Alginate– and gelatin-based bioactive photocross-linkable hybrid materials for bone tissue engineering

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A B S T R A C T

The paper presents the synthesis, the physico-chemical and the biological properties of novel hybrid materials prepared from photo-crosslinked gelatin/alginate-based hydrogels and silica particles exhibiting potential for the regeneration of bone tissue. Both alginate and gelatin were functionalized with methacrylate and methacrylamide moieties, respectively to render them photo-crosslinkable. Submicron silica particles of two sizes were dispersed within three types of polymeric sols including alginate, gelatin, and gelatin/alginate blends, which were subsequently photo-crosslinked. The swelling ratio, the gel fraction and the mechanical properties of the hybrid materials developed were examined and compared to these determined for reference hydrogel matrices. The in vitro cell culture studies have shown that the prepared materials exhibited biocompatibility as they supported both MEFs and MG-63 mitochondrial activity. Finally, the in vitro experiments performed under simulated body fluid conditions have revealed that due to inclusion of silica particles into the biopolymeric hydrogel matrices the mineralization was successfully induced.

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1. Introduction

Tissue engineering is an interdisciplinary and multi-disciplinary field of research aiming at the regeneration of living tissue, when a loss or damage has occurred as a result of injury or disease (Roether et al., 2002). To fulfill the diverse needs in tissue engineering, various materials have been exploited as scaffolds for tissue regeneration (Peter, 2008). Preparation of the scaffold that would meet all rigorous criteria for maintaining cell growth is very challenging. The scaffold has to serve as a temporary extracellular matrix (ECM), mimic matrix architecture, guide cells, facilitate their growth and provide mechanical support. (Martinez, Blanco, Davidenko, & Cameron, 2015). It is also required that scaffolds, especially those dedicated for bone reconstruction, will not only mimic the natural extracellular matrix but they should also be bioactive ensuring favourable interactions with tissue and providing the natural environment and support for bone regeneration (Lee & Mooney, 2001; Wei, He, Sun, Zhu, & Feng, 2005). Hydrogels play the pivotal role in that area as they are characterized by mechanical and structural properties similar to many tissues and the synthetic extracellular matrix (ECM) and can be delivered to the body in a minimally invasive manner (Lee & Mooney, 2001). Hydrogels are water-insoluble, 3D networks of crosslinked hydrophilic polymers which exhibit a high degree of swelling in aqueous environments (Wei et al., 2005). The highly swollen state of these materials facilitates transport of nutrients into and cellular wastes away from the hydrogels. Moreover, their biodegradability and non-toxicity as well as the 3D structure, which can provide space and mechanical stability for new tissue formation, render hydrogels promising biomaterials for tissue engineering (Jeon, Bouhadir, Mansour, & Alsberg, 2009; Nieto-Suárez, López-Quintela, Lazzari, 2016).

Various cross-linking techniques have already been used to synthesize biodegradable hydrogels. Recently there has been a growing interest in photo-crosslinked hydrogels, mainly due to the fact that aqueous macromer suspensions containing cells and/or bioactive...
factors can be delivered to the proper tissue in a minimally invasive manner (e.g., by injection) followed by rapid crosslinking in situ under physiological conditions and/or upon brief exposure to ultraviolet (UV) light (Jeon et al., 2009).

Alginate – a linear unbranched polysaccharide derived from seaweed constituting the repeating units of 1,4-linked D-mannuronic acid and a-L-guluronic acid is one of the most versatile natural materials known to form hydrogels (Jeon et al., 2009). This naturally derived polymer is structurally similar to the natural ECM and exhibits low toxicity after purification. Alginate hydrogels are currently used in a broad range of medical applications including cell encapsulation, drug delivery as well as tissue engineering – for which their function is focused on providing mechanical integrity, while simultaneously transmitting initial mechanical signals to the cells and the developing tissue (Van Vlierbergh, Dubrul, & Schacht, 2011; Drury, Dennis, & Mooney, 2004). Gelatin is the basic building block of collagen, a major component of the ECM. Due to the innocuous nature of gelatin, it is one of the most commonly used proteins for creating cellular scaffolds (Graulus et al., 2015; Yung et al., 2007; Van Nieuwenhove et al., 2016).

