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Impact of modification with cetylpyridinium chloride – a potential cariogenic microflora inhibitor, on selected physical-mechanical properties of the water-activated glass-ionomer

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Purpose: Teeth caries is one of predominant civilization diseases. Dental fillings with antimicrobial addition might allow prevention of secondary caries. The purpose of this study was to evaluate hardness and tensile strength of cetylpyridinium chloride modified water activated glass-ionomer cement. *Methods*: Samples with diameter of 6 mm and height of 3 mm made of water-activated glass-ionomer cement were control group (0.0%). Test groups were series of samples of the same dimensions, with addition of cetylpyridinium chloride antibacterial in concentrations of 0.5, 1.0, 1.5 and 2.0%. Two subgroups were prepared in each group to determine Vickers Hardness and Diametral Tensile Strength after 1 and 24 hours of sample storage in distilled water. *Results*: During hardness studies, no strong effect of antibacterial concentration on hardness of samples was observed. Higher hardness values after 24 hours were demonstrated for all groups, compared to the samples tested after 1 hour. The exception was the group with the addition of 1% cetylpyridinium chloride, in which no statistically significant differences were observed. Diametral Tensile Strength values for samples tested after 1 hour decreased with increasing antibacterial concentration. A similar relationship was noticed for samples tested after 24 hours. No statistically significant differences were found between test samples after 1 or 24 hours. *Conclusions*: There was no significant effect of cetylpyridinium chloride concentration on the hardness of the samples that significantly increased during the study. With the increase in antibacterial concentration a decrease in diametral tensile strength value was observed, but these values did not change over time.

Key words: *hardness, diametral tensile strength, glass-ionomer*

1. Introduction

Caries disease still remains a major public health problem that besets human race. Oral cavity accommodates different bacteria living in a balanced ecosystem. Changes in this homeostasis can induce shifts of benign biofilm microflora towards pathologies, such as teeth caries. Secondary caries remains a serious problem which causes additional costs and it is bothersome and uncomfortable for the patients [11]. Remineralization is one of the best strategies to manage with this problem [23]. Attempts to produce bioactive restoratives were prompted by the concept that fluoride-releasing biomaterials materials exert useful effects in the body. In Mount's [14] opinion, favourable dental restorative characterized by self-adhesion, no setting shrinkage, minimal thermal expansion, optimum fluoride release or other ions recharge, is nothing other than Glass Ionomer Cement (GIC).

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GICs have been developed since late 1960s by a research group led by – chemist Alan Wilson [21] and dentist John McLean [13]; these cements are biomaterials that gel and set as the result of interaction of Al^{3+} and Ca^{2+} ions extracted of the basic glass powder, with COO^{-} polyanions in water-soluble poly(acrylic acid). Taking into the consideration the chemical composition of GICs, conventional (CGIC), metal-reinforced (MRGIC), fast setting (FSGIC), cermet-ionomers, anhydrous (WAGIC), high viscosity (HVGIC), visible-light-activated (VLAGIC), resin-modified (RMGIC – dual and tree-cured DCRMGIC and TCRMGIC) and recently developed glass carbomers (GCC), can be chronologically distinguished [10].

Partial carious dentin removal (PDR) in one session does not ensure total microorganisms removal. This problem may be solved by the use of dental materials that inhibit bacterial growth and, thus, termed Interim Therapeutic Restoration (ITR) [7]. The ability of GICs to ions exchange may lead to the hypothesis that GICs can potentially be used as templates for the release of other active microbial inhibiting agents – antimicrobials (AMb) [5]. Among lots of AMb [18], amphiphilic quaternary ammonium salts (QAS) are used one of which is cetylpyridinium chloride (CPC) characterized by a high efficiency. It disrupts bacterial cell metabolism, thereby inhibiting cell growth as the consequence that the negatively charged cell membrane loses electrical balance with positively charged nitrogen groups and bursts under its osmotic pressure. Cetylpyridinium chloride has the molecular formula $C_{21}H_{38}NCl$ and at room temperature it has the form of pure white powder (solid state). It is soluble in water but insoluble in acetone, acetic acid, or ethanol [17].

Microbial inhibiting factors – antimicrobials (AMb), have been incorporated into a variety of dental materials including orthodontic adhesives, denture base materials, and dentinal bonding agents [22], but there is still absence of them in dental restorative materials. GICs require an optimum amount of these agents, which should not jeopardize the basic properties of parent materials [12]. The idea is to enhance cariogenic flora inhibiting properties of GICs with balanced dose of additives incorporated into their compound, without causing biological and physic-chemical changes of a biomaterial. Taking it into the consideration, we started our project with current study evaluating surface hardness (HV0.3/15) and diametral tensile strength (DTS) of a water activated glass ionomer cement (WAGIC) modified with increasing mass concentration of cetylpyridinum chloride (CPC) cariogenic microbiota inhibitor.

