Preliminary Assessment of Photoactivated Antimicrobial Collagen on Bioburden in a Murine Pressure Ulcer Model

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Abstract

Objective and background data: Overcoming bacterial antibiotic resistance requires alternative strategies. The ability of photoactivated collagen-embedded flavins (PCF) to reduce bioburden in infected pressure ulcers was investigated. **Design and methods:** Two pressure ulcers were created on the dorsum of female BALB/C mice ($n=40$, 35 g) maintaining a 5 mm skin bridge between lesions. Ulcers and surrounding skin were covered with Tegaderm™ and inoculated with 0.1 mL of 1×10^5 colony-forming units (CFU)/mL methicillin-resistant *Staphylococcus aureus* (MRSA). Fluid was permitted to reabsorb for 10 min. In experiment 1, one wound from each animal was treated using PCF and photoradiation (PCF+R, $n=12$) or photoradiation alone (R, $n=11$). Composite dressing-treated wounds received 1×1 cm PCF discs. Overlying Tegaderm was excised, and PCF was placed over the wound and again covered with Tegaderm. Wounds were irradiated at 455 ± 5 nm (350 mW, 1 cm spot diameter, 15 min) using a diode laser 10 min after placement. Controls received no PCF or photoradiation (C, $n=12$). Animals were euthanized 24 h post-therapy. Quantitative bacterial counts (CFU/g tissue) were determined. In experiment 2, composite dressing-treated wounds were irradiated at 455 ± 5 nm (350 mW, 1 cm spot diameter, 15 min) using a diode laser 10 min after daily PCF placement (0, 1, 2, or 3 treatments, $n=8$ /group). Controls received no treatment. Wounds were cultured daily. Animals were euthanized on day 7 post-infection. Quantitative bacterial counts were determined. **Results:** PCF+R significantly reduced bacterial counts at 24 h (experiment 1, $p < 0.0001$; experiment 2, $p < 0.05$). The bacterial counts in rats receiving photoradiation alone were no different from those of untreated controls (experiment 1, $p = 0.24$). PCF+R produced a 2–3 log reduction in bacterial counts (experiment 2, $p < 0.001$). Antibacterial effects increased with number of treatments, and persisted for several days post-therapy ($p < 0.002$). **Conclusions:** PCF+R inhibited bacterial growth in this model. This effect increased with successive treatments, persisting several days post-therapy. Further studies to optimize this treatment modality are warranted.

Introduction

MILLIONS OF SURGICAL, TRAUMATIC AND IATROGENIC wounds occur on an annual basis. Each requires appropriate management to facilitate healing, reduce the potential for infection, and minimize disability and scarring. Wound infection and related complications increase the cost of care significantly, prolong hospital stays, and result in increased morbidity and mortality.^{1–5} At the same time, bacterial resistance to antibiotics is increasing, with community acquired methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence approaching 50% in several communities, based on wound culture data.⁶

Antibiotic resistance has become a global problem, with some strains of bacteria that are nearly untreatable.^{6–8} Emerging patterns of resistance have stimulated interest in the search for new strategies.

Riboflavin (vitamin B2) and its byproducts are redox-active chromophores found in enzymes and photoreceptors.⁹ These compounds are photoreactive and photodecompose with exposure to UV and visible light. They have the potential for use as high efficacy antimicrobial agents with low toxicity and low probability of inducing protein damage. The chemical mechanism for the rapid photodecomposition of riboflavin and how to protect it has been extensively studied.^{10–12} Riboflavin is

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capable of interacting with and damaging nucleic acids of pathogens after UVA exposure. Damage may occur by direct electron transfer, production of electrophilic singlet oxygen, or generation of hydrogen peroxide with formation of hydroxyl radicals.¹² Both pathogen DNA and RNA are affected in the absence of oxygen. This strategy has been proven in pre-clinical investigations to be effective against bacteria, intracellular HIV-1, West Nile virus (WNV), and porcine parvovirus.^{11,12}

The therapeutic efficacy of using riboflavin solutions and UVA light at 365 nm and 3 mW/cm² for ulcerative keratitis has also been investigated in multidrug-resistant clinical isolates.¹³ The combination of riboflavin and UVA was effective against oxacillin-resistant *Staphylococcus epidermidis* (ORSE), penicillin-resistant *Streptococcus pneumoniae* (PRSP), pan-resistant *Pseudomonas aeruginosa* (PRPA), oxacillin-susceptible *S. epidermidis* (SE), MRSA, oxacillin-susceptible *S. aureus* (SA), and susceptible *P. aeruginosa* (PA).

