JB&JS OPEN ACCESS

Reduced Bacterial Burden of the Skin Surrounding the Shoulder Joint Following Topical Protocatechuic Acid Application

Results of a Pilot Study

Omid Jalali, MD, Molly Best, MD, Alison Wong, MD, Brett Schaeffer, MD, Brendon Bauer, MD, and Lanny Johnson, MD

Investigation performed at Loma Linda University, Loma Linda, California

Background: Reducing the rising health-care burden associated with shoulder surgical site infection (SSI) is of paramount importance. The purpose of this study was to investigate the antimicrobial efficacy of protocatechuic acid (PCA) as a topical reagent for surgical skin antisepsis surrounding the shoulder joint.

Methods: This was a 2-phase skin-disinfection trial involving the human shoulder. The shoulders of healthy volunteers were randomized to topical treatment with PCA (a 10% concentration of PCA in Phase I [11 subjects] and a 17% concentration in Phase II [12 subjects]), with a control of isopropyl alcohol (IPA) applied to the contralateral shoulder. Mechanical scraping was performed for skin harvest following reagent application, and samples were sent for aerobic and anaerobic culture. Sterilization rates and bacterial counts were determined for each treatment group, and the proportion of subjects with persistent *Cutibacterium acnes* colonization following topical application of PCA was determined using DNA sequencing analysis.

Results: The topical application of 10% PCA was associated with significantly higher aerobic and anaerobic sterilization rates (90.9% and 81.8%, respectively) compared with treatment with IPA ($p = 0.0143$ and $p = 0.0253$, respectively). The topical application of 17% PCA was associated with a significantly higher anaerobic sterilization rate (83.3%) and trended toward a significantly higher aerobic sterilization rate (91.7%) compared with treatment with IPA ($p = 0.0143$ and $p = 0.083$, respectively). *C. acnes* was identified in 18.2% and 0% of subjects following treatment with 10% and 17% PCA, respectively.

Conclusions: The topical application of PCA was associated with a reduction in the bacterial burden of human shoulder skin and demonstrated dose-dependent antimicrobial activity against *C. acnes* in young, healthy subjects. Clinical studies in a shoulder surgical population are warranted to determine the potential for application in surgical skin antisepsis to reduce shoulder SSI.

Level of Evidence: Therapeutic Level II. See Instructions for Authors for a complete description of levels of evidence.

Surgical site infection (SSI) following shoulder surgical procedures leads to worse health-care outcomes and increased costs[1]. *Cutibacterium acnes,* formerly *Propionibacterium acnes,* is a gram-positive anaerobe responsible for the majority of postoperative shoulder infections[2-4]. Insufficient skin-penetration properties of existing shoulder skin antisepsis protocols have been hypothesized to contribute to the persistence of *C. acnes* in the dermal layer of the skin[5] despite prophylaxis with intravenous[6,7] and topical antimicrobials[8-10]. There is a need for alternative antimicrobials for skin disinfection in shoulder surgery, with skin-penetration properties and efficacy against shoulder-joint pathogens.

Disclosure: The authors indicated that no external funding was received for any aspect of this work. On the **Disclosure of Potential Conflicts of Interest** forms, *which are provided with the online version of the article*, one or more of the authors checked "yes" to indicate that the author had a relevant financial relationship in the biomedical arena outside the submitted work (one author, L.J., has a website that sells the test reagent that is the subject of the study) and "yes" to indicate that the author had a patent and/or copyright, planned, pending, or issued, directly relevant to this work (http://links.lww.com/JBJSOA/A189).

JBJS Open Access • 2020:e19.00078. openaccess.jbjs.org

Natural substrates with antimicrobial properties have been identified as potential sources of novel antimicrobials[11]. In preclinical work by our group, we demonstrated the broad-spectrum, in vitro antimicrobial efficacy of protocatechuic acid (PCA), a phytochemical found in food plants[12]. PCA demonstrated laboratory efficacy against skin and wound pathogens and known drug-resistant organisms[12]. Daily application of topical PCA was then shown to result in a dose-dependent reduction in dermal *C. acnes* loads in a mouse model of dermal skin infection[12].

Building on this groundwork, the objective of the present study was to investigate the antimicrobial efficacy of PCA in human shoulder skin disinfection. We hypothesized that topical application of PCA would decrease the bacterial burden of the skin surrounding the shoulder joint and demonstrate antimicrobial activity against *C. acnes* in this region.

