Evaluating The Effectiveness Of Light Cured SDF And Its Penetration: An *In Vitro* Study

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THESIS

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LIST OF ABBREVIATIONS

AAPD	American Academy of Pediatric Dentistry
ADA	American Dental Association
ANOVA	Analysis of Variance
ECC	Early Childhood Caries
EDX	Energy-dispersive X-ray spectroscopy
FDA	Food and Drug Administration
GA	General Anesthesia
GAO	Government Accountability Office
IRB	Institutional Review Board
ITR	Interim Therapeutic Restorations
PI	Principal Investigator
SEM	Scanning Electron Microscope
SDF	Silver Diamine Fluoride

Summary

SDF has demonstrated caries arresting potential for years and is widely accepted as a treatment modality in pediatric dentistry. Many additions and modifications have been made to the manufacture protocol to increase effectiveness of SDF. Currently, no protocol makes mention of light curing SDF. In a randomized clinical trial performed by Zhi, et al. 2012, anterior teeth had higher rates of arrest. Anecdotal evidence suggest that surfaces exposed to light may result in more active silver precipitation leading practitioners to believe light curing after drying can improve arrest in posterior areas not exposed to natural light, as light-cured surfaces turn dark immediately. The purpose of this study was to evaluate *in vitro* the effect of light cured SDF on the penetration of silver ions and color change. 60 non-carious primary molars were inoculated with Streptococcus mutans and treated with SDF. Experimental group was light cured and samples evaluated under scanning electron microscopy (SEM) to determine penetration depth of silver ions. Light cured samples resulted in immediate color change, allowing for chairside education and confirmation of the arresting lesion. SEM analyses saw no significant difference in the penetration of silver ions between light and non-light cured samples. There was no observable difference in the percentage of silver ions detected in each sample. Due to lack of statistical data, no inference can be made on the effects of light cured SDF. Immediate color change appears to be the only additive benefit of light cured SDF, one of which does not appear necessary as there was no addition in the penetration of silver ions detected. Therefore, we reject the null hypothesis that light curing SDF halters the penetration of silver ions. More studies are needed to evaluate the arresting potential of light cured SDF.

1. Introduction

1.1 Background

Silver diamine fluoride, also candidly name “The Silver Fluoride Bullet” was introduced into the field of dentistry around the 1900’s in countries such as Japan, Mexico, and Australia as a method of caries prevention. SDF is gaining a lot of interest in pediatric dentistry due to its’ ease of application. SDF allows caries prevention without administering local anesthesia, which is ideal for patients who lack cooperation or are pre-cooperative. Evidence supports a paradigm shift from surgical to non-surgical caries management in children. The advantages of caries treatment with SDF include its’ ease of use, low material cost, non-invasive nature and minimal requirement for personnel time and training. SDF is a colorless aqueous solution containing silver and fluoride ions. It is composed of 24.4%-28.8% silver and 5.0-5.9% fluoride ions. Solution concentration ranges from 3.8% - 38%. Advantage Arrest is the only commercially available SDF product approved by the Food and Drug Administration (FDA) within the United States. It is manufactured by Elevate Oral Care at a proven, arresting and prevention concentration of 38%. Currently, SDF is only approved by FDA in its’ use in treating dental hypersensitivity. However, SDF has been proven to inhibit carious processes and aid in the prevention of secondary caries through numerous clinical trials and studies. Visible black staining is believed to provide clinical evidence of arrest due to SDF’s byproduct, silver phosphate. Clinicians are seeking to accelerate black staining by light curing SDF following application. To date, this is the only study evaluating the effects of light cured SDF on the penetration of silver ions.

1.2 Purpose of this Study

The purposes of this study are:

1) To evaluate *in vitro* the effect of light cured SDF on the penetration of silver ions on primary molars.

2) To determine if light curing SDF accelerates color change.

1.3 Hypothesis

We hypothesize that light curing SDF reduces the penetration of silver ions and therefore decreases its' effectiveness.

2. Literature Review

2.1 Dental Caries: An Epidemic

The US Centers for Disease Control and Prevention reports that 28% of all US toddlers and preschoolers are affected by caries and nearly half of US children experience caries before entering kindergarten (Dye BA, Tan S, Smith V, Lewis B, Barker L, Thornton-Evans G, 2007). Dental caries is the most common chronic disease in US children aged 5-17, and the number is five times higher than that of children who suffer from asthma (Bagramian RA, Garcia-Godoy F, 2009). Early childhood caries (ECC) still remains a global health epidemic despite the numerous advances made in dentistry through techniques and medications. ECC is the presence of 1 or more decayed, missing due to caries, or filled surfaces (dmfs), in any primary tooth in a child age six and under (American Academy of Pediatric Dentistry, 2016). Although dental caries has been linked to poor diet, oral hygiene, and bacterial species, a grave amount of dental decay is due to poor parental education, adverse socioeconomic conditions, low family income, having a single parent household, and some medications (Chu 2000). Bagramian, et al., 2009 reported that 50% of children aged 5-9 suffered from ECC within the United States. Untreated dental caries can lead to tooth pain and infection and can affect ones' quality of life. There's been many studies linking dental caries in the primary dentition to have detrimental effects on the permanent dentition.

There has been a significant improvement in the oral health of Americans over the last 50 years. Much success is due to effective prevention and treatment efforts such as community water fluoridation and school-based sealant programs. Community water fluoridation benefits about 7 out of 10 Americans who get water through public water systems (CDC 2010). However, there still exist a considerable number of individuals who do not have access to preventive

programs. People who have the least access to preventive services and dental treatment have greater rates of oral diseases. Social determinants that affects ones' ability to access oral health care are education level, income, race, and ethnicity. Lack of access to dental care for all ages remains a public health challenge. This issue was highlighted in a 2008 Government Accountability Office (GAO) report that described difficulties in accessing dental care for low-income children (GAO 2008). In 2013, GAO reported an increase in dental services among children who were Medicaid and CHIP beneficiaries, but children still visited the dentist less often than privately insured (GAO 2013).

Conventional dental treatment poses another obstacle to families seeking care due to financial constraints and/or patient behavior. Uncooperative patients are not well suited for traditional dental practices and require behavior management. Behavior management modalities have been employed to aid in relieving discomfort, reducing anxiety, and improving safety in pediatric patients such as oral sedation and general anesthesia. Oral sedation and general anesthesia are not always readily available or affordable for low-income families. AAPD recognizes Interim Therapeutic Restorations (ITR) to be beneficial and best utilized as part of comprehensive care in the dental home, when conventional dental treatment is not a viable option.

ITR may be used to restore and prevent further decalcification and caries in young patients, uncooperative patients, or patients with special health care needs or when traditional cavity preparation and/or placement of traditional dental restorations are not feasible and need to be postponed. ITR may be used for caries control in children with multiple carious lesions prior to definitive restorations of the teeth (American Academy of Pediatric Dentistry, 2008). SDF is a form of ITR that has become more widely accepted among the pediatric population in the United

States due to ease of application, atraumatic experience, no local anesthesia, or operative removal of decay. For years, SDF has been widely used to deal with high caries prevalence by haltering the rate of caries progression by other countries, including Australia and China. In Japan, it has been accepted as a therapeutic agent by the Central Pharmaceutical Council of the Ministry of Health and Welfare for dental treatment for more than 40 years (Chu & Lo, 2008).

2.2 Evolution of Silver Diamine Fluoride (SDF)

Silver in dentistry, dates back as early as the 1800's. Silver nitrate was used by early American dentists to instantaneously cauterize carious lesions (Barillo 2014). Silver nitrate remained a popular caries arresting medicament and was utilized throughout the era of G.V. Black. Ammonia was added to the silver nitrate solution in 1917 and marketed as an antimicrobial product (Lansdown 2006). This "Howe's solution" was used until the 1950's to sterilize lesions after cavity preparation. In the 1970's, the Western Australia School Dental Service used silver fluoride as the initial part of a minimally invasive treatment process for a cohort study of disadvantage young children in New South Wales, which found sliver fluoride to inhibit carious growth of existing lesions (Peng, et al., 2012). The application of stannous fluoride was added to silver fluoride as a reducing and caries prevention agent.

The development of SDF arose due to the influx of studies implementing the caries prevention of silver + fluoride. SDF was first investigated by Mizuho Nishino in 1969. She sought to combine the powerful antimicrobial properties of silver with the benefits of a high dose of fluoride. She reported a 74% reduction in dental caries in school-aged children (Nishino, et al., 1969). Soon after her discovery, the Central Pharmaceutical Council of Ministry of Health and Welfare and Welfare of Japan granted approval of SDF as a cariostatic agent. It was marketed under the name Saforide. In vitro and in vivo studies of SDF as an alternative dental

treatment initially emerged from dental public health researchers in the developing world, where access to oral health was extremely limited. The primary groundwork populated from countries such as Argentina, Brazil, China, Cuba, Japan, and Nepal (Yamaga, et al., 1972).

In 2014, FDA cleared SDF as a Class II medical device, approved to treat dentin hypersensitivity in adults aged 21 and older. It was classified as a medical device, rather than a drug; due to its' ability to occlude dentinal tubules. This allowed expedited approval. In 2016, FDA awarded SDF the designation of "breakthrough therapy" based on its arrest of dental decay in children and adults, a first for an oral health therapy (FDA 2017). FDA recognizes and acknowledges "preliminary clinical evidence" demonstrating substantial benefits over existing therapies and lack thereof. This marked the first time in history where oral disease had been categorized as a serious medical condition, elevating its importance as a significant public health epidemic. Clinical evidence supports the off-labeled use of SDF as a caries arresting and prevention medicament in children and adults (Gao, et al., 2016).

Currently, Elevate Oral Care is the only approved US manufacture of SDF. SDF is a colorless topical agent comprised of 24.4-28.8% silver and 5.0-5.9% fluoride at pH 10, marketed as Advantage Arrest (Horst, et al., 2016). Clinical trials suggest 38% SDF is effective and efficient in arresting and preventing carious lesions. Currently, SDF is only available in the US at a concentration of 38%. Single application appears insufficient for sustained effects, while annual re-application results in remarkable success, and even greater effects with semi-annual application (Zhi, et al., 2012). Therefore, SDF is recommend in biannual applications, only to carious lesions without excavation, for at least the first two years.

2.3 Mechanism of action of SDF

In vitro studies suggested that silver- fluoride regimens inhibit S. mutans growth (Thibodeau EA, Handelman SL, 1978), metabolic activity of dental plaque (Oppermann, et al., 1980), and caries lesion depth progression (Klein U, Kanellis MJ, 1999). Bedi and Infirri (1999) pointed out four main advantages of SDF as: control of pain and infection, affordable cost, simplicity of treatment, and minimal support needed. The silver component acts as an anti-microbial agent killing bacteria and preventing the formation of new biofilm, while the fluoride acts to prevent further demineralization of tooth structure.

In teeth with dental caries, SDF reacts with hydroxyapatite to form fluoroapatite, and the by-product is silver phosphate. Fluoroapatite is less acid-soluble than hydroxyapatite, inhibiting the decay process. Silver phosphate (which is responsible for the black staining) subsequently reacts with bacterial amino and nucleic acid thiol groups to form silver amino and nucleic acids. Silver amino and nucleic acids are unable to carry out metabolic and reproductive functions, leading to bacterial killing. Studies have indicated that silver interacts with sulfhydryl groups of proteins and with DNA, altering hydrogen bonding and inhibiting respiratory processes, DNA unwinding, cell-wall synthesis, and cell division (Oppermann, et al., 1980). At the macro level, these interactions effect bacterial killing and inhibit biofilm formation (Wu, et al., 2007).

In teeth with exposed sensitive dentin, topical application of SDF results in the development of a squamous layer of SDF, plugging the dentinal tubules. Decreased sensitivity in treated surfaces is consistent with the hydrodynamic theory of dentin hypersensitivity (Horst, et al., 2016). Upon application of silver diamine fluoride to a decayed surface, the squamous layer of silver protein conjugates forms, increasing resistance to acid dissolution and enzymatic digestion (Mei, et al., 2017). The treated lesion increases in mineral density and hardness while the lesion depth decreases. Meanwhile, silver diamine fluoride specifically inhibits the proteins

that break down the exposed dentin organic matrix: matrix metalloproteinases, cathepsins and bacterial collagenases (Rosenblatt, et al., 2009).

2.4 Clinical Trials of SDF and clinical efficacy

In 2002, Chu, et al. performed a clinical trial investigating the effectiveness of topical fluoride applications in arresting dentin caries in upper primary anterior teeth in 375 Chinese pre-school children (Chu C, Lo E, 2002). Children were placed in 5 treatment group: groups 1 and 2 received annual applications of SDF, groups 2 and 3 received sodium fluoride varnish every three months, and group 5 served as the control with no treatment. In both the first and third groups, soft carious tissues were removed prior to fluoride application. 308 children returned for the 30 month follow-up. At the 30 month follow-up, there were statistically significant differences in the mean number of arrested carious tooth surfaces among the five treatment groups. There was no significantly difference found in groups where soft dentinal caries were removed compared to groups were no excavation was performed. Concluding that caries excavation is not warranted. Children who received an annual application of SDF had more arrested caries lesions in their upper anterior teeth than did children in other groups. They found that children with no treatment, developed more new caries than any other group.

A 36-month controlled clinical trial was conducted in a cohort of 373 schoolchildren in Santiago de Cuba by Llodra, et al., 2005. The school children received an application of 38% SDF solution every 6 months on the caries lesions of primary teeth and permanent first molars. There was significantly more surfaces with inactive caries and fewer new caries in children who received SDF. Resulting in roughly 77% of active lesions becoming inactive (Llodra et al., 2005). Chu et al 2002, found SDF to be effective after 30 months, resulting in no increase in the

risk of a tooth becoming non-vital. In Japan, a 30-month field study of SDF on 220 young children was carried out, and a 52% reduction in caries severity was found in children receiving SDF compared with that of children receiving no treatment (Hihara, et al., 1994).

Gao, et al., 2016 performed a systematic review to investigate the effectiveness of SDF. The initial search found 1,123 publications. After manually screening the remaining studies by title, abstract, and full text when necessary, 829 of the 850 remaining publications were removed because they were literature reviews, case reports, laboratory studies, or clinical studies on caries prevention, hypersensitivity, or endodontic treatment. Finally, 19 studies were reviewed in detail, including 8 studies published in English, 4 studies in Chinese, 3 studies in Portuguese, 1 study in Spanish, and 3 studies in Japanese. Meta-analysis was conducted on 8 studies, which used 38% SDF to arrest dentine caries in primary teeth in children and had properly reported data. The results showed that the caries-arresting rate of SDF treatment was 86% at 12 months, 78% at 18 months, 65% at 24 months, and 71% at or beyond 30 months(Gao, et al., 2016). The overall proportion of arrested dental caries after SDF treatment was 81%. It is noteworthy that the application frequency of SDF varied in different studies. Apart from staining the arrested caries lesion black, the 19 clinical trials did not report any significant complication of SDF use among children. All studies using SDF with high concentration (38%) reported a statistically significant caries-arresting effect on children. Although the fluoride concentration was high (44,800 ppm in 38% SDF), no significant complication was reported in these studies. Studies of SDF used not only different concentrations but also different application frequencies. The application frequency could be one-off or repeated applications every 3, 6, or 12 mo. One study reported that increasing the application frequency increased the caries arrest rate of SDF application (Zhi , et al., 2012).

