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ORIGINAL RESEARCH

Proposal for experimental *in vitro* model to assess morphological alterations in erythrocytes exposed to 5.25% NaOCl

Propuesta de un modelo experimental in vitro *para evaluar alteraciones morfológicas de eritrocitos expuestos a NaOCl 5.25%*

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ABSTRACT

Introduction: Sodium hypochlorite (NaOCl) is the chemical agent most frequently used as irrigation solution during endodontic therapy. When extruded to periapical tissue, it is highly toxic. In endodontics, hemolysis caused by NaCOl has been proven using different models, nevertheless, there is little or no evidence of morphological alterations in the cellular membrane of erythrocytes. **Objective:** To propose an experimental model which might allow to assess morphological alterations suffered by erythrocytes when they are exposed to NaOCl used in the dental practice by means of high resolution scanning electron microscopy (SEM). **Materials and methods:** In the present study, 20 mL of peripheral blood were obtained and deposited in tubes with EDTA (ethylenediaminetetraacetic acid) anticoagulant. Rinses were conducted with a phosphate buffer solution (Evan's solution). Several dilutions of the erythrocyte sample were prepared (1:1, 1:2, 1:4, 1:8 and 1:16); 100 μL of each of these dilutions was obtained to be then confronted with 100 μL of dental use 5.25% NaOCl (Viarzoni-T, Medental®); 0.5 μL of these samples were taken to then be deposited in a sample holder made of Zn-Cu alloy which was subjected to a process of Cu ion metallization bath, following the old Spluttering method. Microphotographs were obtained with SEM. **Results:** Erythrocytes with alteration type anisocytosis and poikilocytosis (stomatocytes, elliptocytes and discocytes) were observed. Some structural characteristics of NaOCl crystals were equally observed. **Conclusion:** This experimental model allowed assessment of morphological changes experienced by erythrocytes when exposed to 5.25% NaOCl.

Key words: Experimental model, sodium hypochlorite, erythrocytes.

RESUMEN

Introducción: El hipoclorito de sodio (NaOCl) es el agente químico más utilizado como solución irrigadora durante la terapia endodóntica. Es altamente tóxico cuando se extruye a tejido periapicales. En endodoncia la hemolisis causada por el NaOCl ha quedado demostrada utilizando diferentes modelos, sin embargo poca o ninguna evidencia se tiene de las alteraciones morfológicas en la membrana celular de los eritrocitos. **Objetivo:** Proponer un modelo experimental que permita evaluar las alteraciones morfológicas que sufren los eritrocitos cuando son expuestos a NaOCl utilizado en la práctica odontológica mediante microscopia electrónica de barrido de alta resolución (MEB). **Material y métodos:** Se obtuvieron 20 mL de sangre periférica y se depositaron en tubos con anticoagulante EDTA (ácido etilendiaminotetraacético). Se realizaron lavados con solución amortiguadora de fosfatos (solución Evan's). Se prepararon diferentes diluciones de la muestra de eritrocitos (1:1, 1:2, 1:4, 1:8 y 1:16). Se obtuvieron 100 μL de cada una de estas diluciones y se confrontaron con 100 μL de NaOCl 5.25% de uso odontológico (Viarzoni-T, Medental®). Se tomaron 0.5 μL de estas muestras para depositarse en un portamuestra de aleación Zn-Cu, el cual se sometió a un proceso de metalización de baño de iones de Cu por el método antiguo llamado *Sputtering*. Obteniendo microfotografías por MEB. **Resultados:** Se lograron observar eritrocitos con alteración de tipo anisocitosis y poiquilocitosis (estomatocitos, eliptocitos, esferocitos y discocitos). También se observaron algunas características estructurales de cristales de NaOCl. **Conclusión:** Este modelo experimental permitió evaluar los cambios morfológicos que sufren los eritrocitos cuando son expuestos a NaOCl 5.25%.

Palabras clave: Modelo experimental, hipoclorito de sodio, eritrocitos.

