Epoxy Networks for Medicine Applications: Mechanical Properties and *In Vitro* biological properties

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ABSTRACT: This work describes the physicochemical, mechanical, and *in vitro* biological properties of three epoxy networks based on diglycidyl ether of bisphenol-A (DGEBA) epoxy prepolymer cured with triethylenetetramine, 1-(2-aminoethyl)piperazine (AEP) and isophoronediamine. The mechanical properties were evaluated with respect to impact and flexural tests. Functionality rules the mechanical behavior of epoxy networks by increasing the crosslink density and the flexural modulus, increasing T_g and decreasing the chain flexibility and the impact resistance. The biological interactions between the obtained epoxy polymers and blood were studied by *in vitro* methods. Studies on the protein adsorption, platelet adhesion, and thrombus formation are presented. The protein adsorption assays onto polymeric surfaces showed that the epoxy networks adsorbed more albumin than fibrinogen. The results about platelet adhesion and thrombus formation indicated that DGEBA-IPD and DGEBA-AEP networks exhibits good hemocompatible behavior. The materials revealed no signs of cytotoxicity to Chinese hamster ovary cells, showing a satisfactory cytocompatibility. In this way, we can assume that the epoxy polymers are biocompatible materials. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 1215–1225, 2009

Key words: epoxy-amine networks; mechanical properties; blood compatibility; cytotoxicity

INTRODUCTION

The technological advances in medical sciences of the developed countries have leads the ageing of the population by the use biomedical implants based on synthetic materials, named biomaterials.[1] The successful insertion of a biocompatible material into a living tissue has few evidence of rejection revolutionizing the medicine and dentistry today.

Biomaterials are either natural or synthetic materials that are used to direct, supplement or replace the functions of living tissues or organs of human body.[2] The purpose of biomaterial is to replace a part or function of the body in a safe, reliable and physiologically acceptable manner.[3] The biomaterial must be biocompatible, it should not elicit an adverse response from the body, and it should be nontoxic and noncarcinogenic. Additionally, the biomaterial should possess adequate physical and mechanical properties to serve as augmentation or replacement of body tissue and at same time should be amenable to being formed or machined into different shapes, have relatively low cost and be readily available.[4]

Modern medical devices are made from a wide range of materials including polymers, metals, ceramics and composites. Today, polymer composites represent the most important and largest family of biomaterials being used for many unique applications in medicine and modern dentistry. These interesting hybrid organic–inorganic materials may be used directly or indirectly for restorations, prostheses or for production of appliances in dentistry field.[5]

Composites based on epoxy resins of diglycidyl ether of bisphenol-A (DGEBA) may be pointed as the most widely used organic matrix materials for preparing reinforced materials for orthopedic and dentistry applications.[6,7] Recently, the successful use of nanoclay reinforcement technology to improve the performance of epoxy resins would represent a major technical achievement in the development of advanced structural materials with high potential for use in medicine and dentistry.[8]

The recent advances in the development of low-toxicity onium initiators have facilitated the development of a wide range of ring-opening polymerization processes such as cycloaliphatic epoxy resins.[9] These materials can be formulated to produce highly reactive matrices, which low shrinkage and high

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strength, suitable for the development of composites with interesting mechanical and physical properties for dentistry and orthopedic applications.

Over the past two decades, epoxy polymers have been used in implantable devices such as coatings for titanium, bioactive epoxy/sodium bioglass ceramic composites, and biosensors.[10–12]

The epoxy requirement for the biomedical applications varies markedly according to the application being considered. One of the major problems encountered in medical applications is blood compatibility since many of these devices either handle blood directly or come into contact with blood in subcutaneous or implantable devices. With respect to implants in contact with physiological fluids such as blood, the synthetic surface are exposed to a mixture of protein adsorbates that dominates the available surface area as dynamically forming surface layer, activated or passivated by the underlying synthetic surface.[13] Considering the implants for blood-contacting applications, protein adsorption has focused on fibrinogen and albumin.[14] The importance of fibrinogen adsorption comes from the close relationship between the adsorbed fibrinogen layer on implant surface and the activation of blood platelets by the adsorbed fibrinogen.

Although numerous studies have been published about the biocompatibility property of diepoxy compounds and epoxy polymers,[15–18] to our knowledge, no work has been undertaken about the blood compatibility of epoxies materials based on epoxy-aliphatic amines systems.

The purpose of this study was to examine the blood compatibility, cytotoxicity and mechanical properties of three epoxy-amine networks based on DGEBA epoxy prepolymer cured with three aliphatic amine epoxide hardeners. The objective is the improvement of the resistance to the fracture acting on the structure of the amine epoxide hardener with the aim of producing epoxy polymers with the necessary biocompatibility properties for orthopedic or dentistry applications.

To maintain a high functionality in the aliphatic amine one ethyleneamine such as triethylenetetramine (TETA) was chosen. The two other amine epoxide hardeners are cycloaliphatic amines based on 1-(2-aminoethyl)piperazine (AEP) and 5-amino-1,3,3-trimethylcyclohexanemethylamine (isophoronediamine, IPD), having both cyclic structures.

The mechanical properties of the epoxy polymer were evaluated with respect to impact and flexural properties and its *in vitro* blood compatibility and cytotoxicity properties are addressed and discussed.

