

Jurnal Info Kesehatan

Vol. 21, No. 4, December 2023, pp. 795-803

P-ISSN 0216-504X, E-ISSN 2620-536X

DOI: [10.31965/infokes.Vol21Iss4.1339](https://doi.org/10.31965/infokes.Vol21Iss4.1339)Journal homepage: <http://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****Cytotoxicity of Sodium Bicarbonate Solution to Human Gingival Fibroblast Cells**Erma Mahmiyah^{1a*}, Jojok Heru Susatyo^{1b}, Neny Setiawaty Ningsih^{1c}¹Department of Dental Health, Poltekkes Kemenkes Pontianak, Pontianak, West Kalimantan, Indonesia^a Email address: erma.mahmiyah@gmail.com^b Email address: drg.jojok@gmail.com^c Email address: nenysetiawaty26@gmail.com

Received: 13 September 2023

Revised: 31 December 2023

Accepted: 31 December 2023

Abstract

Immunoglobulin A (IgA) is a crucial antibody originating in mucosal lymphoid tissue, actively distributed across the epithelium. It plays a vital role in binding to and neutralizing microbes that threaten organisms through mucosal organs, thereby contributing to mucosal or secretory immunity. This research aims to determine the Cytotoxicity of Sodium Bicarbonate Solution to Human Gingival Fibroblast Cells. The research method used to investigate the safety and efficacy of various sodium bicarbonate concentrations, we conducted a laboratory experimental study utilizing a post-test-only control group design. Sodium bicarbonate solutions with concentrations of 1%, 2%, 3.5%, 7%, 10%, 15%, and 20% were tested. The results of the study using analysis through ANOVA followed by Tukey HSD revealed that solutions with concentrations of 20%, 15%, and 10% exhibited comparable non-toxicity to fibroblast cells, as they shared the same column. In contrast, concentrations of 7%, 3.5%, 2%, and 1% were found to have toxicity levels that exceeded the IC50 threshold. Further examination using the Tukey HSD test showed that the 2% and 3.5% concentration groups did not show significant differences. In conclusion, the Sodium bicarbonate solutions with concentrations of 7%, 3.5%, 2%, and 1% are not toxic to fibroblast cells and can be used as a basis for further research applications based on sodium bicarbonate materials. It is recommended for future studies to conduct further examinations with different concentrations.

Keywords: Baking Soda (Sodium Bicarbonate), Cytotoxicity, Fibroblasts.***Corresponding Author:**

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1. INTRODUCTION

The oral cavity serves as the primary gateway for microorganisms, making it imperative to employ effective defense mechanisms against pathogenic bacteria (Kitamoto, et al., 2020). Various practices, such as regular teeth brushing, mouth rinsing with antiseptics, interdental cleaning using dental floss, tongue cleaning, and gum chewing, are implemented to mitigate bacterial population in the oral cavity. Caries prevention encompasses multiple strategies, including successful fluoride-based procedures and prolonged restriction of cariogenic sugar consumption, resulting in a substantial reduction in caries (Ahmed, et al., 2022). However, the impracticality of limiting sweets without offering viable alternatives poses challenges. Individual immunity, an intrinsic defense mechanism, also plays a crucial role in preventing bacterial infections.

Sodium bicarbonate, recognized for its antibacterial properties and alkaline nature, can neutralize the oral cavity's pH, inhibiting bacterial metabolic processes that produce acid. The alkaline properties stimulate ion exchange mechanisms, affecting cations like potassium and sodium in extracellular fluids such as saliva. This ion exchange becomes particularly relevant during conditions of increased extracellular hydrogen ions, leading to pH reduction and potassium redistribution. Moreover, sodium bicarbonate's hypertonic nature influences osmotic pressure, causing bacterial cells to lose water, ultimately dehydrating and potentially killing them (Khorolsuren, et al., 2021).

Interventional gargling using sodium bicarbonate introduces a chemical stimulus, derived from its taste, that activates parasympathetic nerves originating from the superior and inferior salivatory nuclei of the brainstem. This stimulation, triggered by tactile and taste stimuli on the tongue, oral cavity, and pharynx, results in an increased salivary flow rate. Sodium bicarbonate, known for stimulating salivary flow and possessing natural alkaline elements, holds promise for xerostomia therapy. Its high buffer capacity maintains pH close to normal limits, enhancing its therapeutic potential (Abbate, et al., 2014).

