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Investigation of the anti-biofilm properties of purified biosurfactants in Enterococcal biofilms

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Introduction

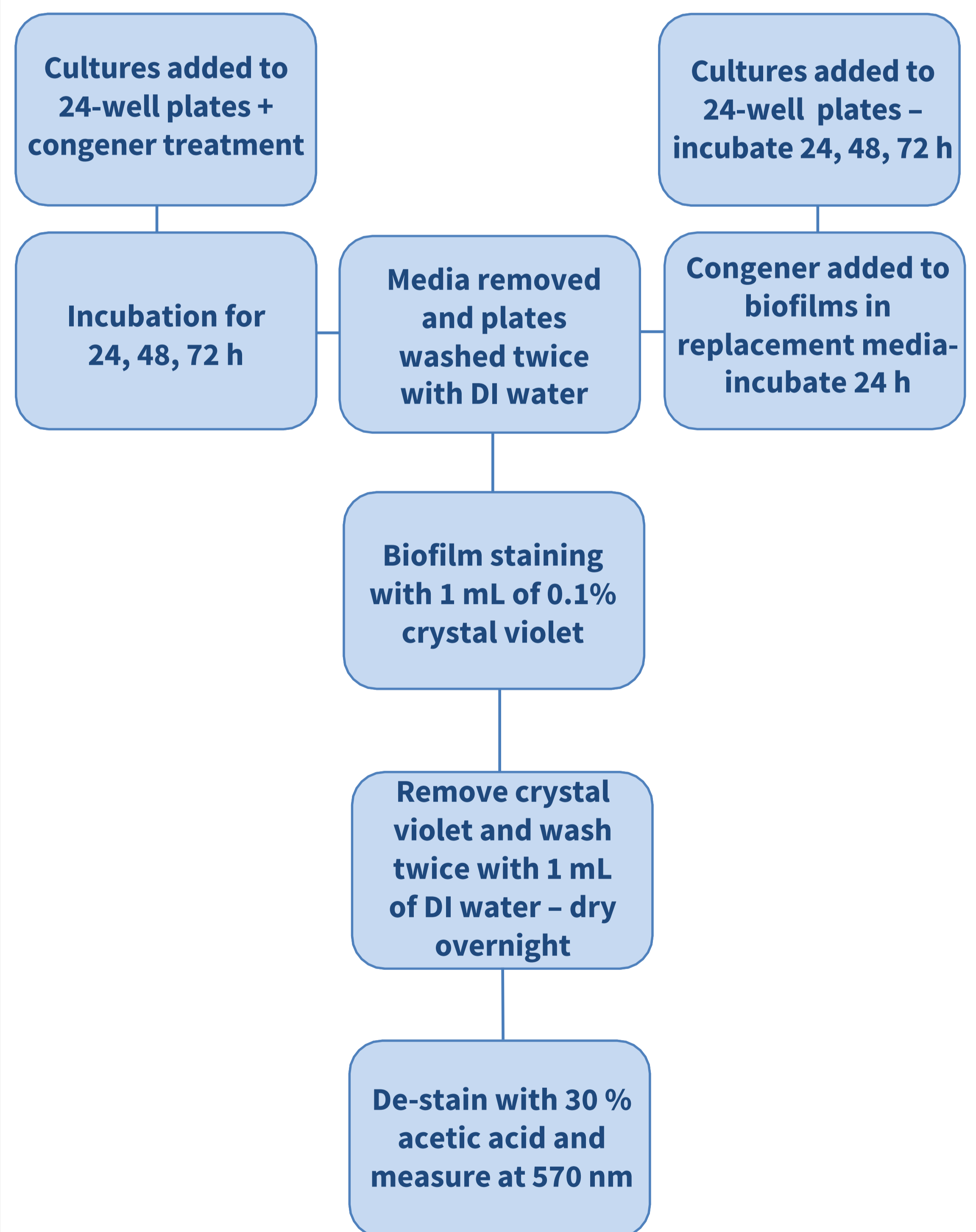
- Enterococcus* spp. have become a leading causative of healthcare-associated infections (HAIs) ranging from bloodstream infections to UTIs¹. Some 30% of healthcare-associated enterococcal infections are caused by vancomycin-resistant enterococci (VRE), requiring prolonged antibiotic therapy or becoming resistant to antibiotic monotherapy².
- Biofilms, structured biological communities that grow enveloped in an extracellular matrix of protective polysaccharides that subsequently prevents the action of antimicrobial agents³, can develop on an array of surfaces, from indwelling medical devices such as catheters to infected heart valves, facilitating chronic infections⁴.
- Biosurfactants are unique amphiphilic molecules of microbial origin that are capable of interacting with the lipidic components of microorganisms⁵. Biosurfactant interactions with different surfaces can affect their hydrophobic properties; their ability to alter microorganisms' adhesion abilities and consequent biofilm formation⁶ could make biosurfactants suitable for targeted use in medical and pharmaceutical applications⁷.

