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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии  
საქართველოს სამედიცინო სიახლენი

## GEORGIAN MEDICAL NEWS

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**GMN: Georgian Medical News** is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

**GMN: Медицинские новости Грузии** - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

**GMN: Georgian Medical News** – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებშიდან.

### WEBSITE

[www.geomednews.com](http://www.geomednews.com)

## К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman (Кириллица)**, для текста на грузинском языке следует использовать **AcadNusx**. Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - <http://www.spinesurgery.ru/files/publish.pdf> и [http://www.nlm.nih.gov/bsd/uniform\\_requirements.html](http://www.nlm.nih.gov/bsd/uniform_requirements.html) В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректур авторам не высылаются, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

**При нарушении указанных правил статьи не рассматриваются.**

## REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or computer-printed on a single side of standard typing paper, with the left margin of 3 centimeters width, and 1.5 spacing between the lines, typeface - **Times New Roman (Cyrillic)**, print size - 12 (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: [http://www.nlm.nih.gov/bsd/uniform\\_requirements.html](http://www.nlm.nih.gov/bsd/uniform_requirements.html)  
[http://www.icmje.org/urm\\_full.pdf](http://www.icmje.org/urm_full.pdf)

In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

**Articles that Fail to Meet the Aforementioned  
Requirements are not Assigned to be Reviewed.**

## ავტორთა საქურაღებოლ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დაიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx**. შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედეგის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფხიხლებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

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## PH VALUE AND ANTIBACTERIAL EFFECT OF ALKASITE RESTORATIVE MATERIALS

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### Abstract.

**Background and aims:** This (in vitro) investigation was conducted to evaluate PH value and antibacterial effect of Alkasite restorative materials against important oral pathogens *Streptococcus mutans* and *Lactobacillus plantarum*.

**Methods:** Four groups were made of three different type ion releasing materials Cention N= group 1, Primer free Cention Forte=group 2, Primer applied Cention Forte=group 3 and Fuji IX= group 4. A total number of 72 discs in form samples (2 mm height and 5mm diameter) were constructed in polyethylene mold divided according to the evaluating parameters. 32 specimens for PH measurements (n=8) and 40 specimens were utilized for antibacterial effect (n=5) for each bacterial species. The antibacterial properties of groups were assessed by direct contact test. An adjusted diluted broth culture of each bacterium (*Streptococcus mutans*) and (*Lactobacillus plantarum*) were prepared.

**Results:** At all intervals of time both Cention N and Primer free Cention Forte has significantly higher PH value in comparison with Fuji IX. Primer applied Cention Forte. There was no significant difference between Cention N, Primer free Cention and Fuji IX in terms of their effect in reducing viable colony count in both bacterial species.

**Conclusion:** Along period of (28day) Alkasite material groups (Cention N and Primer free Cention Forte) showed the ability to increase the storage solution PH value. Also, both groups have antibacterial effect against (*Streptococcus mutans*) and (*Lactobacillus plantarum*) by inhibiting their numbers. Applying Cention primer showed negative effect on both PH value and the antibacterial effect of the material.

**Key words.** PH value, antibacterial effect, Alkasite restorative materials, *streptococcus mutans* and *lactobacillus plantarum*.

### Introduction.

Dental caries well known as one of the widely prevalent chronic oral related diseases in humans. there are several factors able to cause tooth caries including bacteria, host immune system response and also the eating habits, another etiology is related to microecological harmony of the highly complicated bacterial biofilm [1,2]. *Streptococcus mutans* is known as the initial bacteria that responsible for the onset of carious lesion, while *Lactobacillus* considered the main pathogen that responsible for both progression of the caries and secondary caries initiation [3]. These bacteria metabolize the ingested dietary sugars and produce acids as byproduct, leading to lower the oral cavity PH to a limit that is less than dental hard tissues solubility limit [4,5]. Different types of direct filling materials are available to use in the contemporary dental practice ranging from amalgam and through to the bulk fill composite [6].

