Characteristics of experimental resin-modified glass-ionomer cements, containing alternate monomers to HEMA

Amani Agha[1*], Sandra Parker[1], Eric Kenneth Parkinson[2], Mangala Patel[1]

[1] Dental Physical Sciences Unit, Queen Mary University of London, E1 4NS.

[2] Clinical and Diagnostic Oral Sciences, Queen Mary University of London, E1 2AT.

Abstract

Objective. Resin-modified glass ionomer cements (RMGICs) present several advantages (e.g. fluoride release), but their reported cytotoxicity has been associated with hydroxyethyl methacrylate (HEMA) monomer release. Therefore, different monomers were tested for use in RMGICs in order to improve their biocompatibility and reduce monomer release.

Methods. Eight experimental liquid compositions were prepared replacing different percentages of HEMA (conventional monomer used in commercial RMGICs) with hydroxypropyl-methacrylate (HPM) and/or tetrahydrofurfuryl-methacrylate (THFM), which are known to have better biocompatibility. Moreover, two commercial materials (Fuji-Plus and RelyX) and two compositions, based on these (home), were included as controls. Monomer release of all materials (commercial, home and experimental) were tested using high-performance liquid chromatography (HPLC) methods after immersing discs in deionized-water (DW) or ethanol:DW. Cytotoxicity of the materials extracts was tested on normal human oral fibroblast line (NHOF-1) using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay.

Results. Three experimental materials containing THFM (F3, R3 and R4) showed less or similar monomer release compared to corresponding commercial products. Furthermore, two experimental materials (F3 and F4) showed similar effects on NHOF-1 cells compared to the negative control medium.

Significance. The lower monomer release and higher cell viability of some experimental THFM compositions are encouraging. THFM partially replacing HEMA is potentially a suitable alternative for producing biocompatible RMGICs.

Keywords: Resin modified glass ionomer; hydroxyethyl-methacrylate; tetrahydrofurfuryl-methacrylate; hydroxypropyl-methacrylate; high-performance liquid chromatography; cytotoxicity

1 Introduction

Resin modified glass ionomer cements (RMGICs) were introduced in order to overcome some of glass ionomer cements (GICs) limitations, mainly moisture sensitivity and to improve the cement's mechanical properties [1]**.** Resin containing luting cements (e.g. resin composites and RMGICs), undergo polymerization, which can be activated chemically, by light, or both. During this process, the monomers form

polymers through conversion of the double bonds (C=C) to single bonds and cross-linking with other monomers to form a cross-linked resin matrix. Theoretically, all monomers should be converted to polymers, but this is not the case in all dental cements, and conversion ranges between 33-50% [2]. Therefore some unconverted monomers may leach out from the cement matrix and into the oral environment [2,3]. Similarly to GICs, RMGICs release small amounts of ions from the glass matrix as well (Al^{+3}, F^{-}, Sr^{+2}), where the released amount does not reach cytotoxic limits in the mouth. If released in high amounts, these ions could be cytotoxic. The reported cytotoxic effect of RMGIC was shown to be associated mainly with the release of unconverted hydroxyethyl methacrylate (HEMA) monomer and its effect on the pulp [4]. Due to its low molecular weight and hydrophilicity, the monomer can easily diffuse through the dentinal tubules to the pulp tissue, and subsequently might cause irreversible inflammation [5,6].

Hamid et al [6] studied the diffusion of HEMA through 1.6 -2 mm thickness of dentine using High Performance Liquid Chromatography (HPLC). The authors demonstrated that during the first day, HEMA was able to diffuse from samples through dentine and into the pulp space. It was also concluded that this release might contribute to the 'adverse pulp response' following application [6]. This agrees with a study carried out by Beriat and Nalbant [7], which also showed that un-converted HEMA monomers diffused from polymerized RMGICs.

It has been documented that HEMA may jeopardize the biocompatibility of RMGICs and potentially cause cytotoxic effect on cells, as shown through in-vitro testing [4,8–10]. It was reported that HEMA released from restorative dental materials can disrupt the function of the fibroblasts in the pulp and, even in low concentrations, cause severe

damage [11]. Conversely, clinical studies on RMGICs did not show adverse reactions in patients and clinicians. However, these cements do demonstrate compromised biocompatibility properties when compared to GICs due to the incorporation of HEMA [12].

Different monomers have been suggested as replacements for HEMA in RMGICs, in an attempt to improve the properties of commercial products. According to Agha *et al.* [14], tetrahydrofurfuryl methacrylate (THFM) was considered as a good alternative to HEMA for the fabrication of RMGICs liquids since it reduced the water uptake of the set cement [14]. Some physicochemical properties of THFM and hydroxypropyl methacrylate (HPM) in RMGICs were also studied and compared with commercial products. These included polymerization shrinkage, exotherm, degree of conversion and mechanical properties [13,14]. The experimental THFM-containing compositions generally showed similar polymerization shrinkage and degree of conversion compared with commercial materials [14], and improved mechanical properties compared with control materials containing HEMA as a monomer [13]. HPM could also be used to partially or fully replace HEMA. Propyl is the next member following ethyl in the homologous series, and therefore, HPM has an extra CH_2 group between the hydroxyl and methacrylate groups. There have been only two further reports describing the use of HPM in dental applications. Atai *et al.* [15] confirmed the lower shrinkage (strain) rate of HPM compared to higher molecular weight monomers (e.g. urethane methacrylate, UDMA), but no differences were found compared to HEMA. This monomer also demonstrated a lower setting exotherm and reduced water uptake compared to HEMA, in poly (ethyl methacrylate)(PEM) systems [16].

Two patents [17,18] also included THFM as a potential monomer for the fabrication of

RMGICs. Although Mitra [17] mentioned that THFM could be incorporated in RMGICs, details of the composition were not provided in the patent. Anstice et al. tested THFM and other monomers for use in RMGICs [18]. These authors demonstrated that different compositions incorporating THFM could be used (in place of HEMA), but they did not report on residual monomer release and/or biocompatibility.

THFM has also been patented and studied for use in different biomedical applications, such as fluoride releasing biomaterials [19], a tissue repair material [20] and temporary crown and bridge materials [21]. The latter use, where THFM was mixed with poly(ethyl methacrylate) (PEM) and cured at room temperature, showed no irritation in monkey's dental pulp tissues [22].

Hence, since THFM and/or HPM in different applications have demonstrated improved properties compared to HEMA, they emerge as potential alternatives for the fabrication of new experimental RMGICs.

Therefore, the aim of this study was to investigate the cytotoxic nature of experimental resin-modified glass-ionomer cements, containing THFM and HPM that partially or fully replaced HEMA.

The objectives of this study are summarized as follows:

- To measure the amount of monomer(s) released from experimental, control and commercial RMGICs samples, by replacing HEMA fully or partially with HPM and/or THFM, using HPLC.
- To compare the cytotoxicity of experimental, control and commercial RMGICs on normal human oral fibroblast line NHOF-1 fibroblast cells.

2 Materials and methods

2.1 Materials

This study included two commercial, chemically cured RMGICs, Fuji Plus (FP, GC Corporation, Tokyo, Japan) and RelyX Luting (RX, 3M-ESPE, St Paul, MN, USA; Table 1). Control home liquids were prepared following the manufacturer's materials safety data sheets. The powder used in all cement formulations was the corresponding commercial powder (Table 2).