Aim/Objectives

- To determine the inhibitory effects of 24-hour biosurfactant congener treatment (Mono Rhamnolipid) on 24, 48 and 72 hour *E. faecalis* biofilms.
- To determine the biofilm disruption effects of the congeners on established 24, 48 and 72 hour *E. faecalis* biofilms.
- To determine minimum inhibitory and biocidal concentrations of the congener by broth microdilution.

Methods

Biofilm inhibition



Conclusions

- Mono-RL** shows significant ($P < 0.001$) efficacy in biofilm inhibition and disruption. Results show a **dose-dependent response**, with higher concentrations of biosurfactants leading to greater inhibition/disruption of biofilm growth.
- Preliminary results suggest that biosurfactants may offer a promising alternative or adjunctive treatment option against enterococcal biofilms.

Mono-Rhamnolipid structure

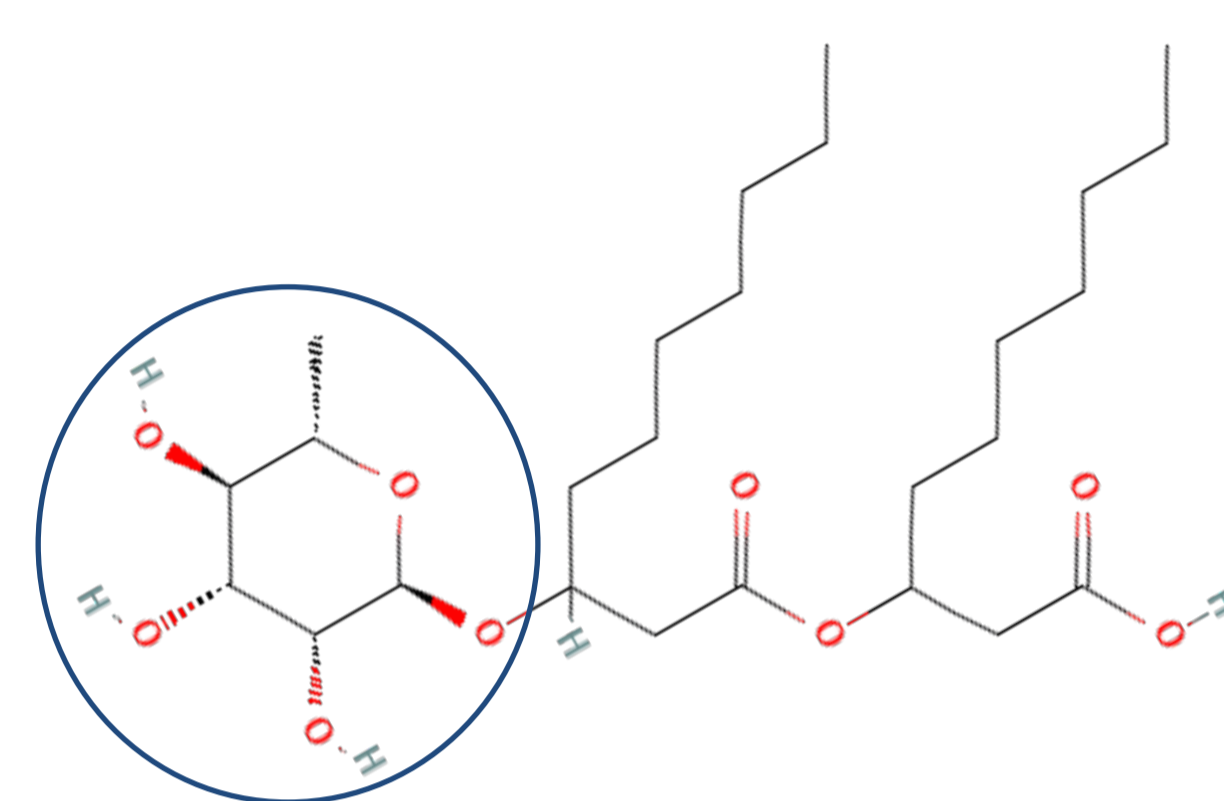


Fig 1. Rha-C₁₀-C₁₀. Molecular weight = 504.7 g/mol. Structure of RL consists of 2 β -hydroxyl fatty acids attached by a glycosidic linkage to 1 rhamnose sugar (PubChem, 2021).

