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RESEARCH PROTOCOL
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Title: The *in vitro* effect of a tooth bleaching agent on coffee and wine stained teeth.

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SUMMARY

Title: The in-vitro effect of a tooth bleaching agent on coffee and wine stained teeth.

Key words: Tooth discoloration / staining, tooth bleaching, spectrophotometer.

Aim: The aim of this laboratory study is to assess the efficacy of a tooth bleaching agent by evaluating the degree of color change with the use of a spectrophotometer and not by the usual subjective, visual methods.

Methodology:

Twenty specimens of human teeth will be collected, polished and divided into two groups. A baseline color measurement by the CIE L* a* b* with a spectrophotometer against a white background will be taken before one group is immersed in coffee and the other in red wine for two weeks. Bleaching of the specimens will be done according to manufacturer's instructions for two weeks. Color readings will be taken before bleaching, weekly during bleaching and 1 and 2 weeks after the bleaching treatment. Color change (ΔE) will be calculated mathematically as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. An observation of whether the baseline color reading will be regained by the bleaching process will be made.

Results:

Data collected will be recorded on an Excel spreadsheet. Advice from a qualified statistician will be sought to analyze the data. Results will be discussed in comparison with the existing literature on this subject.

FULL PROTOCOL:

INTRODUCTION

Esthetics has become a priority to patients. Patients now increasingly want to improve their esthetic appearance. With the introduction of home bleaching using trays in 1989, whitening of the anterior teeth has become a common request (Haywood and Heymann, 1989). Tooth bleaching has hence become a routine treatment option for discolored teeth. The good esthetic result with night guard vital bleaching and the safety of the dentist-supervised products has made bleaching a popular treatment modality (Matis *et al* 2000 and Attin *et al* 2004). It is a more conservative means of lightening teeth compared to the more aggressive veneers or full coverage crowns previously recommended (Haywood, Leonard and Dickinson 1997 and Braun, Jepsen, and Krause, 2006).

Staining is not the only reason for whitening teeth. Patients with normal tooth color can be dissatisfied with the color of their teeth and request tooth whitening of their teeth for a better, 'whiter' smile. There is also a hope of improving their self esteem with whiter teeth. Other reasons include when getting married, changing jobs or for a youthful experience (Sulieman, 2004).

Color is one of the crucial factors influencing esthetics. Dentists, technicians and patients themselves visually assess color change of an esthetic treatment. A common method used in assessing color is by visual color determination (van der Burgt *et al*, 1990). Color perception is very subjective as it is difficult to quantify color and there are individual variations. Technology in the science of color has brought the ability to digitally express color from objects and quantifying color parameters with the spectrophotometer and colorimeter. These instruments eliminate the subjective errors in color assessment. It has been used in color measurements of dental materials, porcelain, denture teeth and shade guides (O'Brien, 2002). Its use in the evaluation of color change in bleached teeth is now of increasing interest and use.

The outcome of bleaching treatment has mostly been evaluated by subjective color matching techniques in the past (Brunton, Ellwood, and Davies, 2004 and Swift Jr *et al*, 1999). Johnson and Kao, 1989 found accurate measurement of color and color differences with colorimetric devices *in vitro*.

Therefore the aim of this study is to employ a more objective color matching technique with the use of a spectrophotometer in assessing the efficiency of a home bleaching agent currently on the market.

LITERATURE REVIEW

Staining of teeth.

Dentists may regard tooth staining as unimportant but to many patients, it is a great concern and interest. Discoloration and staining of teeth has a negative effect on appearance. To improve the appearance or esthetics of discolored teeth is a common reason for dental visits. It is paramount for practitioners to recognize and know the cause of the discoloration to arrive at a correct diagnosis and hence successful treatment. The blue, green and pink tints of enamel and yellow to brown shade of dentine determines the normal color of teeth. Tooth discoloration has been historically classified according to the site of the stain as intrinsic or extrinsic discoloration (Watts and Addy, 2001).

Intrinsic Stains

These occur after a change in the structure, composition or thickness of the dental hard tissues during their development which changes the tooth's property of transmitting light. Systemic factors such as metabolic disease as well as local factors including an injury can result in discoloration (Watts and Addy, 2001).