Materials and Methods

Materials

In both phases, the use of PCA was compared with the use of a control of 70% isopropyl alcohol (IPA) alone. A 10% concentration of PCA dissolved in 70% IPA was used for Phase-I testing (Table I). In Phase II, the concentration was increased to 17% PCA, and propylene glycol (PG) and essence of peppermint oil (EPO) were added to optimize transdermal skin penetration[13-15].

Study Design

This study was a 2-phase, human clinical trial. Following institutional review board approval, healthy volunteers ≥18 years of age were recruited to participate; consent was obtained from participants. All Phase-I testing was performed in April 2014, and all Phase-II testing was performed in March 2015. Similar study designs were employed in each phase.

The skin surrounding the proximal aspect of the deltopectoral groove at the anterior part of the shoulder was selected for testing[5,7,9,10,16,17]. To isolate the antimicrobial effects of test reagents, the subjects were instructed to avoid showering or bathing on the day of the study, and they were excluded if they had used antibiotics in the previous 30 days, had a diagnosis of diabetes, or reported tobacco use. Each subject had 1 shoulder randomized to treatment with PCA; the contralateral shoulder was treated with 70% IPA monotherapy as a control (Table I). Skin bacterial samples were collected at 2 time points for each shoulder: immediately prior to treatment application and 20 minutes following treatment application. A 20-minute interval was selected to approximate the time between application and incision that would be expected in surgical preparation. The index sample was taken from a designated 6 × 6-cm region of the surface of the skin with a moist cotton swab. The assigned treatment was applied throughout the demarcated region with a single swab application for 30 seconds, avoiding brushing, scrubbing, or pressure to avoid the potential for confounding with the use of particular application techniques (e.g., scrubbing at different speeds or intensities when using a surgical preparation stick).

The area was allowed to dry. Twenty minutes following treatment, the back of a sterile scalpel was rubbed with moderate pressure over the demarcated skin surface to maximize the harvesting of microbes on, and deep to, the surface of the skin[17], and a second swab was used to sample the harvested skin layers and appendages. Care was taken to ensure that posttreatment culture samples were harvested at least 3 cm from the index sample site to ensure anatomic separation of the harvest sites.

TABLE I Phase-I and Phase-II Study Design, Material, and End Points*

	Phase I	Phase II
Sample		
No. of subjects	11	12
No. of shoulders	22	24
Culture time points†	Immediately prior to treatment application and 20 min after application	Immediately prior to treatment application and 20 min after application
Test reagents		
PCA concentration‡	10%	17%
Formulation additives	None	EPO, PG
Control agent	70% IPA	70% IPA
End-point analysis (same for both phases)		
Activity against aerobic bacteria	Aerobic bacteria sterilization rates, mean aerobic bacterial counts by treatment	Aerobic bacteria sterilization rates, mean aerobic bacterial counts by treatment
Activity against anaerobic bacteria	Anaerobic bacteria sterilization rates, mean anaerobic bacterial counts by treatment	Anaerobic bacteria sterilization rates, mean anaerobic bacterial counts by treatment
Activity of PCA against *C. acnes*	*C. acnes* colonization rates following treatment with topical PCA	*C. acnes* colonization rates following treatment with topical PCA

*PCA = protocatechuic acid, IPA = isopropyl alcohol, EPO = essence of peppermint oil, and PG = propylene glycol. †Time points defined in relation to topical treatment application. ‡PCA was dissolved in a vehicle of 70% IPA in both phases.

JBJS Open Access • 2020:e19.00078. openaccess.jbjs.org

Culture Methods and Bacterial Identification

Harvested specimens were placed in 0.5 mL of Dey-Engley broth, stored in individual transport bags on ice, and sent to an independent laboratory for aerobic and anaerobic culturing (WuXi AppTec in Marietta, Georgia). For each sample, a Pasteur pipette was used to inoculate 2 culture media of brain heart infusion agar. To determine growth of aerobic organisms, 1 culture medium from each sample was incubated aerobically at 37°C for up to 3 days. To culture anaerobic organisms, and optimize the yield of *C. acnes*[18], the other was incubated at 37°C in an anaerobic environment for 21 days, with MycoSeal sealant (Hardy Diagnostics) applied to the exterior of the agar plates to prevent desiccation during incubation.

