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Residual monomers released from glass-fibre-reinforced composite photopolymerised in contact with bone and blood

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Purpose: The aim of this study was to determine the quantity of residual monomers of glass fibre-reinforced composite released into water from the composite that had been photopolymerized in contact with bone and blood. *Materials and methods:* E-glass fibre reinforced composite (FRC) made of E-glass fibre veil and the bis-GMA-TEGDMA-PMMA resin system was used in the study. In the first group, pieces of non-polymerised FRC were photopolymerised (40 s) in air which influenced the oxygen inhibited resin layer (positive control). In the second group, the FRC was polymerized between two glass plates allowing both surfaces to be well polymerized (negative control). In the test groups, the FRC was polymerized in contact with bone or in contact with blood. FRC specimens from all four groups were incubated in three milliliters of deionised water at 37 °C for three days. At the end of the incubation period, the residual monomers were extracted from the water with dichloromethane, and the residual monomers of TEGDMA and bis-GMA quantitatively analysed by HPLC. The degree of monomer conversion was measured by FTIR from the surface of the test specimen. Differences between the groups were analysed using one-way ANOVA ($p < 0.05$). *Results:* The total quantity of residual monomers released from FRC polymerized in contact with bone was lower (0.55 wt%) than in the positive control group (0.97 wt%) ($p = 0.021$), and only slightly exceeded that of the negative control group (0.42 wt%) ($p = 0.717$). The total quantity of monomers released from FRC polymerized in contact with blood was at the level of the negative control group. The main residual monomer released was TEGDMA. The surfaces of the positive and negative controls showed a clear difference between the degree of monomer conversion, 34.0 and 62.8%, respectively, when analysed with FTIR ($p < 0.001$). *Conclusion:* The surface of the bone or contact with blood did not significantly inhibit the photoinitiated free radical polymerisation of the dimethacrylate monomer system of the FRC.

1. Introduction

In the development of new polymeric composite materials for biomedical use it is important to know the extent of residual monomer release from the surface of the polymerised composite, because of the possible harmful effects of the residual monomers on tissues [1–10]. It is well known that certain factors such as the presence of oxygen inhibits the free radical polymerisation of monomers of the resin. The inhibited surface layer contains unreacted monomers and oligomers [11, 12]. On the other hand, polymerisation reaction by light activation or autopolymerisation in a clinical situation does not result in complete conversion of all carbon-carbon double bonds. The monomers that had not reacted (i.e. residual monomers) can leach out from the polymer. The existence of residual monomers of acrylic polymers is well known in orthopaedics: poly(methylmethacrylate) (PMMA) based bone cements are known to contain and release unpolymerized methyl methacrylate (MMA) [13–16]. Polymeric and composite biomaterials, such as fibre-reinforced composites (FRC), have been developed [17–30]. The monomeric components of currently used FRCs are based on bisphenol-a-glycidyl-dimethacrylate (bis-GMA) and triethyleneglycol dimethacrylate (TEGDMA) (Figs. 1(a) and (b)). Addition of reinforcing fibres to such monomer systems can be used to improve the mechanical strength of the material.

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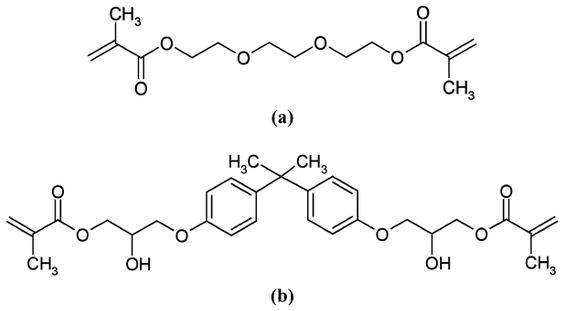

Figure 1 (a) The chemical structure of (a) TEGDMA (b) BisGMA.

The mechanical properties of FRC can be tailored to match the properties of bone in terms of strength and modulus of elasticity [20]. The resin matrix of the glass FRC has been made of *semi*-interpenetrating polymer networks (*semi*-IPN) of dimethacrylates and polymethylmethacrylate, which resulted in a slightly plasticised cross-linked polymer matrix for FRC [21]. Carbon/graphite FRC has also been tested as endosseus implant material [22, 24, 25]. Adequate polymerisation of the resin matrix of FRC is needed in terms of biocompatibility and biomechanical properties. The question has arisen of whether photopolymerisation of the dimethacrylate system in contact with bone or blood can inhibit free radical polymerisation and result in increased quantities of residual monomers. The hypothesis to be tested was whether the contact with bone or blood has an impairing effect on the photopolymerisation of the dimethacrylate-based FRC.

