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Photodynamic effects of methylene blue-loaded polymeric nanoparticles on dental plaque bacteria

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Abstract

Background and Objectives—Photodynamic therapy (PDT) is increasingly being explored for treatment of oral infections. Here, we investigate the effect of PDT on human dental plaque bacteria *in vitro* using methylene blue (MB)-loaded poly(lactic-co-glycolic) (PLGA) nanoparticles with a positive or negative charge and red light at 665 nm.

Study Design/Materials and Methods—Subgingival plaque samples were obtained from 14 patients with chronic periodontitis. Suspensions of plaque microorganisms from seven patients were sensitized with anionic, cationic PLGA nanoparticles (50 µg/ml equivalent to MB) or free MB (50 µg/ml) for 20 min followed by exposure to red light for 5 min with a power density of 100 mW/cm². Polymicrobial oral biofilms, which were developed on blood agar in 96-well plates from dental plaque inocula obtained from seven patients, were also exposed to PDT as above. Following the treatment, survival fractions were calculated by counting the number of colony-forming units.

Results—The cationic MB-loaded nanoparticles exhibited greater bacterial phototoxicity in both planktonic and biofilm phase compared to anionic MB-loaded nanoparticles and free MB, but results were not significantly different ($p > 0.05$).

Conclusion—Cationic MB-loaded PLGA nanoparticles have the potential to be used as carriers of MB for PDT systems.

Keywords

Dental plaque bacteria; methylene blue; polymeric nanoparticles; photodynamic therapy

INTRODUCTION

Dental plaque is a structurally and functionally organized multi-species biofilm (1). This biofilm colonizes tooth surfaces and epithelial cells lining the periodontal pocket/gingival sulcus and can lead to development of periodontal diseases (gingivitis or periodontitis).

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DISCLOSURE OF PROPRIETARY INTERESTS

We certify that we have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript (e.g., employment, consultancies, stock ownership, honoraria).

Mechanical removal of dental plaque is currently the most frequently used method to treat periodontal diseases. Antimicrobial agents are also used, but bacteria growing in biofilms exhibit several resistance mechanisms (2).

Photodynamic therapy (PDT) has been suggested as an alternative to chemical antimicrobial agents to eliminate subgingival species and treat periodontitis (3). This method is based on the concept that a photosensitizing agent (a photosensitizer) can be preferentially localized in certain tissues and subsequently activated by light of the appropriate wavelength in the presence of oxygen to generate singlet oxygen and free radicals that are cytotoxic to cells of the target tissue (4).

Several clinical studies have been carried out to investigate the effects of PDT mediated by methylene blue (MB) in human periodontitis (recently reviewed and summarized in 5). Single sessions of PDT as an independent treatment or as an adjunct to scaling and root planing did not show any beneficial effects over scaling and root planing alone (6–8). A recent meta-analysis on the effect of PDT for periodontitis supports these findings (9).

The treatment of biofilm-associated bacterial infections poses challenges due to several antimicrobial resistance mechanisms of biofilms (10). The reduced susceptibility of biofilms derived from human natural dental plaque to MB-mediated PDT *in vitro* has been demonstrated recently as well (11). There are several possible explanations for the reduced susceptibility of oral biofilms to PDT, including the inactivation of photosensitizer (12), the existence of biofilm bacteria in a slow growing or starved state (13), and the expression of certain phenotypes by organisms growing within the biofilm (14). The restricted penetration of MB in oral biofilms (15) and ability of bacterial cells to expel MB via multidrug resistance pumps (16) also contribute to the incomplete eradication of biofilm microorganisms. One way to overcome the latter two deficiencies is to develop a delivery system that significantly improves the pharmacological characteristics of MB.