2.5 AAPD Guidelines on the usage of SDF (Crystal, et al. 2017)

The guideline intends to inform the clinical practices involving the application of 38 percent SDF to enhance dental caries management outcomes in children and adolescents, including those with special health care needs. These recommended practices are based upon the best available evidence to-date. Recognizing the following:

(1) Untreated dental decay in young children remains a challenge and confers significant health and quality of life impacts to children and their families and is marked by pronounced disparities.

(2) Surgical-restorative work in young children and those with special management considerations (special health care needs) often requires advanced pharmacologic behavior guidance modalities which have additional health risks and limitations and are often not accessible, at all or in a timely manner.

(3) The cost of managing severe early childhood is disproportionately high, especially when hospitalization is necessary. The need to treat children with rampant decay or special health care needs under general anesthesia in a hospital sedation is quite common in the US. Studies have found that children from “less affluent” regions have higher dental surgery rates than those from “more affluent” communities, which results in an economic burden for communities already impacted by the effects of poverty-related health problems.

(4) With caries lesion arrest rates upwards of 70 percent, SDF presents as an advantageous modality. SDF is favored over other modalities of treatment because its’ less invasive in nature and inexpensive.

(5) The undesirable effects of SDF – mainly black discoloration – are outweighed by its desirable properties in most cases, while no toxicity or adverse events associated with its use have been reported.

In a randomized clinical trial performed by Fung, et al., in 2016, SDF was proven to be more effective at arresting dentin caries in primary teeth of preschool children at 38% rather than 12% concentration and when applied biannually rather than annually. In 2017, AAPD performed a thorough systematic search using PubMed®/MEDLINE, Embase®, Cochrane Central Register of Controlled Trials, and gray literature databases to identify randomized controlled trials and systematic reviews reporting on the effect of silver diamine fluoride. The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach was used to assess the quality of the evidence and the evidence- to-decision framework was employed to formulate a recommendation (Crystal, et al., 2017). The panel made a conditional recommendation regarding the use of 38% SDF for the arrest of cavitated caries lesions in primary teeth as part of a comprehensive caries management program and developed the following protocol, suggesting 38% SDF with biannual applications based upon best clinical practices:

(1) Removal of gross debris from lesion to allow better SDF contact with denatured dentin.
(2) Place a protective barrier such as cocoa butter or petroleum jelly on gingiva tissues and membranes or isolate with cotton rolls to prevent pigmentation or irritation.
(3) Dry affected tooth surfaces with gentle flow of compressed air or dry with cotton rolls/gauze.
(4) Apply SDF directly to affected tooth surface.
(5) Dry with gentle flow of compressed air for at least one minute

(6) Remove excess SDF with gauze, cotton roll or cotton pellet and continue to isolate site for up to three minutes when possible.

2. 6 Use of light curing SDF

The application of SDF is a learning curve as new attributes to the chemistry and new uses in conjunction with restorative work are discovered. Protocol's released by Advantage Arrest, UCFS, and AAPD make no mention of light curing SDF. Through a literature review, zero evidence-based articles providing evidence on the effectiveness of light curing SDF were found. When "silver diamine fluoride + light curing" was searched, zero results were produced. Anecdotal evidence reports that in clinical settings, the use of a curing light after drying seems to improve arrest in posterior areas that are not exposed to natural light, as light-cured surfaces immediately turn dark. Reports make mention that arrested lesions generally occur more often in the anterior region due to frequent exposure to natural light for the precipitation of silver ions and ease of cleaning. This led practitioners to believe that light curing SDF was beneficial to provide clinical evidence of arrest and correct application of SDF. Silver ions are sensitive to light. Light curing results in the oxidation of SDF, resulting in the precipitation of silver ions out of the solution. Precipitation of silver ions out of the solution theoretically results in lesser silver ions available to penetrate through the depth of the lesion, rendering the solution less effective. Elevate Oral Care LLC, the manufacturer for the only FDA approved 38% SDF product (Advantage Arrest) released a notion stating that SDF should not be light cured (Care, 2017). Despite the omission of light curing from recommended protocol, this study is aimed at evaluating the effect of light cured SDF on the penetration of silver ions.

2.7 Scanning Electron Microscope

The Scanning Electron Microscope (SEM) was developed in the 1950's. SEM utilizes the same basic principles as light microscopes but uses focused beams of energetic electrons opposed to photons, to magnify an object. SEM provides detailed surface information by tracing a sample in a raster pattern with an electron beam and the beam's position is combined with the detected signal to produce an image. The electron gun generates a beam of energetic electrons down the column and onto a series of electromagnetic lenses. Theses lenses are referred to as solenoids, which are wrapped in coil. The coils can be adjusted to focus the electron beam onto the sample (Anderson, n.d.). The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition. Specimens can be observed in high vacuum in conventional SEM, or in low vacuum or wet conditions in variable pressure or environmental SEM, and at a wide range of cryogenic or elevated temperatures with specialized instruments. The beam focuses onto a stage, where a solid sample is placed. Adjustments results in fluctuations in the voltage, increasing or decreasing the speed at which the electrons come into contact with the specimen surface. The beam can be adjusted to change magnification as well as to determine the surface area to be scanned.

Various types of signals are produced including secondary electrons (SE), reflected or back-scattered electrons (BSE), characteristic X-rays and light (cathodoluminescence) (CL), absorbed current (specimen current) and transmitted electrons. Secondary electron detectors are standard equipment in all SEMs, but it is rare that a single machine would have all detectors available. In secondary electron imaging, secondary electrons are directed very close to the specimen surface. Therefore, SEM can produce very high-resolution images of the sample's surface, revealing details less than 1 nm in size. BSE are reflected from the sample by elastic scattering. They emerge from deeper locations within the specimen and therefore resolution of

BSE images are less than SE. BSE signal is strongly related to the atomic number of the specimen and images can provide information about the distribution of different elements in the sample. Characteristic X-rays are emitted when the electron beam removes the inner shell from the sample releasing energy. These characteristic X-rays can be used to identify the composition of the element in the sample. Biological samples have to be prepared prior to imaging.

Samples must be completely dry. Living cells, tissues, and whole, soft bodies organisms require chemical fixation to preserve and stabilize their structure. Fixation is usually performed by incubation in a solution of a buffered chemical fixative, such as glutaraldehyde. The fixed tissues are then dehydrated to prevent collapse and shrinkage. The preparation of samples can result in artifacts. The negative impact can be minimized with knowledgeable experience researchers being able to identify artifacts from actual data as well as preparation skill. There is no absolute way to eliminate or identify all potential artifacts (Anderson, n.d.). Samples must be small enough to fit inside the vacuum chamber. The vacuum chamber is designed to prevent any electrical and magnetic interference which aids in reducing the chance of radiation escaping the chamber.

2.8 Energy-dispersive X-ray spectroscopy (EDX or EDS)

Energy-dispersive X-ray spectroscopy (EDX or EDS) is an analytical technique used for the elemental analysis or chemical characterization of a sample. Each element in the periodic table has a unique atomic structure which results in a unique set of peaks on its electromagnetic emission spectrum. This is the fundamental principle of EDS, its' ability to characterize elements. In order to stimulate the emission of characteristic X-rays from our samples, particles such as protons and electrons are charged using high energy beams which are focused onto our sample. Atoms at rest contain ground state electrons in discrete energy that are bound to the

nucleus. As electrons and protons are charged they move to and from different valance levels and the difference in energy between the higher-energy shell and the lower energy shell can be released in the form of X-rays. The energy and number of the x-rays emitted from a specimen can be measured by EDS. Because the energies from the X-rays are characteristic of the difference in energy between the two shells and of the atomic structure of the emitting element, EDS allows the elemental composition of the specimen to be measured.

3. Methods

3.1 Study Design and Procedures

Institutional Review Board (IRB) approval was granted for this study to be conducted at The University of Illinois at Chicago. A protocol for this study was adopted from Willershausen, et al., 2015, who evaluated the penetration of silver ions from SDF solution into dentin using SEM analysis. A pilot study was conducted using 10 samples and the protocol modified accordingly. Multiple comparisons of pairs power analysis using simulation was performed and it was determined 4 groups of 15 would provide sufficient statistical power to detect a clinical meaningful difference between the treatment and control groups. Groups were randomly assigned and treated accordingly (Figure 1).

FIGURE 1

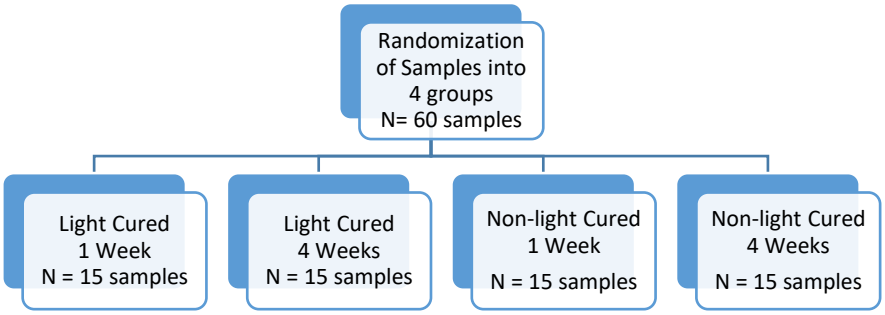


FIGURE 1: STUDY GROUPS

3.2 Collection and Storage of Teeth

Non-carious primary molars were collected from an existing storage of teeth at the University of Illinois at Chicago Department of Pediatric Dentistry. Inclusion criteria was non-carious primary molars while any carious primary molars were excluded from the study. Teeth were stored in 0.05% thymol solution to inhibit fungi and bacterial growth. This solution was changed once a month. Teeth were removed from thymol solution and washed thoroughly with sterile water. Using an IsoMet 1000 precision saw, a diamond blade was used to section samples.

3.3 Sample Preparation

Teeth were mounted onto acrylic blocks using sticky wax and sectioned from their roots to produce coronal samples. These coronal samples were sectioned in half mesial-distally to create 2mm buccal and lingual samples. Buccal and lingual surfaces were chosen due to larger surface area than interproximal surfaces. Teeth were polished using EcoMet 3000 variable speed grinder-polisher with grits 600, 800, and 1200. Teeth were examined to ensure that enamel was still present.

3.4 Streptococcus mutans inoculation

A 3x3mm inoculation window was created on each sample using tape. Teeth were covered with acid resistance nail varnish to control site of inoculation. Teeth were sterilized using ethylene oxide to ensure sterility. Specimens were inoculated with streptococcus mutans (strain UA159) using brain heart infusion agar (BHI) medium with 1% sucrose and incubated for time intervals of 1 week and 4 weeks at 37*C with 5% carbon dioxide to grow biofilm. Medium was

changed daily. SDF surface was gently wiped dry to remove any debris. Teeth were randomly assigned to control and experimental groups as assigned in figure 1.

3.5 Silver Diamine Fluoride Application

Teeth were gently cleaned with a Kim wipe. SDF was applied to each standardized lesion per manufactures guidelines. Excess removed. The control group set aside, within a hood with minimal visible light and monitored for color change and recorded immediately. The experimental group was light cured for 20 secs and set aside and monitored for color change and recorded immediately. Color change was recorded with the following identifiers: 1: immediately (less than 30 seconds), 2: chairside color change (less than 5 minutes), 3: after dismissal color change (<24 hours), and 4: next day color change (>24 hours). Samples were mounted with sticky wax onto acrylic blocks and crossed-sectioned. Samples were mounted in epoxy resins for SEM (Hitachi S-3000N) evaluation.

3.6 SEM and EDX Analysis

Samples were examined using SEM at 10 KV with x500 magnification. Elemental data analyses were performed using EDX (point and ID and mapping) to identify and measure the percentage and penetration of silver ions. The percentage of silver was traced from the edge of the enamel treated SDF surface into dentin. Silver ions were traced until a minimal detectable threshold and depth was recorded. Following SEM evaluation, acrylic resin blocks were polished using EcoMet 3000 variable speed grinder-polisher with grits 600, 800, and 1200. The depth of decay for each sample was measured via an optical microscope and recorded. Statistical analysis performed using IBM SPSS Statistics.

4. Results

4.1 Results of color change

100% of light cured samples resulted in an immediate color change regardless of 1 week or 4 weeks of carious growth. Non-light cured samples varied with 4-week samples resulting in next day color change and 1-week samples more disperse: 53% >24hours and 40% <24 hours. An observable difference was detected in the growth of decay in 1-week samples vs. 4-week samples, however, no statistical significance in the difference in growth was noted. The results from color change are outlined in figure 2.

FIGURE 2

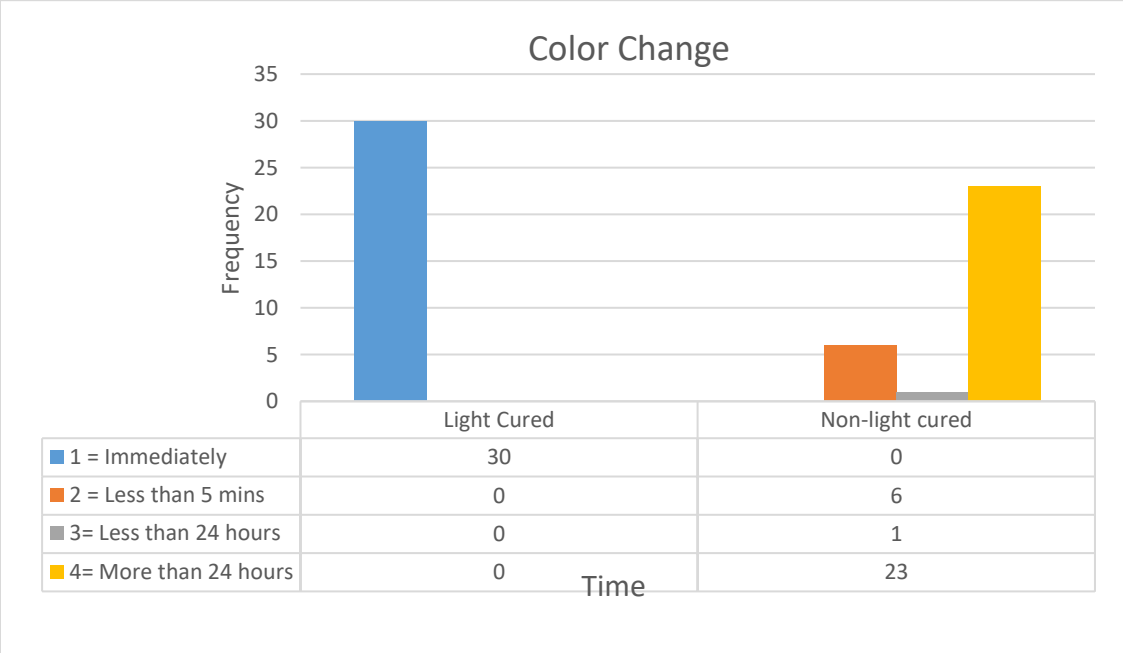


FIGURE 2: RESULTS OF COLOR CHANGE

4.2 Results of Ag penetration:

The results of Ag penetration are outlined in figure 3. 66% of non-light cured samples resulted in 0*µm of Ag penetration. 53% of light cured samples detected 0µm of Ag ions. The average depth of penetration of Ag ions for non-light light cured samples was 60µm and light cured samples 87µm. There was no significant difference between the penetration of silver ions in light cured samples vs non-light cured samples (t-test, P>0.05). The average depth of penetration of Ag ions for 1-week samples was 99µm and 46µm for 4-week samples. There is no significant difference between the penetration of silver ions detected in 1-week samples vs 4-week samples (t-test, P>0.05).*