Despite the major improvements in the esthetic and mechanical characteristics of composite resins through the past couple

decades, the attempts to find new alternative able to limit the secondary caries development that occurs around or beneath the restorative materials are still ongoing, these materials are considered neutral not capable to initiate any physiological responses in the host tissues they are only fill the cavities that follows the loss of tooth structure and not able to prevent the subsequent complications like the developing of secondary caries as a result to the acids produced by the bacteria [7].

Restorative materials that have antibacterial properties considered essentially important in order to inhibit recurrent carious lesions, very popular example in this regard are glass ionomers which were recommended due to their ability to release fluoride and their adhesive characteristic to the dental hard tissue, but due to their relatively poor mechanical strength and their reduced resistance to wear [8]. Comprehensive research in the modern field of dentistry been able to create several advancements that led invent a new dental restorative material with improved esthetic, physical properties and also inhibitory properties for caries [1].

Recently, a new class of the restorative materials is alkasite restorative material that categorized as a subgroup of resin composite [9]. This material contains alkaline glass which able to release several different ions including (calcium, hydroxide, and fluoride ions) [9]. Cention N consist of (78 %) inorganic fillers by weight. The alkaline glass makes of (24%) from the final weight of the product [10]. This material consists of (powder and liquid) it differs from the resin composite snice their monomer has no (Bis-Gma, TEGDMA and HEMA) [11]. This material similar to glass ionomer cement as they also release fluoride. Cention N showed an improvement in the aesthetic characteristics, which showed reasonably much more transparency in comparison to other glass ionomers products. Additionally, it owns superior compressive strength as a result Cention N provides a combination of the best qualities of both GICs and amalgam [12].

A new product of the alkasite material introduced in (2021) by the same manufactures (Ivoclar Vivadent, Liechtenstein) called Cention Forte it was provided in the form of capsules. The manufacturers also claimed this material release (fluoride (F<sup>-</sup>), calcium (Ca<sup>+2</sup>), and hydroxide (OH<sup>-</sup>)) ions, Cention Forte comes along with a primer which designed to be only used with Cention Forte. The primer is both self-curing and self-etching which offer a foundation in order to increase the bond between Cention Forte/tooth [13,14]. The two of the alkasite materials (Cention N and Cention Forte) have both self and light curing property. Both are considered radiopaque and basic filling material that used to restore the cavities in the anterior and posterior teeth. Wich is used as a bulk to fill several cavity types (class I, class II, and class V cavities) in the permanent and deciduous teeth.



The growth inhibitory potential of ion-releasing materials is related to their ability to release different ions with fluoride ions considered the most recognized ions in addition, hydroxide ions from these restorative materials could also help in neutralizing the excess amount of the acids created by cariogenic microbes, thus preventing demineralization [15]. Both of these factors will work along to enhance the anticariogenic potentials of the bioactive Cention-N and Cention Forte. Also, hydroxide ions on the material surface able to control the disease of dental caries, through neutralization of the acids from the bacteria could aid maintaining the microbial harmony inside the bacterial biofilm [2,16].

This investigation aimed to assess antibacterial effect of two alkasite restorative material (Cention N), (Cention Forte) and (Cention Forte with their matching primer (Cention primer)) against two bacterial species (*S. mutans*) and (*L. plantarum*) separately and their ability to elevate PH of the storage solution.

### Materials and Methods.

Restorative materials used in the study are listed with their compositions in Table (1).

