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AUTOMATION OF MEASUREMENT OF WETTING ANGLE OF HUMAN ENAMEL WITH ANTISEPTIC SOLUTIONS USING MATLAB

Abstract: *The need for simple methods for measuring the contact angle increases in various scientific disciplines. There are algorithms that help users enter the droplet limits themselves, and the algorithm calculates the required data. Interaction with the user introduces uncertainty into the objectivity of the results. The goal was to create an algorithm in Matlab, intuitive to use, automated with minimal user interaction. Data were collected by an experiment that analyzed the enamel wetting with artificial saliva and mouthwashes solutions. The images were taken after complete droplet propagation, transferred to ImageJ software, which calculated the contact angle, and then the contact angle was calculated using an algorithm in Matlab and comparable results were obtained.*

Key words: *contact angle, wetting, Matlab, algorithm*

1. INTRODUCTION

The interaction between saliva and anatomical structures in the mouth is crucial for overall oral health and the maintenance of homeostasis. The surface of biomaterials, enamel or restorative materials, is important in the interaction between enamel and biological conditions prevailing in the oral cavity, where all structures are exposed to the phenomenon of adhesion and plaque removal [1].

The use of antimicrobial agents in cariology has been proposed having in mind the infectious nature of the disease. The goal of this approach is to achieve a caries-preventive effect by suppressing the cariogenic flora. For the purpose of use as mouthwash solutions intended for use in dental health care. American Reference Institutions, American Dental Association (ADA) and the Food and Drug Association (FDA) have adopted guidelines for testing the efficacy and safety of antiseptic agents with significant review by the expert community and formed a consensus that, in addition to fluoride solutions, only products containing chlorhexidine, cetylpyridinium chloride or from the group of approved essential oils (such as Listerine) may be approved in the category of mouthwash and bear the affirmative seal of acceptance [2]. In order to be effective on both smooth and solid structures in the oral cavity, mouthwash solutions should be completely spread on the surfaces, theoretically they should have the lowest possible contact angle [3].

However, the relationship between the chemical composition of the antiseptic solution is not directly correlated with their achieved clinical effect and precisely because of that, the research

of physico-chemical properties of antiseptic solutions used in dentistry is increasingly represented.

The measure of the ability of a solid body to be wetted by a liquid is called the contact angle and is denoted by Θ .

The contact angle is actually defined by three different interfacial surfaces: solid-liquid; solid-gaseous; liquid-gaseous, which results in the existence of three different interfacial tensions that meet at one point. The contact angle is the angle between the tangent drawn from that point to the circle that represents the contour of the droplet of the tested liquid and the surface of the solid body that the liquid moistens, which actually represents the solid-liquid interfacial voltage.

The most commonly used technique for measuring the contact angle is the direct measurement of the tangent angle at the three-phase contact point on the profile of the settled droplet [4]. This optical method requires small amounts of liquid and a small surface area of the solid substance. The contact angle of a liquid droplet on an ideal horizontal solid surface is determined by the mechanical equilibrium of the droplet under the action of three intermediate voltages of the liquid

2. MATERIALS AND METHODOLOGY

The labial and buccal surfaces of three human donated intact teeth were cut with a diamond disc and washed under running tap water with abrasive paper of fineness 800 to 1200 and polished with Al₂O₃ powder in distilled water.

After that, the polished surface was washed with demineralized water. In this research,

artificial saliva based on carboxymethyl cellulose made according to the recipe of the Pharmacy Institution Belgrade (registered under the main preparations of the Republic of Serbia) was analyzed. The glycerine / citric acid solution is prepared separately. For 1 kg of solution, 388 g of glycerine and 25 g of citric acid were used according to the recipe Pharmacy institutions Novi Sad (registered under master preparations of the Republic of Serbia). with four mouthwashes:

- 1) 0.1% chlorhexidine solution (Eludril Classic, Pierre Fabre Medicament, Bologna, France);
- 2) Elmex, mouthwash (Gaba International, France) where active ingredients were 100 ppm amine fluoride and 150 ppm sodium fluoride,
- 3) Listerin (Cool Mint Listerine Johnson & Johnson, New Brunswick, NJ)
- 4) Dentadent, (the alcohol-free formulation containing a combination of aluminium lactate, sodium fluoride and chlorhexidine (Lilly)

In this study, a total of 14 different solutions (artificial saliva solution, saliva stimulant solution, 4 mouthwash solutions, as well as 8 solutions (1: 1) solution of each mouthwash solution with artificial saliva and saliva stimulant) were tested on the surface of intact enamel.

The wetting angle measurement experiment was performed using the following apparatus and the procedure to be described below. Namely, a tripod is fixed on the anti-vibration surface, both for the camera and for the surface on which the preparation is placed. The camera is adjusted at a level a few cm from the upper limit of the specimen behind which the light source is placed. The camera is connected to a laptop, via a USB port. Drip is performed with an automatic pipette. After the first contact with the substrate, wait 10 s, until the drop subsides.

