

Water-Soluble Aldehyde-Bearing Polymers of 2-Deoxy-2-methacrylamido-D-glucose for Bone Tissue Engineering

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ABSTRACT: In this study the methods of synthesis of hydrophilic aldehyde-bearing polymers and copolymers based on 2-deoxy-2-methacrylamido-D-glucose have been developed. Polymers with controllable aldehyde group content were synthesized via free-radical polymerization and subsequent polymeranalogous modification. The water-soluble polymers obtained were investigated in view

of their capacity to be adsorbed on two commercial mineral supports used in bone tissue engineering. Besides, cytotoxicity of synthesized polymers was tested. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 108: 2386–2397, 2008

Key words: water-soluble polymers; aldehyde group; adsorption; bone tissue engineering

INTRODUCTION

Water-soluble biocompatible polymers represent attractive objects from the point of view of their potential application for construction of composite three-dimensional supports (3D scaffolds) for hard tissues regeneration.¹ In dominant number of published articles the biodegradable polymers play the role of biologically functional constituents, while an inorganic component, which is inserted to increase a mechanical stability, is usually dispersed in a polymer.^{2–4} Recently, the new approach for construction of composite polymer-inorganic scaffolds has been suggested.⁵ The strategy supposes the use of bioabsorbable polymers as a base for development of 3D composite scaffolds for bone tissue construction where a suitable mineral support can be covered by biocompatible but nondegradable macromolecular compounds. In this case, a mineral support^{4–9} provides necessary mechanical stability whereas a polymer component modified with bioligands^{10,11} promotes enhanced cell adhesion, anchorage, and growth.

Evidently for the case described, the choice of polymer constituent is a crucial point. There are few basic requirements that have been accepted for the

water-soluble nondegradable polymers to be used in biomedical applications.¹² First, the polymer should be biocompatible. Second, it has to possess required molecular mass (MM). It means that, on the one hand, the polymer has to be of comparatively high MM to reveal itself as a macromolecular compound, and, on the other hand, the polymer MM should not prevent its removal from the body. Consequently, the optimal value of MM was found as 10,000–30,000. Finally, the polymer has to have a sufficient adsorption capability with regard to the chosen mineral support and contain the reasonable amount of reactive groups to bind covalently necessary biofunctional ligands.

Recently, a few reactive *N*-vinylpyrrolidone (*N*-VP) copolymers were tested as organic part of developed composite materials.⁵ However, the desire to have more choices and ranges of specific polymer properties has provided the stimulus to consider other candidates, among those a new type of polymers known as polyvinylsaccharides^{13–15} can be taken into consideration. The latter represent carbon backbone polymers containing carbohydrate residues as side groups. The properties of these macromolecular compounds were intensively studied concerning their physical peculiarities,^{16,17} problems of enzyme stabilization,¹⁸ and drug delivery,^{19,20} as well as their noncytotoxicity and bioactive character were established.^{13,19–21} The methods of synthesis to obtain linear, block- and graft-copolymers with vinylsaccharides were developed.^{21,22} The presence of saccharide groups in such polymers gives a possibility to form a structure similar to cell membrane surface glycoprotein that, in its turn, may induce a significant

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increasing of specific interactions. Although there are numerous techniques of immobilization of bioligands on polymer surface,^{21,23–29} for the case of construction of composite supports, the covalent binding of biomolecules, such as peptides and proteins, are preferable. However, to prevent any negative consequences, the side-products of such reaction have to be non-toxic. From this point of view, the use of aldehyde polymers, allowing covalent binding of amino-bearing ligands at mild physiological conditions, looks very promising. The reaction results in formation of Schiff's base that can be easily converted into more stable C—N bond by use of equimolar quantity of sodium borohydrate.

In this study the synthesis of polymers based on vinylsaccharide, namely, 2-deoxy-2-methacrylamido-D-glucose (MAG), was performed. There are two ways to introduce aldehyde groups into polyvinylsaccharide chain. The first is a copolymerization of vinylsaccharide with aldehyde-containing, or easily converted into aldehyde-containing (like diethylacetal acrolein, DAAc), monomer. The second one is periodate oxidation of polymer itself. The latter technique is in common practice with respect to modification and structural studies of natural polysaccharides such as cellulose and dextran.^{30–34} Both methods were utilized in present work. Besides, the investigation of adsorption of synthesized polymers on commercial mineral supports, namely, hydroxyapatite (HA) and Sponceram[®] (Sp), as well as cytotoxicity estimation were also carried out.

EXPERIMENTAL

Materials and instruments

All commercially available chemicals were used without additional purification. If necessary, the solvents were distilled and dried by common methods. (+)-D-glucosamine hydrochloride used as initial reagent for monomer synthesis was purchased from Aldrich (Munich, Germany). *N*-vinylpyrrolidone (VP) and DAAc (Vekton, Saint-Petersburg, Russia) were distilled under reduced pressure prior to use. Azo-bis-isobutyronitrile (AIBN) and sodium periodate were purchased from Fluka (Germany). Fuchsin-sulfurous acid (Schiff's reagent) used for aldehyde groups quantification was synthesized, purified, and characterized according to general procedure.³⁵

The dialysis bags Spectra/Pore[®] (MWCO: 1000) used for polymer desalting were purchased from Spectra (USA).

For adsorption studies of obtained polymers, the macroporous monolithic Sponceram[®] (Sp) (doped ZrO₂ ceramic material; pore size 600 μm; surface area ~ 1.4 m²/g) (Zellwerk GmbH, Berlin, Germany) and HA powder (particle size 20 μm; average pore

size 7 μm) (Pentax, Tokyo, Japan) were used as mineral supports.

The ¹H NMR and IR spectra of polymers were measured using a Bruker AV400 and FTIR spectrophotometer (Bruker, Rheinstetten, Germany), respectively. For UV-vis measurements the Full-spectrum UV/Vis Spectrophotometer NANO-DROP ND-1000 (peQLab Biotechnologie GmbH, Erlangen, Germany) was used. The Zetasizer Nano ZS (Malvern Instruments, Herrenberg, Germany) apparatus equipped with 4 mW He-Ne 633 nm laser source was used to perform light scattering measurements.

