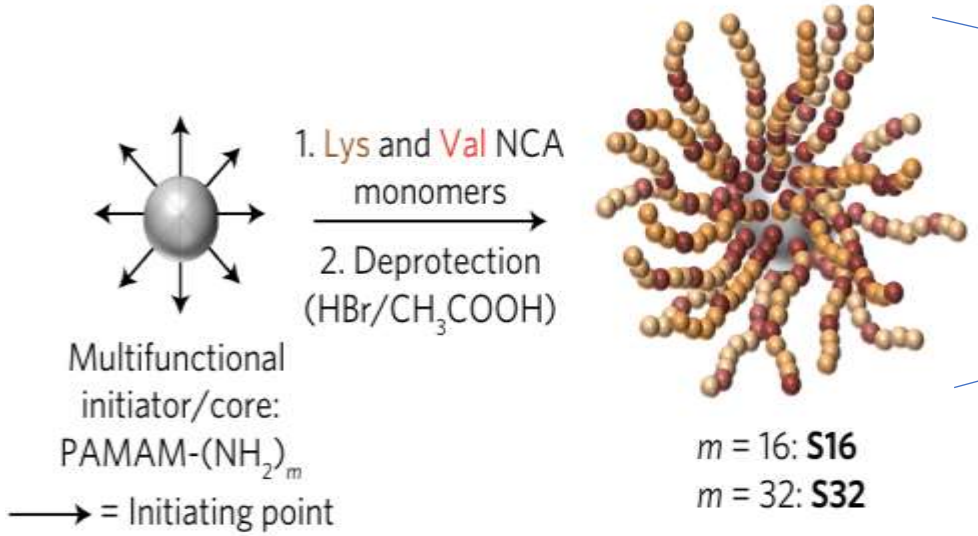
COMPARISON OF SURFACES

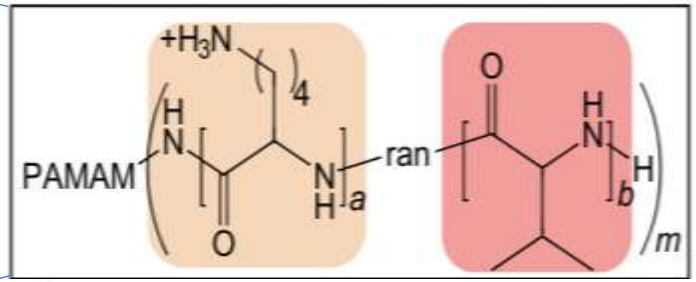


	Anti-adhesive	Biocide release	Contact active
Broad-spectrum against bacteria and fungus	✗	✓	✓
Low level of induced resistance	✓	✗	✓
Environment friendly	✓	✗	✓

ANTIMICROBIAL NCA POLYPEPTIDES

- *Structurally Nanoengineered Antimicrobial Peptide Polymers (SNAPPs)* [2]
  - *Star polypeptide arms*
  - *Stable architectures even when diluted*
  - *Antimicrobial against MDR bacteria*





(1) Jiang, Y. et al. (2021). *Advanced Drug Delivery Reviews, 170, 261–280.*
(2) Lam, S. J. et al. (2016). *Nat Microbiol 2016, 1.*

STRUCTURALLY NANOENGINEERED ANTIMICROBIAL PEPTIDE POLYMERS

- *Great efficacy in antimicrobial activity*

Table 1 | Antimicrobial activity of SNAPPs and other peptides against a range of Gram-negative pathogens.

Antimicrobial type	Code/name	Medium	MBC* (μM)					
			E. coli	*P. aeruginosa*	*K. pneumoniae*	*A. baumannii*	CMDR *P. aeruginosa*	CMDR *A. baumannii*
SNAPP	S16	MHB	0.72 ± 0.06	1.42 ± 0.08	1.54 ± 0.08	0.85 ± 0.05	1.38 ± 0.03	1.61 ± 0.23
	S32	MHB	0.72 ± 0.54	0.97 ± 0.05	0.83 ± 0.14	0.79 ± 0.02	1.00†	0.85 ± 0.03
AMP	Ovispirin‡	MHB	8.39 ± 0.44	95.49 ± 9.73	11.49 ± 4.86	2.21 ± 0.88	Not tested	Not tested
	Magainin II‡	MHB	47.85 ± 6.08	55.96 ± 2.84	154.59 ± 9.32	19.87 ± 3.24	Not tested	Not tested
	Melittin‡	MHB	33.71 ± 5.18	29.37 ± 8.24	109.25 ± 20.43	0.91 ± 0.09	Not tested	Not tested