The system is then photographed three times, adjusting the brightness, contrast and focus on the camera itself (Fig 1). The obtained images were saved, and the drop is wiped from the substrate, then the process is repeated five times, for each solution.

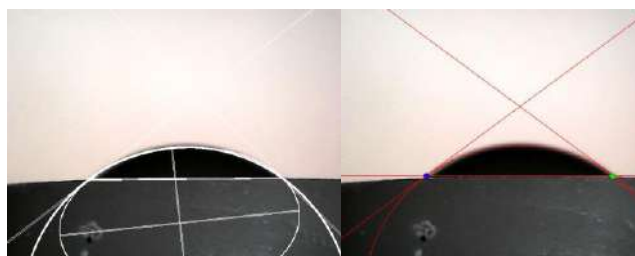


Fig. 1. Wetting angle

All images were divided by solutions and the wetting angle was automatically calculated using the algorithm which is described in detail in our previous work [5], with one change. Due to the amount of images, the algorithm is executed over the entire folder, taking each image from the same and calculating the left and right wetting angles. The improved algorithm is shown in Fig. 2.

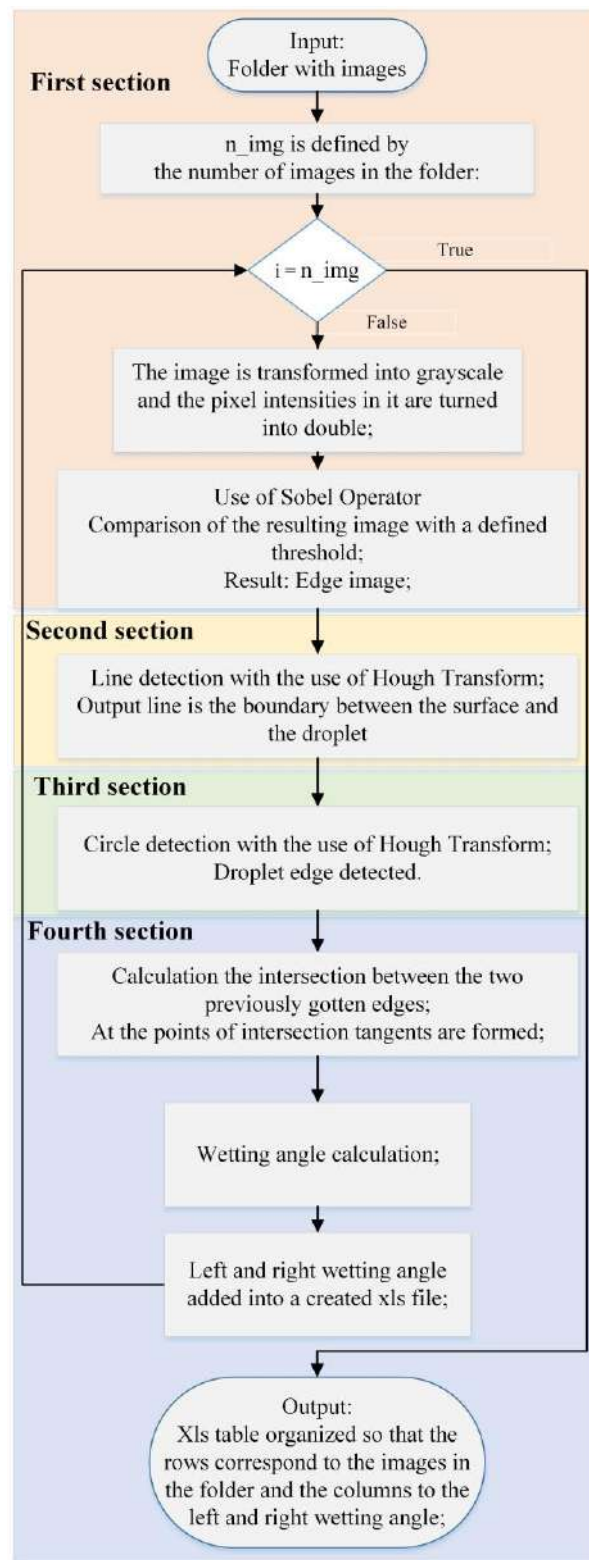


Fig. 2. Main algorithm

When it comes to statistical analyses, the data were presented in the form of mean values and standard deviation. To evaluate how far data were from normality, the Shapiro–Wilk test was used. In cases when data were with disturbed, distribution normality, the significance of the difference between the examined research groups, was tested by the Wilcoxon signed-rank test, while for the data that exhibited normal distribution one-way ANOVA and paired samples T-tests were used. The level of statistical significance was set at 5% ($p < 0.05$). For all statistical calculations Jamovi software (version 0.9.2.8) was used.

3. RESULTS

Fig 3 shows the mean values of the left and right contact angles measured using all three methods used on all 14 tested fluids and the obtained results show mutual agreement (manual, Image J and Matlab measurements included).