Amelogenesis imperfecta is a hereditary condition that disturbs the mineralization or matrix formation of enamel resulting in a thin hard yellow to brown enamel. Dentinogenesis imperfecta may be of hereditary or environmental etiology and hence can

be classified as Types I, II and III. Type I is hereditary and both dentitions are affected, the primary being more severe. The teeth are blue-brown in color with an obliterated pulp and show an opalescence on transillumination. Enamel often chips away exposing the dentine to excessive wear. Type II is associated with osteogenesis imperfecta, a disorder of type I collagen or mixed connective tissue. The primary teeth resemble those of type I and the secondary dentition presentation varies. The enamel is not prone to fracture and the pulp is not occluded by dentine, a difference from type I radiographically. This type has a better prognosis. Type III is thought to be related to type II, with the primary dentition having many pulpal exposures. Dentine production is deficient and teeth appear like 'shell teeth' on radiographs.

Tetracycline staining occurs with the deposition of the tetracycline stain within the dental hard tissues and bone when it is used during the developmental stage of these tissues. Tetracycline can cross the placenta and is thus contra-indicated in pregnant and breast feeding women as well as children below the age of 12 years. Dentine is more affected with tetracycline staining compared to enamel and presents with a yellowish or brown grey appearance that is worse on eruption. There are reports of tooth color change with prolonged tetracycline use in adults as well as with the use of minocycline (synthetic compound of tetracycline) used in the treatment of acne (Patel, Cheshire and Vance, 1998).

Fluorosis was reported to be due to a high intake of fluoride by Dean in 1932. The source of the fluoride can be from a natural occurrence like in water supplies (greater than 1 ppm), foods or from fluoride delivering agents like toothpastes, mouthwashes and tablets. Both dentitions are affected in endemic areas. Affected enamel varies from flecking to mottling opacities with a color range from chalky white to dark brown to black. It is thought the black appearance is due to the internalization of extrinsic stains on the porous enamel as the discoloration is post eruption (Watts and Addy, 2001).

Enamel hypoplasia is another intrinsic stain due to any type of disturbance to the developing tooth germ including trauma (localized hypoplasia) or systemic disease such as maternal vitamin D deficiency, rubella and drug intake during pregnancy. It manifests

as pitting or grooving which facilitates extrinsic staining on the affected part that then becomes internalized.

Pulpal hemorrhagic products due to severe trauma can cause discoloration by the hemoglobin group of red blood cells combining with the putrefying pulpal tissue to form a black iron sulphide.

Ageing discoloration is due to the normal laying down of secondary dentine that affects light transmission of teeth which gradually darken.

Extrinsic Stains

Extrinsic stains occur outside the tooth structure lying on its surface or on the acquired pellicle. The causes can be divided into two categories, those chromogens incorporated into the pellicle producing stains as a result of its color and those that stain due to a chemical reaction on the tooth surface (Watts and Addy, 2001). Direct staining is from dietary sources that are taken up by the pellicle or plaque on the tooth surface and its color determines the stain. Tobacco smoking and chewing, drinks such as tea and coffee, mouth rinses and some medicaments also stain teeth. The stain is derived from the polyphenolic compounds that give food its color. The extent of staining of teeth is associated with the dietary habits of the individual (Bagheri, Burrow and Tyas, 2005).

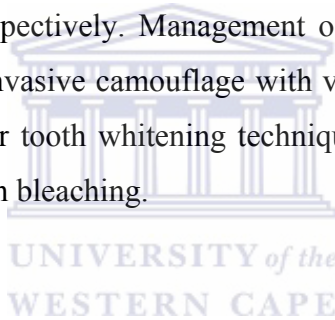
Indirect tooth staining is associated with an agent that does not have color or the stain produced from it is of a different color from the agent. It is seen in antiseptics and metal salts. A number of metal salts are associated with staining salts such as copper salts in mouth rinses cause a green discoloration. Potassium permanganate, stannous fluoride and silver nitrate salts cause a violet to black, golden brown and grey stain respectively when used in mouth rinses. Use of chlorhexidine has resulted in an increase of tooth staining (Watts and Addy, 2001) and more research with chlorhexidine is currently being done to study the mechanism of stain formation.

Internalised Stains

Watts and Addy, 2001 included Internalized discoloration in their review of tooth discoloration and staining that was recently introduced. Internalized stains are derived

from the extrinsic stains that are incorporated within the tooth substance following tooth development. It occurs in the enamel defects and the porous surfaces of the exposed dentine.

