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Release of chlorhexidine digluconate and flexural properties of glass fibre reinforced provisional fixed partial denture polymer

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The objective of this study was to determine the flexural properties and the release of chlorhexidine digluconate (CHX) of CHX laced unidirectional E-glass fibre reinforced provisional fixed partial denture polymer. Bar shaped test specimens ($3.3 \times 10.0 \times 65.0$ mm) were fabricated from provisional fixed partial denture polymer (mixture of poly[ethylmethacrylate] powder and *n*-poly[butyl methacrylate] monomer liquid) with E-glass fibre reinforcements. Poly(methyl methacrylate) preimpregnated continuous unidirectional glass fibre reinforcement was laced with CHX. The glass fibre reinforcements were incorporated into the polymer and the polymerised to the form of test specimens. In addition test specimens without CHX in glass fibre reinforcement were made for comparison. Control specimens did not contain glass fibres in the test specimens. Flexural strength and modulus of test specimens ($n = 6$) was tested with three-point bending test after storing the specimens dry or in water (two weeks). Released CHX was determined with high performance liquid chromatography during 180 days water immersion. In dry conditions, the flexural strength and the modulus of the polymer was 43 MPa and 1.7 GPa, and with glass fibre reinforcement 96 MPa and 3.5 GPa. With the reinforcement laced with CHX, the strength was 92 MPa and the modulus was 3.2 GPa. The water storage of test specimens did not weaken the reinforced polymer. The majority of the CHX released from the glass fibre reinforced polymer during the first days of storage in water. Flexural properties of provisional fixed partial denture polymer were increased using glass fibre reinforcement. The fibre reinforcement that was laced with CHX resulted in similar reinforcing effect.

1. Introduction

The physical properties of polymers have been improved for decades by using fibres [1–5]. In dental technology, this has been utilized, until recently, to reinforce polymers for example glass and carbon/graphite or aramid fibres [5, 6–10]. The use of fibre-reinforced composites (FRC) is rapidly growing in dentistry and it offers number of new treatment alternatives from single and multiple tooth replacement to removable dentures and tooth fillings [11–15].

The use of glass fibre-reinforcement that had been preimpreganted with polymer in order to obtain well impregnated FRC was tested in provisional fixed partial dentures (FPD). It was shown that the glass fibre reinforcement considerably increased the fracture resistance of the interim FPD [16, 17]. This is of great importance since the provisional FPD polymers of poly(methyl methacrylate) (PMMA), poly(ethyl methacrylate) (PEMA), or poly(butyl methacrylate) (PBMA) do not offer high enough flexural strength for multiple unit tooth replacements [17, 18].

Chlorhexidine digluconate (CHX) has been widely used in clinical dentistry. It is an effective disinfectant in direct exposure and in addition has an ability to adhere to various substrates resulting in a long-term wide-spectrum antimicrobial efficacy [19, 20]. CHX confounds also microbial adhesion to polymer surfaces. Earlier *in vitro* studies have shown that number of adherent of yeast cell decreases when the denture base polymer has incubated with CHX [21, 22]. It has previ-

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ously been shown that pretreating the porous polymer preimpregnated glass fibre reinforcement with CHX results in reduction in the number of adherent yeast cells on the surface FRC material [22]. However, to our knowledge there is no information available concerning the influence of CHX to mechanical properties of fiber-reinforced composites. Furthermore, release rate of CHX from such material is not known.

The aim of this study was to determine flexural properties of provisional FPD polymer that had been reinforced with glass fibre reinforcement laced with CHX. In addition, release of CHX from the fibre reinforced test specimen into water was determined during 180 days water storage.

2. Materials and methods

2.1. Fabrication of test specimens

Materials used in this study are listed in the Table I. Five groups ($n = 6$) of test specimens were made of autopolymerizing provisional FPD polymer of PEMA powder and PBMA monomer liquid and glass fibre reinforcements containing E-glass fibres and porous PMMA preimpregnation polymer [23]. Additionally, the fibre reinforcement was immersed with CHX—water solution for minute [22]. The fibre reinforcements were then dehydrated in desiccators for one week before using them in the test specimen's fabrication. The test specimens in group 1 (control) were prepared using plain polymer, the test specimens in group 2 were prepared using the polymer and the glass fibre reinforcement, and the test specimens in groups 3, 4 and 5 were prepared using the polymer and fibre reinforcement laced with CHX (Fig. 1).

