

## Effect of addition of antimicrobial triclosan on selected properties of water-activated glass ionomer cement

### Wpływ dodatku przeciwdrobnoustrojowego triklosanu na wybrane właściwości cementu szklano-jonomerowego aktywowanego wodą

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#### Abstract

**Introduction.** Failure of dental restorations is commonly associated with residual infected dentine underneath. Dental materials containing antimicrobials may overcome the problem of recurrent caries without jeopardizing their physicomachanical characteristics. **Aim of the study.** To evaluate hardness and diametral tensile strength (DTS) of a water-activated glass-ionomer (WAGIC) enriched with increasing mass concentration of triclosan (TRC) – the antimicrobial agent. **Material and methods.** TRC was added to the powder of WAGIC in weight concentrations of 0.5%, 1.0%, 1.5%, 2.0% served as experimental groups, and 0.0% as a control group, respectively. In each group two subgroups were defined to record Vickers hardness (HV0.3/15) and DTS after 1 and 24 hours of storage in distilled water. Shapiro-Wilk test of normality, parametric (F-test or t-test) or non-parametric (Kruskal-Wallis test or Mann-Whitney test) were used for the purpose of statistical analysis ( $p = 0.05$ ); the equality of variance was checked with Levene's test.

#### Streszczenie

**Wprowadzenie.** Uszkodzenia wypełnień dentystrycznych są powszechnie związane z aktywnością próchnicową pozostawionych w zębinie bakterii. Stosowanie materiałów stomatologicznych zawierających dodatki przeciwdrobnoustrojowe, które nie wpływałyby negatywnie na ich właściwości wytrzymałościowe mogłoby pozwolić na rozwiązanie problemu powikłań związanych z próchnicą wtórną. **Cel pracy.** Celem badania była ocena twardości i wytrzymałości na rozciąganie średnicowe (DTS) szkło-jonomeru aktywowanego wodą (WAGIC) wzbogaconego triklosanem (TRC). **Materiał i metody.** Próbkę wykonaną z WAGIC stanowiły grupę kontrolną (0,0% TRC). Grupy badane stanowiły serie próbek z dodatkiem TRC w stężeniach wag. 0,5%, 1,0%, 1,5% i 2,0%. W każdej z grup przygotowano dwie podgrupy dla oznaczenia twardości i DTS po 1 i 24 godz. przechowywania próbek w wodzie destylowanej. Testy normalności Shapiro-Wilka, parametryczny (test F lub t-test) lub nieparametryczny (Kruskala-Wallisa)

#### KEYWORDS:

glass-ionomer, triclosan, hardness, DTS

#### HASŁA INDEKSOWE:

szkło-jonomer, triklosan, twardość, DTS

**Results.** HV0.3/15 was significantly higher after 24 h than after 1 h. In both cases addition of 0.5 % of TRC caused significant decrease of hardness. DTS values were significantly higher after 24 h with addition of 0.5 % TRC, but generally it was slightly decreased in comparison to the control group. **Conclusions.** Except 0.5 % TRC, there was no negative influence of antimicrobial addition on HV0.3/15 and DTS, so TRC does not deteriorate strength of WAGIC.

## Introduction

Amalgam and composite restorative materials are not natural ones and have many drawbacks. An alternative should be both easy to use and apply without complex procedures. In all cases, it should provide a reliable seal for the lesion brought by the decay process. The material should be as natural as possible in order to allow a powerful and natural remineralization process to further restore the defect and minimize the necessary preparation.<sup>1</sup> Clinical trends themselves ensure, following Goldberg, that there will continue a sustained reduction in the use of dental amalgams in clinical practice across the European Union.<sup>2,3</sup> In Mount's opinion, favourable restorative dental material that can adhere to the tooth and help in further prevention of caries by continuous fluoride release is none other than Glass Ionomer Cement.<sup>4</sup>