These defects are developmental in origin and are responsible for their own discoloration as mentioned in intrinsic stains. Acquired defects due to physiological wear and tear of teeth throughout life and their restorations may discolor teeth directly or indirectly. Internalization of extrinsic stains can be taken up on exposed dentine due to tooth wear, gingival recession and trauma causing enamel cracks or loss of enamel. Caries also influences tooth color, an early lesion is seen as an opaque, white spot area due to its increased porosity and refractive index. Black is seen when there is a hard arrested carious lesion, the stain of the lesion is said to be from exogenous origin. Materials such as amalgam and eugenol used in endodontics also stain teeth via tin migration into tubules and their pigments respectively. Management of stained teeth ranges from the removal of the surface stain, invasive camouflage with veneers and, crowns to the more conservative tooth bleaching or tooth whitening techniques (Sulieman, 2004). However not all stains can be treated with bleaching.



Bleaching of teeth.

Heymann, 2005 stated that vital tooth bleaching when done correctly, is one of the safest, most effective, conservative, esthetic procedure available to patients today. Much attention has been directed to this treatment as it is easy to execute and non-invasive.

For over a century, bleaching has been used to attain a better tooth color with lime being the agent used in the mid 1800s. Agents used were aluminium chloride, oxalic acid, sodium peroxide and cyanide of potassium. Hydrogen peroxide and its precursors are the agents that bleaching is based on (Sulieman, 2004). The active ingredient in these agents is an oxidizing agent that acts on the organic portion of the stain. These agents can be applied onto the external surfaces of teeth called “vital bleaching” or inside the pulp chamber called “non-vital bleaching”. The agents can be delivered “in-office” at the surgery or “at-home, nightguard” bleaching. A higher concentration of the agent is used in the office application for a shorter period of time while at home a lower concentration of the bleaching agent is used for a longer period by means of a custom made tray. Under

ideal conditions of case selection, the at-home and in-office bleaching can be combined to attain significant teeth whitening (Deliperi, Barnwell and Papathanasiou, 2004).

Indications for bleaching include generalized staining, ageing, dietary stains (tea, coffee, wine), smoking, pulpal damage, tetracycline and fluorosis stains. Tetracycline stains will need more time to attain an acceptable tooth color while severe staining will need additional treatment such as microabrasion or veneers. Also bleaching of mild to moderate fluorotic stains may need a combination of bleaching and microabrasion (Bodden and Haywood, 2003). Severely fluorotic teeth will require veneers or crowns.

The chemistry involved in bleaching is not well understood but it is thought to take place when oxidizers gain access to dentine via micropores on the enamel. They then react chemically with the organic pigment molecules situated on the enamel and dentine. The double bonds of the pigment molecules are broken down to smaller molecules that diffuse out of the tooth or absorb less light hence giving the tooth a lighter shade (Feinman, Goldstein and Garber, 1987 and Sulieman, 2004).

Carbamide peroxide is a well accepted bleaching agent that is dispensed in a gel form (Attin *et al* 2004). It breaks down into urea and hydrogen peroxide which is the oxidizing agent. Due to its low molecular weight, it easily diffuses into the organic matrix of the enamel and dentine where the stain is present and facilitates the chemical reaction. Ten percent (10%) of carbamide peroxide was a standard concentration but higher concentrations are used to try to increase its efficiency (Haywood 1997 and Matis *et al* 2000). Higher concentrations have shown to whiten teeth faster but similar effects are ultimately attained despite the concentration used (Braun, Jepsen and Krause, 2006).

Effects of bleaching agents on the tooth structure have been studied with the SEM and little or no change in the enamel morphology was reported (Splading, Taveira and DeAssis, 2003). Different reports on tooth sensitivity have been documented. Sulieman 2004 states that two thirds of patients experience sensitivity with home bleaching while other studies have reported no hypersensitivity. It is difficult to predict the outcome of

bleaching teeth however good results are reported in elderly patients with small pulps and whose stains are largely due to ageing and dietary products. Nicotine stains are difficult to bleach as well as fluorotic and tetracycline stains (Sulieman, 2004). Moderate tetracycline stains have responded to prolonged bleaching of 3 to 6 months. Recurrence of the stain is common despite the use of in-office or at-home modalities (Deliperi, Barnwell and Papathanasiou, 2004). Matis *et al* 1998 showed a color relapse occurred with a 10% carbamide peroxide in the first month post-bleaching. The development of color science has made it possible to quantify tooth color and objectively compare and monitor the color change resulting from bleaching.

