Fibre-reinforced composite implant: *in vitro* mechanical interlocking with bone model material and residual monomer analysis

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The aim of this study was to examine *in vitro* the mechanical interlocking of an experimental implant made of E-glass fibre-reinforced polymethyl methacrylate (PMMA)-based composite (FRC) to dental stone. FRC implants with a porous surface were embedded into the dental stone, which was chosen to simulate bone ingrowth into the porous surface of the implant, after which push-out tests were performed. PMMA cylinders with smooth and grooved surface were used as controls. In addition, the release of residual methyl methacrylate monomer (MMA) into water from FRC and control implants with different compositions and fabrication methods was determined using high performance liquid chromatography (HPLC). The highest push-out force (2149 ± 263 N) was measured for the implants with grooved surface and the lowest value for the implants with smooth surface (194 ± 68 N). The push-out forces were over five times higher for FRC implants with a porous surface (958 ± 217 N) than for implants with smooth surface. During the first day of testing, the MMA release into water was 1.4–2.8 times higher from the FRC implants than from the control PMMA implants, depending on fabrication method. With time, the difference between the implants diminished.
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1. Introduction

Within the field of current biomaterials research, one of the major orthopaedic ambitions is to achieve a proper biological fixation of the implant in the surrounding bone. Polymers, especially fibre-reinforced composites have been under inspection due to biomechanical properties [1–5]. In the previous study, we have introduced a polymethylmethacrylate (PMMA) based surface porous fibre-reinforced composite (FRC) intended for use as an endosseus implant [6].

A commonly used and simple method for evaluating the strength of a bone-implant interface is the push-out test, which measures the force that is necessary to move the implant in the surrounding bone. The most common applications for push-out tests include testing for the effects of implant material, surface texture, cross-sectional geometry and surface composition in the context of cementless fixation by bone ingrowth or bone apposition to the implant. Usually, the push-out test is based on a cylindrical implant that is placed in cortical or trabecular bone [7, 8]. However, synthetic bone model materials like polyurethane foams (with a density and mechanical properties similar to those of cancellous bone) have also been used in some studies [9–11].

In this study, interface mechanics between surface porous FRC and dental stone, which was chosen to simulate bone ingrowth into the porous surface of the composite, were evaluated by measuring the maximum push-out forces. PMMA implants with smooth and grooved surfaces were used as controls.

Another aim of this study was to determine the release of residual MMA monomer from the surface porous FRC implant. This information is needed for evaluation biocompatibility of the material produced in the surface porous form.

2. Materials and methods

2.1. Push-out measurements

The materials used in the study are listed in Table I. For the push-out tests polymethylmethacrylatey (PMMA)

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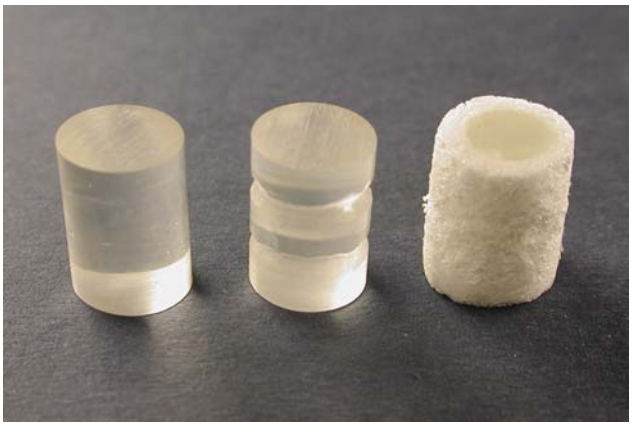


Figure 1 The test implants used in the push-out tests: (a) smooth surface (b) grooved surface and (c) porous surface. The implant with porous surface contained 10 wt% of chopped E-glass fibres and the IPF-process.

