Use of high-resolution microendoscopy (HRME) in patients with adenocarcinoma in-situ (AIS) of the cervix

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1.0 Objectives

1. To determine the feasibility of acquiring *in-vivo* high-resolution microendoscopy (HRME) images of cervical adenocarcinoma *in situ* (AIS) immediately prior to conization.

2. To determine accuracy of HRME images in distinguishing AIS from normal cervical tissue.

2.0 Background

Cervical adenocarcinoma *in situ* (AIS):
Cervical AIS is a precursor to invasive cervical adenocarcinoma, which accounts for approximately 25% of cervical cancers in the United States (1). While the incidence of squamous cell carcinoma of the cervix has been on the decline in the U.S. due to widespread screening, detection, and treatment of precancerous lesions, the incidence of both AIS and invasive cervical adenocarcinoma has been rising for unclear reasons (2). Similar to squamous cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma, cervical AIS and invasive adenocarcinoma are caused by persistent cervical infections by carcinogenic or high-risk human papillomavirus (HPV). AIS is histologically characterized by atypical columnar epithelial cells with no invasion present. Unlike squamous CIN lesions, 10 to 13% of patients with AIS have multifocal disease, with foci of AIS separated by at least 2 mm of normal mucosa ("skip" lesions) (1,3-5).

The majority of patients are asymptomatic and the diagnosis of AIS is usually made following an abnormal Pap test. There are no clearly distinguishable clinical, cytological or colposcopic features of AIS. The lesion is usually located at the transformation zone with contiguous involvement of the endocervical canal (1,6). Cervical cytology and colposcopy-directed biopsy can miss up to 60% of AIS cases, however, the addition of endocervical curettage (ECC) significantly improves the detection rate (7). Cervical conization is usually required to make the diagnosis of AIS. Cold knife conization is preferred over loop electrosurgical excision procedure (LEEP) as it minimizes the risk of thermal artifact and allows the pathologist to determine the presence of invasive disease and margin status (8-10).

AIS is difficult to manage conservatively for several reasons. The lesion may be located high in the endocervical canal or be multicentric with "skip" lesions present. The sensitivity of cervical cytology and ECC is therefore only approximately 50%, making it difficult to adequately monitor women with AIS managed conservatively (4,11). In addition, the incidence of residual AIS or invasive adenocarcinoma following conization for AIS is high (3-5, 9, 12-14). Given these challenges, the standard treatment of AIS in women who have completed childbearing is simple hysterectomy. However, if the cervical cone margins are positive, a repeat cervical conization is performed prior to proceeding to simple hysterectomy to confirm negative margins and rule out an invasive occult cancer that could require different treatment. For women who desire future fertility, conservative management with cervical cone is an alternative provided the conization margins and ECC sample are negative. In women with a positive margin or ECC, repeat conization to remove the entire lesion and exclude the presence of invasive disease is recommended.

High-Resolution Microendoscopy (HRME) Imaging:
The HRME imaging system is a low-cost, innovative cervical visualization technique to evaluate epithelial cell morphology *in situ*. Proflavine, a topical contrast agent is applied to the cervix in a manner similar to the application of acetic acid at the time of colposcopy. The tip of a small fiber-optic probe is then placed directly onto the cervix, and the fluorescence from the proflavine-stained epithelium is immediately transmitted back to the HRME unit and displayed on a laptop computer, tablet or cell phone screen (Fig. 1). Morphologic features typically evaluated by pathologists including nuclear crowding, pleomorphism, and nuclear-to-cytoplasm (N/C) ratio are assessed *in vivo* in real-time. Image analysis software is then used to quantify nuclear morphology parameters and calculate N/C ratio. This approach allows for real-time, point-of-care detection of high-grade precancerous cervical lesions without a biopsy being performed. The technique has been shown to be effective in the evaluation of squamous lesions and is being studied as part of a "See & Treat" approach in limited resource settings where patients can be screened and treated at the same visit with cryotherapy or LEEP.