FIGURE 3

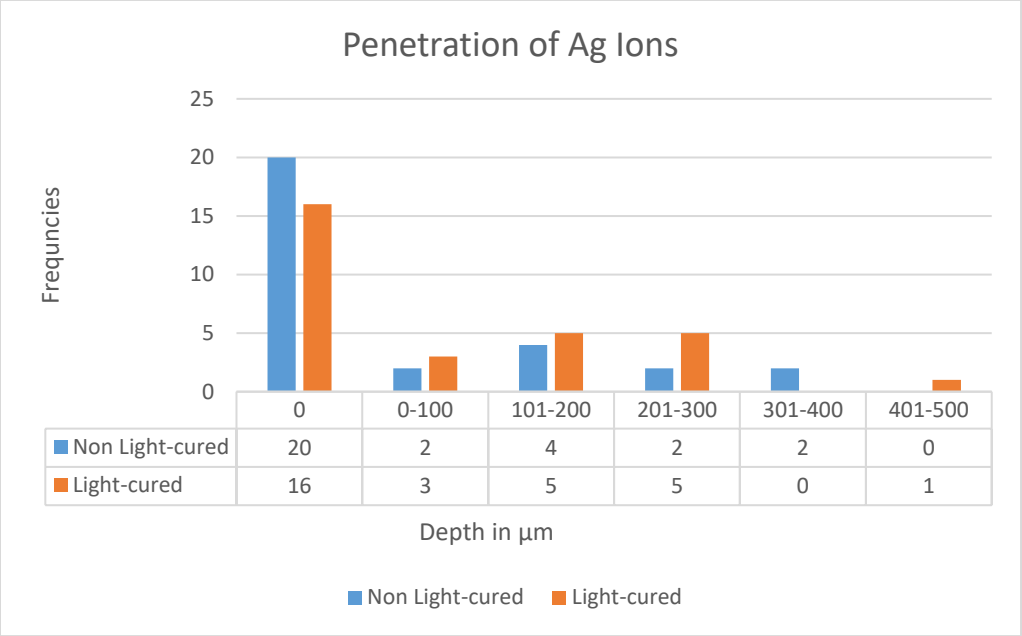


FIGURE 3: RESULTS OF PENETRATION

5. Discussion

5.1 Effects of light-curing

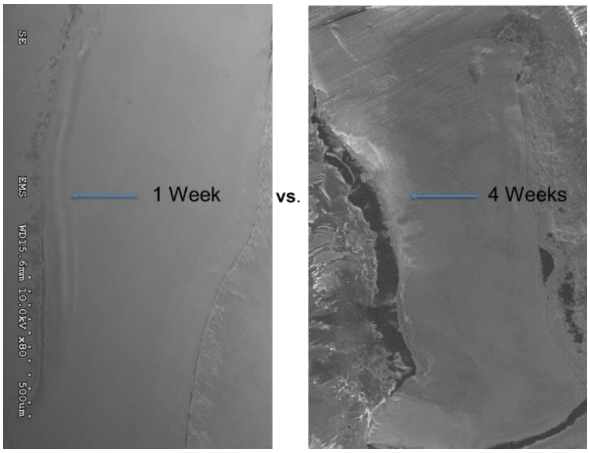
Practitioners who wanted to ensure that SDF was active developed the implied notion that light curing SDF aids with product placement and arrest. There is no concrete data that supports such claims. Our results show that 100% of light cured samples resulted in immediate chair-side color change. Samples that were not light cured, eventually darken within 24 hours, although not instant, color change did result. Signifying that light curing SDF is not a necessary step to have color change occur. It is not evident that light curing SDF results in activation of silver ions or caries arrest. As reported earlier, silver ions are light sensitive and can become oxidized out of the solution. Our results showed that there was no significant difference in the penetration or percentage of silver ions in our samples because of light curing. SDF is favored for its ease of application and minimal requirement of equipment. In pediatric dentistry, an additional 20 secs of light curing per SDF treated surface along with 3 minutes of isolation and drying could result in additional chair time for an already uncooperative patient.

5.2 Demineralization of lesions

We observed an increase in the growth of demineralization in 4-week samples compared to 1-week samples (figure 4). Indicating that prolonged exposure to s. mutans resulted in further tooth demineralization. Streptococcus mutans is a Gram-positive, non-motile, non-spore forming, catalase- negative, facultative anaerobic cocci bacterium commonly found in the human oral cavity. It is a significant contributor to tooth decay (El-sherbiny 2014) resulting in demineralization of enamel and dentin tissues. Zhao et al, reported dentine surfaces treated with SDF had significantly less growth of strep. mutans than those without SDF treatment. In

demineralized tissues, there is a net loss of minerals in enamel and dentin surfaces. During demineralization, calcium release precedes phosphate release from enamel, dentin, and cementum. In our demineralized samples, there was a decrease in the amount of calcium and phosphate ions detected. In samples with increased phosphate, there was a decrease in Ag ions. There was a significant difference in phosphate ions compared to calcium and silver. Mei, et al. 2017, reported that minerals such as phosphate are less soluble; therefore, results in the release of Ag ions. This could describe why majority of our demineralized samples resulted in a minute amount of Ag ions detected. Liu, et al. 2012 demonstrated that SDF increased the mineral density of demineralized enamel lesions, yet the inhibitory effect of SDF on demineralized dentine is yet to be studied.

Picture 1: Depth of decay in 1-week vs 4 weeks samples



5.3 Penetration of Ag ions

Results showed a decrease in silver ions detected in 4 weeks samples compared to 1-week samples. An inverse relationship was found between depth of decay and penetration of silver ions. As depth increased, the penetration potential of the silver ions decreased. These findings were similar to those reported by Willershausen, et al. 2015, who examined the penetration potential of SDF. SEM results showed that silver deposits could be found in a covering on the dentin layer (1.7%) and in occluding dentinal tubules to a depth of 20μm but diminished after 40μm. This data suggests SDF's limited potential to penetrate deep carious lesions. More studies are needed to determine the effects of SDF on the pulp. In respect to penetration depths in light cured samples vs controls, no significant difference was found. Light curing SDF did not increase or halter the penetration potential of silver ions when compared to non-light cured samples. More studies are needed investigating the caries arresting potential of light cured SDF.

5.4 Study Strengths and Limitations

A major strength of this study was the successful development of an in vitro microbial caries model. This model was modified to produce natural carious lesions in primary molars. This study effectively introduced streptococcus mutans, standardizing all samples with the same strain of microbes. This study successfully demonstrated the penetration potential of silver ions in varied carious depths. .Varied depths of decay were achieved via varied incubation times of 1 week and 4 weeks. This study added value to a limited database on the effects of light curing SDF.

A viable cell count would have been beneficial to ensure that samples had similar counts of microbes. In vitro studies are limited in their capabilities to replicate the natural oral environment and therefore cannot account for factors such as oral hygiene, diet, saliva, etc. Another major limitation of this study was cost and time.

5.5 Future Studies:

The evidence that supports the carious arresting success of SDF has been implemented and tested in several different clinical studies. There is no question that this "magic silver bullet" can help to decrease the caries epidemic globally but more studies are needed to determine its' effectiveness in deep carious lesions and lesions approximating the pulp. More in-vitro studies are needed to evaluate the penetration of silver ions through dentin. More SEM analyses are needed to examine histologically the mechanism of action of SDF. The restorative capabilities of SDF as a definitive treatment modality needs more investigating. This study should be carried out on interproximal and occlusal surfaces of primary molars, as the enamel and dentin thickness differ in these locations and the effect of SDF might be different.

6. Conclusion:

Within the limitations of an in-vitro study, the following was concluded:

1. Light curing SDF resulted in an immediate color change but did not result in an increase in the penetration of silver ions. Therefore, we can not confer that light curing results in further activation of silver ions.

2. The penetration of silver ions diminished as the depth of decay increased, suggesting SDF's limited potential to potentate deep carious lesions. Caution should be taken when altering manufactures guidelines, therefore; an in-depth examination of light cured SDF effectiveness in caries arrest is warranted.

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Appendices

Determination Notice
Research Activity Does Not Involve "Human Subjects"

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

May 17, 2017

Jasma McDonald, DDS
Pediatric Dentistry
801 S. Paulina Street, Room 269-D, M/C 850
Phone: (312) 996-7532 / Fax: (312) 413-8006

RE: Research Protocol # 2017-0513
"Evaluating the effectiveness of light cured SDF and its' penetration: An In Vitro Study"

Sponsor(s): None

Dear Dr. McDonald:

The above proposal was reviewed on May 17, 2017 by OPRS staff/members of IRB #7. From the information you have provided, the proposal does not appear to involve "human subjects" as defined in 45 CFR 46. 102(f).

The specific definition of human subject under 45 CFR 46.102(f) is:

> *Human subject* means a living individual about whom an investigator (whether professional or student) conducting research obtains
>
> (1) data through intervention or interaction with the individual, or
> (2) identifiable private information.
>
> *Intervention* includes both physical procedures by which data are gathered (for example, venipuncture) and manipulations of the subject or the subject's environment that are performed for research purposes. *Interaction* includes communication or interpersonal contact between investigator and subject. *Private information* includes information about behavior that occurs in a context in which an individual can reasonably expect that no

observation or recording is taking place, and information which has been provided for specific purposes by an individual and which the individual can reasonably expect will not be made public (for example, a medical record). Private information must be individually identifiable (i.e., the identity of the subject is or may readily be ascertained by the investigator or associated with the information) in order for obtaining the information to constitute research involving human subjects.

Specifically, this research will involve the analysis of de-identified specimens collected during routine dental extractions that would be otherwise discarded as waste.

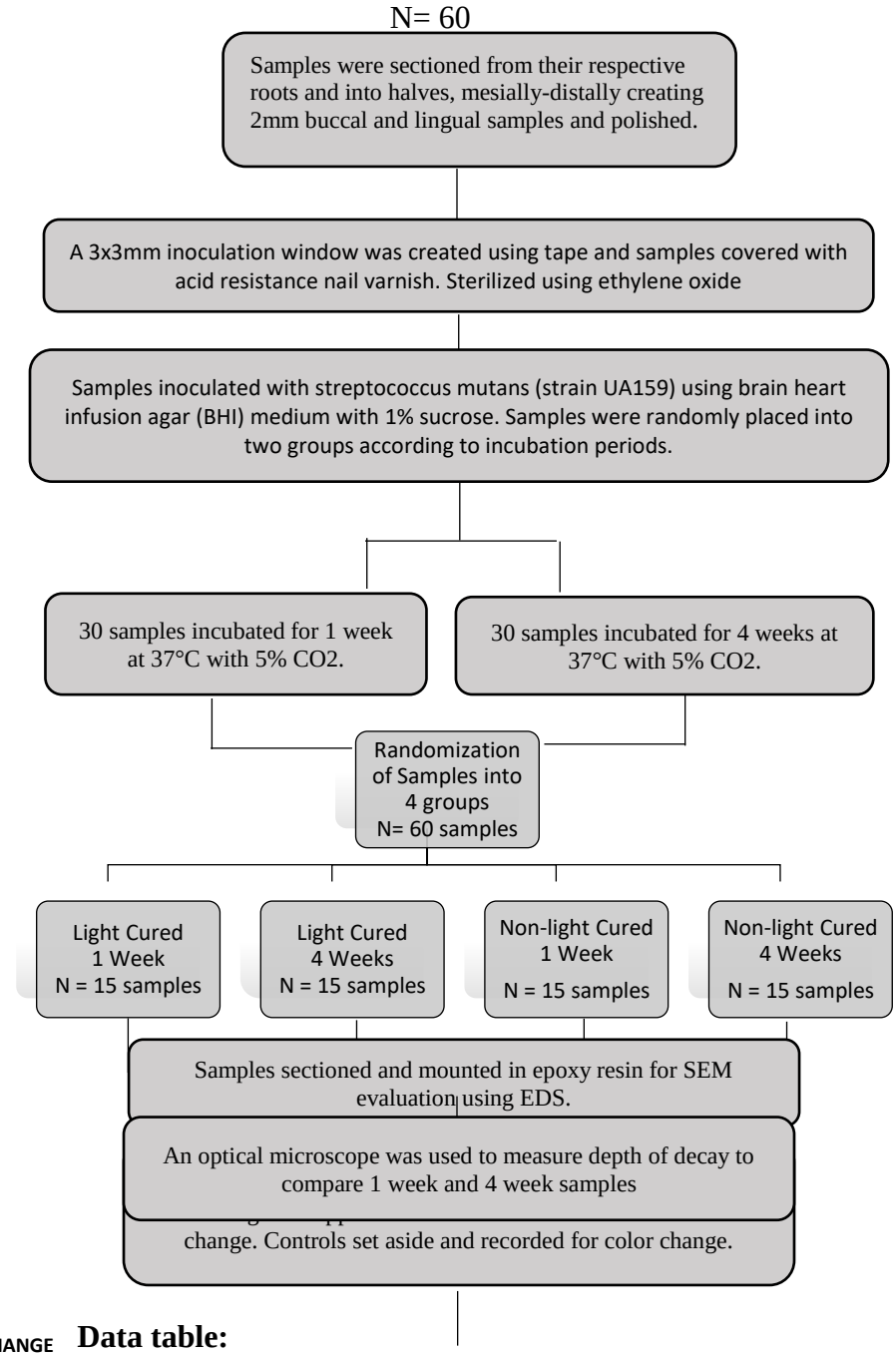
All the documents associated with this proposal will be kept on file in the OPRS and a copy of this letter is being provided to your Department Head for the department's research files.

If you have any questions or need further help, please contact the OPRS office at (312) 996-1711 or me at (312) 355-2908.