**Table 1.** The materials used in this assessment.

Materials name	Compositions
Cention N (Ivoclar Vivadent, Schaan, Liechtenstein)	Powder: fillers (ytterbium trifluoride, Isofiller, barium aluminium silicate glass, calcium barium aluminium fluorosilicate glass and calcium fluorosilicate (alkaline glass) filler, initiator and pigments. Liquid: dimethacrylates (aliphatic aromatic UDMA, (UDMA)) and initiators
Cention Forte (Ivoclar Vivadent, Schaan, Liechtenstein)	Powder: inert barium alumino-boro-silicate glass, ytterbium fluoride, calcium fluoro-alumino-silicate glass, and reactive SiO <sub>2</sub> -CaO-CaF <sub>2</sub> -Na <sub>2</sub> O glass fillers Liquid: aliphatic-aromatic UDMA, DCP, UDMA, and PEG-400-DMA Initiator system including hydroperoxide, Ivocerin, and acyl phosphine oxide
Cention Primer (Ivoclar Vivadent, Schaan, Liechtenstein)	Methacrylate-modified polyacrylic acid, MDP, HEMA, Bis-GMA D3MA, silicon, ethanol, dioxide, potassium hydroxide and campherquinone
Fuji IX GP (GC Corporation, Tokyo, Japan)	Fluoroaluminosilicate glass, Polyacrylic acid and polybasic carboxylic acid

**Grouping of specimens:** A total number of 72 samples were fabricated by using a specially constructed standardized Polyurethane mold, each material type was packed to make samples with disc shape (2 mm height and 5 mm diameter). The samples then were divided according to the parameters of assessment into 32 specimens for PH measurement and 40 specimens for antibacterial effect (N=20 for each bacterial spp.).

#### For PH measurement (n=8):

- Group 1- Cention N
- Group 2- Primer free Cention Forte
- Group 3- Primer applied Cention Forte
- Group 4- Fuji IX (control positive)

#### For antibacterial effect evaluation (n=5 for each bacterial species)

- Group 1- Cention N
- Group 2- primer free Cention Forte
- Group 3- Primer applied Cention Forte
- Group 4- Fuji IX (control positive)

The mold was positioned in the middle of a glass microscope slide and a single mylar strip was placed in between them, each of the restorative material was packed into the sterile mold with a slight overfilling the mold. Each one of the different material types were manipulated in regard to the manufacturers guidelines instructions.

For the Primer applied Cention Forte (group 3) after loading the material into the mold the primer was loaded into a sterile container then, an inter-proximal carver (composite filling instrument), was immersed into the primer for 1 second then each one side of the instrument was swept for 3 seconds on the container edge in order to remove the excess of the primer. Then the instrument with the primer was adapted on the top surface of the uncured material sample with a slight of pressure to obtain flat and smooth surface. An additional strip was applied for covering the samples top surfaces in order to inhibit the construction of oxygen inhibiting layer, on top of it a second glass microscope slide was applied. 500 gm weight was applied to compress the mold for 30 sec. in order to extrude out the excess material, to achieve good packing and also, suitable consistent sample surface. All of the fabricated specimens were let to set for (15) minutes at room temperature without using light curing device.

**Storage of the specimen for PH measurements:** Each individual specimen of the materials groups was immersed in plastic container that contain five ml of deionized water D.W (PH 6.8). The immersed samples were then stored at 37 °C in an incubator with 95% humidity. The PH measurements have taken place at the end of 1, 7, 21 and 28 days of the incubation periods. At the measurements time, each individual specimen was carefully taken out the container and the storage solution were utilized for the evaluation, then the samples were replaced into a new container with fresh five ml of D.W and stored to the followed measurement time at 37 °C and 95 % relative humidity, the same procedure was done at all of the measurements time intervals.

**PH measurement:** The pH values of material groups were measured by a glass electrode of a digital pH meter (EUTECH INSTRUMENT, ECPH70042S, Singapore) that was previously calibrated with a serial of standard buffer solutions (10.0, 7.0, and 4.0 PH) respectively, the measurement was performed at a constant temperature of 25 °C of each incubation times (1, 7, 21 and 28) days. After removing the sample and thoroughly stirring of the solution for (5 seconds) the glass probe of the pH meter device was immersed in the testing solution until the reading appeared on the device screen became stable. The mean of pH value of each material group and for control positive group were recorded and calculated for comparison over times.