Eight experimental liquid formulations were also prepared, four based on each of the commercial products (RX, FP or R and F respectively for sample codes), where HEMA was replaced with either 100% HPM (F1 and R1), 70%/30% HPM/THFM (F2 and R2), 50%/50% THFM/HEMA (F3 and R3) or 30%/70% THFM/HEMA (F4 and R4). 100% THFM was trialed, but due to phase separation of the liquid, this composition was not included in the study.

2.1.1 HPLC solvents:

HPLC grade acetonitrile (ACN; Merck Millipore, MA, USA) and deionized water (DW) were used as solvents in the HPLC study.

2.2 Methods

2.2.1 Monomer release study

The release of four monomers (HEMA, THFM, HPM and UDMA, used in RMGICs) was analyzed using HPLC in DW and 75:25% ethanol: DW. Different concentrations of standard solutions of each monomer were prepared (ranging from 0-300 ppm).

2.2.1.1 Specimen and solution preparation

One hundred and forty-four discs were prepared according to the manufacturer's

instructions (2:1 g:g of FP powder to liquid ratio for FP group materials and 1.6:1 g:g for the RelyX Luting group materials). Six discs of each material (10 mm diameter and 1 mm thick) were prepared by placing the mixed materials into a poly(tetrafluoroethylene) (PTFE) mould placed on a glass slide covered with acetate sheet. The surface of the material was also covered with acetate sheet, pressed with a glass plate and left in an incubator at 37°C to set for one hour. After setting, the samples were de-moulded, checked for any irregularities and then immersed in individual amber glass test tubes. The tubes were filled with either 10 mL DW or 10 mL ethanol:DW and left in an oven at 37°C at least 24 hours prior to immersion of the samples.

1mL of immersion solution was extracted at pre-determined time points; 1 hour, 4 hours, 24 hours and 168 hours after initial immersion. The solution was changed at the last time point (168 hours) and an additional extract was taken after 672 hours (4 weeks). It should be noted that no further release of monomers was recorded at this latter time point. Amber glass vials were used to store the extracted solutions, which were stored in the refrigerator at 4°C±1°C prior to HPLC analysis. 1 mL of DW was added to the glass test tube each time an aliquot (1 mL) was extracted, in order to maintain the volume of solution at 10 mL.

2.2.1.2 HPLC method for monomer release of FP group

HPLC with UV-Vis spectrometric detector was used to analyze and quantify the release of monomers from commercial, home and novel RMGICs used in this study.

The HPLC column was a Zorbax Eclipse® XDB-C18 4.6×100 mm 5 μm column (Agilent Technologies, USA). The mobile phase consisted of ACN and DW at a flow rate of 1 mL/min, injection volume 10μL; the UV detector absorbance was at 210 nm; the column temperature was set at 30°C and the run time was 30 minutes.

The HPLC method used gradient conditions as follows: 0min, ACN 30%, 15min, ACN 70%; 17min, ACN 70%; 20min, ACN 100%; 21min, ACN 30%; 30min, ACN 30%. Under all these conditions, ACN was combined with DW. Contamination of the column with hydrophobic elements was noticed during pilot studies, and therefore it was necessary to wash the retained components following each sample analysis. This process involved running of the initial mobile phase (ACN 30%) for 9 minutes, at the end of each test sample, to eliminate any possibility of compounds carrying over following separation.

2.2.1.3 HPLC method for monomer release of RX group

The same parameters mentioned above were used for identification and quantification of monomer release from the RX group. The only differences between the two methods (FP and RX) were the gradient conditions and run time. The gradient conditions for the RX group were as follows: 0min, ACN 30%, 5min, ACN 35%; 6min, ACN 35%; 6:05 ACN 30%; 8min ACN 30%, runtime 8 minutes (shallow gradient). All HPLC parameters for both methods are presented in Table 3.

2.2.1.4 Treatment of HPLC data

Measurements were taken twice for each of the extracts and calibration solutions and then the mean of the peak heights was calculated. For every batch of samples at each time point (n=6), calibration curves were calculated by plotting the known concentration of each standard solution (ppm) versus the peak height obtained. The concentration of all monomers was then calculated using the correlation coefficient obtained from linear regression analyses of the calibration curves. Means of monomer release were then statistically compared using SPSS IBM statistics version 22 (Chicago, IL, USA) One-way ANOVA, followed by post-hoc Tukey test at a significance level

of p=0.05.

2.2.2 Cell culture

2.2.2.1 Cells

The normal human oral fibroblast line NHOF-1 fibroblast cells were obtained from a buccal mucosa biopsy, under ethical approval of the Dental Teaching Hospital, Peradeniya, Sri Lanka, following informed patient consent (Ethical Clearance Certificate No. FDS-ERC/2008/02/TIL) [23]. The NHOF-1 were tested for mycoplasma following isolation with the MycoplsmaAlert® (Lonza, Switzerland) and found to be negative, and re-tested at the time of use. The cells were cultured and maintained at 37^{o}C in a humidified atmosphere of 10% CO_2/90% air in Dulbecco's Modified Eagle's Medium (DMEM), 4.5g/l glucose (Lonza) supplemented with 10% vol/vol hyclone II fetal bovine serum (FBS – Thermoscientific), penicillin, streptomycin 50units/ml (Life Technologies) and 2mM glutamine (Life Technologies). Cells were sub-cultured and prepared for experiments by washing once with 0.02% EDTA (ethylenediaminetetraacetic acid) in calcium- and magnesium-free phosphate buffered saline (PBS) and then incubating with 0.01% trypsin/0.01% EDTA in PBS at 37^{o}C, until the cells detached. Following this, the trypsin/EDTA mixture was neutralized with three times the volume of DMEM/FBS medium, and the cells counted in a haemocytometer prior to resuspension, dilution and plating for the experiments. Cells were seeded in a 96 well plate at a density of 9.32 x 10^3/cm^2 in each well.

2.2.2.2 Sample preparation

The same procedure described for HPLC specimen fabrication was carried out here but with one difference, the cement was packed in a PTFE mould with internal dimensions of 5mm diameter and 2 mm thickness. Each sample had a surface area of 70.7 mm^2 and

a surface to liquid ratio of 47.13 mm^2/mL. Following setting, the samples were removed from the moulds and each sample was then placed in a well containing 1-1.5 mL DMEM in a 24 well plate. Afterwards, the 24 well plate containing one sample of each material (commercial FP or RX, Home, or experimental RMGIC) and one well containing only medium, which served as negative control, were placed in an incubator maintained at 37°C and 10%CO_2/90% air for 24 hours. This experiment was repeated three times.

2.2.2.3 Cytotoxicity testing procedure

Following 24 hours, the medium containing the samples, and the negative control medium, were filter sterilized using 0.22µm Sterile Syringe Filter (VWR, Pennsylvania, USA). The medium in the wells containing the cells was gently aspirated and then 200 µL of the materials supernatants were placed in each well to a total of 4 wells per material; this was also performed for the negative control medium. The treated cells were then left in an incubator for 72 hours at 37°C and 10% CO_2/90% air. This experiment was repeated 3 times to ensure reproducibility. Following incubation, the cell viability and number were tested using the MTT assay.

