Development of Macromolecular Iron Chelators Capable of Eradicating Biofilms

Arshdeep Gill[1,3], Elijah Tongol[2] and Jayachandran N. Kizhakkedathu*[1,2,3,4]

Centre for Blood Research[1], Department of Pathology and Laboratory Medicine[2] , and Department of Chemistry[3] , School of Biomedical Engeneering[4], University of British Columbia, Vancouver, BC

Introduction

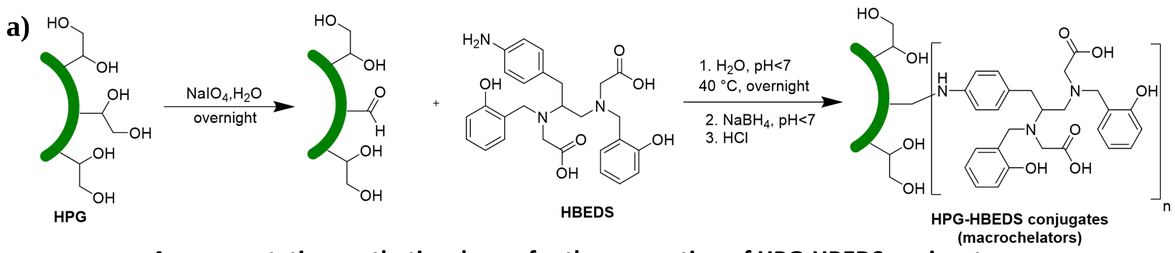
- Millions of resistant nosocomial infections occur every year
- Severe lack of new antibiotics being developed + biofilms confer even more resistance
- These infections become systemic & require harsher antibiotics →worse side-effects
- Unique approaches needed to combat this persistent problem.
- **Our method takes advantage of iron & its role in infection.**
    - Upon infection → war for iron acquisition between the host & bacteria
    - Bacteria secrete small iron binding molecules, *siderophores,* to bind + uptake iron
    - Siderophores vary in structure, size, and $K_{complex}$.
    - Iron → essential nutrient for most bacterial metabolisms
        - **Iron sequestration should limit/eradicate bacteria growth**

- Iron sequestration has been attempted with various siderophores
    - Literature results poor
        - Easy uptake & high dosage for tested small chelators
        - Macromolecular approaches limit uptake
            - complex synthesis + solubility issues

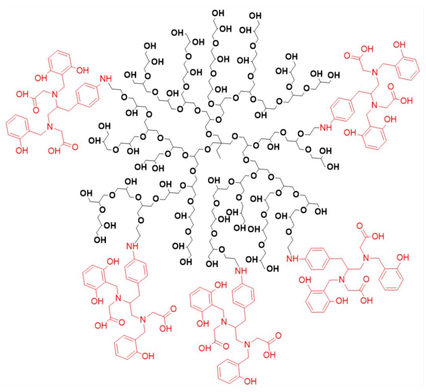
- Ideal macrochelator requirements:
    - Be able to compete with siderophores ($K_{complex}$ between 10^{30} to 10^{40})
    - Not be up-taken by bacteria
    - Won't harm host
    - Highly soluble
    - Easily Synthesized

- **Our Approach**:
    - High-molecular weight polymeric scaffold (Hyper-Branched Polyglycerol, *HPG*) conjugated to a modified high-affinity iron chelator, *HBEDS*
    - Investigated inhibition/eradication of biofilms + effect of molecular weight of macrochelator

Approach



- **A representative synthetic scheme for the generation of HPG-HBEDS conjugates**



- **Structure HPG-HBEDS conjugates. Black is the HPG scaffold. Red is the conjugated HBEDS chelator**

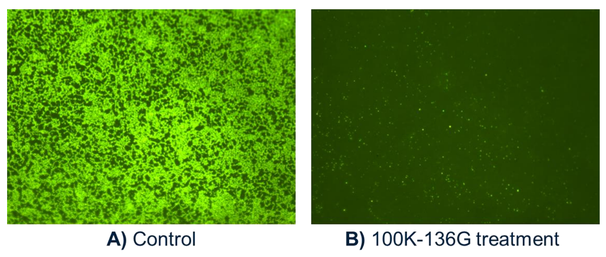
Methods

- Library of macrochelators created by conjugating hyperbranched polyglycerol (HPG) (100kDa and 31kDa) to a modified small molecule chelator, *HBEDS*, via reductive amination
- Macrochelators characterized via NMR, IR, & UV-Vis spectroscopy to confirm conjugation & number of HBEDS groups
- S. *aureus* lux strain (Xen36 Lux) was main strain studied
    - Biofilm eradication assays performed by growing a biofilm first (100ul of 10^6 CFU culture) for 24 hours in polypropylene 96 well plate in 50% TSB media
    - Media was drained, water washed 3x, & treated with macrochelator
    - Crystal violet assay conducted as described in literature
    - For inhibition assays, conjugate was added with bacteria on day one
- Modified eradication protocol used for confocal studies with syto9 stain for imaging
    - Our molecules labelled with AZDye 405 cadaverine via reductive amination.
- Data normalized to control
- Error bars represent standard deviations of 3 biological replicates, N=3

Results

Destruction of Pre-formed Biofilms

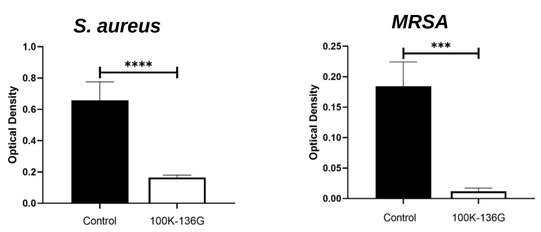
1. Fluorescence Microscopy of S. aureus biofilm after conjugate treatment



