Articles

Biocompatibility Testing of Branched and Linear Polyglycidol

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Polyglycidols are flexible hydrophilic polyethers that are potentially biocompatible polymers based on their similarities to the well-studied poly(ethyleneglycol). Polyglycidols can be prepared as branched or linear polymers by suitable synthetic methods. Biocompatibility testing of these polymers conducted in vitro as well as in vivo are reported here. The in vitro studies included hemocompatibility testing for effects on coagulation (PT and APTT), complement activation, red blood cell aggregation, and whole blood viscosity measurements. In vitro cytotoxicity experiments were also conducted. The results were compared with some of the common biocompatible polymers already in human use. Results from these studies show that polyglycidols are highly biocompatible. Hyperbranched polyglycidols were found to be well tolerated by mice even when injected in high doses.

Introduction

Poly(ethyleneglycol) (PEG) is one of the most studied biocompatible polymers and is widely used in the pharmaceutical and biomedical fields. Grafting ("PEGylation") or coating of PEG onto blood-contacting surfaces decreases protein adsorption, platelet adhesion, and thrombogenicity.[1−3] PEGylation of many drugs or drug delivery candidates, such as polypeptides and liposomes, leads to better plasma half-lives, reduced immunogenicity, and therapeutic efficiency.[4−8] Polyglycidol (PG) is a flexible hydrophilic aliphatic polyether polyol that can be prepared in branched or linear forms. Because of the structural similarities with PEG, polyglycidols are also expected to be biocompatible.[9]

It is widely accepted that protein adsorption is the primary event when a foreign material comes into contact with blood, usually followed by a series of biochemical reactions such as the complement cascade. It has been theoretically predicted that, at equivalent areas per molecule, a branched polymer will be more efficient in protein rejection than linear grafts.[10] Recently, Haag and co-workers reported that hyperbranched polyglycidol self-assembled monolayers (SAM) are as protein resistant as PEG SAMs.[11] Moreover, hyperbranched PG is thermally and oxidatively more stable than PEG.[11] This suggests that these robust polymers are promising as biomaterials. We are exploring the use of hyperbranched polyglycidols in preparing protein or drug conjugates. As a first step, we report here some biocompatibility evaluations of hyperbranched and linear polyglycidols. The biological activity of branched polymers might be different from their linear counterparts, as the more globular structure of the hyperbranched material may hinder interactions with biomolecules compared to a flexible linear chain.[12] The PG results will be compared with those of some common biocompatible polymers currently in clinical use. In vitro experiments such as coagulation (PT and APTT), platelet activation, complement activation, red blood cell aggregation, whole blood viscosity, as well as cytotoxicity experiments, were conducted. Hyperbranched polyglycidols were tested for toxicity in mice.

Experimental Section

Materials and Methods. All chemicals were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON) and used without further purification except the following. Glycidol (96%) was purified by vacuum distillation and stored over molecular sieves in a refrigerator (2−4 °C). Hetastarch was (M_n 150 000, PD = 3.6) obtained from Aldrich as a 6 wt % solution.

NMR spectra were recorded on a Bruker Avance 300 or 400 MHz NMR spectrometer by using deuterated solvents (Cambridge Isotope Laboratories, 99.8% D) with the solvent peak as a reference. Molecular weights and intrinsic viscosity values for polyglycidol samples were determined by gel permeation chromatography (GPC) on a Waters 2690 separation module fitted with a triple detector from Viscotek Corporation, which utilizes refractive index, 90° light scattering and intrinsic viscosity determinations. An aqueous 0.1 N $NaNO_3$ solution was used as the mobile phase at a flow rate of 0.8 mL/min. An Ultrahydrogel linear column with bead size 6−13 μm (elution range 10^3−5 × 10^6 Da) and an Ultrahydrogel 120 with bead size 6 μm (elution range 150−5 × 10^3 Da) from Waters were used. The d*n*/d*c* value for polyglycidol was determined to be 0.12 in aqueous 0.1 N $NaNO_3$ solution and was used for molecular weight calculations.

Synthesis of Hyperbranched Polyglycidol. Hyperbranched polyglycidol (HPG) was synthesized according to the literature procedure by using trishydroxymethylpropane (TMP) as initiator.[13] The polymer was purified by dialysis against water by using a regenerated cellulose membrane (MWCO 1000 g/mol, Spectrum Laboratories Inc.). The degree of branching and degree of polymerization of the hyperbranched

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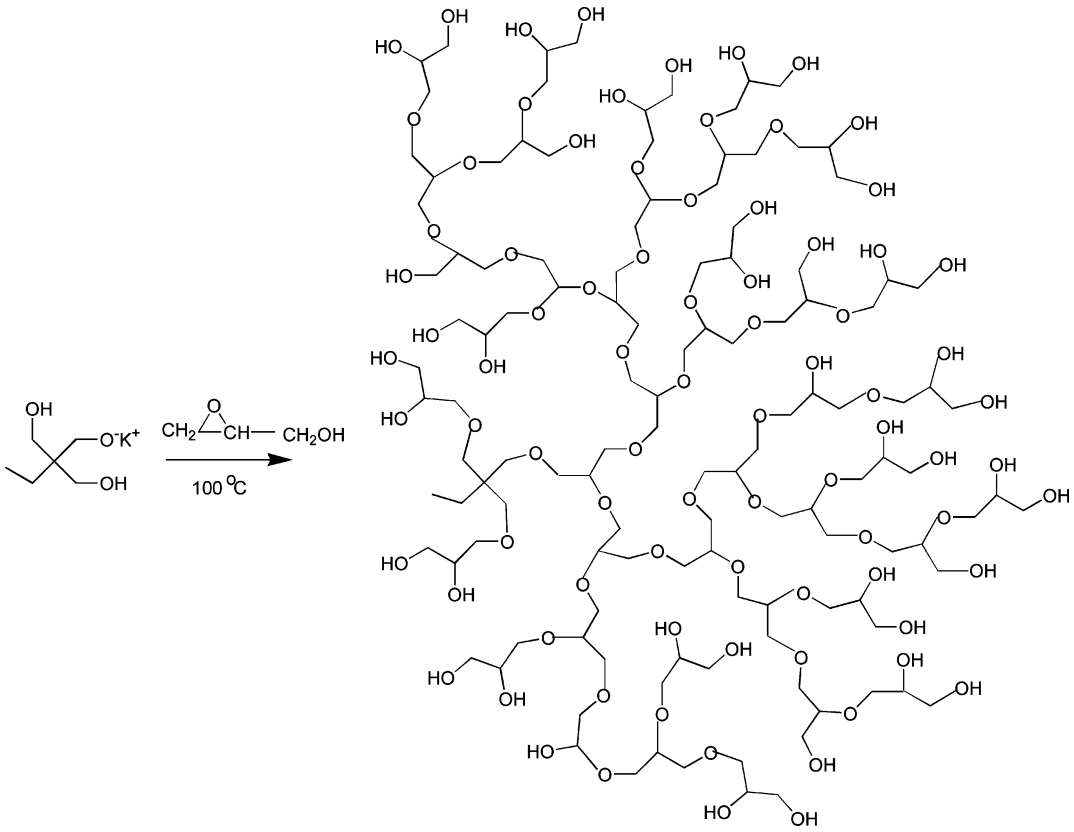
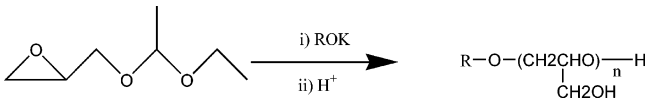
Scheme 1. Synthesis of Hyperbranched Polyglycidol



Scheme 2. Synthesis of Linear Polyglycidol



polymer determined from ^{13}C NMR under quantitative conditions (inverse gated) was 59 and 40, respectively. The structure of the polymer is shown in Scheme 1. The resulting polymer was found to have M_n of 6400, $M_w/M_n = 1.28$, $R_g = 2.82$ nm, and $R_h = 2.16$ nm. ^1H NMR (DMSO-d_6): δ 3.3−3.7 (br, CH_2 and C*H*), 4.4−4.7 (br, *OH*).

Synthesis of Linear Polyglycidol. Linear polyglycidol (LPG) was synthesized following a reported procedure (Scheme 2).[14] Ethoxy ethyl glycidyl ether (10 g) dissolved in 15 mL of THF was added dropwise over a period of 24 h to a solution of 120 mg of KO^{t}Bu in diglyme at 110 °C by using a syringe pump. The reaction mixture was stirred for another 12 h. After removing the solvent, the polymer was dissolved in 200 mL of THF and stirred with 25 mL of 35% HCl. The precipitated polymer was washed several times with THF and acetone. The polymer was further purified by repeated precipitation of methanol solution in acetone. The polymer was additionally purified by dialysis against water by using a regenerated cellulose membrane (MWCO 1000 g/mol) and freeze-dried. ^1H NMR (DMSO-d_6): δ 3.3−3.7 (br, [-CH_2−C*H*CH_2-OH−O]$_n$), 4.0 (br, *OH*). The resulting polymer was found to have M_n of 6400, $M_w/M_n = 1.6$, $R_g = 2.82$ nm, and $R_h = 2.16$ nm.

In Vitro Experiments. The following in vitro experiments were conducted for the assessment of biocompatibility. The polymer solutions of required concentrations were freshly made in 150 mM aqueous NaCl solution and filtered through 0.2 μm filters before use.