Methods

Synthesis of 2-deoxy-2-methacrylamido-D-glucose

The synthesis of methacrylic derivative of glucosamine was carried out similar to published procedure.^{16,21} Glucosamine hydrochloride (0.14 mol, 30 g) was dissolved in 450 mL of MeOH. Then 21 mL (0.15 mol) of triethylamine, which was used for eliminating hydrochloric acid fixation, was supplemented and the mixture was cooled up to -5°C. Under vigorous stirring, methacryloyl chloride (0.17 mol, 16 mL) and 21 mL (0.15 mol) of triethylamine were added simultaneously during 1 h, while the temperature was kept at -5 to -2°C. The stirring was continued for 3 h at 5–10°C and then for further 3 h at the room temperature. Finally, it was left at room temperature overnight. Then, after filtration, the reaction mixture was evaporated under reduced pressure. The rest was washed three times with chloroform, dried in vacuum, and recrystallized from 500 mL of ethanol. The yield of MAG was 24.5 g (72%), mp 197–198°C (mp_{lit} 198–199°C¹⁶). Elemental analysis data: N 5.88%, 5.84%; C 48.64%, 48.57%; H 7.09%, 7.02%. Calculated values (C₁₀H₁₇NO₆): N 5.7%; C 48.6%; H 6.9%.

Polymerization of MAG

The polymerization of MAG was performed similar to the previously published method.²¹ MAG (6 g, 0.024 mol) was dissolved in 57 mL of DMF and 0.3 g (5 wt % from MAG amount) of AIBN was added. The reaction mixture was purged with nitrogen. Polymerization was carried out in a sealed ampule at 60°C for 24 h. Polymer was precipitated in diethyl ether. After filtration and vacuum drying 5.7 g (95%) of polymer was obtained.

Synthesis of poly(MAG-co-VP)

The synthesis of poly(MAG-co-VP) was performed as follows: 0.5 g (0.002 mol) of MAG [M₁], 0.21 ml (0.002 mol) of VP [M₂], and 0.029 g (4 wt % from

TABLE I
Polymerization of MAG and Oxidation of polyMAG and poly(MAG-co-VP)

Sample N	Polymer	[MAG] : [NaIO ₄] (mol)	[CHO] (mol %)
1	polyMAG	2	55
2	polyMAG	1	40
3	polyMAG	0.7	30
4	polyMAG	0.3	10
5	poly(MAG-co-VP)	2	30

Polymerization conditions: [MAG] = 10 wt %, [I]₀ = 5 wt %, DMF.

Yield: 98%, MM characteristics: [η]₂₅ = 0.11, M_w (light-scattering) = 2.58 × 10⁴.

[M₁ + M₂]) of AIBN were dissolved in 6.9 mL of DMF, and purged with nitrogen. Free radical copolymerization was carried out in a sealed ampule at 60°C for 24 h. The polymer was isolated by precipitation in diethyl ether, filtrated, and dried in vacuum. The yield of poly(MAG-co-VP) was 94%.

Oxidation of polyMAG and poly(MAG-co-VP)

The synthesis of dialdehyde derivatives of polyMAG was carried out as follows: 0.5 g (0.002 mol) of polyMAG was dissolved in 80 mL of water and cooled up to 5°C. Then the sodium periodate was added corresponding to the desirable amount of aldehyde groups to be obtained (Table I). The reaction was allowed to proceed for 24 h in dark at 5°C. Then the product was purified by dialysis against water (24 h) and dried by lyophilization. The oxidation of poly(MAG-co-VP) was performed in similar way. The resulting aldehyde group content is shown in Table I.

Synthesis of poly(MAG-co-VP-co-DAAc)

The samples of poly(MAG-co-VP-co-DAAc) were synthesized by radical polymerization of comonomers in the presence of AIBN in DMF solution as described earlier. The comonomer ratios [MAG] :

[VP] : [DAAc] = 30 : 30 : 40 or 15 : 45 : 40 mol% were used. The copolymerization proceeds for 72 h. The resulting polymer products were purified via dialysis for 24 h against water and then lyophilized. Their characteristics are given in Table II.

Activation of poly(MAG-co-VP-co-DAAc)

The activation, e.g. the removal of acetal groups, of poly(MAG-co-VP-co-DAAc) was carried out in aqueous solution of hydrochloric acid containing 5 mg/mL of polymer at 50–60°C for 2 h, pH 2.0. After the reaction completed, the solution of activated polymer was dialyzed for 12 h against water and lyophilized.

Characterizations

¹H NMR spectroscopy. The ¹H NMR spectra of polymers were measured in DMSO (d⁶). The spectra obtained allowed qualitative description of synthesized polymers and copolymers structure.

IR-spectroscopy. Infrared spectra of synthesized polymers were recorded from 5000–200 cm⁻¹ in KBr tablets. This method was used to determine the presence of CHO-groups via characteristic ν_{HC=O} adsorption band.

Viscosimetry. Determination of the viscosity of polyMAG was performed using Ubbelode viscometer. The measurements were carried out in 0.1M Na₂SO₄ solution to ensure that no polyelectrolyte effect that might occur by impurities of methacrylic acid in the polymers could falsify the results.

The MM of polyMAG was calculated using previously established Mark-Houwink relationship:²²

$$[\eta]^{25^\circ\text{C}} = 8.29 \times 10^{-4} M^{0.49}$$

Light scattering. The light scattering MM measurements were carried out according to standard Debay method to evaluate the molecular masses of synthesized polymers. The experiment was performed at 25°C. Deionized water was used from Millipore Alfa-Q purification system equipped with 0.22 μm filter. The 0.2 μm filter was used to purify the samples prior to the light scattering experiments.

TABLE II
Copolymerization of MAG (M₁) with VP (M₂) and DAAc (M₃)

Sample N	Copolymer	Polymerization mixture			Copolymer		
		Monomer ratio (mol %)	[M ₁ + M ₂ (+M ₃) (mass %)	[AIBN] (mass % from [M ₁ + M ₂ (+M ₃)])	Yield (%)	[m ₁] : [m ₂] (:[m ₃]) (mol %)	M _w × 10 ⁻⁴ (light-scattering)
6	poly(MAG-co-VP)	50 : 50	10	4	94	77 : 23	2.40
7	poly(MAG-co-VP-co-DAAc)	30 : 30 : 40	40	1	39	82 : 15 : 3	Not determined
8	poly(MAG-co-VP-co-DAAc)	15 : 45 : 40	40	1	22	75 : 18 : 7	1.35

Quantification of aldehyde groups (reaction with Schiff's reagent). The evaluation of molar amount of aldehyde groups was performed using the reaction of polymers with very specific fuchsinsulfite reagent (Schiff's reagent). For this purpose, the calibration curve was plotted using glutaric aldehyde as a standard and the extinction coefficient of CHO-group was calculated. The analysis was carried out as follows: to a sample of 0.5 mL of aldehyde containing substance 2.5 mL of Schiff's reagent was added and the absorbance of colored complex was measured in 40 min at $\lambda = 550$ nm.

