Novel Method of *Acanthamoeba* Movement Quantification to Determine Efficacy of Contact Lens Care Solutions

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Introduction

Acanthamoeba keratitis is a serious ocular infection which is extremely challenging to treat.[1]

May lead to permanent corneal damage and blindness.

Infection occurs when it comes in contact with contact lenses, usually via water, and is transported to the eye.

Habits of *Acanthamoeba* during both contamination and disinfection have remained elusive.

By quantifying *Acanthamoeba* motility, we may be able to better understand disinfection efficacy of contact lens products.

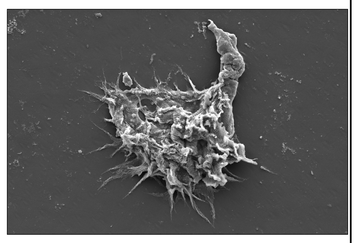

Figure 1. *Acanthamoeba* trophozoite, Alcon Research, LLC 2021

Methods

Two *Acanthamoeba* strains were used (*A. polyphaga*, American Tissue Culture Collection (ATCC) 30461, and *A. castellanii*, ATCC 50370).

Acanthamoeba were scaled up 24 hours prior to testing in axenic culture media to create homogenous trophozoite populations.[2, 3]

Acanthamoeba were seeded in a sterile aluminum flow cell at 7.5 x 10^3 to 3x10^4 CFU/mL and allowed to adhere to the surface for 30 minutes.

The flow cell solution was then changed to one of the following:

Control solutions:

1. ¼ Ringer's solution, on glass
2. ¼ Ringer's solution with heat-killed *E. coli*, on glass
3. Axenic culture media (AC6), on glass
4. Non-nutrient amoeba saline (NNA), on agar

Contact Lens Care Solutions (CLCs):

1. PHMB (polyhexamethylene biguanide)
2. PAPB (polyaminopropyl biguanide)/Polyquad (polyquaternium)
3. Polyquad/Aldox (myristamidopropyl dimethylamine)
4. Polyquad/Alexidine

Images taken once every 24 seconds for up to 12 hours at 4x magnification.

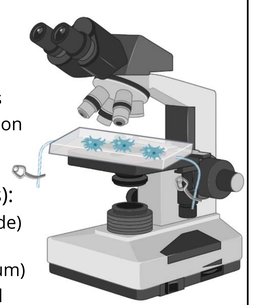

Figure 2. Depiction of a sterile aluminum flow cell with *Acanthamoeba* visible through the glass coverslip. Both ends are clamped during recording to create a closed environment with no flow or shear stress.

Methods

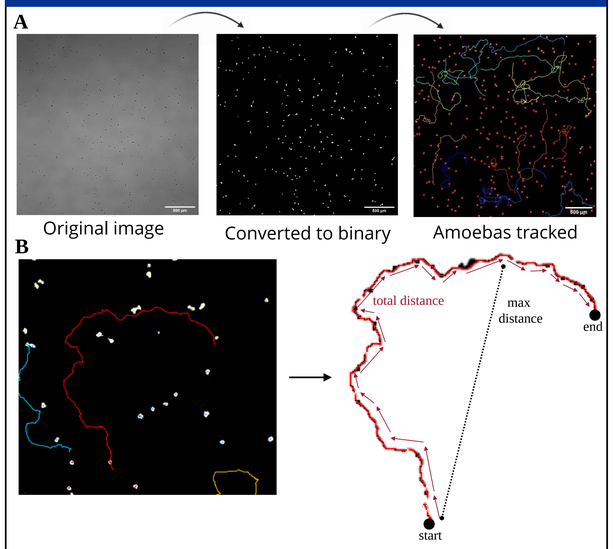

Figure 3. Representative images of quantitative analysis. A) Conversion of brightfield image to binary, followed by tracks identified for each moving amoeba. **B)** From each track, total distance, max distance (the maximum distance achieved from the start point), and speed were quantified in microns. 3 replicates were collected for each group/timepoint/strain, and 20-200 tracks per replicate.

Results

Acanthamoeba Continue Movement in Absence of Nutrients

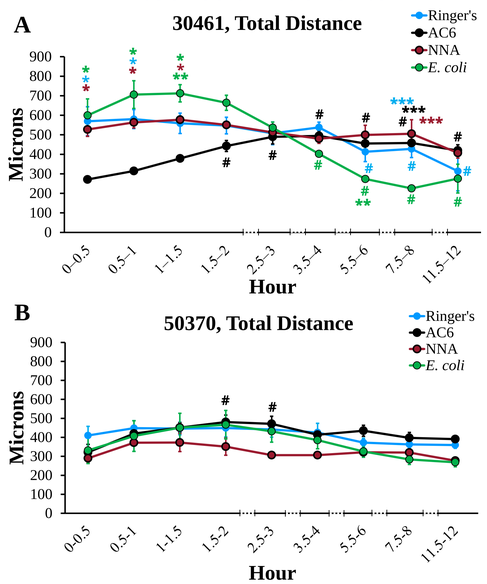

Figure 4. Quantification of total distance of A) ATCC 30461 and **B)** ATCC 50370. * $p < 0.05$ vs. AC6, ** $p < 0.05$ vs. NNA, *** $p < 0.05$ vs. *E. Coli*, and # $p < 0.05$ vs. 0-0.5 time point within the same condition. Color of each statistical symbol corresponds to the stated comparison.

