National Jewish Health
Breathing Science is Life.

JOURNAL CLUB

Article Summary by: Jennifer R. Honda, PhD

ARTICLE

"*Mycobacterium abscessus* biofilms have viscoelastic properties which may contribute to their recalcitrance in chronic pulmonary infections."Gloag, *et al*., Scientific Reports, 11:5020, 2021 (1). https://pubmed.ncbi.nlm.nih.gov/33658597/

CLINICAL QUESTION

It is currently unknown why *Mycobacterium abscessus* causes persistent airway infection in people with cystic fibrosis (pwCF). This basic science study investigates the possibility that *M. abscessus* persistence in the CF lung is caused, in part, by the viscoelasticity of *M. abscessus* biofilms.

SUMMARY

Background: *M. abscessus* are known biofilm formers, but the lack of understanding of the biology of these structures impedes the exploration of new treatment strategies.

This research group has a strong history of applying physical mechanic assays to understand the biological significance and mechanical properties of different microbial biofilms, including *Pseudomonas aeruginosa* (2), *Streptococcus gordonii* (3) and in the current report, *M. abscessus.* Importantly, the significance of *M. abscessus* smooth and rough colony variants remain contentious. Smooth variants are typically regarded as biofilm-forming *M. abscessus* and are associated with the presence of glycopeptidolipids (GPL). On the other hand, rough isolates devoid of GPL are known to be incapable of biofilm formation, yet biofilm aggregates have been reported. While the source of the *M. abscessus* isolates used in this study were not discussed, this team compared the viscoelasticity of smooth *M. abscessus* and rough *M. abscessus* isolate biofilms using mechanical indentation and shear rheometry.

Methods and Results:

1. *Hypothesis 1 (Figure 1):* Biofilms of smooth and rough *M. abscessus* variants differ in mechanical properties. To test this hypothesis, uniaxial indentation and shear rheology was performed on 4 day cultures, confirming *M. abscessus* biofilms maintain their distinct morphologies of smooth and rough *M. abscessus* variants.
2. *Hypothesis 2 (Figure 2): Biofilms of rough M. abscessus variants are thicker and stiffer than biofilms of smooth M. abscessus variants.* To test this hypothesis, uniaxial indentation testing was performed on 4 day cultures of *M. abscessus* using normal force. Biofilms of rough and smooth *M. abscessus* variants showed equivalent thickness. However, biofilms of rough *M. abscessus* variants were stiffer than smooth *M. abscessus* variants after applying the Young's modulus.
3. *Hypothesis 3 (Figure 3): Biofilms of smooth M. abscessus are more resistant to stress compared to rough M. abscessus.* To test this hypothesis, spinning disc rheology was used where a standard force was applied parallel to the biofilms and the resulting stress quantified. Elasticitiy, as quantified as storage (G'), and viscosity as quantified as loss (G") moduli were quantified using incrementally increasing oscillatory strain sweeps. Both *M.*

abscessus biofilms were viscoelastic, but the smooth *M. abscessus* variant was more pliant and endured compression under shear stress compared to the rough isolate.

4. *Hypothesis 4 (Figure 4): M. abscessus biofilms resist clearance from the lung.* To test this hypothesis, values from the frequency sweep analyses were correlated with the mucociliary clearance index (MCI) and the cough clearance index (CCI). The MCI and CCI were developed in the 1980's by King *et al.,* (4) to understand the role of mucous viscoelasticity in cough clearance. While the calculated MCI and CCI scores were similar between the smooth and rough *M. abscessus* variants, they were less than what has been reported for sputum collected from people with CF (5).
5. *Hypothesis 5 (Figure 5): M. abscessus biofilms are more resistant to lung clearance compared to other pulmonary pathogens.* To test this hypothesis, oscillatory frequency sweeps were performed for *M. abscessus* biofilms and compared to 4 day cultures *of Pseudomonas aeruginosa* and MCI and CCI analyzed. MCI and CCI were significantly less than *P. aeruginosa* biofilms, suggesting *M. abscessus* biofilms are indeed more resistant to mechanical clearance from the lung compared to other important lung biofilm formers.

Conclusions:

- Biofilms of rough *M. abscessus* variants are stiffer compared to smooth *M. abscessus* variants,
- Yet, biofilms of smooth *M. abscessus* variants are more pliable compared to rough *M. abscessus* variants,
- *M. abscessus* biofilms, no matter the morphotype, are more viscoelastic compared to other bacterial pathogens such as *P. aeruginosa*,
- *M. abscessus* biofilms resist clearance from the lung by coughing to promote their recalcitrance in chronic pulmonary infections.

In toto, the new data presented helps to understand how *M. abscessus* biofilms may respond to chemical and mechanical forms of eradication.

Remaining questions:

- The source of the smooth and rough isolates were not disclosed. Whether these were clinical isolates from non-CF, CF, or other is not known. Has this been confirmed by testing more *M. abscessus* isolates and does the source of the isolate matter?
- How the biofilms were made is unclear – referenced a prior paper, but referred to *Pseudomonas*, not NTM.
- Biofilms composition between smooth and rough should be elucidated in future studies.
- Rough *M. abscessus* was found to be stiffer than the smooth variant, which was more pliant, and resisted more deformation and compression, yet the role of GPL remains unaddressed.
- Clinical implications from these studies and how they may translate into the natural biofilms in the airway remains to be elucidated.

GROUP OPINION

While greatly needed, novel insights into how *M. abscessus* smooth and rough variants resist clearance from the lung by coughing are lacking. This research group applied standard biofilm testing protocols and well-established concepts of mucous viscoelasticity used to study other bacteria such as *P. aeruginosa* to *M. abscessus* in order to understand the mechanical nature of *M. abscessus* biofilms and how these biofilms may differ between smooth and rough variants. The type of *M. abscessus* biofilm information will also help to predict the effectiveness of therapeutics such as liposomes, lipid nanoparticles, and lipid carriers for pulmonary infections to improve antibiotic accessibility and targeting.

NTM Journal Club: njhealth.org/NTMJournalClub

References

1. Gloag ES, Wozniak DJ, Stoodley P, Hall-Stoodley L. Mycobacterium abscessus biofilms have viscoelastic properties which may contribute to their recalcitrance in chronic pulmonary infections. *Sci Rep* 2021; 11: 5020.
2. Gloag ES, German GK, Stoodley P, Wozniak DJ. Viscoelastic properties of Pseudomonas aeruginosa variant biofilms. *Sci Rep* 2018; 8: 9691.
3. Gloag ES, Wozniak DJ, Wolf KL, Masters JG, Daep CA, Stoodley P. Arginine Induced Streptococcus gordonii Biofilm Detachment Using a Novel Rotating-Disc Rheometry Method. *Front Cell Infect Microbiol* 2021; 11: 784388.
4. King M. The role of mucus viscoelasticity in cough clearance. *Biorheology* 1987; 24: 589-597.
5. Dasgupta B, King M. Reduction in viscoelasticity in cystic fibrosis sputum in vitro using combined treatment with nacystelyn and rhDNase. *Pediatr Pulmonol* 1996; 22: 161-166.