Sincerely,
Charles W. Hoehne, B.S., C.I.P.
Assistant Director, IRB #7
Office for the Protection of Research Subjects

cc: Marcio Da. Fonseca, Pediatric Dentistry, M/C 850
Sahar Alrayyes, Pediatric Dentistry, M/C 850

STUDY DESIGN AND PROCEDURE



Data table:

TABLE 1: SCORING FOR COLOR CHANGE

1	IMMEDIATELY
2	LESS THAN 30 MINUTES
3	LESS THAN 24 HOURS
4	MORE THAN 24 HOURS

Sample	Color change	Average % of Ag Ions (SDF)	Average % of Ag ions (Control)	Average of PEN. Of Ag Ions (nm)	Average of PEN. Of Ag Ions nm (control)	Avg. Depth of Decay (mm)	Light Cured (1=no 2-yes)	Week (1=1wk, 4=4wk)
1	2	0.07	0.00	144	0	0.18666667	1	1
2	3	0.02	0.01	303	25.95	0.35777778	1	1
3	5	0.05	0.02	220	89.25	0.23666667	1	1
4	5	0.00	0.00	0	0	0.27444444	1	1
5	2	0.10	0.00	104.35	0	0.16444444	1	1
6	5	0.05	0.00	101.5	0	0.27	1	1
7	2	0.00	0.00	0	0	0.21888889	1	1
8	2	0.02	0.00	168.5	0	0.09666667	1	1
9	5	0.00	0.00	0	0	0.35555556	1	1
10	5	0.00	0.00	0	0	0.21111111	1	1
11	5	0.02	0.00	54	0	0.13777778	1	1
12	2	0.00	0.00	0	0	0.21111111	1	1
13	2	0.00	0.00	0	0	0.14888889	1	1
14	5	0.00	0.00	0	0	0.19444444	1	1
15	5	0.01	0.00	70	0	0.09444444	1	1
16	1	0.01	0.00	129.5	0	0.18777778	2	1
17	1	0.00	0.00	0	0	0.07555556	2	1
18	1	0.00	0.00	0	0	0.09444444	2	1
19	1	0.02	0.00	52.5	0	0.33666667	2	1
20	1	0.00	0.00	0	0	0.12444444	2	1

21	1	0.05	0.00	111.5	0	0.24555555 6	2	1
22	1	0.10	0.00	186.5	0	0.23888888 9	2	1
23	1	0.02	0.01	260	30	0.26777777 8	2	1
24	1	0.04	0.00	232.5	0	0.14666666 7	2	1
25	1	0.07	0.00	270.5	0	0.21333333 3	2	1
26	1	0.01	0.00	78	0	0.21444444 4	2	1
27	1	0.01	0.00	88	0	0.37777777 8	2	1
28	1	0.00	0.00	0	0	0.15555555 6	2	1
29	1	0.02	0.00	421	0	0.20444444 4	2	1
30	1	0.00	0.00	0	0	0.25444444 4	2	1
31	5	0.00	0.00	0	0	0.83	1	4
32	5	0.04	0.00	364.95	0	0.71444444 4	1	4
33	5	0.07	0.00	204.4	0	0.77666666 7	1	4
34	5	0.00	0.00	0	0	0.86111111 1	1	4
35	5	0.00	0.00	0	0	0.61888888 9	1	4
36	5	0.00	0.00	0	0	0.74333333 3	1	4
37	5	0.00	0.00	0	0	0.26777777 8	1	4
38	5	0.00	0.00	0	0	0.55	1	4
39	5	0.00	0.00	0	0	0.60888888 9	1	4
40	5	0.00	0.00	0	0	0.61333333 3	1	4
41	5	0.00	0.00	0	0	0.56111111 1	1	4
42	5	0.00	0.00	0	0	0.78333333 3	1	4
43	5	0.00	0.00	0	0	0.39111111 1	1	4

44	5	0.00	0.00	0	0	0.71333333	1	4
45	5	0.00	0.00	0	0	0.76555556	1	4
46	1	0.00	0.00	0	0	0.73222222	2	4
47	1	0.00	0.00	0	0	0.70666667	2	4
48	1	0.00	0.00	0	0	0.64444444	2	4
49	1	0.00	0.00	0	0	0.76222222	2	4
50	1	0.00	0.00	0	0	0.81	2	4
51	1	0.02	0.00	261.35	0	0.58222222	2	4
52	1	0.02	0.00	135.55	0	0.68444444	2	4
53	1	0.03	0.01	276.35	116.65	0.72222222	2	4
54	1	0.01	0.00	159.5	0	0.79	2	4
55	1	0.00	0.00	0	0	0.68444444	2	4
56	1	0.00	0.00	0	0	0.62	2	4
57	1	0.00	0.00	0	0	0.63888889	2	4
58	1	0.00	0.00	0	0	0.72333333	2	4
59	1	0.00	0.00	0	0	0.72111111	2	4
60	1	0	0.00	0	0	0.69	2	4

4 Week Light Cured Samples

Sample	C%	O%	Na%	Al%	P%	Mg%	Cl%	Ca%		
1	40	24	0.8	1	10	0	0	0		
2	60	17	0.2	2	0.2	0	0	0		
3	59	38	0	2	0	0	0	0		
4	29	52	1	1	15	0.4	0.3	0.3		
5	61	30	0	8	0.4	0	0	0		
6	66	19	0	10	6	0	0	0		
7	55	30	0	13	0.2	0	0	0		
8	48	15	0.1	4	9	0	1	8		
9	55	28	0	14	0.5	0	0.2	0		
10	63	25	0	11	0.75	0	0.2	0		
11	50	35	0	9	5	0	0	0		
12	52	15	1	4	8	0	0	0		
13	48	31	0.3	17	5	0	0	1		
14	51	34	0.2	12	3	0	0	0		
15	55	34	0	9	1	0	0	0		

4 Week Non-light Cured Samples

Sample	C%	O%	Na%	Al%	P%	Mg%	Cl%	Ca%	Br%	
1	64.5	31	0	3.5	0	0	0	0		
2	64	19.5	0	7.5	2	0	2.5	2.5		
3	59	20	0	6	2.5	0	1.3	3		
4	36	29	0	6	11.5	0.5	0	18.5	0.7	
5	41	51	0.5	0.5	6	0	0	0		
6	62	32	0	6	0	0	0	0		
7	67	30	0	3	0	0	0	0		
8	32	16	0	2	0	0	0	0		
9	38	54	0	0.5	7	0	0	0		
10	59	36	0	3.5	1 / 2	0	0	0		
11	72	27.5	0	1	0	0	0	0		
12	45	52	0	0	3	0	0	0		
13	54	40	0	6	0	0	0	0		
14	70	14	0	3	0	0	0	0		
15	71	24	0	6	0	0	0	0		

1 Week Light Cured Samples

Sample	C%	O%	Na%	Al%	P%	Mg%	Cl%	Ca%	Br%	
1	70	22	0	7	0.5	0	0.5	0		
2	56	36	0	5.5	3.5	0	0	0		
3	60	32	0	3.5	3	0	0	0		
4	47	41	0	4	4.5	0	1	0.5		

5	52.5	37	0	6.5	3.2	0	0	0		
6	0	0	0	0	0	0	0	0		
7	27	31	0	6	12	0	2	8	5	
8	42	41	1	3	11	0	2	1		
9	45	32	0	4	0	0	0	0		
10	0	0	0	0	9	0	2	5		
11	50	31	0	20	0.25	0	0	0		
12	94	49	0	3.5	1	0	0	0		
13	61	29	0	20	0	0	0	0		
14	53	30	0	13.5	2	0	0	0		
15	48	49	0	2	1.5	0	0	0		

1 Week Non-Light Cured Samples

Sample	C%	O%	Na%	Al%	P%	Mg%	Cl%	Ca%		
1	44	30	0	6.5	8	0	1	4.5		
2	58	26	0	11	0	0	2	0		
3	55	24	0	12	2	0	2	.5		
4	55	39	0	2.5	2	0	0	0		
5	50	26	0	8	3	0	2	0		
6	52	20	0	16	0	0	0	6		
7	73	25	0	2	0	0	0	0		
8	40	35	0	8	10	0	0	2		
9	70	27	0	2	.5	0	0	0		
10	56	29	7	8	1	0	0	0.5		
11	67	23	0	8	0	0	1	0		
12	72	26	0	2	0	0	0	0		
13	71	24	0	4	0	0	0	0		
14	63	31	0	6	0	0	0	0		
15	68	23	0	7.5	1	0	0	0		

```
FREQUENCIES VARIABLES=COLORCHANGE AgIons-SDF AgIons-SDF_A AgIons-control
AgIons-control_A PenAgionsSDF PenAgionsSDF_A PenAgionsControl
PenAgionsControl_A LightCured Week MeanPercentIons2observationsLightCured
MeanPercentIons2observationsControl
    MeanPenetration2observationsLightCured
MeanPenetration2observationsControl
  /STATISTICS=STDDEV MINIMUM MAXIMUM MEAN MEDIAN MODE
  /HISTOGRAM
  /ORDER=ANALYSIS.
```

Frequencies

Notes

Output Created		13-APR-2018 10:32:36
Comments		
Input	Data	H:\LeHew\First Year Residents 2016\Jasma'\Data Final.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	60
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data.

Syntax		FREQUENCIES VARIABLES=COLORCHANGE AgIons–SDF AgIons–SDF_A AgIons–control AgIons–control_A PenAgionsSDF PenAgionsSDF_A PenAgionsControl PenAgionsControl_A LightCured Week MeanPercentIons2observationsLightCured MeanPercentIons2observationsControl MeanPenetration2observationsLightCured MeanPenetration2observationsControl /STATISTICS=STDDEV MINIMUM MAXIMUM MEAN MEDIAN MODE /HISTOGRAM /ORDER=ANALYSIS.
Resources	Processor Time	00:00:02.76
	Elapsed Time	00:00:02.66

Statistics

	COLOR CHANGE OVER TIME	% Ag Ions – SDF	% Ag Ions – SDF	% Ag Ions – control	% Ag Ions – control	Pen Ag ions - SDF	Pen Ag ions - SDF	Pen Ag ions - Control	Pen Ag ions - Control	Light Cured	Week	Average Percentage of Silver Treated Sections	Average Percentage of Silver Control Sections	Average Depth of Silver Penetration Treated Sections	Average Depth of Penetration Control Sections
N Valid	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Missing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean	2.67	.014752	.014345	.001432	.000263	81.413	65.178	4.840	3.888	1.483	2.500	.01454833	.00084750	73.2958	4.3642
Median	1.50	.000000	.000000	.000000	.000000	.000	.000	.000	.000	1.000	2.500	.00000000	.00000000	.0000	.0000

Mode	1	.0000	.0000	.0000	.0000	.0	.0	.0	.0	1.0	1.0[a]	.000000	.000000	.00	.00
Std. Deviation	1.893	.0250373	.0287120	.0056213	.0016002	121.3025	125.8254	24.9577	30.1189	.5039	1.5127	.0249103897	.0029100362	109.86785	19.31164
Minimum	1	.0000	.0000	.0000	.0000	.0	.0	.0	.0	1.0	1.0	.000000	.000000	.00	.00
Maximum	5	.1061	.1395	.0305	.0118	467.9	654.0	178.5	233.3	2.0	4.0	.098700	.015250	421.00	116.65

a. Multiple modes exist. The smallest value is shown

Frequency Table

COLOR CHANGE OVER TIME

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Immediate	30	50.0	50.0	50.0
	Less than 30 Minutes	6	10.0	10.0	60.0
	More than 30 Minutes	1	1.7	1.7	61.7
	More than 24 hours	23	38.3	38.3	100.0
	Total	60	100.0	100.0	

% Ag Ions – SDF

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.0000	37	61.7	61.7	61.7
	.0094	1	1.7	1.7	63.3
	.0100	1	1.7	1.7	65.0
	.0114	1	1.7	1.7	66.7
	.0120	1	1.7	1.7	68.3
	.0138	1	1.7	1.7	70.0
	.0149	1	1.7	1.7	71.7
	.0161	1	1.7	1.7	73.3

	.0190	1	1.7	1.7	75.0
	.0211	1	1.7	1.7	76.7
	.0286	1	1.7	1.7	78.3
	.0294	1	1.7	1.7	80.0
	.0347	1	1.7	1.7	81.7
	.0373	1	1.7	1.7	83.3
	.0389	1	1.7	1.7	85.0
	.0432	1	1.7	1.7	86.7
	.0500	1	1.7	1.7	88.3
	.0521	1	1.7	1.7	90.0
	.0559	1	1.7	1.7	91.7
	.0574	1	1.7	1.7	93.3
	.0600	1	1.7	1.7	95.0
	.0655	1	1.7	1.7	96.7
	.0983	1	1.7	1.7	98.3
	.1061	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

% Ag Ions – SDF

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.0000	40	66.7	66.7	66.7
	.0052	1	1.7	1.7	68.3
	.0063	1	1.7	1.7	70.0
	.0072	1	1.7	1.7	71.7
	.0086	1	1.7	1.7	73.3
	.0099	1	1.7	1.7	75.0
	.0146	1	1.7	1.7	76.7
	.0184	1	1.7	1.7	78.3
	.0254	1	1.7	1.7	80.0
	.0341	1	1.7	1.7	81.7
	.0347	1	1.7	1.7	83.3
	.0370	1	1.7	1.7	85.0
	.0384	1	1.7	1.7	86.7
	.0423	1	1.7	1.7	88.3

	.0497	1	1.7	1.7	90.0
	.0716	1	1.7	1.7	91.7
	.0741	1	1.7	1.7	93.3
	.0753	1	1.7	1.7	95.0
	.0771	1	1.7	1.7	96.7
	.0913	1	1.7	1.7	98.3
	.1395	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

% Ag Ions – control

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.0000	55	91.7	91.7	91.7
	.0036	1	1.7	1.7	93.3
	.0087	1	1.7	1.7	95.0
	.0171	1	1.7	1.7	96.7
	.0260	1	1.7	1.7	98.3
	.0305	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

% Ag Ions – control

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.0000	58	96.7	96.7	96.7
	.0040	1	1.7	1.7	98.3
	.0118	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

Pen Ag ions - SDF

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.0	36	60.0	60.0	60.0
	105.0	2	3.3	3.3	63.3
	106.0	1	1.7	1.7	65.0
	107.0	1	1.7	1.7	66.7

	108.0	1	1.7	1.7	68.3
	116.0	1	1.7	1.7	70.0
	120.8	1	1.7	1.7	71.7
	126.7	1	1.7	1.7	73.3
	136.0	1	1.7	1.7	75.0
	140.0	1	1.7	1.7	76.7
	145.0	1	1.7	1.7	78.3
	156.0	1	1.7	1.7	80.0
	158.0	1	1.7	1.7	81.7
	176.0	1	1.7	1.7	83.3
	188.0	1	1.7	1.7	85.0
	231.0	1	1.7	1.7	86.7
	246.0	1	1.7	1.7	88.3
	258.0	1	1.7	1.7	90.0
	271.1	1	1.7	1.7	91.7
	278.3	1	1.7	1.7	93.3
	319.0	1	1.7	1.7	95.0
	376.0	1	1.7	1.7	96.7
	444.0	1	1.7	1.7	98.3
	467.9	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

Pen Ag ions - SDF

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.0	43	71.7	71.7	71.7
	87.0	1	1.7	1.7	73.3
	101.7	1	1.7	1.7	75.0
	118.0	1	1.7	1.7	76.7
	143.6	1	1.7	1.7	78.3
	153.0	1	1.7	1.7	80.0
	162.0	1	1.7	1.7	81.7
	165.0	1	1.7	1.7	83.3
	201.0	1	1.7	1.7	85.0
	207.0	1	1.7	1.7	86.7

	209.0	1	1.7	1.7	88.3
	215.0	1	1.7	1.7	90.0
	262.0	1	1.7	1.7	91.7
	274.0	1	1.7	1.7	93.3
	274.4	1	1.7	1.7	95.0
	288.0	1	1.7	1.7	96.7
	396.0	1	1.7	1.7	98.3
	654.0	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

Pen Ag ions - Control

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.0	57	95.0	95.0	95.0
	51.9	1	1.7	1.7	96.7
	60.0	1	1.7	1.7	98.3
	178.5	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

Pen Ag ions - Control

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.0	59	98.3	98.3	98.3
	233.3	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

Light Cured

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.0	31	51.7	51.7	51.7
	2.0	29	48.3	48.3	100.0
	Total	60	100.0	100.0	

Week

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.0	30	50.0	50.0	50.0
	4.0	30	50.0	50.0	100.0
	Total	60	100.0	100.0	

Average Percentage of Silver Treated Sections

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.000000	36	60.0	60.0	60.0
	.005000	1	1.7	1.7	61.7
	.005700	1	1.7	1.7	63.3
	.008050	1	1.7	1.7	65.0
	.008300	1	1.7	1.7	66.7
	.008600	1	1.7	1.7	68.3
	.016800	1	1.7	1.7	70.0
	.017350	1	1.7	1.7	71.7
	.019200	1	1.7	1.7	73.3
	.020150	1	1.7	1.7	75.0
	.023750	1	1.7	1.7	76.7
	.023900	1	1.7	1.7	78.3
	.023950	1	1.7	1.7	80.0
	.024750	1	1.7	1.7	81.7
	.031650	1	1.7	1.7	83.3
	.039800	1	1.7	1.7	85.0
	.043500	1	1.7	1.7	86.7
	.048200	1	1.7	1.7	88.3
	.050900	1	1.7	1.7	90.0
	.054100	1	1.7	1.7	91.7
	.065000	1	1.7	1.7	93.3
	.068550	2	3.3	3.3	96.7
	.098450	1	1.7	1.7	98.3
	.098700	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

