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INFECTION

Bactericidal efficacy of hydrogen peroxide on *Cutibacterium acnes*

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Objectives

The purpose of this study was to examine the bactericidal efficacy of hydrogen peroxide (H_2O_2) on *Cutibacterium acnes* (*C. acnes*). We hypothesize that H_2O_2 reduces the bacterial burden of *C. acnes*.

Methods

The effect of H_2O_2 was assessed by testing bactericidal effect, time course analysis, growth inhibition, and minimum bactericidal concentration. To assess the bactericidal effect, bacteria were treated for 30 minutes with 0%, 1%, 3%, 4%, 6%, 8%, or 10% H_2O_2 in saline or water and compared with 3% topical H_2O_2 solution. For time course analysis, bacteria were treated with water or saline (controls), 3% H_2O_2 in water, 3% H_2O_2 in saline, or 3% topical solution for 5, 10, 15, 20, and 30 minutes. Results were analyzed with a two-way analysis of variance (ANOVA) ($p < 0.05$).

Results

Minimum inhibitory concentration of H_2O_2 after 30 minutes is 1% for H_2O_2 prepared in saline and water. The 3% topical solution was as effective when compared with the 1% H_2O_2 prepared in saline or water. The controls of both saline and water showed no reduction of bacteria. After five minutes of exposure, all mixtures of H_2O_2 reduced the percentage of live bacteria, with the topical solution being most effective ($p < 0.0001$). Maximum growth inhibition was achieved with topical 3% H_2O_2.

Conclusion

The inexpensive and commercially available topical solution of 3% H_2O_2 demonstrated superior bactericidal effect as observed in the minimum bactericidal inhibitory concentration, time course, and colony-forming unit (CFU) inhibition assays. These results support the use of topical 3% H_2O_2 for five minutes before surgical skin preparation prior to shoulder surgery to achieve eradication of *C. acnes* for the skin.

Cite this article: ***Bone Joint Res* 2019;8:3–10.**

Keywords: *Cutibacterium acnes*, Shoulder, Hydrogen peroxide, Infection, Treatment, Shoulder arthroplasty

Article focus

- The purpose of this study is to investigate the antibacterial potential of H_2O_2 for the eradication of *C. acnes*.

Key messages

- Hydrogen peroxide has both bactericidal as well as bacteriostatic properties against *C. acnes*. The minimum time for hydrogen peroxide to produce its bactericidal effect on *C. acnes* is five minutes.

Strengths and limitations

- This is the first investigation to examine the bacteriocidal properties of hydrogen peroxide for *C. acnes*.
- This is a proof-of-concept pilot study and therefore only utilizes lab isolates of *C. acnes*; it is unknown if hydrogen peroxide is equally as effective with clinical isolates.
- The tissue toxicity of hydrogen peroxide at the minimal inhibitory concentration found in this investigation is unknown.

Introduction

Cutibacterium acnes (*C. acnes*), formerly named *Propionibacterium acnes*, is one of the most common causative organisms causing infection following shoulder surgery.[1-4] Multiple attempts have been made to provide consistent, reproducible methods of

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doi: 10.1302/2046-3758.81.BJR-2018-0145.R1

Bone Joint Res 2019;8:3–10.

decolonization and/or eradication of this troublesome organism preoperatively in an effort to reduce the risk of surgical site contamination and infection.[5-10] *Cutibacterium acnes* is a slow-growing, facultative anaerobic Gram-positive bacillus commonly residing in the deep dermal layer of the skin within the pilosebaceous glands and hair follicles.[4,5,9,11,12] This location not only makes *C. acnes* difficult to eradicate during surgical skin preparation because of poor penetration, but also puts the patient at risk of contamination of the shoulder joint due to repeated contact with this layer beneath the epidermis.[2,4-6,10,11,13,14] It has been hypothesized that *C. acnes* inoculates the surgical wound once incision is made through the pilosebaceous glands.[11,15]

Additional attempts have been made to decolonize the skin utilizing antibiotics, either systemically or via topical application, with varying degrees of success.[5,8,16-18] This bacteria has been shown to be susceptible to several antibiotics including penicillin G, amoxicillin, cephalothin, ceftriaxone, clindamycin, doxycycline, and rifampin.[16,17] The issue that arises following administration of antibiotics is the emergence of resistance, with several recent reports of increasing resistance to clindamycin, doxycycline, tetracycline, minocycline, and erythromycin.[17,19-22] Despite this susceptibility, recent studies[4,15] have demonstrated prophylactic intravenous antibiotics prior to skin incision to be ineffective and to continue to result in positive *C. acnes* cultures. Thus far, routine skin preparation and intravenous perioperative antibiotics have not been shown to provide antimicrobial protection against *C. acnes*.

