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The Effect of Resorcinol on Alginate Tear Strength

Balancing Antimicrobial Functionaly and Mechanical Integrity

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Abstract

The development of dental impression materials with antimicrobial efficacy is a significant biomedical research target. Alginate impression material is widely used in dental practice, and endeavours to enhance its properties remain a persistent research objective. Incorporation of antimicrobial agents into alginate can compromise alginate's mechanical properties and increase the risks of tearing, thus harmonising between these contradictory effects seems an appealing aim to study. Benzene-1,3-diol known as resorcinol was utilized to create a potentially antimicrobial alginate impression material without reducing its tear strength below acceptable levels. This study was conducted to determine the least but effective antimicrobial concentration through incorporation of resorcinol into alginate using different concentrations. The disk diffusion test to evaluate the activity against different microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans* and *Candida albicans*) was utilised. V-notched alginate-resorcinol samples (n=10) were fabricated from standard tear strength plastic mold and its riser with the following dimensions was used in this study (101.6mm, 19.5mm,2 mm, length, width and thickness, respectively) with a 90 degree v-notched region and tested using an Instron testing device with tensile action. The study showed that 2% and 2.5% resorcinol-alginate concentrations demonstrated antimicrobial activities against all the microbial species tested. Therefore, these concentrations were tested for tear strength and showed a statistically significant reduction in tear strength (0.83 &0.79 N/mm, mean) for 2% & 2.5% concentrations, respectively, in comparison to the control group (0.94 N/mm), nevertheless, these results were very close to accepted tear strength range of the modern reinforced alginate (0.8-1.2 N/mm). The incorporation of resorcinol fortified the alginate impression material against multiple microbial species and preserved the tear strength as a representative of mechanical integrity within an optimum accepted specification level.

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Introduction

Irreversible hydrocolloid impression material has been successfully used in dental practice over several decades. The hydrophilicity, low cost, and accepted accuracy level (if it is properly handled) persevere its worldwide popularity [1,2]. Alginate is commonly employed in prosthodontic and orthodontic fields to fabricate study and diagnostic casts, guards, bleaching trays [3]. Diverse microbial community that densely populates the oral cavity can instigate cross-infection because of daily dental practice and laboratory procedures, and it represents a high hazardous jeopardy, indeed, alginate can be easily contaminated by oral fluids during impression making [4,5]. A plethora of methods have been employed to prevent the potential cross-infection happening through using of these materials, but associated adverse effects on alginate properties were mostly encountered, necessitating the quest for further innovative methods [5-7]. Chlorhexidine, alcohol, sodium hypochlorite, glutaraldehyde, quaternary ammonium chemical compounds have been extensively

explored for disinfection of dental impression materials via immersion or surface-spraying techniques, nevertheless, no single chemical has a universal consensus to use it in the dental field [8,9]. Production of self-disinfecting impression is a potentially effective strategy, given the reduced efforts, condensed time consumed as well as the avoidance of negative effects on the material properties resulting from immersion and spraying, especially with highly sensitive materials to heavy wet environments such as alginate [5,6,10,11]. Resorcinol (Benzene-1,3-diol) is a synthetic, colourless to whitish chemical compound with low cytotoxicity and disinfectant properties. It has been known for its use in dyes, cosmetics production and dermatological associated therapies. Acne, psoriasis, hidradenitis suppurativa and dyspigmentation were managed by resorcinol derivative compounds [12-14]. Moreover, resorcinol has a potential antifungal activity; it showed antifilamentous action against the transformation of *Candida albicans* from an oval-shaped yeast form to more adherent hyphal filamentous form, which is considered a considerable virulence mechanism for tissue evasion and the formation of resistant microbial biofilm [15]. Resorcinol derivatives demonstrated an antidermophytic action against *Microsporum gypseum* fungal species [16], and inhibitive activity against *Mycobacterium tuberculosis*, the causative agent of the highly contagious disease tuberculosis [17]. Addition of active agents to alginate did not negatively affect tear strength, thus production of activated alginate is a potential target for research [18]. To the best of our knowledge, no studies have investigated the effect of incorporation of resorcinol into alginate. The objective of this study is to create a potentially self-disinfecting alginate impression material without plummeting the tear strength mechanical property.