Average Percentage of Silver Control Sections

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.000000	54	90.0	90.0	90.0
	.003800	1	1.7	1.7	91.7
	.004350	1	1.7	1.7	93.3
	.005900	1	1.7	1.7	95.0
	.008550	1	1.7	1.7	96.7
	.013000	1	1.7	1.7	98.3
	.015250	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

Average Depth of Silver Penetration Treated Sections

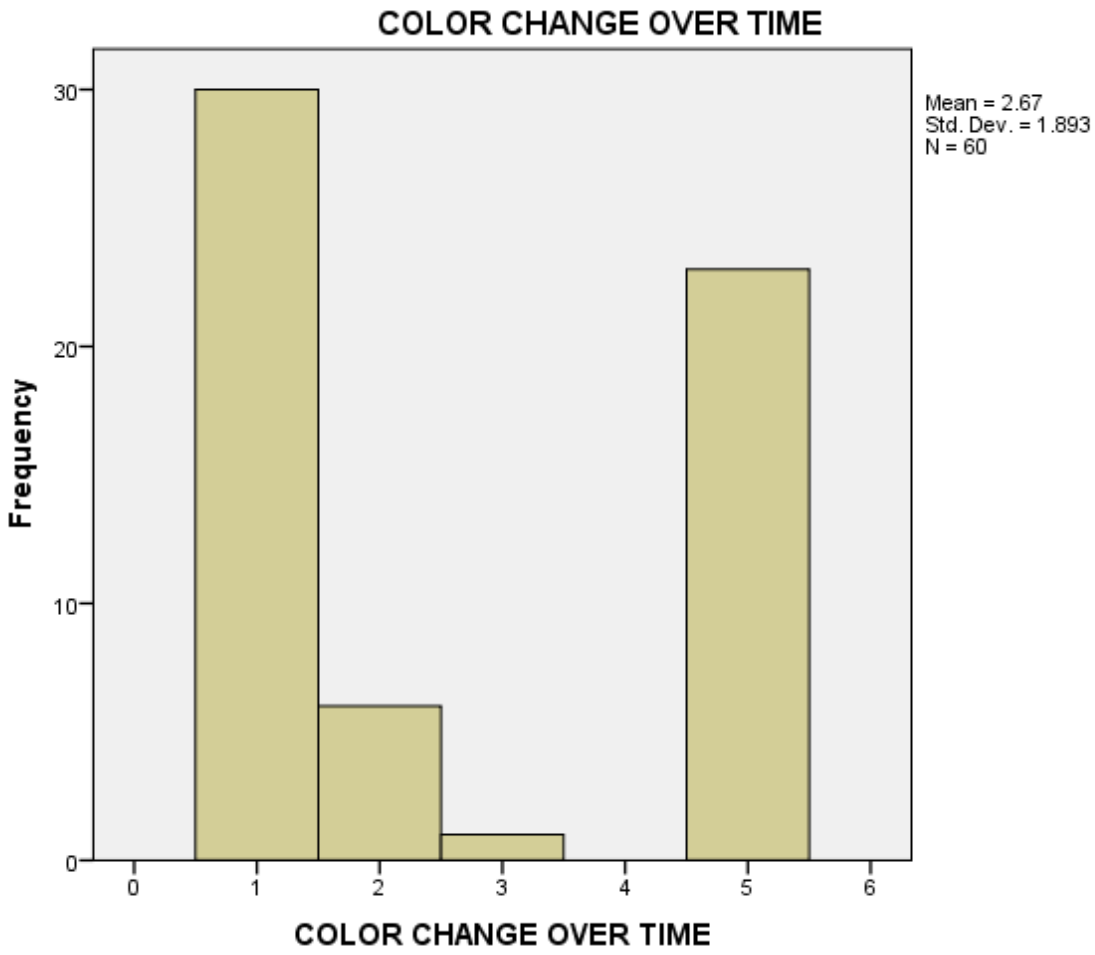
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	36	60.0	60.0	60.0
	52.50	1	1.7	1.7	61.7
	54.00	1	1.7	1.7	63.3
	70.00	1	1.7	1.7	65.0
	78.00	1	1.7	1.7	66.7
	88.00	1	1.7	1.7	68.3
	101.50	1	1.7	1.7	70.0
	104.35	1	1.7	1.7	71.7
	111.50	1	1.7	1.7	73.3
	129.50	1	1.7	1.7	75.0
	135.55	1	1.7	1.7	76.7
	144.30	1	1.7	1.7	78.3
	159.50	1	1.7	1.7	80.0
	168.50	1	1.7	1.7	81.7
	186.50	1	1.7	1.7	83.3
	204.40	1	1.7	1.7	85.0
	220.00	1	1.7	1.7	86.7
	232.50	1	1.7	1.7	88.3
	260.00	1	1.7	1.7	90.0
	261.35	1	1.7	1.7	91.7
	270.50	1	1.7	1.7	93.3

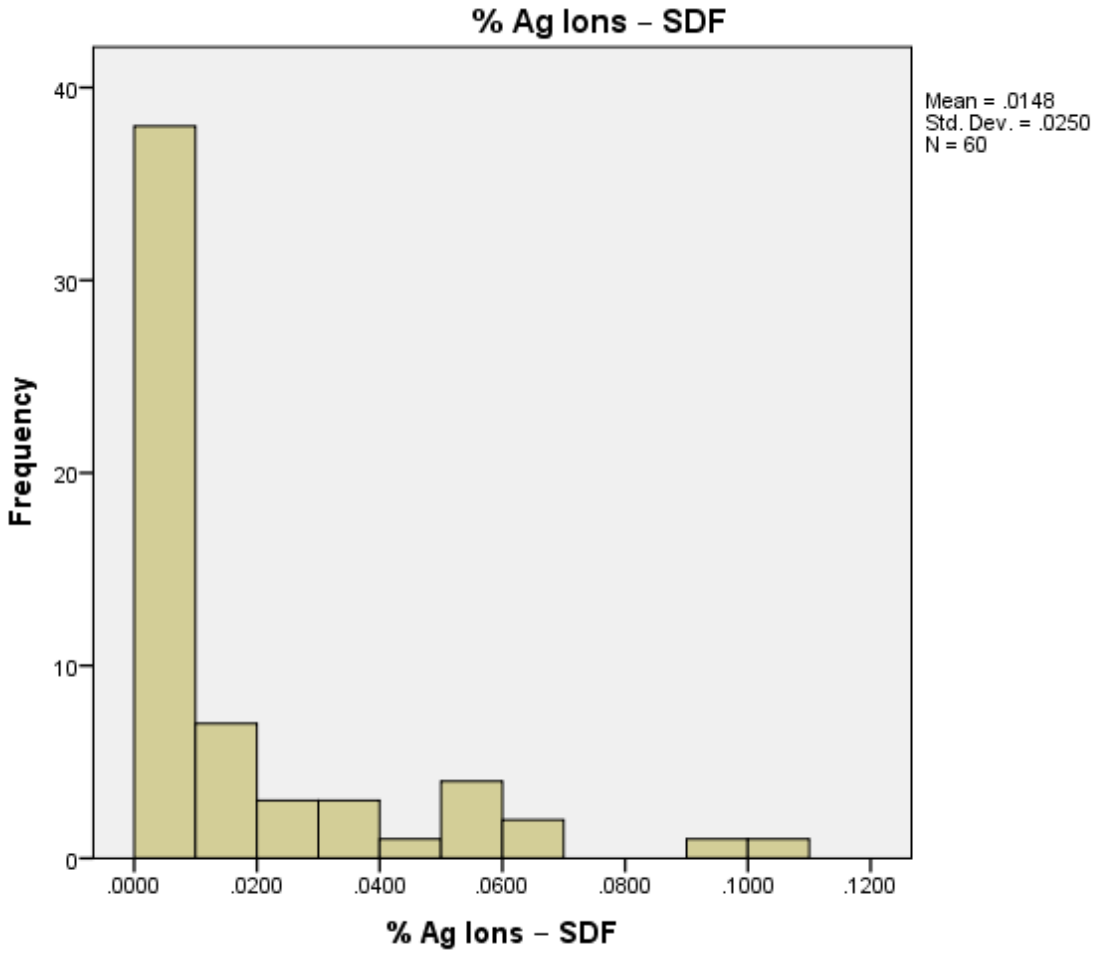
	276.35	1	1.7	1.7	95.0
	303.00	1	1.7	1.7	96.7
	364.95	1	1.7	1.7	98.3
	421.00	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

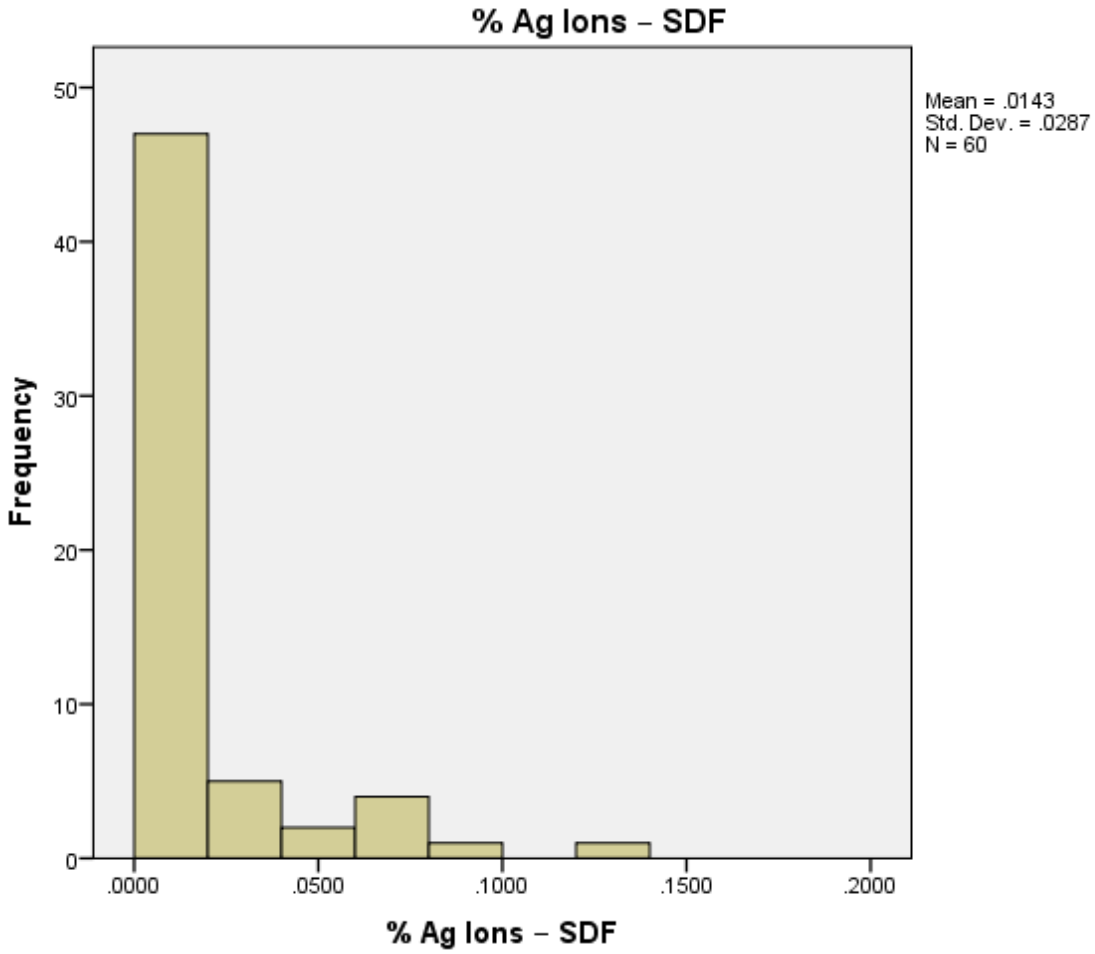
Average Depth of Penetration Control Sections

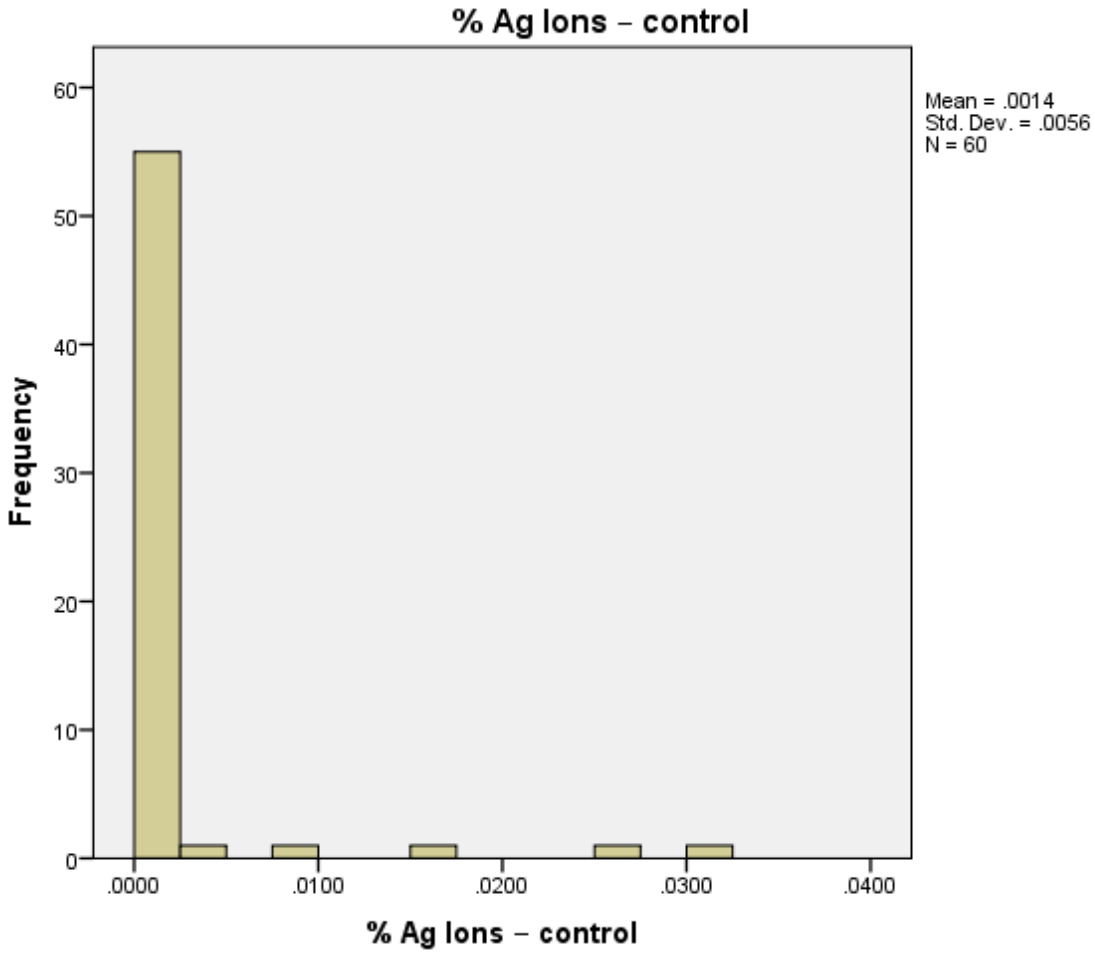
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	56	93.3	93.3	93.3
	25.95	1	1.7	1.7	95.0
	30.00	1	1.7	1.7	96.7
	89.25	1	1.7	1.7	98.3
	116.65	1	1.7	1.7	100.0
	Total	60	100.0	100.0	