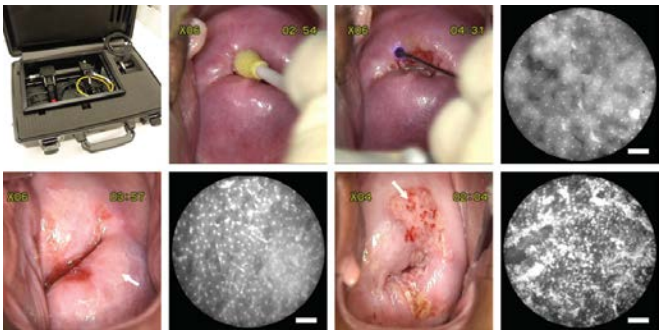

Fig. 1 (a) HRME system; (b) Proflavine is applied; (c) fiber optic probe; (d) high-resolution image is displayed on a laptop computer in real-time; (e) colposcopic view of lesion at 5:00, and (f) resulting HRME image. Histologic diagnosis was normal, consistent with the HRME image showing small, evenly spaced nuclei. (g) Colposcopic view of lesion at 1:00, and (f) resulting HRME image. Histologic diagnosis of this site was CIN3, consistent with the HRME image showing large, crowded, pleomorphic nuclei.

3.0 Preliminary Data and Rationale

Cervical adenocarcinoma in-situ (AIS):
Our group recently performed a large, retrospective study evaluating the outcomes of 188 patients with AIS (15). The mean age at diagnosis was 33.8 years (range 17.6-76.1), with many women of childbearing age. One hundred and seventy-two of the 180 women had at least one cone biopsy performed, with 110 (64.0%) undergoing a cold knife cone (CKC), and 62 (36.0%) undergoing a LEEP as their initial method of treatment. Positive margins were noted in 35.0% of patients undergoing CKC compared with 55.6% undergoing LEEP (p=0.017). Seventy-one patients ultimately underwent hysterectomy with residual disease noted in 10 patients (14.1%), 8 patients (11.3%) with residual AIS and 2 patients (2.8%) with invasive carcinoma. Of the 101 patients who did not undergo hysterectomy, 2 patients (2.0%) developed recurrent AIS at a median of 27.5 months (range 18- 37 months) from the last cone, and none developed invasive carcinoma. However, 28.6% of patients required more than one conization to achieve negative margins. The study concluded that conservative, fertility-sparing management of AIS with CKC alone is feasible but that two or more CKCs may be required to obtain negative margins.

High-Resolution Microendoscopy (HRME) Imaging:
A pilot study evaluating HRME imaging for pre-invasive cervical neoplasia was recently

performed in 174 women in rural China (16). All patients underwent HPV testing, VIA, colposcopy and HRME imaging. Of the 69 women noted to have abnormalities on colposcopy, only 12 (17%) showed high-grade disease on biopsy. However, HRME imaging correctly classified all 12 high-grade areas (100%) as abnormal, and correctly classified 38 of the remaining 57 (67%) as normal. Furthermore, when patients were stratified based on a positive high-risk HPV DNA test, HRME imaging correctly identified 100% of the patients with CIN2 or greater. Of the 30 patients with a positive high-risk HPV DNA test but no histologic evidence of disease, only 6 patients (20%) were incorrectly identified as abnormal on HRME imaging. These preliminary data suggest that HRME has improved specificity over VIA and colposcopy, potentially leading to more accurate identification of patients needing treatment as part of a *See & Treat* protocol. Our collaborative group also recently completed a pilot study comparing HRME to colposcopy and biopsy in 60 patients with cervical dysplasia at Barretos Cancer Hospital in Brazil with final data analysis pending.

Our group is also evaluating HRME for use in conservative, fertility-sparing surgery for women with endometrial cancer. We are evaluating if HRME can help distinguish tumor margins in women undergoing resection of the endometrial tumor without hysterectomy. We recently completed a prospective pilot study evaluating the use of HRME in patients with endometrial cancer. Following hysterectomy, the uterus was examined *ex-vivo* to identify whether HRME could distinguish between tumor and normal endometrium following the application of proflavine. Preliminary results show a 74% concordance rate between HRME and final pathology, which is significantly higher than the 59% concordance rate between gross evaluation and final pathology. A prospective trial evaluating HRME *in-vivo* in women with endometrial cancer was recently approved and accrual will begin in the near future.

Rationale:
Cervical AIS is a significant pre-invasive disease that affects women of childbearing age. For women who desire future fertility, conservative management with cervical conization is considered a feasible option. However, the treatment is challenging as lesions are often located high in the endocervical canal, and are multicentric with “skip” lesions present. Repeat conizations must therefore be performed until negative margins are obtained. Given the microscopic nature of the disease, the surgeon typically cannot visually distinguish AIS from normal tissue in order to obtain negative margins. Furthermore, frozen section is difficult and inaccurate in assessing AIS margins. This lack of accurate intraoperative assessment results in 25-30% of patients undergoing one or more repeated conizations to obtain negative margins. In addition, an unnecessarily large specimen is sometimes removed in an attempt to be certain of obtaining negative margins. These large and repeat cervical conizations are known to be associated with adverse obstetrical outcomes, including preterm delivery and very low birth weight infants (17-19).