*MBC is defined as the minimum drug concentration that causes quantitative bacterial cell death (see Methods, Supplementary Fig. 9 and Supplementary Table 2 for further clarification). All data are expressed as mean and s.d. of four replicates ($n = 4$) completed in two independent experiments; †MBC values were identical across all replicates; ‡The amino acid sequences of ovispirin, magainin II and melittin are KNLRRIIRKIIHIIKKYG-COOH, GIGKFLHSAKKFGKAFVGEIMNS-$CONH_2$ and GIGAVLKVLTTGLPALISWIKRKRQQ-COOH, respectively. Ovispirin, magainin II and melittin were synthesized using standard solid-phase peptide synthesis protocols for Fmoc (9-fluorenylmethoxy carbonyl) chemistry (see Methods for details) and their antimicrobial activities were evaluated as per SNAPPs.

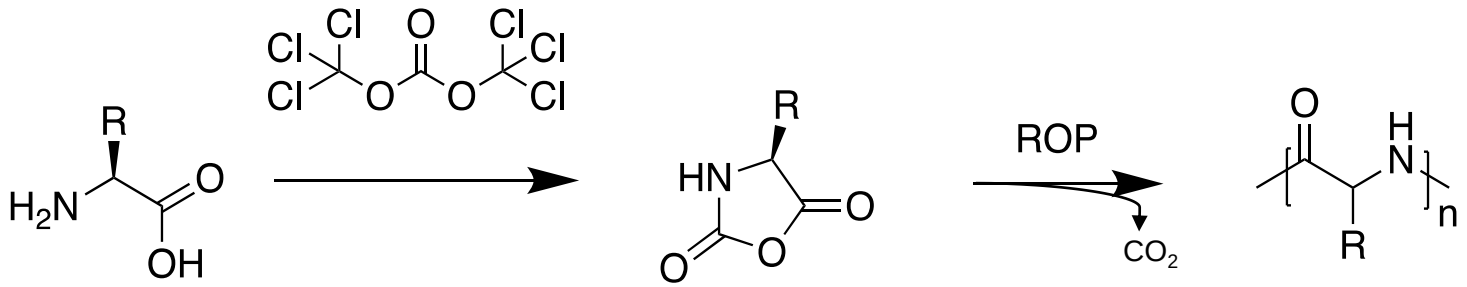
Lam, S. J. et al. (2016). *Nat Microbiol 2016, 1.*

SCIENTIFIC QUESTION

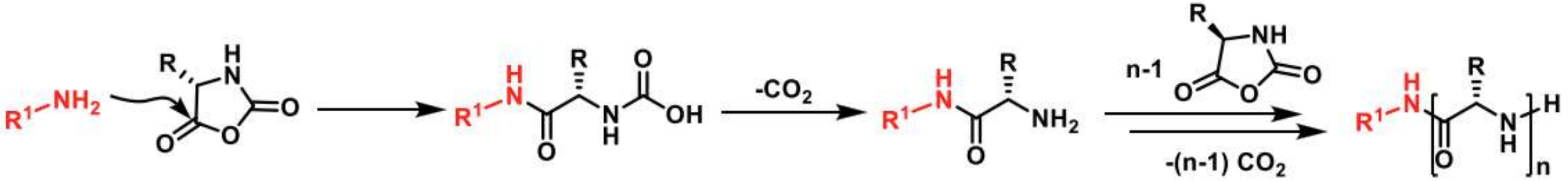
- ***Will surface confined N-Carboxyanhydride (NCA) Polypeptide Brushes show antimicrobial activity as was found in solution?***

NCA-DERIVED SYNTHETIC POLYPEPTIDES

- *Ring-opening polymerization of α-Amino Acid N-Carboxyanhydride is a facile route to synthesize well-defined polypeptides with complex macromolecular architectures*



α-Amino Acid | α-Amino Acid NCA | NCA derived polypeptide



- *Polypeptides display secondary structure and offer considerable (bio)chemical diversity*