Analysis of Culture Results

For each phase, sterilization rates and bacterial loads before and after treatment were determined and compared for shoulders treated with topical PCA or topical IPA. Sterilization was defined as a negative bacterial culture result following treatment application among subjects with a positive culture prior to treatment. All positive specimens then underwent enumeration of colony-forming units (CFUs), transformed to a decimal logarithm. At the microbiology laboratory we used, the upper limit for accurate counting was 200 CFUs, and specimens with counts of ≥200 CFUs were labeled "too numerous to count" ("TNTC"). Because of the lack of an established method by which this semiquantitative result could be combined and compared, all specimens labeled "TNTC" were assigned a cutoff value of 200 CFUs for quantitative analysis.

Identification of C. acnes

To determine the number of subjects who were positive for *C. acnes* following treatment with topical PCA, species-level identification and quantification was performed on a subset of anaerobic subject culture samples using a previously described technique[18]. Briefly, polymerase chain reaction (PCR) amplification and DNA sequencing of 16S ribosomal RNA genes were performed on extracted bacterial DNA. All subjects with positive anaerobic culture results following PCA treatment in either phase were included in DNA analysis. In Phase II, 2 additional subjects with high pretreatment anaerobic bacterial burdens and sterile anaerobic cultures following PCA treatment were also selected for testing.

Subjects with positive anaerobic culture results following PCA treatment and the identification of *C. acnes* on DNA sequencing analysis were considered positive for persistent *C. acnes* colonization. Subjects with positive anaerobic culture results following PCA treatment but negative for *C. acnes* on DNA sequencing analysis, and subjects with a sterile anaerobic culture (i.e., no growth of any anaerobic organisms) following treatment were considered to be negative for persistent *C. acnes* colonization. To determine baseline *C. acnes* burdens, DNA sequencing analysis of pretreatment culture samples was performed for the same subjects.

Statistical Analysis

With an alpha of 0.05 and 80% power, a sample size of 12 subjects in each phase would be required to detect at least a 50% absolute risk reduction in the rate of positive cultures between groups[19]. A McNemar test was used to compare the proportion of negative cultures following each treatment, and a paired t test was used to compare mean log bacterial CFUs. Power analysis calculations were done using G*Power (Heinrich-Heine Universität Düsseldorf), and statistical analysis calculations were performed using Stata/MP software (version 13.1; StataCorp). A p value of <0.05 was considered significant.

Results

Twelve subjects were recruited for each phase of the study. On the day of Phase-I testing, 1 subject declined to participate, thus leaving 11 subjects in Phase 1 and 12 in Phase 2 included in the final analysis. The subjects ranged in age from 22 to 27 years. Six male and 5 female subjects participated in Phase I, and 8 male and 4 female subjects participated in Phase II. No subject was included in both test phases.

A total of 184 culture samples were collected over both phases of the study. All pretreatment culture samples (92 of 92, 100%) were positive for bacteria, regardless of treatment assignment or culture condition. There were no differences in baseline aerobic or anaerobic bacterial burdens between the 2 treatment groups in either phase (Table II). No side effects of topical PCA or IPA were observed.

TABLE II Baseline (Prior to Treatment) Aerobic and Anaerobic Bacterial Burdens*

	Phase I			Phase II		
	10% PCA (N = 11)	IPA Alone (N = 11)	P Value	17% PCA (N = 12)	IPA Alone (N = 12)	P Value
Aerobic cultures						
Log CFUs	1.93 ± 0.64	2.01 ± 0.53	0.766	1.44 ± 0.80	1.28 ± 0.71	0.368
Anaerobic cultures						
Log CFUs	1.68 ± 0.72	1.84 ± 0.53	0.448	1.55 ± 0.72	1.57 ± 0.66	0.913

*The values are given as the mean and standard deviation. PCA = protocatechuic acid, IPA = isopropyl alcohol, and CFU = colony-forming unit.

