# TESTING OF NEW EXPERIMENTAL GIOMERS: WATER SORPTION, CONVERSION DEGREE, RADIOPACITY, MICROSTRUCTURE AND BIOLOGICAL BEHAVIOR

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**ABSTRACT.** Two experimental giomers (G1 and G2) were obtained and then evaluated for water sorption- by weighing the specimens before and after water immersion, for conversion degree (DC)- by Fourier Transform Infrared Spectrometry, for radiopacity- by using the intraoral sensor system Ez Sensor 1.5 Vatech E-Woo (Korea), in relation to their microstructural characteristics assessed by Scanning Electron Microscopy (SEM). They were compared to a commercial giomer: Beautifil II. Both experimental and commercial giomers were also tested by subcutaneous and intramuscular implantation tests, to establish and compare their biological behavior. Intensity of the inflammatory reaction, tissue repair status and the presence of the capsule were the main criteria assessed. After 7 days, the mean values of DC were: 65.8% (Beautifil II), 62.2% (G2) and 60.5 (G1). DC increased after the initial polymerization. Giomers showed mean values of water sorption, below 40 µg/mm3. A certain swelling of the polymer matrix without dislocations of particles could be seen on SEM images. The mean values of radiopacity were between 2.15-2.99 [mmAl]. Giomers were slight to moderate irritants for soft tissues, with no significant difference between the samples. Promising results make G1 and G2 possible alternative to Beautifil II, that can be further improved.

*Keywords: giomer, conversion, water sorption, radiopacity, SEM, biocompatibility*

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## INTRODUCTION

Giomers represent a new class of direct restoration materials, with fluoride controlled release, offering both protection against caries, but also, superior aesthetic properties, high radiopacity, and easy handling. These are hybrid materials that combine the characteristics of composite resins with those of glass ionomers. The main characteristic of giomers consists in the pre-reacted glass ionomer from their composition, previously obtained after a chemical reaction between a fluoro-aluminosilicate glass and a polyacrylic acid [1], [2]. Giomers are available on the dental materials market in different shapes, with different consistencies, depending on their area of applicability (for reconstruction of tooth anatomy, for marginal ridges, cavity liners, and small restorations, etc.) [3-7].

The degree of conversion (DC) is mostly influenced by the structure and relative ratio of each monomers of polymer matrix. DC is also influenced by the filling component of the material. Fillers based on Ba or Zr can improve radiopacity, but at the same time they can decrease the degree of conversion due to the fact that light can no longer penetrate so deep into the material. The proportions and type of components of the initiation system, but also the light source used, the time and the distance of polymerization are just some factors that can influence the DC of composite resins, respectively of the giomers. As the data in the literature show, the conversion is never complete, with a reported DC of 50% to 75% for the conventional composites and respectively of 50% to 81% for the bulk-fill composite materials [8].

Sorption can lead to the swelling of the polymeric matrix and to release of unreacted monomers or filler ions, but it may also affect the optical properties, so the longevity of restorations materials may be reduced. Therefore, although the fluoride-releasing property by the giomers is supported by the ability of water diffusion, a large amount of water can lead to plasticization of the polymer network negatively influencing the properties of the material [5], [9].

Radiopacity is one of the essential properties of all restorative materials including giomers. Adequate radiopacity of the material allows the clinician to differentiate secondary caries formation from restoration and surrounding tooth structure, to evaluate and detect voids, overhangs and open margins. In addition, studies conclude that, for optimum contrast, a restorative material with a radiopacity slightly greater than or equal to that of enamel is ideal for the detection of secondary caries on radiographs [10], [11].

The aim of this study was to evaluate and compare water sorption, conversion degree and radiopacity of two experimental giomers (G1 and G2) in relation to their microstructural characteristics, compared to the commercial giomer: Beautifil II (Shofu, Japan).

## RESULTS AND DISCUSSION

### 1. Degree of conversion (DC)

The degree of conversion (DC) represents a fundamental parameter governing mechanical properties and biocompatibility of giomer materials, also influencing the water sorption.

After polymerization, a crosslinked three-dimensional polymer network which contains a significant amount (percent) of unreacted methacrylate groups (residual double bonds, RDB) is formed. Most of the RDB are pendant methacrylate groups attached to the polymer network and a small proportion of them (ca. 10%) represents the free residual monomer [12]. The frequently used technique for DC determination is FTIR. DC is determined by the proportion between remaining aliphatic C=C double bonds' concentration in the cured giomers reported to the total number of C=C bonds in the uncured giomers [13].