EXPERIMENTAL

Materials

The basic structures of the monomers used in this work are presented in Table I. The epoxy prepolymer used was a DGEBA (DER 331 from Dow Chemicals, Brazil), with an epoxide equivalent weigh equal to 187.5 g eq^{-1} determined by chemical titration.[19] It was carefully dehydrated before use. The aliphatic amine epoxide hardeners triethylenetetramine (TETA; DEH 24 from Dow Chemical, Brazil), AEP and IPD (or 5-amino-1,3,3-trimethylcyclohexanemethylamine) (both from Aldrich (São Paulo, Brazil), 99% purity) were used without further purification.

Preparation of epoxy networks

The networks were prepared by carefully weighing the epoxy amine hardener at the stoichiometric amount (ratio amino-hydrogen to epoxy, $a/e = 1$).[20] All mixtures were gently stirred for 1 min at room temperature to ensure dissolved of the hardener. Then, the mixtures were degassed under vacuum for 10 min to remove trapped air, poured in a silicon mold and cured using a following thermal cycle: 24 h at room temperature (25°C) for all system, and for the formulations based on AEP, TETA and IPD were pos-cured at 120, 130, and 160°C during 2 h, respectively. The cure schedule chosen for each formulation were optimized by calorimetric studies (Shimadzu, Model DSC-60) to obtain the cured-fully networks.[19,21,22]

The cured materials were allowed to cool slowly to room temperature. Then, the materials were removed from the mold and machined for subsequent mechanical characterization, to reach final dimensions and improve surface finishing.

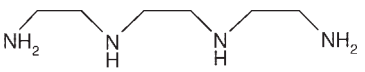
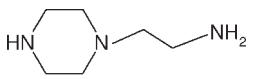
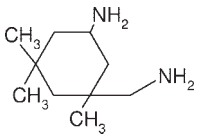
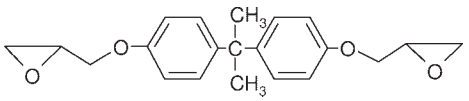
Thermal analysis

A Shimadzu Model DSC-60 was used to determinate the glass transition temperature (T_g) of the epoxy-amine networks under dry nitrogen atmosphere. Glass transition temperatures, defined at the middle-point of the change in specific heat, were determined during a subsequent scan at 10°C min^{-1}.

Mechanical properties measurements

The prepared epoxies materials were tested under flexural conditions. The mechanical tests were carried out at room temperature (23°C) in EMIC universal testing machine, model DL 2000, at a crosshead speed of 10 mm min^{-1}. The values of flexural stress, flexural modulus, and flexural strain were measured in three-point bending using samples dimensions equal to 3.3 mm in depth, 12 mm in width, and 65 mm in length. The length between supports was equal to 50 mm as recommend by the ASTM D 790 protocol.[22] The samples were tested to fracture and the maximum stress (σ_y), maximum strain (ε_y), and

TABLE I
The Basic Structure of the Monomers Used in This Work

Name	Designation	Chemical structure	Functionality
Triethylenetetramine	TETA		6.0
1-(2-Aminoethyl)piperazine	AEP		3.0
Isophoronediamine	IPD		4.0
Diglycidylether of bisphenol A	DGEBA		2.0

the flexural modulus (E_y) were calculated according to ASTM D 790 from the load-defection curves.

The Izod notched impact test was carried out using a pendulum-type impact test (Tinius Olsen, model 892) with a striking velocity of 3.46 m s^{-1}. Rectangular samples were used (62 mm × 12.9 mm × 6 mm) as recommended by ASTM D 253 protocol.[23] The impact test was carried out at 23°C and impact energy was reported in J m^{-1}. A minimum of 10 samples was mechanically assayed and the average value was reported.

Contact angle measurement

It is well known that the free surface energy of a material should be able to provide the interaction between the energy required for protein or blood cell adhesion.[24,25] Thus, the surface roughness must be characterized and controlled to achieve good biocompatibility. Especially, the surface characteristics of biomedical materials in water are important because they are generally used in wet state or aqueous solution. The free surface energy components of the epoxies networks were calculated from the contact angle data of various liquids by using the van Oss-Good methodology.[26]

Contact angle measurements (at least 10 determinations for each liquid and for each material) were performed automatically by using an image analysis system installed in a standard contact angle apparatus. The images were transmitted by a video camera to a personal computer. All the measurements were performed by the sessile drop method at room temperature (25°C). Both advancing and receding contact angles and water (*W*), formamide (*F*), and α-bromonaphthalene (α-*B*) were used. The degrees of hydrophobicity (ΔG_{lwl}) of the epoxy polymers (*l*) were evaluated expressing the free energy of interaction between two entities of that material when immersed in water (*w*). The interaction of the epoxies materials with water suggest that if the interaction between the two entities is stronger than the interaction of each entity with water, the material is considered hydrophobic ($\Delta G_{lwl} < 0$). The thermodynamic potential ΔG_{lwl}, for the epoxies networks surfaces was calculated through the surface tension components of the interacting entities, according to the equation[27]:

$$\Delta G_{lwl} = -2\left(\sqrt{\gamma_l^{LW}}\sqrt{\gamma_w^{LW}}\right)^2 + 4\left(\sqrt{\gamma_l^+\gamma_w^-} + \sqrt{\gamma_l^+\gamma_w^-} - \sqrt{\gamma_1^+\gamma_l^-} - \sqrt{\gamma_w^+\gamma_w^-}\right) \quad (1)$$

where γ^{LW} accounts for the Lifshitz-van der Waals component of the surface free energy and γ^+ and γ^- are the electron acceptor and electron donor parameters, respectively, of the Lewis acid-base component (γ^{AB}), with $\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-}$.