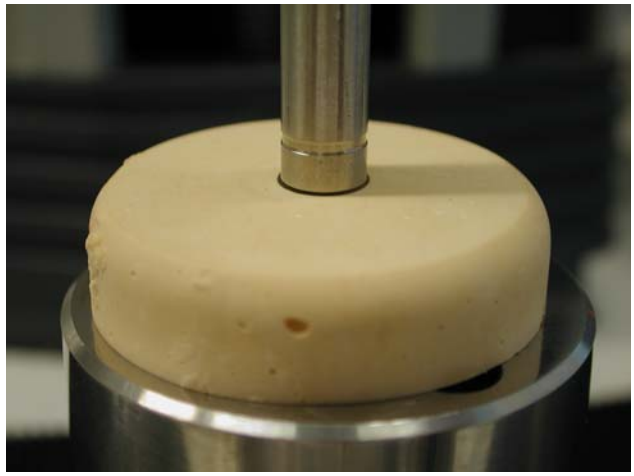


Figure 2 The test set-up for the push-out test.

implants with smooth and grooved surfaces (Groups 1 and 2, respectively) and porous PMMA based fibre-reinforced composite implants (Group 3) were prepared as follows. The different implants are shown in Fig. 1.

PMMA powder(Palapress®)containing benzoyl peroxide intiator and methylmethacrylate (MMA) monomer containing 2 wt% N,N-dinethyl-p-toluidin as activator were mixed together (liquid-to-powder ratio 1:1). The PMMA-MMA mixture was poured into a syringe (ONCE disposable syringe 2 ml, CODAN Medical ApS, Rødby, Denmark) to fabricate cylindrical implants and the mixture was polymerised in a pressure-curing device (Ivomat, Typ IP 2, Ivoclar AG., Schaan, Liechtenstein) at a pressure of 400 kPa, at a temperature of $90 \pm 3^{\circ}$C, for 20 minutes. The cylindrical implant (diameter: 8.6 mm) was taken out of the syringe after polymerisation and rods were sawed into pieces (length: 10 mm). For group 2, two grooves (groove depth: ∼0.5 mm) were drilled horizontally around the implants. Group 3 contained 10 wt% of polymethyl methacrylate preimpregnated chopped E-glass fibres (length: 2–3 mm), which were inserted o a syringe before polymerisation. After polymerisation, composites were taken out of the syringe and inserted into the solvent tetrahydrofuran (THF) for one hour in order to obtain swelling and dissolving of the PMMA on the surface of the implant at room temperature. A porous surface for the test implant (diameter: 8.3 mm) containing PMMA and glass fibres was obtained by solidification of the swollen and dissolved PMMA layer and evaporation of the solvent THF, called IPF (interfacial porosity formation) process [6].

Dental stone was used as a simulated bone model in the push-out test. All implants were first treated with a surface tension decreasing agent and then embedded into the dental stone (GC Fujirock® EP) using the powder-to-liquid ratio of 100 g powder/20 ml water recommended by the manufacturer. Excess dental stone extending on the top of the implant was removed using SiC paper and a grinding machine (LaboPol-21, Struers A/S, Rødovre, Denmark). Implants were left to set for three days at room temperature.

The push-out test for the implants embedded into dental stone (Fig. 2) was performed on a universal testing machine (Lloyd, model LRX, Lloyd Instruments, Fareham, England) at a loading speed of 1 mm/min, and a force-displacement curve was recorded. Twelve implants from each group were used for determining the maximum push-out force (N). The clearance of the hole in the support jig was at least 0.8 mm for all implants.

2.2. HPLC-analysis

For the residual monomer analysis, twenty implants containing 0 and 10 wt% of chopped glass fibres were prepared as above (groups 1 and 3) and after polymerisation the rods (diameter: 8.6 mm) were cut into pieces (length: 5 mm). Half of the implants in each group were treated with THF for one hour to create a porous surface. To analyse the release of residual MMA from the test implants ($n = 5$), the implants were incubated in 10 ml of deionised Milli-Q water (electrical resistivity 18.2 MΩ cm) at the temperature of 37°C for up to 2 weeks. Fig. 3. shows the implants used in the HPLC analysis.