As shown in Figure 1, the conversion degree increases between the first and seventh day after the initial polymerization. This increase in conversion is more pronounced in the case of G1 (8.3%), G2 (7.8%), compared to Beautifill II (4.5%).
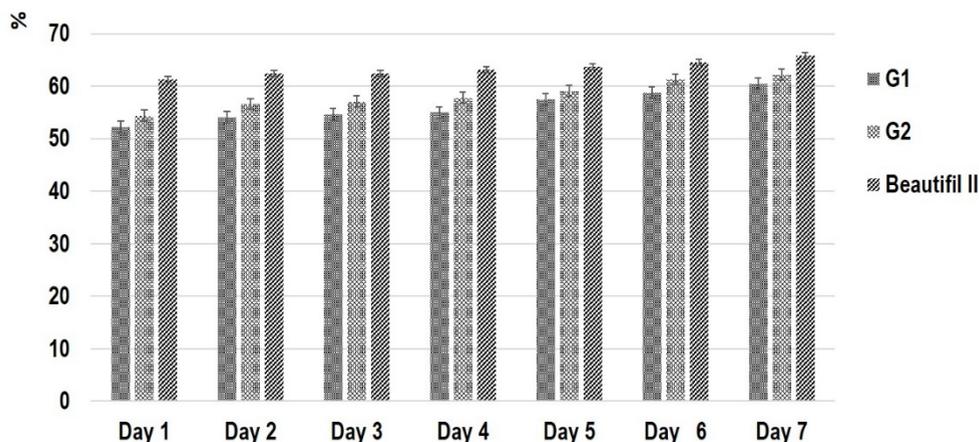


**Figure 1.** Degree of conversion of investigated giomers

For all the tested giomers (Figure 1), a daily increase of DC was found until the end of the investigation period, except on the 3rd day when the same value of DC was recorded as in the previous day, for the Beautifil II giomer.

The highest degree of conversion was recorded in case of Beautifil II, of 61.3% after the first day post polymerization and 65.8% after the 7th day post polymerization. After the first day, for the experimental giomer G2 a DC of 54.4% was registered and for G1 a DC of 52.2%. After 7 days from the polymerization a DC of 62.2% was registered for G2 respectively 60.5% for G1. It can be seen that the polymerization continued during the 7 days with a more significant increase from the first to the 7th day after polymerization for the experimental giomers.

## 2. Water sorption

The water sorption phenomena are mainly influenced by the structure of the polymer network, the nature of the inorganic fillers and the quality of the polymer/ filler interface. Small water molecules, associated by hydrogen bonds, can interact with the polar groups of the polymer, water sorption being influenced by the position of these groups in the three-dimensional polymer network. In case of giomers, the diffusion of water or aqueous solutions like saliva is a requirement in order to achieve the performance of continuous release of fluoride ions. The release of fluoride ions is conditioned by the ability of the material to allow the diffusion of water in its structure [14], [15].

The current study compared the water sorption behavior of a commercial giomer material with two different experimental giomers of varying composition. This was done over a period of 1 week. Mean values of water sorption/day for each giomers are presented in Figure 2.
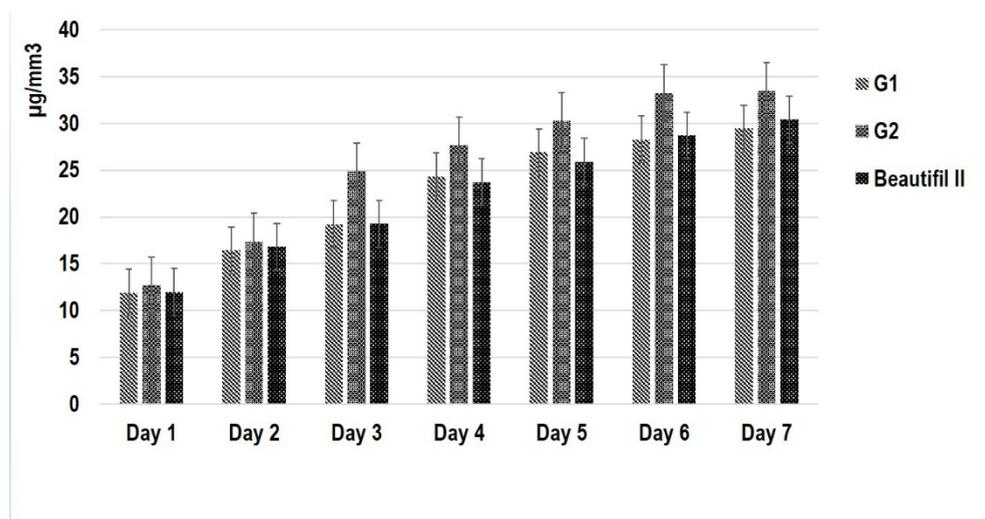