Strategies that would improve the ability to deliver riboflavin and photoactivate it in a broad range of tissues and clinical scenarios were sought. The absorbance spectra of the flavin chromophores riboflavin and lumiflavin in water demonstrate a peak at 450–455 nm.¹⁴ Other investigators have demonstrated that blue light photoradiation is capable of inhibiting bacterial growth *in vitro*.^{15–18} Previous work with derivatized collagen solder formulations demonstrated that high concentration preparations could be cast into films for subsequent sectioning into strips for application to the wound site.^{19–23} Collagen is biocompatible and bioabsorbable, and can be used as a carrier for biologically relevant molecules and chromophores.

Preliminary studies were undertaken using this patented collagen technology impregnated with flavin chromophores to optimize the application of a precise dose and controlled release of the antimicrobial agent.^{24,25} *In vitro* studies were undertaken to determine whether visible light activation of collagen-embedded and non-embedded flavin mixture exhibited bactericidal activity. Strains of MRSA, SA, *Escherichia coli*, and PA were cultured using standard Mueller Hinton and Poloxamer biofilm culture models. Photoirradiation of collagen-embedded and non-embedded flavin mixtures at 455 nm was bactericidal on these strains of bacteria in both standard culture and biofilm models.²⁴

Pilot studies using an acute infection model were conducted to investigate the potential for wound decontamination.²⁵ Treated animals received 1×1 cm collagen-flavin composite wafer implants after having incisional wounds infected with MRSA, SA, *E. coli*, or PA. The wounds were irradiated at 455 nm (300–500 mW output, 1 cm spot diameter) for 15 min using a diode laser 10 min after implant insertion. This pilot study demonstrated that photoradiation at 455 nm after placement of collagen-embedded flavin mixture inhibited bacterial growth in an acutely infected wound model.

The present studies were undertaken to verify the observations from the pilot *in vitro* and *in vivo* studies as well as to determine whether multiple treatments and treatment of chronic wounds were feasible and effective using this treatment paradigm.

Materials and Methods

Flavin-collagen composite film preparation

Collagen films were prepared using Conversion Energy Enterprise's patented methods.²⁶ Purified, telopeptide-poor

type I collagen was modified using an acylating reagent such as glutaric anhydride. The degree of derivatization is selected so that the modified collagen remains soluble at physiologic pH. Following chemical modification, the collagen solution was lyophilized. Films were prepared by dissolving collagen solids in flavin solutions. The collagen concentration ranged from 30% to 50%, and flavin concentration varied between 0.5% and 1%. The resultant viscous solution was centrifuged at elevated temperature and poured into molds. After molding, thin films were extracted from the mold, weighed, and cut to shape and size. The composite films (photoactivated collagen-embedded flavins [PCF]) were vacuum packaged in opaque packaging, terminally sterilized by standard electron beam methods, and stored at 4°C until use.

The experimental protocols described subsequently were conducted in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Rochester General Hospital.

