Assessment of Photodynamic Destruction of *Escherichia coli* O157:H7 and *Listeria monocytogenes* by Using ATP Bioluminescence

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Antimicrobial photodynamic therapy was shown to be effective against a wide range of bacterial cells, as well as for fungi, yeasts, and viruses. It was shown previously that photodestruction of yeast cells treated with photosensitizers resulted in cell destruction and leakage of ATP. Three photosensitizers were used in this study: tetra(*N*-methyl-4-pyridyl)porphine tetratosylate salt (TMPyP), toluidine blue O (TBO), and methylene blue trihydrate (MB). A microdilution method was used to determine MICs of the photosensitizers against both *Escherichia coli* O157:H7 and *Listeria monocytogenes*. To evaluate the effects of photodestruction on *E. coli* and *L. monocytogenes* cells, a bioluminescence method for detection of ATP leakage and a colony-forming assay were used. All tested photosensitizers were effective for photodynamic destruction of both bacteria. The effectiveness of photosensitizers (in microgram-per-milliliter equivalents) decreased in the order TBO > MB > TMPyP for both organisms. The MICs were two- to fourfold higher for *E. coli* O157:H7 than for *L. monocytogenes*. The primary effects of all of the photosensitizers tested on live bacterial cells were a decrease in intracellular ATP and an increase in extracellular ATP, accompanied by elimination of viable cells from the sample. The time courses of photodestruction and intracellular ATP leakage were different for *E. coli* and *L. monocytogenes*. These results show that bioluminescent ATP-metry can be used for investigation of the first stages of bacterial photodestruction.

Photodynamic destruction of cells was first discovered in the early 20th century. Since then, there have been many attempts to use this phenomenon for targeted elimination of cancer cells. The method is based on the principle that nontoxic dyes (photosensitizers) activated by low-intensity visible light generate singlet oxygen and free radicals that are cytotoxic to most live cells. Because of the very short lifetime of both singlet oxygen and free radicals, only those molecules that are in close proximity to the cell surface trigger extensive cell damage, resulting in cell death. Mechanisms of cell damage have been investigated for different photosensitizers, and it was established that they range from disruption of the cell membrane to inactivation of enzymes and DNA damage, depending on the type of cell and the nature of the photosensitizer and its concentration. This approach was shown to be effective against a wide variety of mammalian cells and is currently used for treatment of certain types of cancer (photodynamic therapy) (14). Photosensitizers activated by red light are preferred because of the ability of red light to penetrate deeper into the tissue sample.

Antimicrobial photodynamic therapy research has increased in the last 20 years because of concerns resulting from the emergence of antibiotic-resistant bacterial strains (16). Bacterial cells, as well as fungi, yeasts, and viruses, treated with

photosensitizers were shown to be successfully killed by visible light (15). No resistance to photodynamic destruction has been reported to be acquired by bacteria (7). However, not all of the photosensitizers commonly used for cancer treatment are universally effective against bacteria (5, 15). Currently, the effectiveness of photosensitizers is determined by using the traditional plate count technique, which is very time-consuming and labor-intensive. Previously, it was shown that photodestruction of yeast cells resulted in leakage of intracellular contents, including ATP. The latter was detected by a bioluminescence method, and it was observed that the amount of ATP released depended on the light intensity used during photoinactivation, the time of illumination, and the concentration of the photosensitizer used (11). An *in vivo* method for monitoring of photodynamic destruction of bacteria in mice was proposed recently in which recombinant luminescent strains of bacteria were used. It was shown that there was a dose-dependent loss of luminescence upon illumination of treated cells with red light that was related to bacterial killing (3). However, application of this method is limited by the availability of bioluminescent strains of target bacteria.

The goal of the present study was to use two food-borne pathogens, *Escherichia coli* O157:H7 and *Listeria monocytogenes*, to establish a correlation between ATP leakage from photoinactivated cells and cell viability, as well as to determine whether this can be used as an indicator of cell death. The resulting information would help to determine the mechanisms involved at the early stages of bacterial cell photodestruction by different photosensitizers.

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FIG. 1. Absorption spectra of the photosensitizers used. 1, MB; 2, TBO; 3, TMPyP.