In the present study, our hypothesis was that MB-loaded poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles of either positive or negative charge and with a diameter of <220 nm would exhibit a greater photodynamic effect than would free MB in suspensions of human dental plaque bacteria as well as in biofilms derived from human dental plaque inocula. The nanoparticle matrix PLGA is a polyester co-polymer of poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) that has received approval by FDA due to its biocompatibility and its ability to degrade in the body through natural pathways (17–19). PLGA nanoparticles have been used successfully in drug delivery of photosensitizers (20,21). Once encapsulated within PLGA, the excited state of the photosensitizer is quenched, which results in loss of phototoxicity. When the nanoparticles are incubated with cells, they show a time-dependent release of the photosensitizer, which then regains its phototoxicity and results in an activated photodynamic-nanoagent (22). Recently, we explored the use of PLGA nanoparticles as carriers of MB in antimicrobial PDT (23). Sensitization of *Enterococcus faecalis* species in planktonic phase with MB-loaded nanoparticles followed by exposure to red light led to approximately 2 log₁₀ bacterial killing. In addition, the synergism of nanoparticles and light exhibited approximately one order of magnitude killing of *E. faecalis* biofilm species in experimentally infected root canals of human extracted teeth (23). In the present study, we also hypothesized that cationic PLGA nanoparticles would serve as better alternatives to free MB. There is strong evidence that the positive charge of a photosensitizer enhances its uptake and phototoxicity on bacterial species (24–26).

METHODS

Enrollment of subjects and Sample collection

Samples of dental plaque were collected from 14 subjects. The research for this study complied with all relevant federal guidelines and institutional policies and Forsyth Institute's Institutional Review authorized the protocol. Informed consent was obtained from all participants. Inclusion criteria were a diagnosis of chronic periodontitis with probing depths greater than 5 mm. None of the subjects had used antibiotics or had undergone treatment for periodontitis during three months prior to sampling. Dental plaque samples were taken from supragingival and subgingival mesiobuccal aspects of premolars or molars in each subject using individual sterile Gracey curettes at the Forsyth Dental Clinic. Samples were immediately placed in a sterile pre-reduced anaerobic Ringer's solution (Anaerobe Systems, Morgan Hill, CA, USA). The microorganisms from the plaque samples were dispersed by sonication and repeated passage through Pasteur pipettes. Aliquots of the dispersed bacteria were transferred to 1 mL cuvettes and the optical density of the bacterial suspensions was measured in a spectrophotometer (one optical density unit was considered as approximately 10^9 cells/mL at 600 nm). Samples from seven subjects were exposed to PDT in suspension, whereas samples from seven subjects were used for the development of biofilms, which were also exposed to PDT 48 hrs later.

Preparation of blood agar culture plates and biofilm development

Blood agar culture plates were prepared using 20 g/L of trypticase soy agar (BBL, Cockeysville, MD), 26 g/L of brain-heart infusion (BHI) agar (Difco Laboratories, Detroit, MI), 10 g/L of yeast extract (BBL) and 5 mg/L of hemin (Sigma Chemicals Co., St. Louis, MO), autoclaved and cooled to 50 °C. Under sterile conditions, 5% defibrinated sheep blood (Northeast Laboratory Services, Waterville, ME), 5 mg/ml of menadione (Sigma Chemicals Co) and 10 mg/ml of N-acetylmuramic acid (Sigma Chemicals Co) were added to the autoclaved mixture. Aliquots of 1.5 mL of the agar medium were dispensed into wells of 96-well plates and allowed to dry in the anaerobic chamber in the presence of 80% N₂, 10% H₂, 10% CO₂ at 35°C. For biofilm development, 150 µL of dental plaque/BHI broth (Beckton, Dickinson and Co., Sparks, MD, USA) inocula containing approximately 10^7 bacteria per mL were carefully pipetted to fill four blood agar wells in each 96-well plate. The plates were then incubated anaerobically for 48 hrs.

Biofilm characterization

a) Counts of biofilm bacteria—On 2nd day of their development, biofilms were gently scraped from blood agar from each well using a sterile bacteriological loop to remove the entire visible biomass. Then, spectroscopy at 600 nm was performed to determine the total bacterial load.

b) Confocal scanning laser microscopy—To observe the distribution of dead/live bacterial cells in biofilms, a Leica SP2 confocal scanning fluorescence microscope (Leica Inc., Malvern, PA, USA) was used with a 20x or a 40x water-dipping objective lens. To accommodate for the width of the confocal microscope objective, biofilms were grown on 1.5 cm high blood-agar medium for two days in 24-well plates anaerobically as described above. For optimum biofilm development, the plaque/BHI inoculum contained 10^9 cells/mL. Live and dead biofilm bacteria were simultaneously viewed using the reagents SYTO 9 stain and propidium iodide in the LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probes, Inc., Eugene, OR, USA) according to the manufacturer's instructions. Biofilms were stained in the dark at room temperature for 15 min. An argon laser (476 nm) was used as the excitation source for the reagents, and two separate emission filters at 500 nm (SYTO 9) and 635 nm (propidium iodide) collected the emitted fluorescence light. Sections were collected

at 4- μ m intervals and analyzed using image-processing techniques to assess the distribution of dead/live bacteria within the biofilm matrix.