Spectrophotometer assessment of colour change.

The esthetic appearance of teeth or restorations is governed by color, translucency, gloss and fluorescence. These factors are perceived by the observer (dentist, technician or patient) which in turn is influenced by the light source and the optical properties of the teeth or the restoration in question and the observer's interpretation of the color (O'Brien, 2002). The esthetic aspect of tooth color is difficult to quantify and different people perceive color differently (Watts and Addy, 2001).

The wavelength of light is associated with hues and is commonly known as color. There are different color systems used in Dentistry:

Munsell Color System.

This system uses the parameters of hue, value and chroma. Hue, which is the color, is associated with the wavelength of the light seen. Value is the lightness or darkness of the color. This is the most important factor in tooth color matching. A non-vital tooth appearing gray is said to have a low value. Chroma is the color intensity or the amount of hue saturation in a color.

Commission Internationale de l'Eclairage (CIE) Color System.

This system was defined by the Commission in Paris in 1978 and uses L*, a* and b* parameters to define color, referred to as CIELAB. According to this system, color is

obtained from a mixture in certain proportions of three basic colors; red, blue and green and is widely used in dental research (Johnson and Kao, 1989 and O'Brien, Groh and Boenke, 1990). These color coordinates provide a numerical description of the color relative to a three dimensional color space.

L* symbolizes the value in the Munsell system or lightness. It ranges from 100 (white) at the top to 0 (black) at the bottom. The a* and b* relate to the chromatic component along the red-green axis and the yellow-blue axis respectively. Component a* measures red (+) at one end to green (-) at the other with gray (0) in the middle. The b* component also measures yellow (+) from one end to blue (-) at the other with gray (0) in the middle.

As a result unit change in these parameters can be calculated. A color difference (or color match), ΔE is derived with the following formula (O'Brien, 2002):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Where ΔL^* , Δa^* , and Δb^* are the differences between the CIE L*a*b* of the samples being tested. The color difference, ΔE can be used as a reference guide to clinical color matching. A color difference greater than 1 can be perceived by the human eye under controlled conditions but a color difference (ΔE) between 2.2 and 4.4 is visually perceivable clinically (Johnson and Kao, 1989). Fay, Walker and Powers, 1998 concluded that a color change greater than or equal to 3.3 was visually detectable. Despite the calculation of this color difference, people still view color differently hence disagreement between the dental team and patient can still occur.

Color measurements can be made with a spectrophotometer and colorimeter. The former measures the amount of light reflected at each wavelength. Color parameters are recorded from responses of a double beam spectrophotometer from the object and a standard reference. Colorimetry with this instrument is more accurate than visual assessment with the naked eye in detecting small differences in color. This machine has been used to measure color parameters in restorative resins, denture teeth, shade guides and porcelains (Paul SJ *et al*, 2004). It is now being used in bleached teeth to compare effects of different concentrations of bleaching agents (Braun, Jepsen and Krause, 2006 and Matis *et al* 2000).

AIMS AND OBJECTIVES

The aim of this laboratory based study is to assess the efficiency of a tooth bleaching agent by measuring the degree of color change with a spectrophotometer (Konica Minolta, CM 2600d) and not by the usual subjective visual guide methods.

Objectives of the study are:

1. To determine tooth shade with a spectrophotometer prior to staining the tooth (baseline).
2. To determine which insult causes the most discoloration numerically.
3. To measure the efficiency of the bleaching agent used in the study with periodic color change spectrophotometer readings.
4. To assess if the baseline tooth shade can be regained by the bleaching agent.

NULL HYPOTHESIS.

1. There is no significant difference in the stains produced by red wine and coffee on extracted teeth.
2. There is no significant difference in the color of teeth before and after being subjected to a stain once they have bleached with carbamide peroxide.

MATERIALS AND METHODS

Study Design

An *in vitro* laboratory based study.

Study Sample

Twenty (20) extracted wisdom teeth or teeth extracted for orthodontic purposes will be used for the study with the following inclusion and exclusion criteria.

Inclusion Criteria: Teeth with intact crowns, without any restorations or carious lesions.

Exclusion Criteria: Teeth with restorations, caries, fractures, root canal treatments, crowns and any other damage.