**Microorganism isolation:** Two cariogenic bacterial species: *Streptococcus mutans* (*S. mutans*) and *Lactobacillus plantarum* (*L. plantarum*) were obtained from laboratory of microbiology of Basic Dental Science at collage of dentistry/university of

Mosul each isolate was recultivated in brain heart infusion broth, incubated at 37°C for (18 hours) then cultured on their corresponding selective medium (Mitis salivarius agar) (MSA Himedia, India) and (Rogosa agar) (Rogosa SL agar, Himedia, India) for (*S. mutans*) and (*L. plantarum*) respectively. A pure single colony of each bacterial species from their selective agar plate was inoculated in tryptic soy broth (TSB) tube and incubated at 37°C for (18 hours), the incubated cultures were adjusted in order to obtain dilution equal to 0.5 McFarland standards scale ( $1.5 \times 10^8$  CFU/ml). The turbidity of the broths was assessed visually by comparing the (*S. mutans*) and (*L. plantarum*) (TSB) culture with tube 0.5 McFarland against striped (black, white) card behind them.

**Specimens inoculation:** To assess and compare the anti-bacterial effect of Alkasite bioactive restoration and high viscosity GIC (Fuji IX GP) against two types of bacterial species (*S. mutans*) and (*L. plantarum*). The samples from each material group were positioned at the bottom of a 96 well flat bottom plastic micro titer plate. 10 µL of the adjusted TSB broth culture of each bacteria spp. (*S. mutans*) and (*L. plantarum*) was separately transferred by a sterile micropipette to each disc surface. The discs containing microtiter plates were sealed and incubated at 37 °C for (30 minutes) in anaerobic conditions to make sure a direct contact between the discs and the bacteria. After that, a 190 µL of sterile Tryptic Soy Broth (TSB) was loaded to each well. Then, the microtiter plates with the specimens were incubated at (37 °C) in anaerobic conditions for 24 hours. 10 µl of TSB bacterial suspension with 190 µl sterile broth were added as mentioned above to five-disc free wells for each bacterial spp. to serve as a control negative.

**Direct contact test:** The ability of the bioactive Alkasite material and the high viscosity GIC to influence the viability and growth of two cariogenic bacterial spp. (*S. mutans*) and (*L. plantarum*) were evaluated by counting the (colony forming unit/ml) of the planktonic bacterial phase on petri dishes containing selective agar base (Mitis salivarius agar for *S. mutans*) and (Rogosa agar for *L. plantarum*). After 24-hour incubation of microtiter plates 10 µl broth culture of planktonic phase from each bacterial spp. was retrieved by micropipette tenth dilution were used on the retrieved suspension by transferring 10µl from the planktonic bacterial suspension from each disc containing well to test tubes contain 990 µl of sterile deionized water, three serial dilutions were performed, then the diluted solutions were used to inoculate the prepared agar plate. The diluted solutions separately seeded on the surface of agar plates and spread evenly on the agar base by cotton swabs. The same amount of the suspension also removed from wells that did not contain restorative material discs to serve as control negative, after 24 hours incubation at 37 °C under an anaerobic conditions bacterial colonies formed on each plate were counted. The plates with 30 to 300 typical colonies of (*S. mutans*) and (*L. plantarum*) were counted visually. For this test (n=5) for each bacterial spp.

**Statistical analysis:** Statistical analysis was applied using (SPSS) version.20.0. Data for PH value was analyzed with one-way (ANOVA) followed by Duncan test for intergroup analysis. For antibacterial effect assessment, the data was analyzed with Kruskal Wallis that followed by Dunne's pairwise comparison. The statistically significant was set at (p-value ≤0.05).

## Results.

**PH Measurement analysis:** Table 2 shows the (mean and ±SD) of PH value of the groups in various observation periods. Alkasite (Cention N and Primer free Cention Forte) at all intervals of time showed statistically significant higher PH value compared to positive control (Fuji IX GP). At (1,7) day respectively there was no significant difference between Cention N and Primer free Cention Forte, while at (21,28) day Primer free Cention Forte showed PH value that was significantly higher than Cention N.

**Table 2.** mean, ±SD and significance of the groups pH value in various observation periods.

Periods	Material Groups	Means	±SD	F test	P-value	Sig.
1 day	Group 1	7.0300	0.04840	257.385	0.001	C
	Group 2	6.9800	0.07801			C
	Group 3	4.8000	0.35335			A
	Group 4	6.2613	0.03314			B
7 day	Group 1	7.1800	0.09532	20.818	0.001	B
	Group 2	7.1475	0.11744			B
	Group 3	6.7325	0.33161			A
	Group 4	6.5375	0.14290			A
21 day	Group 1	7.3025	0.12792	59.217	0.001	B
	Group 2	7.6450	0.10677			C
	Group 3	7.5775	0.14685			C
	Group 4	6.9300	0.08783			A
28 day	Group 1	6.9638	0.06844	36.905	0.001	B
	Group 2	7.3400	0.20270			C
	Group 3	7.3325	0.07265			C
	Group 4	6.8125	0.10152			A

Means value have different letters (vertically) have significant differences at (p ≤ 0.05). Group1=Cention N, Group2=Primer free Cention Forte, Group3=Primer applied Cention Forte, Group4=Fuji IX (positive control).

Primer applied Cention Forte tend to reduce D.W (storage medium) at (1,7) day respectively and it showed statistically significant less PH value in comparison with Fuji IX at day 1, but there was not significant difference in day 7. While at (21,28) day respectively Primer applied Cention Forte showed elevation in PH value and it was significantly higher than Fuji IX (positive control).

Cention N has PH value that was significantly higher than Primer applied Cention Forte at (1,7) days. While, at (21,28) days Primer applied Cention Forte showed PH value that was significantly higher than Cention N.

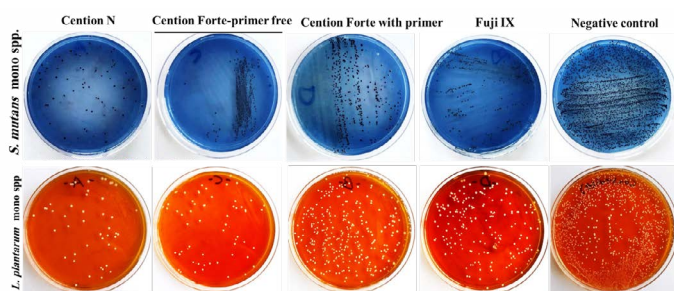
Primer free Cention Forte have PH value that was significantly higher than PH value of Primer applied Cention Forte at (1,7) day, but there was no significant difference in PH value for both groups at (21, 28) day.

**Direct contact test analysis:** Means values of the number of the planktonic viable bacterial colonies of (*S. mutans*) and (*L. plantarum*) of the material groups, positive control (Fuji IX) and control negative (only the bacterial isolate) represented in Table (3). Figure (1) represent the numbers of (*S. mutans*) and (*L. plantarum*) after 24 hours incubation on agar plates of the material groups and control negative.

**Table 3.** Mean  $\pm$ SD and significance for comparison the effect of the material groups on inhibition the numbers of planktonic viable colonies of (*S. mutans*) and (*L. plantarum*) at incubation period of 24 hour.