To advance basic research on the impact of sodium bicarbonate on salivary IgA levels, it is crucial to assess its safety on oral tissues. Preliminary investigations should focus on the substance's toxicity, ensuring biocompatibility and non-inhibitory properties to the surrounding living tissue. A cytotoxicity test, such as the MTT assay, is instrumental in evaluating dental materials' potential impact on fibroblast cell cultures. Fibroblasts, integral to oral mucosa, are pivotal for understanding the substance's effects on cell metabolism and overall safety within the oral environment. This research aims to determine the Cytotoxicity of Sodium Bicarbonate Solution to Human Gingival Fibroblast Cells.

2. RESEARCH METHOD

This study employed an experimental laboratory design with a post-test-only control group. The experiment was conducted at the Pusvetma Surabaya laboratory, utilizing various tools including water baths, digital shakers, rotary evaporators, ELISA readers, 0.2 μ m minisart filters, incubators, vortexes, and plastic sample molds with dimensions of 5 mm in diameter and 2 mm in height. Materials involved in the study comprised baking soda, BHK-21 fibroblast cells, Phosphate Buffer Saline (PBS), Minimum Essential Medium Eagle Alpha Modification (Alpha MEM) culture medium, dimethyl sulfoxide (DMSO), MTT powder (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), and trypsin EDTA.

Sample preparation involved molding on a glass slab with celluloid strips on the mold base. The material weight was determined according to group divisions and stirred on a paper pad for 1 minute until homogeneous. The resulting dough was placed into the molds, allowed to set, removed, and weighed using an analytical balance. Subsequently, samples were ground into fine powder using a mortar and pestle to ensure uniformity across groups. The powdered

samples were then collected, and the specified weight was exposed to human gingival fibroblast culture cells.

Each sample was added to sterile Eppendorf tubes, and 600 μ l of Alpha MEM culture medium was introduced to each Eppendorf. After vortexing, the samples were refrigerated for 24 hours, sterilized with 0.2 μ m minisart filters, and placed in Eppendorf tubes according to group assignments.

Human gingival fibroblast cell cultures were seeded in petri dishes and incubated in a CO₂ incubator for 24 hours. The cells were observed using an inverted microscope at 100x magnification. The cell culture was then transferred to 96-well microplates at a density of 3-5 x 10³ cells per well and further incubated for 24 hours. The microplates were divided into control and treatment groups, with 50 μ l of samples added to designated wells. Incubation continued at 37°C for 24 hours.

Following incubation, 25 μ l of MTT solution (5 mg/ml PBS) was added to each well and incubated for an additional 4 hours. The Alpha MEM culture medium was discarded, and 100 μ l of DMSO was added to each well. Formazan absorbance on human gingival fibroblast cells was measured spectrophotometrically using an ELISA reader at 595 nm. The percentage of live cells was calculated using the formula:

$$\% \text{ of live cells} = \frac{\text{OD of treatment} - \text{OD of media}}{\text{OD of control cells} - \text{OD of media}} \times 100\%$$

Note:

- % of live cells: Percentage of live cells after treatment.
- OD Treatment: Formazan OD (optical density) value of each sample after testing.
- OD Media: Formazan OD (optical density) average for each media control.
- OD Cell: Formazan OD (optical density) average for control cells.

This research has also received ethical approval from the Ethics Commission of the Poltekkes Kemenkes Pontianak with No.176/KEPK-PK.PKP/VII/2022.

3. RESULTS AND DISCUSSION

Table 1. Optical density of the MTT test.

Repetition	Cell Control	1 %	2 %	3,5 %	7 %	10 %	15 %	20 %	Control of Media
1	0,592	0,538	0,569	0,601	0,086	0,067	0,067	0,077	0,054
2	0,552	0,479	0,524	0,545	0,069	0,067	0,065	0,069	0,119
3	0,579	0,645	0,587	0,55	0,076	0,067	0,066	0,071	0,066
4	0,626	0,625	0,602	0,565	0,131	0,07	0,067	0,076	0,081
5	0,651	0,434	0,52	0,484	0,057	0,068	0,067	0,074	0,056
6	0,552	0,538	0,533	0,511	0,111	0,07	0,071	0,076	0,083
7	0,566	0,416	0,454	0,442	0,064	0,069	0,07	0,075	0,054
Mean	0,5883	0,5250	0,5413	0,5283	0,0849	0,0683	0,0676	0,0740	0,0733

Table 1 show that the Optical density of the MTT test with mean cell control 0,5883 and 0.0733 control of media.

Table 2. Percentage of viable cells after exposure to various concentrations of sodium bicarbonate solution.