After the predetermined storage period of 1, 3, 7 and 14 days, the MMA content of the immersion water was analysed by Shimadzu's (LC-2010) modular high performance liquid chromatograph (HPLC) system (Shimadzu Corporation, Kyoto, Japan). The incorporated columns used in the system were Phenomex's C18 precolumn (Phenomex, Torrance, CA, USA) and Phenomex's C18 analysis columns (type: RP18, length: 150 mm, internal diameter: 2 mm, particle size: 5 μm). The analysis was carried out as an isocratic run, in which the flow rate was 0.3 ml/min and the mobile phase was methanol:water (70 vol%/30 vol%) (Methanol HPLC grade, Rathburn Chemicals Ltd, Walkerburn, Scotland). The used wavelength of UV light was 205 nm. The MMA concentration was measured by HPLC analysis using a standard

Figure 3 The implants used in HPLC residual monomer analysis: (a) 0 wt% of E-glass fibres, (b) 0 wt% of E-glass fibres, after the IPF process, (c) 10 wt% of chopped E-glass fibres and (d) 10 wt% of chopped E-glass fibres, after the IPF process.

calibration curve ($R^2 > 0.97$) in which MMA concentrations of 1, 3, 5 and 10 μg/ml served as calibration samples. The concentrations of MMA were calculated from the areas under the curve at the peak produced by the MMA. The amount of released MMA was estimated in ppm per 1.00 g of PMMA per day during the storage period. After the residual monomer analysis PMMA of the FRCs was combusted at $+700^{\circ}$C for one hour and the fibre content (wt%) was calculated from the initial weight of the implant. The weight of the glass fibres was excluded from calculation of the release of residual MMA from the implants that contained fibres.

2.3. SEM observations

The thickness of the porous surface layer of the FRC implant after solvent treatment was measured, and the penetration depth of the dental stone was examined by a scanning electron microscope (SEM, JSM-5500, JEOL, Tokyo, Japan). The specimens were coated with a gold layer using a sputter coater (BAL-TEC SCD 050 Sputter Coater, Balzers, Liechtenstein).

2.4. Statistical analysis

For determining the push-out forces, the Weibull analysis was carried out using Weibull ++ 6.0 (Reliasoft Corporation, USA) with median ranks for estimated fracture probability.

$$P_f = 1 - \exp\left[-\left(\frac{x - x_u}{x_0}\right)^m\right] \quad (1)$$

Where m = Weibull modulus (also known as shape factor), a constant that determines the slope of the distribution function and characterizes the spread of the failure data with respect to x axis. x_0 = characteristic push-out force (i.e. the push-out force level at which 63% of the implants have failed) and x_u = theoretical failure force ($= 0$).

The statistical analysis of MMA release was performed using the SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, USA) software for Windows with univariate ANOVA, followed by Tukey's post hoc analysis.

3. Results

3.1. Push-out tests

The results of the push-out tests are shown in Fig. 4. The highest shear force, 2149 ± 263 N, was measured for the implants with grooved surface. This value is actually the

TABLE I

Brand	Manufacturer	Lot no.	Type of material
Palapress® powder (clear)	Heraeus Kulzer GmbH & Co KG, Hanau, Germany	032200	PMMA powder[a]
Methyl Methacrylate	Fluka Chemie GmbH, Buchs, Switzerland	424318/1	Monomer
N, N-Dimethyl-p-toluidine 99%	Sigma-Aldrich Chemie GmbH, Steinheim, Germany	23208-058	Activator
Stick®	Stick Tech Ltd, Turku, Finland	10001023-R-0056	Preimpregnated glass fibres[b], length 2–3 mm
Tetrahydrofuran	Sigma-Aldrich Laborchemicalien GmbH, Seelze, Germany	11660	Solvent
GC Fujirock® EP	GC Europe N.V., Leuven, Belgium		Type 4 dental stone

[a]Polymethylmethacrylate, Mw 220.000.
[b]Silanated E-glass fibres with polymethylmethacrylate preimpregnation.