Materials to be used:

Twenty (20) extracted human teeth will be collected at the Faculty of Dentistry, University of the Western Cape. The teeth will be preserved in a solution of normal saline with 1% thymol crystals. The teeth will be debrided of any deposits such as plaque and calculus and polished with a prophylaxis paste. A hole will be drilled onto the roots of the teeth with a diamond bur and floss will be used to suspend the teeth in a container filled with a staining media. (or just wholly immersed in a container)

Two 500 milliliter containers will be used to store the staining products. Coffee, red wine and tea have been shown to cause more stains to teeth and restorative materials compared to other food simulating solutions such as soy sauce and cola (Bagheri, Burrow and Tyas, 2005). Cola does not cause much discoloration perhaps due to its lack of a yellow colorant that is present in tea and coffee (Bagheri, Burrow and Tyas, 2005). Um and Ruyter, 1991 explained that tea and coffee had a yellow colorant with different polarities. Higher polarity components found in tea are eluted first and low polarity components in coffee are eluted later. Discoloration with tea is by adsorption and with coffee by both adsorption and absorption of colorants onto the tooth surface.

These agents are of common use and have been used in many color stability studies (Abu-Bakr *et al*, 2000, Fay, Walker and Powers 1998, Gross and Moser, 1977 and Luce and Campbell, 1988). Coffee and red wine will be used in this study.

Spectrophotometer Evaluation

A spectrophotometer (Konica Minolta, CM 2600d) will be employed to determine the shade of the coronal aspect of the teeth without any influence from variables such as visual perception, the time of day or the lighting in the office / laboratory. A white background will be used against the specimen during the measurements and calibration of the spectrophotometer will be done before the measurements are taken.

The shades of the crowns of the specimen teeth will be determined in the CIE L* a* b* color parameters before and after staining and sequentially during and after bleaching.

Methodology

Twenty (20) specimen teeth will be debrided from any deposits and polished with a rubber cup and a prophylaxis polishing paste. A baseline color measurement will be made according to the CIE L*a*b* color system (L_0, a_0, b_0) with a spectrophotometer. The teeth will be randomly divided into two groups with the one group being immersed in red wine and the other group in coffee for a period of three (2) weeks. The temperature of the staining solutions will be maintained at 37°C and will be changed every 48 hours. The specimens will be rinsed with tap water for ten (10) seconds, blotted dry with tissue paper and a second color measurement (L_i, a_i, b_i) will be made to assess the color change due to the staining solution.

The stained specimens will then be subjected to a bleaching procedure for two weeks with a common bleaching agent commercially available. The bleaching will be according to manufactures' instructions. Matis *et al* 2000 showed a significant tooth lightening *in vivo* after 2 weeks of bleaching and no significant difference at 6 weeks.