Iodine test. The molar amounts of VP in poly(MAG-co-VP) and poly(MAG-co-VP-co-DAAc), as well as the concentration of this copolymer in adsorption studies, were determined using iodine test. For that, 3 mL of 3 mM iodine in 0.2M sodium acetate buffer, pH 4.6, was added to 0.5 mL of polymer solution. Immediately, the absorbance of colored complex was measured at 460 nm. The calibration curve applied for quantification of VP in the copolymers was plotted with use of PVP as a standard.

Adsorption studies

All experiments concerning the study of adsorption kinetics and isotherms building were performed at static conditions.

Despite the further experiments on covalent modification of aldehyde-bearing polymers by amino-containing ligands will be carried out in 0.01M sodium borate buffer, pH 10.0, it is essential for adsorption studies to be performed at physiological conditions, e.g. at pH 7.0. Thus, 0.01M sodium borate buffer, pH 10.0 adjusted to pH 7.0 by boric acid called further as borate solution pH 7.0 was used in all experiments.

Adsorption kinetics and desorption studies. Polymer solution (1.5 mL) with concentration of 1 mg/mL in borate solution pH 7.0, were added to 50 mg of mineral support. The adsorption process occurs at slight stirring at 25°C for 3 h. At regular intervals, the suspension was centrifuged and the amount of adsorbed polymer was estimated by measurements of its concentration in a supernatant using the above-described methods (reaction with Schiff's reagent and/or iodine test).

The control of possible polymer desorption was carried out during 14 days. In these experiments, the sample of composite material (mineral support with adsorbed polymer) was stored in PBS buffer, pH 7.0, and the concentration of probably desorbed polymer was measured in a supernatant using the same quantitative methods.

Isotherms construction. For this purpose, several samples each containing 50 mg of mineral support were

used. The concentration of polymer solutions in borate solution, pH 7.0, varied from 0.25 to 3.5 mg/mL. The samples of Sp and HA were incubated for 2 h (the time corresponding to the plateau on the kinetics curves) at 25°C in polymer solution and the amount of adsorbed polymer at fixed concentration was determined as mentioned earlier.

Cell culture experiments

Cultivation of MC3T3-E1 cells

MC3T3-E1 cells were stored in Dulbecco's modified eagle medium (DMEM), supplemented with 10% fetal bovine serum and antibiotics. For assays in 96-well plates, 5000 cells per well were seeded. For cell seeding onto Sp, 15–20 mg pieces of Sponceram[®] were shaken (350 rpm) with 20 μ L of a cell suspension (1 mio cells/mL) for 30 min at 37°C, 5% CO₂. The Sp pieces were sterilized by autoclaving and incubated with cell culture medium for at least 2 h before cell seeding.

MTT assay

MTT assays were performed with $n = 5$ and one negative control. Matrices were placed into new wells to measure exclusively the viability of the cells adherent to the Sp not those on the bottom of the well. Cells/matrices were incubated with 100 μ L of cell culture medium and 10 μ L MTT (5 mg/mL in PBS) for 4 h at 37°C, 5% CO₂. Formazan crystals were dissolved overnight with 10% SDS in 0.01M HCl. The formazan absorption was measured at 570 nm subtracted by the 630 nm absorption.

Statistical analysis

All values were given as mean ones and standard error of a mean value (SEM) was calculated. A normality test and a test for equal variances was performed before running a Student's two-tailed *t*-test to compare results between two groups. In the case of unsatisfied correlation of the data, the normality Mann-Whitney-Rank-Sum test was performed. A *P*-value < 0.05 was considered as statistically significant. To confirm the dependence of one variable on another (e.g. whether cell viability is dependent of polymer concentration), a linear regression was performed, considering a *P*-value < 0.01 as statistically significant.

RESULTS AND DISCUSSION

Polymer synthesis and modification

This work was aimed to obtain aldehyde-bearing hydrophilic polymers of MAG with controlled

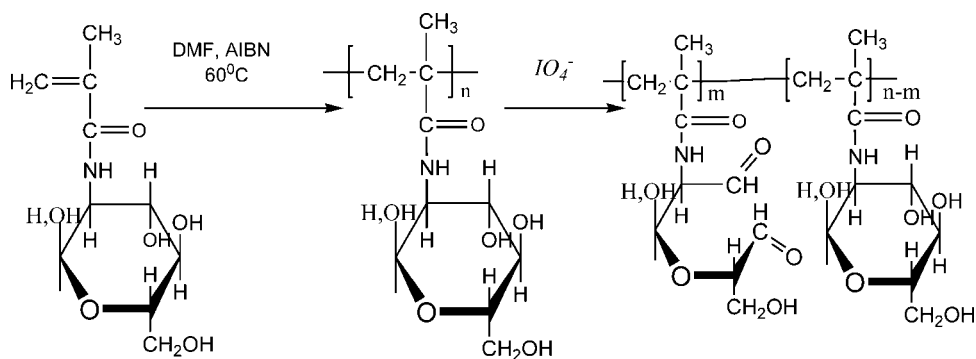


Figure 1 Synthesis and oxidation of polyMAG.

amount of reactive groups in a polymer chain. As mentioned earlier, this goal could be reached by copolymerization of MAG with DAAC, as well as using oxidation of MAG polymers.

Synthesis and modification of polyMAG

The synthetic ways to obtain MAG homopolymer and its dialdehyde derivative are presented in Figure 1.

Polymerization of MAG was carried out similarly to previously published methods^{16,21} that allowed us to obtain the polymer with high yield (94%) and low MM. The value of its intrinsic viscosity was measured to be $[\eta] = 0.11 \text{ dL/g}$ (0.1M Na_2SO_4 ; 25°C) that corresponds to $\text{MM}_n = 21,000$.¹⁶ In parallel, the MM_w value obtained from light-scattering measurements was found as 25,800. Evidently, both the values are nearly similar.

The oxidative cleavage of α -glycols via reaction with IO_4^- ion is a common method used for the structure studies and modification of polysaccharides. The mechanism of this reaction was thoroughly investigated and commonly established as proceeding through the cyclic intermediate complex.^{30,33} In our work we apply this technique to the new type of synthetic polymers, namely, polyvinylsaccharides. As there is only one *trans*- α -glycol in glucose residue, we were supposed to carry out the reaction for longer time than it might be needed in the case of *cis*- α -glycol.

The data obtained in polyMAG oxidation experiments are collected in Table I. The process was performed at different molar ratios of MAG and sodium metaperiodate. The appearance of aldehyde groups in oxidized polymer was registered by IR-spectra (Fig. 2). There are two small peaks, 1755 and 1735 cm^{-1} , at the foot of strong amide carbonyl band, which might be referred to the absorption of two aldehyde groups arising from α -glycol cleavage.

The molar amount of introduced aldehyde groups was determined via reaction with Schiff's reagent.

As it was expected, the introduction of larger amounts of metaperiodate results in the increase of aldehyde groups contents.

Copolymerization of MAG with VP and DAAC and generation of aldehyde groups

To realize the second method of obtaining aldehyde-bearing polymers, the attempts were made to synthesize the copolymers of MAG with DAAC. However, it was established that only MAG homopolymer containing the trace amounts of DAAC was formed in all experimental cases. It is known that DAAC is very inactive monomer; however, its copolymers with VP can be easily obtained.⁵ Thus, it was of special interest to synthesize poly(MAG-*co*-VP-*co*-DAAC) because such copolymers, as well as poly(MAG-*co*-VP) ones, might give the possibility to combine and compare advantages of both MAG and VP polymers regarding to their application at the construction of composite scaffolds.

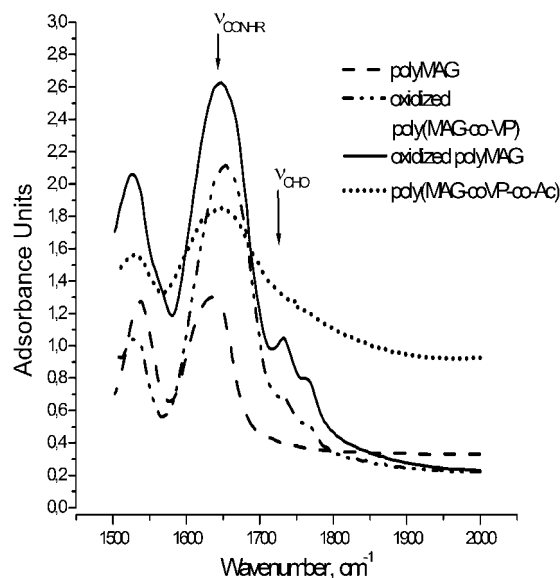


Figure 2 IR-spectra of polyMAG and oxidized/activated polymers.

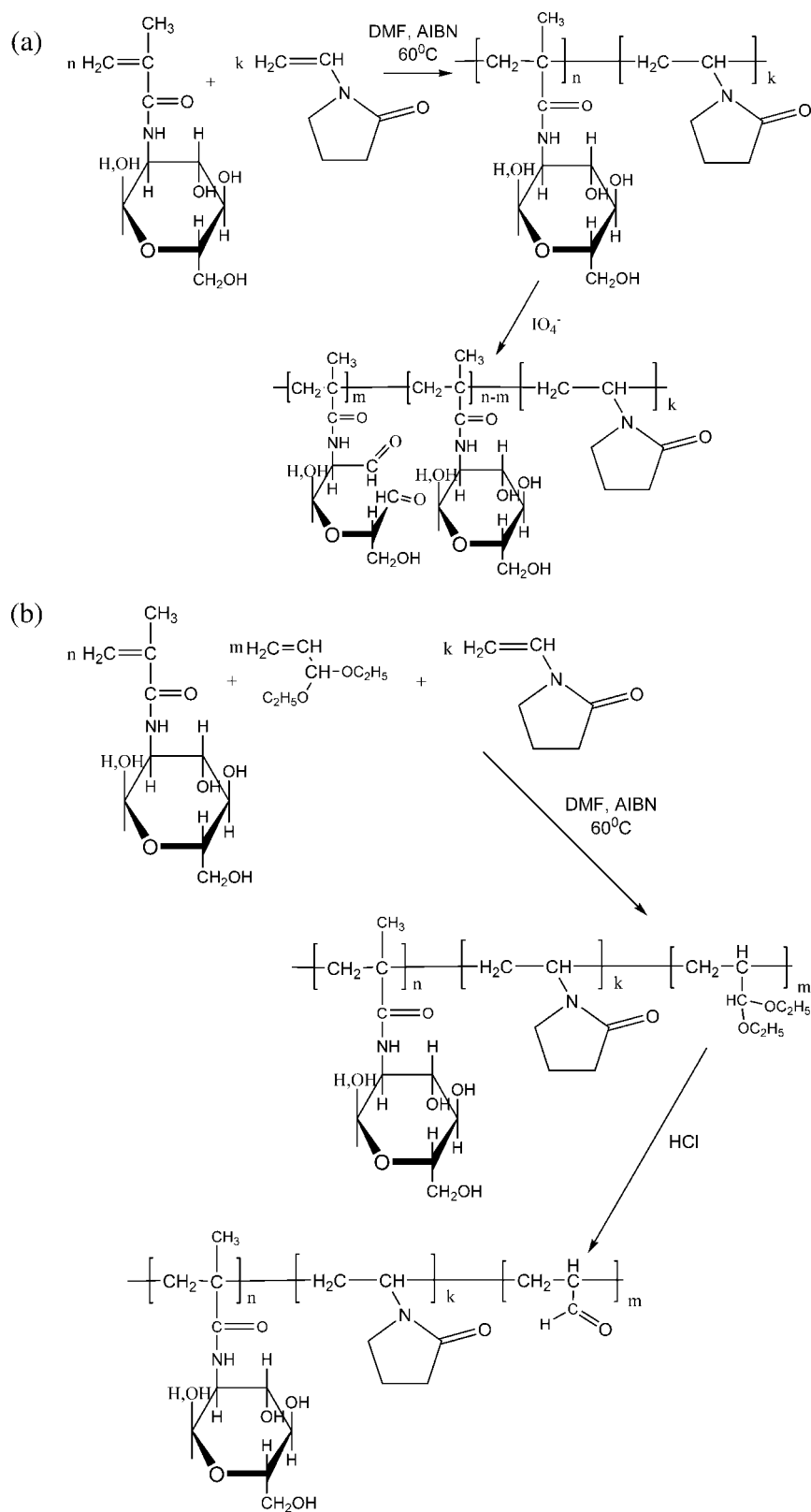


Figure 3 (a) Synthesis and oxidation of poly(MAG-co-VP); (b) synthesis and activation of poly(MAG-co-VP-co-DAAc).

The radical copolymerizations were carried out in DMF solution and were initiated with AIBN. The schemes of synthesis of poly(MAG-co-VP) and poly

(MAG-co-VP-co-DAAc), and their modification resulting in aldehyde derivatives formation are presented in Figure 3(a,b), respectively. The copolymerization

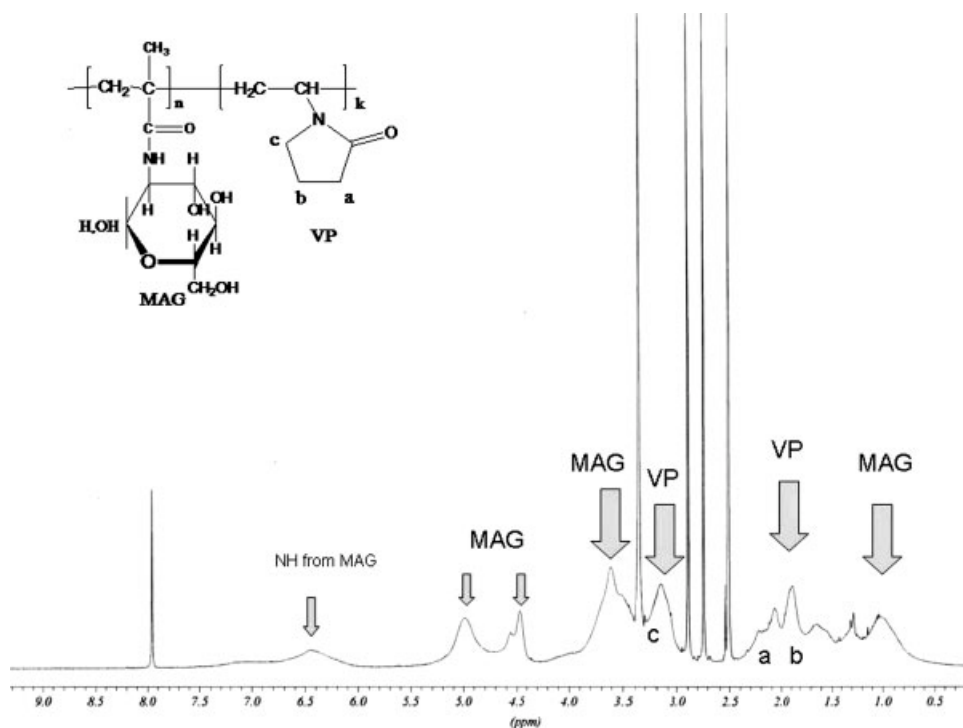


Figure 4 ^1H NMR spectra of poly(MAG-co-VP).

conditions and characteristics of obtained copolymers are presented in Table II. As one can see, the obtained copolymers possess low MM values.

The formation of poly(MAG-co-VP) and poly(MAG-co-VP-co-DAAc) copolymers was proved by one-dimensional ^1H NMR and COSY spectra investigations, respectively. In ^1H NMR spectra of poly(MAG-co-VP) the regions corresponding to the protons of MAG and VP rings were revealed (Fig. 4). In ^1H NMR COSY spectra of poly(MAG-co-VP-co-DAAc) the cross peaks corresponding to the presence of EtO-groups of DAAc and lactame ring of VP were found and congruent protons were correlated (Fig. 5).

For the analysis of copolymers composition, the iodine test for VP determination was used. For ternary copolymers the evaluation of DAAc quantity was carried out via the reaction of Schiff's reagent with aldehyde groups appeared in target polymer after elimination of protective group. The amount of MAG in copolymers was calculated as a result of deduction of VP and DAAc parts.

As it can be seen from the data in Table II, the yield of poly(MAG-co-VP) is quite high. Observing the composition of resulting copolymers, one can find that all polymer products are enriched mostly with MAG and less with VP, as compared with monomer mixture composition. The DAAc content in ternary copolymers is much less than that in initial monomer mixture. It is known that DAAc, being an allyl type monomer, causes the degradation trans-

fer during the polymer chain growth. As a result, the yield and MM of forming polymer decreases and the content of DAAc is appeared to be poor. We obtained very similar results (Table II). Besides, it was searched out, that the quantity of aldehyde groups in the goal polymers can be varied by comonomer ratio change. The increase of VP and decrease of MAG contents in initial monomer mixture at the

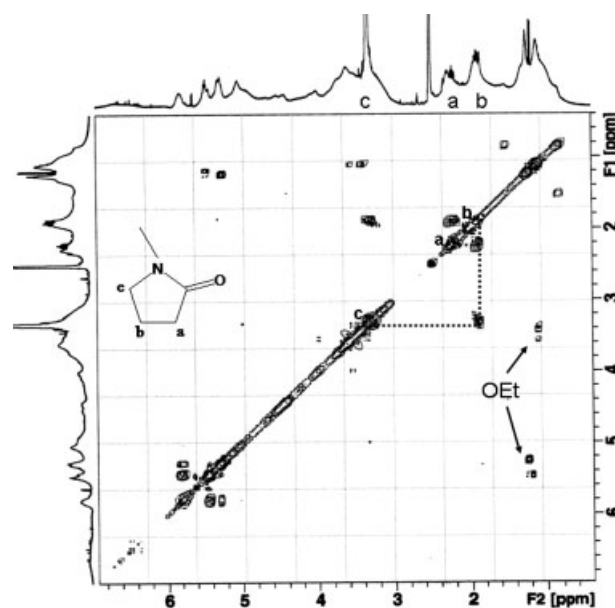


Figure 5 ^1H NMR COSY spectra of poly(MAG-co-VP-co-DAAc).