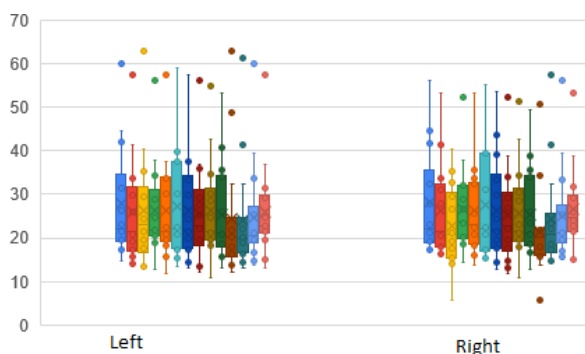


Fig. 3. Right and left contact angles

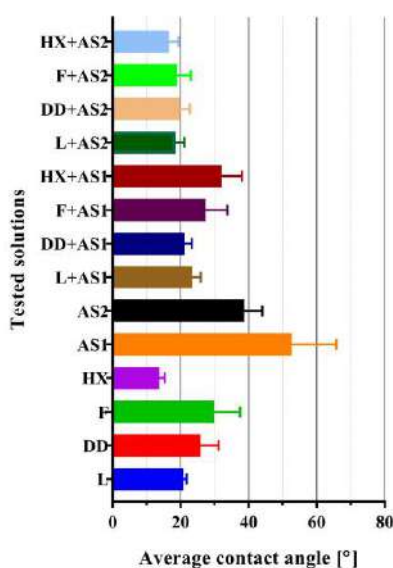


Fig 4. Contact angles – average values

The obtained mean values of the contact angle ranged from 15 degrees (observed with

chlorhexidine solution) to 53 degrees with artificial saliva, shown in Fig. 4. A statistically significant difference in the values of the contact angle ($p < 0.05$) was observed between all examined liquids.

In all 4 tested antiseptic solutions, lower values of contact angles were observed during mixing with the saliva stimulant compared to artificial saliva, and this difference was statistically significant ($p < 0.05$).

4. DISCUSSION AND CONCLUSION

There are significantly variable results in the literature when it comes to the values of the contact angle with the enamel. In a significant number of studies, not human but bovine enamel was used, some studies were performed in vivo, some in vitro. The reasons for such a wide range of contact angles of different enamel liquids can be explained by all three components of the experimental design, method of measurement, nature of the liquid being analyzed and preparation of the substrate (enamel) Due to all this, it may be more expedient to monitor the differences in the values of contact angles within one experimental protocol than to compare the obtained values with the absolute values obtained in other studies. Theoretically, the values of the contact angles of antiseptic values should have the lowest possible values, which would justify their role in cleaning tooth surfaces, and the effective bioavailability of the active components of the mouthwash solution.

Indeed, there are reports in the literature about really low values of the contact angle of the mouthwash, significantly lower than those shown in the conducted research. However, in a study conducted by Perdock. and associates published values of contact angles in the range that fully corresponds to the contact angles measured in our study, ranging from 37 to 54 degrees, at pH values from 3.1 to 7.5. Significantly higher values for the chlorhexidine contact angle, even over 50 degrees, have been reported by Alves et al.

It should be noted here that it is not recommended to compare different formulations of the same antiseptic solutions because the real concentrations of active substance in them are extremely low, often not exceeding 0.1%, so the contact angle will not depend on the active substance, but more on detergents, solvents and other substances. present in commercial mouthwashes.

Matlab is a software platform designed specifically for engineers and scientists to analyze and design systems. The basis of Matlab is the

Matlab programming language, which is based on matrices.

This fact is especially important when looking at image processing because each image is, at its core, a matrix. In addition to the fact that the manipulation of the matrix or image value is facilitated due to the very nature of the programming language, Matlab offers many implemented functions that are used for image processing. The methods and functions used gave satisfactory results and proved to be important when it comes to solving problems of this type. It has been shown that it is possible to solve complex problems of image segmentation obtained during a biomedical experiment using built-in Matlab functions.

The algorithm used in this paper can also be used with input images obtained in a different way, ie by changing the experimental setting. The development of software tools that would automate the process of determining the contact angle shows considerable potential.

The development of such a tool would reduce people's subjective influences on results and would also significantly shorten the time required for assessment.

Comparing the add-on to the ImageJ software for calculating the wetting angle and the mentioned algorithm encoded in Matlab, user participation is completely reduced to running the application in Matlab. Namely, due to the large number of images, the algorithm described in [5] has been modified to load all images from folders, unlike the ImageJ plugin for calculating the wetting angle which requires loading each image individually and manually marking the edges of the droplet.

Due to the possibility of blurred edges in the images from the experiment, the variability is further increased by using ImageJ software. On the other hand, when calculating the edges of an image, in Matlab, a step is added that thins it out, in order to reduce the variability introduced by the blurred image.

By loading the whole folder, the lack of low speed of the algorithm from [5] was overcome, because now the application can count in the background and process all the images, while the user is doing something else.

Biomedical engineering encompasses a wide range of scientific areas using different techniques. It is well known that measurements on humans need to be as non-invasive as possible with physical separation of signal acquisition hardware and the rest of the equipment. With this in mind, it is preferred for the measuring processes to be as automated as possible. Thus, the need for

automatization in this sphere of research is essential.

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