Letters to the Editor

Enhanced Growth of Surface-Attached *Acanthamoeba polyphaga*

*Acanthamoeba* keratitis remains a problematic infection, especially for contact lens wearers. Current treatment is not always effective, and *Acanthamoeba* spp. may persist on contact lenses despite the use of disinfector. Growth of *Acanthamoeba polyphaga* in culture medium without bacteria has already been studied (3), but we know of no previous studies of the effect of surface attachment on this organism’s growth. Attachment can have a profound effect on the growth, survival, and disinfector sensitivity of other microorganisms (1), so we have studied cultures of *A. polyphaga* on cellulose fibers compacted into small cylinders (20 mm in length and 10 mm in diameter) termed Sorbarods (Ilacon, Kent, United Kingdom). Sorbarods were originally developed for the growth of plant cells (2). Both light microscopy and wet scanning electron microscopy clearly demonstrated acanthamoebae adhering to the cellulose fibers.

To each of 15 Sorbarods 7.5 $\times$ 10$^5$ *A. polyphaga* trophozoites in 1 ml of peptone-yeast extract-glucose (PYG) medium were added, which completely wetted the Sorbarod. To prevent drying, the Sorbarod cultures were incubated suspended above 1 ml of sterile water in a sealed container. For each Sorbarod a control culture consisting of 1 ml of PYG medium in a bijou (5-ml glass screw-top bottle) was inoculated and incubated in the same way as the Sorbarod. Cultures were incubated aerobically for up to 45 days at 30°C, with pairs of cultures being removed and counted for *A. polyphaga* content about every 3 days. *A. polyphaga* counts in control cultures were determined by microscopy using a modified Fuchs-Rosenthal counting chamber. Sorbarods were first broken up and treated with Triton X-100 to release the amoebae, which were then pelleted by centrifugation and resuspended in 1 ml of Pages’ saline, and the amoebic content was counted in the same way as the control cultures. This process probably underestimated the counts in the Sorbarod cultures.

Counts of trophozoites from the Sorbarods ranged from 8.8 $\times$ 10$^5$ to 4.5 $\times$ 10$^6$ per ml, with a median of 3.2 $\times$ 10$^6$, while counts of cysts ranged from 3.0 $\times$ 10$^4$ to 2.9 $\times$ 10$^5$, with a median of 1.6 $\times$ 10$^5$. Counts of trophozoites from control cultures ranged from 3.0 $\times$ 10$^5$ to 1.4 $\times$ 10$^6$, with a median of 5.1 $\times$ 10$^5$, and were in all cases lower than those from the paired Sorbarod cultures ($P = 0.001$ [Wilcoxon’s signed rank test]). Counts of cysts from control cultures ranged from 1.1 $\times$ 10$^4$ to 1.0 $\times$ 10$^5$, with a median of 4.0 $\times$ 10$^4$. Counts of cysts in the Sorbarods were higher ($P = 0.001$) than those in the control cultures except in two pairs; in one pair both cultures had 0 cysts, and in the other pair both cultures had 1.0 $\times$ 10$^5$ cysts.

The presence of cellulose fibers enhances the growth and possibly the persistence of *A. polyphaga* in culture, probably due to the provision of a wide surface area for attachment to cells. Surface-attached cultures of *Acanthamoebae* may be of value for efficacy studies of treatment agents and of contact lens disinfectants.

REFERENCES


Malcolm Armstrong
James Soothill
Department of Medical Microbiology
2nd Floor Clinical Sciences Building
Manchester Royal Infirmary
Oxford Road
Manchester M13 9WL, United Kingdom