2. Materials and methods

A silanized E-glass fibre (diameter 11 μm; elemental composition presented in Table I) reinforced veil that had been preimpregnated with a photopolymerisable resin system of bis-GMA and PMMA (experimental material, Stick Tech Ltd., Turku, Finland) was used as the test material in this study. The thickness of the veil was 1.5 mm, and it was supported by a laminate of woven fibre with a thickness of 0.06 mm on one side (Figs. 2(a)–(c)). Micrographs of the veil were taken with a scanning electron microscope (SEM), (JSM-5500, Jeol Ltd., Tokyo, Japan). The resin matrix contained 1 wt% of camphorquinone and DMAEMA (N,N-dimethyl aminoethyl methacrylate) as the photo-initiator. The slightly porous fibre veil was further-impregnated by light-polymerizable bis-GMA-TEGDMA resin prior the use (Stick Resin, lot 304683, Stick Tech Ltd., Turku, Finland) to make the veil adapt better to the surface of the test substrates. The further-impregnated veil was referred to the "veil prepreg." The fibre veil was cut into 10 × 10 mm^2 pieces. Further-impregnation resin was dropped onto the veil pieces, spread with forceps, and left untouched for about 2 min, after which the extra resin was absorbed into blotting paper. The amount of further-impregnation resin left in the test specimen was 5% of the final weight. The veil prepreg was then photopolymerised for 40 s with a lightcuring unit (Optilux 501, SDS, Kerr/Demetron, Danbury, CT, USA). Irradiation intensity was 800 mW/cm^2, and wavelength 400–500 nm. The photopolymerisation was done when the veil was in contact with the various substrates according to the following groups. There was three veil prepregs in each group.

TABLE I The elemental composition of E-glass fibres (wt%) according to manufacturer's information

Oxide	E-glass
SiO_2	54,5
CaO	22,9
Al_2O_3	14,2
Na_2O	0,1
MgO	0,7
K_2O	0,7
B_2O_3	6,3

Negative control: The veil prepreg was photopolymerised for 40 s between objective glasses. Neither of the sides of the veil was exposed to atmospheric oxygen and no oxygen-inhibited surface layer was formed.

Positive control: The veil prepreg was held with forceps in air and photopolymerised for 40 s. Both sides of the veil were exposed to atmospheric oxygen, which caused an oxygen-inhibited resin layer on both sides of the prepreg.

Bone contact group: The veil prepreg was gently pressed onto the compact bone surface of frozen (−20 °C) and thawed (RT) pig maxillofacial bone cubes with some moisture on the compact bone surface. The veil side of the prepreg was in contact with the bone. There was a Mylar® film and an objective glass on one side of the prepreg through which the sample was photopolymerised for 40 s. There was no exposure to atmospheric oxygen on either side of the veil prepreg in this group.

Blood contact group: A drop of whole human blood (Finnish Red Cross, licence 2.4.2003, Turku, Finland) was placed on an objective glass and the prepreg with the veil side was placed on this glass and gently pressed into contact with the blood. The other side was then covered with a Mylar® film and an objective glass and photopolymerised for 40 s. No atmospheric oxygen access was allowed so as to inhibit the polymerisation reaction on neither side of the veil prepreg.

Three replicates from each group were then incubated in 3 ml of de-ionised water (Milli-RO Plus 30 deionised water, 18 MΩ cm, Millipore, Helsinki, Finland) at 37 °C for three days.

After the incubation period (3 days), the residual monomers were extracted from the water using dichloromethane (Riedel-de Haen, analytical reagent, Lot 02790, Seelze, Germany), (3 × 25 ml), that was evaporated and the residual monomers were dissolved in 3 ml of HPLC-grade tetrahydrofuran (Rathburn Chemicals Ltd., Walkerburn, Scotland, UK). Before the analysis of the released monomer contents, the HPLC samples were filtered using a 0.45 mm GHP membrane syringe filter (Pall Gelman Laboratory, Ann Arbor, MI, USA).