The hydrogels obtained from the mixture of gelatin and alginate are interesting materials for the preparation of scaffolds because of their chemical similarity to the ECM, their flexibility ensuring fast diffusion of hydrophilic nutrients and metabolites, as well as the low content of dry mass, which reduces irrigation and lowers the amount of degradation products (Yan et al., 2005). The most important requirement for these materials to be applied for bone regeneration is their ability to form a bone–like apatite layer on their surface in the body environment providing a bond with natural bone (Lewandowska-Ła´ncucka, Fiedjasz, Rodzik, Latkiewicz, & Nowakowska, 2015). That ability can be determined in in vitro experiments carried out using simulated body fluid conditions, originally developed by Kokubo et al. (Kokubo & Takada, 2006).

We have previously shown that submicron silica particles improve the bioactivity of polymeric materials. (Lewandowska-Ła´ncucka, Fiedjasz, Rodzik, Koziel & Nowakowska, 2015). It was proposed that the formation of the apatite is induced by silanol (Si-OH) groups on the surface of these materials. In the presence of SBF, the Si–OH group is negatively charged (Si–O–) and interacts with positively charged Ca2+ ions to form a Ca-rich positive thin layer. That layer combines with negatively charged PO3–3 ions forming amorphous calcium phosphate, that eventually transforms into apatite in SBF (Takadama, Kim, Kokubo, & Nakamura, 2002).

This paper presents the results of our studies concerning the physico-chemical and the biological properties of the novel materials fabricated from photo-crosslinked alginate/gelatin-based hydrogels and silica particles that can serve as scaffolds for bone tissue regeneration. Alginate and gelatin were functionalized with methacrylate and methacrylamide moieties, respectively allowing their photo-crosslinking. Synthesized by the Stöber method, sub-micron silica particles of two sizes were dispersed into three types of polymeric solids including alginate, gelatin, and gelatin/alginate, which were subsequently photo-crosslinked. One can assume that using various polymeric matrices and playing with silica particles sizes and concentrations the scaffolds characterized by different physico-chemical and biological properties can be obtained. Therefore, the swelling ratio, the gel fraction and the mechanical properties of the materials developed were examined and compared to plain hydrogel matrices in the absence of silica particles. The mineralization process was conducted in an in vitro environment in the presence of 1.5 SBF. The materials obtained were exposed to simulated body fluid solution for 7 days and then the morphology and the chemical composition of the minerals formed were studied by means of SEM and EDS measurements. The hybrids were synthesised in order to prepare novel scaffolds, which after swelling can fill up bone defects and provide the conditions favourable for bone tissue regeneration. Considering that application, biological tests were also performed. The cytocompatibility of the resulting hybrids was evaluated using XTT test against two different cell lines including osteoblasts-like cells (MG-63) and mouse embryonic fibroblasts (MEFs). To the best of our knowledge, as yet, there has been no studies presented in literature on the effect of SiO2 particles on bioactivity, cytocompatibility and mechanical properties of the photo-crosslinked alginate/gelatin-based hydrogels designed for tissue engineering applications.

2. Experimental section

2.1. Materials

Tetraethoxysilane (TEOS, >98%, Fluka), gelatin B (the bloom strength: 254 g; 6.67% w/w gelatin – 10 °C (Sigma Aldrich, Bornem, Belgium), sodium alginate (MG ratio of 2.3, Mw of 76 kDa and a D of 4.6; Sigma Aldrich, Bornem, Belgium), methacrylic anhydride (Sigma Aldrich, Bornem, Belgium). The photo-initiator 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one (Irgacure® 2959) was a kind gift from Ciba Speciality Chemicals N.V. (Groot-Bijgaarden, Belgium). In Vitro Toxicology Assay Kit, XTT based (Sigma). Osteoblasts-like culture, MG-63 (ATCC® CRL-1427™), Organism: Homo sapiens, human; Tissue: bone; Disease: osteosarcoma and Mouse Embryonic Fibroblasts (MEF) were cultured in Dulbecco’s Modified Eagle’s Medium – high glucose with 4500 mg/L glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture (DMEM/High, Sigma) supplemented with 5% fetal bovine serum (FBS; HyClone) and 100 IU/ml penicillin and 100 µg/ml streptomycin (HyClone) in a humidified atmosphere with 5% CO2 at 37 °C.