Two recent studies[5,9] evaluated the effectiveness of topically applied benzoyl peroxide perioperatively. These studies demonstrated an almost 50% reduction in positive superficial cultures[5,9] and, when combined with topical clindamycin, positive deep cultures decreased from 19.6% to 3.1%.[5] Topical benzoyl peroxide has been proven to be an effective treatment, as it is lipophilic and can penetrate the deep dermal layer as well as release free oxygen radicals.[23-26] These free radicals result in oxidation of proteins in bacterial cell membranes with subsequent cell death. While this method seems to be effective, it utilizes an antibiotic and can develop resistance to treatment. Furthermore, these treatments require patient compliance with the perioperative topical treatment regimen. Allhorn et al[27] describe a novel antioxidant enzyme, radical oxygenase of *Propionibacterium acnes* (RoxP), which is produced by the bacteria to protect against oxidation and is hypothesized to facilitate its survival on skin. We are unaware of any studies describing the effectiveness of this enzyme when the bacteria have not had time to adapt to exposure to a new strongly oxidative product.

Hydrogen peroxide (H_2O_2) is cheap, widely available, and can be applied at the time of surgery. Given that H_2O_2 is not an antibiotic, there is no risk for development of resistant *C. acnes* strains. In theory, the aerobic environment created in the presence of H_2O_2 should be detrimental to anaerobic bacteria. It has been well established that polymorphonuclear leucocytes (PMNs) eliminate microorganisms through the generation of reactive oxygen free radicals, and utilize H_2O_2 to facilitate this respiratory burst mechanism resulting in cell death.[28]

The purpose of this study is to investigate the antibacterial potential of H_2O_2 for the eradication of *C. acnes*. To our knowledge, no study has been performed evaluating the efficacy of H_2O_2 in reducing the burden of *C. acnes*. We hypothesize that H_2O_2 will be an effective bactericidal treatment against *C. acnes*.

Materials and Methods

Cutibacterium acnes (ATCC 6919) was cultured in tryptic soy broth (TSB; Becton, Dickinson and Company, Franklin Lakes, New Jersey) with 5% defibrinated sheep blood (DSB) and plated on TSB agar with 5% DSB (TSB-DSB agar), at 37°C under anaerobic conditions using Gas-pak (Becton, Dickinson and Company). Both liquid media and agar plates were pre-reduced for 24 hours before *C. acnes* culture. All pre-inoculums were made from a single colony in 2 ml broth.

Two inoculums of 25 ml each were started with a 1:100 dilution from 24-hour pre-inoculums (10^7 colony-forming units (CFUs)). The cell number was calculated after a colony count of serial dilutions. Serial dilutions were prepared from 100 ml samples taken at 0, 6, 24, 30, 48, 54, 78, and 97 hours of growth, and cultured in duplicate on TSB-DSB agar. The plates were incubated at 37°C for five days under anaerobic conditions. After the incubation period, viable colonies were counted and the number of CFUs per millilitre of culture (CFU/ml) was determined. The generation time (gt) was calculated according to Hall et al,[29] using the following formula, where $\frac{CFU_f}{ml}$ and $\frac{CFU_i}{ml}$ are final and initial CFU per ml, t_f and t_i are final and initial times in hours, and $K = \log 2 = 0.301$:

$$gt = \frac{K[t_f - t_i)]}{\log(\frac{CFU_f}{ml}) - \log(\frac{CFU_i}{ml})}$$

Dilutions of 1%, 3%, 4%, 6%, 8%, and 10% H_2O_2 were freshly prepared with Milli-Q water or 0.85% sodium chloride (NaCl) (saline) from a 30% H_2O_2 solution (Fisher Bioreagents, Pittsburgh, Pennsylvania) and compared with a commercial solution of stabilized 3% topical H_2O_2 in water (topical solution) (Henry Schein Inc., Melville, New York). Pre-inoculums and 1:100 dilution inoculums were grown in TSB-DSB broth. At 50 hours of culture (10^9 CFUs), bacteria were centrifuged at 4300 × g for ten minutes, resuspended in saline and divided into 15 tubes. Each tube was treated for 30 minutes at room temperature

Table I. Results of a power analysis of the five-minute data from the time course experiment