Coagulation. Blood was drawn from a healthy unmedicated donor into an evacuated siliconized glass tube (Becton Dickinson, Franklin Lakes, NJ) containing 3.2% sodium citrate (nine parts blood to one part anticoagulant). Plasma was isolated by centrifugation at 2000 × g for 15 min at room temperature and used immediately. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured with a coagulation analyzer, using mechanical end point determination (ST4, Diagnostica Stago). For the PT determination, the extrinsic and common coagulation was activated by incubating plasma with Innovin reagent, and the clotting time was then measured. Innovin (Dade Behring) is a lyophilized reagent consisting of recombinant human tissue factor and synthetic phospholipids (thromboplastin), calcium ions, a heparin-neutralizing compound, buffers, and stabilizers (bovine serum albumin). For the APTT determination, the intrinsic and common coagulation pathways were activated by adding a partial thromboplastin reagent (Dade Behring) and calcium chloride to plasma, and the clotting time was measured. The effect of each polymer solution on coagulation was studied after mixing plasma and the polymer solution in the cuvette strips for 5 min at room temperature before adding the coagulation reagents. Control experiments were done adding identical volumes of 0.15 M saline solution. Each experiment was repeated three times. The results were tested statistically by using a Student's *t*-test at 95% confidence levels.

Whole Blood Viscosity. The viscometer used was a Couette bob-in-cup type (Contraves Low Shear 2) equipped with a guard ring to prevent surface film artifacts. The cup rotates around the bob at a series of constant rates, shearing the sample occupying the gap between the two surfaces. The bob remains stationary but is coupled to a torque measuring circuit. At the bob surface, the induced torque, proportional to the shear stress, is measured. The output was logged on a chart recorder, and the records were analyzed manually. The temperature of the sample is controlled by water circulated through the rotor of the viscometer.

The viscosity was determined at 37 °C on approximately 400 μL of sample. Polymer solutions in isotonic saline were added to freshly drawn, EDTA-anticoagulated blood to a final concentration of 20 mg/mL of plasma. The final blood hematocrit (cell volume fraction) was 40%, and the final plasma protein concentration was 67% relative to that of the blood prior to addition of polymer solution. The final cell, plasma, and polymer concentrations were achieved by adding 0.5 mL of 60 mg/mL polymer in saline to 2.0 mL of blood adjusted to a hematocrit of 50% v/v. Testing was completed within 4−6 h of collection of the blood.

The peak in the shear stress−time record, which occurs within a few seconds of commencement of shear,[15] was used as the basis for the true shear rate and viscosity calculations. The procedure for the calculation of true shear rates was that of Kreiger and Elrod[16] and is described in detail previously.[15] Plasma viscosity was determined at 37 °C and averaged over shear rates in the range of 20−60 s^{-1}. The viscometer torque measurement was calibrated with Canon S6 standard oil at 37 °C.

Red Blood Cell Aggregation. EDTA-anticoagulated blood was incubated for 20 min at 37 °C with polymer solution to a final polymer concentration of 20 mg/mL in whole blood. After incubation, the red blood cells isolated by centrifugation were resuspended in plasma and examined by transmitted bright field light microscopy (Zeiss Axioskop 2plus) using wet-mounted slides. Samples obtained after viscosity analysis were also examined. Images were captured with a microscope-mounted black-and-white CCD camera (Qimaging Retiga 1300, exposure times less than 1 ms).

Complement Activation. To assess complement activation, the cleavage of complement component C3 was monitored by measuring the formation of its activation peptides, C3a and C3a des arg, using a commercial C3a enzyme immunoassay kit (Quidel, San Diego, CA). Activation studies were performed by using pooled plasma isolated by centrifugation from whole blood donations. The plasma was anti-coagulated in 3.8% sodium citrate. Equal volumes of plasma and polymer solution in saline at concentrations ranging from 5 to 20 mg/mL were incubated at 37 °C and samples withdrawn after 10 min, 30 min, and 2 h. Briefly, the samples were diluted with the dilution buffer provided in the kit and added to a microtiter plate coated with a monoclonal antibody specific for human C3a and C3a des arg. After a 1 h incubation at room temperature to allow any C3a in the sample to bind to the monoclonal antibody, the plates were washed and incubated with peroxidase-conjugated rabbit anti-C3a for 15 min. Following a final wash step, the chromogenic substrate was added to detect bound C3a. Absorbance was measured at 450 nm. The sample C3a concentrations were calculated by using a standard curve with net absorbance values plotted on the *y*-axis for each C3a concentration indicated on the *x*-axis. Sample values were accepted as valid if they fell on the standard curve; sample values above the top end of the curve were retested following further dilution. The measurements were done in duplicate. Values reported are the means of duplicate measurements.

Platelet Activation. To measure platelet activation, blood was collected from normal donors into sodium citrate anticoagulant and the platelet-rich plasma (PRP) isolated by centrifugation. PRP (50 μL) was then incubated at 37 °C with an equal volume of polymer dissolved in saline at concentrations ranging from 5−20 mg/mL. Aliquots of the incubation mixture were removed at 10 and 30 min to assess the activation state of the platelets by using fluorescence flow cytometry. Expression of the platelet activation marker CD62P and the pan-platelet marker CD42 were detected by using a Coulter Epics-XL (Miami, FL) and a double-staining method. Briefly, 10 μL of postincubation polymer/platelet mix was diluted in HEPES buffer and incubated with 5 μL of monoclonal anti-CD42-FITC and 5 μL of monoclonal anti-CD62P-PE (both from Immunotech, Marseille, France). The addition of 1 U/mL of bovine thrombin (Sigma) was used to activate platelets as a positive control. Mouse IgGs conjugated to the same chromophore (FITC or PE) were used as the nonspecific binding controls. After 30 min incubation, the samples were fixed with 1 mL of formyl saline. Samples were analyzed within 2 h; instrument gates were sent to count 5000 platelets as defined by their forward scatter profile. Data are reported as the percentage of platelets positive for both of the bound antibodies.