The surface tension components of the epoxies networks were obtained by measuring the contact angles of three pure liquids (one apolar and two polar) with well-known surface tension components followed by resolution of three equations simultaneously in according to the following equation[28]:

$$(1 + \cos\theta)\gamma_1^{TOT} = 2\left(\sqrt{\gamma_s^{LW}\gamma_1^{LW}} + \sqrt{\gamma_s^+\gamma_l^-} + \sqrt{\gamma_s^-\gamma_l^+}\right) \quad (2)$$

where θ is the contact angle and $\gamma^{TOT} = \gamma^{LW} + \gamma^{AB}$.

In vitro properties studies

Over the past two decades, several investigators have sought to evaluate the epoxies networks as

potential implant materials.[15–18] The majorities of these studies are based on histological observations and morphologic evaluations and are directed toward understanding the cellular organization immediately adjacent to the implant. Our efforts over the past several years have been directed toward developing a better understanding of the relationships between polymer structure and blood compatibility.[24,29,30] This obviously involves both an appreciation of the blood response to the material as well as the material response to cells, proteins and other mediators that may be present at the interface.

In this work, the *in vitro* blood compatibility of the prepared epoxies networks were studied by four *in vitro* bioassays: protein adsorption, blood platelet adhesion, thrombus formation and cytotoxicity. These *in vitro* assays are sensitive, reliable, reproducible and quantifiable to verify the blood compatibility of epoxies networks without use animal experimentation.

Protein adsorption

The nature of the proteinaceous film deposited on the epoxies materials following implantation is a key determinant of the subsequent biological response. Two proteins were selected for the study: human serum albumin (HSA) and human serum fibrinogen (HFb). HSA is the preponderant protein in blood and its abundance outweighing all the rest of plasma proteins.[31] Adsorption of this protein has a profound influence on the succeeding events of the blood coagulation cascade and a reduced activated platelets adhesion has been reported for polymers that adsorb relative amounts of HSA.[32] Fibrinogen plays a central role in hemostasis participating not only in the coagulation cascade, but it also promotes adhesion of platelets and activates then when adsorbed onto certain solid surfaces.[33]

To quantify the surface concentrations of HSA and HFb adhering to the epoxies networks surfaces, solutions of 10 mg mL^{-1} HSA (Sigma-Aldrich, 99% purity) and 1 mg mL^{-1} HFb (Sigma-Aldrich, 99% purity) were prepared separately in a phosphate-buffered saline (PBS) at pH 7.2 and ionic strength of 0.01*M*. The epoxy specimens were transferred into appropriate Teflon® tubes and a volume of 6 mL of PBS were injected to displace the air and then thermally equilibrated at 37°C. Any air bubbles, which would adhere to the samples, were removed by allowing the samples to cross the air-buffer interface several times. Aliquots of 6 mL of the HSA or HFb solutions were then introduced into the tubes. After the protein solution was in contact with samples at 37° for 2 h.

The amount of adsorbed protein was determined by measuring spectrophotometrically the difference between the amount of HSA or HFb in the solution before and after contact with the epoxies surfaces. The spectroscopic analytical methods for protein dosage is based on the reaction of HSA or HFb with Coomassie brilliant blue (Fluka, 99% purity) dyestuff to record the absorbance of the protein-Coomassie brilliant blue complex in according to Bradford's method.[34] The protein concentration samples were measured at the wavelength of 595 nm, using an UV/VIS spectrometer (Cary 50, Varian). The results were compared to reference curves for the specific protein with a detection limit of 7.0 μg mL^{-1}. From the protein concentration resultant in the supernatant, the total amount of protein added in the beginning, the protein amount adsorbed was calculated.

Platelet adhesion

Blood compatibility of the epoxy polymer was evaluated by the open-static platelet adhesion test with whole human blood.[35] Epoxies specimens were cut in plates (10 mm × 10 mm × 1 mm), polished, cleaned and sterilized by gamma rays at a radiation dose of 25 kGy. The tests were performed by depositing 2 mL of fresh blood onto each of the five test surfaces. After contact times of 180 s, the surfaces were washed with saline solution (0.1*M*, NaCl) under carefully controlled conditions to remove all blood components that did not adhere to epoxies materials surface. The specimen was treated with glutaraldehyde (2%) and dehydrated by dilution with ethanol (25–100%).

Scanning electron microscope

The platelet amount was measured from SEM microphotographs (Phillips XL 30). The average number of adhered platelets was obtained from five photographs of different surface areas (1 cm^2) of the same specimens.

Epifluorescence microscope

After addition of 10 μL of acrydine orange the epifluorescence (BX60, BX-FLA, Olympus, using a combination of optical lenses 100× objective lens, 2× intermediate lens, and 10× contact lens) of the labeled platelets were detected onto epoxy specimens by using a digital camera (DN 100, Nikon Co.) coupled on the front part of the microscope. A vapor mercury lamp selectively filtered within the range 450–490 nm was used as excitation light. A long-pass filter allowed the fluorescence signal to be detected for wavelengths longer than 515 nm. The platelet morphology, platelet density and pseudopodium leaching were observed and evaluated.