CREATION OF GRAFTED SURFACES

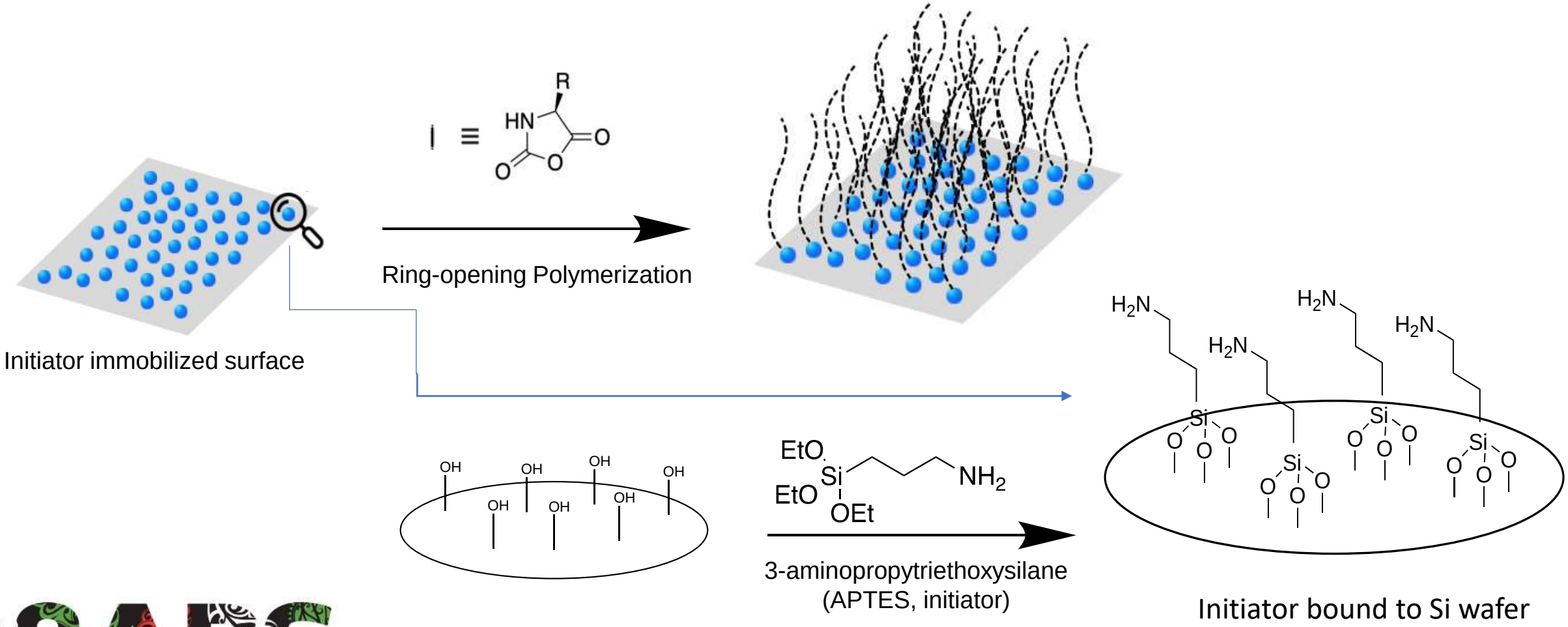
- *Grafting From*
  - *Polymerization from immobilized initiators*

- *Grafting To*
  - *Direct coupling of existing polymer molecules to the surface*

Comparison	Grafting from	Grafting to
Advantage	High grafting density	Defined composition film
Disadvantage	Characterization	Low grafting density