Cytotoxicity Testing. The cytotoxicity testing was carried out by the Advanced Therapeutics group at the B. C. Cancer Research Centre, Vancouver, BC, Canada. The cell lines were obtained from the American Type Culture Collection (ATCC), USA. The in vitro cytotoxicity of the branched and linear polyglycidol against human umbilical vein endothelial cells (HUVEC) and L-929 fibroblast cell lines were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[17,18] Adherent cell lines were plated into 96-well plates 1 day prior to adding polymers to allow the cells to adhere to the plate. The cell density was 7000 per well. The plates were left in an incubator overnight. The following day, the polymers were added in increasing concentration to the wells. Following 3 days incubation, cell viability was determined by the MTT assay. The absorbances were measured at 580 nm and compared with those of controls. Fibroblast culture media consisted of minimum essential medium with 2 mM l-glutamine and Earle's BSS adjusted to contain 1.5 g/L of sodium bicarbonate, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate (90%), and fetal bovine serum (FBS) (10%). The endothelial cell growth medium purchased from Clonetics consisted of modified MCDB 131 formulation with 10 ng/mL of hEGF, 1.0 μg/mL of hydrocortisone, 50 μg/mL of gentamicin, 50 ng/mL of amphotericin B, 3 mg/mL of bovine brain extract, and 2% v/v FBS.

Table 1. Polymer Characterization Data

polyglycidol	M_n	M_w/M_n	intrinsic viscosity (mL/g)	R_g (nm)	R_h (nm)
branched	6400	1.28	4.7	2.36	1.81
linear	6400	1.60	7.3	2.82	2.16

The results were analyzed statistically by using ANOVA for multiple comparisons and Student's *t*-test for two-group comparisons at 95% confidence levels.

Animal Studies. Animal studies were carried out by the Advanced Therapeutics group at the B. C. Cancer Research Centre, Vancouver, BC, Canada. Only the hyperbranched polyglycidol was tested for toxicity. Polymers dissolved in isotonic saline were injected into Balb/C mice at different concentrations, and the animals were followed for 28 days before termination.

Results and Discussion

A hyperbranched polyglycidol (PG) sample with number-average molecular weight of 6400 ($M_w/M_n = 1.2$) and a linear polyglycidol ($M_n = 6400$, $M_w/M_n = 1.6$) of similar molecular weight were synthesized following reported procedures (Schemes 1 and 2).[13,14] The polymers are easily soluble in polar solvents such as water and methanol. They were characterized by NMR and GPC, and the data are shown in Table 1. The [η] (intrinsic viscosity) value of the hyperbranched polymer was 4.7 mL/g for M_n of 6400, while that of the linear analogue was 7.3 mL/g, consistent with the branched nature and more compact structure of the former.

Coagulation Studies. Polymer samples were tested for blood compatibility by using conventional clinical coagulation assays. Prothombin time (PT) is used to evaluate the extrinsic and common coagulation pathway, and the results are expressed in seconds required for a fibrin clot to form after tissue thromboplastin (Innovin) was added to a citrated blood plasma sample. The activated partial thromboplastin time (APTT) is used to evaluate the intrinsic and common coagulation pathway, and the results are expressed in seconds required for a fibrin clot to form in the plasma after a partial thromboplastin reagent (actin) and calcium chloride were added to the sample. The results are shown in Figures 1 and 2. Neither the branched nor the linear polyglycidols were found to affect the coagulation significantly, as the values were close to that of the control. However, a small increase in coagulation time (PT and APTT) with increasing polymer concentration was observed in the case of hyperbranched polyglycidol ($p < 0.05$). A small increase in APTT time was observed for LPG ($p < 0.05$), which did not increase with concentration. Hyperbranched polyglycidols were earlier reported to be inactive in coagulant activities when used in low concentrations up to 0.1 mg/mL.[19]

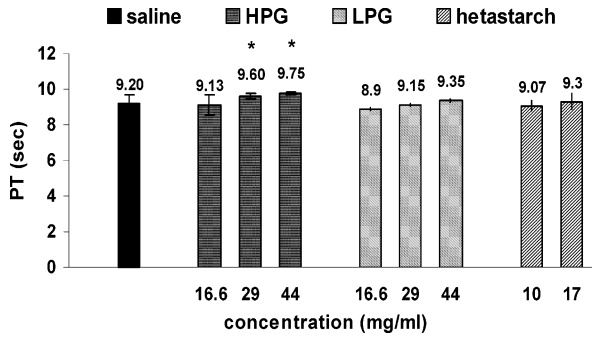


Figure 1. Effect of polymer concentration on the prothrombin time (PT). Significant results compared to saline control are indicated by ∗.