The bar shaped test specimens ($3.3 \times 10.0 \times 65.0$ mm) were fabricated according to ISO 1567. Before incorporation of the fibre reinforcements into the polymer, the reinforcements were further impregnated with slurry viscosity mixture of PEMA powder and PBMA monomer liquid for 10 min. During the further-impregnation period, the monomers of the BMA penetrated into the preimpregnation polymer of PMMA and dissolved it partly [16, 17]. After the further-impregnation, two 65 mm length fibre reinforcements were placed into the mould and the mixture of PEMA/PBMA was poured on the reinforcements. The power-to-liquid ratio of the resin mixture was 2 g to 1.2 ml. The resin was polymerized in water at $(55 \pm 1)\,^{\circ}$C for 15 min under air pressure of 300 kpa (Ivomat Type IP2, Ivoclar A.G. Schaan-Liechtenstein). The polymerized test specimens were finished to predetermined dimensions by wet grinding with silicone carbide grinding paper (No. 1200 Fepa, Struers A/S, Rodovre, Denmark).

Fibre content as a percentage by volume (%), in the test specimens with fibre reinforcement, was calculated based on the weight of the fibres, density of E-glass (2.54 g/cm^3) and the volume of the specimens. Fibre volume fraction was 3%.

TABLE II Classification of the test groups

Group	Specimens type and fibre content by volume (vol%)	Type of test
Group 1	PEMA/PBMA polymer	Flexural test for dry specimens
Group 2	PEMA/PBMA polymer + glass fibre reinforcement (3 vol%)	Flexural test for dry specimens
Group 3	PEMA/PBMA polymer + glass fibre reinforcement laced with chlorhexidine digluconate (3 vol%)	Flexural test for dry specimens
Group 4	PEMA/PBMA polymer + glass fibre reinforcement laced with chlorhexidine digluconate (3 vol%)	Flexural test for specimens stored 2 weeks in water
Group 5	PEMA/PBMA polymer + glass fibre reinforcement laced with chlorhexidine digluconate (3 vol%)	Release of chlorhexidine digluconate

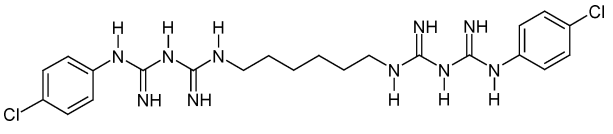

Figure 1 The chemical structure of chlorhexidine (i.e. 1,1′-hexamethylenebis[5-(4-chlorophenyl)biguanide]) (CHX).

2.2. Flexural strength and modulus test

The flexural strength and flexural modulus of the test specimens in the groups 1, 2 and 3 were tested after dry storage whereas the test specimens in the group 4 were stored in water at 37 °C for two weeks before testing. The three-point bending test was made with Lloyd LRX (Lloyd LRX, Lloyd Instruments Ltd., Fareham, UK) universal testing machine (Fig. 2). The specimens were loaded at a crosshead speed of 5.0 mm/min and the force-displacement curve was registered with Nexygen 4.0 software (Lloyd Instruments Ltd, Fareham, UK).

TABLE I Materials used in this study

Brand	Manufacturer	Lot number	Type and code
Chlorihexidin diglugonat sol 20%	University Pharmacy, Helsinki, Finland	YA9901028	Chlorhexidin diglugonat (CHX)[a]
Stick	Stick Tech Ltd, Turku, Finland	1010321-R-0058	PMMA[b] preimpregnated E-glass fiber reinforced composite (FRC)
Dentalon Plus Powder (P) & liquid (L)	Heraeus Kulzer GmbH, Wehrheim, Germany	P: 040270, 150270. L: 010193, 060199	Provisional fixed partial denture polymer (PEMA[c] and PBMA[d])

[a]chlorhexidine (i.e. 1,1′-Hexamethylenebis[5-(4-chlorophenyl)biguanide].
[b]poly(methyl methacrylate).
[c]poly(ethyl methacrylate).
[d]poly(butyl methacrylate).

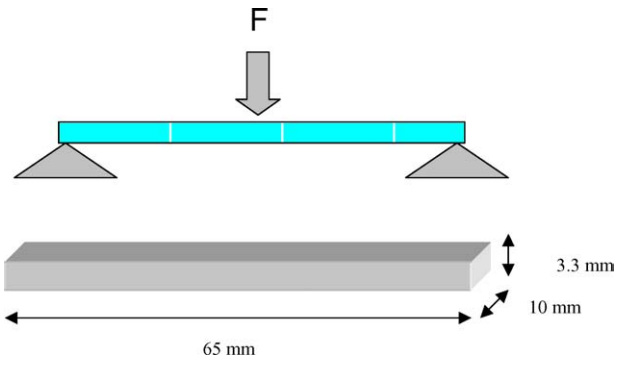

Figure 2 Schematic diagram of the three-point bending test with a bar shaped test specimen (F = force).