Materials and Methods

Commercially available, normal set, dust free alginate impression material was used in this research (Cavex alginate CA37, Netherlands). Resorcinol C6H6O2 polyphenolic compound, (ACS,99% purity, molecular weight 110.11, highly soluble in water) product was supplied as flakes through Sisco Research Laboratories Pvt.Ltd. India Batch No. 3898357. Growth medias (broth and agar, HIMEDIA Inc, India) were used for antimicrobial tests, where Mueller-Hinton broth and agar for *Staphylococcus aureus, streptococcus mutans and Escherichia coli* bacterial species and sabouraud dextrose broth and Agar for *Candida albicans* fungal species.

Alginate samples (control group) and alginate/resorcinol experimental samples were prepared for each tested microbial species to conduct pilot studies for determination and selection of the lowest two antimicrobial concentrations that ranged between (0.5-2.5% at 0.5% increment) (n=5). The concentration of resorcinol was determined according to the alginate powder/ resorcinol weight-by-weight percentage, where the weight of the incorporated resorcinol flakes is subtracted from the weight of the alginate powder to formulate the intended concentrations. Five ml of sterile distilled water was mixed with the resorcinol as it dissolves easily in water and vortexed for 30 second to ensure complete solubility, then it was mixed with weighed alginate to form the experimental samples, while for fabrication of control samples, the distilled water was mixed with the weighed alginate directly. The mixing was performed in sterile rubber bowl for 45 seconds according to the manufacturer's instructions using sterile stainless steel spatula. The prepared alginate was loaded immediately in a special mold with 2 mm in thickness and was pressed constantly. Once the material had set, the alginate was cut with a sterile cork borer to obtain symmetrical alginate discs with dimensions of 2 mm in thickness and 8 mm in diameter. The discs were sterilized by exposure to ultraviolet light for 15 minutes on each side in laminar flow cabinets. All the tested microbial species were isolated from patients at the teaching hospital of college of dentistry, university of Baghdad. The tested microbial species were cultivated for 24 hours at 37 °C in Mueller-Hinton broth for the bacterial species and in Sabouraud dextrose broth for *Candida albicans*. The disc diffusion test was conducted separately for each tested microorganism by distributing and affixing the samples (control and experimental) in the agar Petri dish spaced a part at equal distances. Inoculated suspensions from the microorganisms were prepared up to 0.5 McFarland standard turbidity, which is equivalent to 1.5×10^8 colony forming unit (CFU)/ml. Then 15 ml from that standardized inoculum was seeded over the samples in the agar Petri dishes, and left for 60 minutes (at room temperature), then aerobically kept in an incubator for further 24 hours at 37 °C. When the incubation period was completed, inhibition zones were measured.

Instron universal testing machine (WDW-20, Laryee Technology Co. Ltd., China) was used to measure the tear strength (Ts) of study samples (control and resorcinol- impregnated samples). The samples (n=10) were prepared by a custom mold fabricated according to ISO 21563 specifications. The samples were 90º V notched shape (with riser) and had the following dimensions (101.6 mm, 19.5 mm and 2 mm, length, width and thickness, respectively). Each sample was clamped in the Instron device and strained up to failure at a 500 mm/min speed rate. The tear strength (N/mm) was calculated according to the following formula: Ts =F/d, where F: the maximum applied force in Newton leading to propagation of a tear in the sample and d: thickness of the sample.

Data organization, scientific graphing and statistical analysis were performed using GraphPad prism 5 program using one-way analysis of variance with Bonferroni multiple comparison test at a 0.05 level of significance.