Infected pressure ulcer model creation

Pressure ulcers were created on the dorsum of 40 BALB/C mice weighing ~35 g each (Charles River, Wilmington, MA), according to the technique developed in our laboratory.²⁷ Briefly, animals were lightly anesthetized using a 50:50 mixture of CO₂ and O₂ for ~50–60 sec. The dorsal hair was shaved and the area was cleansed with 70% isopropanol after depilation with Nair™. A template was used to mark the location of the magnetic plates to assure a consistent placement on each animal. The skin was gently pulled up and placed between two round ceramic magnetic plates that were 12 mm in diameter and 5.0 mm thick, with an average weight of 2.4 g and 1000 Gauss magnetic force (Magnetic Source, Castle Rock, CO). The resultant "pinch" procedure left a 5.0 mm skin bridge between the two magnets. The underlying carnosus muscle was also captured between the magnets, and sustained ischemia-reperfusion injury. This process created 50 mm Hg pressure between the two plates. A single ischemia-reperfusion cycle (I/R) consisted of a period of magnet placement for 12 h followed by a release or rest period of 12 h. Three I/R cycles were used in each animal to initiate decubitus ulcer formation. Animals were not immobilized, anesthetized, or otherwise treated during the I/R cycles. The animals were allowed food and water *ad libitum*. The animals resumed normal activity within a few minutes of magnet placement and were able to tolerate the additional weight of the magnets without any difficulty. Ulcers, which are typically fully formed at 3 days, were generally 11 mm in diameter. (Fig. 1). The animals were anesthetized with IP xylazine and pentobarbital prior to inducing infection and/or treatments as described subsequently.

The ulcers and surrounding skin were covered with Tegaderm™ (3M Healthcare, St. Paul, MN) and then inoculated with 0.1 mL of a solution containing 1×10⁵ colony-forming units (CFU) per milliliter of MRSA (ATCC, Manassas, VA) in 0.9% saline using a syringe and 25 gauge needle (Figs. 2 and 3). The inoculated fluid was permitted to reabsorb for a minimum of 10 min.

FIG. 1. Formed pressure ulcer. The appearance of the formed pressure ulcer after three ischemia/ reperfusion cycles is shown.

Single treatment experiment 1

One wound from each animal was treated using the collagen composite films and photoradiated using a blue light source (PCF+R, $n=12$) or photoradiation alone (R, $n=11$). Composite dressing-treated wounds received 1×1 cm dressings, which were applied by excising the Tegaderm overlying the ulcer, placing the composite over the wound, and again covering the wound with Tegaderm (Fig. 4). The wounds were irradiated at 455 ± 5 nm (350 mW output, 1 cm spot diameter) for 15 min using a diode laser (Conversion Energy Enterprises, Spring Valley, NY) 10 min after dressing placement. The animals were recovered and individually caged. Control group wounds (C, $n=11$) were handled similarly, but did not receive the implant or photoradiation. Animals were euthanized at 24 h post-therapy with xylazine and pentobarbital overdose. Cultures were obtained using a sterile cotton tip applicator to sample the wound exudate. Quantitative wound bacterial counts (CFU/g tissue) were determined to measure bactericidal efficiency. One wound from each animal served as a control.

Multiple treatment experiment 2

A daily treatment regimen was investigated based on the basic infected pressure ulcer model used in experiment 1. Wounds were treated using PCF and photoradiated (PCF+R) or were untreated (C). Composite dressing-treated wounds received 1×1 cm PCF films, applied by excising Tegaderm overlying the ulcer, placing PCF over the wound, and covering the wound with Tegaderm. Wounds were irradiated at 455 ± 5 nm (350 mW, 1 cm spot diameter, 15 min) using a diode laser (Conversion Energy Enterprises, Spring Valley, NY) 10 min after dressing placement daily (PCF+R). The PCF+R wounds received 0, 1, 2, or 3 treatments at 24 h intervals ($n=8$ /group). Control wounds received neither PCF nor photoradiation (C). Wounds were cultured daily for 7 days. Cultures were obtained using a sterile cotton tip applicator to sample the wound exudate immediately prior to redressing and treating the wound as per the treatment group parameters described (Fig. 5). Animals were euthanized on day 7 by xylazine and pentobarbital overdose and quantitative bacterial counts (CFU/g tissue) were determined.