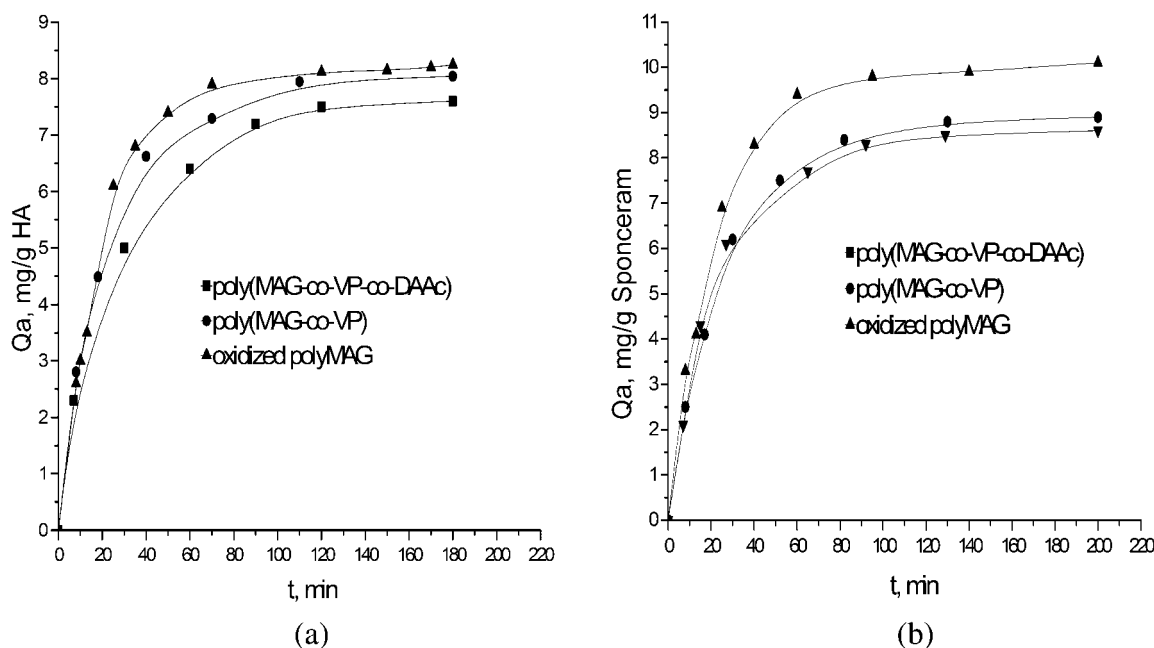


Figure 6 (a) The kinetic curves of oxidized polyMAG, poly(MAG-co-VP), and poly(MAG-co-VP-co-DAAc) adsorption on HA; (b) The kinetic curves of oxidized polyMAG, poly(MAG-co-VP), and poly(MAG-co-VP-co-DAAc) adsorption on Sponceram[®]. Conditions: concentration of polymers solutions—1 mg/mL in 0.01M borate buffer, pH 7.0; 25°C.

same DAAc contents increase the quantity of aldehyde units in final copolymer (Table II, samples 7 and 8).

The aldehyde groups were introduced to poly(MAG-co-VP) via periodate oxidation similar to the procedure carried out in a polyMAG case. For example, at the conditions for sample 1 (Table I) the oxidation of poly(MAG-co-VP) resulted in the polymer product with 30 mol % of aldehyde groups.

The presence of aldehyde groups in target copolymers was verified by IR-spectroscopy (Fig. 2). As in the case of oxidized polyMAG, the two small peaks of lower intensity were found in oxidized poly(MAG-co-VP) spectra. For activated poly(MAG-co-VP-co-DAAc) there is the evident swell (in comparison with polyMAG spectra) at the foot of amide carbonyl band that might be referred with confidence to the absorption of aldehyde groups.

Polymer adsorption on mineral supports

As it was pointed out in Introduction, the general goal of this work was to obtain the polymers which can be used as organic part of composite scaffolds for bone tissue regeneration. The important issue in such constructions is a strong interfacial binding between ceramics and polymer. If the adhesion of two phases is poor, the biological properties of polymers conjugated with biomolecules before adsorption on inorganic matrix, as well as mechanical properties of created scaffold, will definitely suffer. Thus, the ability of synthesized polymers to be

adsorbed on inorganic support is their essential practical characteristics.

The mineral supports used in our experiments, namely, Sp and HA, are differed by their chemistry and porous structure. Nevertheless, both of them have one feature in common—anionic character of a surface. This fact should be taken into account at the development of polymeric part of composite material.

Adsorption kinetics and desorption studies

Experimental curves for adsorption of synthesized polymers on HA and Sp are demonstrated in Figure 6(a,b), correspondingly. Obviously, in both cases, the adsorption process occurs intensively and achieves the maximum in ~ 100 min.

The data obtained in desorption studies are demonstrated in Table III. It was shown that all polymers investigated were not significantly desorbed within 14 days of observation. This experimental time has been chosen regarding to the initial period of cell cultivation.

Adsorption isotherms construction

The plotted adsorption isotherms are shown in Figure 7(a,b) for HA and Sp, respectively. The acquired values of $Q_{a\ max}$, for which the amount of adsorbed polymer corresponding to the plateau on isotherm curve was accepted, are presented in Table III.

TABLE III
Adsorption/Desorption Parameters of Studied Polymers

Copolymer	Adsorption (mg/g sorb.)	Desorption (mg/g sorb.)	
		7 days	12 days
Sponceram			
Oxidized polyMAG	16.5	0.0	0.0
poly(MAG-VP)	15.1	0.2	0.1
poly(MAG-co-VP-co-DAAc)	14.1	0.1	0.0
HA			
Oxidized polyMAG	11.5	0.1	0.0
poly(MAG-co-VP)	11.4	0.3	0.2
poly(MAG-co-VP-co-DAAc)	11.3	0.2	0.1

Thus, considering the results of adsorption kinetics and plotted isotherms, it is possible to conclude that no drastic differences were found both in the trends of curves and adsorption capacity of all polymers studied. Regarding to the previously published data,⁵ we can assert that the presence of MAG is a general cause for all polymers to be adsorbed. This effect might be explained by the ability of MAG to form a considerable amount of hydrogen bonds with slightly negatively charged oxygen atoms on the surfaces of mineral matrices.