It was Sorel who 161 years ago introduced zinc oxide-chloride cement and then phosphate cement – the first synthetic dental cement. After 3 years Rostanigs reported obtaining the “artificial bone”. Today we can say it was only zinc-oxide-phosphate cement but the biomimetic concept in search for substitute materials imitating tooth tissues began to bloom. 142 years ago Fletcher introduced what he called “artificial dentine”, which in fact was only a zinc-sulphate cement; however, 4 years later he applied the first biomaterial for direct restorations, imitating hard dental tissues, “artificial dentine, artificial enamel” – a silicate cement by Steenbok's recipe. 53 years ago Smith introduced zinc-polycarboxylic cement. A very important step in the development of restorative materials in the context of safety, minimal invasiveness and biomimetic idea evolution was the first self-

lub Manna-Whitneya) zostały wykorzystane do analizy statystycznej ( $p = 0,05$ ); równość wariancji badano testem Levena. **Wyniki.** Twardość próbek była znacząco wyższa po 24 godzinach niż po 1 godzinie. Dla próbek 0,5 % była niższa w obu przypadkach w porównaniu z grupą kontrolną 0,0 %. **Podsumowanie.** Za wyjątkiem grupy 0,5 %, nie odnotowano negatywnego wpływu dodatku TRC na HV0,3/15 i DTS. TRC nie zaburza wytrzymałości WAGIC.

adhesive cariostatic glass-polyalkenocarboxylic cement (GIC) biomaterial called ASPA, developed 55 years ago by a team of McLean, Wilson, Kent, Crisp at the Laboratory of the Government Chemist in London, as a material that gels and sets 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 consideration the chemical composition of GICs, conventional (CGIC), semi- and anhydrous (WAGIC), metal-reinforced (MRGIC), visible-light-activated (VLAGIC), resin-modified (RMGIC – dual and tri-cured DCRMGIC and TCRMGIC) ones and newly introduced glass carbomers – HAp enriched, poly(diethylsiloxane) modified GICs, can be distinguished.<sup>5-8</sup>

Dental caries is a public health problem and the most prevalent infectious disease found in the oral cavity in humans. It results in destruction of sensitive dental hard tissues caused by Gram-positive microorganisms: *Streptococcus oralis*, *Streptococcus mitis*, *Streptococcus sanguis*, and mostly by *Streptococcus mutans* and *Lactobacillus acidophilus*.<sup>9-12</sup> Dentine caries occurs in different layers of the tooth, in which the outer layer is highly infected with bacteria, resulting in an impossibility of remineralization. Nevertheless, the inner layer is less frequently contaminated with bacteria and although bacteria may dissolve the mineralized tissue, the cross-banded ultra-structure of the collagen matrix is preserved. Accordingly, the dentine of this carious layer can be preserved and remineralized if these bacteria are removed or killed.<sup>13,14</sup> Also, during the ART procedures, dental hand instruments alone do not remove carious dentine as effectively as rotary burs, which

does not ensure total microorganisms removal, and cariogenic bacteria can survive remaining under GICs restorations. The carious process may progress in the course of time and cause failure of the restoration. This problem may be solved by the use of dental materials that inhibit bacterial growth and thus termed Interim Therapeutic Restoration (ITR).<sup>15-18</sup>

In an attempt to obtain restorative materials that could prevent marginal gaps colonization, materials capable of releasing fluoride and providing antimicrobial activity have been developed, such as glass ionomer cements (GIC), the so-called "compomers" (PMRC) and fluoridated ceramic-polymer composites (RC).<sup>19</sup> There is no doubt that fluoride in drinking water and oral care products serve to protect the teeth and improve oral health at an individual and community level. Clinical data is not supportive of a cariostatic effect of GICs, and there is no clear evidence to support beneficial result of fluoride-releasing restorative materials.<sup>20</sup> In 1988, *Purton* and *Rodda* showed that the cement not only releases fluoride ions, but that it also can release calcium, phosphate, silica, strontium and other ions from glass/bioglass.<sup>21-23</sup>

The ability of GICs to release  $F^-$ ,  $Ca^{2+}$ ,  $PO_4^{4-}$ ,  $SiO_4^{4-}$  ions and their cariostatic activity may have led to the hypothesis that these restorative materials would potentially be used as potential carrier systems/delivery matrix for antimicrobial agents (AMb).

