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The preparation of high conversion polymeric systems containing eugenol residues and their rheological characterization

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Abstract Copolymeric systems bearing eugenol covalently linked to the macromolecular chains have been prepared and characterised in terms of thermal, rheological and in vitro behaviour. Eugenyl methacrylate (EgMA) and ethoxyeugenyl methacrylate (EEgMA) were polymerized and copolymerized with ethyl methacrylate (EMA) in a wide range of feed composition by bulk at high conversion. Glass transition temperature (T_g) increased with the content of EgMA in the copolymer whereas T_g decreased with the content of EEgMA. The presence of any of the eugenyl monomers in the copolymer increased the thermal stability of the materials. Rheological analysis showed that EMA-co-EgMA copolymers present a higher density of cross-linking or entanglements among the chains compared with EMA-co-EEgMA materials, and copolymers containing more than 40% EgMA exhibited a pseudo-solid like behaviour with G' relatively independent of the frequency. The average molecular weight between cross-links (M_c) for the latter copolymers was in the order of 10^4 Da. For EEgMA copolymers, tan δ was lower than unity and G' showed a gradual and slight dependency with frequency over the whole range of composition. In vitro behaviour showed a higher hydrophilicity of the EEgMA containing copolymers manifested in higher water sorption values, lower water contact angle values and higher surface free energy due to an increase in polarity. All systems presented a good cytocompatibility with human fibroblasts assessed through direct and indirect assays.

1 Introduction

Innovations in the field of polymers for therapeutic purposes are increasingly aiming to develop systems with specific functionality. The immobilisation of biologically active molecules in macromolecular chains is a strategy currently used to attain this objective [1]. One immobilisation route is to covalently link the active molecule to a polymeric backbone that has the advantage of providing the stability of the biomolecule in the system. The incorporation of a biomolecule within a hydrocarbonated skeleton can be attained directly or through the introduction of a spacer group. The use of a spacer group provides a higher steric freedom which, consequently will result in a higher efficiency of the activity of the biomolecule, especially in the case of smallest ones [2]. Moreover, the spacer group can contribute to the hydrolytic release of the immobilised molecule during the degradation process [3]. Following this strategy route, the development of polymeric systems in which a eugenol residue has been covalently linked to the macromolecular chains has been attempted successfully [4]. Eugenol, has been used in

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dental materials [5] since at least the 19th century and currently used in combination with zinc oxide as temporary pulp capping agent and as filling systems in root canals, where the eugenol functions as an obturating agent producing a soothing effect on the pulp. It has been reported that the release of unreacted eugenol, in some concentrations, can produce tissue irritation and induce inflammatory reactions over the oral mucous membrane. Furthermore, eugenol also interferes with the free radical polymerization of the dental composites that are often required to be placed. The use of a covalently linked eugenol is advantageous as it reduces the migration of eugenol to the surrounding tissues and improves its hydrolytic stability. On the other hand the covalently linked eugenol does not produce the inhibitory effect of eugenol against free radical polymerization and the new derivative can participate in polymerisation reactions. This approach widens the application of eugenol in the dental and also in the orthopaedic field through the preparation of self-curing resins. In addition, the bactericidal properties are maintained. Thus, two eugenol derivative methacrylates with spacer groups between the methacrylic group and the eugenol moiety were synthesized: eugenyl methacrylate (EgMA), in which the acrylic residue was directly bonded to the aromatic ring of eugenol, and ethoxyeugenyl methacrylate (EEgMA) in which the acrylic and eugenol moieties were separated through an oxyethylene group.

The aim of this study was the preparation and characterisation of high conversion polymers of EgMA and EEgMA and their copolymers with ethyl methacrylate (EMA) in a wide range of feed composition. Due to the presence of two different double bonds in the chemical structure of both the eugenyl monomers, they are susceptible to polymerise in the presence of a free radical initiator and yield polymeric networks that are partially crosslinked. Thus, the degree of crosslinking and average molecular weight between cross-links (M_c) have been evaluated by rheological properties. In addition, thermal properties and the in vitro behaviour of the copolymer systems have been studied through water sorption experiments, contact angle measurements and cellular response to human fibroblast cultures.

2 Experimental procedures

2.1 Materials

Ethyl methacrylate (EMA) (Aldrich) was purified by distillation under reduced pressure. EgMA and EEgMA were synthesized as reported previously [4]. Azobisisobutyronitrile (AIBN) (Merck) was recrystallised from methanol (M.p. 104 °C). The solvents hexane (Scharlau), tert-butanol (Aldrich) and toluene (Merck) were purified by standard procedures.

2.2 Synthesis and characterisation of polymers and copolymers

High conversion polymers of EMA, EgMA and EEgMA and EMA-co-EgMA and EMA-co-EEgMA copolymers for feed EMA/eugenyl monomer molar ratios ranging from 20:80 to 80:20 were obtained by bulk radical polymerization using AIBN (1 wt% with respect to monomer) as a free radical initiator. About 250 mg of the corresponding monomer or monomer mixture were introduced in polypropylene capped cylinder moulds of 1.5 mm of diameter. The reaction medium was saturated with N_2 atmosphere for 15 min and then the tube was capped and heated at 70 °C for 2 h. Subsequently, the temperature was reduced to 60 °C and the reaction was allowed to proceed for 46 h. The polymerised samples were extracted from the moulds, thoroughly washed with hexane and water to remove residual monomers and filed with a sandpaper to obtain 1 mm thickness discs. Finally, the samples were dried under vacuum until constant weight.

The soluble fraction of each material was obtained by Soxhlet extraction in toluene for 48 h. After the treatment, samples were dried under vacuum and soluble fraction was calculated by using Eq. 1:

$$F_{sol} = [100 \cdot (W_i - W_f)/W_i] \tag{1}$$

where F_{sol} is the soluble fraction; W_i the initial weight of the sample before the treatment and W_f is the dried weight of the sample after the treatment. Three samples of each material were tested and results were averaged.

Densities of PEMA, PEgMA and PEEgMA homopolymers were measured by a flotation pycnometric method using an aqueous calcium nitrate solution at 25 ± 0.1 °C. Measurements were taken placing 10 small films of each material in water and adding successive small volumes of a saturated aqueous calcium nitrate solution until the density of the final solution equalled that of the sample. Then, the density of the final solution was determined by pycnometry.

