www.czasopisma.pan.pl PAN www.journals.pan.pl
Chemical and Process Engineering 2022, 43 (3), 405–417
DOI: 10.24425/cpe.2022.142282

Hydrogel microspheres containing dextran-based nanoparticles as novel anticancer drug delivery system

Aleksandra Kulikowska-Darłak[1]*, Agata Stefanek[2], Iga Wasiak-Wojasińska[3], Paulina Wiechecka-Ożdżyńska[4], Tomasz Ciach[5]

Faculty of Chemical and Process Engineering, Warsaw University of Technology,
Waryńskiego 1 Street, 00-645 Warsaw, Poland

Developing an effective and safe cancer therapy could significantly reduce the number of deaths and improve the quality of life of treated patients. Nowadays medicine has developed a wide range of anticancer chemotherapeutics but at the same time there is a lack of effective drug delivery methods. Therefore, the development of the targeted drug delivery system which will selectively release drug into the cancer cells is a key challenge of modern medicine.

The main aim of the presented research was to investigate the targeting effect of a drug delivery system based on the controlled release of dextran nanoparticles containing the anticancer drug – doxorubicin from the alginate microspheres coated with chitosan multilayers.

During the research the physicochemical properties of the alginate microspheres and its stability in the physiological environment were investigated. Moreover, the kinetics of the nanoparticles with doxorubicin release from the alginate microspheres covered with chitosan multilayers was characterized, depending on the thickness of the chitosan layer. Further, the cytotoxicity study of the alginate microspheres covered with chitosan multilayer and containing nanoparticles was performed to determine the therapeutic effect of the released nanoparticles with doxorubicin on the HeLa cells during the *in vitro* cell culture.

Keywords: alginate, dextran, nanoparticles, microspheres, drug delivery

1. INTRODUCTION

With the development of novel therapeutic agents, there has been an increased need for the controlled drug delivery systems that enable the release of drugs at a specific rate over an extended period of time, in order to achieve the desired therapeutic response (Adepu and Ramakrishna, 2021; Tiwari et al., 2012). The advent of polymers that are both biodegradable and biocompatible has led to much research being focused on developing modern polymer drug delivery systems and designing them for sustained drug release applications (Hoare et al., 2012; Zhang et al., 2010). The use of polymer drug delivery systems can overcome the challenges of drug uncontrolled release and degradation while ensuring drug release to its target site

* Corresponding author, e-mail: aleksandra.kulikowska-darlak.dokt@pw.edu.pl

to achieve the maximum therapeutic efficacy and to reduce the risks of under- and overdose and rapid removal of the drug from the circulation (Vargason et al., 2021). The most commonly used biomaterials for drug delivering systems are alginate, chitosan, polylactide (PLA), poly(2-hydroxyethylometakrylane) (PHEMA), cellulose and its derivatives and collagen (Chan et al., 2008; Gui et al., 2014; Weng et al., 2013; Wu et al., 2013).

The controlled-release drug delivery systems which are now widely used include microparticles and nanoparticles made of polymers (Wu et al., 2013). Hydrogel microspheres are the specific type of the polymer drug delivery systems, with many potential applications in regenerative medicine (regeneration of bone and cartilage tissue by grafting encapsulated osteoblasts and fibroblasts with grown factors), and also in the diabetes type I, Parkinson's disease and cancer therapy (by grafting encapsulated cells producing biotherapeutics, for example insulin, neurotropic factors and angiogenesis inhibitors) (Su et al., 2010; Yang and Owusu-Ababio, 2000).

The developed drug delivery systems enable facile drug administration via routes such as oral, vascular, pulmonary, and parenteral, and do not require surgical removal once the drug is fully released. Much interest is dedicated to the vascular route, because of the embolization effect i.e., transcatheter arterial embolization (TEA). TEA is a technique based on the intended occlusion of blood vessels by introducing embolic agent into them using a catheter (Zhou et al., 2014). This embolic agent reduces or completely cuts off the supply of the oxygen and nutrition essential for the survival of the tumor by reducing or blocking the blood flow towards the tumor, thereby causing devascularization and subsequent progressive tumor shrinkage (Weng et al., 2013).

Transcatheter arterial chemoembolization (TACE) utilizing microspheres loaded with a drug demonstrated lower drug-related toxicity and side effects in comparison with the conventional procedure of TACE (Namur et al., 2011). However, the only drug-releasing beads available on the market (DC bead, Biocompatibles, UK) are non-biodegradable, and their presence in the body for an extended period of time has been reported to cause chronic inflammation and thus cause greater tissue damage (Lammer et al., 2010). Therefore, it is imperative to investigate new types of drug-loadable, spherical embolic agents which are biocompatible, and which do not additionally affect the deterioration of the patient's quality of life during treatment (Weng et al., 2013).

Hydrogel microspheres as a novel drug delivery system have been facing the challenge of a wide range of size distribution, relatively high initial burst release and unpredictable drug release kinetics. Recent reports have emphasized that both the size and the shape of the drug delivery systems significantly affect the initial burst effect and the kinetics of drug release (Budhian et al., 2007). Therefore, producing monodisperse nanoparticles and microparticles of a controlled shape and size has become one of the key challenges in the development of controlled-release drug delivery systems (Wu et al., 2013). In this work, we aimed to investigate the effectiveness of a controlled drug delivery system based on polysaccharide nanoparticles containing the anticancer drug (doxorubicin) released from chitosan-coated alginate microspheres.

2. MATERIALS AND METHODS

2.1. Nanoparticles synthesis and size determination

2.1.1. Dextran oxidation

Dextarn (Pharmacosmos, Denmark, MW = 70 kDa) was oxidized according to the procedure described by Mu et al., in order to obtain aldehyde groups along the polysaccharide chain (Mu et al., 2012). Briefly, dextran was dissolved in distilled water at the concentration of 5% (w/v) and the sodium (meta)periodate

(Sigma Aldrich, Poland) was added to the dextran solution in molar ratios of 1:20 (IO-4/glucose units). The mixture was then stirred in the dark at ambient temperature and the ethylene glycol (Chempur, Poland) was added after 1 h to quench the oxidation reaction. Periodate-oxidized dextran was then dialyzed against water with dialysis membrane bags (MWCO 12,000–14,000 Da, Carl-Roth) in ambient temperature for 72 h and the water was changed every 12 h. The resulting product was dried at 60 °C in an incubator for 24 h. The number of aldehyde groups in the resulting polyaldehydo-dextran (PAD) was determined with hydroxylamine-hydrochloride method (Zhao and Heindel, 1991), using dextran without any modification as a reference.