Power for analysis of variance (ANOVA)	
Data source: *5* mins 3% H_2O_2 in saline *vs* 3% topical H_2O_2	
Power	1
Difference in means	65.73
Standard deviation	13.52
Groups, n	3
Group size, n	5
Alpha	0.05
Data source: *5* mins 3% H_2O_2 in water *vs* 3% topical H_2O_2	
Power	1
Difference in means	74.13
Standard deviation	13.52
Groups, n	3
Group size, n	5
Alpha	0.05

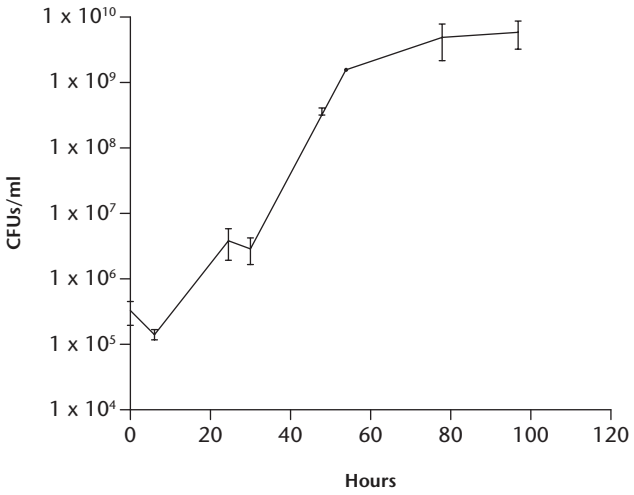

Fig. 1

Growth curve of purified strain of *C. acnes*. Bacteria were grown in tryptic soy broth–defibrinated sheep blood (TSB-DSB) at 37°C under anaerobic conditions. Samples were taken at 0, 6, 24, 30, 48, 54, 78, and 97 hours of growth and culture aliquots were plated on TSB-DSB agar. The calculated generation time (gt) is 4.39 hours. Data are presented as the mean (standard deviation) of two measurements. CFUs, colony-forming units.

with one of the following conditions: saline only, water only, 1%, 3%, 4%, 6%, 8%, or 10% H_2O_2 prepared in saline, the same dilutions prepared in water, or 3% topical solution. Tubes were centrifuged at 10 000 × g for five minutes and resuspended in 1 ml of saline. From this, 10 µl of serial dilutions up to 1/100 000 $^{v}/_{v}$ were plated by duplicate on TSB-DSB agar. After incubation under the same conditions as above, colonies were counted and the minimum inhibitory concentration (MIC) was calculated. From the 1/10 $^{v}/_{v}$ dilution of: a) saline; b) water; c) 3% H_2O_2 prepared in saline; d) 3% H_2O_2 prepared in water; and e) topical solution, 100 µl were plated as lawn in TSB-DSB agar for up to five days.

Fresh H_2O_2 dilutions in water and saline were prepared as previously mentioned for dose response. For this experiment, pre-inoculums and 1:50 dilution inoculums were grown in TSB without blood. Bacteria were centrifuged at 4300 × g for ten minutes, washed in saline once, and divided into 19 tubes. Each one was incubated in one of the following conditions: 3% H_2O_2 prepared in saline; 3% H_2O_2 prepared in water; or 3% topical solution for 0, 5, 10, or 15 minutes at room temperature. Saline-only and water-only controls were incubated for 15 minutes. Bacteria were centrifuged at 10 000 × g for five minutes, which resulted in final incubation times of 5, 10, 15, and 20 minutes. Cells were resuspended in saline and stained with Live/Dead BacLight bacterial viability kit (Molecular Probes, Invitrogen, Waltham, Massachusetts) following the manufacturer's instructions. A standard curve for each experiment was created by mixing the percentages of live and dead bacteria in saline. To obtain dead bacteria, tubes were exposed to 95°C for ten minutes. Plates were exposed to 480 nm for excitation with 520 nm (green) and 620 nm (red) emission.

Statistical analysis. A two-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons of time course data was performed with GraphPad Prism version 7 (GraphPad Software, La Jolla, California). Differences were considered significant when the p-value was less than 0.05. A one-way ANOVA with Dunnett's *post hoc* test was performed to compare timepoints on each 3% H_2O_2 solution. A power analysis of time course data was performed with SigmaPlot 13.0 (Systat Software, Inc., San Jose, California) (Table I).