Preparation of PLGA nanoparticles

PLGA nanoparticles encapsulating MB (10% w/w) were prepared by blending the medical grade PLGA (MW 12 KDa, 50:50 lactide-glycolide molar ratio; Birmingham Polymers, Pelham, AL) with Pluronic® F-108 triblock copolymer (Performance Chemicals Division of BASF, Parsippany, NJ) and fabricating the nanoparticles by a solvent displacement procedure as previously described (27). Briefly, a solution of PLGA (76 mg) and Pluronic® F-108 (14 mg) was prepared in acetone (5 mL) and heated with stirring until it became clear. For the preparation of the MB-loaded nanoparticles, the oleate salt of MB (Sigma Chemicals Co) was dissolved at 10% (w/w) concentration in the acetone solution of PLGA. Pluronic® triblock copolymers were added to the polymer solution in acetone at 20% (w/w) to insure that the formed nanocarriers have a stable hydrophilic surface, which resists aggregation. The solution was introduced into an aqueous (50 mL) solution under vigorous stirring and left to stir overnight. The next day, nanoparticles were centrifuged at 10,000 rpm for 20 min, then washed twice with deionized distilled water and lyophilized under vacuum for 48 hr. To modify surface properties of nanoparticles with cationic or anionic charge, we have used cetyl trimethyl ammonium bromide (CTAB; Sigma Chemicals) or 10% w/w Pluronic F-108® (PEO-PPO-PEO triblock co polymer bought from BASF corp.) as a surfactant, respectively.

Characterization of PLGA nanocarriers

The mean size of PLGA nanoparticles, with and without the encapsulated payloads, was determined via laser light scattering using a ZetaPALS system (Brookhaven Instruments, Holtsville, NY). The surface morphology of the nanocarriers was visualized by scanning electron microscopy (Shimadzu, Japan) following freeze-drying. The surface charge on the nanoparticles, in the presence and absence of encapsulated payload, was determined by zeta potential measurements of the nanocarrier suspensions in PBS (pH 7.4) with ZetaPALS (Phase Analysis Light Scattering) ultra-sensitive zeta potential analyzer. To determine the amount of drug loaded into the nanocarriers (capacity) as well as the percentage of added drug (efficiency), a known amount (~10 mg) of the control and PEO-modified nanocarriers was dissolved in acetone. The amount of encapsulated drug in the nanocarriers was determined by using the UV-VIS absorbance of MB. The release kinetics of MB-oleate salt from the nanoparticles was determined in PBS (pH 7.4). To increase the solubility of MB complex, Tween®-80, a non-ionic surfactant, at 1.0% (w/v) concentration, was added to the release medium. This also prevented the MB-loaded nanocarriers from binding to the container surface. One hundred milligrams of the drug-containing nanocarriers was incubated with 10 mL of the release medium in a shaking water bath (50 rpm). Periodically, 5 mL of the release medium was removed and replaced with 5 mL of fresh buffer to maintain sink conditions. MB in the release medium was assayed by Shimadzu UV-VIS spectrophotometer (Columbia, MD). Cumulative amount and percent drug released was determined from appropriate calibration curves of the respective agents.

Light source and light parameters

A diode laser (BWTEK Inc., Newark, DE, USA), with an output power of 1 W and a central wavelength of 665 nm, was used. The laser possessed a spectral stability of ± 2 nm with an output power stability of 10 mW. The system was coupled to a 1 mm optical fiber that delivered light into a lens, which formed a uniform circular spot, 2 cm in diameter, on the base of the 96-well plate. This spot of light was able to irradiate each time a group of four wells in a 96-well plate from above for 5 minutes in the dark at room temperature. The

power density of incident radiation was measured using a power meter (Ophir Optronics Ltd, Danvers, MA, USA).