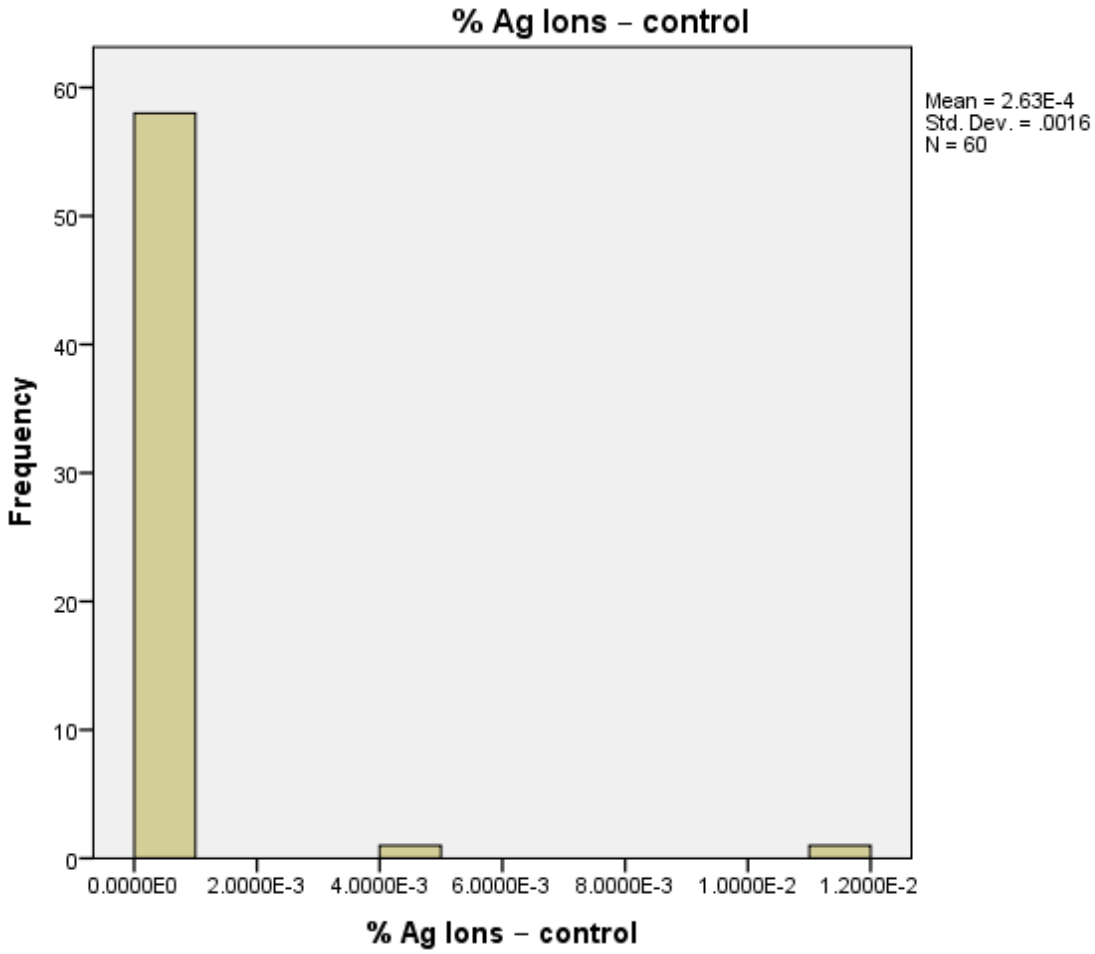
Histogram

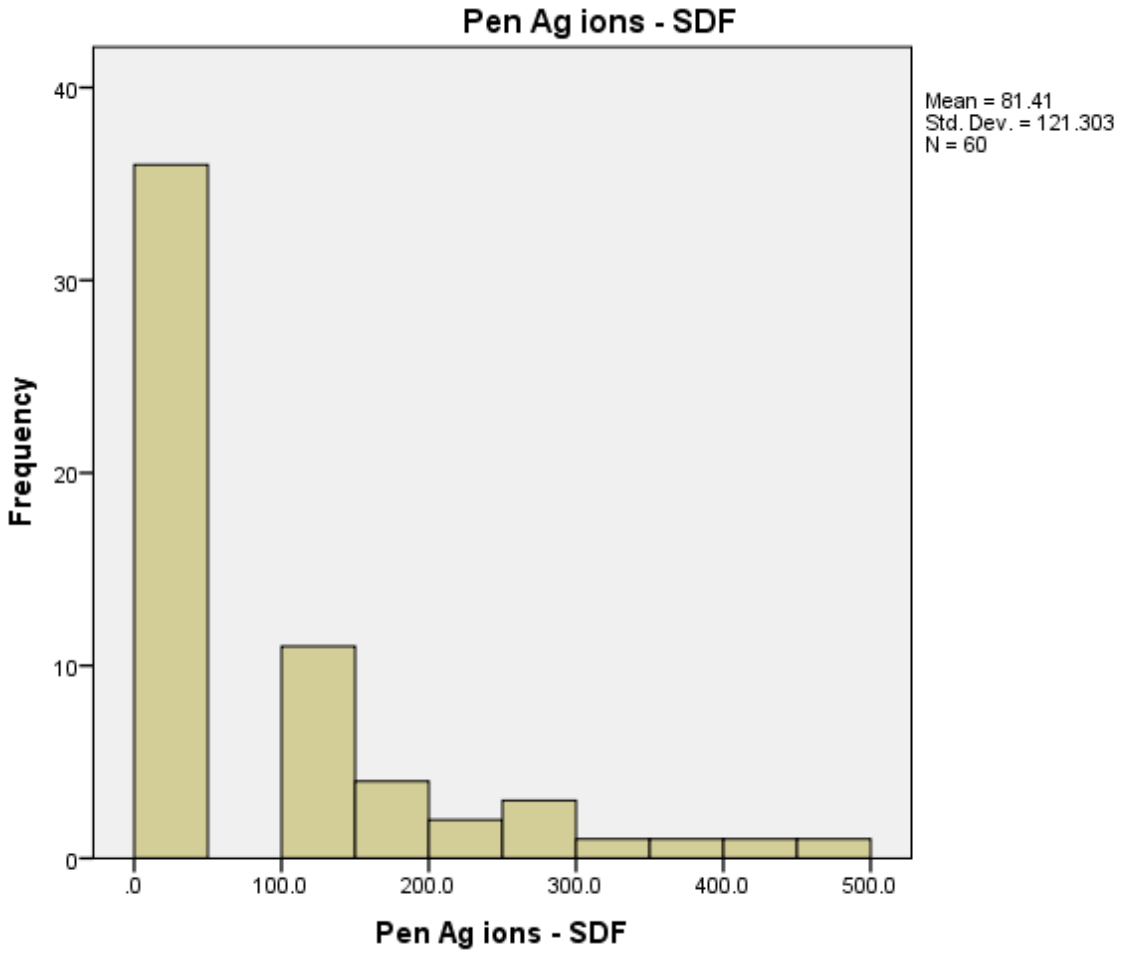

COLOR CHANGE OVER TIME
Mean = 2.67
Std. Dev. = 1.893
N = 60
Frequency
30
20
10
0
0
1
2
3
4
5
6
COLOR CHANGE OVER TIME

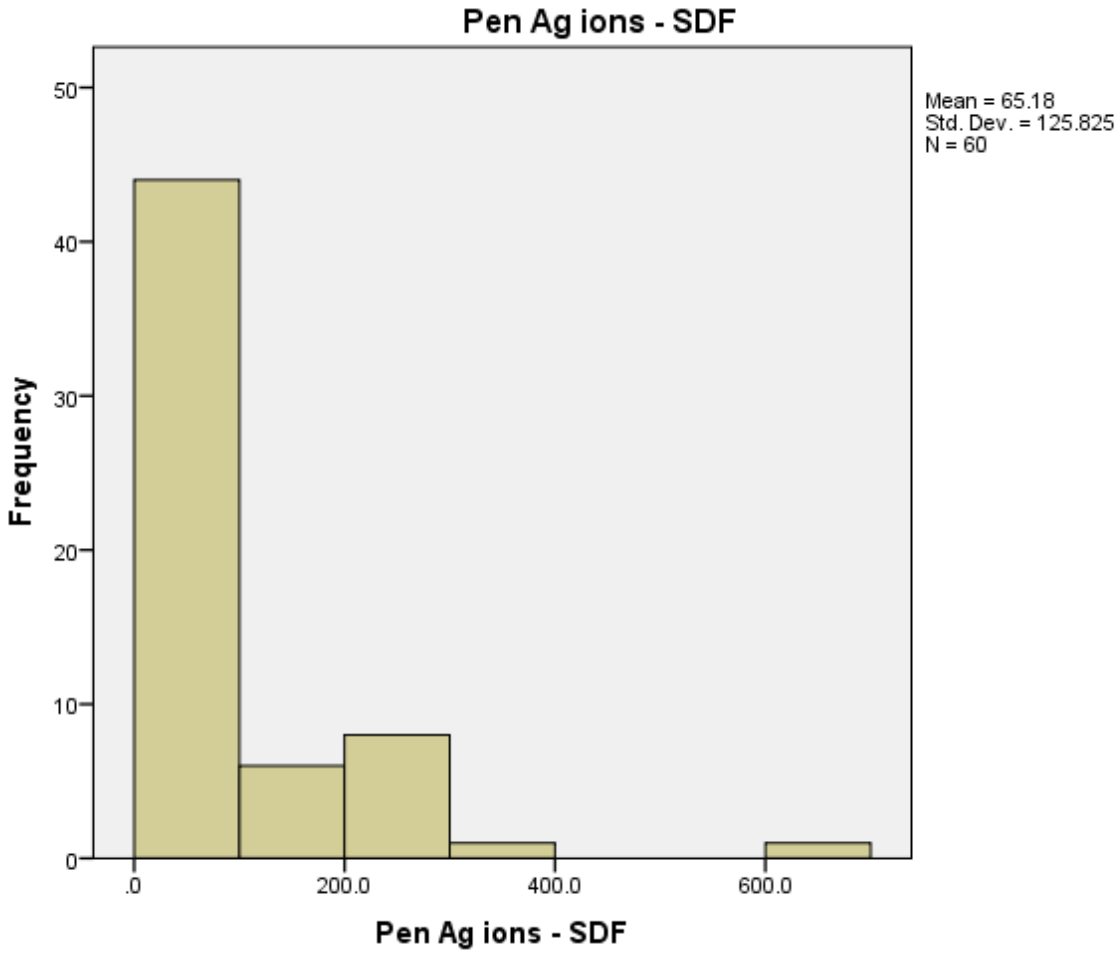

% Ag Ions – SDF
Mean = .0148
Std. Dev. = .0250
N = 60
Frequency
40
30
20
10
0
.0000
.0200
.0400
.0600
.0800
.1000
.1200
% Ag Ions – SDF

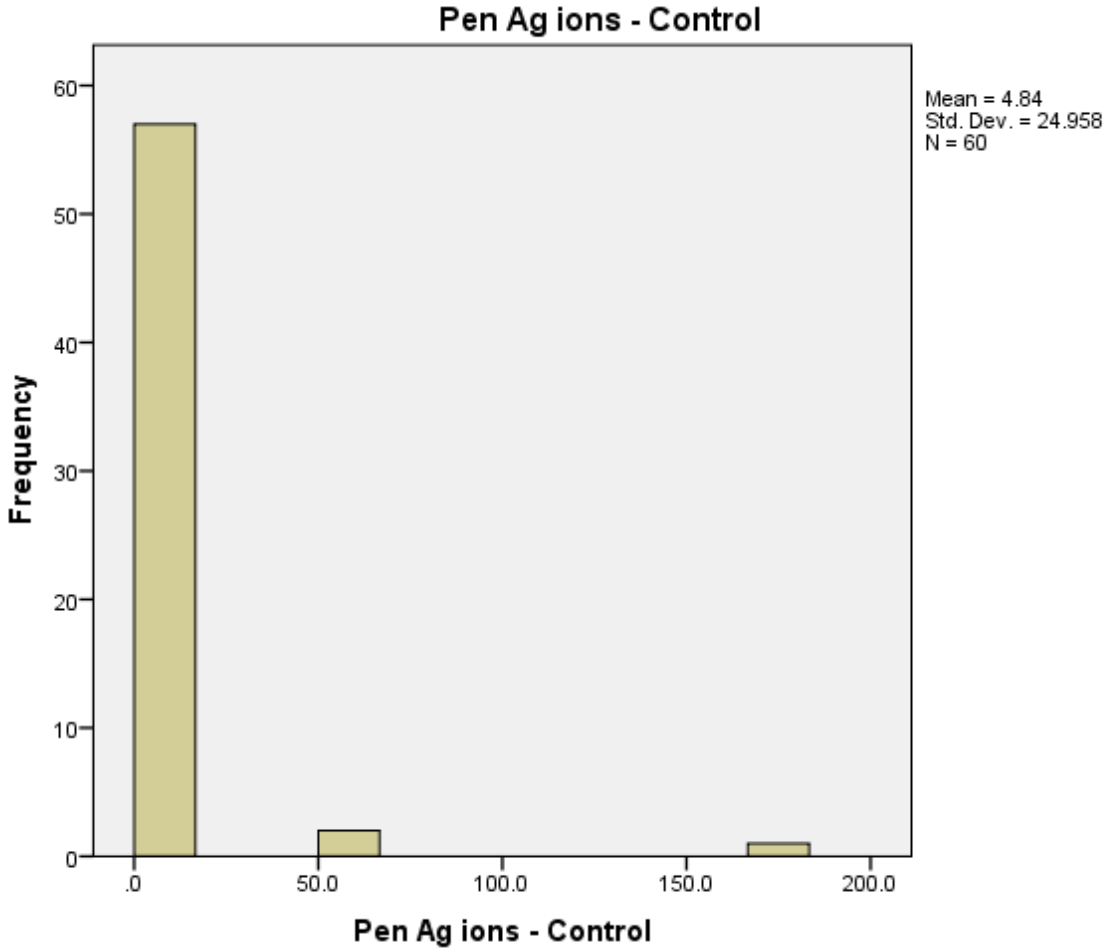

% Ag Ions – SDF
Mean = .0143
Std. Dev. = .0287
N = 60
Frequency
50
40
30
20
10
0
.0000
.0500
.1000
.1500
.2000
% Ag Ions – SDF

