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TITLE: Preoperative Topical Treatment and the Prevalence of Propionibacterium Acnes

STUDY PHASE: Pilot Study

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1.0 Background

Great efforts are undertaken in the perioperative period to avoid infection, a major cause of morbidity and mortality. Still, infection rates can range from 0.6% to 5%, depending on the type of surgery performed.¹ Studies in the orthopaedic literature have shown that organisms usually characterized as more indolent, such as *P. acnes*, a known skin pathogen, cause surgical site infections following shoulder surgery despite proper preoperative surgical preparation.² Matsen et al. hypothesized that *P. acnes* infections do not originate from the superficial epidermis, but from the dermal layer, which may be expected as *P. acnes* is known to reside in the sebaceous glands and hair bulbs.^{2,3}

Lee et al. showed a 70% growth rate of *P. acnes* despite the application of Chloraprep prior to sampling, contradicting previous studies that found only 7% growth in the superficial layer of the skin using the same skin preparation.^{4,5} However, this study was limited as it failed to utilize a control group and only investigated one preparatory technique. Meanwhile, there remains to date no study investigating the effect of topical treatments in eradicating *P. acnes* within the dermal layer. The purpose of this study is to investigate how specific topical treatments affect growth in the dermal layer.

2.0 Objective and Purpose

1. What is the prevalence of Propionibacterium in the dermal layer following topical treatments?
2. Does the type of topical treatment used affect the prevalence of Propionibacterium in the dermal layer?

Primary Outcome:

Positive growth of Propionibacterium acnes on bacterial culture.

Secondary Outcomes:

Positive hemolysis on brucella agar.

3.0 Study Design

Twelve healthy volunteers who are over the age of 18 will be consented to participate in our study. Those individuals with active acne or those who received a course of antibiotics in the last month will be excluded. The subject's upper back will be sectioned into quadrants above the scapular spine, which will be each assigned a different topical treatment: clindamycin alone, benzoyl peroxide alone, clindamycin plus benzoyl peroxide, and a control, with no topical application. Each application will be applied twice daily for 3 consecutive days. Randomization will be performed using a random number generator to draw numbers 1-4 in order to designate a topical treatment to each quadrant. Quadrants will be labeled with surgical markers to ensure the correct treatment is applied. Research subject will be trained on application using a mirror with the research staff during recruitment.

A 3-mm dermal punch biopsy specimen will be obtained at each site using a commercially available kit after prepping with isopropyl alcohol and sterile injection of local anesthesia with 2% lidocaine with epinephrine. The biopsy sites will be covered with sterile gauze and allowed to heal without closure. Unfortunately, due to procedural limitations, the dermal layer of the skin cannot be accessed without penetrating the epidermis. Thus even separating the dermis from the epidermis is not helpful because the dermis will have been contaminated from potential pathogens from the epidermis during the biopsy procedure.

A total of four punch biopsies will be obtained from the subject's back; one for each of type of topical treatment and the control. The four punch biopsy specimens will then be cultured for *P. acnes*, with serotyping, and held for 21 days to assess for growth. Assessment of growth will be made the USC Microbiology Lab, who will be blinded to the treatment condition.

Pipettes will be used to inoculate the fluid onto the following media: blood agar, chocolate agar, brucella agar, and brain heart infusion broth. This same culture technique was used by Lee et al, in their published study investigating *P. acnes* levels in the dermis of the skin.⁴ In order to determine hemolytic subtype, the samples will also be tested on Brucella Blood agar, as described by Wright et al.⁶

4.0 Drug/Device Information

- Clindamycin 1% topical gel
- Benzoyl Peroxide 5% topical gel
- Clindamycin 1% plus Benzoyl Peroxide 5% topical gel
- Lidocaine 2% with epinephrine

5.0 Selection and Withdrawal of Subjects

Inclusion Criteria:

- Age > 18 years

Exclusion Criteria:

- History of antibiotic use in the last month
- Active acne on the back
- Non-English speakers (the study personnel do not have adequate training to converse with and consent in other languages)

6.0 Sample Size

We will recruit participants until there are 12 total subjects.

Power Analysis:

Data from previous studies were used to estimate that 70% of cultures will grow positive for *P. acnes* with no therapy.⁴ For a clinically meaningful response, we would expect only 5% of cultures to grow positive for *P. acnes* following treatment. Thus, for an alpha of 0.05 and power of 80%, we would need a minimum of 12 biopsies per treatment group to have adequate power, based on a McNemar test for paired proportion comparison.

Sample Size:

We will recruit a minimum of 12 total subjects to achieve adequate power. Because each patient is receiving 4 biopsies, there will be a total of 48 samples.

7.0 Study Agent Administration

Study participation will be discontinued if subject experience any of the side effects listed in section 8.0

A participant may always be removed from study participation whenever he/she wishes

8.0 Assessment of Efficacy and Safety

Side effects and toxicities to be monitored:

1. Anaphylactic reaction: symptoms include flushing, itching, swelling, difficulty breathing, and fainting.
2. Nerve Weakness
3. Infection
4. Fever
5. Swelling
6. Abscess formation
7. Dizziness
8. Visual Disturbances
9. Headache
10. Nausea
11. Seizure
12. Diarrhea
13. Abdominal pain
14. Arrhythmia (abnormal heart rhythm)

Because only 2-4 mL of 2% lidocaine with epinephrine will be used there will be no longterm follow-up for these subjects.