**Figure 2.** Water sorption of the investigated giomers

As it can be seen from Figure 2, for all tested giomers the water sorption increases all throughout the investigation period. After the first day, the lowest average value (11.89μg/mm$^3$) was recorded for the experimental giomer G1, similar to that of the Beautifil II giomer (11.99μg/mm$^3$). Also at the end of the investigation period, the lowest value of water sorption was recorded for G1 giomer, (29.44μg/mm$^3$), slightly lower than the average value recorded for Beautifil II (30.4μg/mm$^3$). The average value recorded, after day 7, for the experimental giomer G2 was 33.5 μg/mm$^3$. However, it was found that the water sorbtion for G2 giomer was the highest (24.91 μg/mm$^3$) on the 3rd day, more by 7.53 μg/mm$^3$ than on day 2 and on day 7 it absorbed only 0. 27 μg/mm$^3$ more than on the 6th day. Beautifil II absorbed on the 7th day with 1.7 4μg/mm$^3$ more water than on the 6th day and G1 with 1.14 4μg/mm$^3$ more water than on the 6th day. All this information leads us to the assumption that although the experimental G2 giomer recorded the highest values of water sorption, towards the end of the investigation period, sorption increased much slower compared to the other two giomers, with a alleged tendency to decrease in the following days if the study would have continued.

Thus, after 1 week, all the tested materials showed acceptable water sorption, below 40 μg/ mm3 (the maximum water sorption stated by the ISO 4049) [16].

Therefore, the difference in the water sorption value of the materials was due to the composition of each material. This was in agreement with several studies in terms of the maximum amount of water sorption gained within the first week [17], [18].

The water sorption values for G1, G2, Beautifil they are quite close. This behavior suggests that water sorption is influenced in this case by the nature of the polymer matrix in the giomers, and much less by the nature of the hybrid filling.
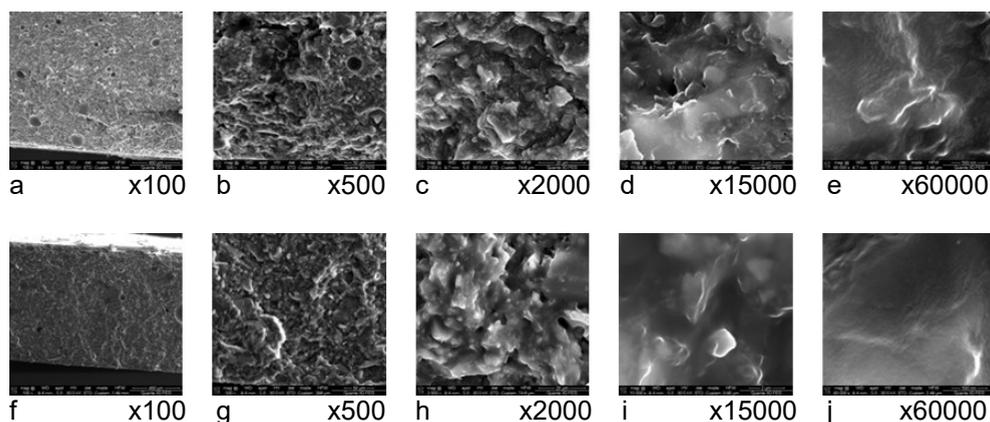
McCabe and Rusby 2004 reported that the nature and hydrophilicity of the resin matrix is a major parameter which may regulate rate and extent of water diffusion. The investigated materials contain bisphenol-A-glycol dimethacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) oligomers in their matrices. These types of polymers are known of their high hydrophilic nature that might be attributed to strong hydrogen bonds formed between their hydroxyl groups and water molecules. This explains their increasing tendency for water sorption. Compared to other oligomers, TEGDMA is more heterogeneous in composition and has higher flexibility. The more heterogeneous the network, the larger are the micropores created between polymer clusters and the larger is the quantity of absorbed water. Being flexible, TEGDMA chains become more liable to swell and accommodate higher amounts of water. This explains the ability of materials to absorb water.

The resin matrix composition may not be the only factor that affects the amount of absorbed water. Giomers in presence of surface pre-reacted glass polyacid zones have the capability to generate an osmotic pressure that potentially increases water sorption [19], [20].

The difference in the composition of G1 and G2 materials is the type of pre-reacted glass. In the case of G2 material, the polyalkenoic acid that enters in the composition of the pre-reacted glass was grafted with methacrylic groups. By grafting, the degree of crosslinking increased and so did the degree of conversion. Also, the fact that meshes formed in the polymer network due to crosslinking, led to the predisposition of the G2 material to a higher release of fluorine, respectively to a higher water absorption, due to the diffusion phenomenon.