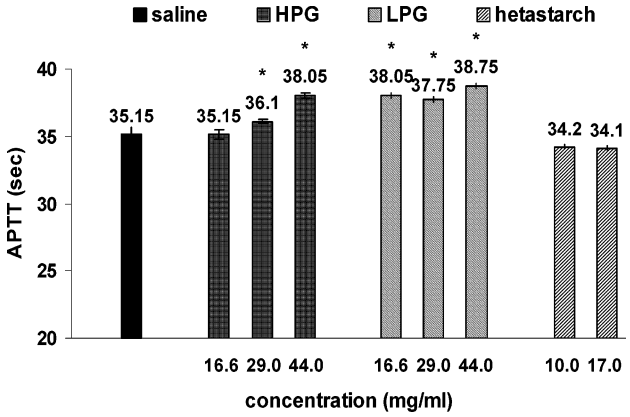


Figure 2. Effect of polymer concentration on activated partial thromboplastin time (APTT). Significant results compared to saline control are indicated by ∗.

Red Blood Cell Aggregation and Whole Blood Viscosity. The apparent viscosity of anticoagulated whole blood depends mainly on plasma viscosity, hematocrit, geometry, and deformability of red blood cells (RBC) and their aggregation.[20] Blood viscosity at high shear rate reflects red blood cell deformability, and the viscosity at low shear rate reflects erythrocyte aggregation.[21] Erythrocytes aggregate naturally in the presence of macromolecules such as fibrinogen or high molecular weight dextran, forming primarily linear aggregates known as rouleaux.[22] Strongly adhesive aggregates can be very difficult to break down in flow due to reversible bridge formation by the polymeric molecules, which adsorb between the erythrocytes. On the other hand, red cells do not aggregate in the presence of albumin or low-molecular-weight dextran (≤40 000) that do not form the bridges due to their smaller size and weaker adsorption.[23] At constant hematocrit, the apparent viscosity of anticoagulated whole blood is high at low shear rates and declines monotonically as the shear rate is increased. Because of the ease with which red cells are deformed in shear, the viscosity of whole blood is much lower than an equivalent concentration of rigidified cells,[24] but aggregation increases the viscosity due to the energy dissipated in breaking aggregates down to allow the suspension to flow.

The response of red cells to polyglycidols when added to whole blood (20 mg/mL) in vitro was studied by microscopic examination and the images of RBCs resuspended in plasma after 2 h of incubation are shown in Figure 3. Both branched and linear polyglycidols were found to have no effect on aggregation after 20 min (images not shown) and 2 h of incubation. In addition, no significant aggregation was observed for the samples subjected to viscosity measurements (~2 h of incubation). However, hetastarch was found to cause enhanced aggregation and the extent of aggregation depended on incubation time. A picture taken after 2 h of incubation is shown in Figure 3. Even though no significant aggregation change was observed after 20 min (image not shown), the cells became distorted and formed tighter clumps than the control after 2 h. Similar aggregation was also observed for the sample subjected to viscosity analysis. These results with hetastarch are in agreement with those published earlier.[21]

Whole blood variable shear rate viscosity studies showed that both branched and linear polyglycidols added at a concentration of 20 mg/mL plasma had little effect on blood viscosity, consistent with the red cell aggregation studies. As shown in Figure 4, the blood viscosities were not different from that of the control in which no polymer was added ($p > 0.05$). However, hetastarch was found to increase the low shear blood viscosity when added at similar concentration (data not shown), as reported earlier.[21] Therefore, both visual examination of RBCs and the blood viscosity studies show that the polyglycidols are highly hemocompatible.

Complement Activation. When a foreign material comes in contact with blood, the complement pathway of the host defense system activates, and a series of chemical reactions take place leading to inflammation. Activation may occur in the presence (classical pathway) or absence (alternative pathway) of antibody specific for the foreign material. Complement activation induced by various biomaterials has been studied.[25−27] Examples of polymers that cause varying degrees of complement activation include dextran, regenerated cellulose, sephadex, nylon, poly(methyl methacrylate), poly(propylene), poly(acrylamide), poly(hydroxyethyl methacrylate), and plasticized PVC. An example of polymer that does not activate complement is poly(*N*-vinylpyrrolidone) (PVP).[28] Surface-induced complement activation has been reported to activate cell adhesion, platelet aggregation, and platelet activation leading to thrombosis.[29−30]