2.2.2.4 The MTT assay

Following 72 hours incubation of the cells, the medium in each well was gently aspirated and 200 µL of MTT solution (0.5 mg MTT to 1 mL DMEM) was added to each well and incubated for 60 minutes, at 37°C and 10% CO_2/90% air. Blank wells containing no cells were processed identically. At the end of one-hour incubation, medium containing MTT was carefully aspirated and then 200 µL of dimethyl sulfoxide (DMSO) was suspended in each well. Then, the color was quantified using a simple colorimetric assay at 570 nm absorbance to determine the optical density (OD) using

an Optima Plate reader. The blank values were subtracted from the experimental values.

Cells viability was expressed as Equation 1.

$$Cell\ viability = {}^{OD\ (Test)}\Big/_{OD\ (Control)} \times 100 \qquad \text{Equation 1}$$

where OD (Test) was the optical density of the experimental medium and OD (Control) was that of the control medium.

As a result of the first experiment, no definite conclusion was drawn regarding the toxicity of the FP group materials, whilst RX group (commercial, home and novel) materials showed a definite cytotoxic reaction. Therefore, another set of experiments was performed which included only the FP group materials. The only difference between the first and the second set of experiments was that the samples had more surface area in the second experiment (282.8 mm^2) and the surface to liquid area was 141.4 mm^2/mL. The toxicity of the materials on cells was tested as neat aliquots with no dilution and was also tested following dilution with a similar volume of DMEM.

In one experiment the MTT assay was confirmed for materials FP, F3, RX and R1 by counting the viable cells on a haemacytometer following disaggregation with trypsin/EDTA, as described above.

2.2.2.5 Treatment of cell culture data

Negative control data were used as 100% cell viability and compared with the viability of cells treated with materials substrate. Results were presented as mean ± standard deviation (SD) for all tested materials. Statistically significant differences were established using the unpaired Student's *t* test at a significance level of $p < 0.05$.

3 Results

3.1 Identification of monomers released from RMGICs

Monomers released from FP samples were detected using an HPLC technique and compared with their respective standard solutions (HEMA, THFM, HPM and UDMA). Peaks for HEMA (retention time ~3.351) and UDMA (retention time ~15.707), acquired from ChemStation software, plotted as absorbance (mAU) against time (minutes), were identified in solutions taken at 1 and 4 hours, 1 day and 1 week following immersion of FP and FP-Home samples in DW. These can be seen in the typical chromatograms in Figures 1 and 2 respectively.

A peak with a retention time of ~8.89 minutes was also detected in FP commercial sample (Figure 1). This peak's retention time did not match with any of the ingredients mentioned in the original manufacturer's MSDS of FP commercial liquid, which was obtained prior to the publication of the newer MSDS version. The newer MSDS version [24] included the component (glycerol dimethacrylate), which upon examination had a retention time of ~8.8, similar to that found in the HPLC trace for FP commercial sample.

A further HPLC method was developed for RX in order to decrease the retention time from 30 to 8 minutes and also to allow for identification of extracted, residual monomers in experimental, home and commercial RX. Extracted solutions from RX samples showed peaks for all monomers included in each corresponding liquid (HEMA, THFM and HPM). RX and RX-Home showed peaks for HEMA only, whereas HPM was present in HPLC spectra of R1 and R2, along with THFM in the latter material; HEMA and THFM were found in R3 and R4 spectra.

3.2 Quantification of monomers released from all materials

Mean cumulative monomer release from FP group samples, following immersion in DW after 1 and 4 hours, 1 day and 1 week are presented in Table 4, which summarizes the monomer release data from all FP materials at the same time points.

Following one-hour immersion in DW at 37^{o}C, FP and FP-Home showed significantly higher release of HEMA compared to F3 and F4 ($p \leq 0.001$), although there was no significant difference between the cumulative release of monomers from these four materials ($p \geq 0.810$). F3 and F4 additionally released the monomer THFM, which was not present in FP and FP-Home. A maximum release was noticed in solutions from F1 samples, at 1 hour, which continued to show significantly higher release than all other materials in the same group, at all-time points ($p \leq 0.001$). Furthermore, at all-time points, F1 showed a significantly higher release of HPM and a higher cumulative monomer release (HPM and UDMA) than F2 ($p < 0.0001$) (Table 4). Lower amounts of residual monomers were released from F3 and commercial FP at 1 day and 1 week compared to all other materials ($p \leq 0.023$).

Similar to the results obtained from samples immersed in DW, when immersed in ethanol:DW, FP and F3 did not show any significant difference in HEMA release at 1 day and 1 week time points ($p \geq 0.124$). F1 samples released greater amounts of HPM than F2 ($p < 0.0001$) and moreover showed higher release compared to all other materials in the FP group ($p < 0.0001$) (Table 5).

In DW, commercial RX released significantly more HEMA than home and novel RX materials at all time points ($p \leq 0.015$). However, similar amounts of HEMA were released from R3 and R4 at all time points ($p \geq 0.403$), which was significantly lower than commercial and home materials after one hour ($p \leq 0.013$). R1 and R2 presented

similar HPM release values at 1 and 4 hours, and 1 day ($p \geq 0.153$). Materials containing THFM in their composition showed variation in the amounts released. As an example, R3 (which contained a higher percentage of THFM monomer in its liquid composition) showed a greater release compared to the other two materials that contained less THFM (R2 and R4) ($p \leq 0.007$). On comparing all monomers released from RX and RX-Home, the post-hoc Tukey test showed no significant differences between RX and RX-Home, at 1 hour and 1 day immersion. However, at 1 week, the concentration of released monomers was lower in the RX- Home solution, which indicates a decrease in the amount of monomers released following 1 day, compared to the corresponding commercial material (Table 6).

In 75:25% ethanol:DW, similar residual HEMA concentrations were noted from commercial RX, RX-Home ($p \geq 0.174$) and from R3 and R4 samples, following 1 day and 1 week immersion ($p \geq 0.944$) (Table 7). Novel compositions R3 and R4 released less HEMA than commercial and home RX materials following 1 day and 1 week immersion in 75:25% ethanol:DW, similarly to their release in DW ($p \leq 0.005$). In 75:25% ethanol:DW, R1 presented a greater release of HPM compared to R2 at all time points ($p < 0.0001$) and the cumulative release of monomers from it was higher than all other materials, at 1 hour ($p \leq 0.001$) (Table 7).

3.3 Cytotoxicity of materials (commercial, home and novel)

3.3.1 First set of experiments on FP and RX group materials

Mean percentage of active cells following exposure to aliquots (control medium in which the samples were immersed) of all RMGICs (commercial, home and novel) and, standard deviations of the experiments (n=3), are presented in Table 8. Table 8 includes the statistical analysis and comparison between i) materials (commercial, home and

novel) versus medium; ii) materials (home and novel) versus commercial; and iii) novel materials versus home. P values less than 0.05 were used to confirm statistically significant differences between materials.

In the RX group, all materials (commercial, home and novel) showed significantly lower cell viability than the negative control. Only F2 in the FP group presented with a lower p value than 0.05 (p=0.028) and less viable cells, following 72 hours of exposure to the material's supernatant compared with the negative control medium. No significant differences were noted between both groups on comparing with the commercial and home counterparts (Table 8).

It is worth noting that the color of the medium containing RX samples changed from pink to yellow, which indicates that this medium was more acidic compared to the medium containing FP samples; the latter did not show a change in color and remained pink throughout the time of the experiment.