ATR-FTIR spectra of all the polymers and copolymers were recorded on a Perkin–Elmer-Spectrum One spectrophotometer, with an ATR attachment.

2.3 Thermal properties

Glass transition temperatures (T_g) were measured using differential scanning calorimetry (DSC) with a Perkin–

Elmer DSC7 interfaced to thermal analysis data system TAC 7/DX. Films of dry samples (15–20 mg) were placed in aluminium pans and heated from −20 °C to 150 °C at a constant rate of 10 °C/min. T_g was taken as the midpoint of the heat capacity transition. Two samples were tested for each material. Thermogravimetric diagrams were obtained in a thermogravimetric analyzer using a TGA Q500 (TA Instrument) apparatus, under dynamic nitrogen at a heating rate of 5 °C/min in a range of 40–600 °C. Samples were tested in duplicate.

2.4 Rheological measurements

The rheological properties were evaluated by using a stress-controlled rotational rheometer (Gemini, Bohlin Reologi A B, Lund, Sweden) using parallel plate configuration. The tests were performed at a controlled temperature of 37 ± 0.1 °C. Swelling of polymer discs in tert-butanol to equilibrium was carried out to study the rheological behaviour of the materials. Three samples of each formulation were allowed to equilibrate for at least 30 min prior to analysis. To avoid tert-butanol evaporation, the cell containing the sample was enclosed in a solvent trap saturated with solvent. The alcohol sorption (AS) was calculated according to Eq. 6.

Small amplitude oscillatory shear experiments were performed to evaluate the viscoelastic properties. This technique has been used successfully to determine the structure-mechanical properties relationship of materials or tissues [6–7]. In dynamic mode the material was subjected to a sinusoidal shear strain:

$$\gamma = \gamma_0 \sin(\omega t) \tag{2}$$

where γ_0 is the shear strain amplitude, ω is the oscillation frequency (which can also be expressed as $2\pi f$ where f is the frequency in Hz and t the time). The mechanical response, expressed as shear stress τ of viscoelastic materials, was intermediate between an ideal pure elastic solid (obeying Hooke's law) and an ideal pure viscous fluid (obeying Newton's law) and therefore out of phase with respect to the imposed deformation as expressed by Eq. 3:

$$\tau = G'(\omega)\gamma_0 \sin(\omega t) + G''(\omega)\gamma_0 \cos(\omega t) \tag{3}$$

where $G'(\omega)$ is the shear storage modulus or elastic modulus, and $G''(\omega)$ is the shear loss modulus or viscous modulus. G' gives information about the elasticity or the energy stored in the material during deformation, whereas G'' describes the viscous character or the energy dissipated as heat.

The dynamic viscosity η' is related to the loss modulus G'' as shown in Eq. 4 [8]:

$$\eta' = \frac{G''}{\omega} \tag{4}$$

It is a measure of the gel resistance to shear flow which is always associated with internal dissipation, generally, as heat; while the loss tangent (tan δ) is defined as:

$$\tan\delta = \frac{G''}{G'} \tag{5}$$

Preliminary strain sweep test at a fixed oscillation frequency (consisting in monitoring the viscoelastic properties while logarithmically varying the strain amplitude γ_0) were performed on the materials to determine the strain amplitude at which linear viscoelasticity is valid. The oscillation frequency ranged from 0.1 Hz to 10 Hz.

2.5 In vitro behaviour

For swelling experiments, discs of polymers were accurately measured and weighed, and introduced in phosphate-buffered solution (PBS), and kept until they reached equilibrium at 37 °C. The values of water sorption (WS) were determined gravimetrically using Eq. 6, where W_w is the weight of the wet sample at time t and W_0 is the weight of the dry sample. Three samples of each material were evaluated and results averaged.

$$WS\,(\%) = 100 \times (W_w - W_0)/W_0 \tag{6}$$

Contact angles were measured on non filed polymer surfaces discs by using the sessile drop technique and employing liquids with known surface tension: water ($\gamma_L = 72.8$ mN/m) and methylene iodide ($\gamma_L = 51.8$ mN/m). γ_L refers to the total surface free energy of the liquid. A minimum of 10 drops were applied on each sample. The surface free energy of homopolymers and copolymers (SFE) was calculated by the Fowkes' [9] and Owens' method [10].

$$\gamma_s = \gamma_s^d + \gamma_s^p \tag{7}$$

$$(1 + \cos\theta)\gamma_1/2 = (\gamma_s^d \gamma_l^d)^{1/2} + (\gamma_s^p \gamma_l^p)^{1/2} \tag{8}$$

where θ is the contact angle, γ_s and γ_l are the surface free energy of solid and liquid respectively and γ_s^{d}, γ_s^{p}, γ_l^{d} and γ_l^{p} γ_l^{p} are the dispersive and polar components of SFE of solid and liquid respectively.

For biocompatibility evaluation, a direct contact assay was performed in which Thermanox® (TMX) as the standard control and discs of the copolymers were placed in a 24-well plate (in duplicate), seeded with human fibroblasts at a density of 1.4×10^5 cells/mL and incubated at 37 °C. After 24 h of incubation, the cultures were washed to remove non-adherent cells and the cells adhered were fixed with 1.5% glutaraldehyde buffered in 0.1 M phosphate buffer. The dried samples were sputter-coated with gold before examination under an ESEM apparatus (Philips XL 30) at an accelerating voltage of 15 KeV.

To evaluate the cytotoxicity of the eluants from the materials, TMX and discs of copolymers were set up in 5 mL of minimal essential Eagle's medium (MEM), foetal bovine serum-free. They were placed on a roller mixer at 37 °C and the medium was removed at 1 and 2 days. The medium containing the extracts of the specimens was added to a sterile six-well culture plate containing human fibroblasts seeded at a density of 1.4×10^5 cell/ml, in complete medium and incubated at 37 °C for 24 h. The culture medium was replaced with the corresponding extract and incubated again at 37 °C for 96 h. Then, the cells were fixed, using 10% formaldehyde solution, and stained using Harris haematoxilin and eosin. The obtained samples, all of them in duplicate, were observed and photographed using an optical microscope NIKON ECLIPSE E600 with a camera NIKON DXM 1200.