Photodynamic treatment

The light parameters used for both planktonic and biofilm experiments were 100 mW/cm² (power density) and 30 J/cm² (energy fluence). The concentration of free MB was 50 µg/mL and the final concentration of cationic or anionic MB-loaded PLGA nanoparticles was 50 µg/mL equivalent to MB. The following experimental groups were used: 1) No light/No photosensitizer (control); 2) treated only with free MB; 3) treated only with anionic MB-loaded nanoparticles; 4) treated only with cationic MB-loaded nanoparticles; 5) treated only with light; 6) treated with light and free MB; 7) treated with light and anionic MB-loaded nanoparticles; and 8) treated with light and cationic MB-loaded nanoparticles. Groups 1, 2, 3 and 4 were kept in plates at room temperature and covered with aluminum foil during irradiation. Each treatment group was carried out in quadruplicates for both the biofilm and the bacterial suspension experiments. The primary endpoint for evaluation was the mean number of colony-forming units (CFU) per group.

Suspensions—For the PDT of microorganisms, aliquots of bacterial suspensions (10⁸/mL) were placed in sterile microcentrifuge tubes and were centrifuged (7,000 rpm for 4 minutes). The supernatants were aspirated and 1 mL of sterile PBS 1X (pH 7.4) with free MB or MB-loaded nanoparticles was then added. Cultures were resuspended with the drug and placed in the wells of 96-well plates for 20 minutes before they were exposed to light. During PDT, 96-well plates remained covered with a lid. Following illumination, bacterial suspensions underwent serial dilutions in BHI broth and 100 µl aliquots were plated on blood agar plates and then incubated under anaerobic conditions for 7 days prior to scoring CFU.

Biofilms—Growth medium was aspirated from each well of 96-well plates carefully and the drug in PBS was added in the wells with the biofilms for 20 minutes followed by exposure to light. During PDT, 96-well plates remained covered with a lid and were not disturbed. After illumination, adherent bacteria were gently scraped from blood agar in each well using a sterile bacteriological loop to remove the biofilm and dispersed in BHI broth. The same experienced researcher removed all of the biofilms to minimize sample-to-sample variability by ensuring that the scrapings collected the entire biofilm. Aliquots were measured in a spectrophotometer at 600 nm in 1 mL cuvettes. Then, serial dilutions were prepared and 100 µL aliquots were spread over the surface of blood agar plates. The plates were incubated anaerobically at 35 °C for 7 days prior to counting CFUs.

Statistical Analysis

Both the planktonic and biofilm experiments involved 7 patients. Aliquots from each patient sample were exposed to all 8 treatments, as described above. The setup constituted a randomized blocks design (28). The endpoint for each patient sample was CFU levels for each of the treatments. For reasons of nonnormality and variance heterogeneity, CFU values from both experiments were log transformed for statistical analysis. The standard analysis of variance for treatments by patient blocks was done for both the planktonic as well as biofilm data. Pairwise comparisons of selected treatment effects (mean log CFU) were done by comparisons of least squares means.

RESULTS

Characterization of Nanoparticles

The diameter of PEO-PLGA nanoparticles ranged from approximately 190 to 220 nm (Table 1). The average surface charge of the nanocarriers, as determined by zeta potential measurements, was -17.5 , -38.3 and 43.5 mV for blank nanoparticles, MB-loaded anionic and MB-loaded cationic nanoparticles, respectively. PLGA nanoparticles were spherical in shape and had a smooth surface (Fig. 1). Inclusion of MB in nanoparticles did not affect the particle size as determined by SEM. UV-visible spectroscopy verified the capacity and efficiency of MB encapsulation in PEO-PLGA nanoparticles. A standard curve of MB was obtained by dissolving the oleate salt in different concentrations ranging from 50 to 250 $\mu\text{g/ml}$ in acetone or PBS and shown to be linear over this concentration range (Fig. 2). Cationic nanoparticles released MB oleate much faster than anionic nanoparticles. After 12 hours, over 80% of encapsulated MB oleate was released in PBS at 37 °C from the cationic nanoparticles and approximately 28% from anionic PEO-PLGA nanoparticles (Fig. 3).

Biofilm confocal studies

The two-day biofilm derived from dental plaque was fully formed and covered the entire well of the 24-well plate. Confocal images (X-Y) showed primarily live microorganisms organized in clusters extending to a depth of 120 μm (Fig. 4). The thickness of biofilms ranged from 75 to 130 μm . Fluorescent signals of free MB in biofilms following their incubation were not possible to observe and measure, possibly due to the low concentrations of released MB.