Figure 3 (a-e) is a representative example showing the effects of materials extracts (supernatant) and negative control in contact with cultured fibroblast cells. Figure 3 (a) shows healthy fibroblast cells with a 'spindle like' shape, which were also present in Figure 3 (b), following exposure to FP aliquots. Cells exposed to RX and R1 samples exhibited a change in morphology from a spindle like shape to a round shape (Figure 3, c and e). F3 showed spindle like cells with long processes, similar to the cell morphology of FP cells and those found in the control medium (Figure 3, d).

3.3.2 Second set of experiments on FP materials

The second set of experiments included testing materials with increased specimen surface area. This experiment was only performed on the FP group, since the RX group

showed high cytotoxicity in the first set of experiments and therefore, it was assumed they would behave in a similar manner, in this experiment.

Table 9 represents mean and standard deviations of viable cells following exposure to neat and diluted FP group solutions. Table 9 gives the p values for commercial, home and novel materials versus medium, home and novel materials versus medium and novel materials versus their home counterparts.

F3 and F4 neat aliquots showed similar effects on NHOF-1 cells when compared with control medium ($p \geq 0.185$). Other compositions including commercial and home neat solutions were shown to be cytotoxic, as the results were statistically significant from the control medium ($p \leq 0.027$). All diluted solutions demonstrated similar effects on cells compared to the control medium, with the exception of F4 that presented with higher viability of cells, after 72 hours exposure to solution ($p=0.023$) (Table 9).

4 Discussion

RMGICs were introduced to overcome the limitations presented by the conventional GICs, while also maintaining the benefits of this conventional cement (e.g. fluoride release and bonding to tooth tissue). This was achieved by incorporating a resin, HEMA which is commonly used in RMGICs, in order to improve their strength and early sensitivity to moisture [25,26].

Theoretically during the setting reaction, the polymerization should continue until all the monomers are converted to polymers, but this is rarely the case [3,27] and unreacted monomers remain. These residual unreacted monomers can leach out into the oral environment, and can pass through the dentine to the pulp, causing cytotoxic effects [9]. Hence, it is crucial to measure the amount of monomers released from polymeric

materials, which can give an indication of the degree of polymerization as well as the biocompatibility and cytotoxicity of the cement.

The US FDA recommendation suggests that 75%/25% ethanol/DW mimics the oral environment more than pure water [28], so this solution was selected as an organic solvent (in which samples were immersed) to collect the residuals from the samples. It has been reported that this solvent penetrates the cement matrix causing swelling of the polymer, hence facilitating the leaching of the unreacted monomers. It may exaggerate what really happens in the oral environment, but it is beneficial as it represents the worst-case scenario of the whole process. Therefore, in this study, two immersion media were used, DW and 75%/25% ethanol/DW. Although glycerol dimethacrylate was present in a very small quantity in the FP liquid composition (1-5%), HPLC LC was able to identify it. A later MSDS [24] for FP included this as an additional monomer. Therefore, the HPLC method used was suitable for analyzing residuals from experimental, home and commercial RMGICs.

Commercial FP showed less monomer release compared to home and novel materials, with the exception of F3 (containing THFM). This indicates higher degree of polymerization occurring in both FP and F3 and agrees with published literature, utilizing the two monomers THFM and HEMA, in a 50/50% ratio. These systems (THFM and HEMA, in a 50/50% ratio, mixed with poly[ethyl methacrylate] or in a RMGIC formulation) also presented with respectively lower water uptake [29] and higher exotherm [14], thus indicating a higher degree of polymerization occurring within.

Experimental F1 showed the highest monomer release compared to all materials, which suggests a lower degree of conversion of the monomer, HPM. This was also true for samples whether immersed in DW or ethanol/DW. The only difference was that

samples immersed in ethanol/DW showed a higher release of monomers, as explained earlier.

All RX samples also showed release of an additional compound, which was detected at a retention time of ~1 minute, using HPLC. Therefore, HPLC-MS was conducted on all samples in this group to identify this compound. It was confirmed that this compound was poly(acrylic acid), which according to the manufacturers MSDS, had a molecular weight of ~2000. It should be noted that the release of poly(acrylic acid) was not evident in the FP compositions. This finding might be related to the level silane treatment of glass (fluoroalumino-silicate) used in commercial RX powder, which rendered it highly hydrophobic. It is postulated that this silane treated glass (hydrophobic) will not react with all of the poly(acrylic acid), which is hydrophilic, and this will result in some of it being released [30].

In the RX group, R3 and R4 presented with similar or lower release of monomers compared to commercial and home materials, in both solutions, similar to F3 in FP group. R1 in ethanol/DW solution showed higher HPM monomer release. Similar to F1, R1 showed a lower degree of polymerization of the HPM monomer, compared to HEMA monomer. This agrees with published literature that showed reduced reactivity of systems containing HPM, compared to similar systems containing HEMA [16].

RX samples showed significantly lower cell viability compared to the control and FP group. Since the HPLC results showed lower monomer release, with evidence of poly(acrylic acid) released from the RX group compared to the FP group, it can be assumed that the cytotoxicity was rather a result of the acid released (and not monomer leached). This was confirmed by the change in color of the culture medium in which the samples were placed, which indicated acidity of the medium, and this has been

documented previously [31]. Therefore, the cytotoxicity was linked to the high acidity rather than cytotoxicity of the monomers.

It can be confirmed that all RX group materials showed highly cytotoxic behavior. This was in agreement with Pontes *et al.* (2014), who concluded that RX produced a very 'intense' cytotoxic effect and moreover confirmed the acidity of RX supernatants [31]. However, our results did not agree with da Fonseca Roberti Garcia *et al.*, who demonstrated that RX is not considered as a cytotoxic material. It was noted in this study that this cement showed reduced odontoblast cell viability compared to the negative control [32].

In the first experiment (sample surface to liquid area = 47.13 mm^2/mL), the FP group did not show differences between materials and control medium, with the exception of F2 that showed lower cell viability, but higher standard deviations. The second set of experiments included higher surface to liquid area (141.4 mm^2/mL; compared to 47.13 mm^2/mL in the first experiment), in an attempt to show if there were any differences between the FP materials in relation to the monomer used in each varying formulation. The diluted supernatants generally showed significantly similar cell viability compared to the control medium. Promising outcomes were obtained for F3 and F4, since they showed comparable results to the control medium (the negative control). All other materials, including commercial and home, showed significantly less cell viability compared to the control medium. The higher cell viability could be a result of the monomer used in this material (THFM), which partially replaced HEMA, and is known to be a biocompatible material in the dental pulp of monkeys [22].

5 Conclusion

The HPLC methods used for analyzing residuals from experimental, home and commercial RMGICs were confirmed to be suitable. Compositions containing THFM (F3, R3 50%/50% HEMA, and R4 70%/30% HEMA/THFM) showed similar or lower release of corresponding monomers (HEMA and THFM, in addition to UDMA from F3), compared to commercial and home materials, in both solutions (DW and 75%/25% ethanol/DW). This indicates a higher, or similar, degree of polymerization of the monomer in experimental compositions compared to commercial materials, which in turn contributed to the enhanced cell viability of one of these cements. Furthermore, promising results were obtained for F3 (50%/50% THFM/HEMA) and F4 (30%/70% THFM/HEMA) with respect to the second cell culture study with an increased surface area, which showed insignificant differences compared to the control medium. Hence, THFM partially replacing HEMA appears to be a suitable alternative for producing biocompatible RMGICs.