## 1. Scanning Electron Microscopy (SEM)

For this evaluation a scanning electron microscope (Quanta 3D FEG) was used. As an example, in Figure 3 SEM images of the G2 giomer are shown: the initial state and the appearance after 7 days of storage in water. A relative degree of swelling of the polymer matrix can be observed without dislocations of particles from the polymer matrix (Figure 3).



a    x100   b    x500   c    x2000   d    x15000   e    x60000

f    x100   g    x500   h    x2000   i    x15000   j    x60000

**Figure 3.** SEM images of the G2 experimental giomer (initial state a-e; after 7 days of storage in water f-j) at magnifications of: x100, x 500, x2000, x 15000, x60000
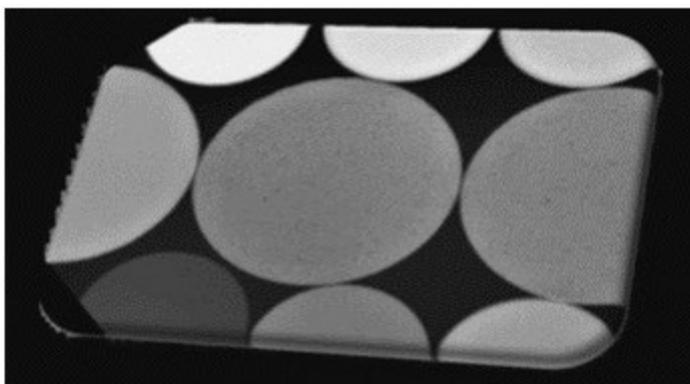
## 2. Radiopacity

Restorative materials should ideally be radiopaque to enable visualization and assessment by radiograph, and all newly developed materials should, therefore, be investigated in this order.

According to the International Standards Organization for Standardization (ISO 4049) [21], the radiopacity of such materials should be equal to or greater than the same thickness of aluminum. The radiopacity values in the case of experimental giomers G1 and G2 were lower than the value obtained for Beautifil II (Table.1, Figure 4.), but higher than the limit imposed by ISO 4049 (1mm Al) [21]. The dentin and enamel reference radiopacity values used in the present study were 1.09 ± 0.0 and 1,84 ± 0.0 mm Al, respectively. The results show that the radiopacity values of all the tested materials were greater than those of enamel and dentin, which means that none of the tested materials could be misinterpreted as dentinal caries on the radiographie.

**Table 1.** Radiopacity mean values of the investigated giomers

| Sample | Beautifil II | G1 | G2 |
|---|---|---|---|
| Radioopacity [mmAl] | 2.99 | 2.15 | 2.21 |



**Figure 4.** Radiopacity of giomers (In the middle line from left to right: Beautifil II, G1, G2 giomer. Third and first lines: The aluminum step wedges (thickness increasing by 1mm for each step to a maximum of 6 mm)

## 3. Implantation tests

Subcutaneous and intramuscular inoculation were well tolerated by all subjects. After the implantation, no changes in general status and behavior were noticed. Healing of the skin wound at the implant site occurred without any complications. Implants were well tolerated with a very short convalescence time, clinically insignificant, without rejection signs. Macroscopically, there was no difference between the groups. Neither necrosis, nor hemorrhage or granulative tissue were noticed around the implants.

Concerning the *subcutaneous implantation*, when the skin was removed, the implant's body appeared attached to the connective tissue and was wrapped in a transparent capsule. The body of the implant was well fixed in a cavity formed by connective tissue proliferation.

From microscopic point of view, the tissues surrounding the implants showed mild to moderate, chronic, inflammatory reaction. In case of G1 the repair process was in an early stage and a thin, fragile, capsule could be identified in contact with the implant (Figure 5). In case of the G2 and Beautifill, the inflammatory reaction was milder, and the surrounding tissue exhibited a moderate repair process.
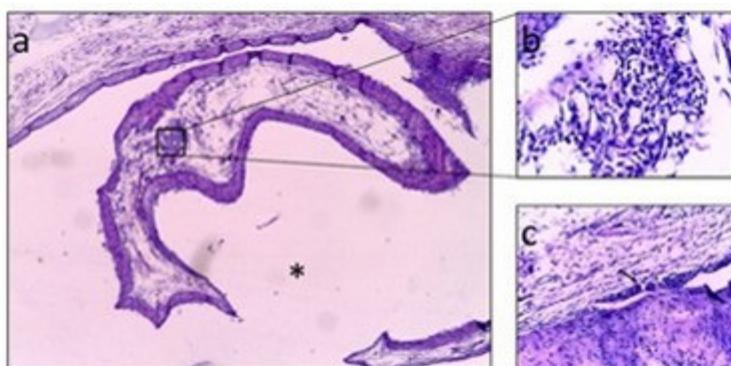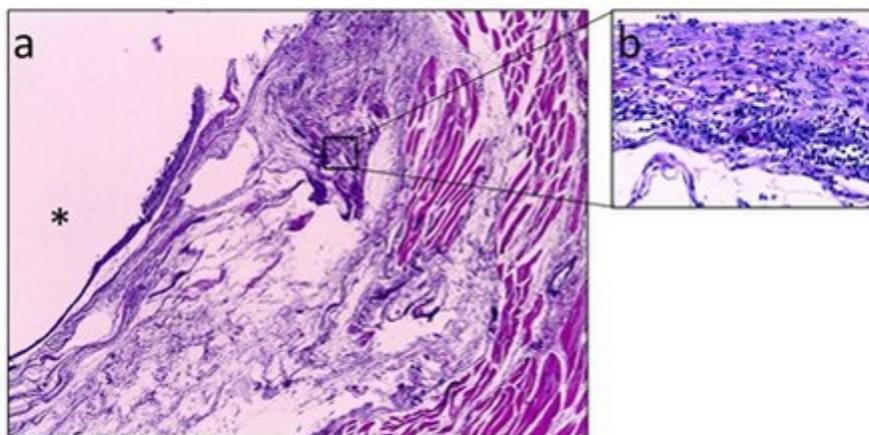