Repetition	1 %	2 %	3,5 %	7 %	10 %	15 %	20 %
1	105,47	96,40	102,71	90,29	3,02	1,64	1,64
2	102,91	87,53	91,67	78,66	1,92	1,64	1,37
3	111,38	99,95	92,66	78,83	2,86	2,06	1,51
4	107,44	102,91	95,61	69,79	2,89	2,06	1,64

5	115,13	86,74	79,65	74,63	2,61	2,058	1,64
6	103,10	89,31	84,97	78,66	2,89	2,33	2,06
7	90,29	73,69	71,37	66,24	2,75	2,20	2,058
Mean	105,103	90,932	88,377	76,729	2,706	1,998	1,703

Table 2 illustrates the mean percentage of viable cells following exposure to different concentrations of sodium bicarbonate solution. The results indicate that at 1%, the mean viability is 105.103%, at 2% it is 90.932%, at 3.5% it is 88.377%, at 7% it is 76.729%, at 10% it is 2.706%, at 15% it is 1.998%, and at 20% it is 1.703%.

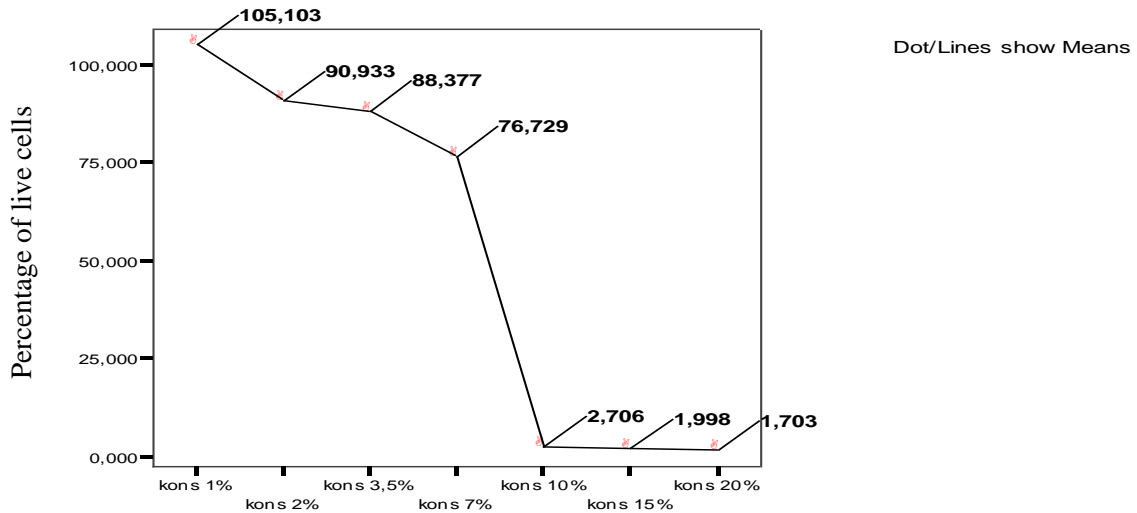


Figure 1. Sodium Bicarbonate.

Table 3. Sample size, mean optical density, percentage of live fibroblasts and standard-deviation.

Group	n	Mean		Standard deviation
		Optical density	Percentage of live	
Sod. bicarbonate 1%	7	0,5250	105,103	0,0885
Sod. bicarbonate 2%	7	0,5413	90,932	0,0499
Sod. bicarbonate 3,5%	7	0,5283	88,377	0,0534
Sod. bicarbonate 7%	7	0,0849	76,729	0,0269
Sod. bicarbonate 10%	7	0,0683	2,706	0,0014
Sod. bicarbonate 15%	7	0,0676	1,998	0,0021
Sod. bicarbonate 20%	7	0,0740	1,703	0,0029
Control cell	7	0,5883	100,000	0,0378
Media controls	7	0,0733	0,000	0,0236

Table 3 illustrates the results of a cytotoxicity test conducted on fibroblast cells, media controls, and cell controls using various concentrations of sodium bicarbonate. The study employed the Inhibitory Concentration 50% (IC50) as the key parameter. IC50 represents the concentration of a substance capable of inhibiting cell proliferation in 50% of the population. Based on the IC50 parameter, it can be inferred that concentrations below 7% are non-cytotoxic to fibroblast cells.