TABLE III Aerobic and Anaerobic Bacterial Counts and Sterilization Rates Following Treatment Application*

	Phase I			Phase II		
	10% PCA (N = 11)	IPA Alone (N = 11)	P Value	17% PCA (N = 12)	IPA Alone (N = 12)	P Value
Aerobic cultures						
Sterilization rate (%)	10/11 (90.9%)	5/11 (45.5%)	0.0143	11/12 (91.7%)	9/12 (75.0%)	0.083
Log CFUs†	0 ± 0	1.062 ± 1.09	0.0092	0.025 ± 0.24	0.393 ± 0.72	0.113
Anaerobic cultures						
Sterilization rate (%)	9/11 (81.8%)	4/11 (36.4%)	0.0253	10/12 (83.3%)	5/12 (41.7%)	0.0143
Log CFUs†	0.064 ± 0.21	0.482 ± 0.59	0.0288	0.050 ± 0.19	0.705 ± 0.45	0.0162
Persistent *C. acnes* (%)	2/11 (18.2%)	—	—	0/12 (0%)	—	—

*PCA = protocatechuic acid, IPA = isopropyl alcohol, and CFU = colony-forming unit. †The values are given as the mean and standard deviation.

Antimicrobial Activity in Human Skin

Following Phase-I treatment, we found that the sterilization rate for shoulders treated with 10% PCA was significantly higher than that for IPA-treated shoulders against aerobic (90.9% compared with 45.5%; p = 0.0143) and anaerobic (81.8% compared with 36.4%; p = 0.0253) organisms (Table III). The use of 10% PCA was associated with significantly lower mean aerobic (p = 0.0092) and anaerobic (p = 0.0288) bacterial counts.

Following Phase-II treatment, the sterilization rate for shoulders treated with 17% PCA was significantly higher than that for IPA-treated shoulders against anaerobic organisms (83.3% compared with 41.7%; p = 0.0143), and trended toward a significantly higher rate against aerobic organisms (91.7% compared with 75%; p = 0.083). The use of 17% PCA was associated with significantly lower mean anaerobic counts compared with IPA (p = 0.0162), and trended toward significantly lower mean aerobic counts (p = 0.113).

Efficacy of PCA Against C. acnes

DNA analysis was performed for subjects with positive anaerobic culture results following Phase-I PCA treatment and those with positive anaerobic cultures following Phase-II PCA treatment. Two additional Phase-II subjects who demonstrated marked reductions in anaerobic bacterial counts following PCA treatment were tested (n = 2 subjects in phase I, and n = 4 subjects in Phase II).

In Phase I, *C. acnes* was identified in 2 (18.2%) of 11 subjects following topical application of 10% PCA. Both of these subjects had >200 anaerobic CFUs prior to treatment, with prominent colonies of *C. acnes* identified. Following treatment, 1 CFU of *C. acnes* was grown on culture for each of these subjects.

In Phase II, 0 (0%) of 12 subjects were positive for *C. acnes* following topical application of 17% PCA. Prior to treatment, prominent colonies of *C. acnes* were identified in 3 of the 4 shoulders of subjects for whom Phase-II DNA analysis was performed. *Staphylococcus epidermidis* was the only organism identified for subjects with positive anaerobic culture results following treatment with 17% PCA.

Discussion

Reducing the rising health-care burden associated with shoulder SSI is of paramount importance. In this proof-of-principle pilot study, we examined the potential of PCA as a topical antimicrobial for antisepsis of the skin surrounding the shoulder joint. Our hypothesis was confirmed, as topical application of PCA was associated with significantly reduced bacterial burden of the skin surrounding the shoulder and resulted in the sterilization of aerobic bacteria in 21 of 23 shoulders and of anaerobic bacteria in 19 of 23 shoulders over 2 phases. In addition, topical PCA demonstrated dose-dependent antimicrobial activity against *C. acnes* in the skin and skin appendages of young, healthy subjects. The present study demonstrates the antimicrobial efficacy of topical PCA in human skin, including activity against shoulder-joint pathogens. Future studies involving a shoulder surgical population are warranted to determine the potential of topical PCA to be included in shoulder skin disinfection regimens.

As one of the biologically active components identified in several natural medicines, PCA has been used for decades and is generally regarded as safe[20-22]. Investigations into the pharmacological profile of PCA have demonstrated antimicrobial properties, although many of these studies focused on plant extracts of which the exact ingredients and dosages are unknown[23,24]. In other work by our group, we performed antimicrobial susceptibility testing of PCA and 7 related phenolic compounds against several common pathogens. Compared with related compounds, PCA demonstrated the broadest spectrum of antimicrobial activity, including efficacy against organisms including *S. aureus, methicillin-resistant S. aureus (MRSA), Pseudomonas aeruginosa,* and *C. acnes,* among others[12]. These findings suggest that PCA was the likely active antimicrobial component seen in prior food-plant-based studies, and help to identify its potential for clinical application in the treatment of skin and wound pathogens. The results of the present study suggest that the broad-spectrum antimicrobial activity of PCA seen in laboratory testing may be maintained when applied as a topical reagent for human skin antisepsis.