**Figure 5.** Subcutaneous implant with G1 giomer
* The place where the implant was a) 4x Magnification – overview -fybroconjunctive capsule in the vicinity of the implant; b) 40x Magnification – lympho- plasmocitary inflammatory infiltrate c) 20x Magnification – giant multinuclear cell

In case of *intramuscular implantation*, specimen analysis revealed moderate fibrosis and slight to moderate inflamatory reaction. Regenerating muscle cells could be seen in the tissue next to the implants, demonstrating the tendency of the muscle to restore to the normal structure.

In case of G1, the tissue around the implant showed foci of inflamatory cells (lymphocytes, PMN and plasma cells) along with few new blood vesselles. The inflamatory reaction was milder in case of G2 was and characterized by the presence of lymphocytes, plasmocytes but also a minimal amount of adipose tissue associated with fibrosis. For Beautifil II fewer lymphocytes, plasmocytes, macrophages and PMNs were observed in the dense fibrous capsule and around the blood vessells (Figure 6).

**Figure 6.** Intramuscular implant with giomer G2
* - the place where the implant was a) 4x Magnification – overview -fybroconjunctive capsule organization around the implant with connective tissue; b) 40x Magnification- inflammatory infiltrate Lympho- plasmocitary (the most representative part)

After evaluating each criterion of inflammation (presence of inflammatory cells, necrosis, neovascularization, fibrosis and adipose infiltration) according to ISO standards and calculating the final score, all tested materials were ranked as slight to moderate irritants for the soft tissues, with G2 and Beautifil II having almost the same scores regarding the biological behaviour. No statistically significant differences were found between the 3 groups regarding the inflammatory response of the subcutaneous or muscular tissue exposed to the experimental materials by implantation tests (Table 2 and Table 3).

**Table 2.** Scores of inflammation and materials ranking after subcutaneous implantation

| Parameter | G1 subcutaneous Mean± SD | G2 subcutaneous Mean± SD | Beautifil II subcutaneous Mean± SD |
|---|---|---|---|
| PMN | 0.67 +/0.58 | 1+/-0.58 | 1+/-0.57 |
| Lymphocytes | 2.33+/-0.58 | 2+/-1 | 2/-1 |
| Plasmocytes | 1+/-0 | 1+/-0 | 1+/-0 |
| Macrophages | 0 | 0 | 0 |
| Giant cells | 0.67+/-0.58 | 0 | 0 |
| Necrosis | 0 | 0 | 0 |
| SUB-TOTAL(x2) | 9.33+/- 1.15 | 6.66+/-2.30 | 7.33+/-1.54 |
| Neovascularization | 1.33+/-0.58 | 1.33+/-0.58 | 1.33+/-0.58 |
| Fibrosis | 1+/-0 | 1+/-0 | 1+/-0 |
| Adipose infiltration | 0 | 0 | 0 |
| SUB-TOTAL | 2.33+/-0.57 | 2+/-0 | 2.33+/-0.57 |
| **TOTAL** | **11.6+/-1.52** | **9 +/- 1.73** | **9.6+/-0.57** |

**Table 3.** Scores of inflammation and materials ranking after intramuscular implantation

| Parameter | G1 im Mean± SD | G2 im Mean± SD | Beautifil II im Mean± SD |
|---|---|---|---|
| PMN | 1+/-0 | 0.33+/-0.58 | 0.33+/-0.58 |
| Lymphocytes | 1.67+/-0.58 | 1.67+/-0.58 | 1.33+/-0.58 |
| Plasmocytes | 1+/-0 | 1+/-0 | 0.67+/-0.58 |
| Macrophages | 0 | 0 | 0.33+/-0.58 |
| Giant cells | 0 | 0 | 0 |
| Necrosis | 0 | 0 | 0 |
| SUB-TOTAL(x2) | 7.33+/-1.15 | 6+/-2 | 5.33+/-2.30 |
| Neovascularization | 1.33+/-0.58 | 1+/-0 | 1.33+/-0.58 |
| Fibrosis | 1+/-0 | 1+/-0 | 1+/-0 |
| Adipose infiltration | 0 | 0.33+/-0.58 | 0 |
| SUB-TOTAL | 2.33+/-0.57 | 2.66+/-1.15 | 2.33+/-0.57 |
| **TOTAL** | **9.6+/-4.35** | **8.3+/-1.52** | **7.6+/-2.86** |

The favorable biological behavior induced by the giomers is due to their chemical and physical properties.