Quantitative culture technique

Quantitative wound bacterial counts (CFU/g tissue) were determined to measure bactericidal efficiency. The wound area was measured and the exudate was collected by sterile cotton swab (cotton tip applicator [CTA]) after the Tegaderm was carefully removed (Fig. 5). The CTA was submerged in 2.0 mL of physiological saline or trypticase soy broth and repeatedly compressed to release the absorbed wound exudate fluid from the swab. Serial dilutions of the resultant bacterial suspension were constructed from 1:1 to $1:10^6$ for each sample, and $10 \mu\text{L}$ was inoculated and spread onto Columbia -CNA agar plates containing 5% sheep blood (Fisher Scientific, Pittsburgh, PA) and incubated at 37°C for 24 h. Plates for the serial dilutions were counted if they

FIG. 2. Wound infection and treatment technique. (A) Formed pressure ulcers are covered with a Tegaderm™ dressing. (B) The ulcer is inoculated with 0.1 mL of a solution containing 1×10^5 colony-forming units (CFU)/mL of methicillin-resistant *Staphylococcus aureus* (MRSA) and the fluid is allowed to absorb for 10 min. (C) The Tegaderm overlying the ulcer is sharply excised to permit wound culture and treatment. (D) Appearance of wounds after placement of photoactivated collagen riboflavin dressing (foreground) or untreated control wound (background).

FIG. 3. Appearance of wounds after infection. **(A)** Appearance of an untreated wound 24 h after inoculation with methicillin-resistant *Staphylococcus aureus* (MRSA). Note the presence of wound exudate. **(B)** Appearance of a photoactivated collagen riboflavin (PCRB) treated wound 24 h after inoculation with MRSA and photoradiation. Note that the wound exudate is diminished as compared with the untreated control **(A)**.

contained between 30 and 300 colonies. The final number of CFU, expressed as CFU/cm² of the wound area, was calculated.

Table 1 provides a summary of the experimental design for both the single and multiple treatment studies. Statistical analysis of the results was determined using ANOVA and *t* test for two samples with unequal variance.

Results

The results for the single treatment regime after wound infection with MRSA are demonstrated in Fig. 6. The composite dressing plus photoradiation (PCF+R) resulted in a statistically significant reduction in bacterial counts at 24 h post-treatment as compared with untreated controls (C) ($p < 0.0001$). However photoradiation alone (R) was no different from untreated controls ($p = 0.24$). These results confirm those obtained in the pilot study using the incisional wound model.²⁵ A single PCF+R treatment resulted in a 2 log reduction in bacterial counts at 24 h post-therapy.

The results for the multiple (daily) treatment study are shown in Figs. 7, 8, and 9. PCF+R resulted in statistically

significant 2–3 log reduction in bacterial counts as compared with untreated controls (C) ($p < 0.001$). As expected, the antibacterial effects increased with the number of treatments administered. This effect was most pronounced in the group receiving three daily treatments. The results at 72 h are shown in Figs. 7 and 8. The antimicrobial effects of PCF+R therapy persisted for several days post-therapy ($p < 0.05$) as demonstrated by quantitative wound culture performed at 168 h (day 7) as is shown in Fig. 8. Figure 9 plots the rate of reduction of bacteria at day 7 versus the number of treatments given.

Discussion

The increasing incidence of antibiotic resistance threatens our ability to successfully manage acute and chronic wounds. Nosocomial infections are a global problem that will require innovative and cost-effective solutions. The spectrum of pathogens that are responsible for nosocomial infections continues to change over time.^{28,29} Antibiotic-resistant strains of both gram-positive and gram-negative microorganisms are being increasingly isolated from

FIG. 4. Wound with photoactivated collagen riboflavin (PCRB) dressing placement. The appearance of a pressure ulcer wound is shown after the PCRB has been replaced and the lesion has been again covered with Tegaderm™.

FIG. 5. Wound culture method. Quantitative wound cultures are performed by collecting wound exudate with a sterile cotton tip applicator after the overlying Tegaderm™ has been removed.