Results

Differences in Strain Speed are Dependent Upon Condition

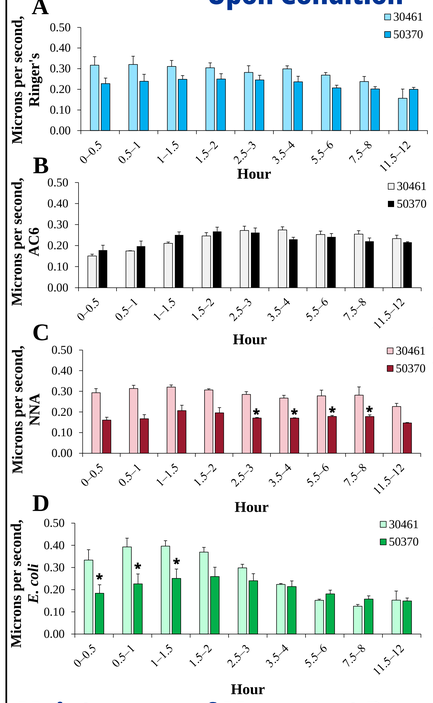

Figure 5. Comparison between strains within each condition and each time point of speed of *Acanthamoeba* movement. A) Ringer's on glass, **B)** Axenic culture media (AC6) on glass), **C)** Non-nutrient amoeba saline on agar (NNA), **D)** *E. coli* in Ringer's on glass. * $p < 0.05$ vs. ATCC 30461.

Maintenance of Movement Dependent on CLC Biocides

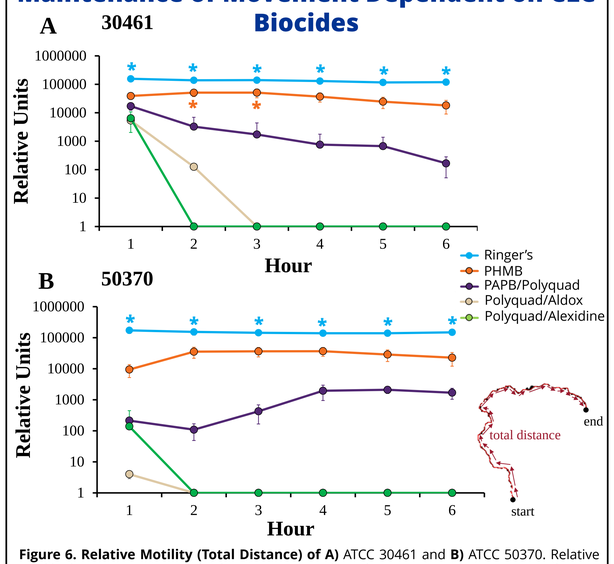

Figure 6. Relative Motility (Total Distance) of A) ATCC 30461 and **B)** ATCC 50370. Relative motility is calculated by multiplying the number of moving amoebas by the average distance moved by all moving amoebas to account for the large number of non-moving amoebas during CLC disinfection. Presented in log scale. * $p < 0.05$ vs. all other CLCs.

Results

Maintenance of Forward Motion Dependent on CLC Biocides

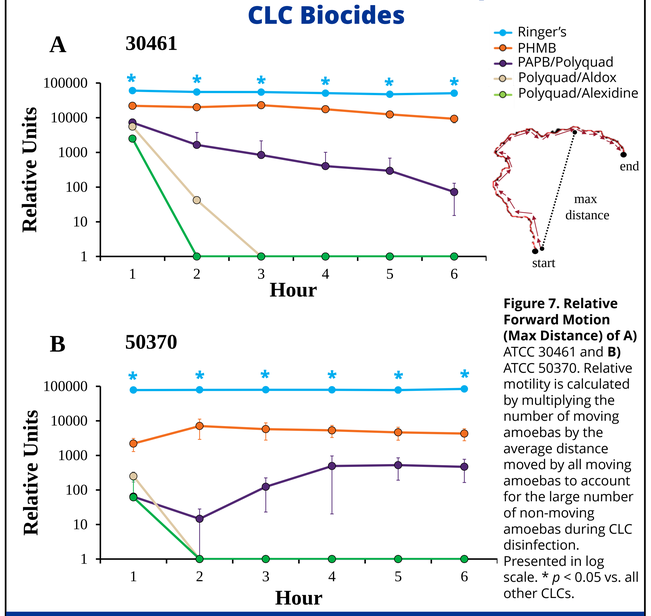

Figure 7. Relative Forward Motion (Max Distance) of A) ATCC 30461 and **B)** ATCC 50370. Relative motility is calculated by multiplying the number of moving amoebas by the average distance moved by all moving amoebas to account for the large number of non-moving amoebas during CLC disinfection. Presented in log scale. * $p < 0.05$ vs. all other CLCs.

Conclusions

Acanthamoeba in all control conditions maintained consistent movement even in the absence of nutrients.

Significant differences between strains within each condition persist.

When CLC solutions were used to disinfect against *Acanthamoeba* trophozoites, solutions containing polyquad/aldox and polyquad/alexidine produced the largest cessation of movement.

CLC solutions relying solely on PHMB had the least effect on movement.

Compared to control conditions, all CLC solutions either completely halted or severely impaired *Acanthamoeba* motility.

References & Disclosures

1. Dart JKG, Saw VPJ, Kilvington S. Acanthamoeba Keratitis: Diagnosis and Treatment Update 2009. American Journal of Ophthalmology 2009;148:487-499.e482

2. Campolo A, Shannon P, Crary M. Evaluating Alternate Methods of Determining the Antimicrobial Efficacy of Contact Lens Care Products against Acanthamoeba Trophozoites. Pathogens 2021;10

3. Walters R, Miller E, Campolo A, et al. Differential Antimicrobial Efficacy of Multi-Purpose Solutions against Acanthamoeba Trophozoites. Optom Vis Sci 2021

All authors are employees of Alcon Research, LLC. This work was funded by Alcon Research, LLC.

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Presented at the Global Specialty Lens Symposium, January 2022