One of the most critical aspects of using polymer based composites for medical purposes is the release of residual monomer, which can induce cytotoxic, inflammatory, allergic and even mutagenic reactions [22]. The optimal conversion degree observed for the experimental giomers correlates with a lesser quantity of residual monomer release. Also, the quantity of residual monomer released by the giomers is influenced by the hydrophilic/hydrophobic character as well as by the amount, size and flexibility of the each monomer's specific molecules. Bis-GMA oligomers have large, rigid molecules and stronger hydrophobic character compared to the other monomers. This leads to a significantly smaller quantity of extracted Bis-GMA monomer in a hydrophilic medium [23], [24].

The biological response to the implanted materials depends not only on the chemical properties of the materials but also on the response to the trauma of surgery. Proper surgical technique, adequate preparation of the samples ensures valid results for the implantation test [25, 26].

## CONCLUSIONS

Seven days after polymerization, a more pronounced increase in DC was found in G1 (8.3%) and G2 (7.8%), compared to Beautifill II (4.5%), which means that the polymerization was efficient and exhibited an ascending course. At the end of the investigation period, the decreasing order of water sorption for the studied giomers was: G2>Beautifil II> G1. Still, the process of water sorption reached a stable level in case of G2, while for G1 and Beutifil II an ascending trend was noted, with the probability of increase in the following days, in case the study would have continued. Comparing the SEM images before and after 7 days of storage in water, a swelling of the polymer matrix was observed, but without detachment of any portions of the material. The highest radiopacity was recorded in the case of the commercial giomer Beautifill II, but also all the tested giomers have values of radioopacity over those of enamel and dentin. All the tested materials were slight to moderate irritants for living tissues, with G2 and Beautifill II inducing almost the same reactions.

Since the difference in the composition of the experimental giomers is given only by the pre-reacted glass used, the small differences between the results obtained from the tests performed are given by the two polyalkenoic acids used in the syntesis of the pre-reacted glasses.

Promising results make G1 and G2 a possible alternative to Beautifil II, that can be further improved.

## EXPERIMENTAL SECTION

### Materials

2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA) camphorquinone (CQ), dimethylaminoethyl-methacrylate (DMAEM), butylated hydroxy toluene (BHT), were purchased from Sigma Aldrich Chemical Co. (Taufkirchen, Germany) and used without additional purification.

Beautifil II giomer - shade A30- was purchased from Shofu, Japan, Bach no. 051215 (PN1420 2015-04).

### Preparation of experimental giomer pastes

The experimental light-curing giomers were prepared as monopastes by mixing the resin matrices with the hybrid fillers.

The experimental resins were formulated using monomer mixtures of 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane (Bis-GMA) as

base monomer and triethylene glycol dimethacrylate (TEGDMA)) as diluting monomer. The ratio between the base monomer and diluting monomer was 70/30. In the composition of the resins, besides the methacrylic oligomers and monomers, a photosensitizer, camphorquinone (CQ) in an amount of 0.5% (by weight), and an accelerator dimethylaminoethyl-methacrylate (DMAEM), in an amount of 1% (by weight), were added. Butylated hydroxy toluene (BHT) was added in a quantity of 650 ppm related to the monomers mixture.

For the obtaining of hybrid fillers, the pre-reacted glass ionomer fillers (PRG1 and PRG2 respectively), fluorohydroxyapatite (FHAP), the silanized radiopaque glass powder and the silanized quartz particles (surface area 6.25 m2/g, particle sizes between 10-40 μm) were mixed and then sifted together.

The method of obtaining and characterization of the barium fluoro-alumino-boro-silicate glass (radiopaque glass) having the composition $SiO_2$ (25%), $B_2O_3$ (11%), $Al_2O_3$ (14%), $BaF_2$ (50%) was described in a previous study [27].

The experimental pre-reacted glass ionomer fillers (PRG1 and PRG2) were obtained following literature procedures [7]. PRG1 was prepared by hand-mixing the 50% aqueous solution of a polyalkenoic acid P(AA-co-IA-co-Leu), synthesized from N-acryloyl-L-leucine (Leu), acrylic acid (AA) and itaconic acid (IA) (molar ratio of 0.5:4:1) with the superficially active glass powder having the oxidic composition $SiO_2$ (49%), $Al_2O_3$ (22%), $CaF_2$ (29%) in a weight ratio of 1/2.4. PRG2 was obtained in a similar manner to PRG1, with the exception of using a polyalkenoic acid grafted with methacrylic groups P(AA-co-IA-co-LeuM) instead of P(AA-co-IA-co-Leu) [28], [29].

The method of obtaining and the characterization of FHAP was shown elsewhere [30].