3 Results and discussion

3.1 Synthesis and characterisation of polymers and copolymers

The free radical polymerization of EgMA and EgMA carried out in bulk at high conversion provided polymers which were insoluble in common organic solvents, with a soluble fraction in toluene as little as 1.4 and 0.8% for PEgMA and PEEgMA respectively. This fact reveals the participation of the two types of double bonds present in the chemical structure of the eugenol monomers, acrylic and allylic double bonds. It has been demonstrated that acrylic double bonds are the first to react giving soluble polymers when the polymerization reaction is carried out at low conversion [4]. Then, as polymerization conversion increases the pendant allylic double bonds contribute to grafting and crosslinking. If the polymerization is carried out in bulk, the conditions involved in this process may contribute to the formation of crosslinks. Bulk polymerization involves heat dissipation, which promotes an exponential increase of viscosity, decreasing the mobility of the macro-radicals that enhances the autoacceleration effect. The density of PEgMA and PEEgMA was calculated and compared with that of PEMA obtained under identical conditions. The values obtained were 1.186 g/mL for PEMA, 1.181 g/mL for PEgMA and 1.093 g/mL for PEEgMA, which was slightly lower for the methacrylate bearing the most voluminous side group. The free radical copolymerization of the eugenol containing monomers with EMA in a wide range of monomer feed composition, also provided copolymers partially insoluble in organic solvents and the soluble fraction increased with the EMA content in the copolymer up to 26% for contents of 80% EMA in the copolymer, reflecting that the presence of allylic double bonds is determinant of the degree of grafting and crosslinking.

PEgMA, PEEgMA and the two copolymers were characterized by ATR-FTIR spectroscopy (see Fig. 1). All the spectra showed the absorption bands characteristic of the eugenyl methacrylates and EMA units [11], at 3,080 cm^{-1} ν

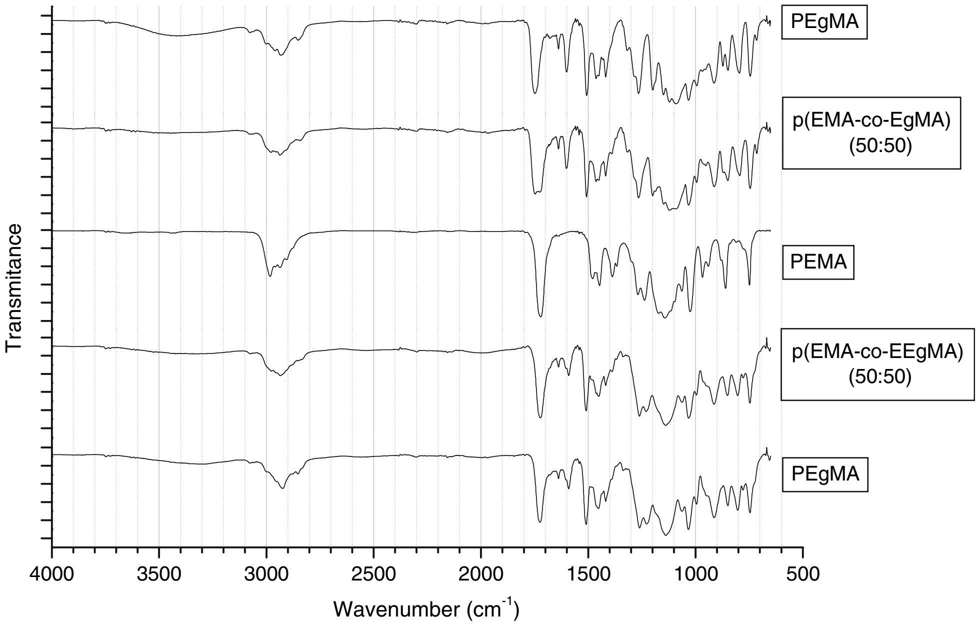


Fig. 1 ATR-FTIR spectra of EMA-co-EgMA and EMA-co-EEgMA systems obtained at high conversion

C=C in the allyl group [12, 13], 3,000–2,900 cm^{-1} ν_s and ν_{as} CH_2 aliphatic, 1,725 cm^{-1} ν C=O ester, 1,650–1,550 cm^{-1} ν C=C in the aryl-ester groups, 1,635 cm^{-1} ν C=C in the allyl group [12, 13], 1,503 cm^{-1} ν in the aromatic ring, 1,415 and 995 cm^{-1} δ C=C in the allyl group [12, 13], 1,360 cm^{-1} ν O–Ph, 700–760 cm^{-1} ν in the aromatic ring.

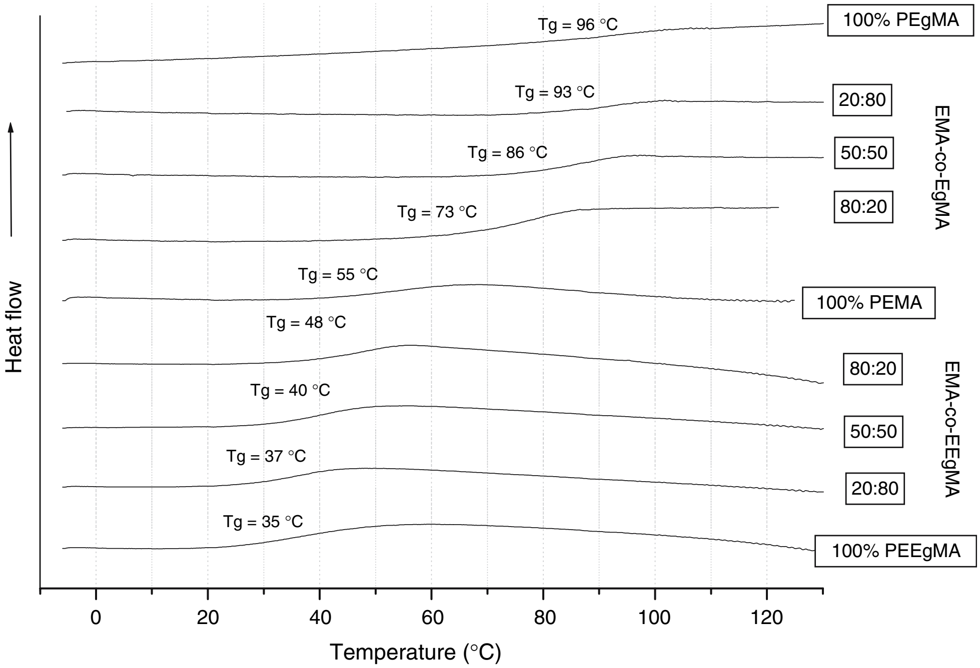
3.2 Thermal properties

Thermal properties of the high conversion polymers and copolymers were evaluated by DSC and TGA. The determination of the glass transition temperature (T_g) provides accurate information about the relative flexibility of polymeric segments and hence of the polymeric network. Values of T_g are shown in Fig. 2 along with the corresponding themograms. T_g values of homopolymers were in the order PEgMA > PEMA > PEEgMA and were consistent with those reported for homopolymers obtained at low conversion [4]. EMA-co-EgMA copolymers exhibited values of T_g in the range of those of the corresponding homopolymers (96–55 °C), with a trend to decrease with EMA content in the copolymer, which reflects the greater rigidity of the EgMA unit, probably associated to interactions involving the substituent groups in the aromatic ring. For EMA-co-EEgMA copolymers, on the other hand, values of T_g decreased with the content of EEgMA units in the copolymer, indicating that the voluminous group of eugenol separated from the backbone by an oxyethylene group contributed to the flexibility of the side group. The values of T_g obtained were in the range 55–35 °C. The thermal stability of the copolymers was evaluated by thermogravimetric analysis and the results are shown in Fig. 3. The thermogravimetric curves of PEMA showed four degradation steps, which was very similar to the behaviour of PMMA that has been well described in literature [14–15]. The first stage of degradation (around 207 °C) originate from the sterically hindered linkages that result from head-to-head coupling during polymerisation, the second stage of degradation (around 274 °C) is attributed to the decomposition of the chain from the vinylidene end formed via disproportionation, and the two subsequent stages (around 350 and 430 °C) are ascribed to the random chain scission of the main chain leading to depolymerisation of the acrylic polymer. However, the eugenol containing polymers and copolymers underwent practically single-step decomposition, which can be attributed to the totally random scission of the main chain. The presence of the eugenyl monomer provided a clear increase in thermal stability, which is reflected in the values of the temperature for maximum rate of decomposition and temperature for 50% weight loss as shown in Table 1.

3.3 Rheological properties

Rheological behaviour of both the high conversion systems were studied using a stress controlled rotational rheometer with parallel plate configuration. To perform these measurements, polymeric and copolymeric discs were swollen in tert-butanol to attain equilibrium and the values of AS

Fig. 2 DSC thermograms of EMA-co-EgMA and EMA-co-EEgMA systems obtained in bulk at high conversion



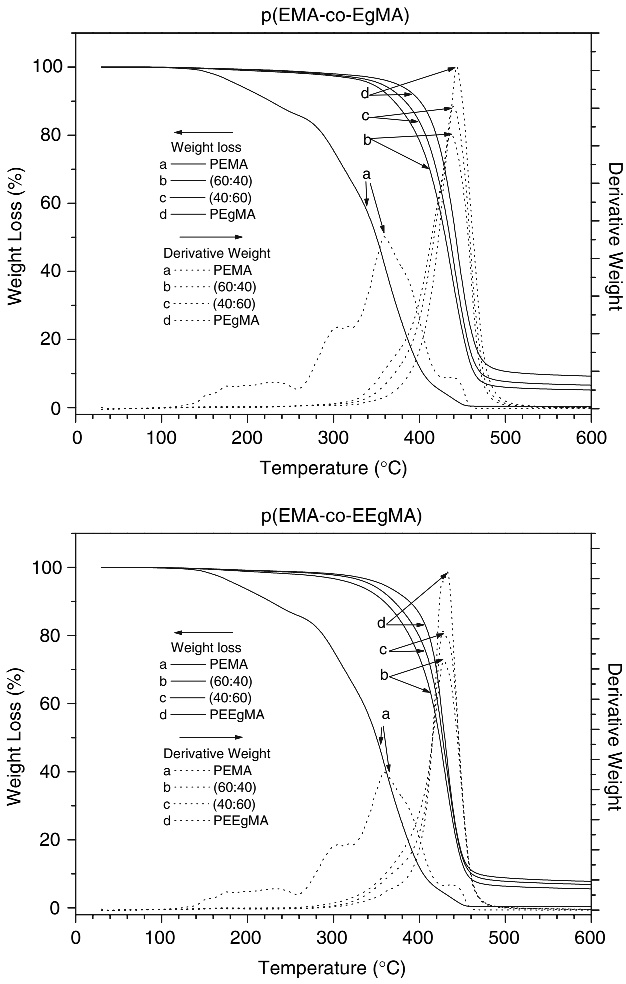


Fig. 3 TGA and DTA curves of high conversion PEMA and eugenol containing polymers and copolymers obtained by bulk polymerization. Upper: EMA-co-EgMA system; lower EMA-co-EEgMA system

Table 1 Values of temperature for maximum rate of decomposition (T_{max}) and temperature for 50% weight loss ($T_{50\%}$) obtained from the TGA curves of PEMA, eugenol containing polymers and copolymers synthesized at high conversion in bulk polymerization

System	Composition (%)	T_{max} (°C)	$T_{50\%}$ (°C)
PegMA	100	444	442
EMA-co-EgMA	40:60	439	435
	60:40	437	429
PEMA	100	359	350
EMA-co-EegMA	60:40	427	423
	40:60	428	427
PEEgMA	100	431	429

Table 2 Values of swelling at equilibrium in tert-butanol, slope of G′ versus frequency [$\log(G') = a + n \log(w)$] and molecular weight between cross-links (M_c) calculated according to the rubber elasticity theory at 1 Hz and 37 °C

System	Composition (%)	AS (%)	*n*	M_c (Da)
EMA-co-EgMA	80:20	22.8 ± 0.8	0.52	–
	60:40	3.6 ± 0.6	0.31	–
	40:60	2.3 ± 0.4	0.05	1.0×10^4
	20:80	1.9 ± 0.5	0.02	1.1×10^4
PEgMA	100	0.8 ± 0.8	0.09	0.6×10^4
EMA-co-EegMA	80:20	26.7 ± 0.9	0.11	–
	60:40	14.5 ± 0.9	0.21	–
	40:60	7.8 ± 0.8	0.13	–
	20:80	7.2 ± 0.9	0.14	–
PEEgMA	100	5.0 ± 0.2	0.17	–

are shown in Table 2. In order to determine the rheological parameters it was necessary to establish the validity range of linear viscoelasticity. To this aim, preliminary stress sweep tests at a fixed oscillation frequency (consisting in monitoring the viscoelastic properties while logarithmically varying the stress amplitude) were performed on the materials. For all polymers and copolymeric systems the dynamic storage modulus G′ reached a constant value for stress higher than 100 Pa, and a stress amplitude of 350 Pa was fixed for further tests.

Figure 4 shows the dependence of the storage modulus G′ for both copolymeric systems upon frequency. EMA-co-EgMA copolymers showed values of G′ one order of magnitude higher than those obtained for EMA-co-EEgMA copolymers indicating that a higher density of crosslinking or entanglements among the chains is present in the former system. It was also observed that EMA-co-EgMA copolymers containing more than 40% EgMA and PEgMA, exhibited a pseudo-solid like behaviour with $G' > G''$ and G′ relatively independent of the frequency range of 0.1–100 rad/s. This fact is reflected in the values of slope of G′ versus frequency which were almost zero, equal to 0.02 for EMA-co-EgMA 20:80, 0.05 for EMA-co-EgMA 40:60 and 0.09 for PEgMA. For EEgMA copolymers, G′ showed a gradual and slight dependency with frequency over the whole range of frequency and for all copolymer compositions, with values of slopes between 0.11 and 0.21, indicating the typical viscoelastic behaviour exhibited by branched systems with a slightly crosslinked degree [16, 17].

The average molecular weight between cross-links (M_c) can be estimated from viscoelastic measurements by applying the rubber elasticity theory [7]. For EMA-co-EgMA copolymers, that show pseudo-solid like behaviour, the values of M_c obtained, using the storage modulus value at 1 Hz and 37 °C, were in the order of 10^4 Da. For PEgMA a lower value of M_c was obtained (0.6×10^4) indicating a highest degree of crosslinking in the final network.

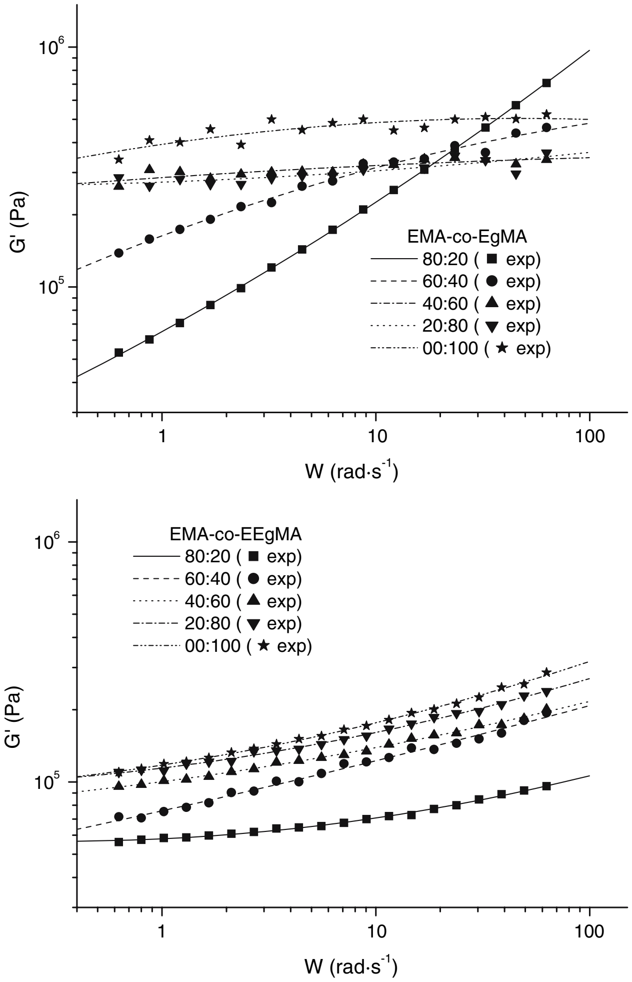


Fig. 4 Dependence of elastic modulus (G′) with frequency of high conversion EMA-co-EgMA (upper) and EMA-co-EEgMA (lower) copolymers obtained by bulk polymerization

A linear relationship between storage modulus (G′) and eugenyl monomer in the copolymer was obtained, and G′ increased with the content of EgMA or EEgMA in the copolymer. Both curves are shown in Fig. 5 where it is evident that the elastic behaviour is different for each copolymeric system.

The relationship between elastic and viscous components ($\tan\delta$) is shown in Fig. 6. It can be observed that for EMA-co-EgMA 80:20 copolymer loss tangent was higher than unity ($\tan\delta > 1$) for over the range oscillation frequencies and $\tan\delta$ decreased with increasing frequency showing a predominant dissipative behaviour in all the frequency range examined. For the rest of EgMA copolymers, $\tan\delta$ was lower than 0.5 and independent of frequency according with a pseudo-solid like behaviour. For EEgMA copolymers, $\tan\delta$ was lower than unity showing a quasi-linear relationship.