The oldest recorded medical use of an antimicrobial agent concerned copper mentioned in the Smith Papyrus; this Egyptian medical text, written 4600-4400 years ago describes the application of copper to sterilize chest wounds and drinking water. Ancient Egyptians and ancient Greeks also used specific molds and plant extracts to treat infection.<sup>24,25</sup> Looking back on the history of human diseases, infectious diseases have accounted for a very large proportion of diseases as a whole. Almost 150 years ago there was no knowledge that microorganisms were found to be responsible for a variety of infectious diseases that had been plaguing humanity from ancient days. Accordingly, chemotherapy aimed at the causative organisms was developed as the main therapeutic

strategy. The first synthetic antimicrobial agent in the world was salvarsan, a remedy for syphilis that was synthesized by Ehrlich 106 years ago. 81 years ago sulfonamides were developed by Domagk and other researchers. These drugs were synthetic compounds and had limitations in terms of safety and efficacy. 88 years ago Fleming discovered penicillin.<sup>26</sup>

Among many antimicrobials incorporated into dental restorative material, organic ones were introduced into restorative dental materials, well-known and tested e.g. bisbiquanides: chlorhexidine digluconate and diacetate (CHG, CHXA), alexedine (ALX), chitosane (CHT), monomers 12-methacryloyloxydodecylpyridinium bromide (MDPB), quaternary ammonium dimethacrylate (QADM), furanone chloride and bromide (GLC), polyquaternary ammonium salt (PQAS), cetrimide (CT), polyethyleneimine nanoparticles (nPEI), zinc oxide nanoparticle (nZO), cetylpyridinium chloride (CPC), glutaraldehyde (GA), benzalkonium chloride (BACH), sodium fusidate (SF), antibiotics (metronidazole, ciprofloxacin and cefaclor).<sup>27-37</sup>

There are only a few papers that deal with triclosan (TRC) as an addition into dental restoratives. Triclosan is a chlorinated aromatic compound (in use since 1972) [5-chloro-2-(2,4-dichlorophenoxy) phenol] with summary formula  $C_{12}H_7Cl_3O_2$  and molar mass of 289.54 g/mol<sup>-1</sup>, synthetic white powdered species, nonionic, broad spectrum antimicrobial and antifungal agent, anionic by nature. The proposed mechanism of action of triclosan suggests the material to be bactericide/also fungi RNA immobilizer, which does not leach from the carrier material, thereby favouring long-term anticariogenic activity. It is slightly soluble in water and very soluble in ethanol, methanol, diethyl ether and very well soluble in basic solutions, e.g. sodium hydroxide.<sup>38-41</sup> Triclosan is present in soaps (0.10-1.00%), deodorants, toothpastes 0.3%, mouthrinses 0.15% and cleaning materials. It is incorporated into an increasing number of consumer products, such as kitchen utensils, toys, bedding, socks, and rubbish bags.<sup>42</sup> At high concentrations triclosan acts as a biocide, with multiple cytoplasmic and membrane targets. At lower concentrations,

however, triclosan appears bacteriostatic and is seen to target bacteria mainly by inhibiting fatty acid synthesis. It demonstrates effective antimicrobial action when added to GICs.<sup>43</sup> TRC does not deteriorate shear bond strength (SBS) and the marginal seal of GICs in dye penetration test, as is further explained. It is important to notice this higher shear bond strength values for triclosan-incorporated glass ionomer cement group than for conventional glass ionomer cement group. Microleakage of 2.5% triclosan-incorporated GIC was compared with conventional type II GIC and the results revealed that microleakage was similar to that of conventional GIC.<sup>44,45</sup>

The idea is to enhance the antimicrobial properties of GICs with a balanced dose of additives incorporated into the compound, without causing biological, physical and chemical changes of a biomaterial. It is suggested that these restorative materials can be used as controlled-release devices, a topic of great interest in modern day pharmacy.<sup>46</sup> Taking it into consideration, the project was started with evaluating surface hardness (HV0.3/15) and diametral tensile strength (DTS) of a water-activated glass ionomer cement (WAGIC) enriched with increasing mass concentration of triclosan (TRC) antimicrobial.