MATERIALS AND METHODS

Reagents and instrumentation. The photosensitizers used in this study were meso-tetra(*N*-methyl-4-pyridyl)porphine tetratosylate salt (TMPyP), toluidine blue O (TBO), and methylene blue trihydrate (MB) (all from the Sigma Chemical Co., St. Louis, Mo.). Physicochemical characteristics and absorption spectra of the photosensitizers used are presented in Table 1 and Fig. 1, respectively.

An array of light-emitting diodes (10 by 28 cm) was used for illumination. It produced red light (660 nm) with an intensity of 7.6 mW/cm² as measured by a model 1825-C power-energy meter (Newport Corp., Irvine, Calif.). During illumination, the light source was placed at a distance of 1.8 cm from the top of the sample plate.

Bioluminescence was measured on a Optocomp I luminometer (MGM Instruments Inc., Hamden, Conn.).

Bacterial cultures. *L. monocytogenes* Lm 353 (Canadian Research Institute for Food Safety culture collection) (gram positive) and *E. coli* O157:H7 ATCC 43889 (gram negative) were grown in brain heart infusion (BHI) broth (BD Diagnostic Systems, Sparks, Md.) at 30 and 37°C, respectively.

Determination of photosensitizer MICs. A microdilution method was used to determine the MICs of the photosensitizers used in this study (12). Twofold serial dilutions in 10 mM phosphate-buffered saline (PBS, pH 7.4) of all of the photosensitizers (100 μl) were made in two identical 96-well microplates (flat bottom; Nalge Nunc International, Rochester, N.Y.). The concentrations of the photosensitizers ranged from 0.049 to 100 μg/ml. An overnight culture of the test bacterium was centrifuged, washed twice with PBS, and diluted to achieve a cell density in the range 10⁵ to 10⁷ CFU/ml. The cell suspension (100 μl) was inoculated into each well of the two microplates to give a total volume in each well of 200 μl. Plates were incubated in the dark with gentle shaking for 60 min at room temperature to allow sorption of the photosensitizer on the cell surface. After incubation, one plate was exposed to the light source for 60 min while the second plate (the control) was kept in the dark. It should be noted that the killing step requires oxygen. To determine bacterial numbers in the absence of photosensitizers, the initial bacterial suspension in PBS was incubated under identical conditions and exposed to the same light source. After illumination, the cell suspension (100 μl) from each well of the microplates was transferred to another two microplates containing 100 μl of double-strength BHI broth. The use of nonselective media would aid the recovery of sublethally injured and stressed

cells. The microplates were incubated for 18 h at 37°C for *E. coli* and 24 to 30 h at 30°C for *L. monocytogenes*. Turbidity was measured with a Wallac 1420 Multilabel Counter (E.G. & G. Inc., Wellesley, Mass.) at a wavelength of 450 nm for cells with TBO and MB and at 630 nm for cells with TMPyP. The lowest concentration of each photosensitizer that prevented bacterial growth was considered to be the MIC of that photosensitizer.

Determination of bacterial counts. Viable bacterial counts were obtained for both experimental and control samples by plating serial dilutions of the samples on BHI agar plates, followed by aerobic incubation at 37°C for 18 h for *E. coli* and at 30°C for 48 h for *L. monocytogenes*.

Determination of intracellular ATP (ATP_{in}) and extracellular ATP (ATP_{out}). Dimethyl sulfoxide (DMSO; Sigma Chemical Co.) was used for extraction of total ATP as described by Romanova et al. (10). The cell suspension (50 μl) was added to 450 μl of DMSO. According to previous results, ATP_{in} is released from both gram-negative and gram-positive bacteria within 1 min by this treatment (90% DMSO) and is stable in this solution for several hours when stored at room temperature. For ATP detection, an internal standard method and bioluminescent ATP reagent, Immolum (Moscow State University, Moscow, Russia), were used (2). This reagent is based on immobilized *Luciola mingei* firefly luciferase-luciferin with enhanced thermostability and is less prone to inhibition by sample components than the soluble enzyme. Standard ATP solutions were prepared fresh daily from ATP disodium salt (Sigma Chemical Co.) in sterile PBS.