Material group	Mean	$\pm$ SD	p-value	Bacterial spp.	Sig.
Group 1	50	10	0.012**	<i>S. mutans</i>	A
Group 2	50.333	9.018499			A
Group 3	312.33	2.516611			B
Group 4	97	4.582575			AB
Control negative	640	36.05551			B
Group 1	40.333	9.504384	0.012**	<i>L. plantarum</i>	A
Group 2	40.667	9.018499			A
Group 3	160.33	0.577350			B
Group 4	89.333	3.055050			AB
Control negative	593.67	7.234170			B

Means value with different letters (vertically) have significant differences at  $p \leq 0.05$ . Group1=Cention N, Group2=Primer free Cention Forte, Group3=Primer applied Cention Forte, Group4=Fuji IX (positive control) and control negative=disc free wells



**Figure 1.** Numbers of viable colonies for Cention N, Primer free Cention Forte, Primer applied Cention Forte, positive control (Fuji IX GP), and control negative. (A) *S. mutans* viable colonies cultured on Mitis Salivarius agar (B) *L. plantarum* viable colonies cultured on Rogosa agar.

Mean value comparison of effect in inhibition the numbers of planktonic viable bacterial colonies of (*S. mutans*) and (*L. plantarum*) among the groups showed highly statistically significant differences in the groups mean values. Post-hoc Dunne's pairwise comparison test showed there was no statistically significant differences between Control positive (Fuji IX GP) group and Control negative group ( $p < 0.05$ ) in inhibition the number of viable colonies in regard to both bacterial spp.

Kruskal Wallis test showed significant difference among means of viable cell count ( $p = 0.012$ ), ( $p = 0.012$ ) for the tested materials for *S. mutans* & *L. plantarum* respectively, Dunne's pairwise comparison compare between each two materials showed statistically significant difference at ( $p \leq 0.05$ ) between Cention N and Primer free Cention Forte that has less mean of viable colonies count compared to control negative, however, both groups showed no statistically significant differences in their mean value compared to the positive control (Fuji IX). There was no statistically significant differences between Primer applied Cention Forte and control negative and positive control (Fuji IX) at ( $p > 0.05$ ) in inhibition the number of both bacterial

spp. viable colonies however, No statistically significant differences between Cention N and Primer free Cention Forte at ( $p > 0.05$ ) in inhibition the numbers of both bacterial spp. viable colonies. While, both materials showed statistically significant difference at ( $p \leq 0.05$ ) less numbers of both bacterial spp. viable colonies in comparison Primer applied Cention Forte. Figure 1 showed the viable colony count for *S. mutans* and *L. plantarum* on their selective media respectively.

### Discussion.

The acid produced by bacterial will cause pH fluctuation that control the  $Ca^{+2}$  and P ions loss or gain from the dental tissue. Whenever the pH drops below the critical pH of (6.5) for dentin and (5.5) for enamel, demineralization process takes place [17]. The result of this study revealed significant difference among the four material groups in their pH value, thus the first null hypothesis was rejected. The result showed that alkasite (Cention N and Primer free Cention Forte) immediately elevated the storage medium (D.W) PH value which grow gradually overtime then followed by a reduction at the end of the observation period (28 day), but generally the findings of this study proved that Alkasite material has the capacity to continuously increase the storage medium (D.W) pH over the period of 28 days. Wiriyasatiangkun et al. (2022) stated that over the observation period of 28-day Cention N continuously released hydroxide ions which in turn responsible for elevating the storage medium PH value [15].

Cention N and Primer free Cention Forte has significantly higher PH value in comparison with Fuji IX at all intervals of time. Ion release from the restorative materials depend strongly on composition of the material [18]. The higher PH value related to the alkaline glass filler (calcium fluoro-silicate glass) in this material which consist of (24.6%) out of the final material filler content this alkaline glass responsible for different ions release including the hydroxide ( $OH^-$ ) ion [13]. While GICs do not release hydroxyl ions ( $OH^-$ ) [19]. Throughout the periods of the observation Fuji IX (positive control) tend to reduce the storage medium PH. Its effect decreased over time and at (21 and 28) day the original PH has been restored. This relies on fact of the presence and accessibility of unreacted carboxylic acid functional groups within the set cement matrix that leached to the surrounding solution. Also the GIC maturation process is slow and may continue over several weeks or even months, the slow maturation involving high number of unreacted carboxyl group to leach to deionized water at the beginning of the observation period days then with the maturation goes on there is only few numbers of the unreacted carboxylic acid functional groups to be diffused into the storage medium lead to decrease the cement effect on lowering the deionized water PH [13].