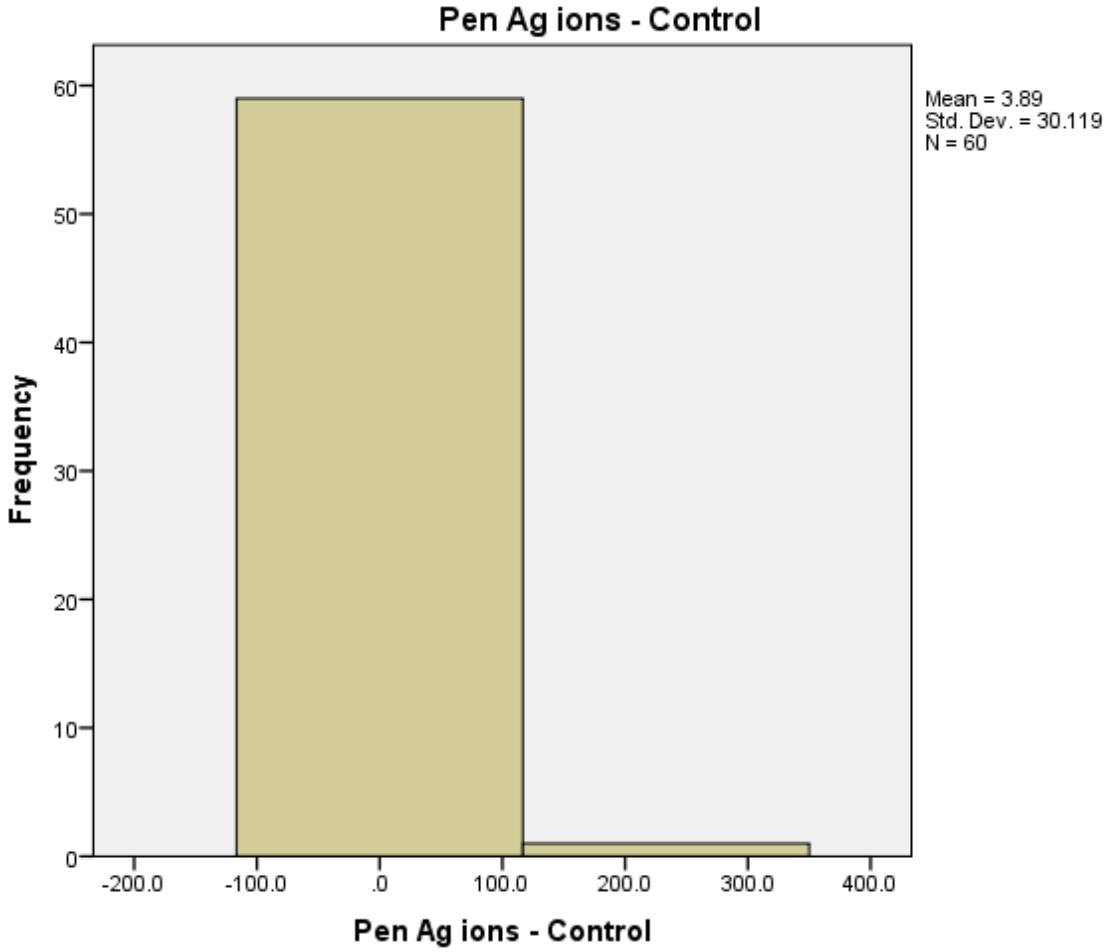

% Ag Ions – control
Mean = .0014
Std. Dev. = .0056
N = 60
Frequency
60
50
40
30
20
10
0
.0000
.0100
.0200
.0300
.0400
% Ag Ions – control

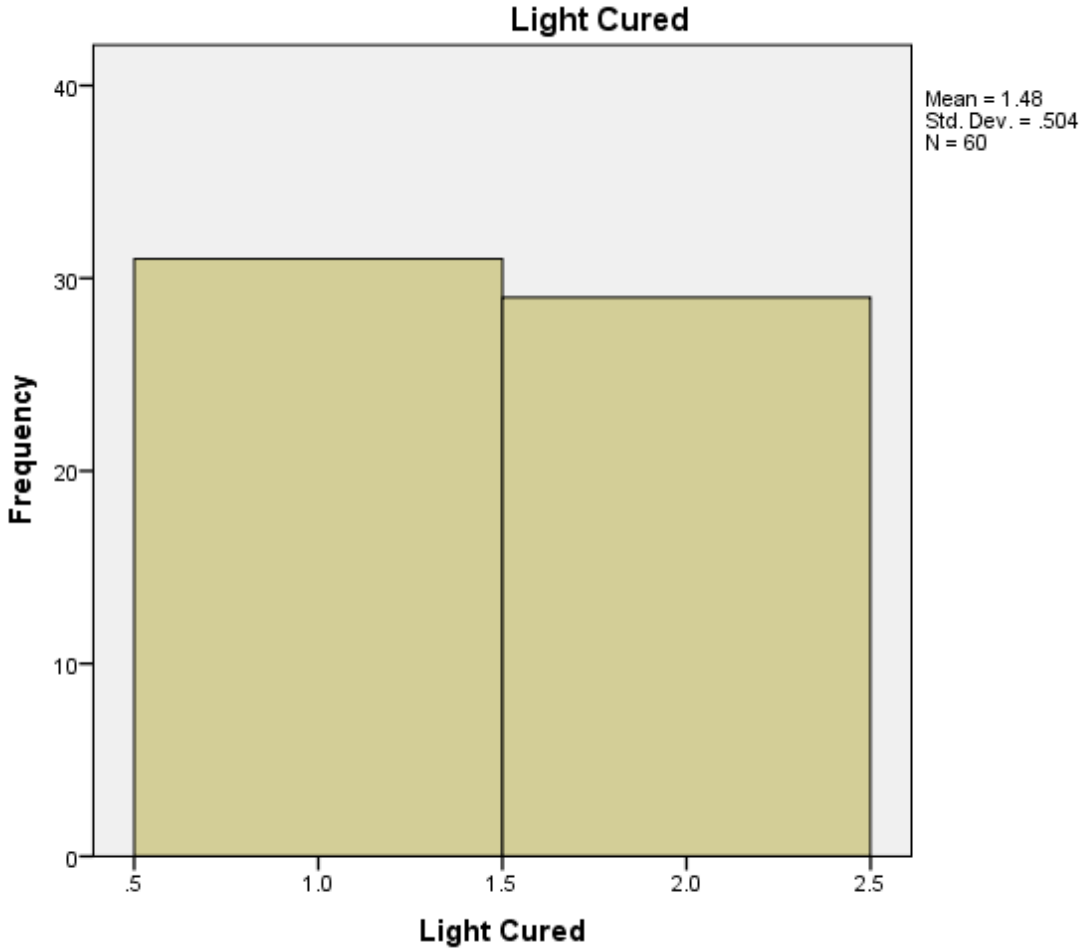

% Ag Ions – control
Mean = 2.63E-4
Std. Dev. = .0016
N = 60
Frequency
60
50
40
30
20
10
0
0.0000E0
2.0000E-3
4.0000E-3
6.0000E-3
8.0000E-3
1.0000E-2
1.2000E-2
% Ag Ions – control

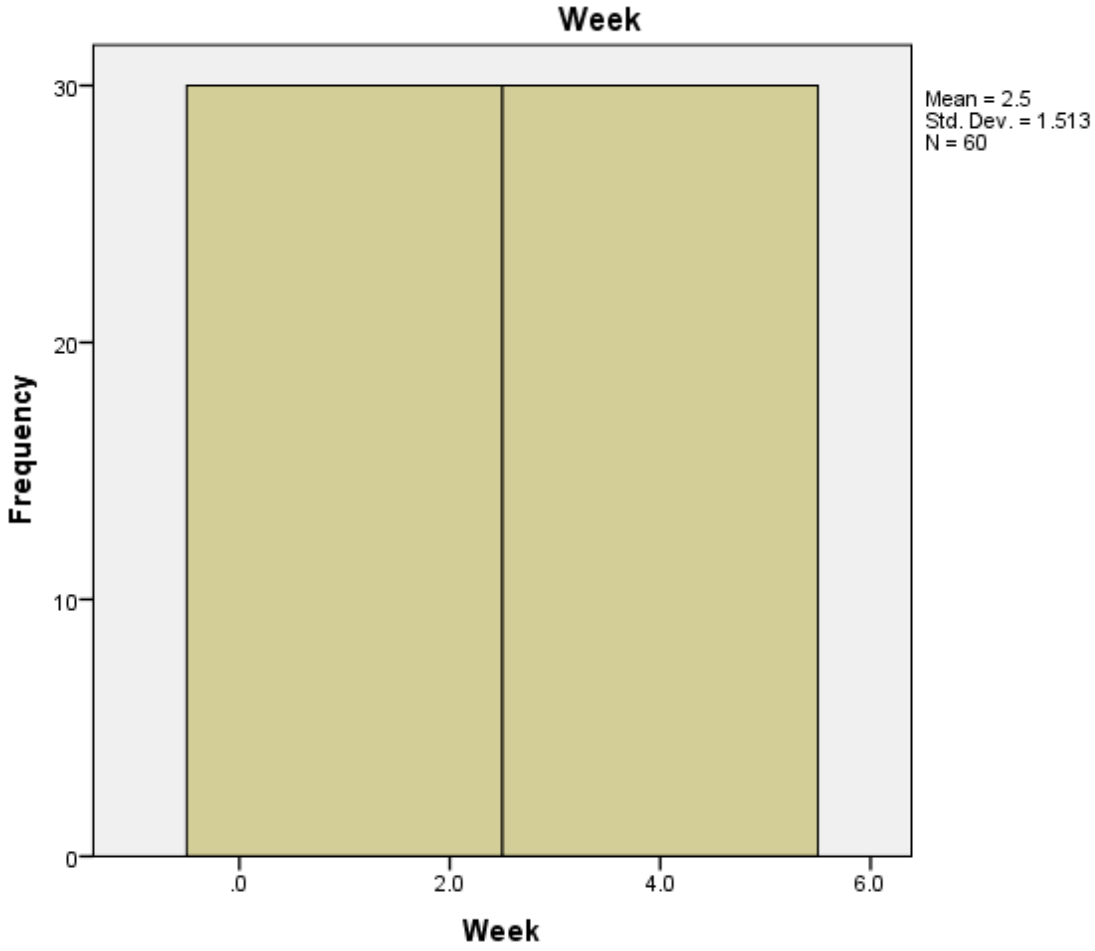

Pen Ag ions - SDF
Mean = 81.41
Std. Dev. = 121.303
N = 60
Frequency
40
30
20
10
0
.0
100.0
200.0
300.0
400.0
500.0
Pen Ag ions - SDF

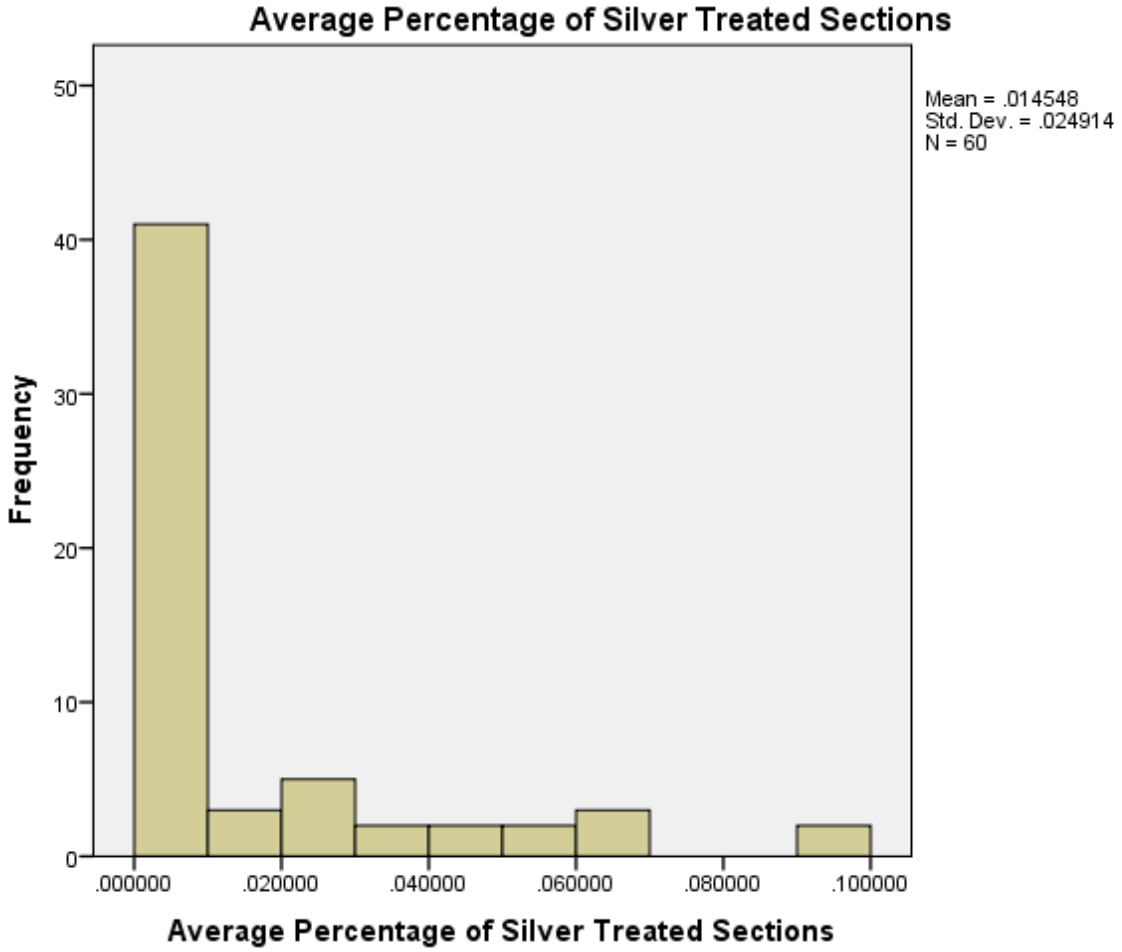

Pen Ag ions - SDF
Mean = 65.18
Std. Dev. = 125.825
N = 60
Frequency
50
40
30
20
10
0
.0
200.0
400.0
600.0
Pen Ag ions - SDF

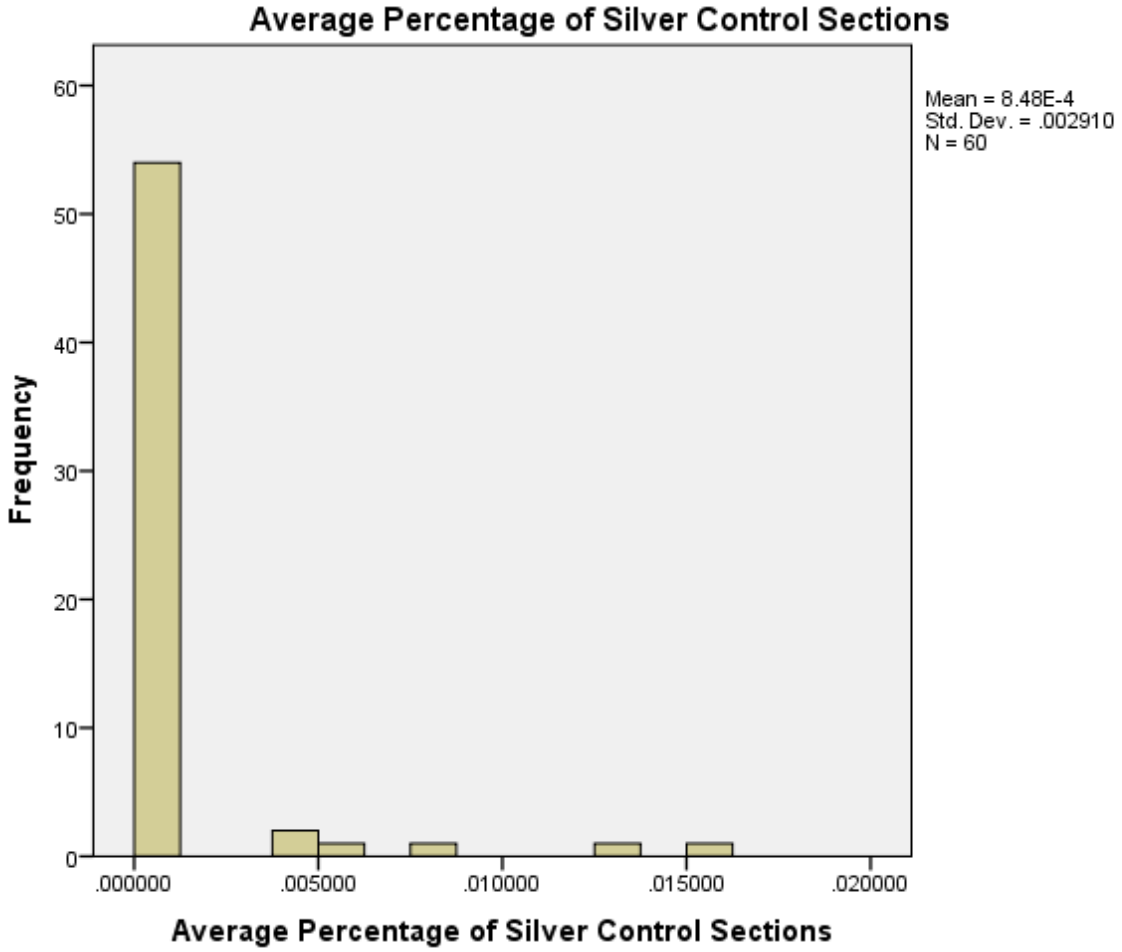

Pen Ag ions - Control
Mean = 4.84
Std. Dev. = 24.958
N = 60
Frequency
60
50
40
30
20
10
0
.0
50.0
100.0
150.0
200.0
Pen Ag ions - Control