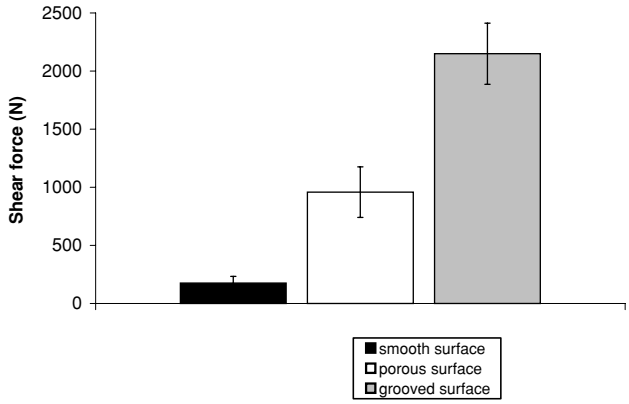


Figure 4 The results of the push-out force measurements.

maximum load value for the gypsum because it cracked up during the test. The shear force of the surface porous FRC implant was 958 ± 217 N and the lowest shear force, 175 ± 58 N, was measured for the implants with smooth surface.

The Weibull values and the Weibull plot for push-out force of are given in Fig. 5. and in Table II. The SEM observation (Fig. 6) shows that the thickness of the porous surface layer was 300–500 μm for FRC implants after one hour of solvent treatment and the penetration of dental stone into the pores illustrated that there was an interconnective porous structure in FRC implants which is crucial in terms of bone ingrowth into the material.

TABLE II

Group	Characteristic push-out force $= x_0$	Weibull modulus $= m$	Correlation r-coefficient
Smooth surface	192.41	3.77	0.95
Porous surface	1026.90	5.76	0.90
Grooved surface	2262.23	9.07	0.97

3.2. Residual MMA analysis

Fig. 7 shows implants used for residual monomer analysis. The mean residual MMA release per day into water was higher with FRC implants (with and without the IPF process) than with control implants ($p < 0.001$). The difference diminished with time. Two-way ANOVA revealed that there was some interaction with independent factors of implant type and solvent treatment (IPF process). During the first day of storage, 125 ± 41 ppm of MMA was released from the surface porous FRC implants, while the control implants after solvent treatment without any glass fibre inclusions released only 46 ± 6 ppm of MMA into storage water. During the 14 days storage period, the total amount of MMA released into water from implants varied between 189 ppm and 379 ppm, depending on the fabrication method of the implants.

4. Discussion

In orthopaedics, there is a need for bone reconstructive load-bearing material that has the ability to bond to the