Results

Reconstituted *C. acnes* was first plated in TSB-DSB agar in anaerobic conditions to produce single colonies. Controls included bacteria grown in aerobic conditions and non-cultured plates in both anaerobic and aerobic conditions. No colonies were observed in any of the controls. All colonies of *C. acnes* in plates shared the same characteristics: circular, convex, smooth, white, and opaque. We then characterized the growth of this *C. acnes* purified strain from ATCC for up to 97 hours of culture (Fig. 1). Our

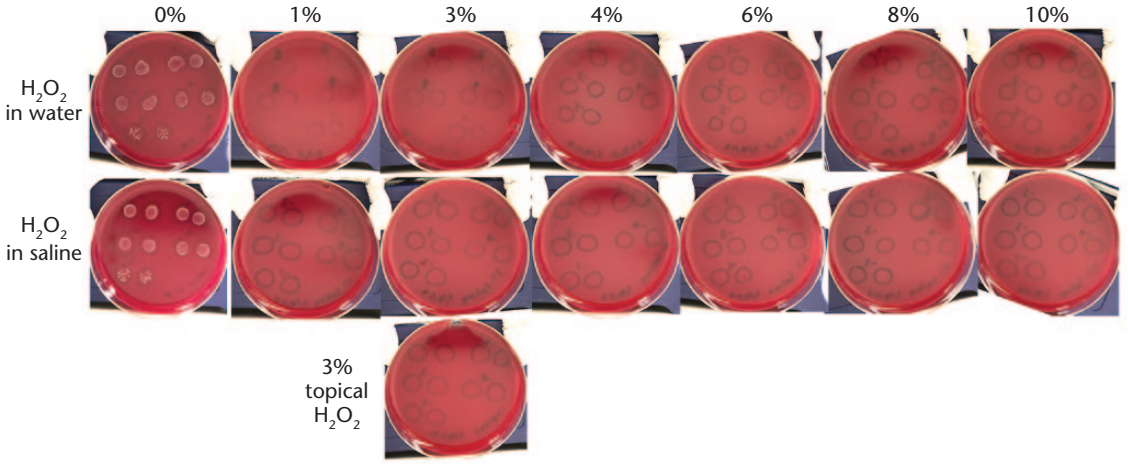

Fig. 2a

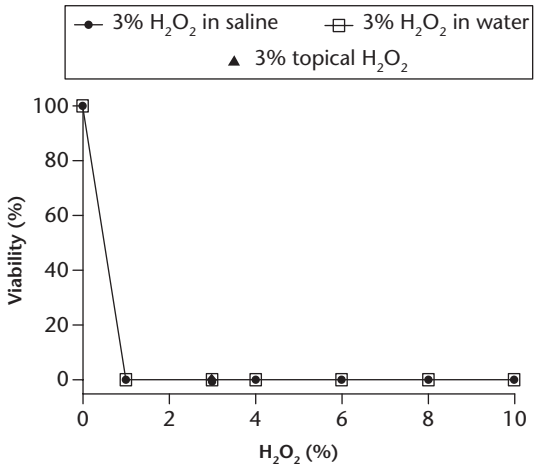

Fig. 2b

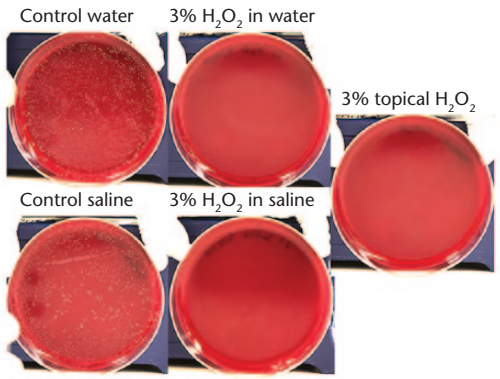

Fig. 2c

Minimum bactericidal concentration (MBC) was performed with a dose response of H_2O_2 prepared in saline and in water and compared with a 3% topical solution of H_2O_2. Bacteria were treated for 30 minutes on each solution (0% to 10%) and serial dilutions were seeded on tryptic soy broth–defibrinated sheep blood (TSB-DSB) agar. Colonies were counted after five days of incubation. All treatments are effective in their bactericidal activity. a) Representative image of three independent experiments. b) Graph of percentage of viability of *C. acnes versus* percentage of H_2O_2 grown in TSB showed a MBC of 1% for H_2O_2 prepared in saline and in water. Topical solution is equally effective. d) *C. acnes* treated for 30 minutes with 3% H_2O_2 prepared in saline, in water, or 3% topical solution were grown on TSB-DSB agar as lawn to further assess for bacterial growth inhibition. None of the treatments produced growth of colonies, while controls saline and water only show normal bacterial growth.

calculations for generation time (gt) were 4.39 hours, similar to the 5.1 hours previously described by Hall et al,[29] who studied intraocular clinical isolates of subjects with chronic postoperative endophthalmitis.

For the consecutive experiments, we calculated that 50 hours of culture were necessary to obtain 10^9 CFUs.