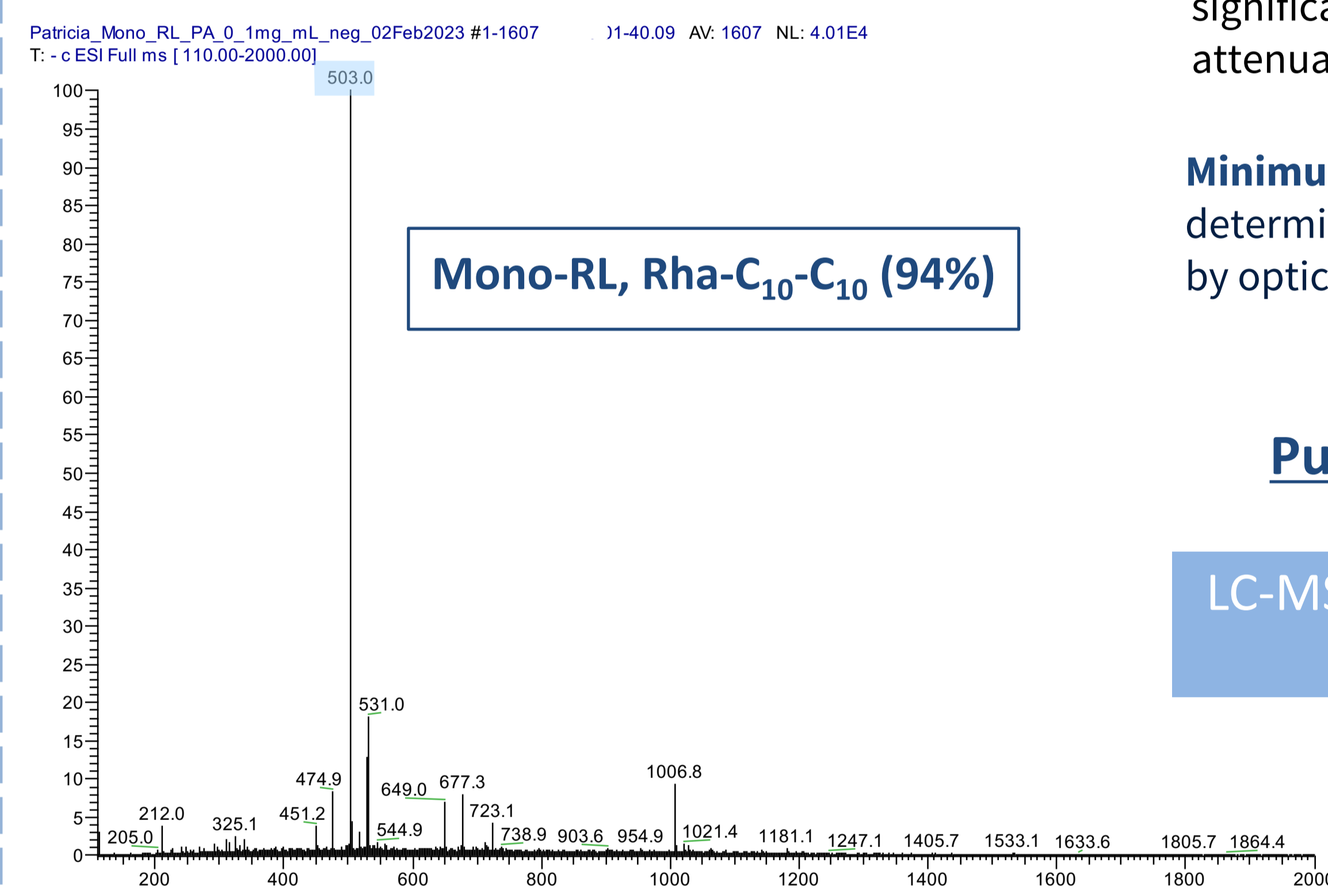


Table 1. MIC and MBC of Mono-RL for *E. faecalis* strains.

| Strain (<i>E. faecalis</i>) | Mono Rhamnolipid ($\mu\text{g/mL}$) | |
|-------------------------------|---------------------------------------|-----|
| | MIC | MBC |
| MW01105 | 250 | 500 |
| MF06036 | 250 | 500 |
| MW02048 | 250 | 500 |

MIC –the minimum concentration at which there is a significant decrease in growth as measured by attenuation (D) at 600 nm.

Minimum inhibitory and biocidal concentrations –determined by broth microdilution, growth measured by optical density at 600 nm.