Thrombus formation

The kinetics of thrombus formation onto epoxies materials surfaces procedure was detailed previously.[36] The whole human blood was added to one part of acid-citrate-dextrose (ACD) for nine parts of blood. The resultant ACD blood was placed on a glass plate and to epoxies networks surfaces. Clotting was initiated by adding aqueous $CaCl_2$ solution, and the thrombus formed during 30 min was weighed. The relative weights of thrombus formed on different samples were determined, taking as reference the 100% of thrombus formed on a glass plate.

Cytotoxicity measurements

The cytotoxicity of biomaterials must be studied *in vitro* before experiments with animals, to reduce the amount of *in vivo* testing to minimum.[33–38] Many studies have examined the cytotoxicity *in vitro* of epoxy networks[15–18] as well as their monomeric precursors.[38–41] They use different cell lines, a variety incubation times (hours to days), and various assay methods. Both cell cultures and implantation studies in animals have revealed that the cytotoxicity of the epoxy networks is most strongly influenced by the presence of residual monomers. However, the effects of cytotoxicity cellular disappear shortly after setting of the material.[42]

Variability in cell culture conditions normally makes it difficult to directly compare the cytotoxicity experiments conducted in different laboratories. To define cytotoxicity of epoxy polymer it is may be appropriate to use high enough concentrations to define the inhibitory concentration diminishing viability by 50% (IC50 value). The cytotoxicity testing has been well addressed by ISO 10993-5, which presents guidelines for the choice of suitable tests and defines important principles underlying these tests.[43]

In this work, the potential cytotoxicity of the epoxy networks was evaluated against Chinese hamster ovary (CHO) cells, ATCC CHO k1 (American Type Culture Collection, ATCC), according to ISO guidelines.[44]

The cytotoxicity assay was performed by adding dilutions of epoxies networks extracts to a CHO cell culture on a Petri plate (15 mm × 60 mm). The positive and negative controls were a 0.02 vol % phenol solution and ultra-high molecular weight polyethylene (UHMWPE),[44] respectively. Because of their very good biocompatibility properties, UHMWPE have found widely application as implant material in joint endoprostheses[45] and have been indicated by ISO 10993-5 guidelines as negative control in cytotoxicity assays.[45]

Preparation of extracts

Epoxies network samples and UHMWPE films (6 cm^2) were sterilized by gamma radiation (25 kGy) and poured into 100 mL glass flasks. Sixty milliliters of the culture medium MEM-FBS (minimum eagle medium with 10 vol % fetal bovine serum and 1 vol % penicillin and streptomycin solution) was added and incubated at 37°C for 48 h. The supernatant was then filtered through a Millipore® membrane with 0.22 mm pores, and serial dilutions were made of the epoxies networks, UHMWPE and 0.02 vol % phenol solution (100, 50, 25, 12.5, and 6.25 vol %) extracts. UHMWPE and phenol solution were used as negative and positive control, respectively.

Preparation of the primary culture

The CHO cells were cultivated in plastic bottles in a MEM-FBS medium, placed in an incubator at 37°C with a humid atmosphere of 5 vol % CO_2, until a cell layer was obtained. The culture medium was then removed from the incubator, and the cells were washed with calcium- and magnesium-free (CMF) PBS solution. A 0.2 wt % trypsin solution was added to detach the cells from the bottle. The cells were washed twice with CMFPBS, re-suspended in MEM-FBS, and the suspension was finally adjusted to 100 cells mL^{-1}.

Cytotoxicity assay

From the above suspension, 2 mL was distributed on each culture plate (15 mm × 60 mm) and incubated for 5 h for cell adhesion. After this period, the culture medium was removed, and 5 mL of the pure extract and 5 mL of each serial dilution (50, 25, 12.5, and 6.25 vol %) were added to the same plates. Fresh medium (MEM-FBS, 5 mL) was then placed on the plate of the CHO cell control. Each concentration of the tested extracts was made in triplicate. The plates were incubated in a humid incubator with 5 vol % CO_2 at 37°C for 7 days, after which the medium was removed and the colonies mixed with a solution of 10 vol % formaldehyde diluted in a 0.9 wt % saline solution and stained with Giemsa. The visible colonies on each plate were counted and compared with the number of colonies of the CHO cell control plate. The cytotoxicity potential of the material was expressed by an index of cytotoxicity, IC_{50} (%), which represents the concentration of the extract that suppresses the formation of cell colonies by 50% in comparison with the control.

Statistical analysis

All data are presented as mean ± standard deviation. At least, four samples are representative for

TABLE II
Thermal and Mechanical Properties of the Epoxy-Amine Networks

Epoxy networks	T_g[a] (°C)	Impact energy (J m^{-1k})	E_y (GPa)	σ_y[a] (MPa)	σ_y (MPa)	ε_y (%)
DGEBA/TETA	123	36.2 ± 6.1	2.44 ± 0.03	117 ± 2.0	117 ± 2.0	7.0 ± 1.8
DGEBA/AEP	115	70.1 ± 4.4	2.40 ± 0.02	53 ± 0.4	97 ± 0.5	7.4 ± 0.2
DGEBA/IPD	155	33.8 ± 2.8	2.83 ± 0.02	120 ± 0.5	123 ± 0.6	7.8 ± 0.2

[a] Determined by DSC, E_y = flexural modulus, σ_y = strain at break, σ_Y, ε_Y = stress, strain at the maximum.

each data point. Statistical analysis for determination of differences in the measured properties between groups was accomplished using one-tailed analysis of variance, performed with a computer statistical program (Student's *t*-test), and *P* values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Mechanical properties and surface characterization

Thermal and mechanical properties of the epoxy networks based on DGEBA cured with the aliphatic amines TETA, AEP, and IPD are shown in Table II and the flexural stress-strain curves are given in Figure 1. The flexural test of DGEBA-TETA network shows the same flexural modulus than the DGEBA-AEP system, while the higher value of T_g is observed in the first. The T_g depends of crosslink density and chain flexibility of the networks.[46] Therefore, it would be expected that the DGEBA-AEP networks exhibit less flexural modulus and more flexibility that DGEBA-TETA. This behavior is also due to the minor functionality present in the structure of the AEP when compared with TETA. It is well known that the elastic modulus in the glassy state depends on the cohesive energy density and the intensity of subglass transition.[46] In this case, we observed the same flexural modulus for both epoxy networks.