2.1.2. Dextran nanoparticle synthesis

Polysaccharide nanoparticle (NP) synthesis was based on the reaction of aldehyde groups in PAD with amino groups in drug (doxorubicin), or aliphatic amine which was used as the coiling agent. To synthetize dextran nanoparticles with doxorubicin (Dox-NPs) according to the method described by Wasiak et al. (2016) 0.5 g of dried PAD was dissolved in 5 mL of deionized water at 30 °C. Subsequently, 1% doxorubicin (Sequoia Research Products, UK) aqueous solution and 2% dodecylamine (coiling agent) aqueous solution were added. The reaction mixture was stirred continuously at 30 °C and after 30 min its pH was measured and gradually increased with sodium hydroxide (NaOH, Chempur) over 1 h, until the pH of the solution reached 10. During the process of increasing the pH, when the pH was above 8, 2% alanine aqueous solution was added. Next, the mixture's pH was reduced to 7.4 with hydrochloric acid (HCl, Chempur). The solution was then dialyzed against water for 60 min in ambient temperature. The resulting Dox-NPs were then lyophilized (Wasiak et al., 2016). The amount of added drug was calculated to constitute 5% by weight of PAD used for the reaction and the amount of added amine was calculated to substitute 50% of the aldehyde groups introduced into the dextran chain. The remaining unreacted aldehyde groups were substituted with alanine.

2.1.3. Nanoparticle size determination

The size distribution and the mean diameter of the resulting Dox-NPs were measured with a NanoSight LM10–HS device (Malvern Instruments, UK) equipped with Nanoparticle Tracking Analysis (NTA) software. The samples were measured three times.

2.2. Alginate microsphere preparation and characterization

2.2.1. Alginate microsphere preparation

Microspheres were produced according to a procedure described by Walczak et al. (2014), using an encapsulator B-395 Pro (Buchi) wherein the laminar flow of the alginate (20 000–40 000 cps (#MKBB8171, Sigma Aldrich) aqueous solution at concentration 1.5% through the nozzle of a defined diameter was mechanically disturbed at the frequency of 1200 Hz (Walczak et al., 2014). At the optimal wavelength described by Weber (1931), spheres of uniform size were formed. The formed spheres passed through the electrode where they were electrostatically charged and dispersed to avoid clotting. Next the dispersed spheres were collected in the container with the polymerization solution (0.1 M calcium chloride ($CaCl_2$) solution, Chempur).

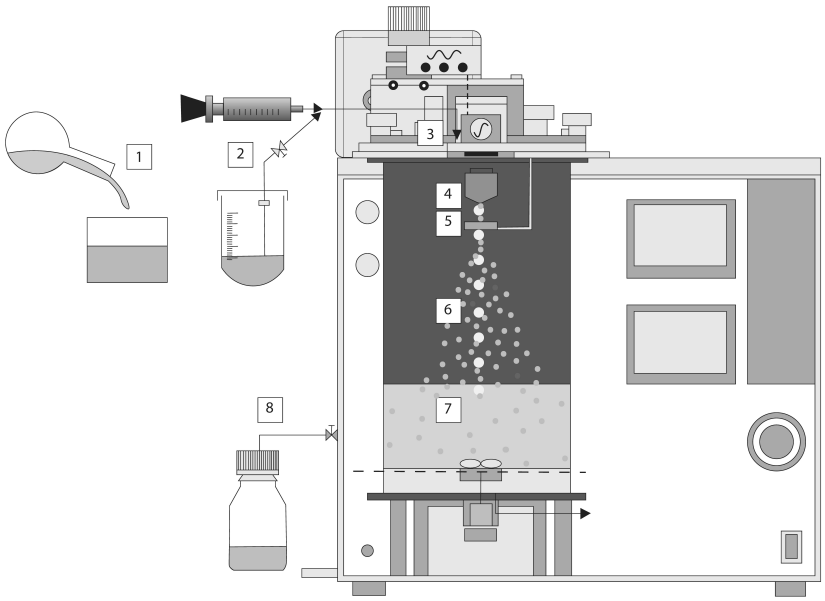

Fig. 1. The scheme of the B-395 Pro encapsulator, 2 – syringe with alginate solution, 3 – pulsation chamber, 4 – nozzle, 5 – electrode, 6 – dispersed spheres, 7 – polymerization solution and magnetic stirrer (BÜCHI, 2016)

2.2.2. Microsphere size determination

The size distribution of the produced alginate microspheres was determined using an optic microscope (Nikon) and the NIS Elements 3.0. software for statistics. Measurements were conducted on the 20 randomly selected microspheres in one sample.

2.2.3. Loading of the Dox-NPs into alginate microspheres

20 mL of alginate microspheres were added to 20 mL of Dox-NPs/PBS (phosphate-buffered saline) solution with a concentration of 2 mg/mL. The mixture was incubated for 4 h at 5 °C under stirring and after this time, the solution from above the microspheres was removed. The concentration of the Dox-NPs inside the microspheres was calculated by measuring the Dox-NPs concentration in the PBS solution before and after incubation with microspheres. The Dox-NPs concentration was measured using a spectrophotometer (Helios γ, Thermo Scientific) at 490 nm according to the obtained earlier standard curve (Yang and Owusu-Ababio, 2000). The same experimental method was used to produce the alginate microspheres with free doxorubicin which were then used as a control in an *in vitro* cytotoxicity study.