Bacteria were treated for 30 minutes with different percentages of H_2O_2 and compared with a commercial topical solution of stabilized 3% H_2O_2. Since the topical H_2O_2 is stabilized in water, we compared a fresh diluted H_2O_2 solution in both saline and water. Plates were incubated for five days in anaerobic conditions to allow the development of colonies. Results show that all the concentrations tested for H_2O_2 have effective bactericidal properties (Fig. 2). Negative controls of saline only and water only showed normal bacterial growth (Fig. 2a). The graph in Figure 2b shows that 1% H_2O_2 is the minimum bactericidal concentration (MBC) for *C. acnes*. The topical solution of H_2O_2 is as effective as 1% of a freshly prepared solution.

To corroborate the bactericidal effect of the 3% H_2O_2 solutions, treated *C. acnes* were seeded as lawn to observe growth inhibition (Fig. 2c). Results show that all H_2O_2 dilutions tested inhibit *C. acnes* growth. These results indicate that H_2O_2 has both a bactericidal and a bacteriostatic effect on this bacterium.

The minimum time required by H_2O_2 to produce its bactericidal effect on *C. acnes* was tested (Table II). The 3% H_2O_2 solutions as described previously were incubated for 5, 10, 15, and 20 minutes, and viability was assayed with Live/Dead BacLight bacterial viability assay (Fig. 3a). The results showed that, starting from five

Table II. Results of the two-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons was performed on the time course data

Test groups	Mean difference	95% CI	Adjusted p-value
Time: 0 mins			
3% H_2O_2 in saline *vs* 3% H_2O_2 water	9.418	–23.07 to 41.9	0.7663
3% H_2O_2 in saline *vs* 3% topical H_2O_2	9.418	–23.07 to 41.9	0.7663
3% H_2O_2 in water *vs* 3% topical H_2O_2	0	–32.48 to 32.48	> 0.9999
Time: 5 mins			
3% H_2O_2 in saline *vs* 3% H_2O_2 in water	-8.396	–40.88 to 24.09	0.8091
3% H_2O_2 in saline *vs* 3% topical H_2O_2	65.73	33.25 to 98.22	< 0.0001*
3% H_2O_2 in water *vs* 3% topical H_2O_2	74.13	41.64 to 106.6	< 0.0001*
Time: 10 mins			
3% H_2O_2 in saline *vs* 3% H_2O_2 in water	-8.674	–41.16 to 23.81	0.7977
3% H_2O_2 in saline *vs* 3% topical H_2O_2	66.75	34.26 to 99.23	< 0.0001*
3% H_2O_2 in water *vs* 3% topical H_2O_2	75.42	42.94 to 107.9	< 0.0001*
Time: 15 mins			
3% H_2O_2 in saline *vs* 3% H_2O_2 in water	-9.61	–42.09 to 22.87	0.758
3% H_2O_2 in saline *vs* 3% topical H_2O_2	66.83	34.35 to 99.32	< 0.0001*
3% H_2O_2 in water *vs* 3% topical H_2O_2	76.44	43.96 to 108.9	< 0.0001*
Time: 20 mins			
3% H_2O_2 in saline *vs* 3% H_2O_2 in water	-10.39	–42.87 to 22.1	0.7236
3% H_2O_2 in saline *vs* 3% topical H_2O_2	63.22	30.73 to 95.7	< 0.0001*
3% H_2O_2 in water *vs* 3% topical H_2O_2	73.61	41.12 to 106.1	< 0.0001*