Silanation of quartz and of $BaF_2$-based glass was carried out with 3-methacryloyloxypropyl-1-trimethoxy-silane (A-174 silane).

The composition of the experimental giomer pastes is presented in Table 4:

**Table 4.** The composition of the experimental giomer pastes

| No. | Resin % | | Hibrid fillers % | | | | |
|---|---|---|---|---|---|---|---|
| | Bis-GMA | TEGDMA | PRG1 | PRG2 | FHAP | BaF$_2$ glass | Quartz |
| G1 | 14 | 6 | 16 | - | 8 | 40 | 16 |
| G2 | 14 | 6 | - | 16 | 8 | 40 | 16 |
| Beautifil II* | 1-10 | 1-5 | Aluminofluoro-borosilicate glass 60-70 % (w) Al$_2$O$_3$ 1-5 % (w) | | | | |

*Beautifil II giomer (A30) - Shofu, Kyoto, Japan.

## Methods for characterizing the investigated material

### 1. Degree of conversion (DC)

The conversion of the resin in giomers was assessed by determination of the residual double bonds (RDB) using the infrared spectroscopy method. The baseline method for determining the peak absorbance was used. The amount of the RDB was determined as percentage of the amount of the originally methacrylic groups present in the monomer mixtures.

The decrease of the absorbance intensity of the methacrylate group C = C absorbance (Ameth) from $1635 - 1640$ cm$^{-1}$ was monitored. The absorption band of the phenyl group (Aarom) from $1605 - 1610$ cm$^{-1}$ was used as internal standard.

The residual double bonds were calculated using the formula (1):

$$RDB\% = (A_{meth} / A_{arom})F / (A_{meth} / A_{arom})I \times 100 \qquad (1)$$

where F means the final state (after curing) and I means the initial state of the material (before curing).

The degree of conversion (DC) was calculated using the relation:

$$DC\% = 100\% - RDB\% \qquad (2)$$

ATR-FTIR spectra of giomers (pastes and solids) were recorded on FTIR spectrophotometer (Jasco FTIR-610) equipped with an ATR (attenuated total reflectance) attachment with a horizontal ZnSe crystal (Jasco PRO400S). The resolution of the spectra was 4 cm$^{-1}$. and scans were repeated 100 times. The appropriate amount of the samples were placed on the ZnSe crystal. and then the FTIR spectrum was measured.

### 2. Water sorption

*Preparation of the Samples*

A total of 30 disc shaped samples were prepared (10 from each giomer). Specimens were placed in cylindrical molds (16mm diameter X 1.0mm thickness) and the material packed was slightly overfilled into a brass ring mold set on a piece of transparent polyester film on a glass microscopic slide. It was then covered with another piece of polyester film while being pressed by another glass slide. The specimens were then light cured for 20 seconds by an LED dental light-curing lamp (Guilin Woodpecker Medical Instruments Co., Guangxi, China) with an irradiance of 950 mw/cm$^2$ of five points on the disc surface. After removing the glass and the celluloid band, the samples were light cured again for 20 sec and polymerisation of the giomers was achieved. Soflex discs (3M ESPE, St. Paul, MN, USA) were used to finish the specimens and to obtain uniform thickness.

Water sorption values were determined according to to ISO 4949:2009 at 1, 2, 3, 4, 5, 6, 7 days of storage in water. The values for water sorption (Wsp), for each of the specimens were calculated using the formula (3):

$$Wsp = M1-M2 / V \tag{3}$$

M1 - the specimen mass after water immersion at a moment in time [μg]
M2 - the final mass of dried specimen [μg],
V- volume of the specimen [$mm^3$].

## 3. Scanning Electron Microscopy (SEM)

The surfaces structure of the giomer materials, before and after storage in distilled water/ after a 7-day period, was recorded on a scanning electron microscope (Quanta 3D FEG).

## 4. Radiopacity

Disc samples of giomers measuring 15 mm in diameter and 1 mm in thickness were made in teflon molds by exposing to a visible radiation generated by LED.E dental lamp for 20 seconds of five points on the disc surface. The giomers samples and the aluminum step wedges (thickness increasing by 1mm for each step to a maximum of 10 mm) were placed on an „Intraoral sensor" in vitro. The images were taken using the intraoral sensor system Ezsensor 1.5 Vatech E-Woo (Korea) and a dental X-ray machine HELIODENT DS Sirona (Germany) at 70 kV, 7 mA, 0.04 sec with a target-sensor distance at 30 cm. The mean gray values of each aluminum stepwedge and selected materials were measured by outlining a region of interest using images software. The regions were selected by avoiding areas containing air bubbles inside the material and the average gray value were recorded for every sample. For each radiograph images the calibration curve generated by the grey scale values as a function of the aluminum thickness was calculated. The radiopacity values of the samples were expressed in terms of the equivalent thickness of aluminum per 1 mm unit thickness of material [31].