Reconstituted ATP reagent (200 μl) was placed into a luminometer cuvette, and the background bioluminescent signal was measured. The ATP extract (50 μl) was added to the same cuvette, and luminescence was measured. The intensity of the sample (*I*_{sm}) was calculated by subtracting the background intensity. A standard solution of ATP (10 μl) was added to the same cuvette, with continued luminescence intensity monitoring. The standard signal (*I*_{st}) was calculated from the difference in luminescence intensity measured after and before addition of the standard solution. The total concentration of ATP in the sample was calculated from the following equation with a sample dilution factor (*N*) of 1.9:

$$[\text{ATP}] = N \times (I_{\text{sm}}/I_{\text{st}}) \times [\text{ATP}_{\text{st}}] \quad (1)$$

where *I*_{sm} is the luminescence intensity of the sample measured in relative light units (RLU), *I*_{st} is the luminescence intensity of the standard (RLU), and [ATP_{st}] is the ATP concentration in the standard solution (10 to 100 nM).

The concentration of extracellular ATP (ATP_{out}) was measured immediately after illumination by adding 50 μl of the cell suspension to 200 μl of ATP reagent and monitoring ATP levels as described above. The ATP concentration was calculated by using equation 1 but substituting a value of 0.2 for the dilution factor, *N*. The ATP_{in} concentration was determined as the difference between the total ATP and ATP_{out} concentrations.

Statistical analysis. All measurements were performed in duplicate, and all experiments were performed twice. The standard deviation of the mean was calculated from the combined measurements.

RESULTS

MICs of MB, TBO, and TMPyP for *E. coli* O157:H7 and *L. monocytogenes*. The bactericidal effect of illumination on the bacterial cells treated with the tested photosensitizers was investigated. Dark incubation of the samples containing TMPyP, MB, and TBO affected the viability of both *L. monocytogenes* and *E. coli*. They caused a two- to fourfold reduction in cell counts during dark incubation for each bacterium (Table 2). Illumination of the samples treated with photosensitizers resulted in at least a 5- to 6-log-cycle reduction in viable bacterial counts. Illumination of the samples with no photosensitizer did not change viable bacterial counts.

The MICs of all of the photosensitizers tested against *E. coli* O157:H7 and *L. monocytogenes* are presented in Table 3. The susceptibility of *L. monocytogenes* to all of these photosensitizers was higher than that of *E. coli* O157:H7. The MICs ranged from 0.195 to 1.56 μg/ml (0.6 to 2.1 μM) for *Listeria* and from 0.39 to 6.25 μg/ml (1.3 to 4.6 μM) for the *E. coli* strain. The effectiveness of photosensitizers (in microgram-

TABLE 1. Physicochemical properties of the photosensitizers used in this study

Photosensitizer	Mol wt	ϵ_{660} ^a (M ⁻¹ cm ⁻¹)	ϕ ^b (pH 7.0)	Reference
MB	373.9	73,513	0.6	13
TBO	305.8	18,974	0.6	8
TMPyP	1,363.6	2,000	0.78	6

^a ϵ_{660} , extinction coefficient at 660 nm.

^b ϕ , quantum yield of singlet oxygen production.

TABLE 2. Viable bacterial counts after treatment with photosensitizers, followed by illumination^a

Photosensitizer and concn (μg/ml)	<i>E. coli</i> O157 H7 (CFU/ml)		<i>L. monocytogenes</i> (CFU/ml)	
	No light	hν ^b	No light	hν
TMPyP				
0	$(4.0 \pm 0.3) \times 10^6$	$(4.0 \pm 0.2) \times 10^6$	$(1.0 \pm 0.03) \times 10^7$	$(1.0 \pm 0.1) \times 10^7$
6.25	$(1.4 \pm 0.2) \times 10^6$	<20	$(3.7 \pm 1.6) \times 10^6$	<20
MB				
0	$(3.4 \pm 0.2) \times 10^6$	$(3.5 \pm 0.3) \times 10^6$	$(3.6 \pm 0.2) \times 10^6$	$(3.6 \pm 0.1) \times 10^6$
3.125	$(8.4 \pm 1.2) \times 10^5$	<20	$(1.2 \pm 0.2) \times 10^6$	<20
TBO				
0	$(3.5 \pm 0.2) \times 10^6$	$(3.5 \pm 0.1) \times 10^6$	$(3.6 \pm 0.2) \times 10^6$	$(3.6 \pm 0.1) \times 10^6$
3.125	$(7.5 \pm 0.8) \times 10^5$	<20	$(1.6 \pm 0.1) \times 10^6$	<20

^a Time of illumination, 60 min; λ = 660 nm.^b hν, after illumination.

per-milliliter equivalents) decreased in the order TBO > MB > TMPyP for both organisms.