2.2. Synthesis procedures

2.2.1. Methacrylation of biopolymers

The synthesis and characterization of methacrylamide-modified gelatin was performed as described earlier (Graulus et al., 2015). A derivative with a degree of methacrylation of 76% was selected in the present work as indicated by 1H NMR spectroscopy. A derivatization of alginate with methacrylic anhydride (MAAH), based on a reaction of the free hydroxyl moieties on the polysaccharide backbone with the anhydride, was performed to incorporate methacrylates enabling subsequent network formation. A 2 mol% sodium alginic solution was prepared in demineralized water using a mechanical stirrer. Subsequently, MAAH was added dropwise to the solution. The added amount corresponded to 2 equivalents of MAAH with respect to the hydroxyl moieties on the alginate backbone. During the reaction, methacrylic acid was released which lowers the pH of the reaction. Therefore, the pH of the mixture was constantly monitored and increased to pH 8 by adding a 5 M sodium hydroxide (NaOH) solution. The reaction was performed in a slightly alkaline environment to improve reactivity. On the other hand, the pH should not be too high in order to avoid hydrolysis of the ester in the reaction product. The mixture was stirred and kept at room temperature for 24 h after addition of the MAAH. Afterwards, dialysis was performed during 72 h while changing the dialysis water twice a day to remove unreacted agents. The resulting solution of modified alginate (algMOD) was frozen and the water removed via lyophilization. The degree of methacrylation was 6% as indicated by means of 1H NMR spectroscopy (Grasdalen, 1983; Grasdalen, Larsen, & Smidsrød, 1979).

2.2.2. Silica particles synthesis

The monodisperse silica particles of two sizes (S1 and S2) were obtained by the Stöber synthesis (Stöber, Fink, & Bohn, 1968) using
the procedure described earlier (Lewandowska-Łańcucka et al., 2015a, 2015b). The resulting particles were characterized in terms of their hydrodynamic diameter and zeta potential values using a Malvern Nano ZS light-scattering apparatus. The zeta potential was measured using the technique of laser Doppler velocimetry (LDV). The mean hydrodynamic diameter for SiO₂–S₁ was 210 ± 0.9 nm whereas the size of the SiO₂–S₂ was about 438 ± 4.8 nm. The zeta potentials (ζ) were –56.9 ± –9.1 mV and –62.9 ± –6.8 mV for SiO₂–S₁ and SiO₂–S₂ particles, respectively.

2.2.3. Preparation of hybrids

Three groups of hybrid materials (see Table 1) were prepared by incorporation of silica particles of two sizes (S₁–210 nm, S₂–438 nm) at various concentrations (C₁–3.8 mg/ml, C₂–1.9 mg/ml) into the gelatin, alginate and gelatin/alginate-based hydrogel matrix, respectively.

The pristine hydrogels were obtained by dissolving an appropriate amount of functionalized biopolymers in 10 ml water at 40 °C (1 g Gel, 0.5 g Alg and in case of G/A hydrogel, 0.5 g Gel and 0.5 g Alg). Subsequently, an aqueous solution of Irgacure 2959 (0.8 w/v%) was added to the biopolymer sols to obtain an initiator concentration of 2 mol% (for Gel) or 4 mol% (for Alg) relative to the number of present double bonds. The samples were protected from light with aluminum foil, mixed about 20 min at 40 °C and then injected between two parallel glass plates covered with a sheet of teflon foil and separated by a 1 mm thick silicone spacer. Such prepared samples were placed between two Long Wave UV lamps model VL-400L with an intensity of 10 mW/cm² and UV-A irradiation (wavelength range of 250–450 nm) was applied in order to initiate chemical cross-linking. After 1.5 h the hydrogel formation was finished, the product was transferred to Petri dish plates, frozen at –20 °C and then lyophilized using a Christ freeze-dryer alpha 2–4–LSC, typically during 24 h.