In order to overcome these challenges associated with the conservative management of AIS, we are proposing to use HRME, a novel cervical visualization technique, to evaluate epithelial cell morphology *in situ* in order to distinguish AIS from normal cervical tissue. *Our overall goal is to eventually use HRME to guide resection margins at the time of cervical conization for AIS*. Our *primary objective* is to determine the feasibility of acquiring *in-vivo* high-resolution microendoscopy (HRME) images of cervical adenocarcinoma *in situ* (AIS) immediately prior to conization. Our *secondary objective* is to determine accuracy of HRME images in distinguishing AIS from normal cervical tissue.

4.0 Eligibility Criteria

Inclusion Criteria**:**
1. Any woman with a confirmed preoperative diagnosis of cervical AIS, including co-existing squamous CIN and/or microinvasive cancer
2. Women undergoing cold knife cone (CKC) of the cervix at MD Anderson
3. Negative pregnancy test for women of child-bearing potential
4. Women who are >/= 21 years of age and < 65 years of age
5. Ability to understand and the willingness to provide informed consent and sign a written Informed Consent Document (ICD)

Exclusion Criteria:
1. Women < 21 years of age and >/= 65 years of age
2. Women with a known allergy to proflavine or acriflavine
3. Women who are pregnant or nursing
4. Patients unable or unwilling to provide informed consent or sign a written Informed Consent Document (ICD)

5.0 Pretreatment Evaluation/Obtaining Informed Consent:

Patients will be screened for the study prior to surgery in the MD Anderson Cancer Center Colposcopy and Gynecologic Oncology clinics. All eligible patients will be approached by the PI or designee and offered participation in the study. Written informed consent will be obtained. The patient will be registered in the Clinical Oncology Research System (CORe). All patients will have their surgery performed at MD Anderson. Patients who are of child-bearing potential will have a pre-treatment pregnancy test (within 7 days) prior to surgery performed as part of their standard of care.

6.0 Research Plan and Methods

Treatment Plan:
The patient will be taken to the operating room and per standard practice, prior to converting the surgical area to a sterile field, an exam under anesthesia will be performed. Five percent acetic acid will be applied to the cervix. Routine colposcopy with visual inspection of the cervix for aceto-whitening will be performed and the findings noted. This will be followed by the topical application of 0.01% proflavine solution to the cervix. The HRME probe will then be applied to areas of the cervix that appear to be both normal and abnormal. High-resolution images will be obtained and the findings noted. Lugol's solution may then be applied to the cervix per standard practice and the findings noted. This may be followed by re-application of 0.01% proflavine solution to the cervix. The HRME probe will then be applied to areas of the cervix that appear to be both normal and abnormal. High-resolution images will be obtained and the findings noted.

The patient and surgical area will then be prepped in the typical sterile fashion. A cold knife cone biopsy will then be performed per standard practice. The investigational procedures are anticipated to add approximately 10 minutes to the patient's time in the operating room.

Immediately following the CKC, the removed surgical specimen will be evaluated. Proflavine will be reapplied to the surgical specimen and repeat evaluation with HRME performed and high-resolution images obtained. Small ink dots will be placed on the tissue surface at each area that was noted to be abnormal as well as normal areas, to enable co-registration of image sites with subsequent histopathology sections. The specimen will then be submitted to pathology for the usual pathological analysis.

A member of the research team will contact each participant within 30 days of the procedure to ask how they are feeling and if they are experiencing any of the symptoms mentioned under the risks and side effects section of the informed consent.

Proflavine:
Proflavine powder will be purchased from Sigma-Aldrich and the solution will be prepared by the MD Anderson Investigational Pharmacy Services using specified instructions.

Data:
The protocol specific data collected will be stored in the Research Electronic Data Capture (REDCap) System/CORe.
Data collected will include the following, as well as all other protocol specific data:

- Age at diagnosis
- Histology
- Date of diagnosis
- Date of surgery
- Operative time
- Duration of time for intraoperative HRME imaging
- HRME imaging findings
- Final pathologic findings

7.0 Potential Side Effects

The HRME imaging system will be used prior to the standard CKC procedure. The standard CKC procedure itself has potential risks including, but not limited to, bleeding, infection and damage to rectum and bladder. These risks will be reviewed with the patient and standard informed consent obtained for the surgical procedure in addition to the study informed consent. There are no known risks associated with the imaging device used in this study.