It is also obvious that Sp has a greater adsorption capacity than HA. This fact is most likely due to the differences in porous structure of tested mineral supports.

The absence of significant desorption measured within 14 days at physiological pH confirms the

strength of adsorption and, thereafter, a possibility to use such kind of polymers for construction of composite scaffolds for cell culture applications without any risk of polymer leakage from a mineral surface.

Cytotoxicity

Initially, every polymer which was considered for adsorption on the mineral matrix was tested for its cytotoxicity in cell culture medium. The dissolved polymer was supplemented to the cell culture medium in three different concentrations (0.1, 0.5, and 0.7 mg/mL). MC3T3-E1 cells were cultured in these media for up to 11 days, MTT assays were performed at four different time points. Standard cell culture medium was used for positive control. The MTT assay [Fig. 8(a)] showed significantly lower cell viability in all polymer supplemented media than in standard culture medium at day 4. In the process of experiment, cell viability in polymer media became comparable to standard medium. At day 6, only oxidized polyMAG and poly(MAG-co-VP-co-DAAc) showed lower cell viability results and at day 8 only oxidized polyMAG supplemented medium resulted in lower cell viability than standard cell culture medium. The comparison of cell viability depending on polymer concentration [Fig. 8(b)] showed a significant reliance of the cell viability to polymer concentration for oxidized polyMAG, poly(MAG-co-VP-co-DAAc) and poly(MAG-co-VP-co-DAAc). In contrast, for

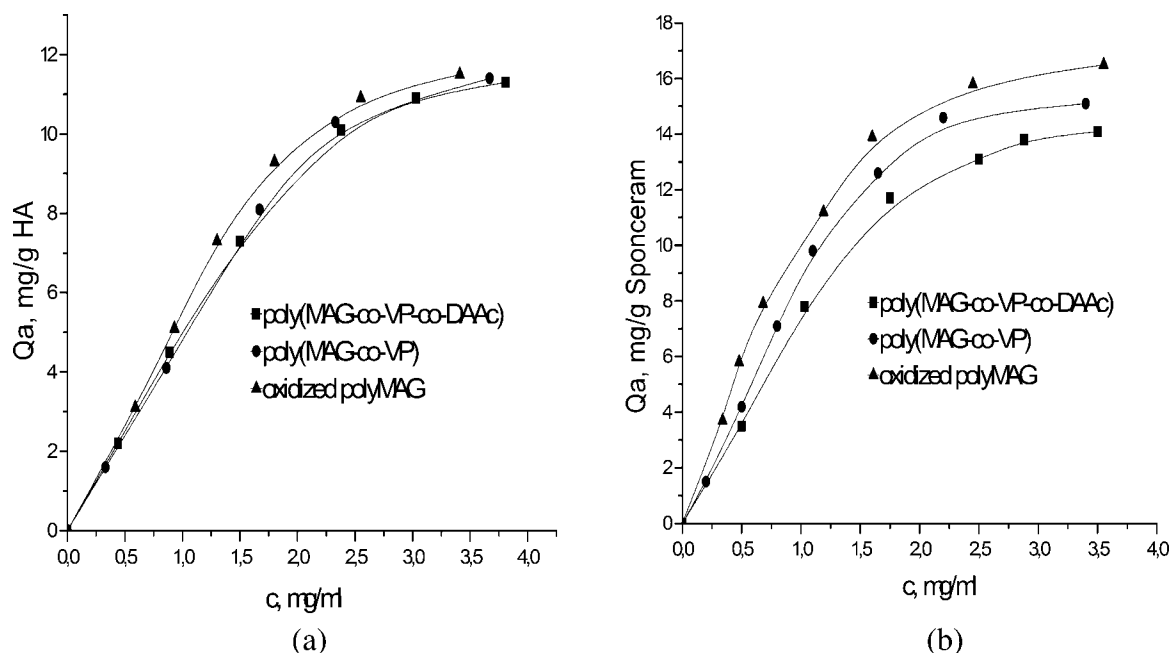
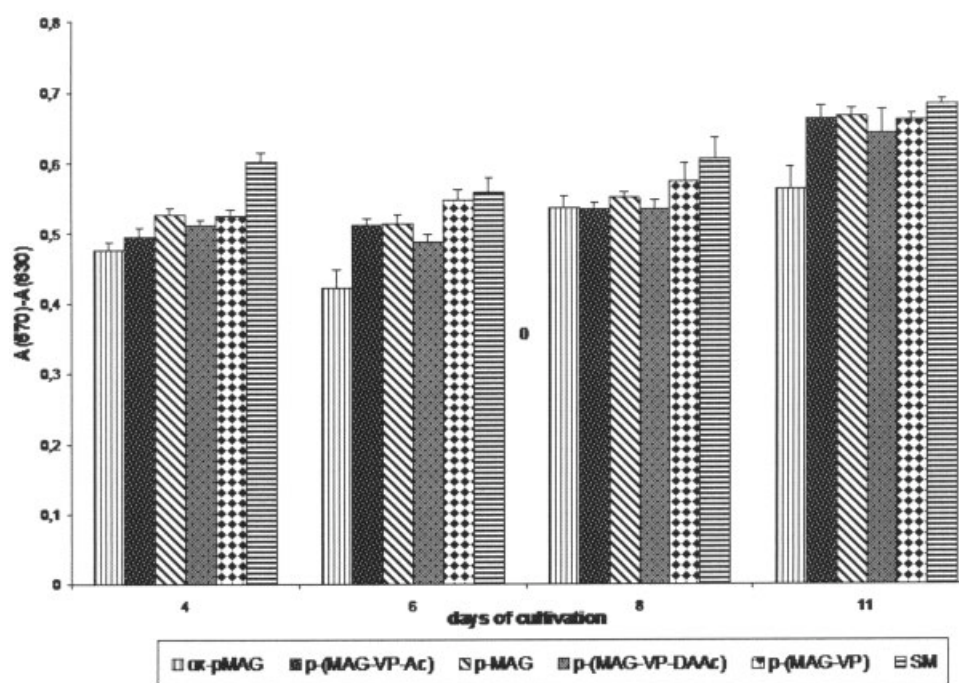
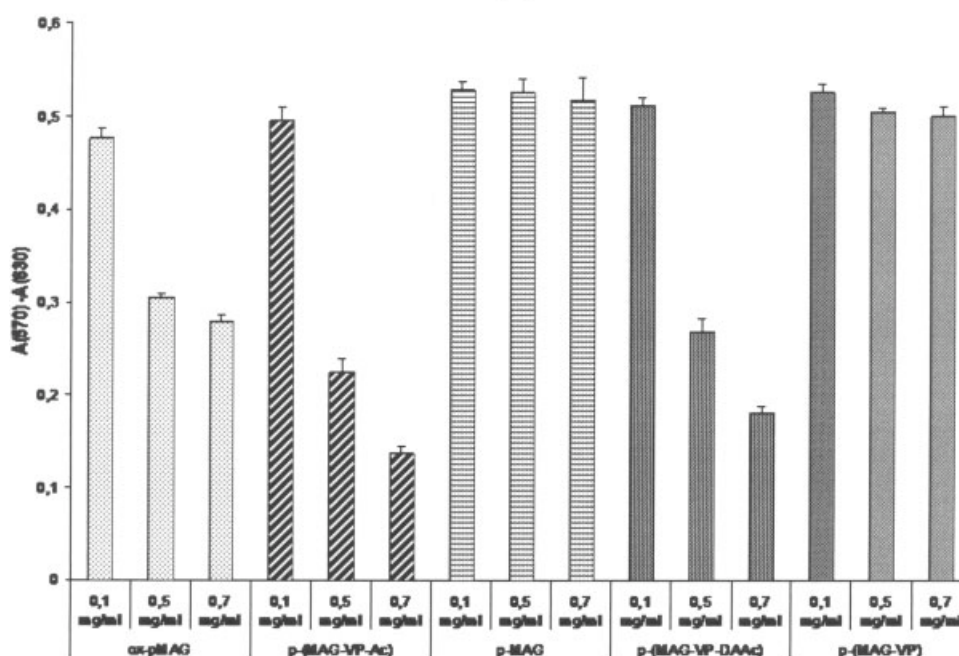