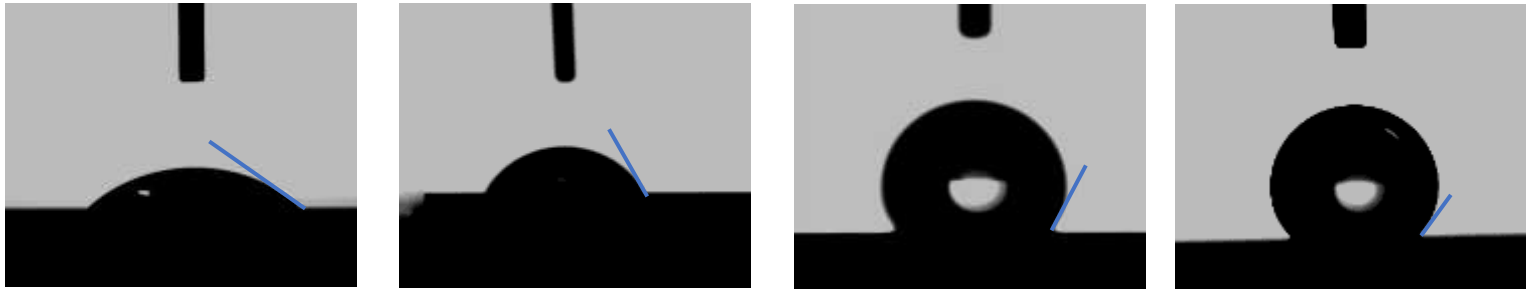
PROJECT SCHEME – GRAFTING FROM

- *Surface initiated (grafting from) ROP of NCA monomer*

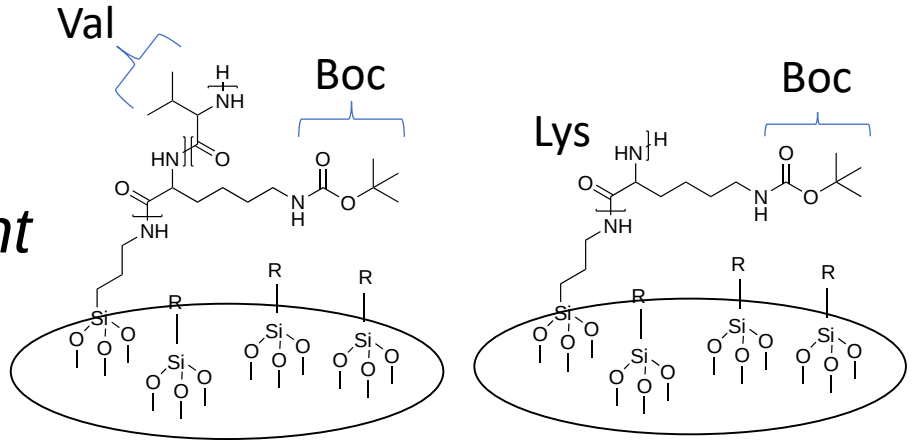


FILM CHARACTERIZATION

- *Surface hydrophilicity by contact angle measurement*



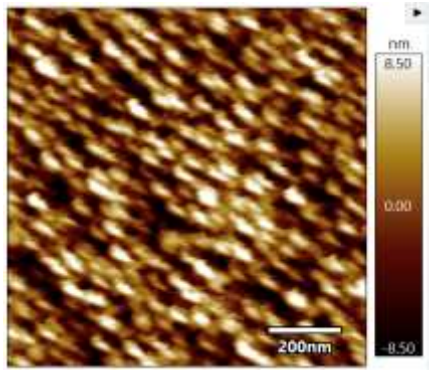
Si | Si-APTES | Si-P(Boc)Lys-co-Val | Si-P(Boc)Lys