## 5. Implantation tests

*Sample preparation*

Cilindrical samples (3 mm high/ 2 mm diameter) of the three giomers (A1, B1 and Beautifill) were prepared for subcutaneous and intramuscular implantation. Each sample was and carefully prepared to avoid any sharp edges. Before implantation, all instrumentsand samples used were sterilized

using plasma (Sterrad, J&J, Irvine, CA, USA). The animal study was carried out following the guidelines of the Ethics Committee of "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca.

### Subcutaneous implantation test

18 male Wistar Rats, weighing about 250g each, were randomly divided into 3 groups of 6 animals, according to the giomer tested. Before surgery, the rats were anesthetized via intraperitoneal ketamine HCL (50 mg/kg) and xylazine (7 mg/kg) injection. When animals became unresponsive, a 30 mm × 30 mm area on the dorsal area was shaved and disinfected with povidone-iodine. Sterilized samples of FRC were placed subcutaneously using a microchip syringe, complying with the asepsis and antisepsis regulations. After placing the samples, the wound area was disinfected with povidone-iodine. No suture was needed [32].

### Intramuscular implantation test

Another 18 male Wistar Rats, weighing about 250g each, were randomly divided into 3 groups of 6 animals, according to the giomer tested. Before surgery, the rats were anesthetized via intraperitoneal ketamine HCL (50 mg/kg) and xylazine (7 mg/kg) injection. When animals became unresponsive, a 35 mm × 35 mm area on the hind leg was shaved and disinfected with povidone-iodine. The skin was sharply incised and the sujacent gluteal muscle exposed. Sterilized samples of giomers were placed intramusculary using blunt longitudinal disection of the muscle. After placing the samples, the wound area was disinfected with povidone-iodine. Suture was performed using resorbable material for the muscle and unresorbable for the skin.

After placing the samples, animals were housed in polysulfone type III-H open-top cages (Tecniplast, Buguggiate, Italy) and had access to filtered tap water in bottles and pelleted feed (Cantacuzino Institute, Bucharest, Romania) ad libitum. The bedding was a standard wood chips aseptic bedding (Lignocel®; J. Rettenmaier & Söhne GmBH + Co. KG, Rosenberg, Germany). The rats were kept in the Laboratory Animal Facility of the "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, at a standard room temperature of 22°C±2°C and a relative humidity of 55%±10%, in a 12:12-hour light: dark cycle (lights on, 7 am to 7 pm), at a light intensity of 300 lx at 1 m above the floor. All experimental protocols were approved by the Ethics Committee of "Iuliu Hatieganu" University of Medicine and Pharmacy and were conducted in accordance with the EU Directive 63/2010 (203/20.04.2015). For environmental enrichment, autoclaved braided cotton dental rolls were used (Celluron®, Hartmann, Heidenhelm, Germany). All

animal-handling procedures were performed according to the European and Romanian guidelines.

During the experiment period, assessments were made regarding the local changes, which occurred at the implant site and their impact upon the general status of the animals. All 30 animals were sacrificed 30 days after implantation, following the guidelines of the Ethics Committee. The skin overlaying the implants was shaved and the tissues surrounding the implants were dissected. Tissues surrounding the implants were macroscopically assessed. Implants were sharply removed together with the adjacent tissues. Tissue samples from the areas that contained the implants were fixed in 10% formalin for 5 days. Implants were gently removed before the tissue samples were embedded in paraffin. Histological sections were cut at 4 μm, mounted on slides and stained with hematoxylin-eosin [33], [34].

The most representative histological sections were evaluated. The number and type of inflammatory cells found (polymorphonuclear cells, lymphocytes, plasma cells, macrophages and giant cells), the presence of new blood vessels, edema, necrosis and the presence of a fibrous capsule (indicating a repair process) were microscopically assessed. Each criterion was evaluated according to ISO standards and irritant ranking of the materials assessed accordingly [35].

The inflammation at the implant site was quantified assessing scores:

0. Absent: no sign of inflammation
1. Mild: 1-5 inflammatory cells of each type/ high powered field (hpf)[×400]/ minimal capillary proliferation (1-3 buds )/ no edema/ early developed capsule
2. Moderate: 5-10 inflammatory cells of each type/hpf[×400]/ groups of 4-7 capillaries with supporting fibroblastic structures/ mild edema/ partially formed capsule
3. Severe: heavy or packed inflammatory infiltrate >20 cells/hpf[×400]/ broad band of capillaries with supporting structures/ severe edema/ completely formed capsule.

The scores given for each criterion were summed up and the results classified each material tested as being:

   -Non-irritant (0 up to 2.9)
   - Slight irritant (3 to 8.9)
   - Moderate irritant (9 to 15)
   - Severe irritant (over 15)

The data were statistically analyzed by one-way analysis of variance (ANOVA), with Tukey's test with the level of significance set at 0.05 in order to determine the significant differences between the mean values of the tested materials.

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