Optimizing Visible-Light Bacterial Eradication through Novel Fluorescent Monitoring Procedures

Hussey, Grant

Bacterial antibiotic resistance has become a concern following this class of drugs' widespread adoption and subsequent misuse. New methods to eradicate bacteria must be discovered and perfected. To begin optimizing bacterial eradication with blue laser light, a method of monitoring cell viability using endogenous and exogenous fluorescent probes after irradiation by light of Methicillin-resistant Staphylococcus aureus (MRSA) was proposed and tested. First, a 405-nm blue laser was used to determine the correct fluence needed to initiate cell death. Second, the autofluorescence of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) was monitored in order to deduce relative concentrations inside of dying MRSA. Analysis was performed for the NADH and FAD concentrations in hopes of finding a correlation between NADH and FAD relative concentration and cell death. Third, MRSA were stained with a dye sensitive to membrane electrical potential, DiOC2(3), and the dye fluorescence emission was monitored over extended periods of time. One of the goals was to find a link between the presence of free radicals and their damaging effect on the cellular membrane. The hypothesis was that after irradiation with a 405-nm laser, cell death would be initiated and, as a result, the redox ratio, calculated as [FAD]/[NADH], will increase while the DiOC2(3) emission spectral shift ratio, calculated as ([Green])/([Green]+[Red]), will tend to 1. No meaningful relationship pertaining to redox ratio was found. However, a tendency for dying bacteria to have a membrane fluorescence ratio of 1 was obvious. Future experiments will employ multiple monitoring procedures hoping they will complement each other as to strengthen the feasibility of each individual probing technique.

Awards Won: