GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 11 (356) ноябрь 2024

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press. Published since 1994. Distributed in NIS, EU and USA.

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 ВИНИТИ 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 ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

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
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. Корректура авторам не высылается, 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 compu-ter-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.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 საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ 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 შეღებვის 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 ფჩხილებში 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.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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Содержание:

GEORGIAN MEDICAL NEWS
No 11 (356) 2024

ANTIMICROBIAL ACTION OF A MODIFIED UNIVERSAL ADHESIVE: AN IN VITRO STUDY

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Abstract.

Background: Resin composites and dental adhesives are widely used to restore carious teeth. A relatively new category of the dental adhesives, the universal adhesives (UAs) is considered user friendly because of its simplicity to use and compatibility with any adhesive strategy. However, the adhesive interface created by these adhesives is highly susceptible to cracking after polymerization which in turn facilitates the initiation of secondary caries. **Objectives:** The present study was set to evaluate the antimicrobial action of All Bond Universal Adhesive after modifying it with chitosan capped cerium nanoparticles (C-CeNPs) against *S. muatns* and *L. acidophilus*.

Materials and Methods: A colloidal suspension of C-CeNPs was synthesized, characterized, and incorporated into the universal adhesive. Two groups of adhesives were used: G I without C-CeNPs and G II with 3% by weight of C-CeNPs. The antimicrobial action of G I and G II was evaluated by agar diffusion test (ADT) and direct contact test (DCT) at 1, 7, and 14-day time intervals.

Results: For ADT, G II resulted in larger inhibition zones against both microbial strains than G I. The inhibition zones for G I and G II where larger against *S. mutans* than *L. acidophilus*. For the DCT, less colony forming units (CFU) of both microbial strains were formed in regard to G II than G I. The number of CFU of both microbial strains increased in line with the time intervals.

Conclusions: Incorporating C-CeNPs into the universal adhesive increased its antimicrobial action against *S. muatns* and *L. acidophilus* microbial strains.

Key words. Universal adhesives, antimicrobial action, chitosan, cerium, nanoparticles.

Introduction.

Nowadays, resin composites and dental adhesive systems are most commonly used to restore decayed teeth as primary direct restorative materials [1]. Currently the so-called universal adhesives (UAs) could represent a potential new trend for dentists as they provide a more straightforward approach to the traditional concept of adhesive technology by reducing procedure's sensitivity, increasing efficiency, and saving time for clinical application [2,3]. UAs are also called multimode adhesives since they can be used with any adhesive strategy and hence, they are considered as user friendly [4]. However, nano-leakage in the adhesive interface of UAs was detected since these adhesives contain hydrophobic and hydrophilic species in the same bottle [5]. Upon polymerization, UAs will shrink giving rise to micro cracks in the adhesive interface [6,7]. These micro cracks can be easily invaded by cariogenic bacteria like *S. mutans* and *L. acidophilus* which eventually leads to secondary dental caries [8,9].

Different antimicrobial agents have been added to UAs adhesives to impart them antimicrobial action with different outcomes observed [10]. A recent study has found that doping an orthodontic adhesive with cerium oxide nanoparticles enhanced its antimicrobial action against *S. mutans* [11]. However, keeping nanoparticles well- dispersed into a suspension is a major issue since nanoparticles tend to agglomerate into clusters of several microns [12]. The agglomeration of nanoparticles can be minimized if suitable capping agents are used [13]. Various capping agents, such as surfactants, tiny ligands, polymers, dendrimers, cyclodextrins, and polysaccharides, have been utilized in the synthesis of nanoparticles [14]. Chitosan is a co-polymer comprising d-glucosamine and N-acetyl-d-glucosamine produced via alkaline deacetylation of chitin naturally occurring in the crustaceans or hydrolysis of chitin by the enzymatic action of deacetylase [15]. Chitosan has been used as a capping agent to stabilize the colloidal dispersion, and to control the morphology and optical properties of metal nanoparticles including gold [16], silver [17], and copper [18]. Regarding biological applications, chitosan has excellent properties like biocompatibility, biodegradability, non-toxicity, and antimicrobial activity [19]. Different modalities have been tried to overcome this issue and provide antimicrobial activities, including resin and seashell [20,21]

To the extent of our knowledge, incorporating chitosan-capped cerium oxide nanoparticles (C-CeNPs) in a universal adhesive, has not been investigated in any study yet. The aim of this study was to evaluate the antimicrobial action of a universal adhesive modified by incorporating chitosan capped cerium oxide nanoparticles. The null hypothesis was that incorporating C-CeNPs would not significantly improve the antimicrobial action of the modified adhesive against *S. mutans* and *L. acidophilus* bacterial strains when compared with the non- modified adhesive.

Materials and Methods.

Synthesis and characterization of chitosan capped cerium oxide nanoparticles colloidal suspension (C-CeNPs):

The synthesis of C-CeNPs was based upon the procedure implemented by Siqi Zhao M.Sc. thesis [22]. The materials used to prepare C-CeNPs were purchased from Sigma Aldrich. After it's synthesis, the colloidal suspension was characterized by Fourier Transform Infrared Spectroscopy, Transmission Electron Microscopy, field emission scanning electron microscopy and energy dispersive X-ray spectroscopy with elemental mapping.

Incorporation of the Capped Nanoparticles into the Universal Adhesives: After characterizing and examining the nanoparticles size distribution, 0, and 3% by weight of the prepared colloidal suspension were incorporated into a universal adhesive. The adhesive agent selected to be used in this study is a commercially available universal adhesive, which is the All-Bond Universal adhesive (Bisco, USA).

After incorporation of the capped nanoparticles, the adhesive bottle was shaken with the aid of mechanical mixing device (VELP Advanced Vortex Mixer; Biobase Biomettch CO. Ltd, China) for 2 minutes in a spiral agitation motion at 2000 rpm speed to allow even blending and dispersion of the capped nanoparticles into the universal adhesive [23].

The bacterial strains used in the study were *Streptococcus mutans* (*S. mutans*) and *Lactobacillus acidophilus* (*L. acidophilus*). The bacterial strains were isolated and identified by specialist microbiologists at Laboratory of Microbiology, Department of Dental Basic Sciences, College of Dentistry, University of Mosul, Mosul, Iraq.

The adhesive groups were:

Group I (G I): All Bond Universal adhesive without C-CeNPs

Group II (G II): All Bond Universal adhesive modified with 3% wt C-CeNPs

Two tests were performed to evaluate the antimicrobial action of the adhesive groups: the agar diffusion test (ADT) and the direct contact test (DCT).

Agar Diffusion Test: Forty Mueller Hinton agar plates were prepared for this test, twenty plates per each group. A micropipette (10-100 µl Accumax PRO; ACCUMAX Lab Devices PVT. Ltd, India) was used to pick up 100µL of each pure microbial strain and spread by the aid of sterile swabs over the entire surface of the plates. Ten plates were used for testing the antimicrobial action against *S. mutans* and another ten plates were used for testing the antimicrobial action against *L. acidophilus*. In each plate, two wells of 6 mm diameter were punched by a sterile cork borer. A micropipette was used to fill the wells with 20 µL of adhesive group. For each plate, one well was filled with G I adhesive while the other well was filled with G II adhesive. A gentle steam of air was applied to the adhesives in the wells for 15 seconds. Then after, the adhesives were light cured with (MaxCure 9 LED curing device, Guilin Refine Instrument. Ltd, China) for 20 seconds. After 24 hours of incubation, the plates were removed out from the incubator and examined for the inhibition zones which were measured around each well in mm using a digital caliper.

Direct Contact Test: This test was performed to evaluate the antimicrobial action of adhesive groups against microbial strains after 1, 7, and 14 days. One 96-well cell plate was used to evaluate the antimicrobial action of adhesive groups against *S. mutans* and another one was used for *L. acidophilus*. From each plate, 48 wells were filled with brain heart infusion (BHI) while the other 48 wells were allocated for the adhesive groups (24 well for each group and 8 well for each time interval). A third 96-well cell plate was used where 16 wells were filled with Control positive groups (microbial suspension without adhesive) (8 wells for each group) and another 8 wells were filled with Control negative group (adhesive without microbial suspension). The plates were incubated at 37° for the determined durations. After incubation, 10 µL from the broth in each well was pulled and transferred to 1 ml of sterile BHI broth. The suspension was agitated for one minute, then subjected to 10-fold serial dilutions from 10^1 to 108. Finally, 0.1 ml of each diluted suspension was dispensed into selective agar plates for each isolate and streaked with an L-shaped glass rod. The culture plates were then incubated at 37 °C for 24 hrs. After incubation, the plates were photographed, and the colonies were counted. The colony forming units (CFU/mL) were calculated using the following formula:

CFU/ml = (no. of colonies x dilution factor) / volume of culture plate

The data were converted to logarithmic transformation (log10).

Statistical Analysis: The statistical analysis was performed using SPSS 25. Data were analyzed by analysis of variance ANOVA) and Duncan's multiple range test.

Results.

Regarding ADT, the inhibition zones created by G I and G II adhesive groups are shown in (Figure 1). The results of ADT including the descriptive statistics are shown in (Table 1) and (Figure 2). G II has resulted in greater inhibition zone values against both microbial strains when compared to G I.

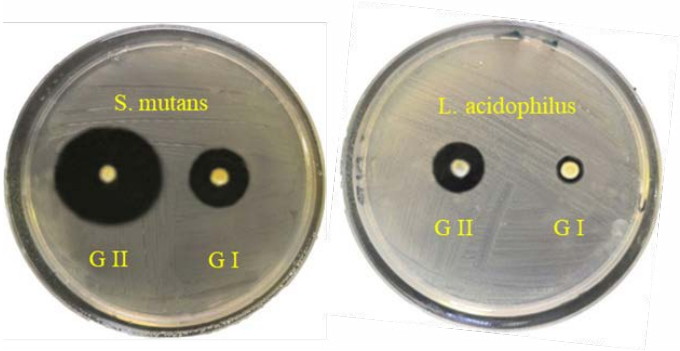

Figure 1. *Inhibition zones created by G I and G II adhesive groups against S. mutans and L. acidophilus.*

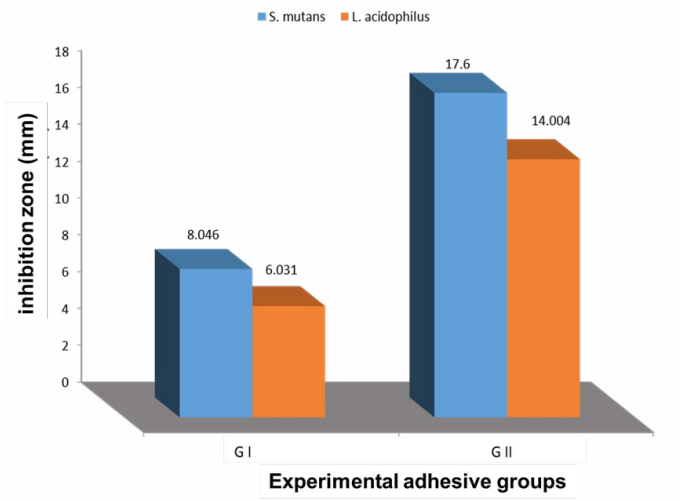

Figure 2. *Graph showing the mean inhibition zone values (± SD) in mm of adhesive groups against S. mutans and L. acidophilus bacterial strains.*

Table 1. *Descriptive statistical results of ADT for adhesive groups against S. mutans and L. acidophilus bacterial strains.*

Adhesive groups	Bacterial strain	Number of samples	Minimum	Maximum	Mean	Std. Deviation
G I	*S. mutans*	10	6.5	9.7	8	0.92
	L. acidophilus	10	4.8	7	6	0.75
G	*S. mutans*	10	16.3	19.1	17.6	1.1
	L. acidophilus	10	12.9	14.9	14	0.75
	Valid N (listwise)	10				

Table 2. *ANOVA test results of ADT for adhesive groups against S. mutans and L. acidophilus bacterial strains.*

		G I	G II	Mean
S. mutans	Mean	8.046 c	17.6 a	12.823 a
	Number of samples	10	10	20
	Std. Deviation	0.92293	1.09828	4.99956
L. acidophilus	Mean	6.0310 d	14.0040 b	10.0175 b
	Number of samples	10	10	20
	Std. Deviation	0.7463	0.75248	4.15459
Total	Mean	7.0385 b	15.8020 a	
	Number of samples	20	20	
	Std. Deviation	1.31749	2.05974	
Different lower-case letters indicate significant differences between treatments at level of significance ≤ 0.01.				

Table 3. *ANOVA test showing the effect of adhesive group, microbial strain type, and the combined effect of adhesive group and microbial strain type on the inhibition zone values of ADT.*

Source	Type III sum of squares	Df	Mean square	F-value	Sig.
Corrected model	852.947	3	284.316	357.493	0.000
Intercept	5216.884	1	5216.884	6.560E3	0.000
Adhesive group	767.989	1	767.989	965.654	0.000
Bacterial strain	78.708	1	78.708	98.966	0.000
Adhesive group * Bacterial strain	6.249	1	6.249	7.857	0.008
Error	28.631	36	0.795		
Total	6098.462	40			
Corrected Total	881.577	39			

The greatest inhibition zone value was for G II against *S. mutans* while the smallest inhibition zone value was for G I against *L. acidophilus*. G I and II resulted in greater inhibition zones against *S. mutans* in comparison to the inhibition zones against *L. acidophilus*.

The results of ANOVA test are shown in (Tables 2 and 3). Table 2 revealed that there were significant differences between adhesive groups regarding their antimicrobial action against the same microbial strain. In addition, the antimicrobial action of adhesive groups against *S. mutans* were significantly higher than their action against *L. acidophilus*. Table 3 revealed that the inhibition zone values were significantly affected by the adhesive group, the type of bacterial strain, and the combined effect of the adhesive group and type of bacterial strain.

Regarding the DCT, the microbial growth of *S. mutans* and *L. acidophilus* bacterial strains after exposure to adhesive groups at different time intervals along with control positive group are shown in (Figure 3). The descriptive statistics are shown in (Table 4 & Figure 4). The mean CFU values of both bacterial strains for both groups increased with increasing time intervals. However, G II resulted in mean values much lower than G I. The highest value was for G I against *S. mutans* at 14 days' time interval while the least value was for G II against *S. mutans* at 1-day time interval.

Duncan's multiple range test (table 5) shows that G II resulted in a significantly lower CFU mean values of both microbial strains than those of G I at all-time intervals. In addition, *S. mutans* CFU mean values of G II, at all-time intervals, were statistically lower than those of *L. acidophilus*. Furthermore, the CFU mean values of each microbial strain for G II were statistically different from each other in respect to time intervals while in G I the difference exists only in respect to the 1-day time interval. ANOVA three-way statistical analysis (table 6) shows the significant interaction between adhesive group and bacterial strain, between adhesive group and time interval, between bacterial strain and time interval, and among adhesive group, bacterial strain, and time interval at level of significance ≤ 0.01.

Discussion.

Secondary caries at the margins of direct composite resin restorations is one of the most common causes of their replacement [24], where bacterial invasion is aggravated by marginal seal breakdown due to the degradation of the adhesive

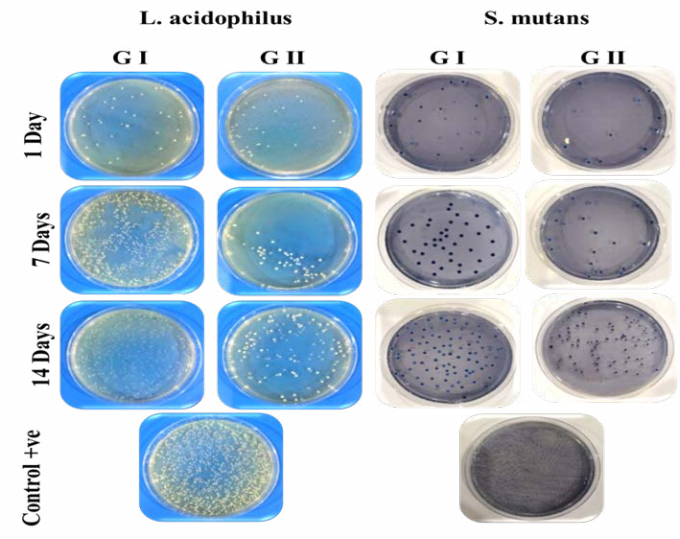

Figure 3. *Microbial growth of S. mutans and L. acidophilus bacterial strains after exposure to adhesive groups at different time intervals along with control positive group.*

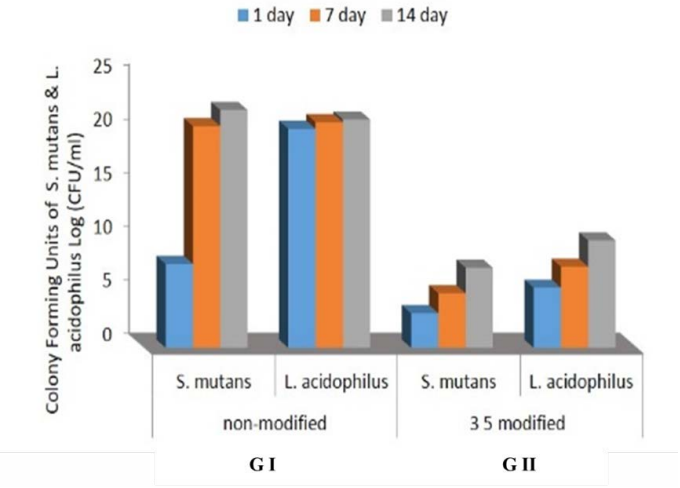

Figure 4. *Graph showing the mean values of log (CFU/ml) S. mutans and L. acidophilus in regard to adhesive groups after different time intervals.*

Table 4. *Descriptive statistical results of DCT of adhesive groups against S. mutans and L. acidophilus bacterial strains at different time intervals.*

Descriptive Statistics							
Group	**Bacterial strain**	**Time interval**	**Number of samples**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
G I	*S. mutans*	1 Day	10	6,7	8.9	7.79	0.86081
		7 Days	10	18.7	22.8	20.59	1.36337
		14 Days	10	18.7	25.2	22.1	2.14372
	L. acidophilus	1 Day	10	19.1	21.5	20.34	0.924
		7 Days	10	19.1	23.7	20.96	1.62221
		14 Days	10	19.1	24.1	21.22	1.86476
G II	*S. mutans*	1 Day	10	2.5	3.8	3.21	0.43321
		7 Days	10	4.4	5.9	5.07	0.46679
		14 days	10	6.1	8.6	7.44	0.96632
	L. acidophilus	1 Day	10	4.9	6.4	5.58	0.50947
		7 Days	10	6.6	8.4	7.53	0.57745
		14 Days	10	8.9	10.7	9.98	0.52873

Table 5. *Duncan's multiple range test of DCT of adhesive groups against S. mutans and L. acidophilus bacterial strains at different time intervals.*

		G I		G II	
		S. mutans	***L. acidophilus***	***S. mutans***	***L. acidophilus***
1 day	Mean	7.7900 d	20.3400 b	3.2100 f	5.5800 e
	Number of samples	10	10	10	10
	Std. Deviation	0.86081	0.92400	0.43321	0.50947
7 days	Mean	20.5900 b	20.9600 b	5.0700 e	7.5300 d
	Number of samples	10	10	10	10
	Std. Deviation	1.36337	1.62221	0.46679	0.57745
14 days	Mean	22.1000 a	21.2200 ab	7.4400 d	9.9800 c
	Number of samples	10	10	10	10
	Std. Deviation	2.14372	1.86476	0.96632	0.52873
Different lower-case letters indicate significant differences between treatments at level of significance ≤ 0.01					

Table 6. *Three-way ANOVA test of DCT of adhesive groups against S. mutans and L. acidophilus bacterial strains at different time intervals.*

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F- value**	**Sig.**
Corrected Model	6346.451	11	576.950	422.935	0.0001
Intercept	19205.230	1	19205.230	1.408E4	0.0001
Adhesive group	4586.797	1	4586.797	3.362E3	0.0001
Bacterial strain	313.957	1	313.957	230.147	0.0001
Time interval	756.411	2	378.206	277.245	0.0001
Adhesive group * Bacterial strain	18.174	1	18.174	13.323	0.0001
Adhesive group * Time interval	120.574	2	60.287	44.193	0.0001
Bacterial strain * Time interval	269.471	2	134.735	98.768	0.0001
Adhesive group *Bacterial strain * Time interval	281.068	2	140.534	103.019	0.0001
Error	147.329	108	1.364		
Total	25699.010	120			
Corrected Total	6493.780	119			

interface [25], and universal adhesives aren't an exception of this dilemma. The antimicrobial action of universal adhesives has been evaluated in several studies [10]. The agar diffusion test is a widely used method to detect the antibacterial action of an agent by its diffusion through the agar plate and subsequent inhibition of bacterial growth being studied [26].

In this study, both adhesive groups, G I and G II, have shown an antimicrobial action against *S. mutans* and *L. acidophilus*. G I, although not modified, showed a slight antimicrobial action against *S. mutans* which may be attributed to the chemical composition of the adhesive since it contains Hydroxyl Ethyl Methacrylate (HEMA), a hydrophilic primer, and 10-Methacryloyloxydecyl Dihydrogen Phosphate (10-MDP), an etchant and adhesion promoting agent [27]. The acidic characteristics of these constituents might result in an inhibited growth of *S. mutans* [28]. However, their effects were weaker against *L. acidophilus* since these bacteria are well- known of their acid tolerance [29].

The antimicrobial action of G II, in addition to its composition, seems to be fortified by incorporating C-CeNPs which is composed from cerium oxide capped by chitosan nanoparticles. Cerium oxide nanoparticles can exert their antimicrobial action by two ways. First; through the redox behavior where cerium oxide nanoparticles switch between two oxidation states: Ce(III) and Ce(IV). This redox behavior allows cerium to generate reactive oxygen species (ROS), which can damage bacterial cell membranes and disrupt metabolic processes. The conversion from Ce(IV) to Ce(III) is particularly important, as it enhances the oxidative stress on bacteria, leading to cell death [30]. Second, through the electrostatic interactions demonstrated by the adsorption of positively charged cerium oxide nanoparticles to the bacterial cell wall and hence impairing cellular respiration, DNA replication, and cell division [31]. In addition to cerium oxide nanoparticles, chitosan has also exerted its own antimicrobial action which can be explained into two ways. First, being positively charged, chitosan can electrostatically interact with the negatively charged bacterial cell membranes. This interaction disrupts the integrity of the cell membrane, leading to increased permeability and ultimately cell lysis [32]. Second, since chitosan used in this study to synthesize C-CeNPs was of low molecular weight this has enhanced its penetration into bacteria, ensured effective disruption of their cell membranes and thereby increased the antimicrobial action [33].

The DCT was also used in this study to detect the antimicrobial action of adhesive groups against bacterial strains for the following reasons. First, it allows for direct exposure of bacterial cells to the dental adhesive. Thus, bacteria will be in close contact with the adhesive material, facilitating a more accurate assessment of its antimicrobial action [34]. Second, it provides quantitative data on bacterial growth inhibition. By measuring the change in bacterial viability before and after contact with the adhesive, precise information can be obtained about the adhesive's effectiveness over time [35]. According to the results of this study, G I has exerted an antimicrobial

action only against *S. mutans* in 1-Day interval which nearly which was nearly vanished at the other two-time intervals. This could be attributed to the diminished effect of HEMA and 10-MDP constituents with time due to their degradation and hydrolysis when the adhesive was mixed with the microbial suspensions and BHI during the DCT procedure [36]. However, G II continued to retard the growth of microbial strain, at all-time intervals because of the incorporation of C-CeNPs with antimicrobial action of cerium dioxide and chitosan previously mentioned [37-39].

Conclusion.

Within the limitations of this study, the antimicrobial action of All Bond Universal against *S. mutans* and *L. acidophilus* can be enhanced by the incorporation of C-CeNPs.

Conflict of interest: The authors have no conflicts of interest to declare.

Acknowledgement and funding: There was no external support for this study.

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