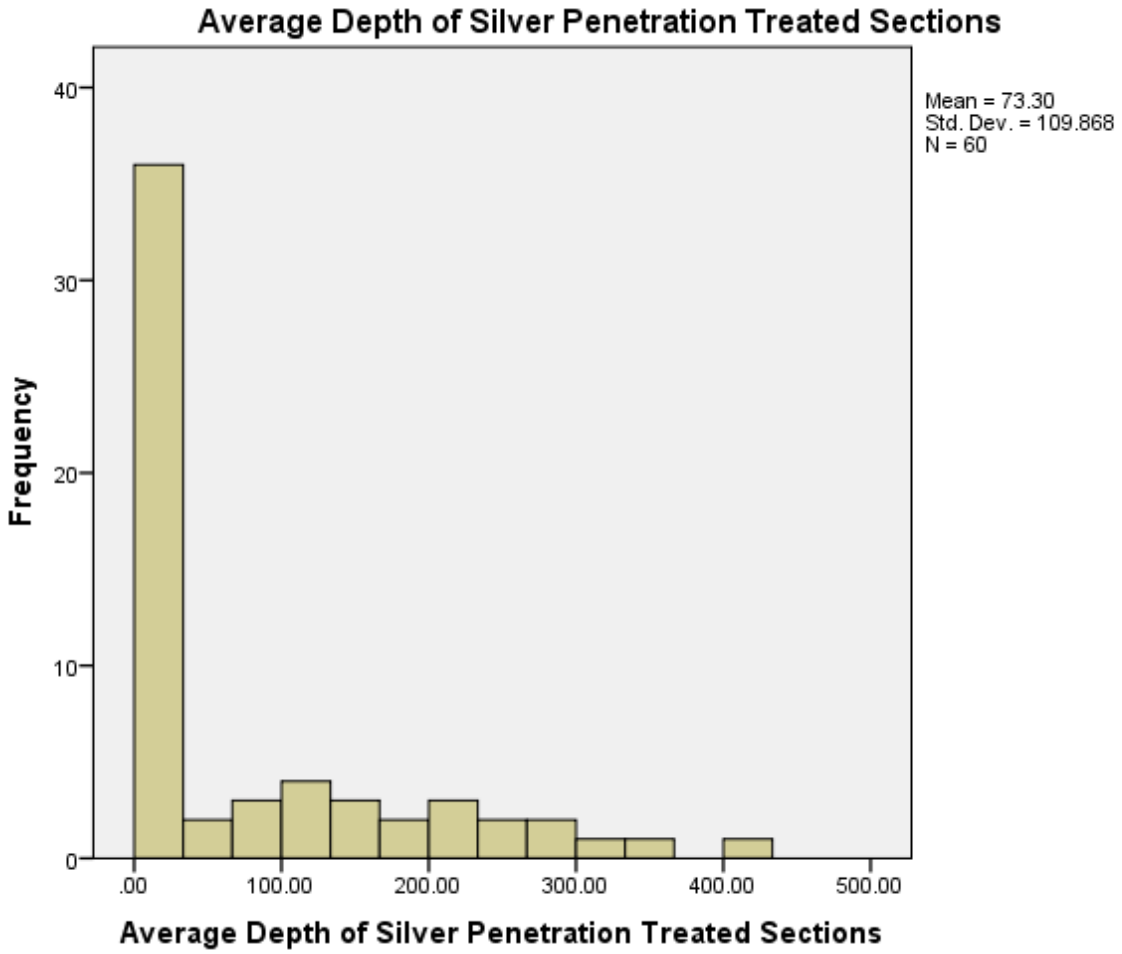

Pen Ag ions - Control
Mean = 3.89
Std. Dev. = 30.119
N = 60
Frequency
60
50
40
30
20
10
0
-200.0
-100.0
.0
100.0
200.0
300.0
400.0
Pen Ag ions - Control

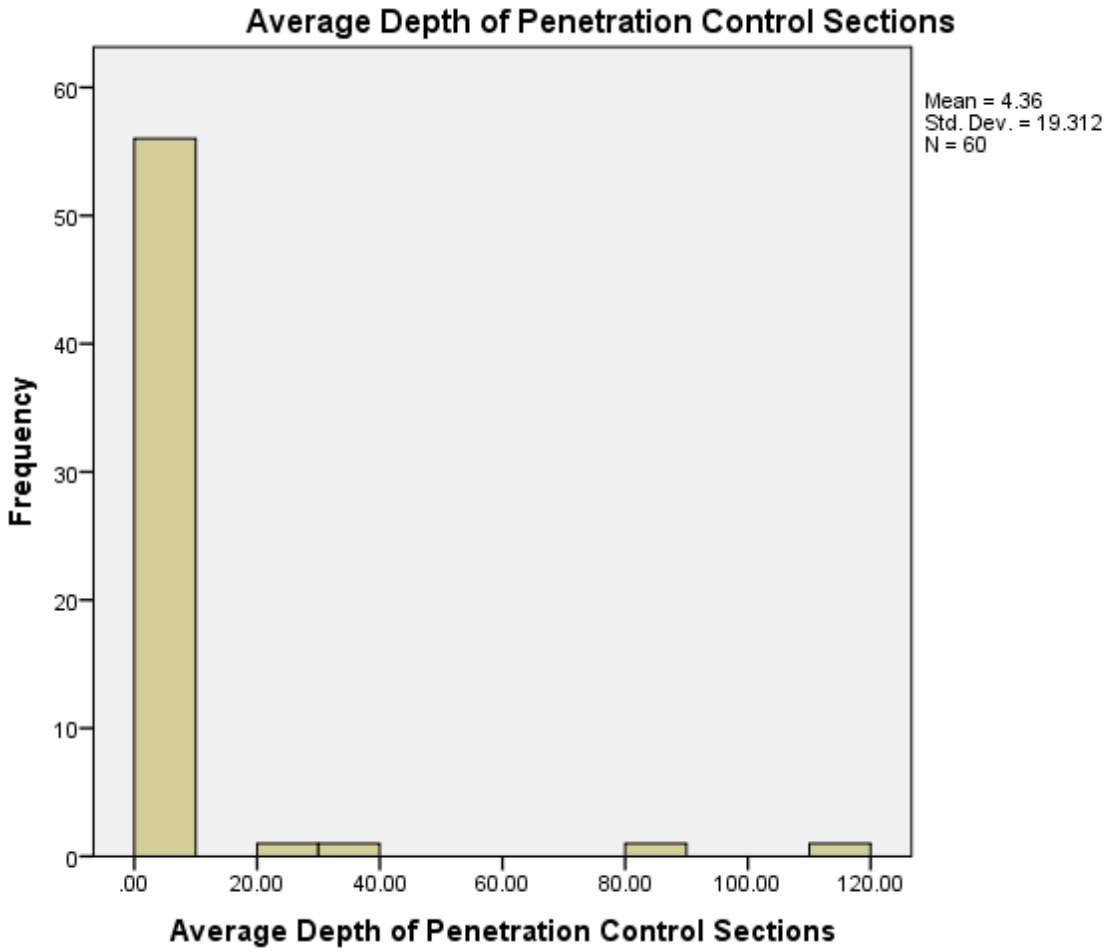

Light Cured
Mean = 1.48
Std. Dev. = .504
N = 60
Frequency
40
30
20
10
0
.5
1.0
1.5
2.0
2.5
Light Cured


Week
Mean = 2.5
Std. Dev. = 1.513
N = 60
Frequency
30
20
10
0
.0
2.0
4.0
6.0
Week


Average Percentage of Silver Treated Sections
Mean = .014548
Std. Dev. = .024914
N = 60
Frequency
50
40
30
20
10
0
.000000
.020000
.040000
.060000
.080000
.100000
Average Percentage of Silver Treated Sections


Average Percentage of Silver Control Sections
Mean = 8.48E-4
Std. Dev. = .002910
N = 60
Frequency
60
50
40
30
20
10
0
.000000
.005000
.010000
.015000
.020000
Average Percentage of Silver Control Sections


Average Depth of Silver Penetration Treated Sections
Mean = 73.30
Std. Dev. = 109.868
N = 60
Frequency
40
30
20
10
0
.00
100.00
200.00
300.00
400.00
500.00
Average Depth of Silver Penetration Treated Sections




```
T-TEST GROUPS=LightCured(1 2)
  /MISSING=ANALYSIS
  /VARIABLES=MeanPercentIons2observationsLightCured
MeanPenetration2observationsLightCured
  /CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		13-APR-2018 10:33:38
Comments		
Input	Data	H:\LeHew\First Year Residents 2016\Jasma'\Data Final.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	60
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=LightCured(1 2) /MISSING=ANALYSIS /VARIABLES=MeanPercentIons2observations LightCured MeanPenetration2observationsLightCured /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.01

Group Statistics

	Light Cured	N	Mean	Std. Deviation	Std. Error Mean
Average Percentage of Silver Treated Sections	1.0	31	.01493226	.026312663	.004725894
	2.0	29	.01413793	.023785720	.004416897
Average Depth of Silver Penetration Treated Sections	1.0	31	60.1452	99.67085	17.90141
	2.0	29	87.3534	119.97996	22.27972

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Average Percentage of Silver Treated Sections	Equal variances assumed	.474	.494	.122	58	.903	.000794327	.006490728	-.012198276	.013786930
	Equal variances not assumed			.123	57.937	.903	.000794327	.006468621	-.012154325	.013742980
Average Depth of Silver Penetration Treated Sections	Equal variances assumed	1.769	.189	-.958	58	.342	-27.20829	28.40337	-84.06380	29.64723
	Equal variances not assumed			-.952	54.588	.345	-27.20829	28.58053	-84.49466	30.07808

```
T-TEST GROUPS=Week(1 4) These results show that there is no significant
difference in the percentage of silver or depth of penetration attributable
to light curing.
  /MISSING=ANALYSIS
  /VARIABLES=MeanPercentIons2observationsLightCured
MeanPenetration2observationsLightCured
  /CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		13-APR-2018 10:34:08
Comments		
Input	Data	H:\LeHew\First Year Residents 2016\Jasma'\Data Final.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>

	N of Rows in Working Data File	60
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=Week(1 4) /MISSING=ANALYSIS /VARIABLES=MeanPercentIons2observations LightCured MeanPenetration2observationsLightCured /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.02

Group Statistics

	Week	N	Mean	Std. Deviation	Std. Error Mean
Average Percentage of Silver Treated Sections	1.0	30	.02266000	.029588766	.005402145
	4.0	30	.00643667	.015848561	.002893538
Average Depth of Silver Penetration Treated Sections	1.0	30	99.8550	113.24931	20.67640
	4.0	30	46.7367	101.35727	18.50522

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Average Percentage of Silver	Equal variances assumed	10.673	.002	2.647	58	.010	.016223333	.006128273	.003956262	.028490405

Treated Sections	Equal variances not assumed			2.647	44.375	.011	.016223333	.006128273	.003875554	.028571113
Average Depth of Silver Penetration Treated Sections	Equal variances assumed	.818	.369	1.914	58	.061	53.11833	27.74809	-2.42551	108.66218
	Equal variances not assumed			1.914	57.301	.061	53.11833	27.74809	-2.43995	108.67662

These results show that the percentage of silver detected decreased from one to four weeks. However, the depth of penetration was not significantly different.

Test Statistics[a,b]

	penetration	time 1 or 4 weeks
Chi-Square	1.251	.000
df	1	1
Asymp. Sig.	.263	1.000

a. Kruskal Wallis Test

b. Grouping Variable: light cured?

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
time 1 or 4 weeks	60	2.5000	1.51266	1.00	4.00
penetration	60	73.2908	109.86457	.00	421.00
light cured?	60	1.5000	.50422	1.00	2.00

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
time 1 or 4 weeks	60	2.5000	1.51266	1.00	4.00
penetration	60	73.2908	109.86457	.00	421.00
light cured?	60	1.5000	.50422	1.00	2.00

Mann-Whitney Test

Ranks

	light cured?	N	Mean Rank	Sum of Ranks
time 1 or 4 weeks	light	30	30.50	915.00
	2.00	30	30.50	915.00
	Total	60		
penetration	light	30	28.27	848.00
	2.00	30	32.73	982.00
	Total	60		

Test Statistics[a]

	time 1 or 4 weeks	penetration
Mann-Whitney U	450.000	383.000
Wilcoxon W	915.000	848.000
Z	.000	-1.119
Asymp. Sig. (2-tailed)	1.000	.263

a. Grouping Variable: light cured?

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
penetration	60	73.2908	109.86457	.00	421.00

percentage of P	59	2.9661	3.87974	.00	15.00
percentage of Ca	60	1.0217	2.94285	.00	18.50
light cured?	60	1.5000	.50422	1.00	2.00

Mann-Whitney Test

Ranks

	light cured?	N	Mean Rank	Sum of Ranks
penetration	light	30	28.27	848.00
	2.00	30	32.73	982.00
	Total	60		
percentage of P	light	29	24.55	712.00
	2.00	30	35.27	1058.00
	Total	59		
percentage of Ca	light	30	31.10	933.00
	2.00	30	29.90	897.00
	Total	60		

VITA

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EDUCATION:

University of Illinois at Chicago, Chicago, Illinois — 2016- 2018
Master of Science

University of Illinois at Chicago, Chicago, Illinois
Certificate in Pediatric Dentistry — 2016-2018
Master of Oral Sciences

Meharry Medical College — 2012-2016
Doctor of Dental Surgery

Alabama A&M University — 2007 –2010
Bachelor of Science
Biology, Major

EMPLOYMENT:

Every Tooth Counts, Chicago, IL — 2017 - current
General Dentist

VOLUNTEER EXPERIENCE:

Give Kids a Smile Day — 2015-2017

LICENSURE:

CITA, Board Certified — August, 2016
Illinois State Dental License — July, 2016

PROFESSIONAL MEMBERSHIP

American Dental Association — 2012- current
American Association of Pediatric Dentists — 2016- current
Illinois Society of Pediatric Dentists — 2016-current