2.2.4. Alginate microsphere coating with chitosan multilayer

The alginate microsphere surface was coated with a chitosan multilayer in the polyelectrolyte complexation process. The negatively charged alginate microspheres were incubated in 0.1% (w/v) chitosan aqueous solution (pH < 6) at 5 °C for 60 min under stirring. The formed alginate-chitosan microspheres were separated and washed with distilled water. The 1-layer microspheres were mixed with 1% (w/v) alginate aqueous solution, and then separated and again washed in the same way to generate alginate–chitosan–alginate (2-layer) microspheres. The multilayer (3-layer) composite microspheres (alginate–chitosan–alginate-chitosan) were obtained by repeating this process (Xiao and Sun, 2013). The thickness of the chitosan layer was determined by the microsphere diameter measurement before and after incubation with polyelectrolyte using an optic microscope (Nikon) and the NIS Elements 3.0. software for statistics. The microsphere diameter was also measured depending on the time of complexation with chitosan, using an AccuSizer 780 Optical Particle Sizer with dynamic light scattering (DLS) technique.

2.2.5. Microsphere degradation

50 mL of the alginate microspheres covered with chitosan were added to 100 mL of PBS solution with pH = 7.4. The mixture was incubated at 37 °C for 30 days under gentle stirring and the microsphere diameter was measured in several time points: 0, 24 h, 48 h, 5 d, 7 d, 14 d, 21 d, 30 days, using an AccuSizer 780 Optical Particle Sizer with dynamic light scattering (DLS) technique. The microsphere degradation assessment was carried out for microspheres not containing Dox-NPs.

2.3. Determination of the kinetics of Dox-NPs release from alginate microspheres

In order to determine Dox-NPs release from alginate microspheres and not free drug release from nanoparticles, the bonds between aldehyde groups in oxidized dextran and amine groups in the drug were reduced using $NaBH_4$ (Sigma-Aldrich, Poland) EtOH solution (Wasiak et al., 2016). As a result of this reaction, redDox-NPs were formed. As the doxorubicin is permanently bounded and cannot be released from redDox-NPs, these NPs were used to characterize the kinetics of NP release from alginate microspheres.

Briefly, 20 mL of alginate-chitosan microspheres containing redDox-NPs were incubated in 20 mL of PBS solution (pH = 7.4) at 37 °C for 24 h under stirring. During incubation, the concentration of the released redDox-NPs was measured using a spectrophotometer (Helios γ, Thermo Scientific) at 490 nm in given time points: 0 min, 2 min, 5 min, 10 min, 30 min, 60 min, 90 min, 180 min, 24 h. The kinetics of redDox-NP release from alginate microspheres was presented as a function of redDox-NPs concentration change over time.

2.4. In vitro cytotoxicity

The prepared microspheres were tested for their cytotoxicity using the MTT viability assays. *Day 1*, HeLa cells – human cervical cancer (Sigma Aldrich, Poland) were seeded in 24-well cell culture plates in a concentration of 10^5 cells/mL using 0.5 mL/well of DMEM culture medium without phenol red (Gibco) supplemented with 1% of L–glutamine (Gibco), 1 % of penicillin/streptomycin (Gibco) and 10% of fetal bovine serum (FBS, Gibco). In order to obtain extracts, 0.4 g of microspheres were incubated with 2 mL of supplemented DMEM for 24 h at 37 °C, 5% CO_2. For details of prepared extracts, see Table 1. *Day 2*, medium was aspirated, and the cells were washed with Dulbecco's phosphate-buffered saline (DPBS, Gibco) and contacted with extracts (1 mL/well) for 24 h at 37 °C, 5% CO_2. *Day 3*, the extracts were removed from all wells. Cells were washed with DPBS and 0.5 mL of 3–(4,5–dimethylthiazol–2–yl)–2,5-diphenyltetrazoliumbromide (MTT) solution (1 mg/mL, Sigma Aldrich) was added to each well. The cells were incubated for 4 h at 37 °C, 5% CO_2 before the MTT solution was aspirated. The MTT-formazan crystals produced by living HeLa cells were then dissolved in 1 mL of isopropyl alcohol. Next, the absorbance was measured at a wavelength of 570 nm (reference 650 nm) utilizing a microplate reader (Epoch, BioTek

Table 1. Type of extracts prepared to determine cytotoxicity of tested microspheres

Microspheres type		
Alginate microspheres	Alginate microspheres + Dox-NPs	Alginate microspheres + Free doxorubicin
Alginate microspheres covered with chitosan	Alginate microspheres covered with chitosan + Dox-NPs	Alginate microspheres covered with chitosan + Free doxorubicin

Instruments). To determine the cell viability (%), the absorbance in each well was compared with a blank – cells cultured with DMEM culture medium without any test substance. The presented data are average values ± SD.

3. RESULTS AND DISCUSSION

3.1. Dox-NPs synthesis and size determination

Dextran oxidation was performed using sodium (meta)periodate ($NaIO_4$) and it resulted in formation of reactive aldehyde groups along the polysaccharide chain. The $NaIO_4$ was added to yield theoretical oxidation of 10%, which means that 10% of glucose residues will be oxidized to form aldehyde groups. The resulting PAD was next examined to determine its final oxidation degree, which reached 13.92%. The obtained oxidation degree differed from the theoretical oxidation degree, but it was consistent with what was previously reported by Muangsiri and Kirsch (2006), that the oxidation factor for the $NaIO_4$ oxidation method can be up to 1.7. In the case described herein, the oxidation factor was approximately 1.4. This means that the oxidation degree should be monitored during each oxidation reaction.

Polysaccharide nanoparticles are formed by the reaction of amino groups present in the drug or aliphatic amine with aldehyde groups present in PAD. During the slow reduction of the reaction mixture pH to 7.4, due to the hydrophobic–hydrophilic interactions, NPs are formed. The NP formation was verified using a light scattering method. Dex-NPs were synthesized from 70 kDa PAD. The amount of added drug was calculated to constitute 5% by weight of used PAD and the amount of added dodecylamine - the coiling agent was appropriate to substitute 50% of the aldehyde groups present in the PAD chain. All the remaining aldehyde groups were substituted with alanine to reduce free aldehyde group toxicity. The diameter of the NPs was measured after synthesis and 30 min of dialysis against the water.