2.3. Analysis of released CHX

In group 5, the test specimens were stored in the contact with 40 ml of de-ionized Milli-Q water at 37 °C (in closed Falcon flasks) for the following time points: 0, 1, 3, 7, 10, 14, 21, and 180 days. The released CHX was measured using high performance liquid chromatography (HPLC). More precisely, all the aliquots of each specimen were separately collected and the supernatants were analyzed.

Shimadzu's (LC-2010) modular HPLC system (Shimadzu Corporation, Kyoto, Japan) was used by using the following components (connected to the computer): a system controller (SCL-10Avp), a liquid chromatograph pump (LC-10Advp), a UV-VIS detector (SPD-10Avp), an on-line degasser (DGU-14A), and an auto injector (SIL-10Advp). The incorporated columns used in the system were Phenomenex's C18 precolumn (Phenomenex, Torrance, CA, USA) and Phenomenex's C18 analysis column (type: RP18, length: 150 mm, internal Ø: 2 mm, and particle size: 5 mm). Finally, the collected data was processed using Shimadzu's CLASS VP software.

The used flow rate was 0.6 ml/min, the run time was 25 min, and the used wavelength (λ) of UV light was 254 nm. A filtered mobile phase was used, and it contained acetonitrile (HPLC grade, Rathburn Chemicals Ltd., Walkerburn, Scotland, UK) and 7 mmol of sodium laurylsulphate (SDS, SERVA Electrophoresis GmbH, Heidelberg, Germany) in Milli-Q water containing glacial acetic acid (0.4 vol%, Merck KGaA, Darmstadt, Germany). The analysis was carried out using a gradient run, where the concentration of acetonitrile was changed from 10 to 90 vol%, while, at the same time, the concentration of SDS solution was changed from 90 to 10 vol% within the run time.

The CHX standards were prepared in the following manner: The CHX—water solution was first evaporated dry using a rotary evaporator (Heidolph, Laborota 4000, Heidolph Instruments GmbH & Co.KG, Schwabach, Germany), then, 250 mg of CHX was weighted and diluted in de-ionized Milli-Q water to give standards with CHX concentrations of 10, 50, and 100 ppm in de-ionized Milli-Q water. A filtered (0.45 μm) standards and samples of each supernatant (100 μl) was injected into the chromatograph and six parallel determinations were done *per* time point. The quantities of released CHX were calculated from the areas under the curve at peaks produced by CHX, in which the retention time was 17 min. A typical chromatogram is given in Fig. 3. From the sample supernatant, the concentration of released CHX ($\mathbf{c}_{\mathrm{CHX}}(\mu\mathrm{g/ml})$) was determinated using linear regression equations obtained from calibration graph ($R^2 > 0.99$). The quantities of released CHX were calculated in ppm.

2.4. Statistical analysis

For the specimens used in flexural tests, the statistical analysis of mean flexural strengths was performed using SPSS (Statistical Package for Social Science, SPSS inc., Chicago, USA) software for Windows with univariate ANOVA, followed by Scheffe's and Dennett's T3 post hoc analysis. The fixed factors were the type of test specimens (unreinforced polymer, glass fiber reinforced polymer, glass fiber reinforced polymer with CHX) and the storing conditions (dry or two weeks in water). The dependent variables were the flexural strength and modulus of the different groups.