The viscosity of a material is a measurement of resistance to flow and it is always associated with the

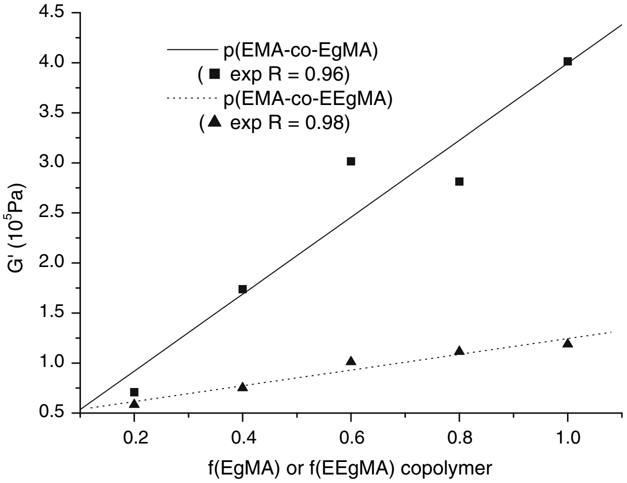


Fig. 5 Dependence of elastic modulus (G′) with eugenyl monomer molar fraction of high conversion EMA-co-EgMA and EMA-co-EEgMA copolymers obtained by bulk polymerization

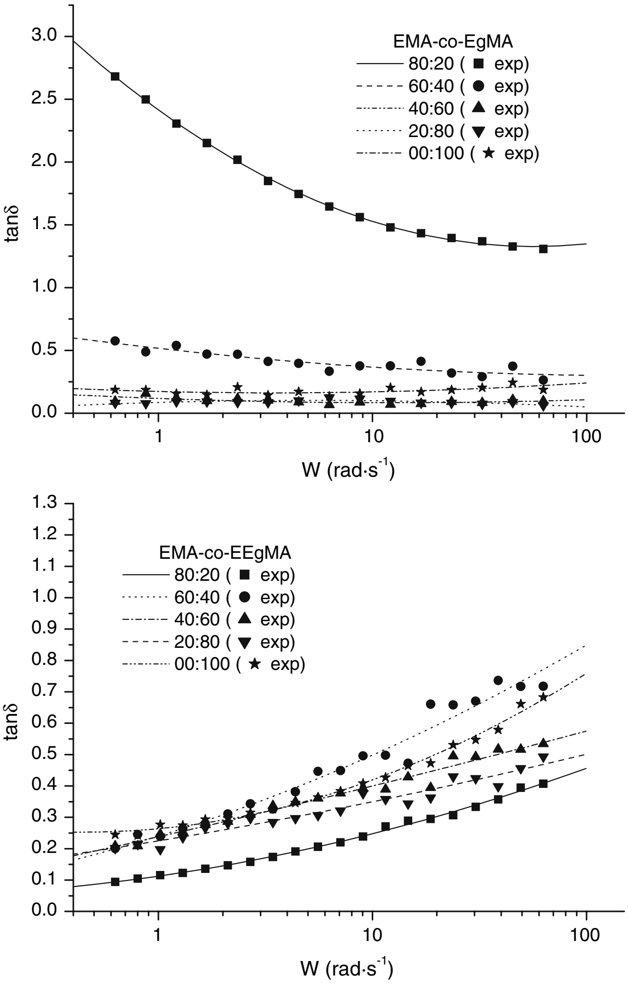


Fig. 6 Dependence of loss tangent (tanδ) with frequency of high conversion EMA-co-EgMA (upper) and EMA-co-EEgMA (lower) copolymers obtained by bulk polymerization

dissipation of internal energy, mainly heating. A low viscosity means that the material slides easily and dissipates little internal energy in the shear stress process. A high viscosity, on the other hand, means that the material slides more slowly and dissipates more energy in the process. That resistance can be approximated by dynamic viscosity. Figure 7 shows the dynamic viscosity as a function of oscillation frequency for all copolymeric systems and eugenyl homopolymers, where the shear thinning effect is manifested. This is a common finding for most macromolecules and is caused by deformation and alignment of the flexible molecules in the streamlines of increasing flow or oscillation frequency. The viscosity decrease with frequency for both systems was approximately linear on the log–log scale, indicating that the relationship between dynamic viscosity and frequency can be adjusted to a power law:

$$\eta' = \mathrm{k}\,\mathrm{f}^{\mathrm{n}} \qquad (9)$$

where f is frequency and k and n are constants. The data shown in Fig. 7 were fitted by the power law equation using a simple linear least-squares regression and the fitting results are shown in Table 3 with the respective values of k, n and correlation coefficient r. The data fitted well the regression equation with values of correlation coefficients of 0.99. Dynamic viscosity decreased with frequency at approximately the same rate for EMA-co-EgMA copolymeric system (values of n around −1.0), except for the 80:20 copolymer, for which the decrease was slower ($n = -0.59$). For EMA-co-EEgMA system the viscosity decrease with frequency was also at approximately the same rate but with lower values (n about −0.45) compared to the former system. The dynamic viscosity values at low frequency (0.1 Hz) decreased with the content of EgMA and EEgMA in the corresponding system.

The rheological characterization of the eugenol derivative copolymers confirmed the participation of the allylic double bonds in the polymerization process providing either branching or crosslinked structures with a range of degrees of crosslinking that are responsible for the elastic or viscoelastic response in the materials.

3.4 In vitro behaviour

Finally, and due to the potential application of the copolymeric systems as biomaterials in dental and bone field, an in vitro study was performed in order to evaluate the water uptake, wettability and biocompatibility of the systems. Water sorption was studied in PBS at 37 °C and results as a function of time are shown in Fig. 8. Water sorption for PEgMA was as little as 0.2%, it was around 0.6 for EgMA containing copolymers whereas for PEEgMA and EEgMA containing copolymers, WS was around 1.7. Although WS was relatively low in all the systems, the differences found between both types of copolymers can be attributed to the different hydrophilicity of the eugenol derivative monomer and to the different crosslinking degree, as observed in the rheological study. The different levels of hydrophilicity are manifested in their interaction with the medium, which was evaluated through contact angle measurements considering two liquids of opposite polarity, water and methylene iodide. Values of contact angles (θ) are summarised in Table 4 along with those of surface free energy (SFE). Water contact angle for PEgMA was relatively high (87 °C) and characteristic of hydrophobic surfaces whereas that of PEEgMA was 10 °C lower (76 °C). EgMA containing copolymers present an intermediate behaviour between both homopolymers but the behaviour of EEgMA containing copolymers approached that of PEEgMA. Surface free energy was around 40 mN/m for EMA-co-EgMA copolymers and around 46 mN/m for