TABLE 1. EXPERIMENTAL DESIGN FOR SINGLE AND MULTIPLE TREATMENT STUDIES

Experiment	Group	n	Laser treatment parameters	PCF	MRSA inoculate (day 1)	Laser treatment delivered			Quantitative wound culture timing
						Day 1	Day 2	Day3	
1	Control (C)	12	0	No	0.1 ml 1×10^5 CFU/mL	-			24 h post-treatment
	Photoradiation (R)	11	455 $\pm$ 5 nm, 350 mW/cm ² , 1 cm spot diameter, 15 min	No	0.1 ml 1×10^5 CFU/mL	X			24 h post-treatment
	PCF+R	12	455 $\pm$ 5 nm, 350 mW/cm ² , 1 cm spot diameter, 15 min	Yes	0.1 ml 1×10^5 CFU/mL	X			24 h post-treatment
2	Control (C)	8	0	No	0.1 ml 1×10^5 CFU/mL	-	-	-	24, 48, 72, 96, 120, 144, 168 h post-treatment
	PCF+R 1	8	455 $\pm$ 5 nm, 350 mW/cm ² , 1 cm spot diameter, 15 min	Yes	0.1 ml 1×10^5 CFU/mL	X	-	-	24, 48, 72, 96, 120, 144, 168 h post-treatment
	PCF+R 2	8	455 $\pm$ 5 nm, 350 mW/cm ² , 1 cm spot diameter, 15 min	Yes	0.1 ml 1×10^5 CFU/mL	X	X	-	24, 48, 72, 96, 120, 144, 168 h post-treatment
	PCF+R 3	8	455 $\pm$ 5 nm, 350 mW/cm ² , 1 cm spot diameter, 15 min	Yes	0.1 ml 1×10^5 CFU/mL	X	X	X	24, 48, 72, 96, 120, 144, 168 h post-treatment

PCF, photoactivated flavin collagen composite film; MRSA, methicillin-resistant *Staphylococcus aureus*; CFU, colony-forming units.

infections in hospitalized and postoperative patients as well as from the environment.³⁰

Emerging evidence indicates that microbial populations colonize wounds and tissues in biofilms. These thin layers of microorganisms adhere to the surface of an organic or inorganic structure, together with their secreted polymers. They are the predominant phenotype of nearly all bacteria in their natural habitat, whether pathogenic or environmental. Current estimates suggest that >65–80% of infections are caused by bacteria growing in biofilms.^{31–33} Biofilms have demonstrated the ability to persist in concentrations of antibiotics and biocides that typically inhibit and completely destroy planktonic cells.³¹ The same strain of bacteria can be two- or threefold more resistant to antibiotics if part of the biofilm community.^{32,33}

Pressure ulcers represent a chronic wound with an eschar that presents a nidus for bacterial proliferation and rapid formation of a biofilm. Chronic infections are fundamentally different than acute infections, and different interventional approaches are necessary to treat these biofilm infections more efficiently. We have shown that photoactivated flavin-impregnated collagen films are capable of significantly reducing bacterial contamination and that this effect increases with successive treatments. The experiments reported herein are the first to our knowledge to utilize the murine pressure ulcer model as a platform to investigate infected or contaminated pressure ulcers in a small animal model. We have demonstrated that these wounds can be reproducibly formed, infected, be treated, and nondestructively monitored over time. Future studies should be undertaken to expand

FIG. 6. Quantitative culture results at 24 h after single treatment. Use of photoactivated collagen-embedded flavins (PCF) plus photoradiation (PCF+R) resulted in a statistically significant reduction in bacterial counts at 24 h as compared with untreated controls ($*p < 0.0001$). Counts for wounds treated with photoradiation alone (R) were no different from those of untreated controls (C) at 24 h ($p = 0.24$).

FIG. 7. Quantitative wound culture results days 1–3 after multiple treatments. Quantitative wound culture results are shown for the first 72 h after wound infection and treatment. Treatment with photoactivated collagen riboflavin (PCRB) plus photoradiation resulted in statistically significant 2–3 log reduction in bacterial counts ($p < 0.001$). Antibacterial effects increased with the number of treatments ($p < 0.002$).

these initial observations and to determine the optimal frequency of treatments that would be required to result in reduction of bacterial counts to zero or near zero.

It is believed that the antimicrobial properties of light-exposed riboflavin and its flavin byproducts occur as a result of the release of oxygen free radicals in combination with the generation of nucleotides that preferentially interrupt the RNA or DNA of pathogens. Development of resistance is unlikely, given the mechanism of action of these compounds. Such a strategy could potentially be effective against a broad spectrum of pathogens including bacteria and viruses.