Hybrid materials were prepared according to a similar procedure with the difference that the biopolymers were dissolved in 8 ml water at 40 °C and silica particles (38 or 19 mg for C₁ or C₂ concentration, respectively) were dispersed separately in 2 ml of water. Next, the silica dispersion was added to the polymeric sols, mixed together for about 20 min and subsequently the solution of photoinitiator was added (its concentration was such as those for pristine hydrogels). Then, the procedure was continued analogously as for plain hydrogels.

2.3. Characterization of the materials developed

(See Supplementary: 2.3.1. Gel fraction measurement; 2.3.2. Swelling ratio determination; 2.3.3. Rheology studies).

2.4. Cytotoxicity testing

(See Supplementary: 2.4.1. Preparation of scaffolds, 2.4.2. Cell culture on hydrogel scaffolds; 2.4.3. XTT test (cell viability assay)).

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Gel 1</th>
<th>GelS1C1</th>
<th>GelS1C2</th>
<th>GelS2C2</th>
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<td>Alg</td>
<td>AlgS1C1</td>
<td>AlgS1C2</td>
<td>AlgS2C2</td>
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<tr>
<td>G/A</td>
<td>G/A</td>
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<td>G/A</td>
<td>G/A</td>
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Table 2

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<th>Gel fraction (%)</th>
<th>Swelling ratio (%)</th>
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<tr>
<td>Alg</td>
<td>66 ± 1</td>
<td>3748 ± 104</td>
</tr>
<tr>
<td>Alg S1C1</td>
<td>67 ± 6</td>
<td>3712 ± 110</td>
</tr>
<tr>
<td>Alg S1C2</td>
<td>71 ± 2</td>
<td>3661 ± 109</td>
</tr>
<tr>
<td>Alg S2C2</td>
<td>75 ± 2</td>
<td>4976 ± 101</td>
</tr>
<tr>
<td>Gel</td>
<td>96 ± 2</td>
<td>863 ± 50</td>
</tr>
<tr>
<td>Gel S1C1</td>
<td>91 ± 2</td>
<td>778 ± 1</td>
</tr>
<tr>
<td>Gel S1C2</td>
<td>96 ± 1</td>
<td>761 ± 16</td>
</tr>
<tr>
<td>Gel S2C2</td>
<td>97 ± 2</td>
<td>1041 ± 17</td>
</tr>
<tr>
<td>G/A</td>
<td>93 ± 3</td>
<td>1596 ± 20</td>
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<tr>
<td>G/A S1C1</td>
<td>91 ± 8</td>
<td>2011 ± 10</td>
</tr>
<tr>
<td>G/A S1C2</td>
<td>94 ± 2</td>
<td>2205 ± 26</td>
</tr>
<tr>
<td>G/A S2C2</td>
<td>82 ± 2</td>
<td>1892 ± 16</td>
</tr>
</tbody>
</table>

2.5. In vitro bioactivity testing

(See Supplementary: 2.5.1. 1.5 SBF preparation; 2.5.2. Incubation of materials developed in SBF).

3. Results and discussion

Novel hybrid materials were synthesised (see Table 1) with the aim to prepare bioactive scaffolds which can potentially be introduced into bone defects which, after swelling, can be perfectly filled. The physicochemical properties, bioactivity as well as cytocompatibility of these materials were evaluated and compared with those obtained for pristine polymeric hydrogels in the absence of silica particles.

3.1. Physicochemical characterization of the hybrid materials

3.1.1. Determination of gel fraction

To assess the level of crosslinking, the gel fractions of the materials were determined. The results, reported as mean values with corresponding standard deviations, are presented in Fig. 1.

The results show that the lowest gel fractions are obtained for alginate hydrogels (66–75%) whereas the gel fractions for Gel and Gel/Alg-based materials are higher. Except for G/A S2C2, no significant differences were noticed (>90%) (see Table 2). Such results show that the addition of silica particles of the applied sizes (S₁ and S₂) and concentrations (C₁ and C₂) does not considerably affect the photo-crosslinking efficiency within the polymeric matrices used. The differences in gel fractions resulted from various degrees of methacrylation for gelatin and alginate, which was equal to 76% and 6%, respectively. Therefore, the amount of photo-initiator (Irgacure 2959) used was accordingly adjusted to the number of double bonds present in the polymeric chains (see Section 2.2.3). Considering the higher degree of gelatin methacrylation, the materials obtained after photo-crosslinking of that biopolymer were more stable as compared to the alginate samples due to the higher number of cross-links.