## Material and methods

### Samples preparation

Triclosan (5-chlor-2-(2,4-dichlor-phenoxy)-phenol, Merck, Germany) was added to the powder of WAGIC (Chemfill Superior, Dentsply DeTrey, Germany) in weight concentrations of 0.5%, 1.0%, 1.5%, 2.0% serving as experimental groups, and 0.0% as a control group. Then, according to manufacturer's instructions, water was added to TRC and WAGIC mixture and mixed to material homogenization. The obtained paste was placed into silicon mould to prepare samples for DTS and HV0.3/15 test and then left 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, the Vickers test was used. In this test 136° diamond square-based pyramid was used as an indenter. Indenter was pressed into the material's surface using load of 300 g. After 15 s the load was removed and diagonal lengths of the indentation were measured. Vickers hardness was calculated from equation (1) as follows:

$$HV = \text{const.} \frac{F}{A} = 0,102 \frac{2F \sin \frac{136^\circ}{2}}{\left(\frac{d_1+d_2}{2}\right)^2} = 0,1819 \frac{F}{d^2} \quad (1)$$

where:

F – load force [N],

A – surface of the indentation [ $\mu\text{m}^2$ ],

$d_1, d_2$  – diagonal lengths [ $\mu\text{m}$ ].

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

### Diametral tensile strength

Diametral tensile strength (DTS) test was performed according to ANSI/ADA standard No. 66. after 1 or 24 hours of storing samples in distilled water. The DTS method is presented schematically in Fig. 1.

In this test, a compression load was placed by peripheral surface of a cylindrical sample (diameter  $d = 6$  mm, height  $h = 3$  mm). In a perpendicular direction to the applied force direction, the tensile forces occurred. In this test, the maximum force was measured by universal testing machine (Z020, Zwick/Roell, Germany) with crosshead speed 0.5 mm/min. The DTS value was calculated with the following equation (2):

$$DTS [MPa] = \frac{F}{S} \frac{[N]}{[mm^2]} = \frac{F}{\frac{1}{2}(2\pi \frac{d}{2} h)} \frac{[N]}{[mm \cdot mm]} = \frac{2F}{\pi d h} \frac{[N]}{[mm^2]} \quad (2)$$

where:

DTS – diametral tensile strength [MPa],

F – maximum force [N],

S – surface [ $\text{mm}^2$ ],

d – sample diameter [mm],

h – sample height [mm].

Results were subjected to statistical analysis.

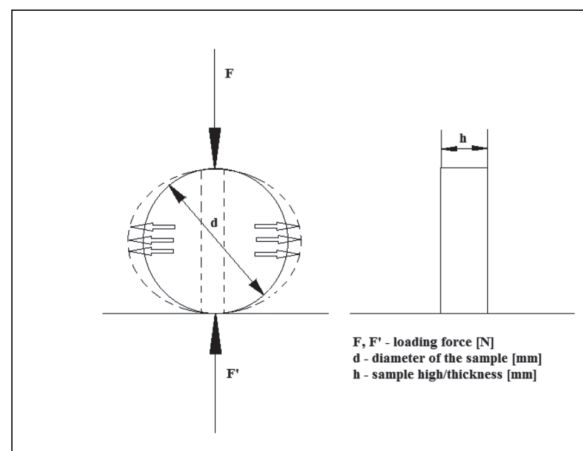


Fig. 1. DTS testing method scheme.

### Statistical analysis

For statistical calculations Excel 2010 (Microsoft, USA) and Statistica v. 12.5 (Statsoft, Poland) were used. Shapiro-Wilk Test of normality, parametric (F-test or t-test) or non-parametric (Kruskal-Wallis test or Mann-Whitney test) tests were used for the purpose of the statistical analysis ( $\alpha = 0.05$ ). Equality of variance was tested with Levene's test.

## Results

### Hardness

HV0.3/15 results for specimens with different concentration of TRC stored for 1 or 24 hours in distilled water in room temperature are shown in Fig. 2.