The experiments to assess biocompatibility by monitoring complement activation were set up under incubation conditions whose duration exceeded those of normal complement studies (2 h vs 20−30 min) in order to optimize any chance of detecting accelerated complement activation. Studies were carried out in two biological media: serum and plasma. Although serum is lacking a number of plasma proteins, including the high-concentration protein fibrinogen, it is more commonly used for tests of in vitro complement activation at the biochemical level. However, as plasma is a much better reflection of the total protein that contacts the polymers in vivo, these in vitro studies were carried out primarily with plasma (see Table 2). The positive control for these studies consisted of incubation of plasma with inulin, a potent activator of the complement system. Negative controls consisted of plasma treated with EDTA diluted in the vehicle control (saline). All control samples were incubated for 2 h. The maximum level of C3a generated in inulin-treated plasma was 47.7 μg/mL; C3a generation in EDTA plasma was on the order of 1.2 μg/mL. Complement activation in plasma showed a time dependence suggesting that the activation process is not an acute event and is unlikely to cause a clinical bioincompatibility. This suggests the absence of the "single hit mechanism" reported for other systems.[26] In this mechanism, the polymer causes a burst in the production of C3a at the time of mixing and subsequently generates lesser amounts. Polymers known to be biocompatible incubated in plasma at the highest concentrations and longest incubations tested caused C3a generation at levels between 11and 25 μg/mL. The activation of complement by polyglycidols ranged from 13 to 18 μg/mL at the same concentrations and incubation times.

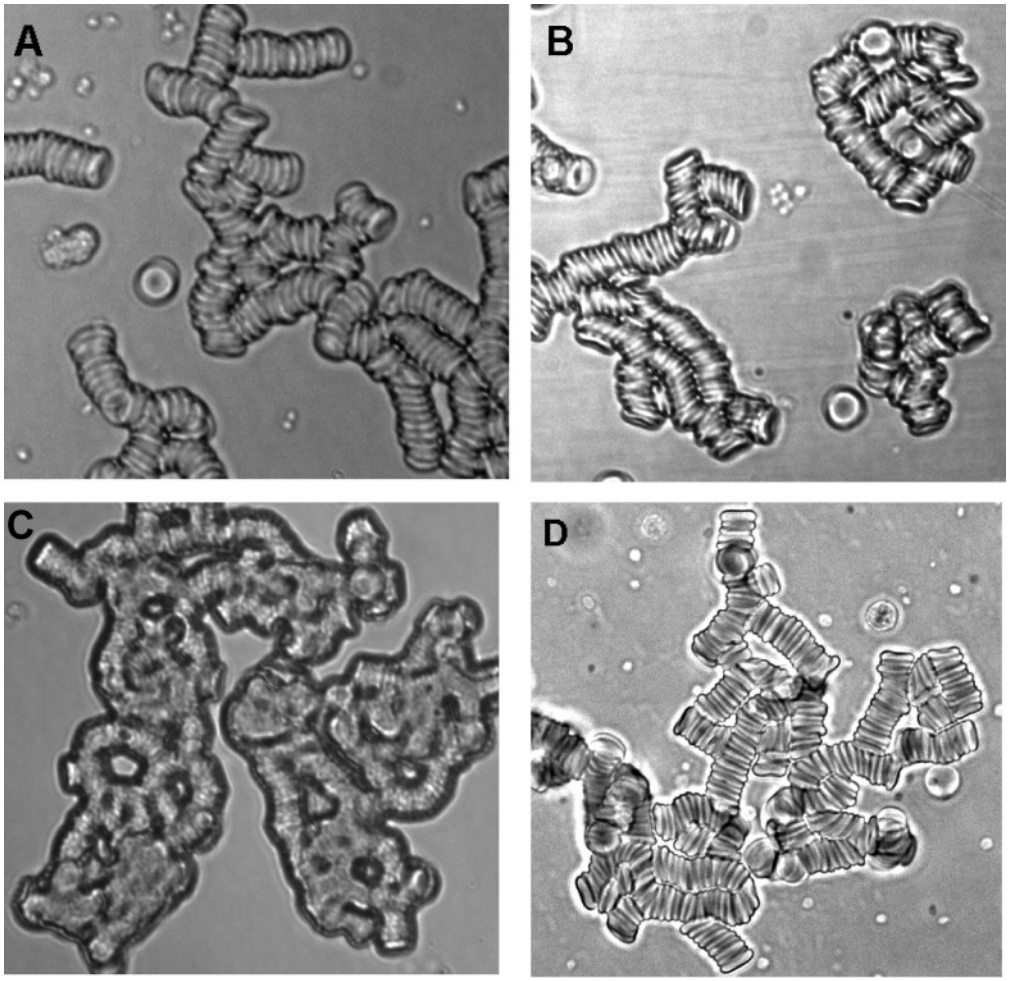


Figure 3. Images of human blood red cells in anticoagulated plasma after 2 h incubation with (A) HPG, (B) LPG, (C) hetastarch, and (D) saline.