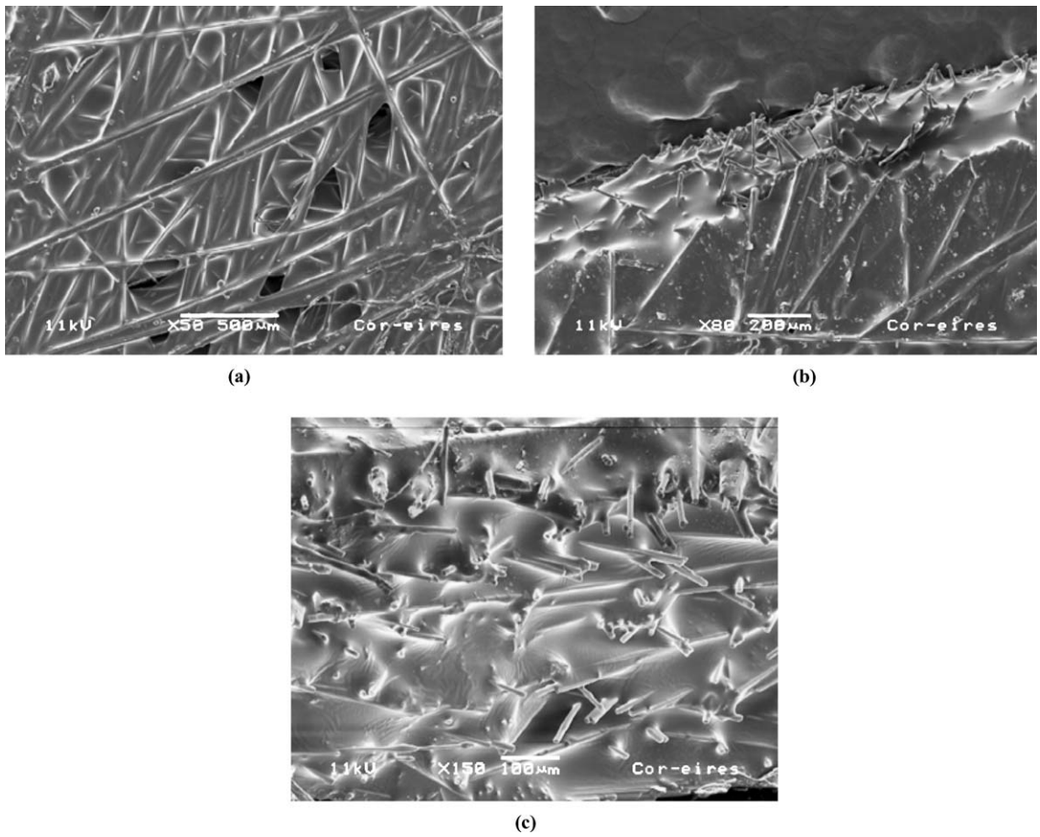

Figure 2 SEM photomicrographs of (a) a side of the veil prepreg showing the random orientation of the fibres (50 × magnification), (b) a cross-section of the veil prepreg (80 × magnification), and (c) a cut surface of the veil prepreg (150 × magnification). (Bar = 500, 200 and 100 μm).

2.1. Analysis of released monomer content

Shimadzu's (LC-2010) modular high performance liquid chromatograph (HPLC) system (Shimadzu Corporation, Kyoto, Japan) was used using the following components (connected to a computer): a system controller (SCL-10Avp), a liquid chromatograph pump (LC-10Advp), a UV-VIS detector (SPD-10Avp), an online degasser (DGU-14A), and an auto injector (SIL-10Advp). In the system, the incorporated columns used 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 μm). Finally, the collected data were processed using Shimadzu's CLASS VP software.

A sample of each extraction solution (5 μl) was injected into the chromatograph and three parallel determinations were done *per* test group. The used mobile phase contained methanol, HPLC grade (Rathburn Chemicals Ltd., Walkerburn, Scotland, UK) and Milli-Q water. The analysis was carried out using a gradient run, where the concentration of methanol was changed from 40 to 90 vol%, while, at the same time, the concentration of Milli-Q water (18 MΩ cm) was changed from 60 to 10 vol% within the run time. The used flow rate was 0.3 ml/min, the run time was 25 min, and the used wavelength (λ) of UV-light was 227 nm.

The amounts of residual monomers of bis-GMA and TEGDMA were calculated from the areas under the curve at peaks produced by the monomers. In the sample solutions, the concentration of residual monomers ($\mathbf{c}_{\text{monomer}}$ (mg/ml), $\mathbf{c}_{\text{bis-GMA}}$ or $\mathbf{c}_{\text{TEGDMA}}$) was determinated using linear regression equations obtained from calibration graphs (Figs. 3(a) and (b)).