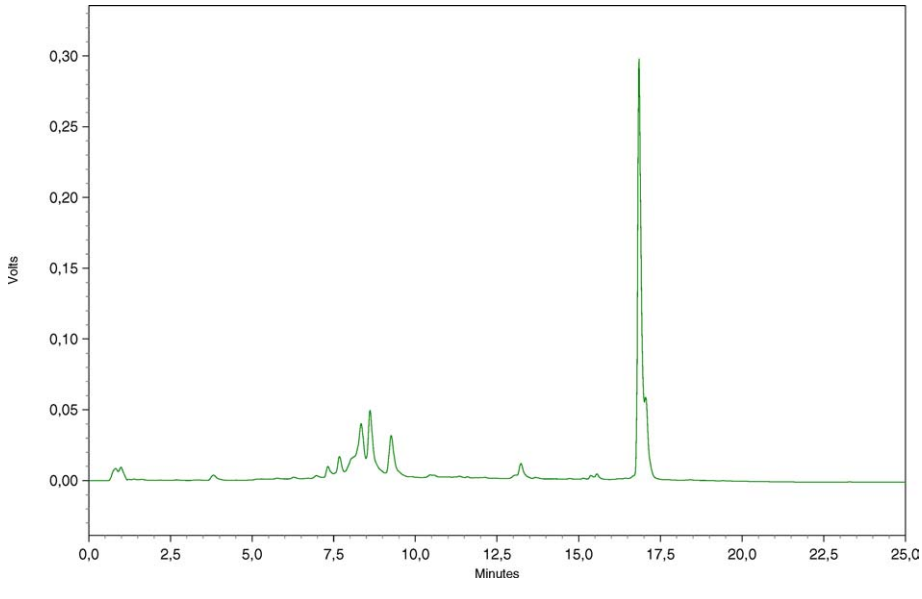

Figure 3 A typical HPLC chromatogram for CHX.

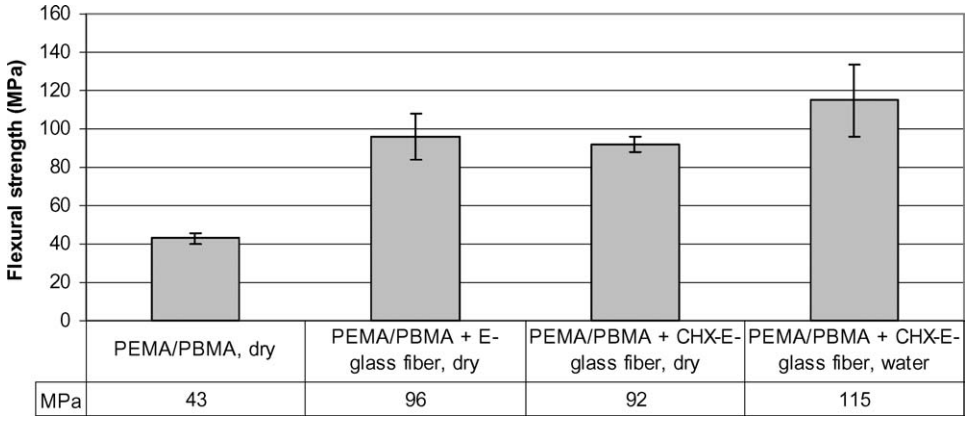

Figure 4 The flexural strength in different groups ($n = 6$).

3. Results

The flexural strength of test specimens is shown in the Fig. 4. The glass fiber reinforcement considerably increased the flexural strength and modulus. The highest flexural strength was in the group 4 (115 ± 19 MPa) (polymer reinforced with CHX laced fibres and stored for 2 weeks in water), and the lowest in the group 1 (unreinforced polymer) (43 ± 3 MPa). The flexural modulus of test specimens are shown in the Fig. 5. The flexural modulus was the highest in the group 4 (3.9 ± 0.6 GPa) and the lowest in the group 1 (1.7 ± 0.2 GPa). ANOVA showed significant differences between reinforced and unreinforced test specimens with regard to flexural strength and flexural modulus ($p < 0.001$).

The CHX was leached out from the test specimens within the first three weeks (from 13 ppm to 36 ppm), as shown in Fig. 6. After 21 days incubation in water, the extracted CHX concentration was ca. 90% from the total CHX concentration after 180 days' incubation.

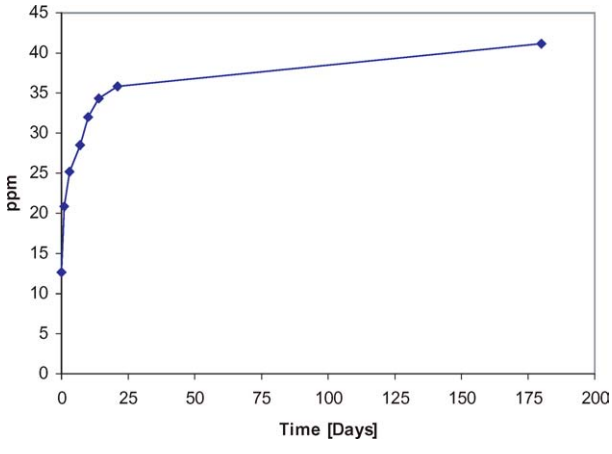

Figure 6 The cumulative quantity of CHX released into water from the test specimens of group 5.