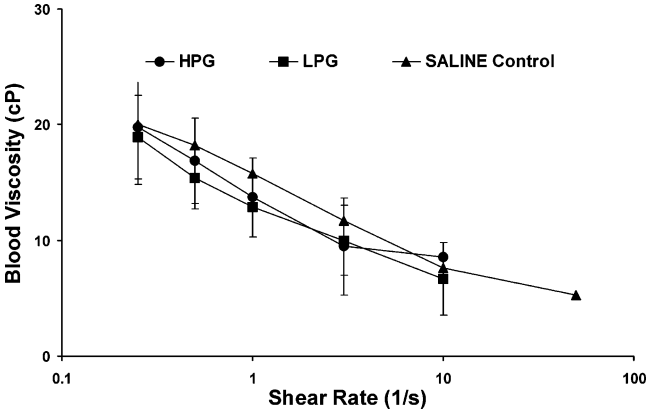


Figure 4. Viscosities of blood−polymer mixtures and control (saline) relative to the corresponding plasma viscosity. Viscosities of test vs saline samples are not statistically different at $p = 0.05$.

Statistical analysis showed no significant difference between the values obtained for controls (saline) and those for HPG (at various concentrations) and LPG ($p > 0.05$). Not surprisingly, upon prolonged incubation of 2 h, all polymers caused marked complement activation in serum, with between 28 and 44 μg/mL of C3a generated. Under these experimental conditions, even saline alone caused marked activation at >22 μg/mL. These data suggest that there is little or no meaningful complement activation caused by the polyglycidols when compared to other polymers that are considered to be biocompatible.

Platelet Activation. Platelets exposed to thrombin (positive controls) showed an anticipated rapid increase in CD62 expression within 10 min to 86.25% of cells analyzed. The effect of various polymers on platelet activation was studied in comparison with that of saline after 10 and 30 min incubation at 37 °C. The PG-based polymers were compared to saline controls in seven separate experiments. The effect of saline dilution on these samples varied from 0.26 to 1.84% of the platelets activated; the values are expressed as the percentage change from the mean control value. For the non-PG polymers, pooled platelets were used. Saline dilution produced a larger effect (18%

Table 2. Complement Activation Caused by the Polymers

polymer	concn (mg/mL)	time (min)	fluid	C3a levels (μg/mL)	SD
inulin (+ve control)	20	120	plasma	47	4.80
EDTA (-ve control)		120	plasma	1.2	0.06
saline		120	plasma	13.1	0.87
HPG	5	120	plasma	13.2	0.11
	10	120	plasma	14.7	0.08
	20	120	plasma	14.1	0.68
	20	10	plasma	0.6	0.04
	20	30	plasma	6.9	0.28
LPG	20	120	plasma	13.4	0.79
PEG-350	20	120	plasma	10.8	0.72
dextran (23k)	20	120	plasma	15.2	0.18
hetastarch	20	120	plasma	25.2	3.21
PVP	20	120	plasma	19.8	0.91
saline		120	serum	22.2	1.42
HPG	20	120	serum	44.3	2.19
LPG	20	120	serum	28.6	3.75

Table 3. Platelet Activation Data, CD62 Expression (% +ve)

polymer	concn (mg/mL)	incubation time (standard deviation) 10 min (SD)	30 min (SD)
negative control		0.07 (0.01)	0.1 (0.08)
positive control		86.3 (2.47)	87.7 (0.28)
saline		1 (0.3)	2.1 (0.08)
HPG	5	1.17 (0.27)	2.10 (0.50)
	10	1.36 (0.38)	1.98 (0.01)
	20	0.84 (0.14)	1.69 (0.23)
LPG	5	0.99 (0.10)	2.63 (0.46)
	10	0.67 (0.21)	2.64 (0.28)
	20	0.99 (0.21)	2.04 (0.13)
PEG-350	10	0.78	0.94
	20	1.05	1.10
hetastarch	10	0.72	1.31
	20	1.04	1.17
PVP	10	0.62	0.88
	20	0.78	0.88
dextran	10	0.69	1.02
	20	0.67	0.89

activation), and the values for these polymers (dextran, PEG 350, hetastarch, and PVP) are expressed as a percentage of the pooled sample saline control (Table 3). The polymers known to be biocompatible, PEG 350, hetastarch, PVP, and dextran, caused CD62 expression at levels ranging from 0.62 to 1.31%. The branched and linear polyglycidols up to 20 mg/mL caused platelet activation expression in the range 0.67 to 1.36%, suggesting that these compounds have little or no direct effect on platelets. These activation levels are consistent with those seen with compounds that are already in human use.[31,32] Statistical analysis of the data indicated no time dependence, as the values obtained after 10 min incubation were similar to those obtained after 30 min. Although there was some CD62 expression seen in all samples other than those incubated only with the control antibodies (0.07%), the polymer-induced activation was no greater than that seen with the biocompatible polymers screened.