The following equation was used to calculate the total amount of bis-GMA or TEGDMA monomers in the sample solutions, $\mathbf{m}_{\text{monomer}}$ (mg) = ($\mathbf{m}_{\text{bis-GMA}}$ or $\mathbf{m}_{\text{TEGDMA}}$).

$$\mathbf{m}_{\text{monomer}}\ (\text{mg}) = [\mathbf{c}_{\text{monomer}}\ (\text{mg/ml}) \times 3\ \text{ml}] \quad (1.1)$$

This value was used to calculate the weight percentage of the residual monomer (bis-GMA or TEGDMA) using the following equation:

$$\text{Residual monomer (wt\%)} = \mathbf{m}_{\text{monomer}}\ (\text{mg}) \times 100/\text{mass of specimen (mg)} \quad (1.2)$$

The degree of monomer conversion (DC%) of the resin matrix of the veil prepreg of negative and positive controls was measured by Fourier transform infrared spectroscopy (FTIR) (Spectrum One, Perkin Elmer, Beaconsfield Bucks, UK) using the DRIFT-sampling accessory (diffuse reflectance infrared Fourier transform). The DC% was calculated from aliphatic C=C of the reactive methacrylate group peak at 1638 cm^{-1}, normalized against the aromatic C=C peak at 1608 cm^{-1}

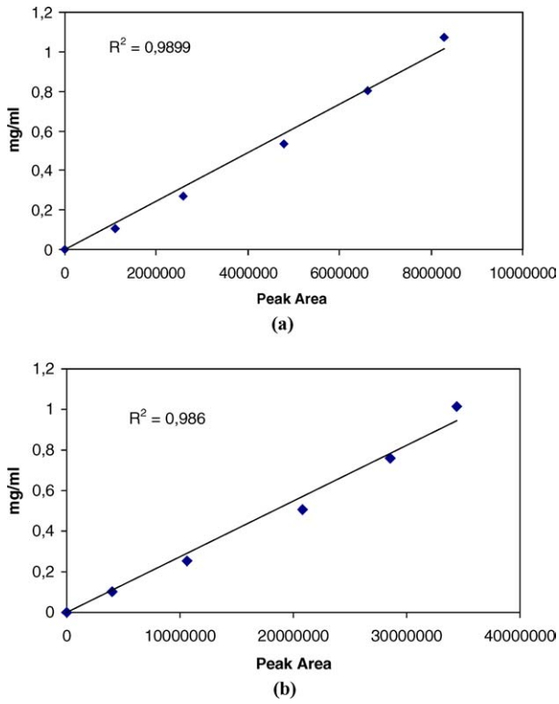

Figure 3 A calibration graph of the HPLC analysis for, (a) TEGDMA and (b) BisGMA.

according to the following equation:

$$\mathrm{DC}\% = \left[1 - \frac{C_{\text{aliphatic}}/C_{\text{aromatic}}}{U_{\text{aliphatic}}/U_{\text{aromatic}}}\right] \times 100\%$$

where $C_{\text{aliphatic}}$ = absorption peak at 1638 cm^{-1} of the cured specimen, C_{aromatic} = absorption peak at 1608 cm^{-1} of the cured specimen, $U_{\text{aliphatic}}$ = absorption peak at 1638 cm^{-1} of the uncured specimen, and U_{aromatic} = absorption peak at 1608 cm^{-1} cm of the uncured specimen.

Each spectrum was recorded with 16 scans using a resolution of 4 cm^{-1}. In addition, three parallel spectra were recorded per test group.

3. Statistical analysis

Statistical analysis was performed using SPSS Systems for Windows. One-way analysis of variance (ANOVA) was used to compare test groups using Tukey's Post Hoc-test. P-values less than 0.05 were considered to be statistically significant in all tests.

4. Results

A typical HPLC chromatogram of immersion water is given in Fig. 4. The total quantity of residual monomers released in the bone contact group (0.55 wt%) was clearly lower than in the positive control group (0.97 wt%) ($p = 0.021$), and only slightly exceeded that of the negative control group (0.42 wt%) ($p = 0.717$) (Fig. 5). Groups negative control, bone and blood did not differ statistically from each other ($p > 0.05$). Released monomers in blood contact group were in the level of negative control (Fig. 5). In all groups the main residual monomer released was TEGDMA, the amount of which clearly exceeded that of released bis-GMA (Fig. 5).