NPs resulting from the above reaction were characterized using a NanoSight LM10–HS device and their diameter was 112 + /−5.2 nm. The analysis result, showing the size distribution of the NPs is shown in Figure 2.The obtained nanoparticles meet the criteria of preferable size range of 50-200 nm, which allows passive diffusion through the leaks of newly formed blood vessels (Dobrovolskaia and McNeil, 2013),

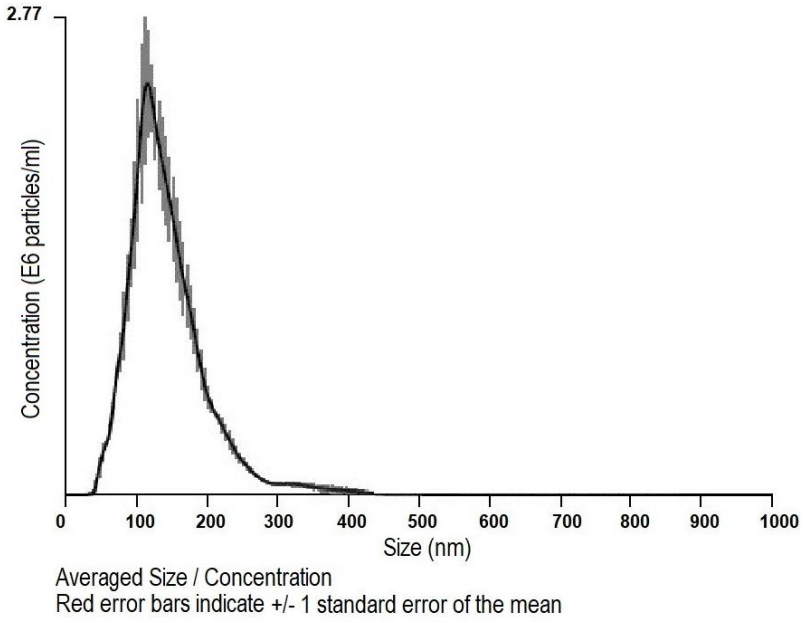

Fig. 2. Size distribution of Dox-NPs

thereby increasing the concentration of particles around the tumor. Moreover, they have a slightly negative zeta potential (−3.97 + /−0.62) that is the charge present on their surface, which may reduce phagocytic uptake, which means that they will not be recognized by mononuclear phagocyte system as "foreign" particles and may exhibit prolonged circulation time in the bloodstream (Honary and Zahir, 2013).

3.2. Alginate microsphere preparation

The method of producing alginate microspheres with controlled size has been developed using an encapsulator 395-Pro (Buchi). The parameters for the production of microspheres are shown in Table 2. The method provided reproducible microsphere formation with high size uniformity and high productivity.

Table 2. Parameters of microsphere production

Nozzle diameter [μm]	Flow rate [ml/min]	Vibration frequency [kHz]	Electrode voltage [V]	Alginate solution concentration [%]	Microsphere diameter [μm]
150	2.8	1200	1700	1.5	318 ± 14
200	4.5	1200	1500	1.5	441 ± 12

The microsphere size distribution was evaluated for two different microsphere dimensions produced using nozzles of two diameters: 150 μm and 200 μm. Measurements were conducted on the 20 randomly selected microspheres in one sample. Pictures were taken using a Nikon optical microscope with NIS Elements 3.0 software.

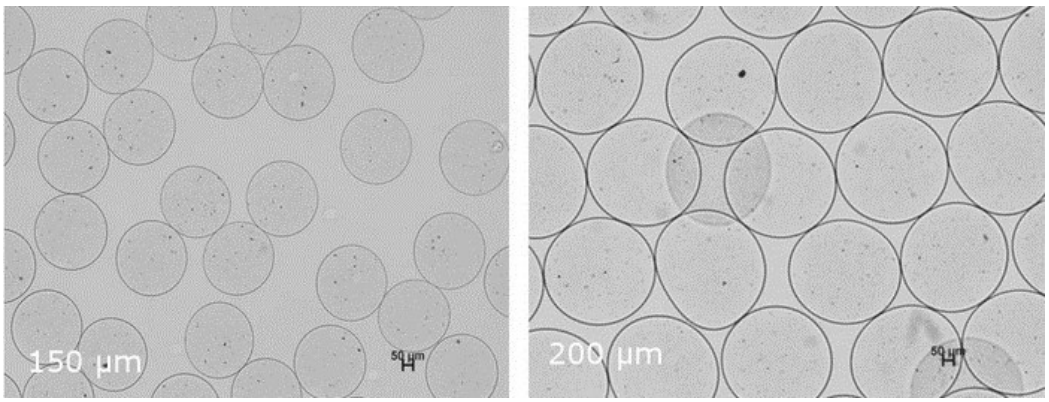

Fig. 3. Alginate microspheres produced using nozzles with diameters of 150 μm and 200 μm. The scale bars represent 50 micrometres (μm)

The produced microspheres were made of natural biomaterial, that can be degraded by the body, therefore, surgical removal is not required (Forster et al., 2010). The presented method of the preparation of alginate microspheres enables the formation of microspheres of various sizes, which allows their size to be adapted for potential use as an embolic agent, depending on the area of application and the diameter of blood vessels, e.g. those supplying the tumor, that we want to embolize. As previously reported (Laurent, 2007; Wang et al., 2020), the microsphere size ranges should correspond to the size ranges of arterioles detectable by angiography and accessible to catheters and microcatheters. The size of commercially available embolic microspheres is usually in the range of 40–1200 μm (Qin et al., 2019). Thus, the obtained microspheres of the diameters of 318 + /−14 and 441 + /−12 μm were considered to meet the clinical needs well.

Chitosan was used to form the outer layer on the microsphere surface because of its positive charge which increases the protein adhesion and at the same time increases the embolic potential of the microspheres (intensified vessel clotting). After the polyelectrolyte complexation process during which alginate microspheres surface was coated with chitosan multilayer, the thickness of the chitosan layer was determined with microsphere diameter measurement before and after incubation with polyelectrolyte, using a Nikon optical microscope with NIS Elements 3.0 software. The microsphere diameter was also measured depending on the time of complexation with chitosan, using an AccuSizer 780 Optical Particle Sizer with dynamic light scattering (DLS) technique.

The diameter of the tested microspheres with the chitosan layer has not changed significantly depending on the time of complexation with the chitosan solution which may indicate that chitosan layer thickness was so small (nanometers) that it did not affect the diameter of microspheres. However, in further studies it should be examined whether the formed chitosan layers are homogeneous on the entire surface of the alginate microspheres.

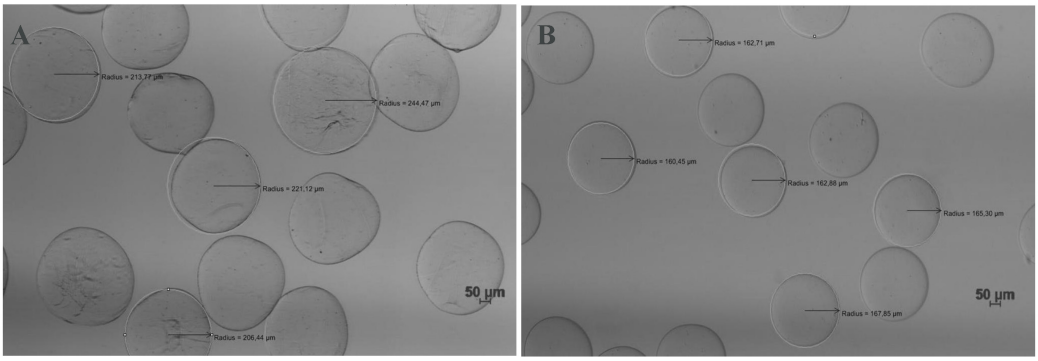

Fig. 4. Alginate microspheres produced using nozzles with diameters: A) 200 µm and B) 150 µm, after 60 minutes of complexation with chitosan. The scale bars represent 50 micrometres (µm)

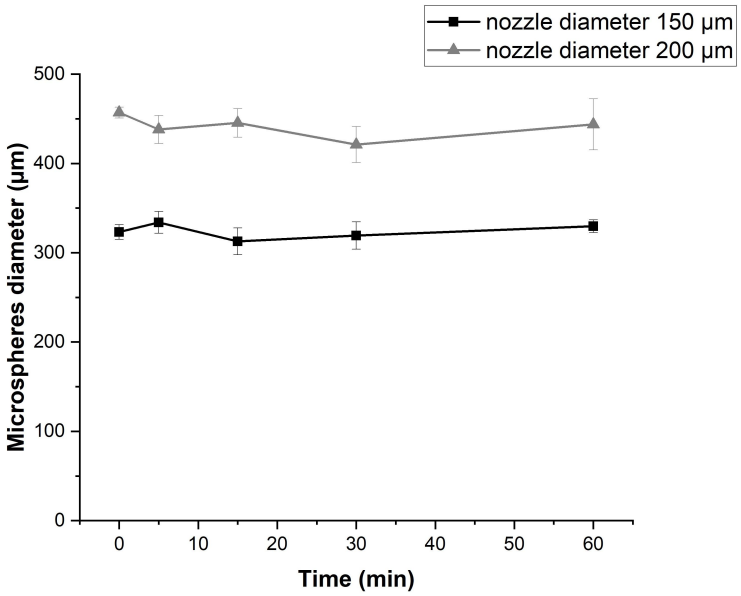

Fig. 5. The microsphere diameter depending on the time of complexation with chitosan

The degradation rate of alginate microspheres covered with chitosan under the physiological conditions was determined by 30-day microsphere incubation in PBS solution (pH = 7.4) at 37 °C and the microsphere diameter measurements in several time points, using an AccuSizer 780 Optical Particle Sizer with dynamic light scattering (DLS) technique.

The duration of microsphere incubation under physiological conditions did not change the diameter of microspheres, which may indicate that alginate microspheres covered with chitosan did not degrade under physiological conditions. Therefore, we believe the proposed microspheres would be a promising tool for long-term vascular embolization.

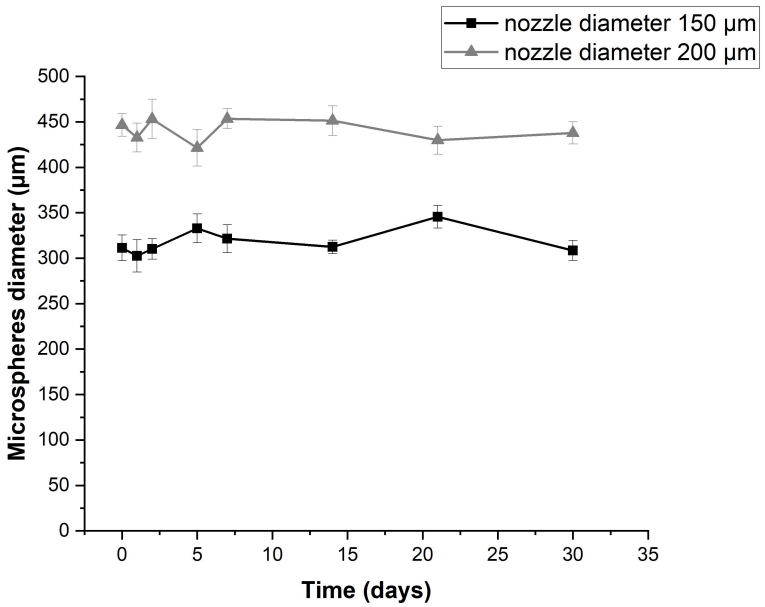

Fig. 6. Degree of degradation of alginate microspheres covered with chitosan under physiological conditions

3.3. The kinetics of Dox-NPs release from alginate microspheres

The kinetics of nanoparticle release from the microspheres was investigated by performing incubation of alginate microspheres with and without chitosan layers containing redDox-NPs in PBS, at 37 °C for 24 h. During the incubation the concentration of the released redDox-NPs was measured.

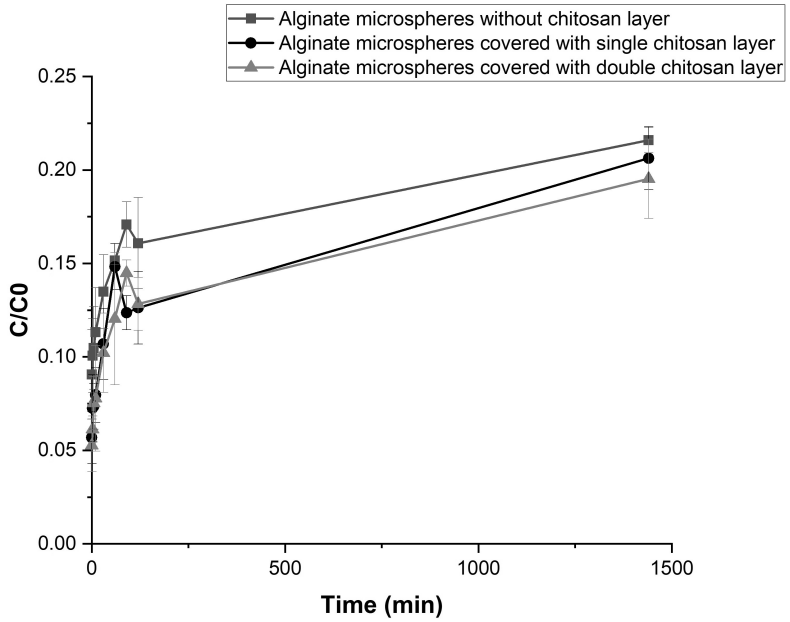

Fig. 7. The kinetics of the redDox-NP release from the alginate microspheres covered with chitosan layer with a diameter of about 300 µm

The obtained results showed that for both microsphere diameters – 300 μm and 400 μm, the kinetics of Dox-NP release was similar, which indicates that the size of alginate microspheres did not affect the rate and number of released redDox-NPs. In addition, the results showed that covering microspheres with chitosan layers did not extend the release time of the redDox-NPs and only caused a slight slowdown in the rate of redDox-NPs secretion in the early stage of the experiment. It is possible that the duration of the experiment was too short to observe the effect of the coating of the microspheres with the chitosan layer on the kinetics of Dox-NPs release. The total amount of released redDox-NPs after 24 hrs was about 20% of the initial redDox-NPs concentration, which means that the release time of redDox-NPs should be extended to investigate if 100% will be released within the longer period of time. Further experiments are required to investigate the release kinetics of non-reduced Dox-NP as well as to determine the amount of doxorubicin released from nanoparticles during this process.

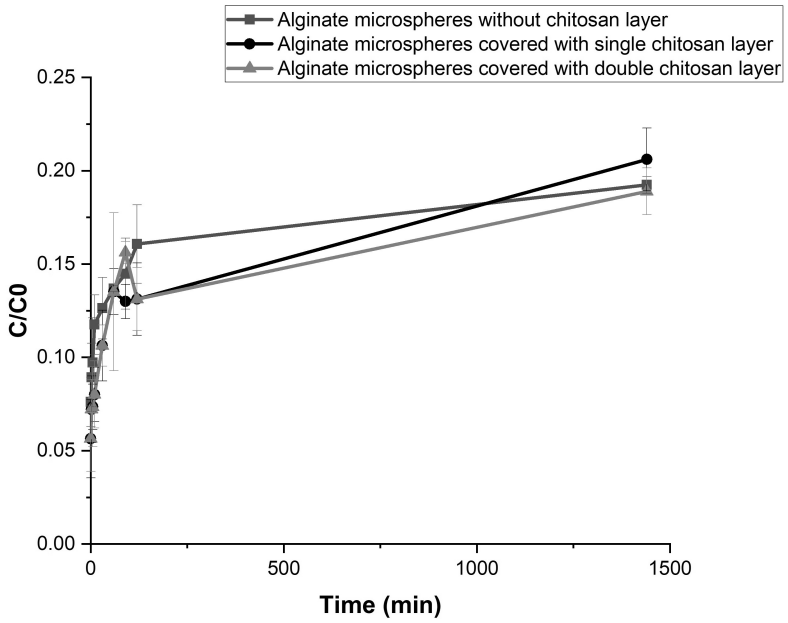

Fig. 8. The kinetics of the redDox-NP release from the alginate microspheres covered with chitosan layer with a diameter of about 400 μm

3.4. Cytotoxicity evaluation

The cytotoxicity of the produced microspheres with and without Dox-NPs was investigated with the i*n vitro* cell culture of the HeLa cells. The extracts (Table 1) made by microsphere incubation in medium for 24 h were contacted with HeLa cells for 24 h. The viability of the cells cultured with microsphere extracts was determined with the MTT assay and was compared to the cells cultured in pure medium.

The results of MTT assay showed that the empty alginate microspheres with a diameter of 300 μm without chitosan layer and empty alginate microspheres with a diameter of 400 μm with and without chitosan layer are non-cytotoxic according to ISO 10993–5 standard, as their cell viability is above 80%. Slightly lower cell viability can be observed for microspheres with 300 μm diameter covered with chitosan (65%) and according to ISO 10993–5 standard, its cytotoxicity is considered as mild. The above results indicate that the alginate microspheres may be a promising tool for future applications such as transarterial embolization or cell encapsulation. Although microspheres containing Dox-NPs regardless of chitosan coverage have slightly reduced cytotoxic properties in comparison to microspheres containing free doxorubicin (positive control), the cytotoxicity of both, microspores containing Dox-NPs and microspores containing free doxorubicin is considered as severe as the cell viability is below 30%. In case of microspheres containing free doxorubicin, the chitosan coating slightly reduced their cytotoxicity.