Acknowledgments:

We acknowledge the help and support from Dr. Lingzhi Gong in developing the HPLC methods in this study. We acknowledge the initial funding from Damascus University, Syria and the remaining funding from Barts and the London School of Medicine and Dentistry, Queen Mary University of London, United Kingdom.

6 Reference:

[1] Smith DC. Development of glass-ionomer cement systems. Biomaterials 1998; 19:467–78.

[2] Kakaboura A, Eliades G, Palaghias G. An FTIR study on the setting mechanism of resin-modified glass ionomer restoratives. Dent Mater 1996; 12:173–8.

[3] Calixto LR, Tonetto MR, Pinto SCS, Barros ED, Borges AH, Lima FVP, de Andrade MF, Bandéca MC. Degree of conversion and hardness of two different systems of the vitrebondTM glass ionomer cement light cured with blue LED. J Contemp Dent Pract 2013; 14:244-9.

[4] Stanislawski L, Daniau X, Lautié A, Goldberg M. Factors responsible for pulp cell cytotoxicity induced by resin-modified glass ionomer cements. J Biomed Mater Res 1999; 48:277-88.

[5] Gerzina TM, Hume WR. Diffusion of monomers from bonding resin-resin composite combinations through dentine in vitro. J Dent 1996; 24:125-8.

[6] Hamid A, Okamoto A, Iwaku M, Hume WR. Component release from light-activated glass ionomer and compomer cements. J Oral Rehabil 1998; 25:94-9.

[7] Beriat NC, Nalbant D. Water Absorption and HEMA Release of Resin-Modified Glass-Ionomers. Eur J Dent 2009; 3:267–72.

[8] Souza PPC, Aranha AMF, Hebling J, Giro EMA, Costa CA d. S. In vitro cytotoxicity and in vivo biocompatibility of contemporary resin-modified glass-ionomer cements. Dent Mater 2006; 22:838-44.

[9] Aranha AMF, Giro EMA, Souza PPC, Hebling J, de Souza Costa CA. Effect of curing regime on the cytotoxicity of resin-modified glass-ionomer lining cements applied to an odontoblast-cell line. Dent Mater 2006; 22:864-9.

[10] Lan WH, Lan WC, Wang TM, Lee YL, Tseng WY, Lin CP, Jeng JH, Chang MC. Cytotoxicity of conventional and modified glass ionomer cements. Oper Dent 2003; 28:251-9.

[11] Bouillaguet S, Wataha JC, Virgillito M, Gonzalez L, Rakich DR, Meyer JM. Effect of sub-lethal concentrations of HEMA (2-hydroxyethyl methacrylate) on THP-1 human monocyte-macrophages, in vitro. Dent Mater 2000; 16:213-7.

[12] Sidhu S, Nicholson J. A Review of Glass-Ionomer Cements for Clinical Dentistry. J Funct Biomater 2016; 7:16.

[13] Agha A, Parker S, Patel MP. The properties of experimental resin-modified glass-ionomer luting cements (RMGICs) containing novel monomers. Dent Mater 2017; 33:1331-9.

[14] Agha A, Parker S, Patel M. Polymerization shrinkage kinetics and degree of conversion of commercial and experimental resin modified glass ionomer luting cements (RMGICs). Dent Mater 2020; 36:893-904.

[15] Atai M, Watts DC, Atai Z. Shrinkage strain-rates of dental resin-monomer and composite systems. Biomaterials 2005; 26:5015-20.

[16] Patel MP, Johnstone MB, Hughes FJ, Braden M. The effect of two hydrophilic monomers on the water uptake of a heterocyclic methacrylate system. Biomaterials 2001; 22:81–6.

[17] Mitra SB. Photocurable ionomer cement systems. US Patent 1992; 5,130,347.

[18] Anstice HM, Kanchanavasita W, Pearson GJ, Schottlander BD, Sherpa AL. Polymerizable cement compositions. US Patent 2001; 6,313,192.

[19] Braden M, Patel MP, Pearson GJF. Fluoride releasing biomaterials. UK Patent application 1995; 95,011,839.

[20] Braden M, Davy KWM, Downes S, Patel MP. The use of biomaterials for tissue repair. UK Patent 1991; 91,244,871.

[21] Glace WR, Ibsen RL. Provisional crown-and-bridge resin containing tetrahydrofuryl methacrylate. US Patent 1981; 4,264,489.

[22] Pearson GJ, Picton DC, Braden M, Longman C. The effects of two temporary crown materials on the dental pulp of monkeys (Macaca fascicularis). Int Endod J 1986; 19:121–4.

[23] Pitiyage GN, Slijepcevic P, Gabrani A, Chianea YG, Lim KP, Prime SS, Tilakaratne WM, Fortune F, Parkinson EK. Senescent mesenchymal cells accumulate in human fibrosis by a telomere-independent mechanism and ameliorate fibrosis through matrix metalloproteinases. J Pathol 2011; 223:604-17.

[24] Fuji Plus GC Europe. Safety Data Sheet; 2018:9. https://europe.gc.dental/sites/europe.gc.dental/files/products/downloads/fujiplus/sds/SDS_Fuji_plus_Capsule_GB.pdf [Accessed 25 May 2021].

[25] Attin T, Vataschki M, Hellwig E. Properties of resin-modified glass-ionomer restorative materials and two polyacid-modified resin composite materials. Quintessence Int 1996; 27:203–9.

[26] Khoroushi M, Keshani F. A review of glass-ionomers: From conventional glass-ionomer to bioactive glass-ionomer. Dent Res J (Isfahan) 2013; 10:411-20.

[27] Kim YK, Kim K-H, Kwon T-Y. Setting Reaction of Dental Resin-Modified Glass Ionomer Restoratives as a Function of Curing Depth and Post irradiation Time. J Spectrosc 2015; 2015:462687.

[28] Altintas SH, Usumez A. Evaluation of TEGDMA leaching from four resin cements by HPLC. Eur J Dent 2012; 6:255-62.

[29] Patel MP, Swai H, Davy KWM, Braden M. Water sorption behaviour of polymeric systems based on tetrahydrofurfuryl methacrylate. J Mater Sci Mater Med 1999; 10:147–51.

[30] Adusei GO, Deb S, Nicholson JW. The role of the ionomer glass component in polyacid-modified composite resin dental restorative materials. J Mater Sci Mater Med 2004; 15:751-4.

[31] Pontes ECV, Soares DG, Hebling J, De Souza Costa CA. Cytotoxicity of resin-based luting cements to pulp cells. Am J Dent 2014; 27:237-44.

[32] da Fonseca Roberti Garcia L, Pontes ECV, Basso FG, Hebling J, de Souza Costa CA, Soares DG. Transdentinal cytotoxicity of resin-based luting cements to pulp cells. Clin Oral Investig 2016; 20:1559-66.

RMGIC	**Composition**	**CAS**	**% by weight**	**Mixing ratio (g)**
FP powder	Fluoroalumino-silicate glass	Not listed	95-100	2
FP liquid	Distilled water	7732-18-5	20-30	1
	Poly (acrylic) acid	9003-01-4	20-30	
	HEMA	868-77-9	25-35	
	Urethanedimethacrylate (UDMA)	72869-86-4	<10	
	Tartaric acid	87-69-4	5-7	
RX powder	Fluoroalumino-silicate glass	Not listed	>98	1.6
	Potassium persulfate	7727-21-1	≤0.2	
RX liquid	Water	7732-18-5	30-40	1
	Copolymer of acrylic and itaconic acids	25948-33-8	30-40	
	HEMA	868-77-9	25-35	
	Ethyl acetate	141-78-6	<2	
	Tartaric acid	Not listed	Not listed	

Table 1 Components of the two commercial materials, their quantity, CAS number and manufacturers recommended powder: liquid mixing ratio.