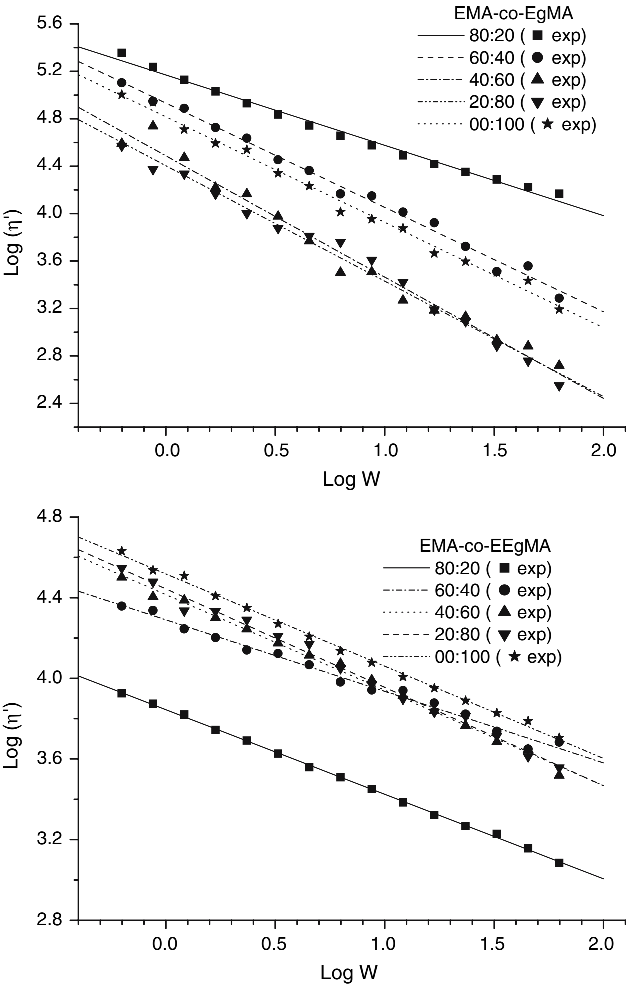


Fig. 7 Dependence of log (η') with the log (W) of high conversion EMA-co-EgMA (upper) and EMA-co-EEgMA (lower) copolymers obtained by bulk polymerization

Table 3 Values of dynamic viscosity at 0.1 Hz and 37 °C (η_0), and results of linear least-squares regression curve fitting by Eq. 9 for high conversion EMA-co-EgMA and EMA-co-EEgMA copolymers obtained by bulk polymerization

System	Composition	η_0(10^5 Pa s)	k	*n*	r
P(EMA-co-EgMA)	80:20	2.27	5.17	−0.59	−0.99
	60:40	1.27	4.93	−0.88	−0.99
	40:60	0.39	4.40	−0.97	−0.99
	20:80	0.37	4.48	−1.02	−0.99
PEgMA	100	1.01	4.81	−0.89	−0.99
P(EMA-co-EEgMA)	80:20	0.84	3.84	−0.42	−0.99
	60:40	0.23	4.29	−0.35	−0.99
	40:60	0.32	4.42	−0.47	−0.99
	20:80	0.35	4.44	−0.48	−0.99
PEEgMA	100	0.43	4.51	−0.45	−0.99

Table 4 Water (θ H_2O) and methylene iodide (θ CH_2I_2) contact angles and solid surface free energies (γ_S) with their dispersive (γ_S^d) and polar (γ_S^p) components for high conversion EMA-co-EgMA and EMA-co-EEgMA copolymers obtained by bulk polymerization

System	Composition	$\theta(H_2O)$ [SD]	$\theta(CH_2I_2)$ [SD]	Surface free energy		
				γ_S (mN/m) [corr.] (%)	γ_S^d (mN/m)	γ_S^p (mN/m)
PEMA	100:00	79.8 [0.6]	42.2 [0.5]	41.1 [99.6]	37.0	4.1
p(EMA-co-EgMA)	60:40	79.7 [1.9]	46.0 [0.5]	40.7 [98.6]	36.5	4.2
	40:60	85.3 [1.1]	43.2 [0.5]	40.2 [99.0]	38.0	2.3
PEgMA	100	86.8 [0.9]	48.2 [2.1]	35.2 [99.2]	35.2	2.2
p(EMA-co-EEgMA)	60:40	72.1 [1.5]	42.8 [1.3]	44.9 [99.7]	38.0	6.9
	40:60	72.3 [1.5]	35.7 [3.6]	46.8 [99.4]	40.6	6.2
PEEgMA	100	75.8 [2.7]	35.9 [0.7]	46.1 [98.6]	41.6	4.5

EMA-co-EEgMA system, due to an increase in polarity. In fact, the latter copolymers showed the highest values in their polar component. Accordingly, PEgMA showed the lowest value of SFE (35 mN/m), reflecting its higher hydrophobic character. Wettability results were consistent with those of water sorption where the WS decreased in the order PEEgMA > EMA-co-EEgMA > PEMA > EMA-co-EgMA > PEgMA. Biocompatibility was analysed by direct contact assay using human fibroblasts culture. Cell morphology and cell-material interactions examined by ESEM revealed a good colonisation of the cells which appeared well spread and flattened over all materials and showing normal morphology (Fig. 9), indicative of a good cytocompatibility [4]. Cytotoxicity coming from the release of extracts of the copolymeric discs to the medium was also evaluated and results are shown in Fig. 10. It is clear that the extracts at 1 and 2 days of any system did not cause alterations on the cellular morphology after 96 h of culture and produced similar cellular densities with respect to TMX.