FTIR analysis of the positive and negative controls showed that the DC% clearly increased when the formation of an oxygen-inhibition layer was prevented by polymerising the veil prepreg between objective glasses (negative control, DC: 62.8% versus positive control, DC: 34.0%) ($p < 0.001$) (Fig. 6).

5. Discussion

In this study, an attempt was made to test the photopolymerisation and possible inhibiting effect of bone and blood on the free radical polymerisation of the dimethacrylate monomer system that had been used in FRC prepregs. The efficiency of photopolymerisation in simulated tissue contact conditions gave preliminary information on the potentiality of using FRC in endosseal implant applications. The quantity of released bis-GMA was low, while the amount of released TEGDMA was clearly at a higher level. This indicated

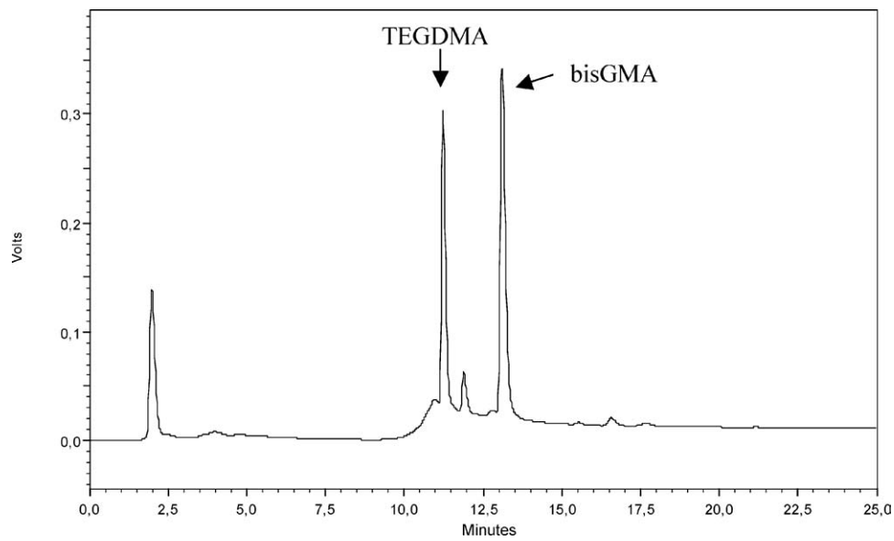

Figure 4 HPLC chromatogram of the positive control sample.

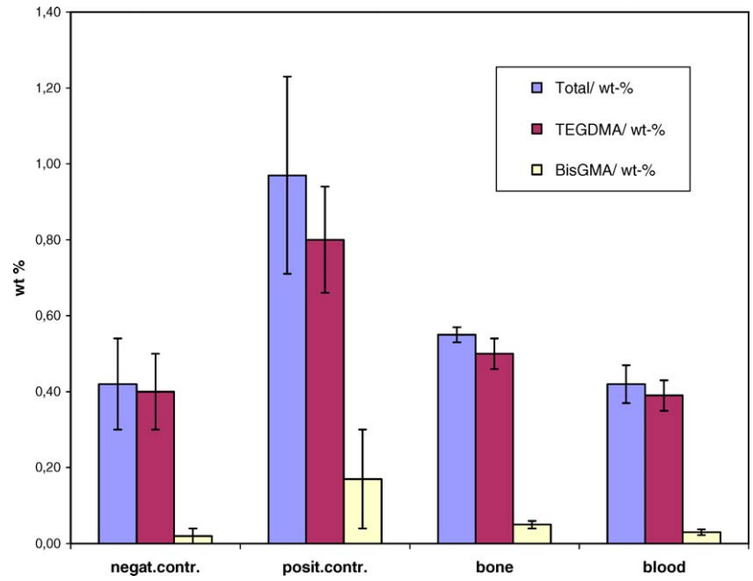

Figure 5 The quantity of released residual monomers as a percentage of the weight of the photopolymerised veil. Bone and blood refer to polymerisation in contact with them.