A possible explanation for this behavior could be based on the chemical composition of the TETA and the possible relation with the local mobility of the network. The local mobility is determined by the totality subglass transition (γ and β between others), those that are determined by the chemical structure that determine the type and intensity of these transitions. For triethylenetetramine (TETA) the term polyethylene-polyamines[47] or ethyleneamines[48] would be more appropriated. This is because the product consist of a mixture of linear, branched, and cyclic (piperazine types) ethyleneamines. Recently, we characterize this hardener (TETA, DEH 24 from Dow Chemical, Brazil) and found four components.[49] The most important is triethylenetetramine, in a concentration of 60 mol % and one component with branched structure, whereas the other two components exhibited cyclic structure; more exactly a combination of linear and cyclic structure similar to AEP molecule. Therefore, the structure based on DGEBA-TETA network is more complex.

The flexural behavior of DGEBA-AEP network gives the best mechanical properties taken into consideration the high value of the flexural strain (ε_y = 7.4%) and the lower value of the flexural stress (σ_y = 97 MPa). This is indicative of a more flexibility of the macromolecular crosslinked epoxy chains when compared with the others networks. This behavior is related to the lower functionality of this hardener. However, DGEBA-IPD network exhibits higher values of the glass transition temperature and worst flexural behavior when compared to the two epoxy networks.

The behavior of the networks based on the cycloaliphatic amines (AEP and IPD) can be explained by the rigid structure of IPD, when compared to the flexible structure in AEP. The cyclic structure in IPD promotes high modulus (E_y) and high glass transition temperature (T_g) when compared to AEP network due to its more rigid skeleton and higher crosslink density. It is interesting to note in Figure 1 the plastic behavior of the DGEBA-AEP system. Experimentally, it was found that the only DGEBA-AEP system had not been broken during the flexion

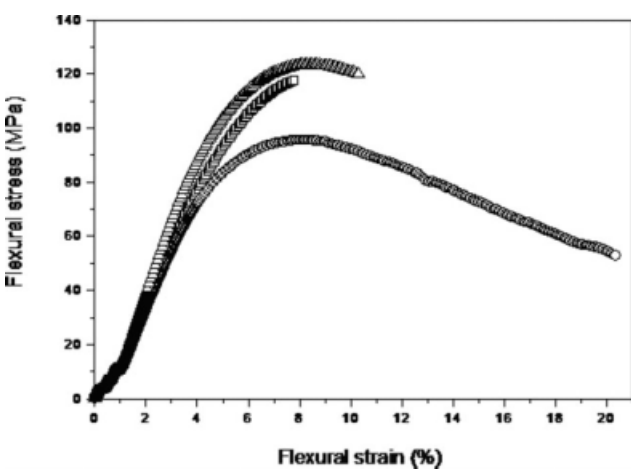


Figure 1 The flexural stress-strain curves obtained by three-point bending test for the IPD (△), TETA (□), and AEP (○) networks.

TABLE III
Contact Angle, Surface Tension Components, and Degree of Hydrophobicity of the Epoxy Networks

	Contact angle			Surface tension components					
Networks surface	θ_W (degree)	$\theta_{\alpha\text{-}B}$ (degree)	θ_F (degree)	γ^{LW}	γ^+	γ^-	γ^{AB}	γ^{TOT}	ΔG_{lwl} (mJ m^2)
TETA	48.3 ± 1.2	20.2 ± 2.1	35.2 ± 1.8	50.6	0.6	24.1	7.6	55.0	−5.1
AEP	65.6 ± 0.9	17.0 ± 1.9	35.8 ± 3.1	51.9	0.1	19.8	2.8	53.5	−6.8
IPD	58.6 ± 2.3	24.62 ± 1.6	48.6 ± 3.1	50.1	0.5	9.7	4.4	54.5	−2.5

θ_W, $\theta_{\alpha\text{-}B}$, and θ_F are the contact angles of water, α- bromonaphthalene, and formamide, respectively.

test confirming the more flexibility of this network. Similar trends were observed in the impact test for the three-epoxy networks studied in this work (Table II). The bigger impact strength was exhibit by the network based on DGEBA-AEP, whereas the epoxy networks based on TETA and IPD shows the same impact energy. The impact strength of a material depends on its ability to absorb or dissipate energy, which requires chain mobility. Therefore, the impact resistance seems to increase when the chain flexibility is increased.