2. Materials and methods

Samples preparation

Hexadecytylpiridinium chloride monohydrate (Sigma-Aldrich, Canada) was added to the powder of WAGIC (ChemFill Superior, Dentsply Sirona, Germany) in weight concentrations of 0.5, 1.0, 1.5 and 2.0%, respectively. These samples were served as experimental groups and specimens with the sample 0.0% CPC as a control group. Then, according to manufacturer instructions, water was added to CPC and WAGIC powder compound and mixed to material homogenization. Obtained paste was placed into silicon mould (diameter d = 6 mm, height h = 3 mm) to prepare samples for DTS and HV0.3/15 test and then put in distilled water for 1 or 24 hours.

Hardness

After predetermined time (1 or 24 hours) hardness measurements were performed using hardness tester (ZHμ, Zwick/Roell, Germany). To evaluate samples hardness, Vickers test was used. In this method, 136° diamond square-based pyramid was used as an indenter. Indenter was pressed into material's surface using load of 300 g. After 15 s the load was removed and diagonal lengths of the indentation were measured. HV0.3/15 was calculated from equation as follows:

$$HV = \text{const.}\frac{F}{A} = 0.102\frac{2F\sin\frac{136^\circ}{2}}{\left(\frac{d_1+d_2}{2}\right)^2} = 0.1819\frac{F}{d^2}$$

$$HV = 0.1819\frac{F}{d^2}:$$

F – load force [N], A – surface of the indentation [μm^2], d_1, d_2 – diagonal lengths [μm].

Each impression point was made in at minimum 3 diagonal lengths distance. On each sample being tested 7 impression points were made in different places. Results were subjected to statistical analysis.

Diametral tensile strength

DTS test was performed according to ANSI/ADA standard No. 66 after 1 or 24 hours storage samples in distilled water. On each, group being tested 11 DTS were measured. In this test, a compression load was placed by peripheral surface of a cylindrical sample. In a perpendicular direction to applied force direction,

the tensile forces were occurred. In this test, maximum force was measured by universal testing machine (Z020, Zwick/Roell, Germany) with crosshead speed 0.5 mm/min. The DTS value was calculated by the following equation:

$$DTS\,[\mathrm{MPa}] = \frac{F}{S}\frac{[\mathrm{N}]}{[\mathrm{mm}^2]}$$

$$= \frac{F}{\frac{1}{2}\left(2\pi\frac{d}{2}h\right)}\frac{[\mathrm{N}]}{[\mathrm{mm}\cdot\mathrm{mm}]} = \frac{2F}{\pi dh}\frac{[\mathrm{N}]}{[\mathrm{mm}^2]}$$

where: *DTS* – diametral tensile strength [MPa], *F* – maximum force [N], *S* – surface [mm^2], *d* – sample diameter [mm], *h* – sample height [mm].

Results were subjected to statistical analysis.

Statistical analysis

For statistical calculations, Excel 2010 (Microsoft, USA) and Statistica v. 12.5 (Statsoft, Poland) were used. The Shapiro–Wilk Test of normality was applied. In analysis between 1 and 24 hours, in particular concentration, parametric *t*-test or non-parametric Mann–Whitney test were applied, depending on distribution. Evaluation of differences between concentrations after 1 hour or 24 hours was performed with parametric *F*-test or non-parametric Kruskal–Wallis test, depending on distribution. Equality of variance was tested with Levene's test. The accepted level of significance was $\alpha = 0.05$.

3. Results

Hardness

Results for specimens with different concentration of CPC stored for 1 or 24 hours in distilled water in room temperature are shown in Fig. 1. No strong negative effect of CPC concentration on the hardness of the samples was observed, while the HV0.3/15 values were higher after 24 hours for all groups, compared to the samples tested after 1 hour, except for the group with the addition of 1%, in which such differences were not shown. On the basis of the *t*-test with independent estimation of variance, a statistically significant difference between 1 and 24 hours for the concentration of 0% (*p*-value = 0.000) and for the concentration of 0.5% (*p*-value = 0.000) was demonstrated. In addition, a difference between 1 and 24 hours for a concentration of 2% (*p*-value = 0.000) was demonstrated on the basis of the *t*-test. On the basis of the Mann–Whitney *U*-test, a statistically significant difference between 1 and 24 hours for the concentration of 1% (*p*-value = 0.025) was demonstrated.

On the basis of the Kruskal–Wallis test, statistically significant differences between the concentrations obtained after 1 hour were demonstrated: 0.5 and 1% (*p*-value = 0.002); 0.5 and 1.5% (*p*-value = 0.000). On the basis of the Kruskal–Wallis test, statistically significant differences were found between concentrations obtained after 24 hours: 0 and 1% (*p*-value = 0.009); 0.5 and 1% (*p*-value = 0.001); 1 and 2% (*p*-value = 0.005).