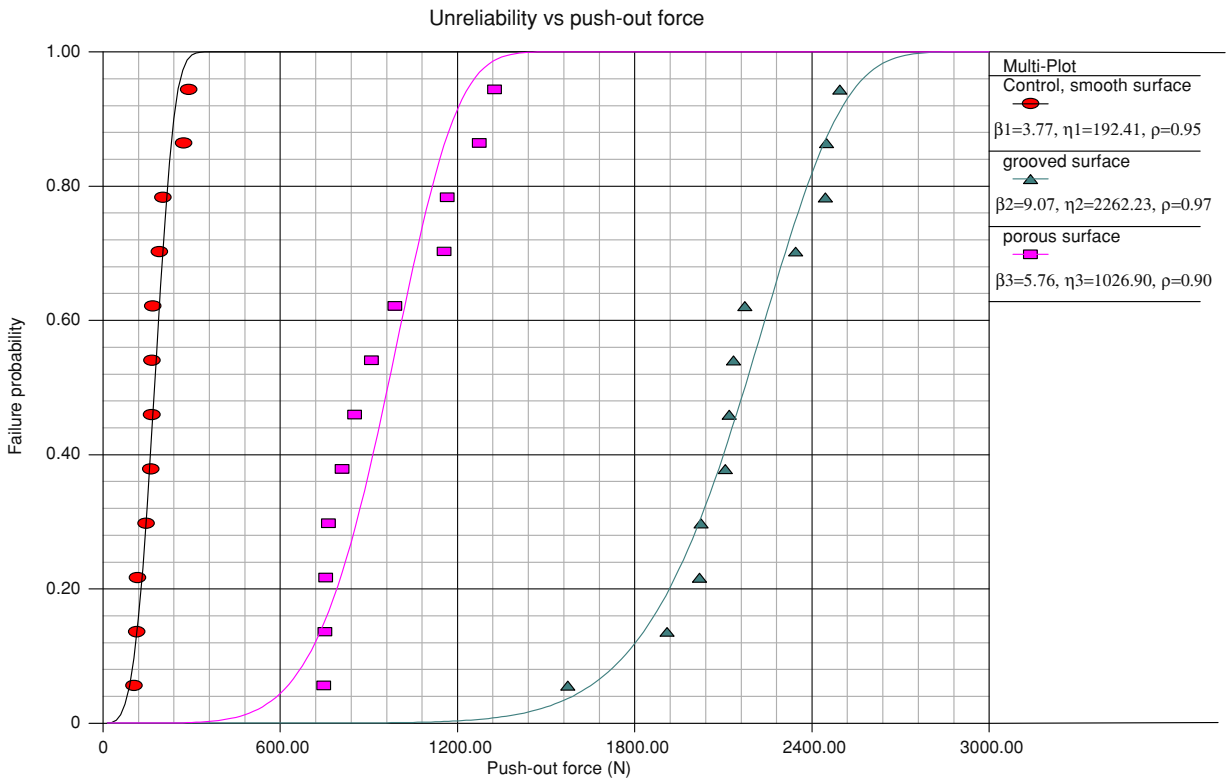
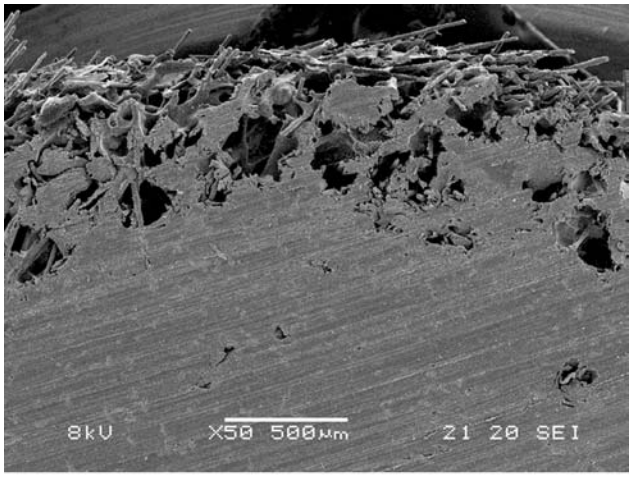
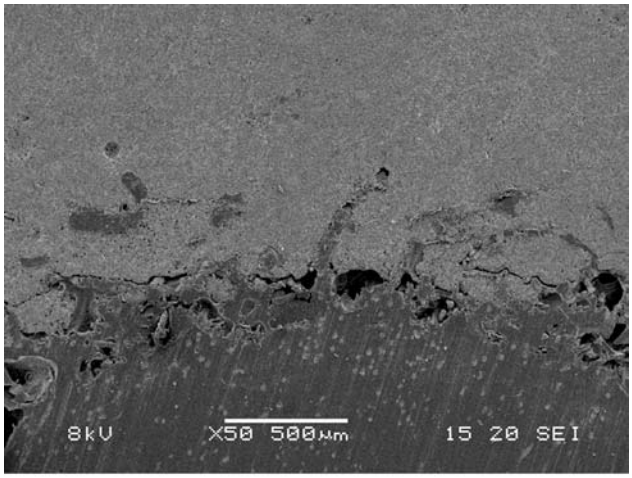


Figure 5 The Weibull plot for push-out force.