3.1.2. Swelling properties of the hybrids

Taking into account the potential application of the developed hybrids as scaffolds for bone tissue regeneration, it was of great importance to determine their swelling properties. The data obtained are presented in Fig. 2, as mean values with corresponding standard deviations.

The results of the swelling experiments confirmed that all synthesised materials are able to absorb large amounts of water (>700%). However, significant differences between them have been revealed. As one can observe, the highest degree of swelling is shown by alginate-based materials (3661–4976%) while gelatin (761–1041%) results in the lowest swelling and materials consist-
ing of alginate and gelatin exhibit an intermediate swelling degree (1596–2205%) (see Table 2). There is no doubt that the silica particles introduced into the hydrogel matrices influenced the swelling properties. However, their impact differs depending on the type of biopolymer. The tendency of the SD changes for alginate and gelatin – based hybrids seems to be quite similar. Only the larger (S2) silica particles at a concentration of 1.9 mg/ml (C2) present in Alg and Gel matrices significantly increased the swelling ratio in both these systems. On the other hand, all hybrids based on gelatin/alginate hydrogels swell more efficiently than pristine G/A hydrogel.

The ability of water retention by the material strongly depends on both its hydrophilicity as well as, on the microstructure (Van et al., 2010). It is also well known that the swelling of hydrogel decreases with increasing degree of cross-linking (Graulus et al., 2015). That corresponds well with our findings since the alginate-based materials with the highest swelling degree have the lowest values of gel fraction reflecting the lowest efficiency of cross-linking. One can conclude that the results of the swelling experiments reveal that all materials studied are capable of absorbing large quantities of water. That is especially important in view of possible tissue engineering applications since the materials with the ability to retain large amounts of water mimic to a greater extent the natural aqueous environment of cells in which they naturally reside (Van Vlierbergh, Dubrueil, & Schacht, 2011).

3.1.3. Rheological evaluation of the hybrids

The mechanical properties of the materials play a very important role in the context of their potential application as an implant or a scaffold for tissue engineering purposes. Therefore, the elastic modulus (G') which is a parameter describing the elastic behavior of a sample was determined for each materials studied herein. G' is related to the number of cross-links in the material and the tendency is that the higher G' values reflect stiffer gels (Graulus et al., 2015). The G' values obtained are presented in Fig. 3.

As illustrated in Fig. 3 the storage modulus values are strongly dependent on the polymeric matrix used. The lowest G' was obtained for alginate-based materials (178–238 Pa), the highest for gelatin-based samples (8770–12300 Pa) whereas in case of mixed polymeric matrices gelatin/alginate, G' in the range of 6880–9030 Pa was observed. These results correspond well with the gel fractions and swelling degrees, confirming that the mechanical properties are related to the number of cross-links created in a material.

When comparing the G' values for plain polymeric matrices with those for hybrids it might be noticed that addition of SiO2 particles influenced the storage modulus significantly, while the effect differs depending on the type of biopolymer used. In case of gelatin-based hybrids the addition of silica particles irrespective of the size and concentration caused a significant increase in G' compared to the value determined for pristine gelatin hydrogels. The storage

![Fig. 1. Gel fractions of the hybrid materials and hydrogel matrices.](image1)

![Fig. 2. Swelling degree of the hybrid materials and hydrogel matrices after immersion in PBS for 24 h.](image2)
moduli found for G/A S1C2 and G/A S2C2 hybrids are definitely higher than the one measured for the pristine gelatin/alginate hydrogel whereas in case of alginate-based hybrids, only Alg S1C1 exhibits a slightly higher $G'$ (difference not significant) than pure Alg hydrogel. Generally it is difficult to notice a defined tendency, however, these findings clearly show that by choosing the polymeric matrix and by playing with the silica particle sizes and concentration, the mechanical properties of the resulting organic-inorganic hybrids might be adjusted according to the specific needs.