Both ultraviolet and blue light wavelengths have been demonstrated to have antimicrobial activity,^{11–13, 15–18} However, the applicability of these wavelengths to clinical use is limited in effectiveness because of lack of penetration and a distance dependency from the light source result in nonhomogeneous microbial inactivation. The PCF strategy increases the concentrations of reactive oxygen species (ROS) at or near the target tissue, which are available for diffusion to deeper layers adjacent to the primary area to which they have been applied. Preliminary *in vitro* studies conducted in

our laboratory using conventional and biofilm bacterial culture techniques²⁴ suggest that the ROS produced after light exposure are stable for a sufficient period of time, such that PCF films which were photoirradiated prior to placement are capable of inhibiting bacterial growth. This may make it possible to treat wounds with these materials without the need to photoradiate the wound itself. However, we did not investigate this alternative in the present studies. Future experiments will explore this strategy.

Collagen is a good source for a drug carrier because it is known to be biocompatible and bioabsorbable. Riboflavin and its light-activated decomposition products are essential nutrients and as such are exploited as a source of ROS, which provide an effective substrate with low toxicity. The chemically modified collagen materials used in these studies have several attributes that are desirable for future clinical use. The material is biocompatible and is pliable.²¹ The material conforms to any surface to which it is applied. It is hydrophilic and behaves as a postoperative adhesion preventative when placed intraabdominally.^{20,22,23} The material can be used as a carrier for biologically relevant molecules and

FIG. 8. Quantitative culture results at 7 days after multiple treatments. Quantitative wound culture results at 168 h demonstrate that treatment with photoactivated collagen riboflavin (PCRB) plus photoradiation results in persistent reductions in bacterial counts ($p < 0.05$) for several days after the last treatment. The effect increases with the number of treatments provided.

FIG. 9. Rate of reduction in quantitative bacterial counts following multiple treatments. The rate of reduction in wound bacterial counts is shown. The rate of reduction is statistically different between treatment groups ($p < 0.05$).

chromophores, and can be designed to dissolve and be re-absorbed over a specific period of time by adjusting collagen photophysical properties. These attributes facilitate the application of a precise dose and controlled release of an optimized flavin mixture as an antimicrobial agent with accurate placement to effectively manage wound bioburden.

There is a need for the development of novel strategies to reduce levels of contamination in acute and chronic wounds. The use of flavins as a photosensitizer that produces ROS after photoactivation with blue light represents an opportunity to achieve antibacterial effects with low toxicity in contradistinction to antibiotics and typical agents utilized for classic photodynamic therapy applications. Future studies are necessary to determine and optimize the frequency of dressing changes and parameters and frequency of light exposure necessary to achieve maximal antimicrobial activity. These studies should also investigate and contrast pretreatment of the collagen-flavin composites with *in situ* activation, as is reported in this series of experiments. Other work should explore the use of this modality as an adjunct to traditional wound care and antibiotic therapy.

The present studies utilized prototype laser sources with blue light emissions spectra within the optimum range for riboflavin photoactivation as documented in our *in vitro* studies²⁴ and the absorption peaks for riboflavin and lumiflavin.¹⁴ Daily photoradiation alone (R) and PCF alone groups were not evaluated in the multiple treatment study (experiment 2) based on the results obtained in our prior pilot studies^{24,25} as well as the fact that there was no difference observed between C and R groups in the single treatment experiment (experiment 1). The results of the multiple treatment study should be confirmed with larger numbers, and with a daily photoradiation group, in light of the work of others demonstrating the antimicrobial effects of blue light alone in some scenarios.¹⁴⁻¹⁷

Wound infection and related complications increase the cost of care and prolong hospital stays. There is increasing pressure to reduce wound infection rates with various strategies, including the Centers for Medicare and Medicaid Services (CMS) "no-pay" initiative for certain hospital acquired infections.^{34,35} Wound care strategies that are easily applied and that could reduce primary or secondary wound

contamination would improve wound care and patient outcomes. Further development of photoactivated collagen technology is warranted.

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Author Disclosure Statement

Drs. Lanzafame and Stadler have intellectual property rights with Conversion Energy Enterprises. Drs. R. Stoltz and B. Stoltz are owners and have an equity position with Conversion Energy Enterprises. Drs. Cunningham, Muhlbauer, and Griggs have no disclosures or conflict of interest with this content.