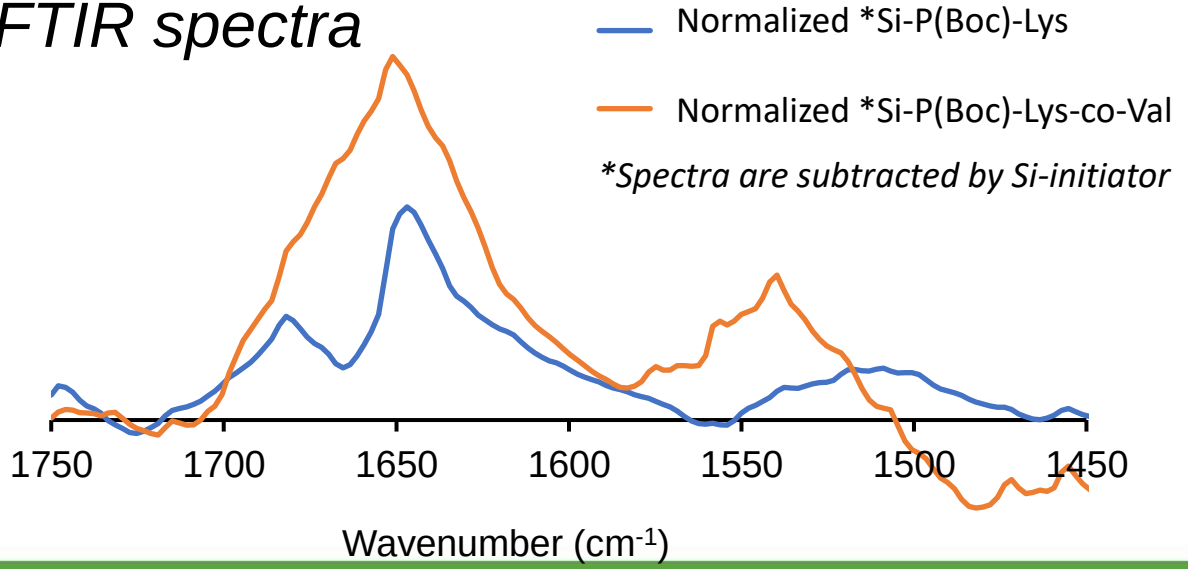
Si-P(Boc)Lys-co-Val | Si-P(Boc)Lys

- *Surface morphology*

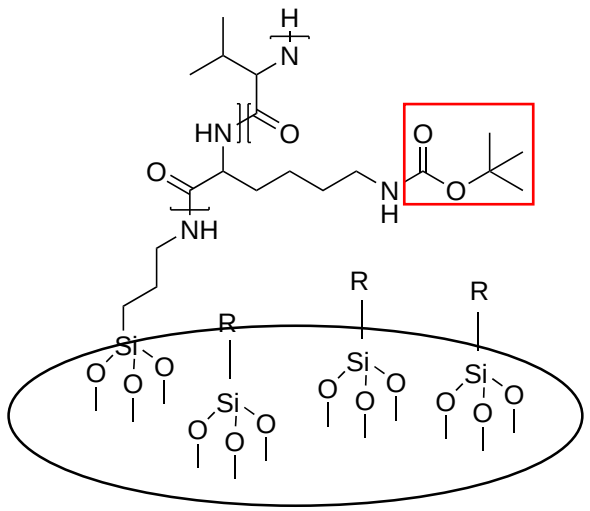
Characterized by Atomic Force Microscopy (Tapping mode in air)