However, there is the rare possibility of a severe allergic reaction to proflavine, the contrast dye used in imaging. Proflavine may be combustible at high temperatures. Proflavine is the principal component of acriflavine and has been used for fluorescent imaging in the European, Asian, and Australian gastrointestinal literature without any adverse effects noted. Proflavine has been clinically used as an antibacterial agent. In neonatal care, Triple dye, a combination of brilliant green, proflavine hemisulfate, and gentian violet is routinely used as a topical antibacterial agent on the umbilical stump of newborn babies (21), with a recent review of the practice categorizing toxicity as rare (22). We propose the use of proflavine solution at concentrations lower than that of the proflavine component in commercial triple dye, 0.11% (w/v) (Kerr Triple Dye, VistaPharm). We have currently done several patients with Barrett's esophagus under an existing protocol and IND and no complications or adverse events have been reported. The

quantity of solution used for diagnostic imaging is likely to be no greater than that used in neonatal care (0.65 ml per single-use swab). Investigational *in vivo* human studies of confocal microscopy for gastrointestinal cancer currently use topical acriflavine at 0.05% concentration (23).

The additional exposure to light which will occur during imaging can also be compared to that received by newborn babies undergoing phototherapy for jaundice. The high-resolution fiber-optic microendoscope proposed for use here delivers 0.5 mW of 455 nm light to the tissue through a 0.8 mm diameter fiber-optic bundle, corresponding to an irradiance level of 100 mW/cm^2. The American Academy of Pediatrics defines intensive phototherapy as a spectral irradiance of at least 30 mW/cm^2 per nanometer over the 430-490 nm spectral band, equivalent to a total irradiance of 1.8 mW/cm^2 (24). Although the irradiance level is over 50-times higher with the fiber microendoscope system, a typical imaging session of 30 minutes (including imaging for routine care) is approximately 50-times shorter than a typical 24 hour (1440 minutes) phototherapy incubation, leading to an equivalent light dose in each scenario.

The imaging procedure will add approximately 10 minutes to the patient's time in the operating room. There is the rare possibility that there may be additional risks from the added time of additional sedation.

8.0 Criteria for Removal from Study:

1. Any patient who is consented for the study but prior to surgery declines to participate in the study.
2. Any patient in whom the surgery is scheduled but not performed.
3. Any patient who withdraws consent for any reason.

9.0 Patient Confidentiality

All pathology specimens, evaluations forms, reports and other patient records will be identified in a manner designed to maintain patient confidentiality. All data and specimens will be entered into REDCap/CORe. Clinical information will not be released without the written permission of the patient or the patient's guardian, except as necessary for monitoring by the MD Anderson IND office or its representative, regulatory authorities, or the IRB.

Study data will be collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at MD Anderson. [24] REDCap (www.project-redcap.org) is a secure, web-based application with controlled access designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless downloads to common statistical packages; and 4) procedures for importing data from external sources. In the case of multi-center studies REDCap uses Data Access Groups (DAGs) to ensure that personnel at each institution are blinded to the data from other institutions. REDCap (https://redcap.mdanderson.org) is hosted on a secure server by MD Anderson Cancer Center's Department of Research Information Systems & Technology Services. REDCap has undergone a Governance Risk & Compliance Assessment (05/14/14) by MD Anderson's Information

Security Office and found to be compliant with HIPAA, Texas Administrative Codes 202-203, University of Texas Policy 165, federal regulations outlined in 21CFR Part 11, and UTMDACC Institutional Policy #ADM0335. Those having access to the data file include the study PI and research team personnel. All protected health information (PHI) will be removed from the data when it is exported from REDCap for analysis or review by the study collaborators at Rice University. All dates for a given patient will be shifted by a randomly generated number between 0 and 364, thus preserving the distance between dates. Dates for each patient will be shifted by a different randomly generated number.

The investigators and all employees and co-workers involved with this study shall not disclose or use for any purpose, other than performance of the study, any data, records or other unpublished, confidential information disclosed to those individuals for the purpose of the study. No patient identifiers will be used when analyzing the data or reporting the results.