According to Kruskal-Wallis test there were statistically significant differences in HV0.3/15 measured after 1 hour between samples with 0.0% and 2.0% ( $p$ -value=0.046), 0.5% and 1.0% ( $p$ -value=0.005), 0.5% and 2.0% ( $p$ -value=0.000), 1.5% and 2.0% ( $p$ -value=0.015) concentration of triclosan. According to the F-test, there were statistically significant differences in HV0.3/15 measured after 24 hours between the following concentrations of TRC in samples: 0% and 0.5% ( $p$ -value=0.003), 0% and 1% ( $p$ -value=0.004), 0.5% and 1% ( $p$ -value=0.000), 0.5% and 1.5% ( $p$ -value=0.000), 0.5% and 2% ( $p$ -value=0.001), 1% and 2% ( $p$ -value=0.010). As shown in Fig. 2 the samples' hardness measured after 24 hours of storage in wet condition was statistically higher than in samples tested after 1 hour.

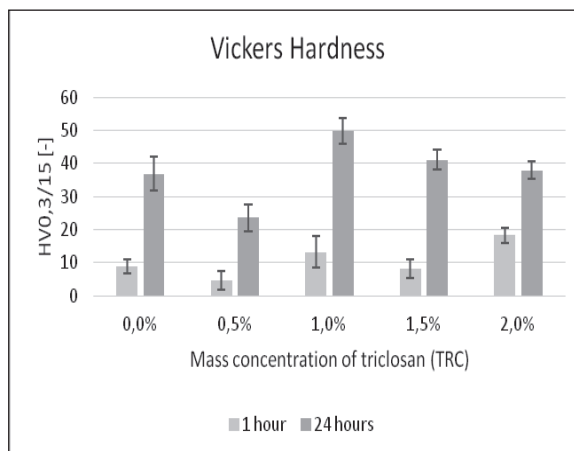


Fig. 2. Vickers hardness results for WAGIC modified with TRC.

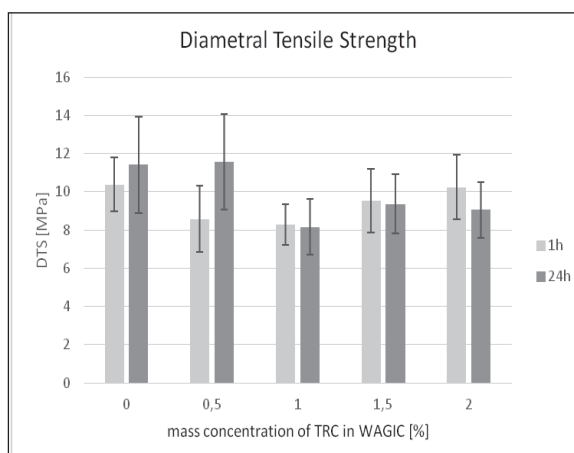


Fig. 3. DTS results for WAGIC modified with TRC.

### Diametral Tensile Strength

Diametral tensile strength results for samples with mass concentration of TRC from 0.0% to 2.0% measured after 1 or 24 hours of storage in distilled water are shown in Fig. 3.

The DTS values measured after storing samples for 1 hour in distilled water was statistically lower for 1.0% addition of TRC compared to samples with 0.0% ( $p$ -value=0.019) and 2.0% ( $p$ -value=0.033). DTS values measured after 24 hours was slightly higher for 0.0% ( $p$ -value=0.009) and 0.5% ( $p$ -value=0.006) in comparison with DTS samples with 1.0% TRC addition. There were no statistically significant differences between DTS



values measured after 1 and 24 hours, except 0.5% TRC addition to WAGIC. In these samples, after 24 hours of storage in water DTS was higher than after 1 hour (p-value=0.012).