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INTRODUCTION

Sodium hypochlorite (NaOCl) is the agent most used as irrigating solution during endodontic therapy,[1,2] this is due to its wide-spectrum antimicrobial activity[3] and its ability to dissolve vital and necrotic tissue;[4,5] it additionally exhibits low viscosity thus facilitating penetration into the root canal system.[6] It is used at different concentrations ranging from 0.5% up to 6.0%.[7] In scientific literature, the most recommended concentrations for irrigation use are 2.5% and 5.25%.[8-10] Its antimicrobial action mechanism is explained by the reactions of aminoacid neutralization and chloramination, while the dissolution of organic matter is caused by saponification processes which are a product of the degradation of lipids and fatty acids.[11]

Contrary to its advantages, NaOCl by itself does not possess the ability to remove smear layers from the canal walls, moreover, it demineralizes dentin and induces corrosion of endodontic instruments.[12-14] Nevertheless, NaOCl cytotoxicity is the property that requires greatest attention from the clinical operator. Extrusion of this irrigating material towards periapical tissue causes hemolysis, ulceration, inhibition of neutrophil migration as well as damage to endothelial cells and fibroblasts,[15-17] which clinically manifests itself as pain, burning sensation, edema and hematoma.[18]

In endodontics, biocompatibility of material is determined by several parameters such as genotoxicity, mutagenicity, carcinogenicity, histocompatibility, antimicrobial effects and mutagenicity. For over 30 years, cell culture studies have been used to assess cytotoxicity reactions induced by endodontic materials.[19]

Among methods used to determine cytotoxicity we can count the following: determination of cell morphology alterations by means of light microscopy, confocal microscopy and scanning electronic microscopy (SEM).[19] Evaluations conducted with SEM have been restricted to mainly showing alterations on periodontal and fibroblast cell lines.[20-23]

In other medical areas, scanning electronic microscopy has enabled evaluation of abnormalities of the red blood cell membrane.[24] In endodontics NaOCl -caused hemolysis has been fully demonstrated using different models,[25] nevertheless there is scarce or no evidence of morphological alterations in the cell membrane of erythrocytes when they come in contact with NaOCl solutions of dental use. Therefore, the aim of the present article was to propose an experimental model which might allow SEM assessment of morphological alterations experimented by erythrocytes when they are exposed to NaOCl used in dental practice.

MATERIAL AND METHODS

Collection of biological sample

A 31 year old male was selected; he reported non-contributory pathological data; 20 mL of peripheral blood were obtained and deposited in sterile glass tubes with EDTA anti-coagulant. Samples were centrifuged at 2,000 rpm/5 minutes, plasma was withdrawn with 100-1,000 μL micro-pipettes

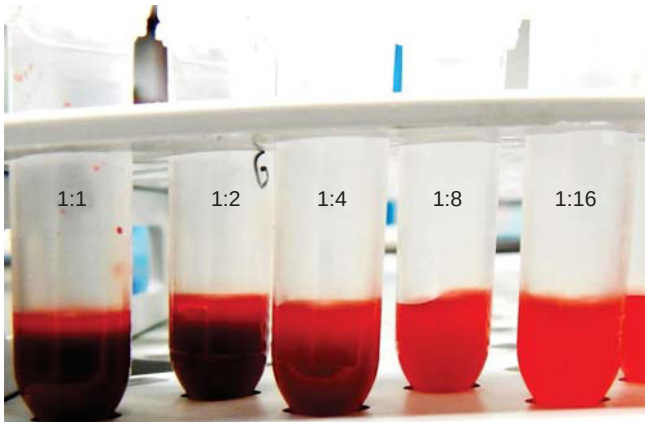

Figure 1. Red blood cell dilutions with PBA.

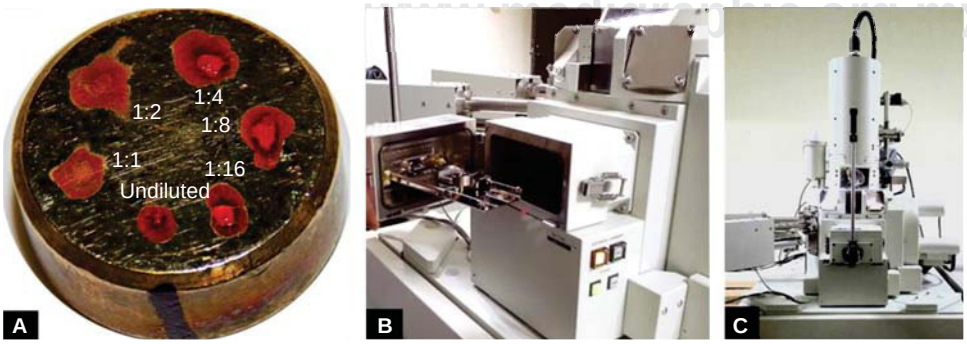

Figure 2.

A) biological sample in An-Cu alloy sample holder of 1 cm height and diameter. **B)** Vacuum chamber of High Resolution Scanning Electron Microscope **C)** JEOL JSM-7600F High Resolution Scanning Electron Microscope.