For this protocol we will only capture adverse events that are determined by the PI to be definitely related to the proflavine and/or HRME imaging. Adverse events and serious adverse events related to the standard of care CKC will not be captured as part of the protocol data

10.0 Statistical Evaluation

We will enroll 20 patients in this exploratory study. We will first examine data from 5 patients to work out the logistical issues in the use of the imaging technology with the surgery as well as evaluation of safety. We will then examine data from the remaining patients in cohorts of 5. After each cohort of 5 patients we will review the logistics of the imaging technology and make adjustments deemed necessary to improve the surgical procedure. We will also evaluate safety.

Our primary objective is to determine the feasibility of acquiring *in-vivo* high-resolution microendoscopy (HRME) images of cervical adenocarcinoma in-situ (AIS) immediately prior to conization. We will use descriptive statistics to summarize the demographic and clinical characteristics of patients. We will estimate the proportion of patients for whom we can successfully acquire *in-vivo* HRME images of AIS immediately prior to conization with a 90% credible interval, assuming a beta(1.8, 0.2) prior distribution. This prior distribution is based on an anticipated success rate of 90%. If we complete the study with 18 patients for whom we can successfully acquire *in-vivo* HRME images of AIS, then our 90% credible interval will be 0.78 to 0.98. If we are able to successfully acquire in-vivo HRME images of at least 18 of the 20 patients enrolled in the study, then we will determine that the methodology is feasible.

Our secondary objective is to determine the accuracy of HRME images in distinguishing AIS from normal cervical tissue. We will tabulate AIS finding by the CKC specimen results as determined by pathology, and we will estimate the concordance of HRME images taken *ex-vivo* (as described in section 6.0) with pathology findings with a 90% credible, assuming a beta (1.8, 0.2) prior distribution for the concordance.

11.0 Serious Adverse Event Reporting (SAE) for MD Anderson-Sponsored IND Protocols

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience - any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.

All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

All life-threatening or fatal events, that are unexpected, and related to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB. Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

All adverse events encountered during the study will be evaluated according to the NCI Common Toxicity Criteria (CTCAE) version 4.03.

The investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning attribution of all events for subjects enrolled.

Reporting to FDA:
Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

12.0 Unanticipated Adverse Device Effect (UADE) Reporting for M. D. Anderson-Sponsored IDE Protocols

Unanticipated Adverse Device Effect

Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects" (21 CFR 812.3(s)).

Events that are related to the surgery will not be reported or recorded in the case report form.

All UADE will be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices".

Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and IRB. Unanticipated Adverse Device Effects will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 812.150.

It is the responsibility of the PI and the research team to ensure unanticipated adverse device effects are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

Device failure

Device failures which occur at the time of participant evaluation and which may prevent a participant from receiving therapy on trial will be kept on a log and will be reported as part of the annual report. Device failures at this point are not expected to affect patient safety, as participants may still receive appropriate therapy as determined by their attending physician.

13.0 References

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Appendix A – Flammability

In order to determine the flammability of acriflavine and proflavine topical dyes during the application of endoscopic thermal devices, we performed the following experiments, using both our contrast agents and both types of endoscopic coagulation/cautery devices:

1. Acriflavine and Proflavine were separately applied to the mucosal surface of a goat esophagus. Acriflavine 0.05% and 0.5% (1X and 10X the proposed clinical concentrations) was used. Proflavine Hemisulfate at 0.01% and 0.1% (1X and 10X proposed concentrations) was used.
2. Immediately, after the dyes were topically applied to the tissue, the two endoscopic coagulation and cautery devices (argon plasma coagulater and monopolar snare-cautery) were applied to the stained tissue at the highest temperature settings used in the esophagus (45-50W).
3. To monitor the change in surface temperature as a result of the thermal devices, a handheld infrared temperature meter was used at each site. Each measurement was performed in triplicate and compared to a control site of unstained mucosa (ie. no contrast agent). A summary of the temperature differences at the different sites is shown below in Figure 1 which reflects application of the argon plasma coagulater (APC) and Figure 2 which reflects application of monpolar cautery (MC).

Figure 1.