Table 4. Tukey Test (HSD) Percentage of Living Cells

Sodium Bicarbonate	n	Subset ($\alpha=0,05$)		
Concentration 20%	7	1,70257		
Concentration 15%	7	1,99829		
Concentration 10%	7	2,70571		
Concentration 7%	7		76,72857	
Concentration 3.5%	7			88,37714
Concentration 2%	7			90,93286
Concentration 1%	7			105,10286
Sig.		1,000	1,000	0,992

Table 4 presents the outcomes of the ANOVA test, followed by Tukey's Honestly Significant Difference (HSD) test. The analysis revealed that sodium bicarbonate solutions at concentrations of 20%, 15%, and 10% shared the same column, indicating that these three concentrations did not exhibit a significant difference in the viability of fibroblast cells. This suggests that the cells may experience cytotoxic effects after 24 hours of exposure, as these concentrations fall below the Inhibitory Concentration 50% (IC50).

Conversely, the concentration groups of 7%, 3.5%, 2%, and 1% displayed viability scores above the IC50. The Tukey HSD test further indicated that the viability of fibroblast cells in the 2% and 3.5% concentration groups did not differ significantly. Consequently, concentrations of 7%, 3.5%, and 1% could serve as a foundation for future research applications. Cytotoxicity test is an initial part of the evaluation of a dental material before it is used in humans (Jiang, et al., 2017; Schmalz, & Galler, 2017; Shahi, et al., 2019; Pagano, et al., 2019; Caldas, et al., 2019). The most frequently used method is the Microculture Tetrazolium Technique Assay (MTT Assay) using MTT 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide reagent (Khoswanto, Arijani & Soesilawati, 2008; Grela, Kozłowska, & Grabowiecka, 2018; Karakaş, Ari, & Ulukaya, 2017; Stockert, et al., 2018; Kamiloglu, et al., 2020; Oh, & Hong, 2022). The MTT method is based on measuring the mitochondrial activity of living cells. Cells that are still alive and whose metabolism is active, can convert the MTT salt which was originally yellow in color to a purple formazan product through a reduction reaction (Riss, et.al. 2016; Bahuguna, et.al. 2017). The color intensity of the formazan crystals in a 96-well microplate was measured using an Elisa reader. The resulting absorbance is directly proportional to the number of viable cells. A darker color corresponds to a higher absorbance value, indicating a greater number of living cells (Emilda, et al, 2014). The MTT Assay method requires reagent incubation with reduced live cell cultures (Riss, et.al. 2016).

The most commonly employed cell culture for cytotoxicity testing of dental materials is the Baby Hamster Kidney 21 (BHK-21) fibroblast cells, derived from baby hamster kidneys. These cells are extensively utilized due to their resemblance in shape and functionality to human fibroblasts, particularly in the production of growth factors. BHK-21 cells are known for their ease of culture, heightened stability, increased sensitivity, and a reduced likelihood of mutation compared to human fibroblast cells (Dewi, 2007; Emilda, et al, 2014; Holland, 2009; Khoswanto, 2008)

This study conducted a cytotoxicity test on fibroblast cells using sodium bicarbonate. Sodium bicarbonate is soluble in water at typical room temperature (approximately 20°C) and insoluble in alcohol. To ensure safe use, it is recommended to dilute sodium bicarbonate in water. The compound remains stable in open air and at normal room temperature, allowing for convenient storage in a closed environment without requiring special handling. Sodium bicarbonate, also known as Sodium Hydrogen bicarbonate, baking soda, bread soda, cooking soda, bicarbonate soda, or bicarbonate soda, dissolves in water and presents as thick white crystals. Its abrasive nature contributes to caries control due to the composition of baking soda-

fluoride. Baking soda consists of hydrate silica, whose action aligns with fluoride, effectively reducing stains and calculus on teeth. In addition to its antimicrobial properties, baking soda crystals have the ability to absorb odors. Baking soda, a chemical compound, is widely utilized to remove stains on teeth. Another benefit of baking soda is its capability to reduce bad breath and whiten teeth by inhibiting bacterial growth and minimizing plaque buildup (Paramita, 2015)

Baking soda (sodium bicarbonate) can be used as an alternative to whitening teeth; besides being easy to find in the community. baking soda is also relatively easy to use. The advantages of baking soda compared to chemical solutions are that baking soda is not irritating and abrasive. Baking soda can also be antibacterial (Schuurs, 2013). It has the unique ability to act as a buffer based on its chemical process. Neutralizers function to maintain a balance or neutralize the pH so that it can be used as a mixed substance in toothpaste and mouthwash to maintain the pH of the oral cavity.