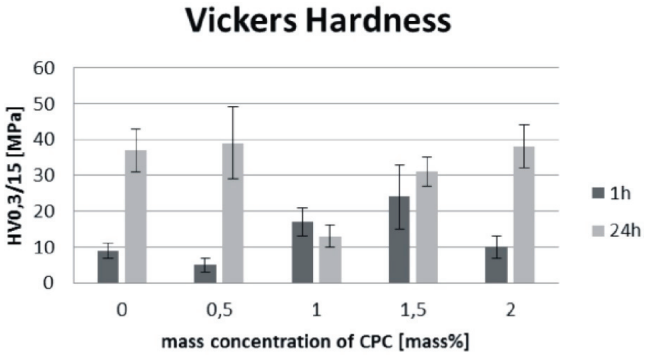

Fig. 1. HV0.3/15 results for CPC modified WAGIC

Diametral Tensile Strength

DTS values for samples tested after 1 hour decreased with increasing CPC concentration from 10.39 ± 1.34 MPa (0.0%) to 6.41 ± 1.28 MPa (2.0%) (Fig. 2). The same observation was made for samples tested after 24 hours where DTS values decrease from 11.42 ± 2.39 MPa (0.0%) to 6.73 ± 1.27 MPa (2.0%) (Fig. 2). There were no statistically significant differences in the *t*-test or Mann-Whitney *U*-test between 1 and 24 hours for individual concentrations. The increasing concentration of CPC in WAGIC decreased DTS, however, it does not change over time. On the basis of the *F* analysis of variance, statistically significant differences were found after 1 hour for concentrations 0 and 0.5% (*p*-value = 0.007); 0 and 1% (*p*-value = 0.000); 0 and 2% (*p*-value = 0.000); 0.5 and 2% (*p*-value = 0.000); 1 and 2% (*p*-value = 0.011). On the basis of the Kruskal–Wallis test, statistically significant differences were found between concentrations obtained after 1 hour: 0 and 1.5% (*p*-value = 0.000); 0.5 and 1.5% (*p*-value = 0.040). On the basis of *F* analysis of variance, statistically significant differences were found after 24 hours for concentrations: 0 and 0.5% (*p*-value = 0.006); 0 and 1% (*p*-value = 0.002); 0 and 1.5% (*p*-value = 0.000); 0 and 2% (*p*-value = 0.000).

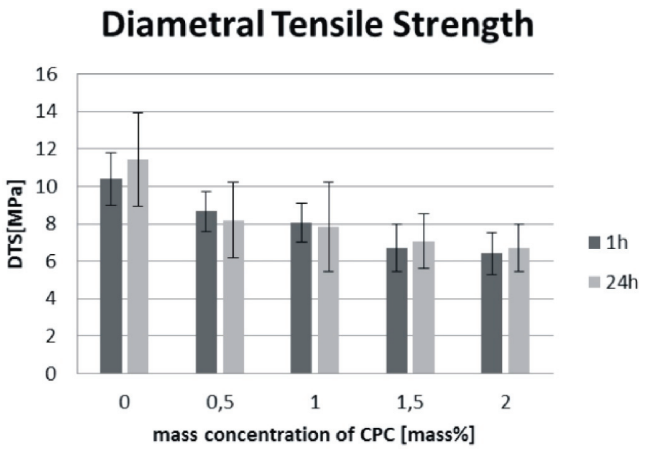

Fig. 2. DTS results for CPC modified WAGIC

4. Discussion

The emerging infectious caries disease and the development of drug resistance in the pathogenic bacteria and fungi at an alarming rate is a matter of serious concern. Therefore, there is a pressing demand to discover novel strategies and identify new cariogenic microbiota balancing agents that re-establish tooth hard tissue mineral stabilization, taking oral and general safety of nowadays Amb into consideration.

There are opposite data regarding antibacterial activity of pure GICs, e.g., Fuji Triage cement inhibited the growth of a broad spectrum of bacteria strains. Fuji IX cement demonstrated the most potent antibacterial activity against *S. sanguis*. Ketac Molar showed antibacterial activity against *S. sanguis* and *S. salivarius*, whereas Ketac Silver was efficient against *S. mutans* as well. Neither of the Ketac cements inhibited growth of the standard *L. casei* strain [8]. On the other hand, there are data indicating that ChemFil Superior WAGIC had no antibacterial activity for cariogenic microorganisms [19]. Even if pure glass-ionomer cement can inhibit the growth of cariogenic flora in biological studies, this is not synonymous with their effectiveness in *in vivo* oral conditions, which prompted our team to this project together with other researchers with similar hypothesis.