Antimicrobial penetration into the deeper layers of the skin is believed to be an important property for the optimization of topical shoulder skin antisepsis protocols[5]. Difficulty in achieving this has been hypothesized to contribute to the persistence of *C. acnes* in the dermal layer of the skin despite prophylaxis with a variety of existing agents[7,9,10,19].

An in vitro study demonstrated potent skin-penetration properties and deep dermal bioavailability of PCA when used as a topical agent[25]. The authors, Zillich et al., compared the skin permeability and dermal availability of 6 topical polyphenols in excised pig skin. Topical PCA demonstrated the greatest skin permeability and dermal penetration when compared with the other polyphenols. In addition, the drug formulation and concentration of test compounds were shown to impact skin penetration[25]. In preclinical work by our group, we tested the skin penetrative properties of topical PCA in living skin using an intradermal injection model of *C. acnes* skin infection in a mouse. Three separate doses of topical PCA were applied at daily intervals, and *C. acnes* skin loads were calculated 96 hours after inoculation. The daily application of 78 mM topical PCA resulted in an approximately 13.8-times lower *C. acnes* load in harvested skin specimens compared with untreated mice. However, both lower doses (39 and 19.5 mM) failed to show an effect[12]. In agreement with the findings of Zillich et al., these experiments suggested that the skin penetrative properties of topical PCA were dose-dependent and that increasing doses could improve the antimicrobial activity of topical PCA against dermal *C. acnes* loads in living skin.

In the present study, 2 different concentrations of topical PCA were tested for their activity against *C. acnes* loads in human shoulder skin. Following Phase-I treatment with 10% topical PCA, persistence of this organism in 2 of 11 subjects was demonstrated, which we hypothesized may be because of insufficient skin penetration. To improve the efficacy of PCA against dermal *C. acnes* in Phase II, we increased the dose and added ingredients to the formulation to enhance transdermal penetration. PG and EPO have previously been shown to enhance transdermal penetration when included in topical drug formulations[13-15].

The Phase-II formulation of topical 17% PCA, PG, and EPO resulted in a *C. acnes* colonization rate of 0% (0 of 12 subjects) following treatment, despite this organism being identified in at least 3 Phase-II subjects prior to treatment (of 4 subjects screened). These findings suggest that the Phase-II PCA formulation was able to eradicate *C. acnes* shoulder skin colonization in healthy subjects, and agree with previous work by demonstrating that the skin penetrative properties and antimicrobial activity of topical PCA against *C. acnes* in human skin may be dependent on the dose and formulation tested. Future studies comparing PCA as an adjunctive or alternative topical agent to existing shoulder skin antisepsis regimens, including benzoyl peroxide (BP), are necessary to determine the potential of PCA to reduce infections following shoulder surgery. Our group plans to perform a randomized controlled trial comparing PCA monotherapy, BP monotherapy, and combination therapy for SSI prevention in a shoulder surgical population.

There were several limitations to the present study. First, our harvesting method for shoulder skin pathogens differs from methods previously reported in the literature, although there is no consensus on harvesting samples for culturing the deeper-lying organisms surrounding the shoulder, such as *C. acnes*[5,18], and the authors of previous studies using mechanical rubbing/scraping protocols for deeper skin exposure have reported similar bacterial colonization rates[17]. In addition, our subjects could not be blinded to treatment because of the differences in smell and appearance between the test agents. However, all samples were collected by the same 2 investigators using an identical protocol to limit sampling error, and organism culturing and identification were performed at an independent laboratory that was unaware of treatment assignments. The subjects were young, healthy volunteers who were not surgical candidates, limiting the generalizability of these findings. Furthermore, we did not test skin regions other than the deltopectoral interval that are known to have high burdens of *C. acnes,* including the chest and upper back[19]. The choice of IPA as a control reagent may also be viewed as a limitation. In performing a pilot study, our intention was not to demonstrate the superiority of PCA over existing antisepsis protocols but to determine whether PCA exhibits sufficient antimicrobial activity against human shoulder skin pathogens to warrant further investigation. Lastly, we assigned a numerical cutoff value of 200 to any culture with ≥200 CFUs. This precluded the analysis of reductions in bacterial burdens with each treatment, as only bacterial counts obtained following treatment maintained the precision for comparative analysis.