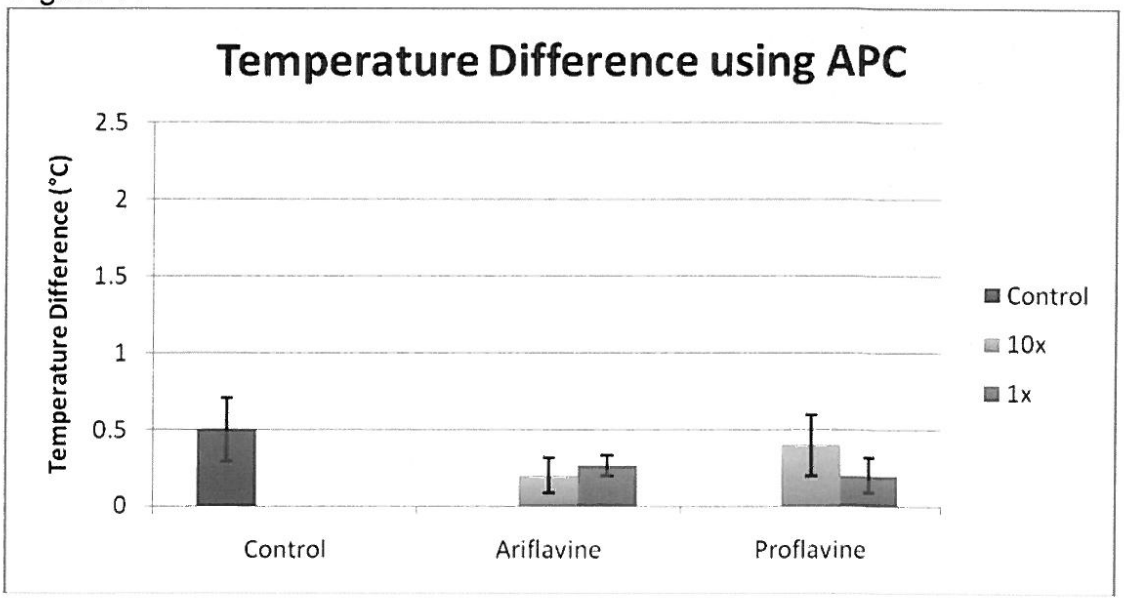

Figure 2.

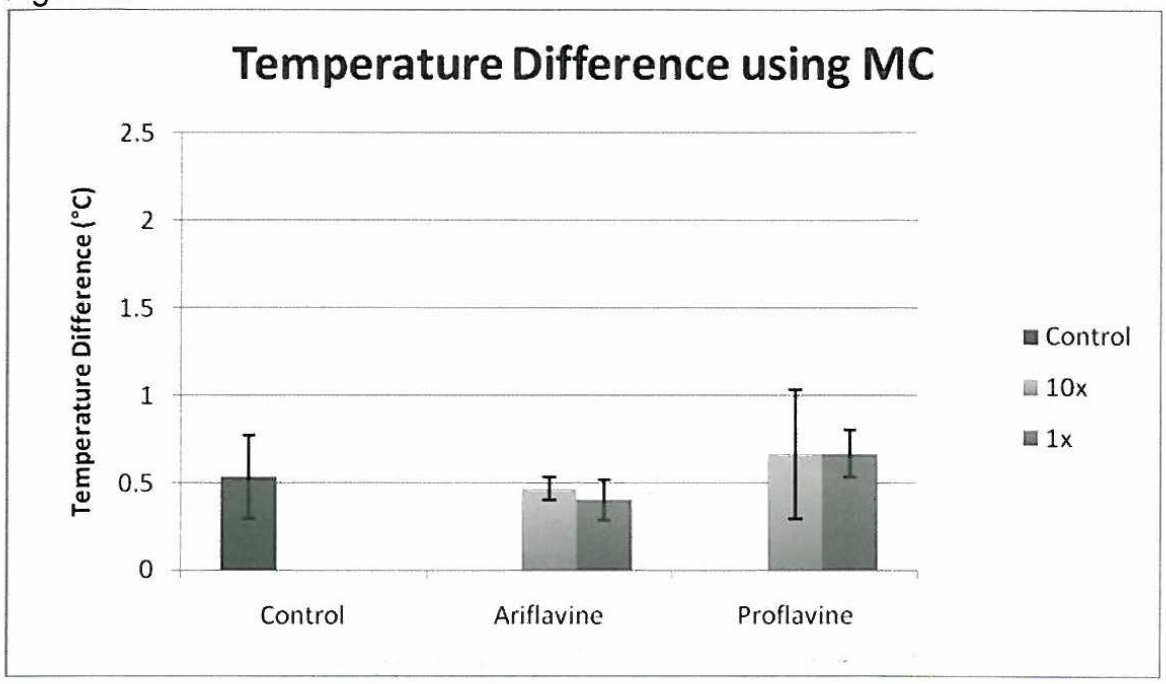

Figure 3