References

- Hollenbeak, C.S., Lave, J.R., Zeddies, T., Pei, Y., Roland, C.E., and Sun, E.F. (2006). Factors associated with risk of surgical wound infections. *Am. J. Med. Qual.* 21(Suppl 6), 29S-34S.
- Perencevich, E.N., Sands, K.E., Cosgrove, S.E., Guadagnoli, E., Meara, E., and Platt, R. (2003). Health and economic impact of surgical site infections diagnosed after hospital discharge. *Emerg. Infect. Dis.* 9, 196-203.
- Cheadle, W.G. (2006). Risk factors for surgical site infection. *Surg. Infect. (Larchmt.)* 7(Suppl. 1), 7S-11S.
- Cutting, K.F., and White, R.J. (2005). Criteria for identifying wound infection—revisited. *Ostomy Wound Manage* 51, 28-34.
- Tang, R., Chen, H.H., and Wang, Y.L. (2001). Risk factors for surgical site infection after elective resection of the colon and rectum: a single-center prospective study of 2,809 consecutive patients. *Ann. Surg.* 234, 181-189.
- Hiramatsu, K., Hanaki, H., Ino, T., Yabuta, K., Oguri, T., and Tenover, F.C. (1997). Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J. Antimicrob. Chemother.* 40, 135-136.
- Seppälä, H., Klaukka, T., and Vuopio-Varkila, J. (1997). The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A *streptococci* in Finland. *N. Engl. J. Med.* 337, 441-446.