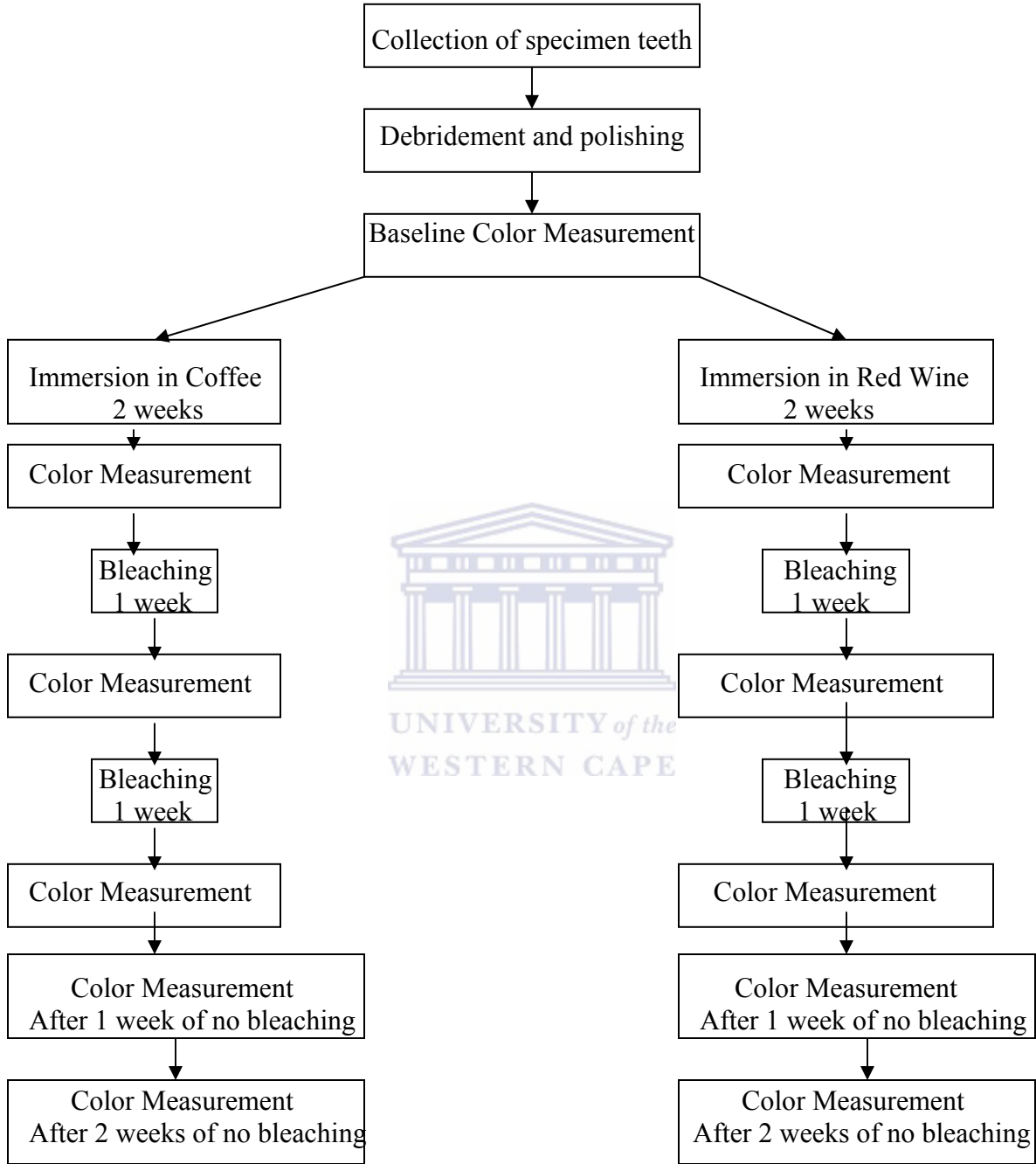
At the end of each week of bleaching, a color evaluation measurement will be done (L_{ii}, a_{ii}, b_{ii} and $L_{iii}, a_{iii}, b_{iii}$) and subsequent measurements two to three weeks after cessation the bleaching.

The difference in the lightness (L) and chroma (a and b) will be determined and the total color change (ΔE) between the recorded readings will be calculated with the following formula:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

The efficiency of the bleaching agent used will be assessed mathematically.

Schematic Presentation of Study Design



Staining solutions	pH	Brands
Coffee (15g/500ml)		Africafe, Tanzania Tea Blenders Ltd
Red Wine (7.5% Vol)		Four Cousins, Van Loveren Wines

Data Analysis

Data will be collected on an Excel spreadsheet. Advice from a qualified statistician will be sought to analyze the data. Results will be discussed in comparison with the existing literature on this subject.

Time Frame

Activity	Dates
Proposal writing and presentation	June, July 2006
Collection of samples and preparation of Experimental materials	July, August 2006
Pilot Study	
Data Collection	August 2006
Data Analysis	August, September 2006
Report Writing	September, October 2006
Thesis submission	October 2006

Limitations

This *in vitro* study is not an ideal situation with regard to teeth staining occurrence *in vivo*. Teeth staining in the oral cavity is due to a combination of staining solutions acting on teeth for a longer period of time than the two weeks the specimen will be immersed. Saliva and other oral fluids dilute the staining effects of agents and tooth brushing also polishes teeth.

Ethical Considerations

Teeth collection: Teeth collected will be will have been extracted for reasons other than those set out in this study.

Teeth disposal: teeth used in the study will be disposed of according to the medical waste disposal practice of the Faculty of Dentistry, Oral Health Centres, University of Western Cape.

Any materials or products used will be for the sole purpose of the study. Report of the results will not be biased nor examined by a supplier prior to its publication.

The author declares he has no financial interest in any of the products used or tested in this study.

Dissemination of Research

An attempt will be made to publish the final report in a reputable dental journal according to its editorial requirements.

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