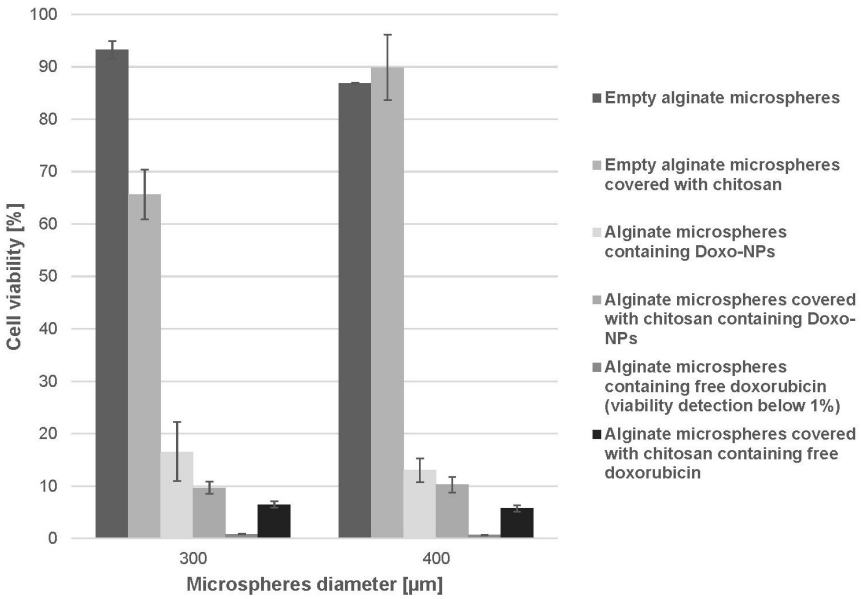

Fig. 9. The MTT assay results

4. CONCLUSIONS

The aim of the presented research was to investigate the targeting effect of a drug delivery system based on the controlled release of dextran nanoparticles containing the anticancer drug – doxorubicin (Dox-NPs) from alginate microspheres coated with chitosan multilayers. The application of known drugs encapsulated in polysaccharide nanoparticles can improve efficacy and decrease side effects of anticancer therapy by increasing drug concentration in cancer tissue. Additionally, the use of hydrogel microspheres as a drug delivery system can allow therapeutic agent to be delivered directly to cancer cells using a novel approach connecting (1) transcatheter arterial embolization (TEA) of the tumor supplying blood vessel by the alginate microspheres with nanoparticles and (2) the structure of dextran-based nanoparticles imitating the free glucose, which cause increased absorption of the nanoparticles in the cancer cells due to their increased glucose requisition (Warburg's effect).

The conducted study showed that the use of the encapsulator B-395 Pro (Buchi) with a concentric nozzle provides reproducible alginate microspheres with high size uniformity and high productivity. Covering the alginate microsphere surface with chitosan layers in the polyelectrolyte complexation process did not affect the size of microspheres and the microsphere degradation study showed that alginate microspheres covered with chitosan are stable under the physiological conditions. The study of the kinetics of redDox-NP release from alginate microspheres showed that alginate microspheres coated with chitosan layers did not show an extended-release time of the redDox-NPs. Moreover, incubation of alginate microspheres with and without chitosan layers containing redDox-NPs in PBS solution at 37 °C for 24 h resulted in the release of only slightly more than 20% of initial redDox-NP concentration, so further research is required to investigate the total degree of NP release. The results of MTT assay showed the lack of cytotoxicity of empty alginate microspheres. The microspheres with encapsulated Dox-NPs resulted in a significant decrease in HeLa cell viability, compared to the empty microspheres. However, the cytotoxic properties of microspheres with encapsulated Dox-NPs were lower in comparison to microspheres containing free doxorubicin, especially for non-chitosan-coated microspheres.

The conducted studies proved that alginate microspheres containing dextran-based nanoparticles have a great potential to be exploited as a novel drug delivery system that will allow to deliver active anticancer drug directly to cancer tissue. Nevertheless, further research is required to improve the drug release rate.

We would like to acknowledge The National Science Centre of Poland for financial support – grant No 2014/13/N/NZ5/01344 and 2014/13/N/ST8/00098.

REFERENCES

Adepu S., Ramakrishna S., 2021. Controlled drug delivery systems: current status and future directions. *Molecules*, 26, 5905. DOI: 10.3390/molecules26195905.

BÜCHI, 2016. Operation Manual, Encapsulator B-395 Pro. BÜCHI Labortechnik AG.

Budhian A., Siegel S.J., Winey K.I., 2007. Haloperidol-loaded PLGA nanoparticles: systematic study of particle size and drug content. *Int. J. Pharm.*, 336, 367–375. DOI: 10.1016/j.ijpharm.2006.11.061.

Chan O.C.M., So K.-F., Chan B.P., 2008. Fabrication of nano-fibrous collagen microspheres for protein delivery and effects of photochemical crosslinking on release kinetics. *J. Control. Release*, 129, 135–143. DOI: 10.1016/j.jconrel.2008.04.011.

Dobrovolskaia M., McNeil S.E. (Eds.), 2013. *Handbook of immunological properties of engineered nanomaterials*. Series: Frontiers in nanobiomedical research, Vol. 1. World Scientific, New Jersey.

Forster R.E., Thürmer F., Wallrapp C., Lloyd A.W., Macfarlane W., Phillips G.J., Boutrand J-P., Lewis A.L., 2010. Characterisation of physico-mechanical properties and degradation potential of calcium alginate beads for use in embolization. *J. Mater. Sci.: Mater. Med*. 21, 2243-2251. DOI: 10.1007/s10856-010-4080-y.

Gui R., Wang Y., Sun J., 2014. Encapsulating magnetic and fluorescent mesoporous silica into thermosensitive chitosan microspheres for cell imaging and controlled drug release *in vitro*. *Colloids Surf., B*, 113, 1–9. DOI: 10.1016/j.colsurfb.2013.08.015.

Hoare T., Young S., Lawlor M.W., Kohane D.S., 2012. Thermoresponsive nanogels for prolonged duration local anesthesia. *Acta Biomater.*, 8, 3596–3605. DOI: 10.1016/j.actbio.2012.06.013.