In conclusion, the topical application of PCA reduced the bacterial burden of the skin surrounding the shoulder joint and demonstrated dose-dependent antimicrobial activity against *C. acnes* colonization in the skin and skin appendages of young, healthy subjects. These data provide a basis for future research employing the application of PCA as a novel topical antimicrobial. Clinical studies involving a shoulder surgical population are warranted to determine its potential for application in surgical skin antisepsis to reduce infection following shoulder surgery. ■

Omid Jalali, MD[1]
Molly Best, MD[2]
Alison Wong, MD[2]
Brett Schaeffer, MD[2]
Brendon Bauer, MD[2]
Lanny Johnson, MD

[1]Keck School of Medicine of USC, Los Angeles, California

[2]Loma Linda Department of Orthopedics (M.B.) and Loma Linda University School of Medicine (A.W., B.S., and B.B.), Loma Linda, California

Email address for O. Jalali: omidjalali3737@gmail.com

ORCID iD for O. Jalali: 0000-0003-2511-4621
ORCID iD for M. Best: 0000-0002-5571-4153
ORCID iD for A. Wong: 0000-0002-0398-6480
ORCID iD for B. Schaeffer: 0000-0002-0784-9141
ORCID iD for B. Bauer: 0000-0002-0666-1411
ORCID iD for L. Johnson: 0000-0002-1433-4410

References

1. Whitehouse JD, Friedman ND, Kirkland KB, Richardson WJ, Sexton DJ. The impact of surgical-site infections following orthopedic surgery at a community hospital and a university hospital: adverse quality of life, excess length of stay, and extra cost. Infect Control Hosp Epidemiol. 2002 Apr;23(4):183-9.

2. Grosso MJ, Frangiamore SJ, Ricchetti ET, Bauer TW, Iannotti JP. Sensitivity of frozen section histology for identifying Propionibacterium acnes infections in revision shoulder arthroplasty. J Bone Joint Surg Am. 2014 Mar 19;96(6):442-7.

3. Portillo ME, Corvec S, Borens O, Trampuz A. Propionibacterium acnes: an underestimated pathogen in implant-associated infections. Biomed Res Int. 2013; 2013:804391. Epub 2013 Nov 6.

4. Qiu B, Al K, Pena-Diaz AM, Athwal GS, Drosdowech D, Faber KJ, Burton JP, O'Gorman DB. Cutibacterium acnes and the shoulder microbiome. J Shoulder Elbow Surg. 2018 Oct;27(10):1734-9. Epub 2018 Jun 13.

5. Matsen FA 3rd, Butler-Wu S, Carofino BC, Jette JL, Bertelsen A, Bumgarner R. Origin of Propionibacterium in surgical wounds and evidence-based approach for culturing Propionibacterium from surgical sites. J Bone Joint Surg Am. 2013 Dec 4; 95(23):e1811-7.

6. Phadnis J, Gordon D, Krishnan J, Bain GI. Frequent isolation of Propionibacterium acnes from the shoulder dermis despite skin preparation and prophylactic antibiotics. J Shoulder Elbow Surg. 2016 Feb;25(2):304-10. Epub 2015 Oct 9.

7. Rao AJ, Chalmers PN, Cvetanovich GL, O'Brien MC, Newgren JM, Cole BJ, Verma NN, Nicholson GP, Romeo AA. Preoperative doxycycline does not reduce Propionibacterium acnes in shoulder arthroplasty. J Bone Joint Surg Am. 2018 Jun 6; 100(11):958-64.

8. Hendley JO, Ashe KM. Eradication of resident bacteria of normal human skin by antimicrobial ointment. Antimicrob Agents Chemother. 2003 Jun;47(6):1988-90.

9. Lee MJ, Pottinger PS, Butler-Wu S, Bumgarner RE, Russ SM, Matsen FA 3rd. Propionibacterium persists in the skin despite standard surgical preparation. J Bone Joint Surg Am. 2014 Sep 3;96(17):1447-50.