Although the difference was not statistically (significant), the result shows that Cention N has higher PH value compared to Primer free Cention Forte at the (1 and 7) days interval of the experiment, while at the (21 and 28) day intervals Primer free Cention Forte has significantly higher PH value than Cention N. This can be related to the formations of bubbles and porosities that produced at mixing time, hence Cention N supplied in powder and liquid (hand mix) by the manufactures. The presence of these microporosities in the Cention N matrix

will lead to more initial diffusion of the hydroxyl ions from the material matrix which in turn led to a higher PH value at the initial time of the observation period [20].

While the mixing regime in the encapsulated form of the alkasite material (Cention Forte) was standardized by the mechanical mixing. Such mixing reduces the air inclusion throughout mixing produce which yield in a reduction in bubbles entrapment or microporosities in the material matrix resulting in a slow diffusion of hydroxyl ions from the Primer free Cention Forte matrix since the initial elution depends on OH<sup>-</sup> ability to diffuse through the material matrix microporosities subsequently Primer free Cention Forte needed longer time to achieve higher PH compared to Cention N [21].

Primer applied Cention Forte at the initial time of the observation period (1 and 7 day) have lowered the (D.W) PH, in a matter of fact at the end of the 1day Primer applied Cention Forte showed significantly the lowest PH value among all other groups. The reduction in storage solution PH of Primer applied Cention Forte is related to the self-etch coat (Cention Primer) which contain high amount of the acidic monomers that contributed to a low PH value. With further time the result showed there was an increase in Primer applied Cention Forte PH value which was significantly higher in comparison to Cention N and Fuji IX at (21 and 28 day), that might be due to the hydrophilic characteristic of the primer because it consisted of monomers having ester bonds like (hydroxylethyl methacrylate) such monomers are ted highly to be hydrolyze with further time in the presence of water, which might be the reason for an increased OH<sup>-</sup> release from the material [22]. Primer applied Cention Forte has PH value was less compared to Primer free Cention Forte PH at (21 and 28) day even so, the difference was not significant, this could be as a result to the presence of the Primer which represent a barrier for the ions to be liberated [23].

In our study and during the measurement of the materials PH value were taken the temperature has been fixed at 25 C, since the PH value was affected by the temperature when temperature is higher than 25 C, lead to a reduction PH value is as a result to the elevated degree of ionization of the water. in contrary, when temperature less than 25 C (PH) reading was elevated as a result of the reduced degree of ionization. *S. mutans* is considered as the main ethological bacteria for tooth caries initiation, and specifically known to be the predominant microorganism in enamel caries. On the other hand, *L. bacillus* is responsible for dental caries progression and secondary caries formation [24,25]. These bacteria metabolize the ingested sugars and produce acids as byproduct, leading to lower the oral cavity PH to a limit that is less than dental hard tissues solubility limit. It was revealed that formation of a new carious lesion is related to the numbers of salivary *S. mutans* in the oral cavity and so, by decreasing the numbers of *S. mutans* will also lead to decrease the caries activity [4,5,26].

In the presented study we used Direct Contact Test (DCT) to evaluate antibacterial effect of two bioactive materials and convention GIC, this test utilized because it relies on a direct and close contact between the restorative material and the pathogenic microbes, and it does not dependent of the diffusion

properties of the chosen dental restoration. And hens it will be more suitable for evaluate and compare the antibacterial properties of the dental restorations and cements. It also simulation to the clinical situation, where the testing materials will come in contact with the microbes [27,28].