Honary S., Zahir F., 2013. Effect of zeta potential on the properties of nano-drug delivery systems - a review (Part 2). *Trop. J. Pharm. Res.*, 12, 265–273. DOI: 10.4314/tjpr.v12i2.20.

Lammer J., Malagari K., Vogl T., et al. 2010. Prospective randomized study of doxorubicin- eluting-bead embolization in the treatment of hepatocellular carcinoma: Results of the PRECISION V study. *Cardiovasc. Intervent. Radiol.*, 33, 41–52. DOI: 10.1007/s00270-009-9711-7.

Laurent A., 2007. Microspheres and nonspherical particles for embolization. *Tech. Vasc. Interventional Radiol.*, 10, 248–256. DOI: 10.1053/j.tvir.2008.03.010.

Mu C., Guo J., Li X., Lin W., Li D., 2012. Preparation and properties of dialdehyde carboxymethyl cellulose crosslinked gelatin edible films. *Food Hydrocolloids*, 27, 22–29. DOI: 10.1016/j.foodhyd.2011.09.005.

Muangsiri W., Kirsch L.E., 2006. The protein-binding and drug release properties of macromolecular conjugates containing daptomycin and dextran. *Int. J. Pharm.*, 315, 30–43. DOI: 10.1016/j.ijpharm.2006.02.016.

Namur J., Citron S.J., Sellers M.T., Dupuis M.H., Wassef M., Manfait M., Laurent A., 2011. Embolization of hepatocellular carcinoma with drug-eluting beads: Doxorubicin tissue concentration and distribution in patient liver explants. *J. Hepatol.*, 55, 1332–1338. DOI: 10.1016/j.jhep.2011.03.024.

Qin X.Y., Liu X.X., Li Z.Y., Guo L.Y., Zheng Z.Z., Guan H.T., Song L., Zou Y.H., Fan T.Y., 2019. MRI detectable polymer microspheres embedded with magnetic ferrite nanoclusters for embolization: in vitro and in vivo evaluation. *Int. J. Nanomed.*, 14, 8989–9006. DOI: https://doi.org/10.2147/IJN.S209603.

Su J., Hu B-H, Lowe Jr. W.L., Kaufman D.B., Messersmith P.B., 2010. Anti-inflammatory peptide-functionalized hydrogels for insulin-secreting cell encapsulation. *Biomaterials*, 31, 308–314. DOI: 10.1016/j.biomaterials.2009.09.045.

Tiwari G., Tiwari R., Sriwastawa B., Bhati L., Pandey S., Pandey P., Bannerjee S.K., 2012. Drug delivery systems: An updated review. *Int. J. Pharm. Investig.*, 2, 2–11. DOI: 10.4103/2230-973X.96920.

Vargason A.M., Anselmo A.C., Mitragotri S., 2021. The evolution of commercial drug delivery technologies. *Nat. Biomed. Eng.*, 5, 951–967. DOI: 10.1038/s41551-021-00698-w.

Walczak A., Monarska A., Ciach T., 2014. Human cell encapsulation in hydrogel microspheres. *EYEC monograph: 3rd European Young Engineers Conference*. Warsaw, Poland, 29–30 April 2014, 119–131.

Wang, C.Y., Hu, J., Sheth, R.A., Oklu, R., 2020. Emerging embolic agents in endovascular embolization: an overview. *Prog. Biomed. Eng.*, 2, 012003. DOI: 10.1088/2516-1091/ab6c7d.

Wasiak I., Kulikowska A., Janczewska M., Michalak M., Cymerman I.A., Nagalski A., Kallinger P., Szymanski W.W., Ciach T., 2016. Dextran nanoparticle synthesis and properties. *PLoS ONE*, 11, e0146237. DOI: 10.1371/journal.pone.0146237.

Weber C., 1931. Zum Zerfall eines Flüssigkeitsstrahles. *Z. angew. Math. Mech.*, 11, 136–154. DOI: 10.1002/zamm.19310110207.

Weng L., Rostamzadeh P., Nooryshokry N., Le H.C., Golzarian J., 2013. In vitro and in vivo evaluation of biodegradable embolic microspheres with tunable anticancer drug release. *Acta Biomater.*, 9, 6823–6833. DOI: 10.1016/j.actbio.2013.02.017.

Wu J., Kong T., Yeung K.W.K., Shum H.C., Cheung K.M.C., Wang L., To M.K.T., 2013. Fabrication and characterization of monodisperse PLGA–alginate core–shell microspheres with monodisperse size and homogeneous shells for controlled drug release. *Acta Biomater.*, 9, 7410–7419. DOI: 10.1016/j.actbio.2013.03.022.

Xiao C., Sun F., 2013. Fabrication of distilled water-soluble chitosan/alginate functional multilayer composite microspheres. *Carbohydr. Polym.*, 98, 1366–1370. DOI: 10.1016/j.carbpol.2013.07.068.

Yang Q., Owusu-Ababio G., 2000. Biodegradable progesterone microsphere delivery system for osteoporosis therapy. *Drug Dev. Ind. Pharm.*, 26, 61–70. DOI: 10.1081/ddc-100100328.

Zhang J.T., Keller T.F., Bhat R., Garipcan B., Jandt K.D., 2010. A novel two-level microstructured poly(*N*-isopropylacrylamide) hydrogel for controlled release. *Acta Biomater.*, 6, 3890–3898. DOI: 10.1016/j.actbio.2010.05.009.

Zhao H., Heindel N.D., 1991. Determination of degree of substitution of formyl groups in polyaldehyde dextran by the hydroxylamine hydrochloride method. *Pharm. Res.*, 8, 400–402. DOI: 10.1023/a:1015866104055.

Zhou X., Kong M., Cheng X.J., Feng C., Li J., Li J.J., Chen X.G., 2014. In vitro and in vivo evaluation of chitosan microspheres with different deacetylation degree as potential embolic agent. *Carbohydr. Polym.*, 113, 304–313. DOI: 10.1016/j.carbpol.2014.06.080.

Received 28 April 2022
Received in revised form 30 July 2022
Accepted 06 September 2022