Results

According to the pilot studies conducted, resorcinol-impregnated alginate revealed antimicrobial activity against all the tested microbial microorganisms at 2 and 2.5% weight/weight concentrations. Thus, these two concentrations were adopted in the tear strength test. Table 1 shows the disc diffusion test results, where all the tested microorganisms demonstrate comparable susceptibility to the experimental discs with a slight superiority for the 2.5% wt./wt. concentration. Figure 1 shows inhibition zones in the Petri dishes in response to the experimental discs for all the tested microorganisms.

The study data showed a statistically significant decrease in the tear strength of the experimental discs in comparison to the control discs (n=10). 2% wt./wt. concentration demonstrated 0.83 N/mm, mean, and 2.5% concentration demonstrated 0.79 N/mm, mean, while control group mean was 0.94 N/mm. Nonetheless, it is noteworthy that these data were highly close to recognized tear strength range of the modern reinforced alginate which ranging between (0.8-1.2 N/mm). Figure 2 graphically shows the effect of resorcinol incorporation into the alginate impression material on its tear strength.

Discussion

Enhancing the properties of alginate, which is one of the most frequently used materials in dentistry, seems to be a significant target for research. Alginate impression material can be a source of cross-infection besides its relatively low tear resistance, thus, the quest for antimicrobial strategies must avoid the deteriorating of the mechanical properties associated with such modifications [1,19]. This innovative report only scratches the surface of such important objective. Resorcinol incorporation into alginate was not studied before, and this is the first study

according to the best of author's knowledge. Although the observed decrease in the tear resistance, this study revealed the potential antimicrobial activity of resorcinol-incorporated alginate against several microbial species (potential habitants of the oral cavity) without causing a sharp decline in the tear resistance to levels below the widely accepted strength. It is noteworthy that tear strength values ≥0.83 N/mm is considered within the range of the modern types of alginate [20]. The only reported usage of resorcinol in the dental field was decades ago, where resorcinol had been blended with formaldehyde in the presence of sodium hydroxide as a catalyst and known as Russian red resin to formulate an endodontic paste/resin for the clinical treatment of difficult root canal cases [21]. Resorcinol has low cytotoxicity, but with long-term of exposure (moderate - high doses), it can disrupt the thyroid function and its hormones [22-24]. This is unlikely to occur with incorporating this material into alginate impression, where low concentration is used and exposure to oral cavity continues for very few minutes. Studies related to formulation of self-disinfecting alginate (used chlorhexidine, povidone iodine, hyopchlorous acid, nanoparticles of silver and titanium oxide, quaternary ammonium) showed the capability of alginate for being antimicrobial [4,5,10,11,25-27, 28], and this study established this notion and reinforced the armamentarium against the possibility of cross-infection in dental and laboratory fields. The study samples were sterilised by a non-chemical process to avoid any premature release of the impregnated material. The antimicrobial mechanism of polyphenols, which resorcinol belongs to, reveals multiple mechanisms such as initiation of oxidative stress, denaturation of proteins, interference with membrane integrity and inhibition of enzymes resulting in a higher effectiveness and lower antibiotic resistance probability [29]. Because of the high solubility of resorcinol in water, it can release from the impregnated discs easily, thus, justifying diffusion test results for the impregnated samples and their antimicrobial activity. Alginate reaction starting by release of calcium ion from calcium sulphate because of contact with water and that calcium reacts with sodium alginate to initiate the polymerization and setting reaction. The resorcinol is a polyphenolic compound, polyphenols have affinity to calcium [30], and therefore it might lead to partial interference to polymerization due to depletion of calcium. Consequently lower resistance to tear crack propagation through the weaker polymerization chains, justifying the slightly reduced tear strength of the experimental group's samples. Moreover, the impregnated samples were fabricated from relatively lower alginate powder/water ratio because of preparation of alginate/resorcinol wt./wt. methodology accounting for the slightly reduced tear strength [20].

Conclusion

It is notable that incorporation of resorcinol at low wt./wt. concentrations into alginate is promising antimicrobial strategy and does not deteriorate the clinically accepted level of mechanical integrity of the alginate impression material, namely tear strength. It represents an innovative endeavour to fortify alginate against microbial contamination. Conducting further studies is highly recommended to confirm the antimicrobial activity and tear strength results, and to investigate the effects on other properties of alginate and even other impression materials or other dental materials.