RMGIC	Composition	CAS	%
FP in-house liquid	Distilled water	7732-18-5	30
	Poly (acrylic acid)	9003-01-4	30
	HEMA	868-77-9	31
	Urethanedimethacrylate (UDMA)	72869-86-4	4
	Tartaric acid	87-69-4	5
RX in-house liquid	Water	7732-18-5	35
	Poly (acrylic acid)	9003-01-4	30
	HEMA	868-77-9	29
	Ethyl acetate	141-78-6	1
	Tartaric acid	87-69-4	5

Table 2 Components of the two home liquids, their quantity and CAS number.

Instrument	**HPLC**
Detector and system controller	Agilent 1100 HPLC with UV detector.
Column	Zorbax Eclipse® XDB-C18
Flow rate	1 mL/min
Volume injected	10 µL
Run time (FP group)	30 minutes
Run time (RX group)	8 minutes
Wavelength	210 nm
Mobile phase	ACN in water (gradient condition)

Table 3 HPLC parameters for the two methods used for FP and RX monomer release studies.

Time	Monomer	FP	FP-Home	F1	F2	F3	F4
1 Hour	HEMA	1.07 (0.55)[a]	1.19 (0.35)[a]	-	-	0.32 (0.10)[b]	0.41 (0.19)[b]
	HPM	-	-	6.60 (1.61)	0.78 (0.45)	-	-
	THFM	-	-	-	0	0.53 (0.24)	0.28 (0.12)
	UDMA	0	0	0	0	0	0
	All monomers	1.07 (0.55)[a]	1.19 (0.35)[a]	6.60 (1.61)	0.78 (0.45)[a]	0.86 (0.34)[a]	0.64 (0.33)[a]
4 Hours	HEMA	3.18 (1.61)[a]	14.21 (4.37)	-	-	1.01 (0.26)[a]	3.11 (0.96)[a]
	HPM	-	-	17.70 (2.60)	3.96 (1.97)	-	-
	THFM	-	-	-	2.07 (0.95)[a]	1.96 (0.35)[a,b]	1.27 (0.43)[b]
	UDMA	0	0	4.31 (0.64)[a]	3.76 (0.30)[a]	0	4.43 (0.43)[a]
	All monomers	3.18 (1.61)[c]	14.21 (4.37)[a]	22.01 (2.86)	9.79 (3.20)[a,b]	2.97 (0.61)[c]	7.34 (2.62)[b,c]
1 Day	HEMA	3.23 (1.59)[a]	30.00 (7.20)	-	-	1.21 (0.24)[a]	12.91 (2.25)
	HPM	-	-	36.24 (7.88)	5.47 (1.99)	-	-
	THFM	-	-	-	2.85 (0.99)[a]	2.25 (0.38)[a]	4.16 (0.91)
	UDMA	1.37 (0.75)[b]	1.70 (0.92)[b]	11.89 (1.04)	6.98 (1.12)[a]	0	6.30 (0.81)[a]
	All monomers	4.60 (2.07)[c]	31.70 (7.41)[a]	48.13 (7.32)	15.31 (3.88)[b]	3.45 (0.62)[c]	23.38 (2.65)[a,b]
1 Week	HEMA	3.88 (1.58)[a]	31.86 (7.46)	-	-	1.60 (0.46)[a]	12.35 (1.83)
	HPM	-	-	37.86 (7.37)	6.06 (1.74)	-	-
	THFM	-	-	-	3.02 (0.82)[a]	2.36 (0.55)[a]	5.48 (1.28)
	UDMA	3.58 (1.22)[b]	2.33 (0.61)[b]	12.43 (1.07)	8.06 (2.27)[a]	2.40 (0.72)[b]	6.98 (0.42)[a]
	All monomers	7.46 (2.76)[b]	34.20 (7.93)	50.29 (6.70)	17.14 (4.55)[a]	5.42 (2.11)[b]	24.81 (2.68)[a]

Table 4 Mean release of each monomer and cumulative release of all monomers (ppm) (SD) from each material in the FP group, in DW at different time points (1 hour, 4 hours, 1 day and 1 week). Similar superscript letters indicate no significant difference (p>0.05).

Time	Monomer	FP	FP-Home	F1	F2	F3	F4
1 Hour	HEMA	1.06 (0.42)^{a}	1.91 (0.29)			0.55 (0.11)^{b}	0.84 (0.24)^{a,b}
	HPM			22.10 (8.38)	6.01 (1.76)		
	THFM				2.66 (0.66)	1.19 (0.20)^{a}	0.69 (0.14)^{a}
	UDMA	0.67 (0.19)^{b}	0.14 (0.23)^{b}	1.44 (0.61)^{a,b}	1.57 (0.34)^{a,b}	0.40 (0.06)^{b}	0.41 (0.07)^{b}
	All monomers	1.73 (0.56)^{a}	2.06 (0.46)^{a}	23.55 (8.55)	10.24 (2.61)	2.15 (0.35)^{a}	1.93 (0.39)^{a}
4 Hours	HEMA	2.00 (0.71)^{a}	15.04 (5.09)			1.15 (0.20)^{a}	2.06 (0.76)^{a}
	HPM			77.66 (22.50)	11.78 (1.84)		
	THFM				6.32 (1.82)	2.10 (0.27)^{a}	1.43 (0.34)^{a}
	UDMA	1.41 (0.33)^{c,d}	1.83 (0.38)^{b,c}	2.63 (0.68)^{a,b}	2.88 (0.89)^{a}	0.66 (0.07)^{d}	0.62 (0.11)^{d}
	All monomers	3.41 (1.00)^{b}	16.86 (4.98)^{a,b}	80.29 (22.65)	20.98 (2.43)^{a}	3.91 (0.53)^{b}	25.34 (5.91)^{a}
1 Day	HEMA	2.56 (0.84)^{a}	75.54 (21.64)			3.21 (0.33)^{a}	14.25 (3.65)
	HPM			266.20 (65.76)	64.83 (9.50)		
	THFM				27.35 (3.66)	5.43 (0.58)	10.13 (2.82)
	UDMA	2.79 (0.48)^{a,b}	5.46 (0.62)^{a}	15.36 (4.56)	5.47 (1.23)^{a}	0.91 (0.06)^{b}	0.97 (0.12)^{b}
	All monomers	5.35 (1.19)^{b}	81.00 (22.11)^{a}	281.55 (70.30)	97.66 (14.24)^{a}	9.54 (0.83)^{b}	25.34 (5.91)
1 Week	HEMA	3.47 (0.75)^{a}	76.41 (20.85)			3.35 (0.28)^{a}	16.39 (3.22)
	HPM			267.05 (56.18)	63.84 (8.54)		
	THFM				26.75 (3.44)	5.04 (0.56)	11.54 (3.19)
	UDMA	4.85 (0.32)^{b,c}	8.29 (1.14)^{a}	17.04 (4.21)	5.96 (1.08)^{a,b}	1.77 (0.06)^{c}	2.46 (0.29)^{c}
	All monomers	8.32 (0.95)^{b}	84.69 (21.90)^{a}	284.10 (60.37)	96.55 (12.92)^{a}	10.16 (0.78)^{b}	30.40 (6.04)