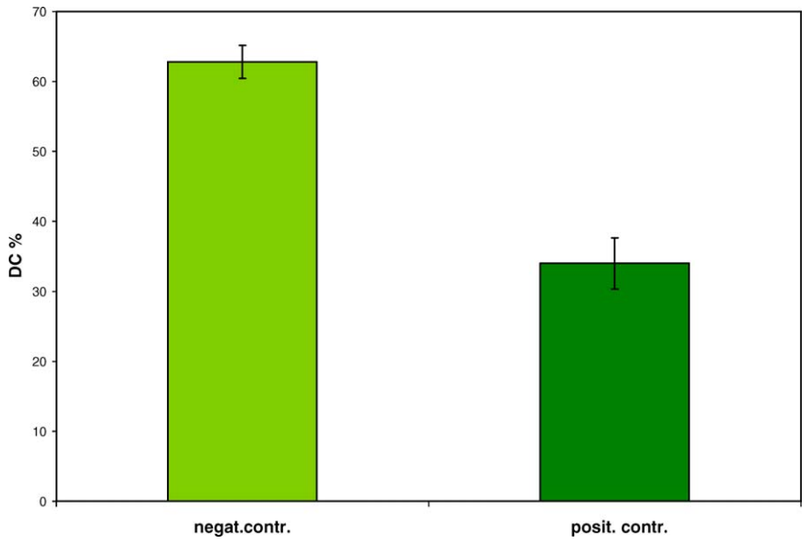

Figure 6 The degree of monomer conversion (DC%) of negative and positive controls measured by FT-IR.

that TEGDMA leaches more easily from the surface of the composite. This is in agreement with the result of Örtengren *et al*. [31].

It should be noted that the residual monomers of the negative control group came actually from the whole polymer matrix of the FRC. It has been shown many times previously that photopolymerisation of dimethacrylate monomer systems in clinical conditions results in a degree of monomer conversion of ca. 65% [32]. Part of the unreacted monomers remain as monomers that can leach out from the polymer matrix. Part of the dimethacrylate monomers reacted only to one of the methacrylate groups and some pendant molecules were present in the polymer matrix [33]. Thus, the residual monomers found in the immersion water of the test samples of this study, contained a background level of residual monomers coming from the whole polymer matrix of FRC. The differences between the groups resulted from the surface layer that was exposed to air or the test substrates (blood, bone).

In this study, information about the polymerisation properties of the composite surface was analyzed by extracting the released residual monomers from water and then quantifying them with HPLC. We used this method instead of FTIR methods, because the proteins from the bone surface and blood tended to stick to the surface of the FRC disturbing the scanning system of the FTIR instrument. Positive and negative controls could be analysed with the DRIFT-IR method, because the veil prepregs had not been in contact with tissue.

Slightly increased residual monomer values were noticed when the prepreg was polymerised in contact with bone. This could be due to the oxygen present in the

microscopically porous surface of the bone which might have inhibited the polymerisation. Also, the moisture contained oxygen to some extent and this might also have had a minor inhibiting effect. Interestingly, no inhibiting effect of blood was noticed although the oxygen in the blood hemoglobin could have had an inhibiting effect on polymerisation.

The effect of polymerisation time was not studied, and the irradiation time was selected because of its general use in dental applications achieving ca. 60% DC with dimethacrylate composites. A polymerisation time of 40 s might also be possible in surgical applications of FRC. Possible surgical applications for FRC might be found in maxillofacial surgery and oral surgery. Because of the good mechanical properties of FRC, some application in load-bearing implants might also be possible [17, 20].

The result of this study was that bone and blood did not have a remarkable disturbing effect on the photopolymerisation of the monomers of FRC. This suggests that the level of the possibly harmful residual monomers, bis-GMA and TEGDMA, was not increased by tissue contact polymerisation. On the other hand, it has also been shown by Shinzato *et al*. [34], that the unpolymerised surface of bis-GMA–based bioactive bone cement seemed to be even useful biologically for exposing the bioactive filler on the surface of the composite, thus effectively inducing bone bonding.

Even though this preliminary *in situ*-polymerization study gave promising results, the potential estrogenicity of residual bis-GMA monomers [35–38] may limit the use of bis-GMA impregnated FRC in endosseous implants. The estrogenical effect of different monomer systems need to be carefully studied before making the choice of any special monomer system for FRC implants. In all monomer systems, the polymerization needs to be optimized in order to diminish the quantity of residual monomers. In some applications, the optimization might be carried out by heat induced postcuring.

6. Conclusions

Bone surface did not significantly inhibit the photoinitiated polymerisation of dimethacrylate monomers of FRC, and nor did contact with blood.

Acknowledgments

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