Influence of illumination on ATP_{in} and ATP_{out} contents in *E. coli* O157:H7 treated with photosensitizers. The influence of the time of illumination on ATP_{out} (ATP_{out}) and ATP_{in} contents was investigated for each photosensitizer to assess the time courses of cell damage and cell death. *E. coli* cells (10^8 to 10^9 CFU/ml) were incubated in the presence of photosensitizers at concentrations equal to their MICs and at concentrations that were four times the MICs, and both ATP_{out} and ATP_{in} levels were measured during the illumination period. At the same time, the bacterial counts (CFU per milliliter) of all of the samples during the illumination period were determined.

Results of *E. coli* illumination in the presence of the MIC of MB (1.56 μg/ml) are presented in Fig. 2a. ATP_{in} decreased during illumination and reached an undetectable level (<0.01 nM) after 60 min of illumination. ATP_{out} increased slightly with time, and after 60 min it reached 10% of the initial ATP_{in} content. Viable cell counts gradually decreased until full measurable cell destruction (corresponding to cell counts of <20 CFU/ml) occurred after 60 min of illumination. In the presence of MB at four times the MIC (6.25 μg/ml), elimination of viable cells and ATP_{in} occurred after 30 min of illumination (Fig. 2b). At that time, the ATP_{out} concentration reached 30% of the initial ATP level. Similar results were obtained with TBO (Fig. 2c and d) and TMPyP (Fig. 3b and c). Thus, the efficiency and rate of photodestruction of *E. coli* O157:H7 cells increased with the photosensitizer concentration used. These data indicated that, for *E. coli*, a dramatic decrease in ATP_{in} content was accompanied by cell death.

To assess the effect of a sublethal photosensitizer concentration on the photodestruction of bacteria, three different

concentrations of TMPyP were used: 0.5 times the MIC, the MIC, and 4 times the MIC. At 0.5 times the MIC, a rapid decrease in ATP_{in} levels occurred during the first 30 min of illumination, and thereafter it stayed at approximately 20% of the initial level (Fig. 3a). The ATP_{out} was very low (less than 6% of initial ATP content) and did not change with time. There was a 7-log-cycle reduction in viable cells in the sample during illumination. At higher concentrations of TMPyP (the MIC and four times the MIC), ATP_{in} and viable cell numbers reached undetectable levels after 60 and 30 min of illumination, respectively. The faster the ATP_{in} concentration decreased, the more ATP_{out} was detected, and the latter reached 30 and 50% of the initial ATP_{in} level in 60 min at photosensitizer concentrations equal to the MIC and four times the MIC, respectively.

TABLE 3. MICs of the photosensitizers used in this study^a

Bacterial strain	MIC, μg/ml (μM)		
	TMPyP	MB	TBO
<i>Listeria monocytogenes</i> Lm 353	1.56 (1.1)	0.78 (2.1)	0.195 (0.6)
<i>Escherichia coli</i> O157 H7 43889	6.25 (4.6)	1.56 (4.2)	0.39 (1.3)

^a Time of illumination, 60 min; λ = 660 nm.

FIG. 2. Time course of the number viable cells (CFU per milliliter, ▲) and concentrations of ATP_{out} (open bars) and ATP_{in} (filled bars) during illumination of *E. coli* O157:H7 ATCC 43889. Concentrations of MB: a, 1.56 μg/ml; b, 6.25 μg/ml. Concentrations of TBO: c, 0.39 μg/ml; d, 1.56 μg/ml.

FIG. 3. Time course of the number viable cells (CFU per milliliter, ▲) and concentrations of ATP_{out} (open bars) and ATP_{in} (filled bars) during illumination of *E. coli* O157:H7 ATCC 43889. Concentrations of TMPyP: a, 6.25 µg/ml; b, 12.5 µg/ml; c, 50 µg/ml.