It was reported that there are many factors like the degree of adhesion between inorganic particles and the polymer matrix, the surface area as well as the packing characteristics of the fillers which affect the mechanical features of inorganic-organic materials. Alvarez et al. (Alvarez et al., 2014) investigated the mechanical properties of silica nanoparticle-collagen composite hydrogels and compared them to plain collagen hydrogels. They have shown that the lowest concentration (0.05 M) of silica particles of a hydrodynamic diameter around 100 nm (SiNP-100) significantly increases the storage modulus from 600±50 Pa for the collagen hydrogel up to 900±50 Pa. However, it was observed that, when a concentration of SiNP-100 reached 1 M, the value of $G'$ decreased to 200±50 Pa. They assumed that by increasing the particle concentration, the formation of silica aggregates was induced which perturbed the collagen fibrillary organization. These structural heterogeneities in the composite’s network represent failure points under mechanical stress and cause lowering of the materials' elastic properties which is in line with previous findings (Yanagioka & Frank, 2008).

Such effects could be also considered in our systems (see Supplementary Figs. S1–S3). Interestingly, in case of gelatin/alginate-based systems for which SiO$_2$ particles of S1 size introduced into hydrogels were at a concentration of 3.8 mg/ml the value of the storage modulus $G'$ decreased whereas the hydrogels with lower C2 concentration of both types of particles exhibit superior mechanical properties, although in both systems the fraction of aggregates was observed (see Supplementary Fig. S3). One can assume that in these mixed matrix, the silica particles at C1 concentration form larger aggregates which affect the spatial organization of crosslinks resulting in the noticeable decrease of the elastic modulus values. Interestingly, in the case of alginate and gelatin-based hybrids the reverse effect has been revealed – the systems with the highest silica concentration are characterized by the highest elastic modulus values. In case of gelatin-based hybrids the silica particles introduced to the gel (of the sizes and concentrations studied) undergo only negligible aggregation, which does not affect the microstructure of that material. Thus, as expected, there is improvement of mechanical properties on addition of silica particles. In a case of alginate based hybrids the aggregation is observed, which diminish the expected improvement of mechanical properties of these systems (see Supplementary Fig. S2). However, based on the results of rheological studies, one can conclude that the gelatin as well as the gelatin/alginate-based hybrids obtained here have satisfactory mechanical properties which, and except for the G/A S1C1 system, they are superior over the pristine hydrogels.

3.2. Biological study

3.2.1. In vitro cell viability, XTT

To confirm the possibility of hybrid application in tissue engineering, biological tests were performed. The cytotoxicity of the materials was examined using the XTT test, which is based on the spectrophotometric measurement of the mitochondrial dehydrogenase activity of living cells. These mitochondrial enzymes present in metabolically active cells reduce the tetrazolium ring of 2,3-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide (XTT), yielding an orange, water-soluble formazan derivative. The absorbance of the orange product at $\lambda = 450$ nm is directly proportional to the number of viable cells. To carry out this colorimetric assay, two different cell lines were applied including mouse embryonic fibroblasts (MEFs) and osteoblast-like cells, MG-63 (ATCC® CRL-1427™). The results obtained are shown in Fig. 4. The data are presented as a mean ± SD based on experiments carried out five times.

As expected, cytotoxicity analysis proved that the addition of the silica particles of two different sizes and various concentrations does not significantly affect the viability of the cell lines studied as compared to pristine hydrogels. A lower viability was observed for alginate hydrogels and the addition of silica particles resulted in an improvement of these materials, although these differences are not statistically significant. In case of osteoblast-like cultures, the XTT test has revealed the same tendency for gelatin and gelatin-alginate materials (Fig. 4A), although MEFs interact definitely better with gelatin surfaces (Fig. 4B).