10. Heckmann N, Sivasundaram L, Heidari KS, Weber AE, Mayer EN, Omid R, Vangsness CT Jr, Hatch GF 3rd. Propionibacterium acnes persists despite various skin preparation techniques. Arthroscopy. 2018 Jun;34(6):e0078-9. Epub 2018 Mar 24.

11. Spellberg B. The future of antibiotics. Crit Care. 2014 Jun 27;18(3):228. Epub 2014 Jun 27.

12. Jalali O, Best M, Wong A, Schaeffer B, Bauer B, Johnson L. Protocatechuic acid as a topical antimicrobial for surgical skin antisepsis: preclinical investigations. JBJS Open Access. 2020. [In press].

13. Chen J, Jiang QD, Wu YM, Liu P, Yao JH, Lu Q, Zhang H, Duan JA. Potential of essential oils as penetration enhancers for transdermal administration of ibuprofen to treat dysmenorrhoea. Molecules. 2015 Oct 7;20(10):18219-36.

14. Jiang Q, Wu Y, Zhang H, Liu P, Yao J, Yao P, Chen J, Duan J. Development of essential oils as skin permeation enhancers: penetration enhancement effect and mechanism of action. Pharm Biol. 2017 Dec;55(1):1592-600.

15. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta. 2009 Nov;1788(11):2362-73. Epub 2009 Sep 3.

16. Padegimas EM, Maltenfort M, Ramsey ML, Williams GR, Parvizi J, Namdari S. Periprosthetic shoulder infection in the United States: incidence and economic burden. J Shoulder Elbow Surg. 2015 May;24(5):741-6. Epub 2015 Jan 13.

17. Kolakowski L, Lai JK, Duvall GT, Jauregui JJ, Dubina AG, Jones DL, Williams KM, Hasan SA, Henn RF 3rd, Gilotra MN. Neer Award 2018: benzoyl peroxide effectively decreases preoperative Cutibacterium acnes shoulder burden: a prospective randomized controlled trial. J Shoulder Elbow Surg. 2018 Sep;27(9):1539-44. Epub 2018 Jul 24.

18. Butler-Wu SM, Burns EM, Pottinger PS, Magaret AS, Rakeman JL, Matsen FA 3rd, Cookson BT. Optimization of periprosthetic culture for diagnosis of Propionibacterium acnes prosthetic joint infection. J Clin Microbiol. 2011 Jul;49(7):2490-5. Epub 2011 May 4.

19. Heckmann N, Heidari KS, Jalali O, Weber AE, She R, Omid R, Vangsness CT, Rick Hatch GF 3rd. Cutibacterium acnes persists despite topical clindamycin and benzoyl peroxide. J Shoulder Elbow Surg. 2019 Dec;28(12):2279-83. Epub 2019 Aug 27.

20. Kakkar S, Bais S. A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacol. 2014 Mar 26;2014:952943.

21. Khan AK, Rashid R, Fatima N, Mahmood S, Mir S, Khan S, Jabeen N, Murtaza G. Pharmacological activities of protocatechuic acid. Acta Pol Pharm. 2015 Jul-Aug; 72(4):643-50.

22. Semaming Y, Pannengpetch P, Chattipakorn SC, Chattipakorn N. Pharmacological properties of protocatechuic acid and its potential roles as complementary medicine. Evid Based Complement Alternat Med. 2015;2015:593902. Epub 2015 Feb 9.

23. Lim HJ, Jeon YD, Kang SH, Shin MK, Lee KM, Jung SE, Cha JY, Lee HY, Kim BR, Hwang SW, Lee JH, Sugita T, Cho O, Myung H, Jin JS, Lee YM. Inhibitory effects of Euphorbia supina on Propionibacterium acnes-induced skin inflammation in vitro and in vivo. BMC Complement Altern Med. 2018 Sep 27;18(1):263.

24. Kuete V, Nana F, Ngameni B, Mbaveng AT, Keumedjio F, Ngadjui BT. Antimicrobial activity of the crude extract, fractions and compounds from stem bark of Ficus ovata (Moraceae). J Ethnopharmacol. 2009 Jul 30;124(3):556-61. Epub 2009 May 18.

25. Zillich OV, Schweiggert-Weisz U, Hasenkopf K, Eisner P, Kerscher M. Release and in vitro skin permeation of polyphenols from cosmetic emulsions. Int J Cosmet Sci. 2013 Oct;35(5):491-501. Epub 2013 Jul 17.