Pawluk concluded research on Chem Flex HVGIC with statement that modification of pure GIC with increasing agents concentration promoted the formation of inhibition haloes for both bacterial strains, statistically greater for *L. casei* than for *S. mutans* ($p < 0.05$). In her research worth emphasizing are results indicating CPC that obtained the highest antibacterial activity with prolongation the setting time or without water loss properties affect [16]. Study also disclosed that as the CPC concentration increased, the hardness of the material slightly decreased, however, it increased over time, which is reflected in our research results HV0.3/15, although we used another glass ionomer type, WAGIC, for which the hardness is twice lower. In addition, we noted a positive impact of both 1.0 and 1.5% CPC on the increase in hardness.

Choosing HV0.3/15 and DTS for analysing of the potential influence of CPC on handling properties of WAGIC, we referred to surface and structural parameters; surface hardness is regarded as an important property to predict the clinical performance of a restorative material, and to assess influence of environment on the materials surface. Hardness refers to the plastic deformation of a solid material when a force is applied. In dentistry, it is commonly used as a parameter for restorations under occlusal stress, and relates to functional parameters, such as abrasive wear resistance [3], [15]. DTS is a critical requirement, because many clinical failures are due to tensile stress. As it is not possible to measure directly the tensile strength of brittle materials like Glass Ionomer Cements (GICs), it has been adopted DTS. In this test, a compressive force is applied to a cylindrical specimen across the diameter by compression plates. While the stresses in the contact regions are indeterminate, there is evidence of a compressive component that hinders the propagation of the tensile crack. Large shear stresses that exist locally under the contact area may also induce a shear failure before tensile failure at the centre of the specimen [2], [12].

In our study we observed that addition of 1.0 and 1.5% CPC improved material hardness in fresh cement after 1h. Best results of HV 0.3/15 after 1 and 24 h, close to control group of pure WAGIC, we reported for 2% CPC group. Moreover, glass-ionomer stored in human saliva has an improved surface hardness as compared to samples stored in water. The reasoning behind this may be that water storage causes only extraction of components, thus resulting in a reduction of strength, whilst storage in saliva can increase the mineral content of glass-ionomer [2].

Opposite data obtained Tüzüner and Ulusu [20], who reported that 2% CPC caused adverse effect on GIC surface micro hardness in 90-days laboratory trial. The same tendency was observed by other researchers detecting influence of CPC on physical properties of GIC [1]. Dimkov with colleagues [4] evaluating impact of CPC on Chem Flex CS, revealed continual drop from 146.5 MPa for pure material to 63.4 MPa for 3% CPC.

Our observations revealed that DTS values were inversely proportional to increasing CPC mass concertation. This diminution of physical property was slight, probably with no clinical relevance. DTS values of GICs are not only influenced by antimicrobial's additions. Extremely important feature is a type of a material. For example, results of DTS obtained by Bresciani's research group [2] were of great difference; Bioglass R: 5.54(0.529) and 6.58(0.808) MPa, Vitro Molar: 8.27(0.475) and 9.43(0.822) MPa, Fuji IX: 7.24(0.699) and 11.96(1.514) MPa, after 1 and 24 hours, respectively. Taking also our results (10.39(1.34) and 11.42(2.39) MPa), into consideration, the choice of pure control material is essential for concept formulation and experiment planning.

In past decade it has been suggested that the cationic compounds based on quaternary ammonium salts, such as benzalkonium chloride and cetylpyridinium chloride, have particular capacity to interact with the poly(acrylic acid) [17]. It may be a possible reason for tolerable decrease in the physical properties, which hamper the setting reaction of the poly(acrylic acid) glasses, thereby extending the setting time, due to an interfered proton attack and leaching of ions from the glasses. Possible equalization of this decrease in physical-mechanical properties may be heat post-curing that could balance any reduction in physical properties of glass-polyalkenoates, particularly modified with antimicrobials. Additional heating of the GIC biomaterials during setting decreases microleakage improving marginal adaptation. Thermo-curing significantly increases flexural strength of high viscosity GIC up to 99 MPa with the light 1000 mW/cm^2, and about 127 MPa for glass-carbomer [6]. It has also an important influence on material properties and may improve the survival rate of GICs in the clinical situation as well as may provide long-term caries protection through formation of fluorapatite [9]. Furthermore, what is to emphasize, as GICs, in opposite to ceramic-resin composites, are inherently degradable/resorbable polyelectrolyte biomaterials of active caries management ability in assumption, their restorative attributes go down into the background.

5. Conclusions

There was no strong dependence between CPC concentration and samples hardness that was significantly increasing with time. DTS decreased with increasing CPC concentration in WAGIC without changes in time.

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