3.2.2. Cell adhesion and morphology

The adhesion and morphology of MG-63 cells seeded on the studied materials were monitored after 24 h of culture using optical microscopy. As can be seen on microscopy images (see Supplementary Fig.S4.), cells cultured on the surface of hydrogels adhere well

Fig. 3. The obtained storage modulus ($G'$) values measured for plain polymeric matrices and the developed hybrids.
and have a flattened morphology, comparable to the control. It can be observed that osteoblasts-like cells were quite well spread on the hydrogels, what can suggest that there is a high compatibility between the obtained materials and the applied cells.

It was reported that there are various factors like surface charge, chemistry, roughness and stiffness which can simultaneously influence the cell behavior. Also the correlation between surface wettability and cell adhesion is intensively studied but it is difficult to draw a conclusion since there are results showing that either more hydrophobic (Hou et al., 2008; Tamada & Ikada, 1994) or hydrophilic (Anselme, 2000; Altankov & Groth, 1994) surfaces are preferable for cell adhesion. Taking into account the results of current studies one may conclude that the obtained materials are cytocompatible and that the introduction of SiO₂ particles into hydrogels does not have a negative effect as compared with the results obtained for the corresponding pristine hydrogels.

3.3. In vitro bioactivity study

Considering the potential application of the synthesised hybrids as scaffolds for bone tissue engineering, in vitro mineralization experiments in SBF medium were carried out. It has been shown that materials forming bone-like apatite on their surfaces in simulated body fluid might also form the apatite in the living body and subsequently bind to living bone through the apatite layer. The ability of the materials to promote the apatite nucleation process was
evaluated after 7 days incubation in 1.5 SBF at 37 °C. The simulated body fluid solution was renewed every day. In order to determine the in vitro bioactivity of the hybrids, the process of mineralization was examined using two techniques: SEM and EDS. The bioactivity of the hybrids was compared with that of the pristine hydrogels.

3.3.1. SEM analysis

Application of scanning electron microscopy provided the possibility to monitor the microstructure of the prepared scaffolds after incubation in 1.5 SBF. Fig. 5 shows SEM images of the investigated materials surfaces with and without silica particles of two various sizes S1, S2 and two different concentrations C1 and C2 after mineralization. In case of all materials number of various regions of studied samples were analyzed and in Fig. 5 the representative micrographs are presented.

In case of gelatin-based materials the mineralization occurs on both pristine and hybrid hydrogels. The new objects created at the surfaces can be clearly noticed. These new phases are in the form of flowerlike microspheres composed of plate-like crystallites. However, as can be seen in Fig. 5, the larger extent of mineralization is particularly noticeable for Gel S2C2. Analysis of this material has clearly proven that the addition of silica particles increased the efficiency of the mineralization process.

Whereas in case of alginate and gelatin/alginate hydrogels, the apatite-like forming ability was strongly correlated with the silica particles addition for plain Alg and G/A hydrogels, there were no mineral phases revealed. Also in case of Alg S2C2 hybrid, the apatite-like structures were not observed.

3.3.2. EDS analysis

EDS analysis was conducted to determine the chemical composition of the mineral structures presented in the SEM images. Application of that technique allows to evaluate the content of two
Table 3

<table>
<thead>
<tr>
<th>Materials developed</th>
<th>Ca/P ratio</th>
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<tbody>
<tr>
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<td>–</td>
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<tr>
<td>Alg S1C1</td>
<td>1.42</td>
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<tr>
<td>Alg S1C2</td>
<td>1.50</td>
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<tr>
<td>Alg S2C2</td>
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<tr>
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promote significantly the cytompatibility of the resulting hybrids with respect to plain hydrogels. Finally, in vitro experiments performed under 1.5 SBF condition have revealed that due to the inclusion of SiO2 into the biopolymeric hydrogel matrices, the mineralization is successfully induced in all hybrids except of Alg S2C2 system.

By means of SEM and EDS measurements the formation of apatite-like structures in hybrid materials were confirmed (except Alg S2C2). These results clearly illustrate that inclusion of SiO2 particles into the hydrogels is beneficial because plain hydrogels (except gelatin) have not induced mineralization under the applied experimental conditions. Taking into account all data obtained one can conclude that gelatin and gelatin/alginate-based hybrids developed within these studies are promising candidates for bioactive scaffolds in tissue engineering.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2016.11.051.

References