Influence of illumination on ATP_{in} and ATP_{out} contents in *L. monocytogenes* treated with photosensitizers. Similar experiments were carried out with *L. monocytogenes*. On illumination of *L. monocytogenes* cells in the presence of MB at a concentration equal to its MIC (0.78 µg/ml), the ATP_{in} decreased during 60 min of illumination from 31 to 0.6 nM and the ATP_{out} increased to levels reaching 90% of the initial ATP_{in} values (Fig. 4a). The number of viable cells decreased rapidly, and after 30 min of illumination, full measurable cell destruction was observed. In the presence of MB at four times the MIC (3.12 µg/ml), numbers of viable *L. monocytogenes* cells were reduced to undetectable levels after 10 min of illumination (Fig. 4b). At the same time, the ATP_{in} content decreased to undetectable levels after 30 min of illumination and the ATP_{out} reached levels that were 25% of the initial ATP_{in}. In the presence of TBO at the MIC (0.195 µg/ml), the ATP_{in} concentration changed only slightly during the first 30 min of illumination and dropped to 40% of the initial value during the

next 30 min of illumination. No significant leakage of ATP from the cells was observed. The cell numbers gradually decreased until full measurable cell destruction was observed after 60 min of illumination (Fig. 4c). In the presence of TBO at four times the MIC, the number of viable cells of *L. monocytogenes* decreased to undetectable levels after 10 min of illumination (Fig. 4d). ATP_{out} concentrations increased within 60 min of illumination and reached 68% of the initial ATP_{in} content. At the same time, the ATP_{in} content decreased to undetectable levels within 60 min.

Three different concentrations of TMPyP were used for photodestruction of *L. monocytogenes* cells: 0.5 times the MIC, the MIC, and 4 times the MIC. In the presence of TMPyP at concentrations less than its MIC (0.78 µg/ml), the content of ATP_{in} more than doubled during 60 min of illumination (Fig. 5). At the same time, no significant cellular leakage of ATP was observed. However, there was a 4-log-cycle reduction in the viable cell count within 60 min of illumination (Fig. 5a). This probably indicated that only a portion of the live bacterial cells was destroyed and the surviving cells continued to grow. Cell contents released into the medium during cell destruction could serve as a nutrient source for growing cells. It is well known that the ATP_{in} content of actively growing cells in nutrient medium is significantly higher than for cells suspended in buffer solution and for cells in the stationary or lag phase of growth (2). Thus, even if the cell number is decreasing, the total ATP content could increase.

In the presence of TMPyP at the MIC, a slight decrease in ATP_{in} content (20 to 30%) and increasing leakage of ATP during 60 min of illumination were observed. The quantity of viable cells decreased by 1 log cycle during the first 30 min, and there was full measurable destruction of cells after an additional 30 min of illumination (Fig. 5b). Treatment of *L. monocytogenes* with TMPyP at four times the MIC led to destruction of cells after only 10 min of illumination (Fig. 5c). The concentration of ATP_{in} decreased fully after 60 min, and at the same time, ATP_{out} levels increased with the time of illumination, eventually reaching 45% of the initial ATP_{in} level.

Unlike that of *E. coli*, for *Listeria* cells, the time required for a maximal reduction in viable cell counts did not coincide with the destruction of ATP_{in}. For all of the photosensitizers used, the ATP_{in} in *Listeria* cells reached undetectable levels approximately 30 min later than the time required to reduce cell numbers to undetectable levels by photokilling.

FIG. 4. Time course of the number viable cells (CFU per milliliter, ▲) and concentrations of ATP_{out} (open bars) and ATP_{in} (filled bars) during illumination of *L. monocytogenes*. Concentrations of MB: a, 0.78 µg/ml; b, 3.12 µg/ml. Concentrations of TBO: c, 0.195 µg/ml; d, 0.79 µg/ml.

FIG. 5. Time course of the number viable cells (CFU per milliliter, ▲) and concentrations of ATP_{out} (open bars) and ATP_{in} (filled bars) during illumination of *L. monocytogenes*. Concentrations TMPyP: a, 0.78 µg/ml; b, 1.56 µg/ml; c, 6.5 µg/ml.