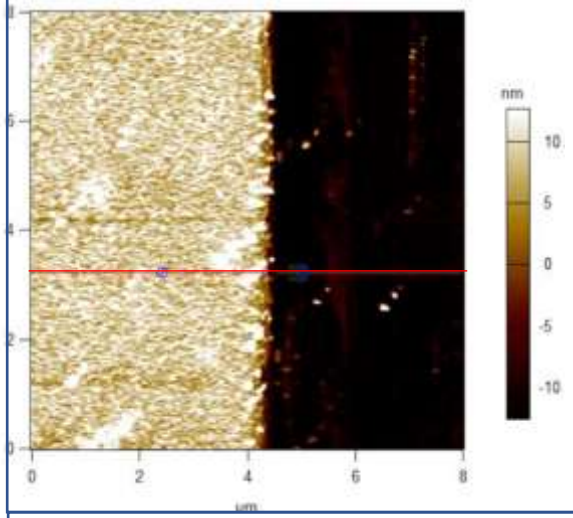
- *ATR-FTIR spectra*



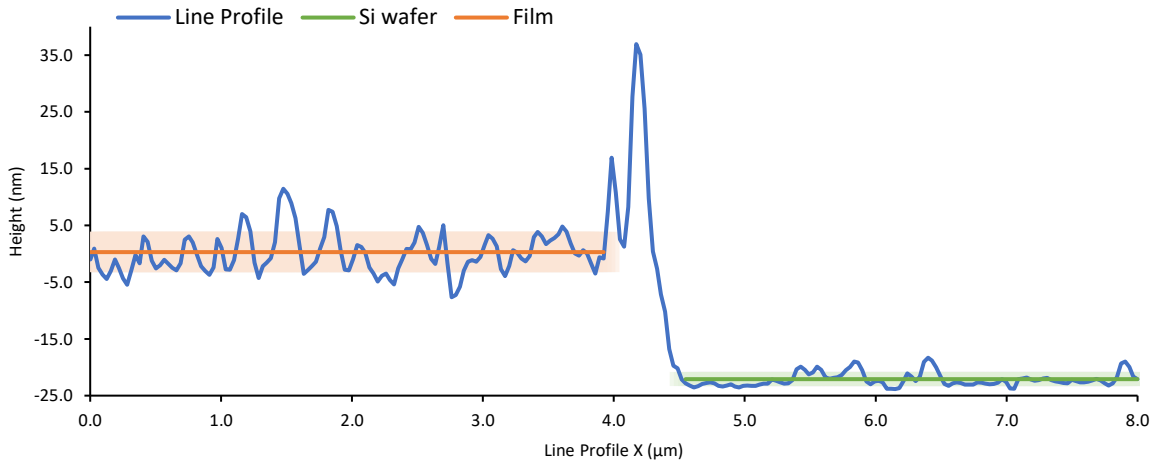
BOC DEPROTECTION – HCl method



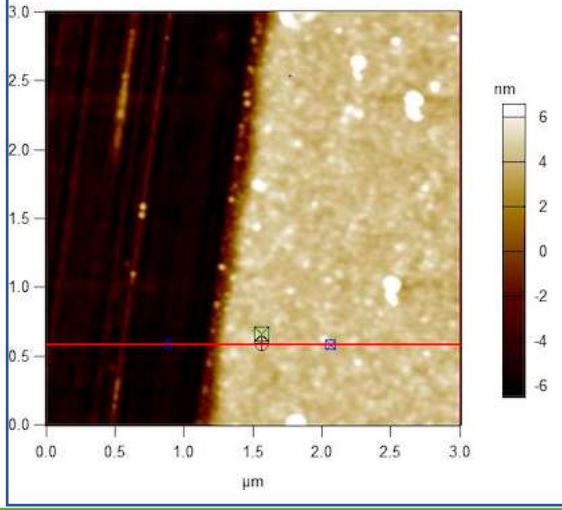
Si-P(Boc)Lys-co-Val



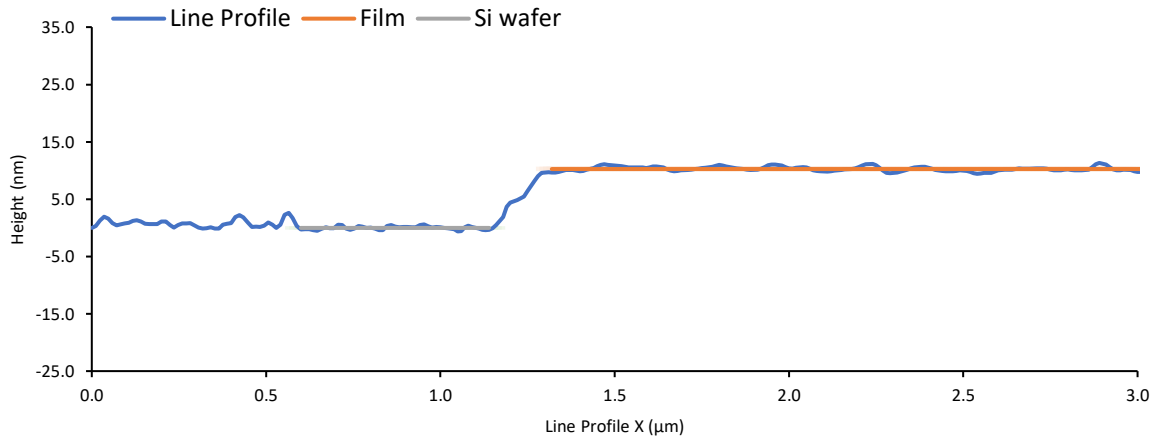
Before Deprotection



Si-P(Lys-co-Val)



After Deprotection



KILLING EFFICACY against *S. aureus*

- *24 h incubation time*
- *Count Colony Forming Unit (CFU)*

$$\text{Killing efficacy} \cdot (\%) \cdot = \frac{C_{control} - C_{sample}}{C_{control}} \cdot \times \cdot 100$$

$$\text{Log reduction} = \text{Log}_{10}(\text{control}) - \text{Log}_{10}(\text{sample})$$


Si-P(Lys)

