Comparison of Quantum Dot- and Fluorophore-conjugated Antibodies for Bacterial Biofilm Research

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The oral cavity is a natural environment for over 500 bacterial species, and oral biofilms have a major role in oral pathology. Quantum Dots (QD) have several attractive properties that make them a promising tool for biofilm imaging, however few direct comparisons between QD-antibody conjugates and traditional fluorophore-antibody conjugates have been made. Here we present such a direct comparison in which primary immunofluorescence labeling of bacterial cells in biofilms by QD-antibody conjugates is compared with that by Alexa Fluor® conjugates. Alexa Fluor® conjugates have been used previously for study of intra- and interspecies interactions in oral biofilms in our laboratory. We used two commercially available QD preparations, here referred to as QDa and QDb, each of which is offered as “ready-for-conjugation” to antibody. These were conjugated to affinity-purified IgG from rabbit serum raised against whole cells of the oral bacterial strain *Streptococcus gordonii* DL1. These polyclonal antibodies recognize streptococcal surface components, including those displayed on *Streptococcus mutans* UA159, an oral bacterium that produces abundant exopolymer. QDa conjugates invariably precipitated and were not studied further. QDb conjugates remained as stable colloidal suspensions and were used for all subsequent experiments. First, the ability of the conjugates to stain streptococcal cells from standard planktonic bacterial cultures was assessed using epifluorescence microscopy; under these conditions, QDb conjugates displayed identical resolution and better bleach resistance than did Alexa Fluor® conjugates. We next evaluated the conjugates for staining of streptococcal cells grown as biofilms. The biofilms were grown in flowcells using 25% saliva or sucrose-supplemented saliva (to encourage exopolysaccharide production) as the sole carbon source. Fluorescence was detected using laser confocal microscopy. Under these conditions, resolution (bacterial cell surface definition) was better with Alexa Fluor® conjugate than with the QDb conjugate at similar concentrations. The concentration of QDb conjugate had to be increased about four-fold to obtain resolution similar to that seen with the Alexa Fluor® conjugate. We further investigated trapping of QDb conjugate in the biofilm exopolymer matrix using Calcofluor to counterstain the matrix. Naked (unconjugated) QDb as well as QDb conjugated to pre-immune IgG behaved identically; neither preparation was retained in the biofilm exopolysaccharide matrix. Thus, non-specific interactions could be ruled out as a cause of reduced resolution seen with the QDb conjugate. Accurate determination of protein concentration is essential for direct comparison of QD conjugates with fluorophore conjugates. Our results indicate that QDb conjugates perform better than do Alexa Fluor® conjugates in certain applications and, while QDb conjugates are usable for biofilm applications, we continue to explore the basis for reduced resolution of the QDb preparation in streptococcal biofilms.