*Statistically significant
CI, confidence interval

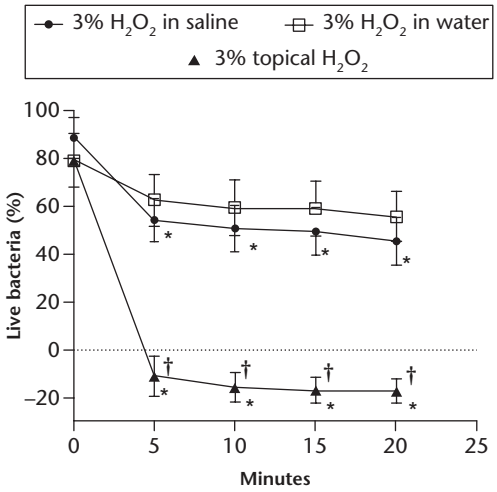

Fig. 3a

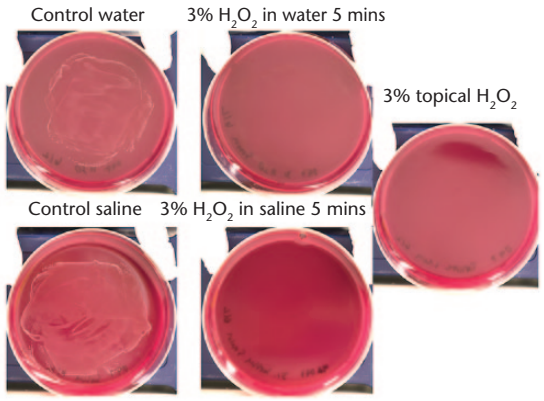

Fig. 3b

Time course of H_2O_2 effect. a) Bacteria grown in tryptic soy broth (TSB) showed that a 3% topical solution is superior to the other solutions in its bactericidal effect, starting from five minutes of incubation. 3% H_2O_2 prepared in saline is not significantly different from 3% H_2O_2 prepared in water in any of the time-points. *$p < 0.0001$ compared with control saline or water; †$p < 0.0001$ compared with 3% H_2O_2 prepared in saline or in water. b) Bacteria grown in tryptic soy broth–defibrinated sheep blood (TSB-DSB) agar and treated with each solution of H_2O_2 for five minutes was grown as lawn to further assess for growth inhibition. All treatment solutions inhibited bacterial growth except the controls with water or saline.

minutes, the 3% topical solution significantly decreases the viability of *C. acnes* compared with the water-only control ($p < 0.0001$), and it was superior in its bactericidal effect compared with 3% H_2O_2 prepared either in saline or water during all times analyzed ($p < 0.0001$). Bacteria treated for five minutes with each of the tested solutions were grown as lawn to further test growth inhibition (Fig. 3b). Only negative controls showed normal growth of *C. acnes* as lawn. None of the treatment solutions displayed any appearance of colonies.

Bacteria stained with Live/Dead BacLight kit were viewed with a Nikon Eclipse Ti fluorescence microscope (Nikon Corp., Tokyo, Japan) to assess the staining profile and investigate the negative values of cell viability obtained for 3% topical solution. The standard curve for live/dead bacteria showed a gradual decrease in green staining (live cells), together with an increase in red staining (dead cells) (Fig. 4a). This was visible for both 3% H_2O_2 in saline and in water. However, topical solutions showed a decrease of fluorescence staining for both green and red (Fig. 4b). The findings for the topical H_2O_2 fluorescence staining indicate that this treatment results in not just cell death, but also complete cell destruction.