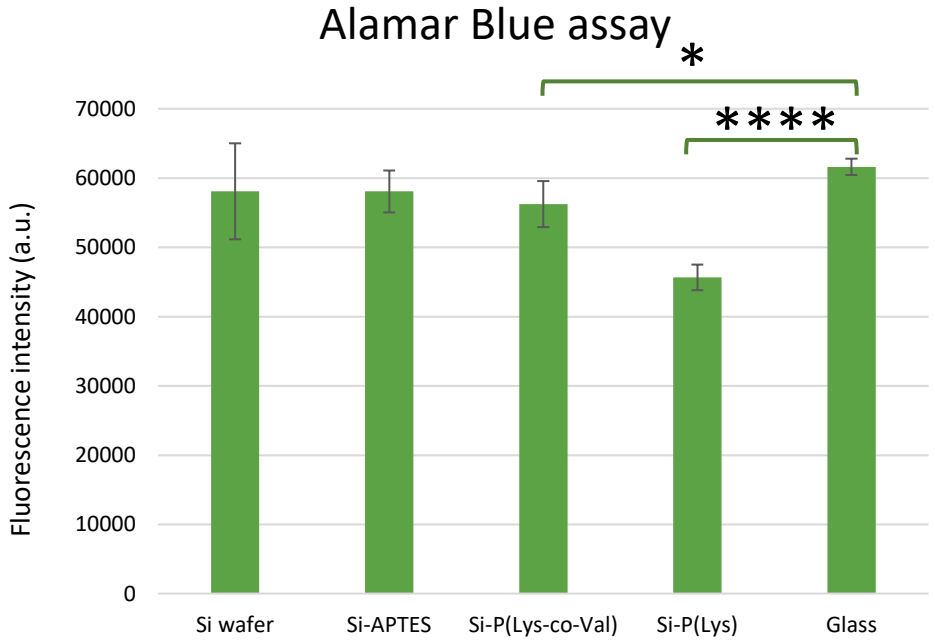

Si-P(Lys-co-Val)


Bare Si


Si-APTES

Sample/Performance	Killing efficacy compared to Si	Log reduction of Si	Killing efficacy compared to Si-APTES	Log reduction of Si-APTES
Si-P(Lys)	99.34 %	2.18	99.94 %	3.22
Si-P(Lys-co-Val)	99.95 %	3.31	99.996 %	4.36

CELL VIABILITY INVESTIGATION with *C2C12* cells



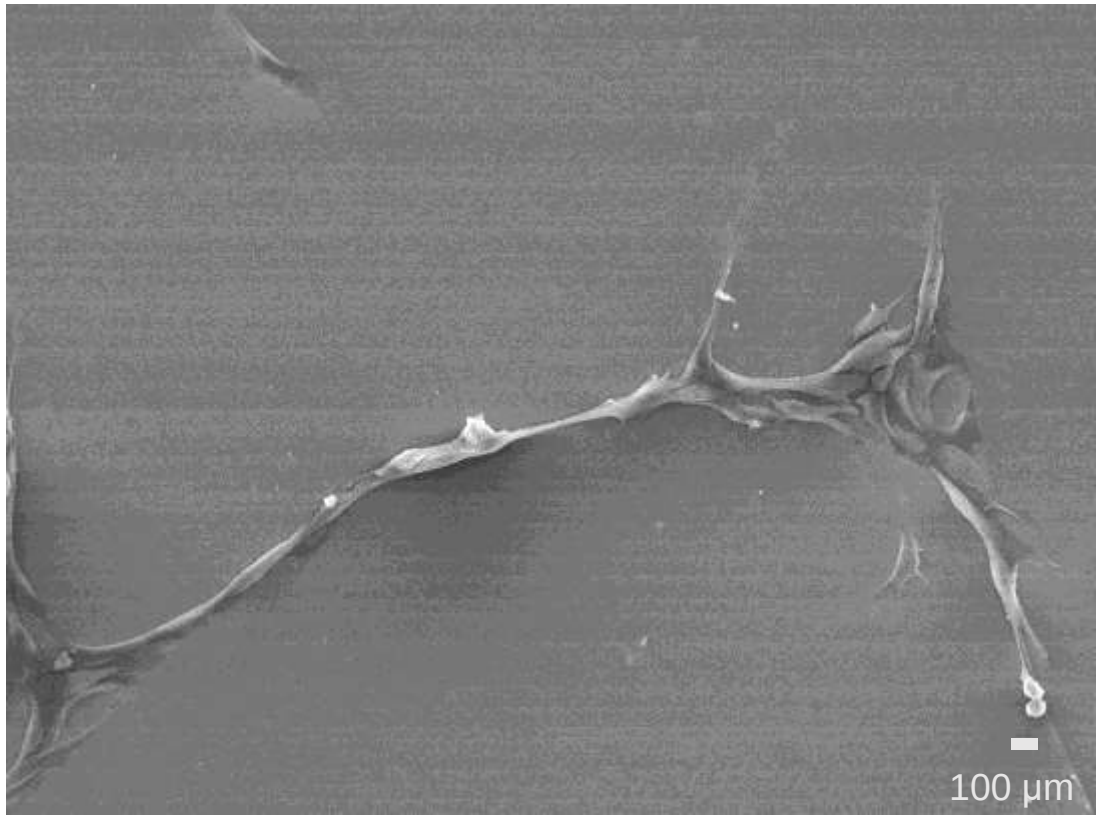
Sample \ Viability (%)	Compared to glass
Si-P(Lys)	72.45
Si-P(Lys-co-Val)	90.75

$$Cell\ viability = (OD_{experimental\ group} - OD_{blank}) / OD_{glass} * 100\%$$