The result revealed significant difference among the groups regarding their antibacterial effect against bacterial spp. therefore, the second null hypothesis was also rejected. All of the tested materials and control positive (Fuji IX GP) showed antibacterial effect against (*S. mutans*) and (*L. plantarum*) through inhibiting the number of the viable colonies compared to the viable colonies count of control negative. Cention N and Primer free Cention Forte showed highest antibacterial effect in term of reducing the number of viable (*S. mutans*) and (*L. plantarum*) colonies which was (statistically significant with control negative but not significant with control positive (Fuji IX)). The inhibitory properties of these materials are relying on their ability to release ions, including F<sup>-</sup> which has multiple anticariogenic influences on teeth, fluoride released from such materials to the dental plaque will adversely affect the growth of *L. bacillus* and *S. mutans* through the interference with the bacterial enzyme systems [29,30]. The reason for an enhanced antibacterial activity of the alkasite material, possibly related to the high amount of F<sup>-</sup> ions released due to the aggressive respond of the material as it placed in an acidic PH that formed from the bacterial lactic acid which led to a quicker wash out of the surface modified layer of the material, leading to baring the material matrix for improved F<sup>-</sup> ions liberation. Additionally, in acidic PH these materials showed increased alkalizing potency. This can be owed to the calcium and hydroxyl ions liberated from alkaline fillers in the Alkasite material, these ions have direct influence on PH levels, and so creating environments where any additional acidity produced from the cariogenic bacterial activity can be countered [4,19,27].

A study done by Kalaivanan and Jaishree (2023) compared the antibacterial activity of Cention N, Fuji IX and other restorative material against *S. mutans*, and their outcome was that alkasite material have significantly more antibacterial effect than the other materials in the study [29]. Fuji IX showed some inhibition ability to the viable colony cells of (*S. mutans*) and (*L. plantarum*) which was lower than the Primer free Cention Forte and Cention N but, higher antibacterial effect than Primer applied Cention Forte and control negative (not statistically significant with all groups). The antibacterial impact of Fuji IX is related to the F<sup>-</sup> release also, additionally, Fuji IX release different type of metal ions such as (calcium, aluminum and strontium) that have a buffering capacity aid in neutralizing bacterial acids [31,32]. Primer applied Cention Forte has the lowest inhibition in the number of viable bacterial colonies among other three groups.

The limited antibacterial activity in the Primer applied Cention Forte group is in alignment with it diminishes ion released as a result of the insulated filler particles from the aqueous medium by the Primer layer. Several authors have confirmed that coat layer will form such a barrier for the ions liberation, and it could impact negatively on the caries preventive potentials of the ion releasing fillings [22,23,33]. Nevertheless, the fact it showed limited antibacterial effect it could be related to the fact in which

the Primer only covered the top surface of the specimen, but the other surfaces were able to liberate ions from the alkaline glass fillers which contributed to inhibitory impact on the numbers of bacterial viable colonies.

This study limitations include the using of standardized specimens while in clinical situations the cavity dimensions are varied. A deionized water was used as a storage media to measure the PH value, while saliva is the media in the oral cavity. This investigation was operated in (in-vitro) conditions on the other hand the oral environment is active and different from the *in-vitro* situations. More advance research needs to be conducted to check the ions recharge ability of Alkaside restorative materials and their antibacterial properties against mixed bacterial species.

### Conclusion.

Cention N and Primer free Cention Forte showed higher PH value than Fuji IX GP on all intervals of time. Cention N and Primer free showed comparable antibacterial effects to Fuji IX in regard to inhibiting the numbers of viable colonies of both (*S. mutans*) and (*L. plantarum*). Applying Cention primer negatively affects OH- release and the antibacterial effect of Cention Forte. Further future investigations should be focused on controlled clinical trials with more complex designs of the experiment consisting of a greater number of reasons that affect the characteristics of dental materials in a real clinical condition to attain more valid conclusions.

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