Purity of Mono-Rhamnolipid

LC-MS/ESI mass spectrum in negative ion mode – purity 94%

Results

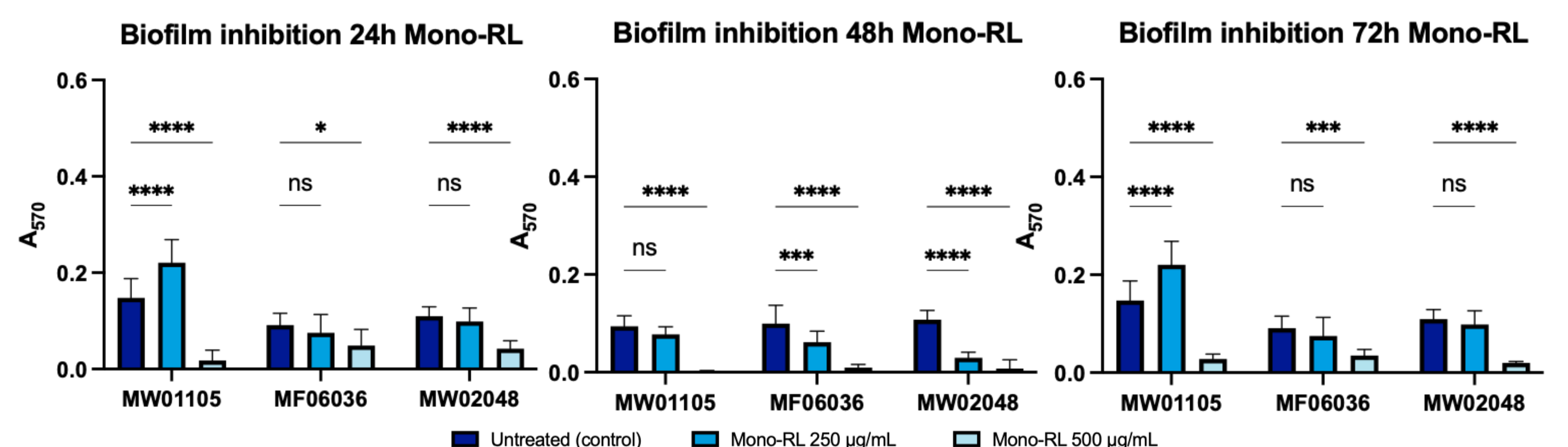


Fig 3. Biofilm biomass after co-incubation for 24, 48 and 72 h with Mono-RL. $n = 9$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

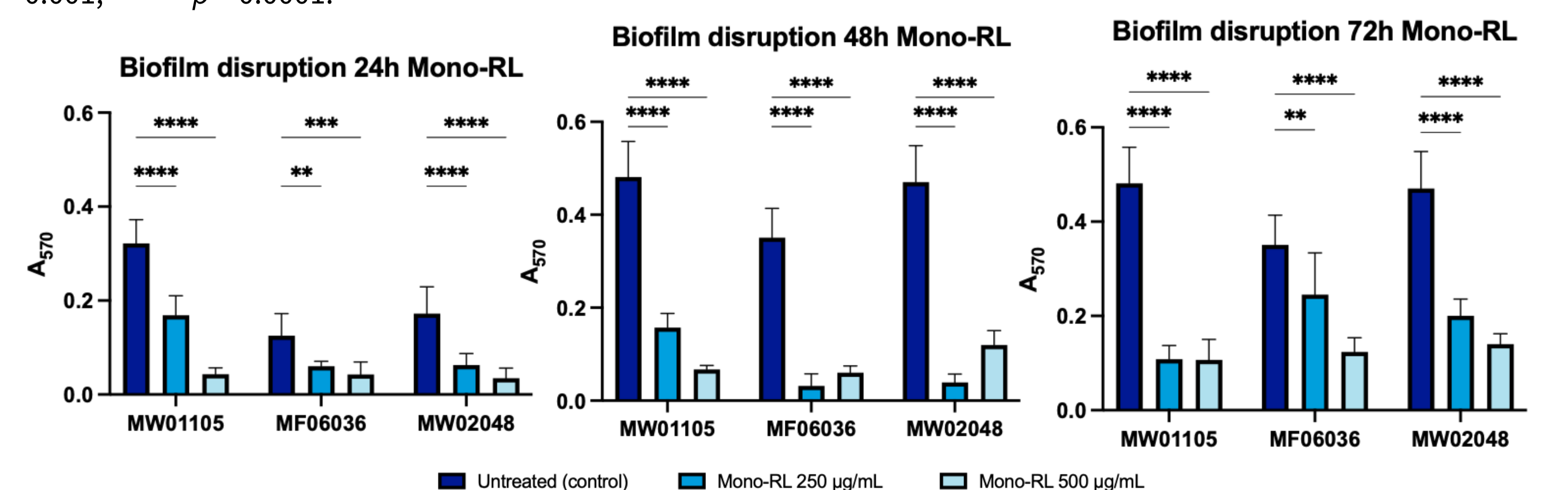


Fig 4. Biofilm biomass after 24, 48 and 72 h with a further 24 h of Mono-RL treatment. $n = 9$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

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