Cytotoxicity Studies. The cytotoxicities of branched and linear polyglycidols against L929 mouse areolar/adipose fibroblasts and human umbilical vein endothelial cells were studied by using MTT assay. Fibroblast cells were selected because they are the predominant tissue type in the body. Endothelial cells were also used because one of the potential uses of these polymers is as intravenous drug delivery systems. The results are shown in Figures 5 and 6. The polymers showed very little cytotoxicity toward fibroblasts, with about 80% cell viability observed even at a high concentration of 10 mg/mL after 72 h of incubation. However, a slight toxicity was observed for these polymers in the case of endothelial cells at high concentrations. Statistical analysis showed no significant difference between

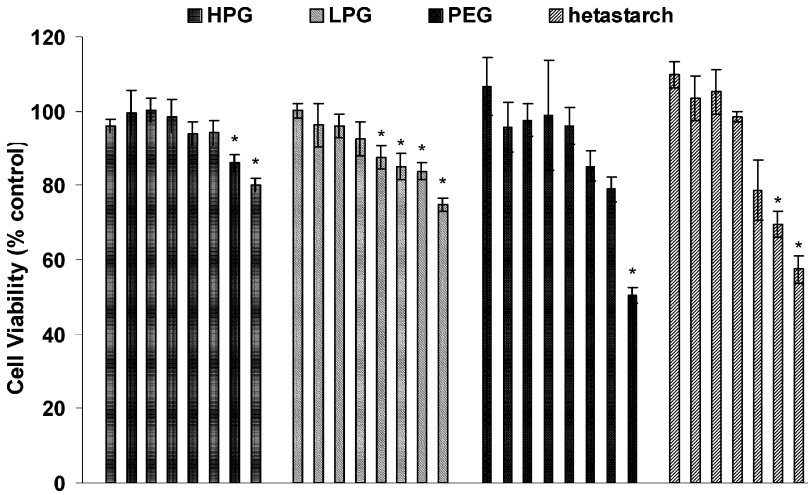


Figure 5. Cytotoxicity of polymers against L-929 cells at increasing concentrations from left to right: 0.0001, 0.001, 0.01, 0.1, 0.5, 1, 5, and 10 mg/mL (up to 5 mg/mL for hetastarch). Significant ($p < 0.05$) results compared to control (without polymer) are indicated by ∗.

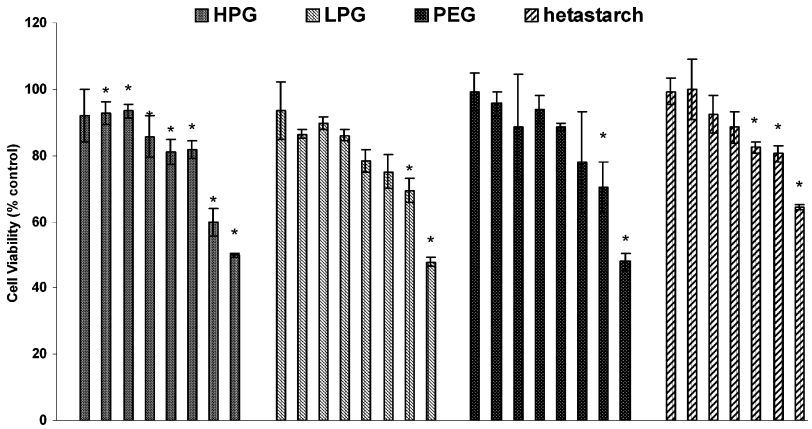


Figure 6. Cytotoxicity of polymers against HUVEC cells at increasing concentrations from left to right: 0.0001, 0.001, 0.01, 0.1, 0.5, 1, 5, and 10 mg/mL (up to 5 mg/mL for hetastarch). Significant ($p < 0.05$) results compared to control (without polymer) are indicated by ∗.

hyperbranched and linear polyglycidols in the concentration range studied for both the cell lines ($p > 0.05$). However, a generalization is not possible, as these polymers are of low molecular weights. Moreover, a comparison of the data with that of PEG or hetastarch showed no significant difference, suggesting that these polymers are highly biocompatible as well.

Animal Studies. Toxicological studies were conducted only for the branched polymer. Two hyperbranched polyglycidols with different molecular weights were tested in mice. These were of M_n 4250 ($M_w/M_n = 1.26$, $[\eta] = 5.7$ mL/g, $R_g = 2.2$ nm, and $R_h = 2.16$ nm) and 15 400 ($M_w/M_n = 6.3$, $[\eta] = 5.2$ mL/g, $R_g = 5.1$ nm, and $R_h = 3.93$ nm). They were injected into Balb/C mice at four concentrations (dosages) from 100 mg/mL (1 g/Kg) to 6.25 mg/mL (62.5 mg/Kg) in isotonic saline, two mice per concentration per compound. The animals were observed and weighed daily for 28 days for signs of toxicity (for example, lethargy, dry eyes, weight loss, scruffy coats). Weight gain was normal and indistinguishable from controls and no untoward indicators were found in any of the animals, even those injected with the high doses of the compound. Hence, the branched polyglycidol compounds were well tolerated by mice. On autopsy, all organs appeared normal.

Conclusions

Both linear and hyperbranched PGs showed very good biocompatibility by a variety of both in vitro and in vivo assays. The topology of the above polymers was found to have no effect, at least for the molecular weights studied. In all aspects of this study, they behaved as well or better than PEG and hetastarch, both polymers commonly used in a variety of clinical settings.

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