## Discussion

The perfect restorative filling material would be the one that can degrade in the mouth and be replaced by natural tooth enamel and dentine. Such an ideal material does not yet exist.<sup>8</sup> This concept is probably the nearest to GICs releasing different species. Also, it can be expected that glass-ionomer-like formulations will play the prominent role in the concept of biomimesis, the exploration of biomaterials that repair or reproduce the original tissues best<sup>47</sup> over maturation times greater than 24 hours.<sup>48</sup> Glass-ionomer cements are considered to be anti-cariogenic. One of the key features that promotes this property is their ability to release fluoride, but it is also aided by their ability to buffer organic acids, such as lactic acid.<sup>49-51</sup>

In the past decade, antimicrobial agents have been incorporated into dental materials to provide them with antimicrobial activity. Many AMb have been used, but they affected the various physical properties of the material at different concentrations.<sup>52,53</sup> *Sainulabdeen et al.*<sup>43</sup> studied the antibacterial effect of triclosan-incorporated glass ionomer cement and concluded that 2.5% concentration of triclosan-incorporated GIC produces optimum antibacterial effect in comparison with 2.5% chlorhexidine.

The incorporation of AMb (e.g. chlorhexidine acetate and gluconate) to glass ionomer cement may result in a dramatic decrease in the physico-mechanical properties of the cement; when added in concentrations above 5.0%, the material tends to deteriorate rapidly, does not contribute to the formation of glass ionomer network, weakens the scaffold, and compromises the mechanical properties of glass ionomer cement.<sup>54,55</sup> Bearing this in mind, it was decided to test lower mass concentration of TRC.

Cationic species like QAS-containing materials did exhibit significant antibacterial activities. However, it has been reported<sup>56</sup> that human saliva

can significantly decrease the antibacterial activity of the QAS-containing restoratives, probably due to electrostatic interactions between QAS and proteins in saliva, as cationic species. Choosing anionic TRC, anionic chemical, would be of interest for long-term action in slow-controlled release.

In the present study, samples hardness was confirmed to be significantly higher after 24 hour than after 1h of storage in distilled water, a well-known GICs property, also obtained for GICs with TRC addition by *Pawluk* in PhD thesis.<sup>57</sup> Addition of 0.5% of TRC caused significant decrease of hardness. DTS values were significantly higher after 24 h with addition of 0.5%, but generally it was slightly decreased in comparison with the control group, that corresponds with *Tüzüner et al.*; antimicrobial agents like CPC, CT, BACH when incorporated into glass ionomer cement affected the clinical performance of the material as they reduced the compressive strength, surface hardness, bond strength but slightly increased the setting time.<sup>58</sup> Opposite DTS data were obtained by the team of *Jaidka et al.* for 0.5% TRC-enriched GIC.<sup>59</sup> A possible reason for those discrepancies might be assigned to the different dimensions of the sample.

The prevalence of caries and the increase in 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 antimicrobial agents from natural and inorganic substances to develop the next generation of drugs or agents to control microbial infections. There are several future aspects of using AMb-enriched GICs in clinic, when planning investigations, where indications/contraindications, dose, and oral/general safety/hazard may play an important role.

As Noort mentioned, there is a subtle distinction between safety and biocompatibility – two important features of dental materials. Safety is concerned with the fact that materials when in contact with the human body should not cause any adverse effect, whereas biocompatibility is the quality of being non-destructive in the biological environment maintaining the beneficial effect to

the patient. So far, few materials can be regarded as completely safe and fully biocompatible in the oral environment. Most dental materials interact with the oral environment and this interaction might involve a release of components with undesirable side effects for oral tissues.<sup>60</sup> From that perspective, it can be stated that two main approaches can be presented when antimicrobial bioactive materials are prepared. One approach is to prepare a substance-release material. Another perspective is to incorporate the antimicrobial to be active being part of its structure without any release of the active component.<sup>61</sup> The proposed mechanism of action of TRC suggests the material to be an immobilized bactericide which does not leach out of the carrier material, thereby favouring long-term anticariogenic activity.<sup>62</sup>

## Conclusion

Except addition of 0.5% TRC into WAGIC, there was no negative influence of antimicrobial addition on HV0.3/15, and DTS values were slightly decreased, so TRC does not deteriorate mechanical properties of WAGIC.

The improvement of anticariogenic properties of a biomimetic direct dental restorative is of great importance, as it would lead to the reduction of probability of reinfection of restored tooth hard tissues, and would prevent the formation of secondary caries.

It is also recommended that further aspects of TRC-incorporated glass ionomer cement should also be researched before recommending it as an effective antimicrobial alternative to conventional glass ionomer cement.

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