PDT of planktonic bacteria

Results obtained from the planktonic PDT experiments are presented in Figure 5a. Red light alone did not have any killing effect on bacterial suspensions. Mean log CFU levels were lower, but not significantly different for light alone versus no light/no drug treatment ($p>0.05$). Free MB as well as MB encapsulated in anionic or cationic nanoparticles reduced bacterial viability by 38.6%, 43.6% and 60.1%, respectively. Mean log CFU levels for these groups were significantly lower than the control treatment (no drug/no light) (all $p<0.01$). Mean log CFU levels for each of the three PDT groups (light plus MB, anionic or cationic nanoparticles) were significantly lower than mean log CFU levels of the groups treated with MB but not light (light and free MB versus MB only, $p=0.04$; light and MB-loaded anionic nanoparticles versus anionic nanoparticles only, $p=0.01$; light and MB-loaded cationic nanoparticles versus cationic nanoparticles only, $p<0.001$). The synergism of light and MB-loaded cationic nanoparticle showed the greatest killing effect (85%). However, differences among PDT groups were not significantly significant ($p>0.05$).

PDT of biofilm bacteria

Results obtained from the biofilm PDT experiments are presented in Fig. 5b. Light alone did not have any effect on bacterial viability. Mean log CFU levels were not significantly different for light alone versus no light/no drug treatment ($p>0.05$). Free MB, anionic and cationic nanoparticles showed similar dark toxicities (approximately 25%). Mean log CFU levels for these groups were significantly lower than the control treatment (no drug/no light) (all $p<0.01$). In the presence of light, free MB, MB-loaded anionic and cationic nanoparticles reduced bacterial viability by approximately, 37%, 42% and 48%, respectively. Mean log CFU levels for light and MB were not significantly different from those of free MB alone ($p<0.05$). On the other hand, mean log CFU levels for light and anionic/cationic nanoparticles were significantly lower than mean log CFU levels of

treatments with nanoparticles alone (both $p < 0.01$). Differences among PDT groups were not significantly different ($p > 0.05$).

DISCUSSION

In the present study, the cationic MB-loaded PLGA nanoparticles exhibited the highest phototoxicity towards bacteria, followed by anionic-MB loaded PLGA nanoparticles and free MB in both suspensions and biofilms. In suspensions, cationic nanoparticles produced approximately 1 log₁₀ killing. In oral microcosm laboratory biofilms, they reduced bacterial viability by 48%. The average percent killings for anionic nanoparticles and free MB were approximately 60% and 40% in suspensions and biofilms, respectively. Although these data were not statistically significant ($p > 0.05$), results exhibited the same trend in all six planktonic or biofilm experiments concerning the greater phototoxicity of cationic MB-loaded nanoparticles.

The planktonic experiments clearly demonstrated the superiority of MB-loaded nanoparticles over anionic nanoparticles and free MB. It is promising that nanoparticles were taken up by microorganisms within a short period of time and were able to release MB amounts that led to bacterial destruction following their exposure to light. Recently, cationic biodegradable PLGA nanoparticles composed of chitosan were studied as gene carriers in the nasal mucosa of mice *in vivo* (29). The results of this study showed that PLGA nanoparticles facilitated gene delivery and subsequent expression with increased efficiency. Also, cationic eudragit containing PLGA nanoparticles showed better adhesion to *Pseudomonas aeruginosa* and *Staphylococcus aureus* than anionic PLGA nanoparticles (30). The enhanced electrostatic interaction between the cationic nanoparticles and the negatively charged residues of the lipid bilayer generate nanoscale holes in the outer membrane (31). This may explain the greater toxicity of cationic nanoparticles in the absence of light in planktonic phase compared with anionic nanoparticles and free MB. It is also possible that the use of MB-loaded nanoparticles limited the ability of microorganisms to pump the MB molecule back out. The faster release of MB by cationic nanoparticles may also have contributed to their greater phototoxicity over anionic nanocarriers. The incomplete photodestruction of dental plaque bacteria in suspension may be related to the phenotypic changes carried by these microorganisms once they were biofilm species (14). However, it is possible that the use of other drug and light parameters (e.g. amount of MB encapsulated in nanoparticles, incubation time, power density, energy fluence) may lead to complete bacterial destruction.