As a hypertonic solution. sodium bicarbonate can facilitate the osmotic movement of water from cells. resulting in shrinkage. plasmolysis. and finally. death of cells. including bacteria. (Madeswaran & Jayachandra, 2018). Mechanism of cell death after exposure to sodium bicarbonate with a time of 24 hours and a certain concentration because sodium bicarbonate has the ability to affect the osmotic pressure of water in cells. The hypertonic nature of sodium bicarbonate causes the hypotonic components of the bacterial cells to lose water, so that the cells will become dehydrated resulting in shrinkage, plasmolysis and eventually killing the bacterial cells. (Strassler, 2013; Madeswaran & Jayachandra, 2018). The author analogizes the mechanism of cell death to fibroblast cells.

Sodium bicarbonate also has alkaline properties which can neutralize the pH of the oral cavity so that it can inhibit the metabolic process of bacteria that produce acid. Another factor responsible for baking soda's antibacterial effect is its ability to change osmotic pressure. The hypertonic nature of baking soda causes the hypotonic components of the bacterial cells to lose water, causing dehydration and killing cells. However, it is said that sodium bicarbonate must interact with bacterial cells for at least 30 minutes so that it can effectively kill bacterial cells (Silhacek, 2004; Hewawaduge, Senevirathne, & Lee, 2020; Jaikumpun, et al., 2020; Saleh, et al., 2022; Karim, & Hossain, 2018).

Currently, sodium bicarbonate is often added to toothpaste to clean teeth from plaque, because it has many beneficial properties, including easy to obtain, cheap price, safe, low abrasive level, soluble in water, acid neutralizing properties, compatible with fluorine, and antibacterial ability (Schuurs, 2013). Sodium bicarbonate besides having anti-microbial properties, sodium bicarbonate crystals also have the ability to absorb odors. Sodium bicarbonate is a chemical compound that is effectively used to remove stains on teeth. Another advantage of baking soda is that it reduces bad breath and can whiten teeth because sodium bicarbonate can inhibit bacterial growth and reduce plaque buildup (Paramita, 2015; Ariani et al., 2023; Garcia, Santiago, & Velasco, 2018).

In the field of dentistry, the effect of sodium bicarbonate in the form of chewing gum, gel, or tablets on the pH of the mouth has been widely studied and proves that sodium bicarbonate helps the buffer capacity of saliva (Abbate, 2013). This unique ability is as a buffer based on the chemical process. Neutralizers function to maintain a balance or neutralize pH so that they can be used as antacids for digestive disorders or neutralize acids in the digestive tract, mixed substances in toothpaste and mouthwash to maintain the pH of the oral cavity (Hurlbutt, 2010).

Research conducted by Ghassemi in 2008 showed that sodium bicarbonate was able to damage the bacterial matrix structure and also damage the bond between bacteria and tooth surfaces. The study compared the antibacterial abilities of baking soda toothpaste and triclosan,

the results showed that sodium bicarbonate toothpaste was more effective in inhibiting plaque bacteria (Ghassemi, 2008). Clinical use of 67% sodium bicarbonate toothpaste can improve periodontal tissue health in gingivitis patients (Taschieri, et.al, 2022; Parkinson, Butler, & Ling, 2023).

The statistical analysis of research data revealed a normal distribution with homogeneous variations, allowing for further investigation using the Anova test followed by Tukey's Honestly Significant Difference (HSD) test to assess group differences. Results indicated that as the concentration of sodium bicarbonate increased, the absorbance value decreased. This suggests a reduction in the number of viable cells or an increase in the number of dead fibroblast cells with elevated sodium bicarbonate concentrations.

In summary, the calculation of the percentage of fibroblast cells that perished after exposure to sodium bicarbonate concentrations of 1%, 2%, 3.5%, 7%, 10%, 15%, and 20% respectively was 0%, 9.1%, 11.6%, 23.3%, 97.3%, 98%, and 98.3%. Based on the Inhibitory Concentration 50% (IC50) parameter, sodium bicarbonate concentrations of 10%, 15%, and 20% fall into the toxic category as the percentage of dead cells exceeds 50%, while concentrations of 1%, 2%, 3.5%, and 7% are deemed non-toxic, with the percentage of dead cells being less than 50%. Additionally, the data indicates that a 10% concentration of baking soda exhibits cytotoxic effects, whereas a 7% concentration demonstrates therapeutic properties. The Tukey HSD test revealed a significant difference between the 10% and 7% concentration groups; however, the specific impact of sodium bicarbonate within the range of 10% to 7% concentration on fibroblast cells remains unclear.

4. CONCLUSION

In conclusion, the Sodium bicarbonate solutions with concentrations of 7%, 3.5%, 2%, and 1% are not toxic to fibroblast cells and can be used as a basis for further research applications based on sodium bicarbonate materials. It is recommended for future studies to conduct further examinations with different concentrations.

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