The principal investigator is the individual responsible for monitoring and reporting the occurrence of adverse events throughout the study. Subjects will be monitored throughout the study. Subjects will be taken off the study participation if they experience any side effects. USC orthopaedic staff will also monitor for any un-anticipated events. Anticipated adverse events are discussed in the Informed Consent Form for this study.

The research team will follow the IRB Adverse Even Policy on mandatory reporting of Serious Adverse Events (SAEs), and we will also report them immediately both orally and in writing to the IRB Program Director within 24 hours of occurrence or recognition.

9.0 Clinical and Laboratory Evaluations

Medical History Assessment Questions:

1. Are you over the age of 18?
2. Are you pregnant or think you could possibly be pregnant?
3. Have you used antibiotics in the last month?

Back Examination:

1. Inspection of the back of any lesions, rash, acne, or deformities.

10.0 Criteria for Evaluation and Endpoint Definitions

All eligible subjects who meet inclusion criteria will be included in the analysis.

The primary outcome will be positive growth of *P. acnes* on culture. Secondary outcome will be subtyping based on hemolysis. The USC Microbiology lab assess the samples and will be blinded to treatment condition. They will only receive the participant ID and corresponding treatment number.

11.0 Data Collection and Monitoring

Protections against risks:

All key personnel have completed courses on protection of human subjects.

Protections against biomedical risks:

Only qualified trained orthopaedic surgeons will be performing the biopsies using sterile technique.

Confidentiality:

To minimize this risk, only the investigators will have access to all data containing personal information. All data will be locked in the PI's office and/or lab. To preserve confidentiality, immediately after enrollment the subjects will be assigned a code by which each research subject will be identified for further analysis.

Data management:

All the study data will be collected by the research team and recorded on data collection form, found in section 16.0. Immediately after enrollment each subject will be assigned a code by which each research subjects will be identified for further analysis to avoid identification by non-qualified individuals.

12.0 Statistical Considerations

Statistical Analysis:

Primary outcomes will be treatment comparisons on the percentage of subjects with positive cultures for *P. acnes*. Primary outcomes will be analyzed using generalized estimating equations to account for the within-subject correlated outcomes, as all 4 interventions will be evaluated on each subject. Given the dichotomous outcome (presence/absence of *P. acnes*), the analysis will use a logistic model (binomial random outcome with a logit link function). Three indicator treatment variables will be specified (e.g., clindamycin, benzoyl peroxide, clindamycin plus benzoyl peroxide; with lidocaine as the referent group). A 3df overall test of the treatment indicators will test for any differences in positivity for *P. Acnes* among the treatments; pairwise comparisons among the treatments will adjust for multiple comparisons. A two-tailed statistical test will be performed, testing at an alpha of 0.05. Analyses will also be performed based on hemolytic subtypes.

Power Analysis:

Data from previous studies were used to estimate that 70% of cultures will grow positive for *P. acnes* with no therapy.⁴ For a clinically meaningful response, we would expect only 5% of cultures to grow positive for *P. acnes* following treatment. Thus, using a McNemar test for paired proportion comparison, with an alpha of 0.05 and power of 80%, we would need a minimum of 12 biopsies per treatment group to have adequate power.

Sample Size:

We will recruit a minimum of 12 total subjects to achieve adequate power. Because each patient is receiving 4 biopsies, there will be a total of 48 samples.

13.0 Registration Guideline

At the time of registration, two copies of a signed and dated patient Informed Consent form with Bill of Rights must be available (one copy for the patient; and the other for the PI's file)

14.0 Biohazard Containment

Biohazardous materials will be disposed of properly in clinic.

15.0 Ethical and Regulatory Considerations

All institutional and Federal regulations concerning the Informed Consent form will be fulfilled. The study will be conducted in adherence to ICH Good Clinical Practice.

16.0 Data Collection Sheet

Pt Code	Age	Sex	Biopsy group (Control vs. Clindamycin vs. Benzoyl Peroxide vs. Clindamycin and Benzoyl Peroxide)	Positive Culture	Hemolytic Subtype	Adverse Events?

17.0 References

1. Dumville JC, McFarlane E, Edwards P, Lipp A, Holmes A. Preoperative skin antiseptics for preventing surgical wound infections after clean surgery. *Cochrane Database Syst Rev*. 2013;3:Cd003949.
2. Mook WR, Garrigues GE. Diagnosis and Management of Periprosthetic Shoulder Infections. *J Bone Joint Surg Am: Incorporated.*; 2014. p. 956-65.
3. 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.
4. 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.
5. Saltzman MD, Nuber GW, Gryzlo SM, Marecek GS, Koh JL. Efficacy of surgical preparation solutions in shoulder surgery. *J Bone Joint Surg Am*. 2009 Aug;91(8):1949-53.
6. Wright TE, Boyle KK, Duquin TR, Crane JK. Propionibacterium acnes susceptibility and correlation with hemolytic phenotyp. *Infectious Diseases: Research and Treatment*. 2016 Oct;9(9):39-44.
8. Hibbard JS. Analyses comparing the antimicrobial activity and safety of current antiseptic agents: a review. *J Infus Nurs*. 2005 May-Jun;28(3):194-207.
9. Butler-Wu SM, Burns EM, Pottinger PS, Magaret AS, Rakeman JL, Matsen FA, 3rd, et al. Optimization of periprosthetic culture for diagnosis of Propionibacterium acnes prosthetic joint infection. *J Clin Microbiol*. 2011 Jul;49(7):2490-5.