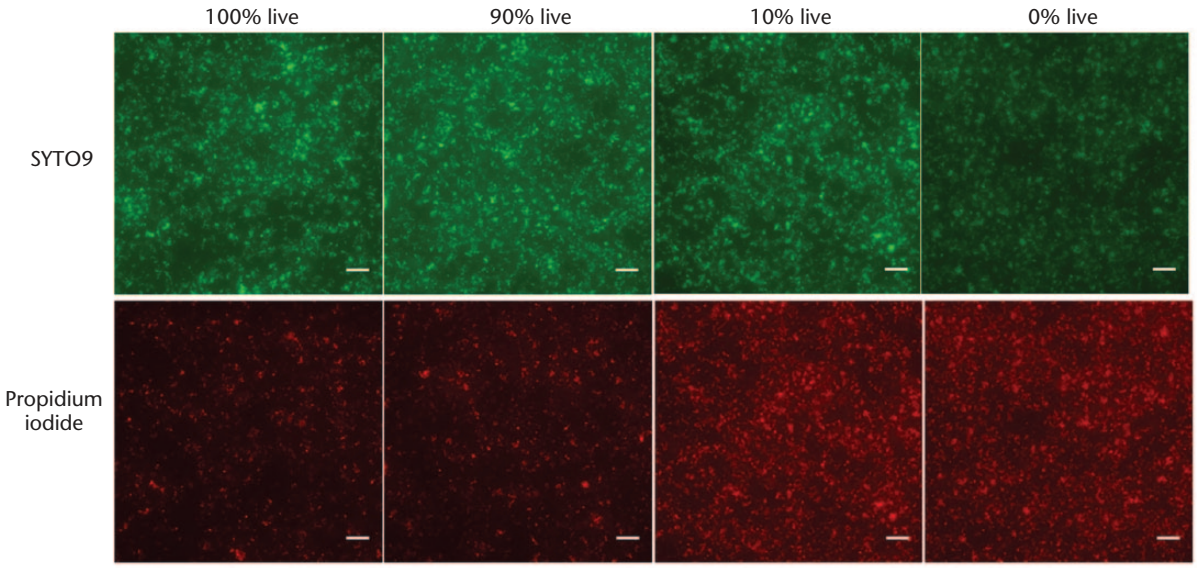

Fig. 4a

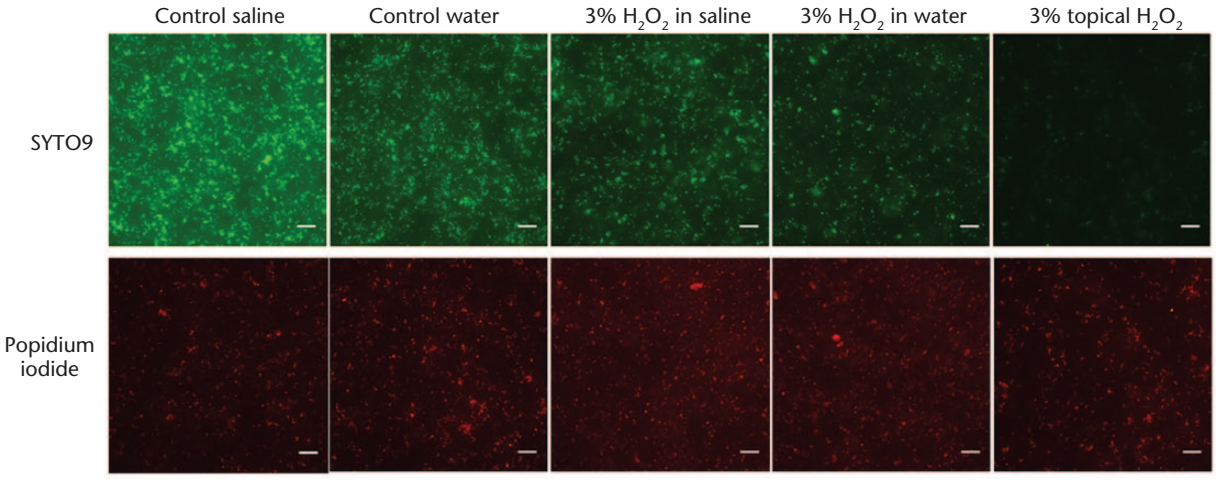

Fig. 4b

Bacterial viability after five minutes of treatment. Viability was tested with Live/Dead BacLight kit after five minutes of incubation with H_2O_2. a) Standard curve used for each experiment was obtained by mixing live bacteria with dead bacteria obtained by incubation at 95°C for ten minutes in saline. Green staining gradually decreased (from 100% to 0% live bacteria) while red increased (from 0% to 100% dead bacteria). b) Both 3% H_2O_2 solution prepared in saline and in water show a similar staining pattern, while 3% topical solution showed a decreased staining for both green and red dyes.

Discussion

The results of this study serve to validate the bactericidal and bacteriostatic properties of H_2O_2 against a specific bacterium, *C. acnes*. After treatment with H_2O_2, the colonies with *C. acnes* showed complete elimination of bacteria within five minutes. The oxidative reaction produced caused extensive damage and even destroyed the bacterial cells. After the treatments with H_2O_2, attempts to regrow the *C. acnes* produced negative results for at up to a week, a timepoint significantly longer than controls. The minimum inhibitory concentration of H_2O_2 for *C. acnes* is 1% solution prepared with either saline or water. More importantly, our results demonstrated that the commercially available topical solution is as effective as the 1% freshly prepared solution, indicating that it is as powerful as the lowest concentration of H_2O_2 solution. Additionally, our results found that the topical solution had a superior bactericidal effect compared with 3% H_2O_2 prepared in either saline or water, and found that the minimum time for bactericidal effect on *C. acnes* was five minutes.

Previous literature has shown that H_2O_2 has both bactericidal and bacteriostatic effect *in vitro* on other bacterium.[28,30] To our knowledge, this is the first study to examine the efficacy of H_2O_2 as it pertains to *C. acnes*. The results of this study are powerful in that the H_2O_2 topical solution is low cost, with an average price of $1.30 (USD) for a 473.18 ml (16 ounce) bottle, and can be easily applied to the surgical field at any time during the surgical procedure. The ease of application provides a means

of delivery to the deep dermal layer where sebaceous glands reside.