4. Discussion

This *in vitro* study demonstrated the effect of E-glass fiber-reinforcement on the fracture resistance and release of CHX from provisional FPD polymer. It was found like in the previous studies [16, 17] that the fracture strength of the FPD polymer increased considerably by adding glass fiber reinforcement with continuous unidirectional fibres. It has been also reported previously that good impregnation of glass fibers with the polymer is essential requirement for the potential for clinical use [23]. High degree of impregnation of glass fibres by highly viscous denture base or provisional FPD polymer can be obtained by preimpregnating the fibres with polymer. It is desired that the polymer in the reinforcement prior the use in highly porous form. The porosities have been shown to allow monomer liquid systems to penetrate into the reinforcement and form multiphase polymer matrix for the fibre-reinforced composite once the resin has been polymerized [23]. The new approach was to reinforce

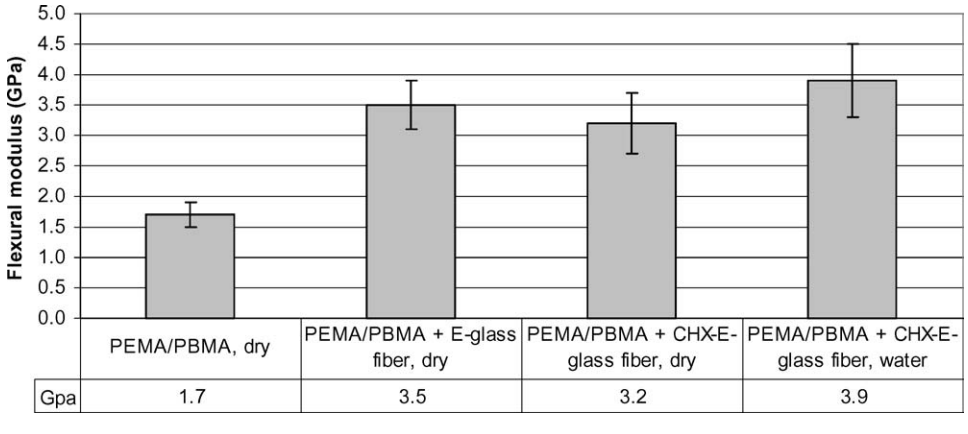

Figure 5 The flexural modulus in different groups ($n = 6$).

the provisional FPD polymer with CHX-laced E-glass fiber reinforcement. The porosities of the preimpregnated fibre reinforcement were reservoirs for the CHX.

The results showed no reduction in the flexural properties of the test specimens when the CHX-laced fibre reinforcements were compared to those of conventional fibre reinforcements. It was hypothesized, that leaching of CHX from the fibre reinforced test specimens could weaken the material. This hypothesis was rejected and the CHX-laced fibre reinforcements can possible also be used in removable denture base polymers such as those suggested by Narva *et al.* [24]. Reason for the unchanged, or even slightly increased flexural properties (flexural strength and modulus) suggests that leaching of CHX from the test specimens was subsequently compensated by water sorption and the net effect on the flexural strength was therefore minor.

The present study demonstrated the expected release of CHX from the fibre-reinforced test specimens. The majority of CHX was released within three weeks of water immersion and thereafter only minor amounts of CHX release could be measured. The release rate shows similar diffusion kinetics as found for instance with residual methyl methacrylate monomers of denture base polymers [25]. The diffusion based release suggests that the therapeutical use of CHX-laced fibre-reinforcement may need to be limited to temporary use only. On the other hand, as was shown, the CHX did not result in any weakening of the material even in longer term and therefore the fibre-reinforced device could technically be used even for a longer period of time.

The antimicrobial agent modified fibre-reinforced materials may also have other clinical applications in dentistry than provisional FPDs. For example, the material could be used in temporary periodontal splinting during the healing phase periodontal surgical operations, or as reservoir for antimicrobial agents in endodontic therapy. However, further investigations are necessary to determine more exactly the antimicrobial activity of CHX and possible development of microbial resistance during long-term use.

5. Conclusions

Within the limitations of the study, it can be concluded that CHX-laced glass fiber-reinforcement can be used with provisional FPD polymer and the release of the CHX occurs within three weeks of water immersion.

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