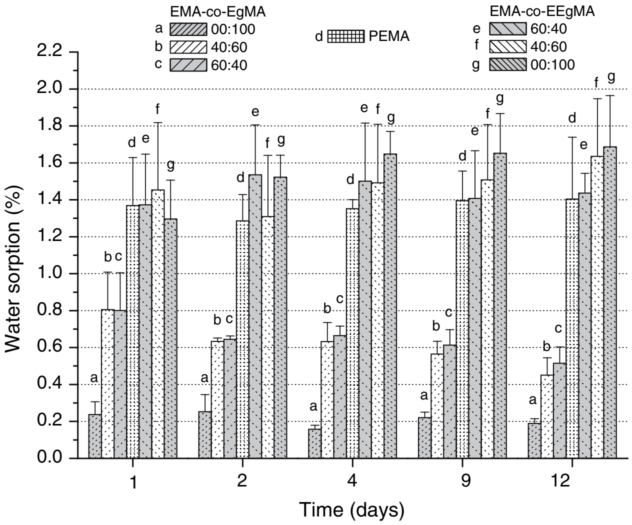


Fig. 8 Evolution of water content with time of immersion in PBS at 37 °C for high conversion EMA-co-EgMA and EMA-co-EEgMA copolymers obtained by bulk polymerization

4 Conclusions

The free radical polymerization of eugenyl methacrylate and ethoxyeugenyl methacrylate was carried out at high conversion to yield slightly crosslinked polymer networks. The thermal properties, rheological measurements, hydrophilicity and water uptake were dependent on the EEgMA

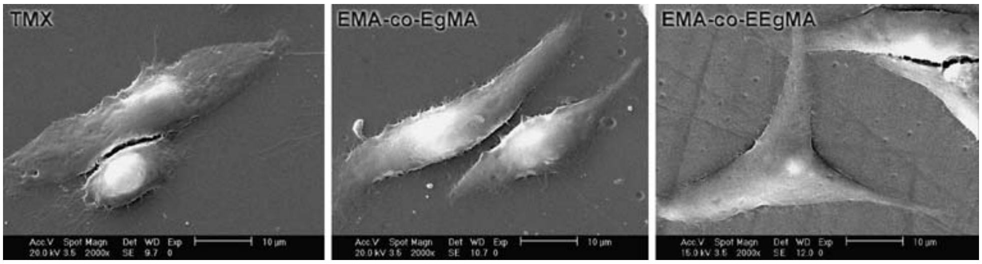


Fig. 9 ESEM images (2,000×) of human fibroblasts colonized over the control TMX (left), EMA-co-EgMA 50:50 (middle) and EMA-co-EEgMA 50:50 (right) copolymers at 2 days

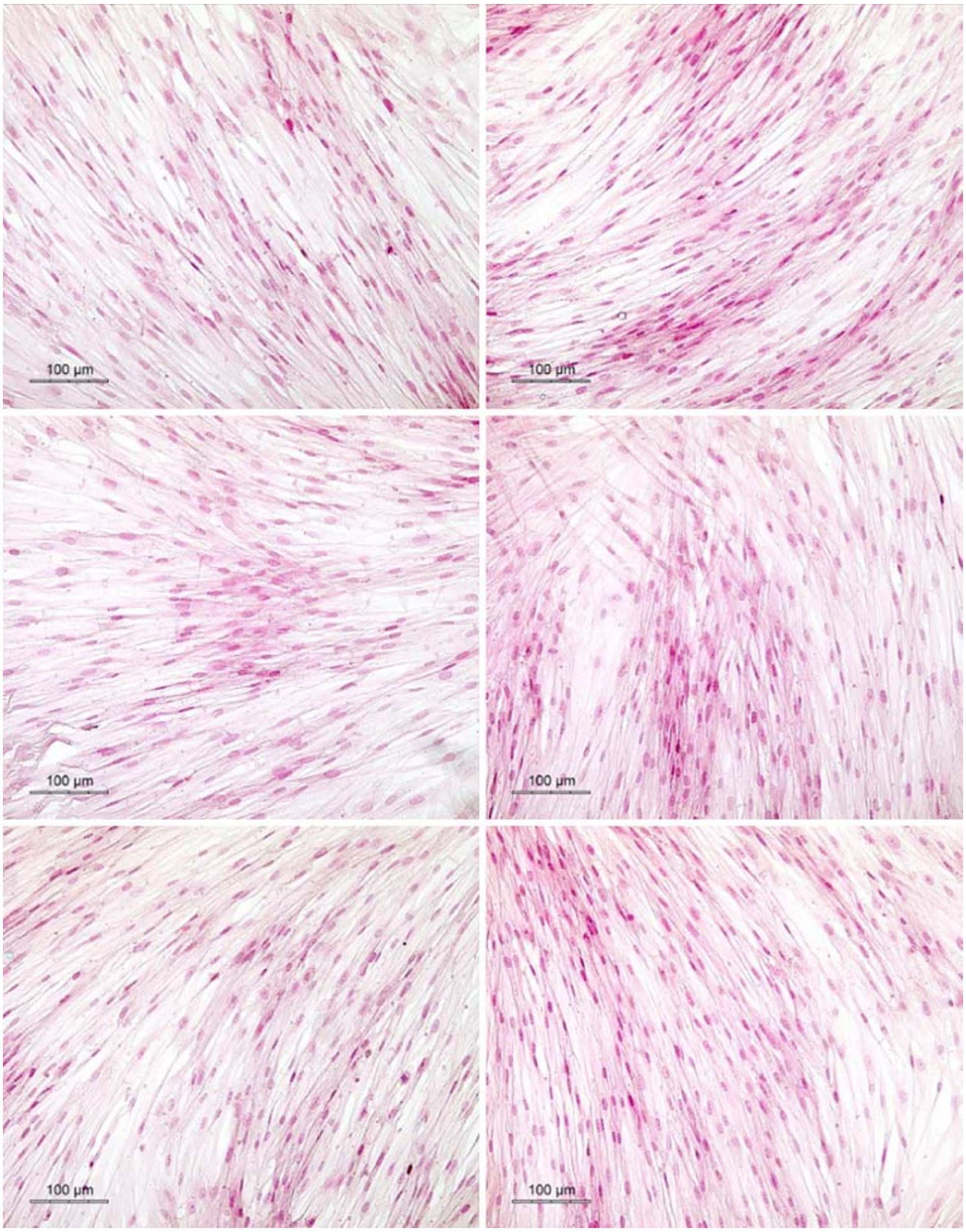


Fig. 10 Microphotographs (200×) showing the influence of the eluted extracts on control TMX (upper), and EMA-co-EgMA 50:50 (middle) and EMA-co-EEgMA 50:50 (lower) copolymers, collected at 1 day (left column) and 2 days (right column) on the cellular culture after 96 h. Harris haematoxylin–eosin dye

and EgMA content in the copolymers. Cytoxicity measurements indicated that all the polymeric systems were viable for in vivo applications.

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