Conflict of Interest

The authors declare no competing interests.

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Table 1. Inhibition zones in millimeters (mm) of resorcinol-incorporated samples for all the tested microorganisms with average and standard deviation (SD) values.

Microbial species	*Streptococcus mutans*		*Escherichia coli*		*Staphylococcus aureus*		*Candida albicans*	
Conc.	2%	2.50%	2%	2.50%	2%	2.50%	2%	2.50%
Sample 1	23	24	24	24	23	26	24	27
Sample 2	19	27	24	26	23	23	21	24
Sample 3	19	24	23	27	21	23	26	27
Sample 4	23	26	21	26	24	26	24	26
Sample 5	26	23	26	26	23	29	21	26
Ave	22	25	24	26	23	25	23	26
SD	2.7	1.8	1.8	1.1	1.1	2.7	2.2	1.4

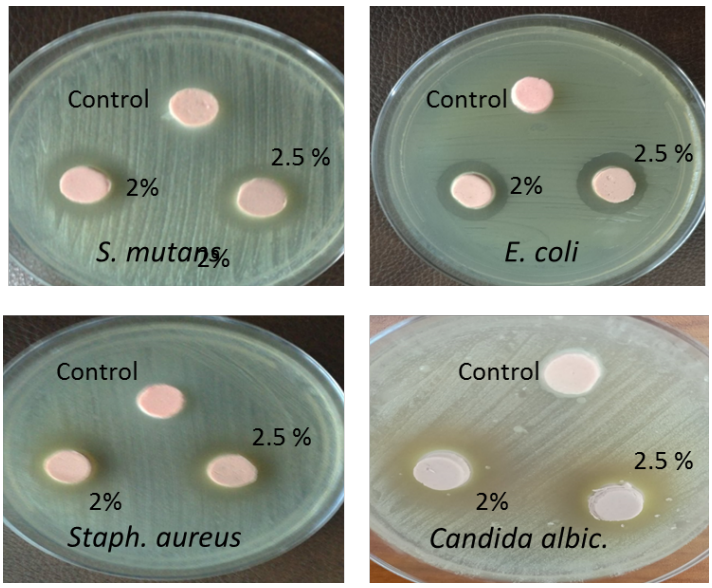

Figure 1. Susceptibility of all the tested microorganisms to the resorcinol-incorporated alginate impression material using the selected concentrations.

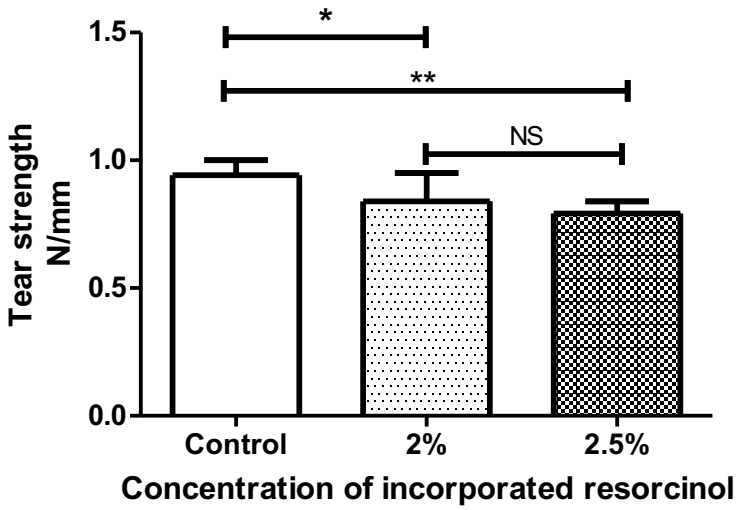

Figure 2. Tear strength of resorcinol-incorporated alginate. Analysis of variance (ANOVA) showed a significant variance among the tested groups (n=10) (P value = 0.0006) and Bonferroni multiple comparison test showed non-significant difference between the experimental groups themselves, while significant difference with the control group.