A) Control **B)** 100K-136G treatment

- **Image shows reduction in biofilm after conjugate treatment relative to untreated control**

2. Crystal violet assay shows biofilm eradication

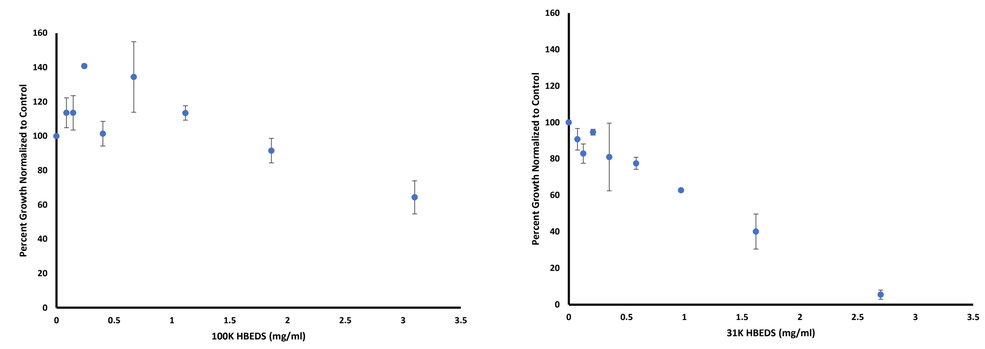


- **Conjugate exhibited biofilm killing against both *S. aureus* and MRSA of up to 75% & 94%, respectively**

Results

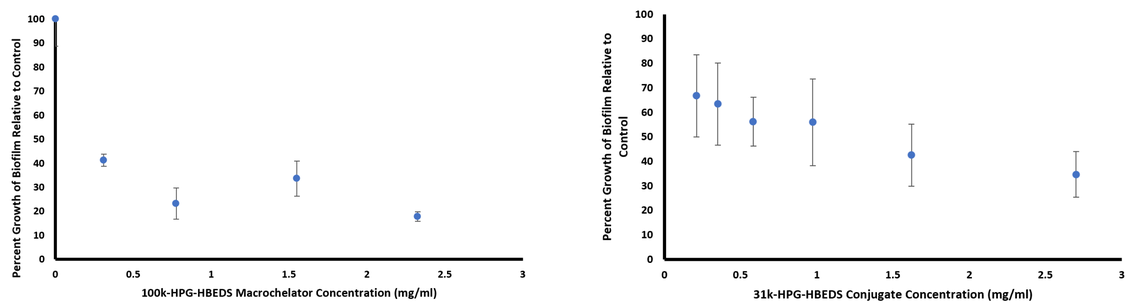
Effect of Molecular Weight of Chelators on Inhibition and Eradication of S. *aureus* Biofilm

3. Biofilm inhibition of S. aureus in presence of macrochelators of 2 different molecular weights (100kDa and 31kDa respectively) at different concentrations



- **31kDa variant seems to perform much better at biofilm inhibition than the 100kDa variant.**

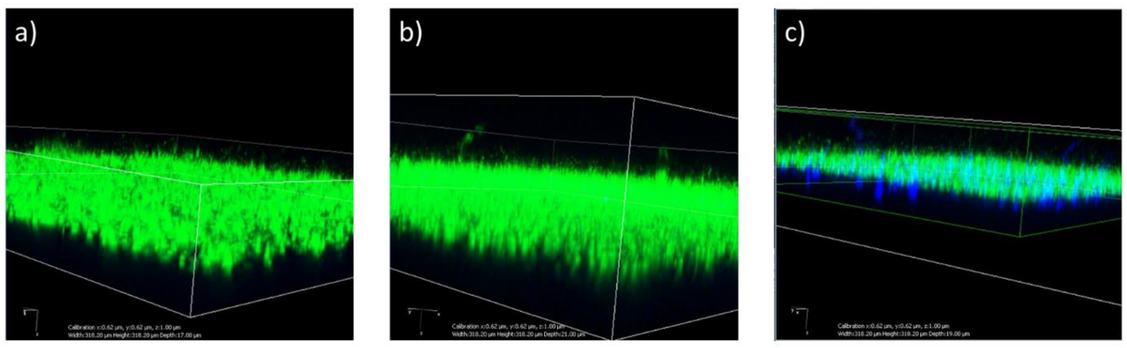
4. Biofilm eradication of S. aureus in presence of macrochelators of 2 different molecular weights (100kDa and 31kDa respectively) at different concentrations



- **100kDa variant is more effective than the 31kDa variant at lower concentrations.**

Effect of Scaffold Molecular Weight on Diffusion of Macrochelator into Biofilm

5. Confocal study of polymer scaffold diffusion into biofilm after 16 hours



Untreated Control | 100k-HPG Treatment | 31k-HPG Treatment

- **Clearly shown that 31kDa scaffold penetrates biofilm while 100kDa scaffold does not**

Conclusions

- **Our macrochelators have biofilm eradication properties against MRSA and *S. aureus*.**
- **31kDa conjugate was much less efficient**
- **100 kDa backbone did not penetrate the biofilm**
- **31 kDa backbone penetrated the biofilm but also inserted itself underneath**
    - **Biofilm penetration may account for differences in respective eradication abilities**
- Studies show importance of further understanding the microenvironment of biofilms in order to effectively combat them
- **More studies underway** on exploring these macrochelators further in the context of biofilm eradication both as solo therapeutics or as adjuvants.

References and Acknowledgements

1. Abbina, S.; Gill, A.; Mathew, S.; Abbasi, U.; and Kizhakkedathu, J.N.; *ACS Applied Materials & Interfaces* **2020** *12* (34), 37834-37844

Contact: jay@pathology.ubc.ca (JNK) ; gill@chem.ubc.ca (AG)