This study was undertaken in order to find a more efficacious form of skin preparation prior to shoulder surgery to reduce the bacterial burden of *C. acnes* and decrease the risk of deep contamination and potential infection. Studies have shown that culture-positive *C. acnes* patients may remain positive in nearly a third following treatment with chlorhexidine, and may be even double this at the end of a surgical procedure.[6] The current 'standard of care' skin preparations are questionably effective in eliminating *C. acnes* colonization, therefore alternative skin preparations must be considered in order to reduce the incidence of postoperative infection by this troublesome bacterium. Although, to date, no study has been performed that looks explicitly at the effects of H_2O_2 on *C. acnes* colonization *in vivo*, there have been studies examining the cultures of shoulders after treatment with benzoyl peroxide, a similarly structured analogue. In this study, Sabetta et al[9] showed that pre-treatment with benzoyl peroxide produced similarly low positive culture rates for *C. acnes* as a control swab, indicating the ability of the substance to penetrate the sebaceous glands and eliminate the bacterium.

A recent study by Namdari et al[8] performed as a randomized controlled trial to evaluate the effectiveness of a preoperative course of oral doxycycline for seven days prior to shoulder arthroscopy to determine whether this treatment would reduce *C. acnes* colonization in males. The authors reported no difference in positive culture rates between the 'no antibiotics' and the doxycycline groups. The most alarming finding was 59.5% positive cultures in the no treatment group and 43.2% in the doxycycline group ($p = 0.245$). The results indicate minimal impact on *C. acnes* and the authors recommended against this type of prophylactic use, as there is potential risk for the emergence of resistance. These results also support the findings reported by Matsen et al[15] and Falconer et al[4] that indicate that current intravenous administered antibiotics given prior to skin incision do not eliminate *C. acnes*. Both of these studies found a 30% culture-positive rate despite perioperative antibiotics, thus supporting the need for additional/other interventions to eradicate this bacteria successfully at the time of surgery.

While this study certainly produces meaningful information, it has some limitations. This study is an *in vitro* analysis of the effects of H_2O_2 on *C. acnes*. Although the results show efficacy in the laboratory, the clinical utility has still not been investigated. In the laboratory, the H_2O_2 comes into direct contact with the bacterial membranes, allowing for aggressive destruction of cells, as demonstrated by the negative curve for the topical H_2O_2 solution in Figure 3a. *In vitro*, the bacteria tend to live in the pilosebaceous glands in the subcutaneous dermal layer of the skin, a location that is not readily accessible to most skin preparations.[4,13] Another limitation is that we used only one bacterial reference strain, and while it is not common clinically to speciate bacterial strains of this organism, we do not know if clinical isolates of *C. acnes* would demonstrate the same susceptibility to H_2O_2. The efficacy of H_2O_2 is a topic for future investigation.

The use/application of H_2O_2 at the time of surgery will need to be at multiple locations to be able to get in contact with the bacteria and be effective. Although this is not an issue during open surgical procedures, H_2O_2 may not be as effective during arthroscopic shoulder surgery because its penetration into the deep dermal layer through topical application is unknown. While benzoyl peroxide has been shown to penetrate the pilosebaceous glands of the skin, no such study has been performed for H_2O_2.[14] It is clear that it is not possible to eradicate *C. acnes* completely, and the focus and goal should be to decrease the bacterial burden in order to prevent contamination of the surgical field and not allow this troublesome bacteria to establish a biofilm deep within the shoulder.

In conclusion, hydrogen peroxide is a very potent antimicrobial against the bacterium *Cutibacterium acnes*. It is both bacteriostatic and rapidly bactericidal, even at low concentrations. The minimum bactericidal concentration of H_2O_2 for *C. acnes* is a 1% solution prepared in either saline or water. The commercially available topical 3% solution is equally as effective as the laboratory-prepared concentration. Within five minutes of exposure to H_2O_2, there is complete eradication of *C. acnes in vitro*. Further studies are needed to establish the depth of penetration through the epidermis to the deep dermal layers. With our current findings, the use of a topical solution of H_2O_2 as part of skin preparation prior to surgery may be a helpful tool to prevent *C. acnes* contamination during shoulder surgery. Additionally, application to the deep dermal layer once a skin incision has been made, and a final application to the deep dermal layer prior to skin closure, may be helpful in the eradication of this troublesome bacterium. Future studies are required in order to validate these recommendations clinically.

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- P. Hernandez: Designed the study, Collected and analyzed the data, Actively involved in the laboratory experiment, Wrote the manuscript.
- B. Sager: Designed the study, Analyzed the data, Wrote the manuscript.
- A. Fa: Collected the data, Actively involved in the laboratory experiment.
- T. Liang: Collected the data, Actively involved in the laboratory experiment.
- C. Lozano: Designed the study, Analyzed the data, Wrote the manuscript.
- M. Khazzam: Developed the hypothesis, Designed the study, Analyzed the data, Wrote the manuscript.

Funding statement
- None declared

Conflict of interest statement
- None declared