The flexural modulus of DGEBA-TETA, DGEBA-AEP, and DGEBA-IPD networks are 2.44 ± 0.03 GPa, 2.40 ± 0.02 GPa, and 2.83 ± 0.02 GPa, respectively. These values are quite close to that of the commonly used implant polymeric materials and would be interesting to design cardiovascular prosthesis such as coatings or stents with good mechanical and biological performances.[50,51]

Contact angle measurements

The values of the contact angles (in degrees) as well as the values of the surface tension components and the degree of hydrophobicity (ΔG_{lwl}) of the epoxy networks assayed in this work are presented in Table III. Water contact angles of the materials were statistically different ($P < 0.05$) among them.

According to the results of the contact angle, all epoxies networks studied are hydrophilic materials with no significant differences in the values for the free energy of self-interaction in water (ΔG_{lwl}). Considering the surface tension parameters, the network based on TETA is a surface predominantly electron donor (higher values of γ^-), with a low electron acceptor parameter (γ^+). This behavior can be related to a number of nitrogen atoms in the TETA molecule that could contribute to the formation of more elastically active network chains. In this sense, the nitrogen atoms could act as electron donors on DGEBA-TETA network, as can be observed by the polar component γ^{AB}. However, a specific role of Lewis acid-base interactions in the adhesion process onto epoxy-amine networks was not possible to hypothesize it. In this way, it is not possible to establish any correlation between the electron donor and electron acceptor capabilities of the interacting surfaces.

In vitro biological properties

Protein adsorption

Despite the extensive literature in the field, the biological basis of the biomaterial/blood interactions is still uncertain, as the adverse events that are initiated when the material is exposed to blood are numerous and complex.[52,53] It is generally believed that, when a synthetic material is exposed to blood, the first event that takes place is the adsorption of proteins onto its surface, followed by platelet adhesion and activation. HSA and HFb adsorption onto DGEBA-TETA, DGEBA-AEP, and DGEBA-IPD networks were studied with the purpose of examining the extent of the surface interaction with proteins in physiological solution.

The protein adsorption measurements of HSA and HFb onto epoxy surfaces are presented in Figure 2. For the epoxy polymers surfaces studied in this work there was a higher HSA adsorption compared

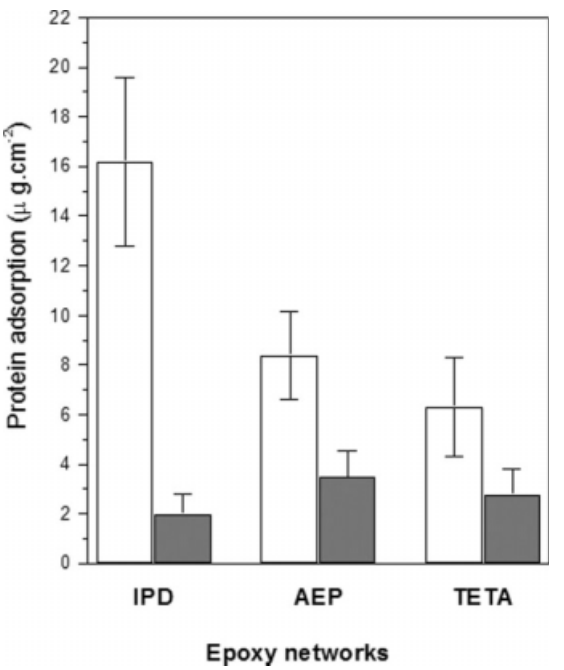


Figure 2 Protein adsorption onto epoxy networks surfaces. In white and gray are represented the HSA and HFb adsorptions, respectively ($P < 0.008$).