DISCUSSION

Analysis of our experimental results demonstrated that all of the photosensitizers tested are effective for photodynamic destruction of both *E. coli* O157:H7 and *L. monocytogenes*. MICs were two- to fourfold higher for the gram-negative bacterium *E. coli* O157:H7 than for the gram-positive bacterium *L. monocytogenes*, which is consistent with previous observations (9). Within each group, the differences in the MIC (molar equivalents) are not very large. To compare the potential efficiency of the photosensitizers used, the relative production of singlet oxygen during illumination with red light was estimated, taking into consideration the MIC for each microorganism, the molar extinction coefficient for each photosensitizer at 660 nm, and the quantum yield of singlet oxygen production at pH 7.0 (Table 1). The calculated relative production of singlet oxygen by photosensitizers at their MICs for *L. monocytogenes* increased at a ratio of 1:4:54 for TMPyP, TBO, and MB, respectively. At the MIC for *E. coli* O157:H7, the calculated relative production of singlet oxygen is 1:2:25 for TMPyP, TBO, and MB, respectively. Such a large difference between photosensitizers is contradictory to our findings, which showed that all of the photosensitizers used were similar in efficacy. This discrepancy may be due to the very short lifetime of singlet oxygen, which results in only molecules bound to the bacterial cell wall being effective for photodestruction of cells. If the photosensitizers have different affinities to cell wall components, the overall efficiency of photodestruction will be different, even if the yield of free radicals and singlet oxygen is similar. Also, a difference in the production of singlet oxygen could be compensated for by a different localization of the photosensitizer. In our case, the affinity of the porphyrin-based photosensitizer to cell walls may be significantly higher than for thiazine dyes (TBO and MB), thus causing greater photodamage than expected. It was shown previously that conjugation of certain porphyrins with polylysine, a compound that has a high affinity for bacterial cell walls, increased the photodestruction of *Prevotella intermedia*, *Fusobacterium nucleatum*, *Peptostreptococcus micros*, and *Actinobacillus actinomycetemcomitans* (4). Taking into consideration that light absorption by TMPyP is very low at the wavelength used (660 nm), use of a light source with shorter-wavelength emission corresponding to absorption peaks of the dye (420, 520, and 585 nm), or use of nonfiltered white light, may increase its phototoxic action severalfold.

The primary effects of all of the photosensitizers tested on live bacterial cells are a decrease in ATP_{in} and an increase in ATP_{out}, accompanied by elimination of viable cells from the

sample. This indicates extensive damage to the cell wall, resulting in leakage of intracellular contents and cell death. A similar effect was observed during the photodestruction of *Porphyromonas gingivalis* in the presence of TBO (1). The higher the photosensitizer concentration, the faster and more extensive is the damage to the cell, and this is supported by the observed increases in ATP_{out} levels accumulating during photodestruction. At lower photosensitizer concentrations, disintegration of the cell wall leads to inactivation of ATP synthesis, but the enzymes involved in ATP hydrolysis remain active. This explains the decrease of total ATP content in the sample. On the other hand, at higher photosensitizer concentrations, both pathways for ATP synthesis and ATP hydrolysis are destroyed simultaneously. As a result, the concentration of ATP_{out} increases with time and reaches levels of up to 90% of the ATP_{in} content within 60 min of illumination.

Differences in the time courses of photodestruction and ATP_{in} leakage between *E. coli* and *L. monocytogenes* could be due to differences in cell wall structure. For the gram-negative bacterium *E. coli*, the cell envelope consists of a cytoplasmic membrane, a thin layer of peptidoglycan (2 to 3 nm), and an outer membrane. Gram-positive bacteria typically have a two-layer cell envelope composed of a cytoplasmic membrane together with a multilayered peptidoglycan cell wall (20 to 80 nm). Taking into consideration that the distance that the free radicals generated from the photosensitizer can travel is very small because of its short lifetime, only molecules close to the photosensitizer would be affected. For gram-negative bacteria, despite the fact that it takes more photosensitizer to destroy the outer layer of the thin cell envelope, it is sufficient to facilitate fast leakage of the intracellular contents, followed by cell death. On the other hand, for gram-positive bacteria, although a lower photosensitizer concentration is needed to destroy vital structures on the cell envelope and kill the cell, leakage of intracellular contents proceeds much more slowly because of the thickness of the cell wall. Thus, it is possible to detect ATP_{in} in *Listeria* samples in which no viable cells are present.

The exact mechanism of photodestruction of bacterial cells is still unknown. From the data obtained, it appears that the primary stage involves disintegration of the cell wall and leakage of intracellular material, resulting in cell death. This effect, in general, is independent of the cell type (i.e., gram positive or gram negative) and the structure of the photosensitizer used (i.e., thiazine based or porphyrin based). However, the photosensitizer concentration that is effective for photodestruction

strongly depends on both the photochemical characteristics of the dye and its ability to bind the bacterial cell wall.

Bioluminescent ATP-metry was shown to be a very useful approach by which to investigate cell photodestruction. It allowed the first stages of the process to be monitored in real time to facilitate photosensitizer testing and process optimization. The rate of change of both ATP_{in} and ATP_{out} concentrations during illumination provided additional information on the mechanisms involved in the photodestruction of bacterial cells.

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