Table 5 Mean release of each monomer and cumulative release of all monomers (ppm) (SD) from each material in the FP group, in 75:25 ethanol:DW at different time points (1 hour, 4 hours, 1 day and 1 week). Similar superscript letters indicate no significant difference ($p>0.05$)

Time.	Monomer	RX	RX-Home	R1	R2	R3	R4
1 Hour	HEMA	1.05 (0.60)	0.33 (0.11)[a]			0.29 (0.18)[a]	0.26 (0.11)[a]
	HPM			0.49 (0.17)[b]	0.69 (0.39)[b]		
	THFM					0.56 (0.26)	
	All monomers	1.05 (0.60)[a]	0.33 (0.11)[a,b]	0.41 (0.25)[a,b]	0.69 (0.39)[a,b]	0.76 (0.49)[a,b]	0.26 (0.11)[b]
4 Hours	HEMA	3.65 (0.81)	1.58 (0.28)			0.82 (0.16)[a]	0.66 (0.23)[a]
	HPM			1.87 (0.57)[a]	2.21 (0.56)[a]		
	THFM				1.06 (0.26)	1.88 (0.35)	0.56 (0.23)
	All monomers	3.65 (0.81)[a]	1.58 (0.28)[c]	1.87 (0.57)[b,c]	3.27 (0.74)[a]	2.70 (0.46)[a,b]	1.22 (0.46)[c]
1 Day	HEMA	3.83 (0.86)	3.05 (0.22)			0.85 (0.16)[a]	1.28 (0.26)[a]
	HPM			1.88 (0.87)[a]	2.13 (0.58)[a]		
	THFM				1.19 (0.32)	2.09 (0.41)	0.66 (0.27)
	All monomers	3.83 (0.86)[a]	3.05 (0.22)[a,b]	1.88 (0.87)[b]	3.32 (0.90)[a]	2.94 (0.53)[a,b]	1.93 (0.52)[b]
1 Week	HEMA	3.34 (0.82)	1.81 (0.25)			0.87 (0.18)[a]	0.74 (0.23)[a]
	HPM			1.34 (0.48)	1.92 (0.53)		
	THFM				0.66 (0.19)[a]	1.44 (0.41)	0.64 (0.27)[a]
	All monomers	3.34 (0.82)[a]	1.81 (0.25)[b,c]	1.34 (0.48)[c]	2.58 (0.71)[a,b]	2.31 (0.39)[b,c]	1.39 (0.49)[c]

Table 6 Mean release of each monomer and cumulative release of all monomers (ppm) (SD) from each material in the RX group, in DW at different time points (1 hour, 4 hours, 1 day and 1 week). Similar superscript letters indicate no significant difference (p>0.05).

Time	Monomer	RX	RX-Home	R1	R2	R3	R4
1 Hour	HEMA	1.10 (0.18)	0.45 (0.09)			0.27 (0.08)[a]	0.18 (0.05)[a]
	HPM			1.61 (0.36)	0.28 (0.09)		
	THFM					0.19 (0.16)	
	All monomers	1.10 (0.18)	0.45 (0.09)[a]	1.61 (0.36)	0.28 (0.09)[a]	0.47 (0.23)[a]	0.18 (0.05)[a]
4 Hours	HEMA	2.96 (0.84)	1.15 (0.38)[a,b]			1.80 (0.88)[a]	0.34 (0.04)[b]
	HPM			3.62 (1.26)	0.99 (0.18)		
	THFM				0.22 (0.18)	0.54 (0.32)	
	All monomers	2.96 (0.84)[a]	1.15 (0.38)[b,c]	3.62 (1.26)[a]	1.20 (0.25)[b,c]	2.33 (1.10)[a,b]	0.34 (0.04)[c]
1 Day	HEMA	6.39 (2.00)[a]	7.08 (0.69)[a]			3.73 (0.39)[b]	3.26 (0.56)[b]
	HPM			7.27 (1.74)	1.91 (0.52)		
	THFM				0.61 (0.27)[a,b]	0.75 (0.23)[a]	0.34 (0.13)[b]
	All monomers	6.39 (2.00)[a,b]	7.08 (0.69)[a]	7.27 (1.74)[a]	2.52 (0.77)[c]	4.47 (0.49)[b,c]	3.60 (0.67)[c]
1 Week	HEMA	5.84 (1.39)[a]	4.90 (0.74)[a]			3.29 (0.29)[b]	3.36 (0.35)[b]
	HPM			6.90 (1.60)	1.97 (0.55)		
	THFM				0.57 (0.30)[a]	0.99 (0.39)	0.36 (0.11)[a]
	All monomers	5.84 (1.39)[a,b]	4.90 (0.74)[b,c]	6.90 (1.60)[a]	2.54 (0.84)[d]	4.28 (0.63)[b,c,d]	3.72 (0.45)[c,d]

Table 7 Mean release of each monomer and cumulative release of all monomers (ppm) (SD) from each material in the RX group in 75:25 ethanol:DW at different time points (1 hour, 4 hours, 1 day and 1 week). Similar superscript letters indicate no significant difference (p>0.05).

Material	Mean cells viability, % (SD)	p values of materials vs. medium	p values of materials vs. commercial	p values of materials vs. home
Medium	100.00 (0.00)	-	-	-
FP	85.95 (29.77)	0.4596	-	-
FP-Home	90.90 (9.29)	0.1651	0.7969	-
F1	75.95 (17.88)	0.0803	0.6440	0.2680
F2	82.96 (8.77)	0.0282	0.8755	0.3422
F3	107.29 (24.26)	0.6302	0.3903	0.3360
F4	94.06 (19.48)	0.6254	0.7131	0.8125
RX	-0.57 (4.67)	0.0001	-	-
RX-Home	0.44 (3.90)	0.0001	0.7878	-
R1	3.82 (1.87)	0.0001	0.2059	0.2478
R2	5.85 (3.80)	0.0001	0.1388	0.1606
R3	1.51 (3.87)	0.0001	0.5849	0.7533
R4	0.96 (4.65)	0.0001	0.7083	0.8894

Table 8 Percentage of viable cells (SD) following treatment with materials' aliquots (commercial, home and novel) for both FP and RX groups (surface area = 70.7mm^2), and p values comparing materials with medium (negative control), commercial and home materials, n=3 (vs. = versus).

Material	Mean cells viability, % (SD)	p values of materials vs medium	p values of materials vs FP commercial	p values of materials vs FP home
Medium	100 (0.00)	-	-	-
FP	25.18 (16.19)	0.0001	-	-
FP-Home	36.88 (43.19)	0.0265	0.6299	-
F1	20.88 (25.11)	0.0007	0.7831	0.5453
F2	13.87 (8.90)	0.0001	0.2667	0.3367
F3	55.04 (60.12)	0.1854	0.3744	0.6411
F4	69.62 (55.77)	0.3177	0.1768	0.3892
Medium 1:1	100 (0.00)	-	-	-
FP 1:1	114.12 (22.35)	0.2534	-	-
FP-Home 1:1	123.33 (45.67)	0.3464	0.7296	-
F1 1:1	47.55 (70.67)	0.1883	0.1226	0.1218
F2 1:1	141.56 (41.97)	0.0949	0.2922	0.5779
F3 1:1	129.23 (37.92)	0.1741	0.5179	0.8489
F4 1:1	154.75 (36.01)	0.0228	0.1036	0.3214

Table 9 The percentage of viable cells (SD) following treatment with materials' aliquots (commercial, home and novel) from the FP group (surface area = 282.8mm^2), both neat and diluted with a similar volume of control medium. Also p values comparing materials vs medium (negative control), home and FP commercial.