(a)



(b)

Figure 6 SEM micrographs of surface porous FRC implant before (a) and after (b) embedding in dental stone and performing push-out test.

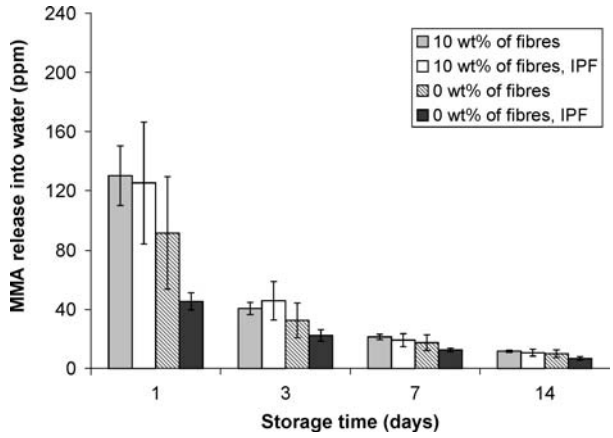


Figure 7 MMA release (ppm) per day.

bone tissue and whose biomechanical properties are similar to bone. FRCs is a group of materials with properties tailorable according to specific needs [12–14]. This study continues the research work with an aim to evaluate biostable FRC materials which may potentially be used as load-bearing implant material.

In this study, mechanical interlocking between the surface porous endosseus implant model and bone simulation material was examined. Implants with grooved and smooth surfaces were used as controls. Shear strength and elastic modulus values could not be measured due to the surface structure of the implants. Penetration of dental stone to surface irregularities was selected to simulate bone ingrowth in the material. The pore size is previously shown to be up to 500 μm, which is optimal for bone ingrowth and vascularization [15, 16]. There was mechanical strength in the porous interface of the implant after it was filled with dental stone, since the FRC implant did not break up into the porous and non-porous sections during the test. It can be hypothesized that in dynamic loading conditions, the porous surface layer containing chopped glass fibres could act as a stress breaking interphase decreasing the stress-shielding. Stress-shielding of the implant has been characterized as one of the most serious shortcomings of implants with high stiffness, i.e. those made of metals or ceramics [17, 18]. The push-out forces were over five times higher for surface porous FRC implants than for implants with smooth surface attaching only with friction. The Weibull analysis showed higher reliability for the push-out force of experimental implants than for grooved implants, although their absolute and characteristic force values were lower. The push-out force of the implants with a grooved surface was higher than the cohesive strength of dental stone. This suggests that optimal implant design may entail a porous surface for microscopic bone attachment and grooves for macroscopic interlocking into bone. To evaluate the usefulness of dental stone bone simulation models, further push-out studies and histological evaluations in animal experimentations are needed. Such studies are also needed for the biological evaluation of the FRC material.

Recently, the toxicity of unreacted MMA at acrylic bone cements has been widely discussed. It has been shown that considerable quantities of MMA are released into the body before and during polymerisation of the acrylic bone cements [19]. In order to diminish the problem related to free MMA, the implants may be fabricated *ex vivo*. In doing so, the polymerisation conditions may be optimised and the potentially harmful free MMA is the residual MMA. It was shown that the majority of residual MMA leached out from the porous surface FRC implants during a few days of water storage. The quantity of released MMA was higher in the FRC groups than in control groups [20]. Generally, the levels of residual monomers detected were considerably lower than those found in chemically cured fibre-reinforced dentures and in modified acrylic bone cements [20, 21].

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