8. Chin, G.J., and Marx, J.E. (1994). Resistant to antibiotics. *Science* 264, 359–393.
9. Holzer, W., Shirdel, J., Zirak, P., Penzkofer, A., Hegemann, P., Deutzmann, R., Hochmuth, E. (2005). Photo-induced degradation of some flavins in aqueous solution. *Chem. Phys.* 308, 69–78.
10. Tsugita, A., Okada, Y., and Uchara, K. (1965). Photo-sensitized inactivation of ribonucleic acids in the presence of riboflavin. *Biochim. Biophys. Acta* 103, 360–363.
11. Samar, B.M., and Goodrich, R.P. (2000). Viral inactivation in plasma using riboflavin-based technology. *Transfusion* 40, S99.
12. Kumar, V., Lockerbie, O., and Kell, S.D. (2004). Riboflavin and UV light based pathogen reduction: extent and consequence of DNA damage at molecular level. *Photochem. Photobiol.* 80, 15–21.
13. Martins, S.A., Combs, J.C., Noguera, G., Camacho, W., Wittmann, P., Walther, R., Cano, M., Dick, J., and Behrens, A. (2008). Antimicrobial efficacy of riboflavin/UVA combination (365nm) in vitro for bacterial and fungal isolates: a potential new treatment for infectious keratitis. *Invest. Ophthalmol. Vis. Sci.* 49, 3402–3408.
14. Kim, H.J., and Min, D.B. (2007). Mechanism of riboflavin destruction under light. Poster. Available at: https://kb.osu.edu/dspace/bitstream/handle/1811/24702/OARDC%20Poster_RF.pdf?sequence=1 Accessed September 18, 2012.
15. Enwemeka, C.S., Williams, D., Enwemeka, S.K., Hollosi, S., and Yens, D. (2009). Blue 470-nm light kills methicillin-resistant *Staphylococcus aureus* (MRSA) in vitro. *Photomed. Laser Surg.* 27, 221–226.
16. Guffey, J.S., and Wilborn, J. (2006). In vitro bactericidal effects of 405-nm and 470-nm blue light. *Photomed. Laser Surg.* 24, 684–688.
17. Guffey, J.S., and Wilborn, J. (2006). Effects of combined 405-nm and 880-nm light on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in vitro. *Photomed. Laser Surg.* 24, 680–683.
18. Kawada, A., Aragane, Y., Kameyama, H., Sangen, Y., and Tezuka, T. (2002). Acne phototherapy with high-intensity, enhanced, narrow-band blue light source: An open study in vitro investigation. *J. Dermatol. Sci.* 30, 129–135.
19. Lanzafame, R.J., Soltz, B.A., Stadler, I., Soltz, M.A., Soltz, R., and DeVore, D.P. (2005). Acute tensile strength analysis of collagen solder for mesh fixation to the peritoneal surface. *Surg. Endosc.* 19, 178–183.
20. Lanzafame, R.J., Stadler, I., Brondon, P., Soltz, B.A., and DeVore, D.P. (2005). Preliminary assessment of postoperative adhesion formation after laser-assisted mesh fixation to the peritoneal surface. *J. Laparoendosc. Surg.* 15, 105–111.
21. Lanzafame, R.J., Brondon, P., Stadler, I., Soltz, R., and Soltz, B.A. (2005). Histologic assessment of mesh fixation following laser-assisted tissue soldering in a lapine model. *Lasers Surg. Med.* 37, 130–137.
22. Lanzafame, R.J., Soltz, B.A., Stadler, I., and Soltz, R.A. (2009). Laparoscopic mesh fixation using laser-assisted tissue soldering in a porcine model. *JSLs* 13, 190–195.
23. Lanzafame, R.J., Soltz, B.A., Stadler, I., and Soltz, R.A. (2009). Transabdominal preperitoneal herniorrhaphy using laser-assisted tissue soldering in a porcine model. *JSLs* 13, 293–310.
24. Lanzafame, R.J., Stadler, I., Cunningham, R., Kasaraneni, J., Khan, F., Soltz, R., and Soltz, B. (2010). Preliminary studies of the antimicrobial effects of light activated collagen-embedded riboflavin-5 phosphate in-vitro. *Lasers Surg. Med.* 42, S22.
25. Lanzafame, R.J., Stadler, I., Cunningham, R., Kasaraneni, J., Khan, F., Soltz, R., and Soltz, B. (2010). Development and preclinical trial of photoactivated antimicrobial collagen for wound care in a murine model. *Lasers Surg. Med.* 42: S22.
26. Patents US 6,939,364; US 6,875,427 and US 6,780,840.
27. Stadler, I., Zhang, R.Y., Oskoui, P., Whittaker, M.S., and Lanzafame, R.J. (2004). Development of a simple, noninvasive clinically relevant model of pressure ulcers in the mouse. *J. Invest. Surg.* 17, 221–227.
28. Hughes, J.M. (1988). Study on the efficacy of nosocomial infection control (SENIC project): results and implications for the future. *Chemotherapy* 34, 553–561.
29. Large, M., Stubbs, E., Benn, R., Beard-Pegler, M.A., Harbour, C., and Vickery, A.M. (1989). A study of coagulase-negative staphylococci isolated from clinically significant infections at an Australian teaching hospital. *Pathology* 21, 19–22.
30. Anderson, B.M., Sortie, D., and Hotvedt, R. (1989). Multiply beta-lactam resistant *Enterobacter cloacae* infections linked to the environmental flora in a unit for cardiothoracic and vascular surgery. *Scand. J. Infect. Dis.* 21, 181–191.
31. Wolcott, R.D., and Ehrlich, G.D. (2008). Biofilms and chronic infections. *JAMA*, 299, 2682–2687.
32. El-Azizi, M., Rao, S., Kanchanapoom, T., and Khardori, N. (2005). In vitro activity of vancomycin, quinupristin/dalfopristin, and linezolid against intact and disrupted biofilms of *staphylococci*. *Ann. Clin. Microbiol. Antimicrob.* 4, 2–10.
33. Walters, M.C., III, Roe, F., Bugnicourt, A., Franklin, M.J., and Stewart, P.S. (2003). Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of *Pseudomonas aeruginosa* biofilms to ciprofloxacin and tobramycin. *Antimicrob. Agents Chemother.* 47, 317–323.
34. O'Reilly, K.B. (2008). Medicare's no-pay events: coping with the complications. *American Medical News*. Available at: <http://www.ama-assn.org/amednews/2008/07/14/prsa0714.htm> Accessed September 18, 2012.
35. O'Reilly KB (2012). Medicare's no-pay rule sharpens infection-control efforts. *American Medical News*. Posted May 14, 2012. <http://www.ama-assn.org/amednews/2012/05/14/prsb0514.htm>, accessed 9/18/12.

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