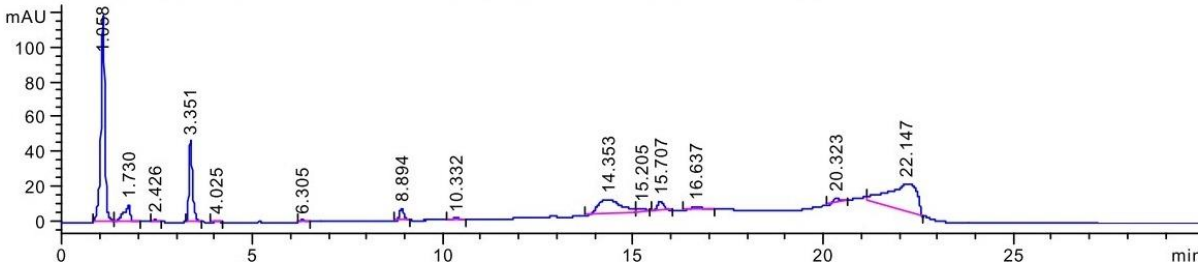

Figure1 A typical HPLC chromatogram of FP sample following 1-day immersion in DW.

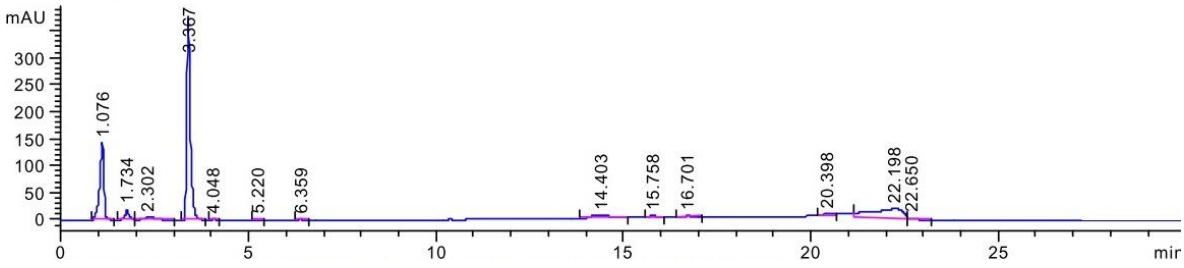

Figure 2 A typical HPLC chromatogram of FP-Home sample following 1-day immersion in DW.

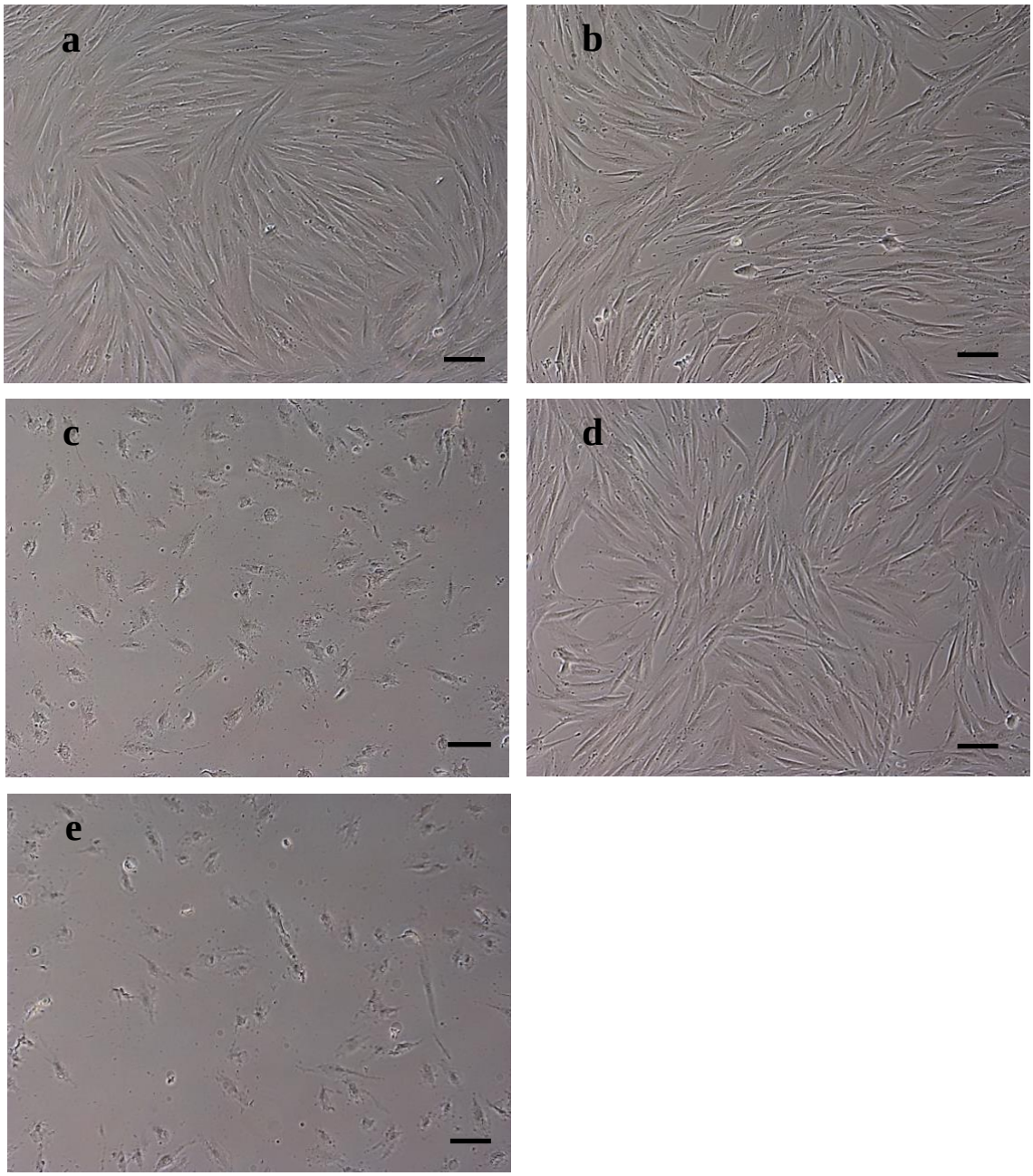

Figure 3 Effects of materials on NHOF-1 cells in culture: (a) medium (negative control), (b) FP, (c) RX, (d) F3, (e) R1. Scale bar equals 100 µm. Viable cell counts: medium 2.0 x 10^5, FP 1.85 x 10^5, F3 1.95 x 10^5, R1 0 (<2.5 x 10^3), RX 0 (<2.5 x 10^3). Mean population doublings in 72 hours: Control 1.05 x 10^5, FP 0.94 x 10^5, F3 1.02 x 10^5, R1 0 (<2.5 x 10^3), RX 0 (<2.5 x 10^3).