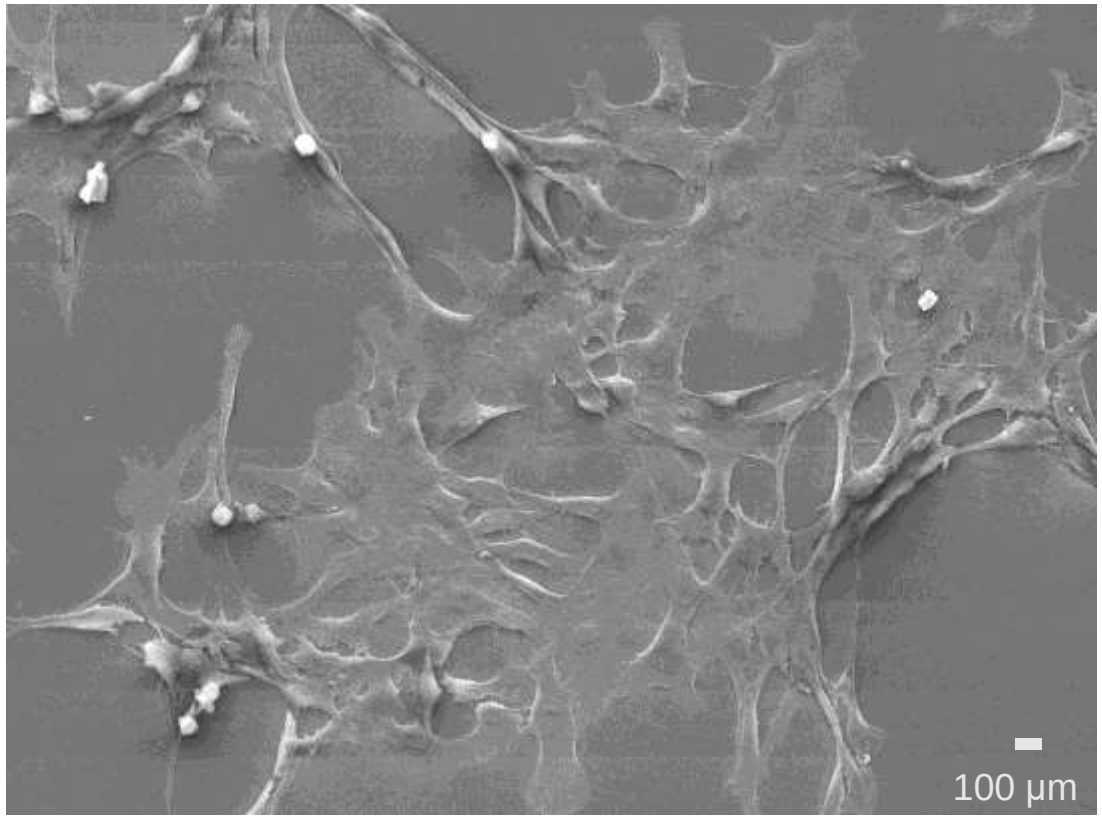
- ***Both Si-P(Lys) and Si-P(Lys-co-Val) showed great antimicrobial activity against S. aureus, while Si-P(Lys-co-Val) is mammalian cell friendly***

C2C12 CELL MORPHOLOGY on surfaces

- *Scanning Electron Microscope imaging*



On Si-P(Lys)



On Si-P(Lys-co-Val)

SUMMARY

- ***Surface-Initiated ROP strategy from solid substrate***
  - *The NCA-derived polypeptide film reaches to 75 ± 26 nm*

- ***Polypeptide grafted surface shows antimicrobial activity***
  - *A composition of Lysine and Valine polypeptide film demonstrates killing efficacy of 99.99% against S. aureus*

- ***Polypeptide grafted surface maintains cell-friendly***
  - *While having excellent bacteria killing efficacy, the co-polypeptide film grafted surface shows low cytotoxicity to mammalian cells*

THANK YOU

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