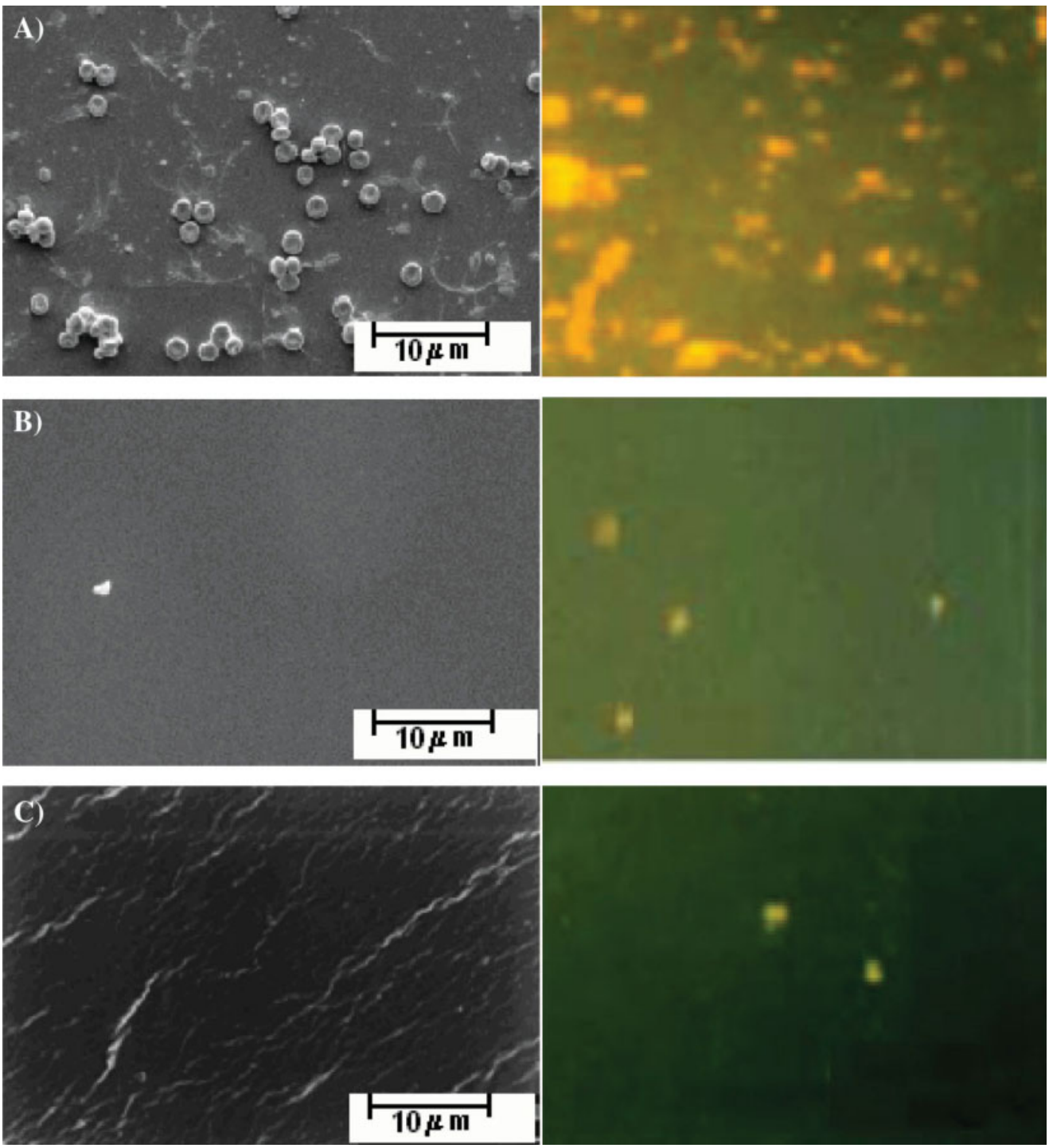


Figure 3 Scanning electron and epifluorescence micrographs of TETA (A), AEP (B), and IPD (C) networks surfaces after incubation with human blood at 37°C for 180 s. The magnification in epifluorescence micrographs is ×2000. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

to the fibrinogen adsorption suggesting a nonthrombogenic behavior of these surfaces.

It is well known that the type and amount of blood proteins adsorbed at the biomaterial/blood interface largely dictate the surface-induced platelet activation. In this sense, the adsorption of fibrinogen is known to accelerate platelet adhesion and activation. However, albumin adsorption on the synthetic surfaces can inhibit platelet activation and, therefore, does not promote clot formation. Thus, the large HSA adsorption relatively to HFb onto epoxy networks may be a good indication of low platelet adhesion and activation by these surfaces.

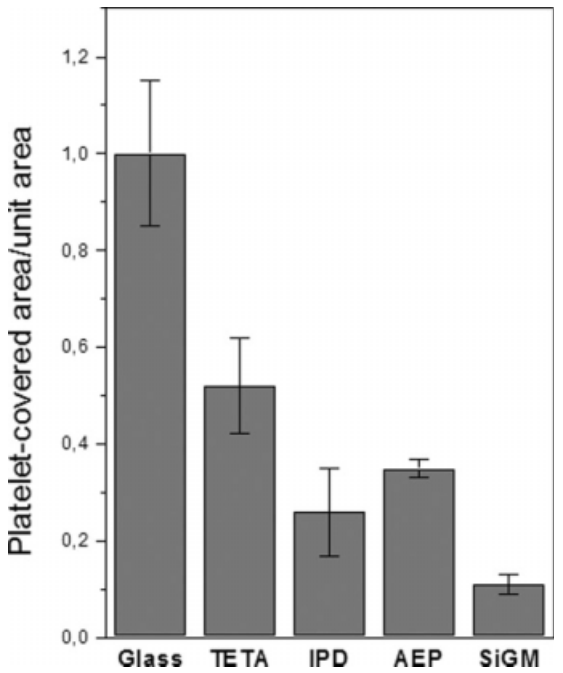


Figure 4 Ratio of platelet-covered area per unit area (100,000 μm^2) on the surfaces of epoxy networks after human blood exposition. Glass and silicone medical grade (SiGM) are used as positive and negative controls, respectively.

Platelet adhesion and activation

Both, scanning electron micrographs and epifluorescence microscopy of the epoxy polymer surfaces after contact with blood showed that TETA network surfaces present a significant thrombogenic activity. The exposition of TETA system surfaces to blood resulted in adhesion of large activated and aggregated platelets and fibrin fibers due to the HFb polymerization on surface of epoxy polymer (Fig. 3). There is no observed adhered activated platelet or fibrin formation in epoxy networks based on IPD and AEP surfaces [Fig. 3(c)]. The epoxies materials based on cycloaliphatic amines (AEP and IPD) surfaces were essentially free from platelet adhesion as well thrombus formation (Fig. 3).

The number of adherent platelets and the platelet-covered area were determined as markers of surface thrombogenicity, using SEM analysis. Both the number of platelets and the changes in morphological shape in active platelets contribute to the platelet-covered surface area of the substrate. Thus, calculating the platelet-covered area/unit area provides an index that reflects platelet adhesion and activation. The ratio of platelets per unit area adhered to the surfaces of epoxy networks surfaces after incubation is displayed in Figure 4. The proportion of platelet-covered area for TETA network surfaces was significantly higher than that for IPD network and silicone medical grade (used as negative control) and this can be ascribed to the high HSA adsorption by these surfaces.