The effect of light resulted in lower reductions of microorganisms within biofilms, which was not surprising (11,32). Biofilm bacteria showed resistance to PDT, with killing not exceeding 48% (for cationic MB-loaded nanoparticles) compared with dark controls. The microcosm biofilm model employed in this study originated directly from the whole-mixed natural dental plaque and showed a structure that resembled that of natural dental plaque as revealed by confocal scanning laser microscopy. This model was validated and tested previously (11,15). Despite the reduced PDT bacterial destruction in biofilms compared with suspensions, the effect was much greater than seen with antibiotic therapy. In planktonic experiments the bacterial killing was two-fold greater compared with biofilms, whereas antibiotics were approximately 250-fold less effective in biofilm state (33). The reduced susceptibility of bacteria in biofilms may be attributed to the negatively charged matrix that hinders penetration of a positively charged agent, such as MB and cationic nanoparticles, because of strong ionic interactions. However, this generalization is difficult to justify because many different factors play a role including the particular system under investigation, the chemical composition of the matrix as well as the physicochemical properties and chemical reactivity of the antimicrobial agent (34) (35). It has been reported

that even when there is strong ionic interaction between a negatively charged matrix and a positively charged antimicrobial agent, diffusion of the agent is not hindered to a great extent and, once all of the binding sites have been filled, the matrix would not present any further barrier to diffusion (36). It is also possible that MB penetration may have been enhanced by either passive targeting or by active targeting via the charged surface of the nanoparticle. Our efforts to study penetration and distribution of nanoparticles into the biofilms by confocal scanning laser microscopy were not successful; it was not possible to detect traces of MB fluorescence in biofilms. The reduced susceptibility of biofilms to PDT using charged nanoparticles may also be due to a failure of nanoparticles to penetrate into the interior of cell clusters by forming aggregates with other nanoparticles as well as sticking to biofilm surface. Aggregation of nanoparticles can form a mass larger than the size of a biofilm channel and therefore block or hinder the entrance of released MB completely. Finally, the increased density of bacterial clusters within biofilms results in a microenvironment with low pO₂ that may be responsible for the reduced PDT effect.

Our findings suggest that cationic PLGA nanoparticles have the potential to be used as carriers of MB for photodestruction of oral biofilms. The greater PDT bacterial killing by cationic MB-loaded nanoparticles showed the ability of nanocarriers to diffuse in biofilms and release the encapsulated drug in the active form. However, it is not certain that the sufficient concentrations of MB were released in order to have the greatest possible effect in eradication of the biofilm organisms. Therefore, future studies should define the physical characteristics of nanoparticles (e.g. size, zeta potential) that are important in determining their intracellular uptake and trafficking. In addition, the optimal PDT parameters for effective elimination of biofilm species should be determined and the safety of PDT should be demonstrated by defining the therapeutic window where bacteria could be killed leaving mammalian cells intact.

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Figure 1. Scanning electron micrograph of blank PLGA nanoparticles. The inset presents higher magnification SEM image showing individual spherical nanoparticles of 190–250 nm in diameter. Scale bars represent 1 μ M.

Figure 2.
Standard curve of MB-oleate; absorbance of MB-oleate at 665 nm under different concentrations µg/ml.

Figure 3.
Release of MB-oleate from nanoparticles. Blue diamond denotes cationic nanoparticles (mean $\pm$ s.e.m); red square denotes anionic nanoparticles (mean $\pm$ s.e.m).

Figure 4.

Images obtained from 2-day dental plaque-derived biofilms grown on agar in 24-well plates using CSLM. Live bacteria (green) with intact membranes are stained with SYTO 9 stain and dead bacteria (red) with damaged membranes are stained using propidium iodide. The depth of the biofilm varied between 80–120 μm. Scale bar represents 100 μm.

Figure 5. Survival in log(CFU) of a) planktonic and b) biofilm-based bacteria after treatments with either no drug, free MB, MB-loaded anionic and MB-loaded cationic nanoparticles in the presence or absence of light at 665 nm.

Table 1

Particle size and surface charge of blank, MB-loaded cationic and MB-loaded anionic nanoparticles.

	Blank nanoparticles	Anionic PLGA nanoparticles	Cationic PLGA nanoparticles
Mean Hydrodynamic Diameter (nm)	189.2 ± 2.2	218 ± 3.1	205 ± 1.3
Mean Zeta Potential (MV)	-17.5 ± 0.74	-38.30 ± 0.17	43.5 ± 1.83