Figure 7 (a) Isotherms of oxidized polyMAG, poly(MAG-co-VP), and poly(MAG-co-VP-co-DAAc) adsorption on HA; (b) Isotherms of oxidized polyMAG, poly(MAG-co-VP), and poly(MAG-co-VP-co-DAAc) adsorption on Sponceram[®]. Conditions: concentration of polymers solutions—0.25–3.5 mg/mL in 0.01M borate buffer, pH 7.0; 25°C.



(a)



(b)

Figure 8 MTT-assays: (a) Polymers just added to standard culture medium (SM) with the concentration of 0.1 mg/mL; (b) Polymers added to standard culture medium (SM) with different concentrations (0.1, 0.5, and 0.7 mg/mL). Each column represents the adsorption of MTT, which is proportional to the number of cells in the well, at certain day of cultivation. Values are given as mean of five samples \pm SEM.

polyMAG and poly(MAG-co-VP) cases, added polymer concentrations did not influence cell viability. One can see, that only aldehyde-bearing polymers cause decrease of cell viability, while their precursors didn't show such negative effect. This fact can

be explained by the presence of aldehyde groups, which might act as crosslinking agents similar to, for example, glutaraldehyde.³⁶

PolyMAG, oxidized polyMAG, and poly(MAG-co-VP-co-Ac) were adsorbed on Sp and the viability of

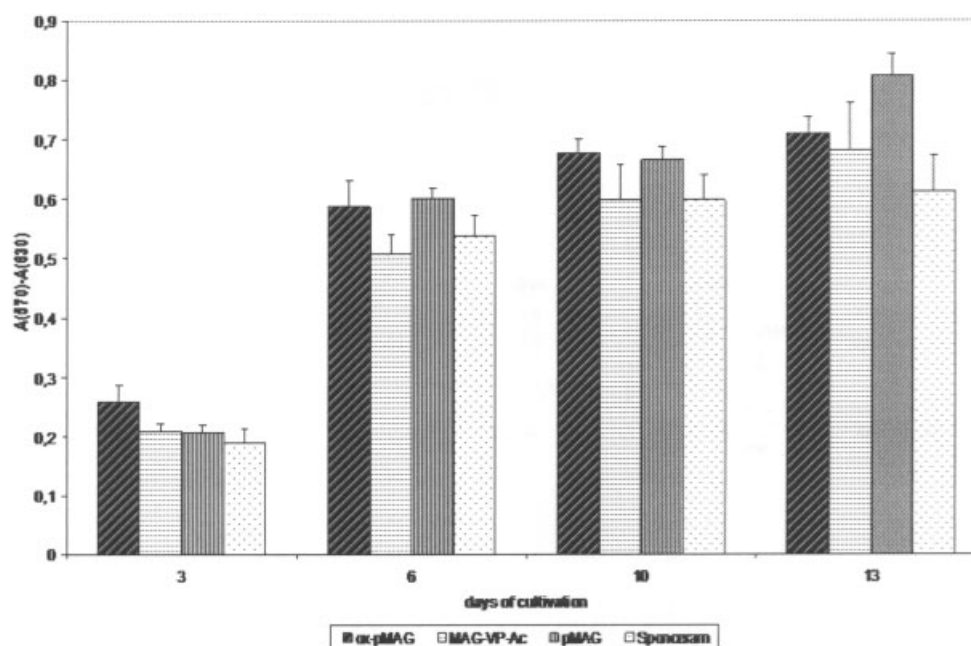


Figure 9 MTT-assay: viability of cells on the Sponceram[®] covered by polymers under study. Values are given as mean of five samples \pm SEM.

MC3T3-E1 cells on these modified materials was investigated. Sp incubated with borate buffer, which was used for the polymer adsorption, served as a control. Cell viability was roughly the same on all tested samples (Fig. 9). Unlike the experiments with polymers supplemented to the cell culture medium, there was no significant cytotoxicity or any difference between polymers containing and free from aldehyde groups.

CONCLUSIONS

The new water-soluble polymers and copolymers based on MAG were synthesized, as well as the methods of controlled introduction of aldehyde groups via copolymerization or polymer postmodification was developed. The study of adsorption/desorption parameters of obtained macromolecular products on two commercial mineral supports differed by their chemistry and structure enabled to consider these polymers as the possible candidates for construction of composite scaffolds for bone tissue regeneration. The first experiments on cells viability on the Sponceram[®] covered with polymers clearly showed that polymers in use are not cytotoxic when adsorbed on the mineral surface.

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