Kinetics of thrombus formation

The kinetics of the thrombus formation after exposure of the epoxy polymer surfaces to human blood is showed in Figure 5. The thrombus formation decreased drastically in both, IPD and AEP networks surfaces, in accordance with the HSA adsorption results. Thus, the lower HFb adsorption appeared to delay the contact activation of intrinsic coagulation in the networks DGEBA-AEP and DGEBA-IPD.

Cytotoxicity

It is well known that the biocompatibility is certainly controlled by the cellular activity at the interface of the synthetic material.[54] In this study, the cellular compatibility of each test sample was evaluated by an *in vitro* cell-culture assay using mammalian cells (CHO). The cytotoxicity level of epoxy specimens was relatively low for all the tested epoxy networks (Fig. 6) and it might be well worth performing further studies of these materials using animal models, to gain insight into the material behavior within the biological media.

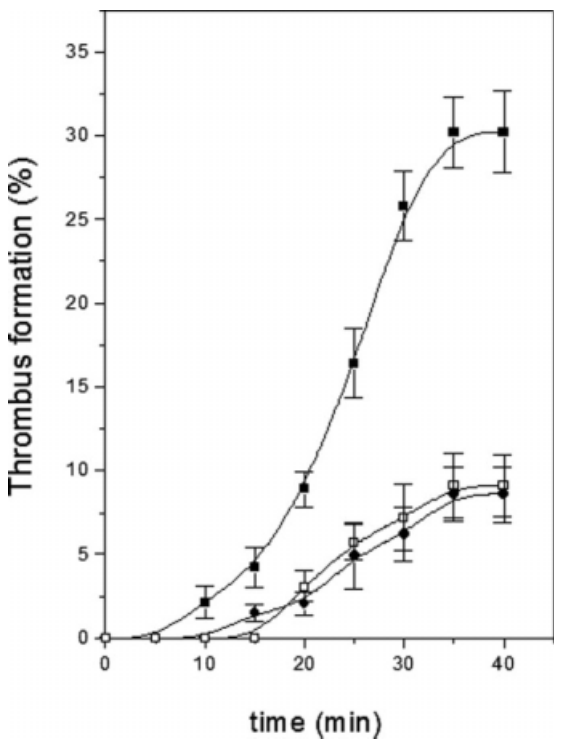


Figure 5 Kinetics of thrombus formation on TETA (■), AEP (●), and IPD (□) epoxy networks.

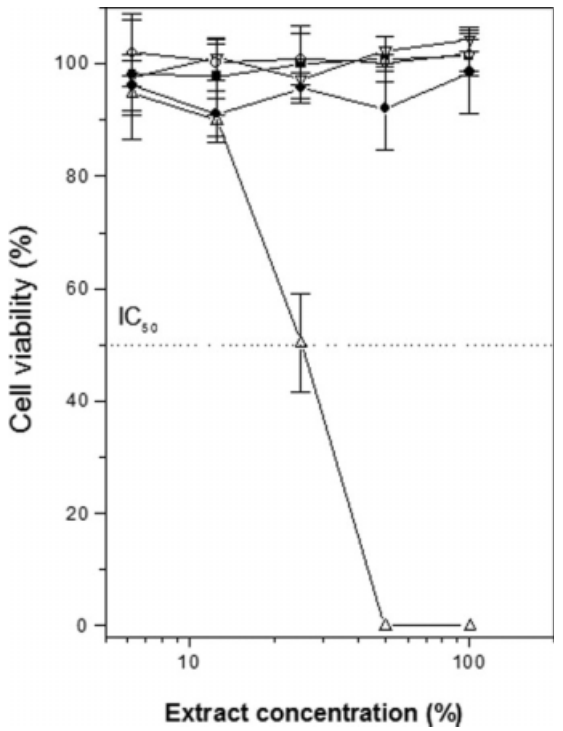


Figure 6 Cytotoxicity of the TETA (■), AEP (●), and IPD (○) epoxy networks extracts, negative control (UHMWPE) (▽), and positive control (phenol) (△) against Chinese hamster ovary cells.

Based on the obtained results and focusing on the application of the epoxy networks based on DGEBA cured with aliphatic amines for use in cardiovascular area in design microspheres for drug carriers or stents the biodegradation behavior, and processing into prosthetic devices are being carried out and the results will be reported elsewhere.

CONCLUSIONS

The mechanical properties, blood compatibility and cytotoxicity of three epoxy-amine networks based on DGEBA cured with triethylenetetramine (TETA), 1-(2-aminoethyl) piperazine (AEP), and IPD were studied and *in vitro* assayed. Functionality rules the mechanical behavior of epoxy networks by increasing the crosslink density and the flexural modulus, increasing T_g and decreasing the chain flexibility and the impact resistance. The higher albumin and lower fibrinogen adsorption as well as the suppression of platelet activation and good cytocompatibility of the epoxy networks based on cycloaliphatic amines (AEP and IPD), indicated that these epoxy networks would be promising materials for cardiovascular applications. The thrombogenicity assays of the network based on TETA suggest that this epoxy network cannot be used in cardiovascular applications without some form of surface modification. The cytotoxicity results indicate that every studied epoxies materials are biocompatible with mammalian cells. Studies of the biodegradation behavior and processing into prosthetic devices from DGEBA-AEP and DGEBA-IPD formulations are currently being investigated in our laboratories and the results will be reported elsewhere.

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