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Effect of surfactants and polymers on stability of superparamagnetic nanoparticles and on immobilization and release of antitumor agents

Hana Buchtelova[1], Zuzana Skubalova[1], Jiri Kudr[1], Vladislav Strmiska[1,2], Vojtech Adam[1,2], Zbynek Heger[1,2]
[1]Department of Chemistry and Biochemistry
Mendel University in Brno
Zemedelska 1, 613 00 Brno
[2]Central European Institute of Technology
Brno University of Technology
Technicka 3058/10, 616 00 Brno
CZECH REPUBLIC

hanabuchtelova@gmail.com

Abstract: The current study demonstrates design, preparation and characterization of biocompatible superparamagnetic iron oxide nanoparticles (SPIONs) coated with three different polymers polyvinylpyrrolidone (PVP) polyoxyethylene stearate (POES) and chitosan (Chit). Such modified nanoparticles were loaded with doxorubicin, as model anticancer drug. Resulting complex has an exceptional stability in physiological conditions. The highest release of complexed Dox was in endosomal environment in case SPIONs with POES. The cytotoxic effects of the complex were tested using breast cancer/healthy epithelial cell lines. Use of SPIONs increased the cytotoxicity of doxorubicin when compared to free doxorubicin and decreased the cytotoxicity in healthy cells. The results demonstrate that modification of SPIONs could have a potential in nanomedicine as versatile nanoplatform to enhance efficiency of anticancer therapy.

Key Words: biocompatibility, SPION, nanomedicine, cytotoxicity

INTRODUCTION

Nanomedicine is a relatively new field of science and a term defines design and testing of functional nanounits in medicine (Boisseau and Loubaton 2011). Material scientists have performed exceptional accomplishments in the design of various types of materials that can be used in nanomedical therefore opens up a vast field of research and application (Linkov et al. 2008).

The frequent drawback of these materials is common systemic toxicity. Superparamagnetic iron oxide nanoparticles (SPIONs) have been used for many years as magnetic resonance imaging (MRI) contrast agents, tissue reparation, or for a drug delivery. Bare SPIONs are often insufficiently stable for a specific accumulation in target tissue (Singh et al. 2010).

Here, we present hybrid SPIONs coated with polyvinylpyrrolidone (PVP), polyoxyethylene stearate (POES) or chitosan (Chit) shell for delivery of conventional cytostatic agent doxorubicin (Dox). Due to unique physical and chemical properties, SPIONs have many usable properties in biomedicine, like tissue repair, magnetic resonance imaging (MRI), detoxification of biologic fluids, drug and gene delivery, biological sensing, and hyperthermia (Naqvi et al. 2010). Nevertheless, the biomedical applications of SPIONs arouse interest about their cytotoxicity (Liu et al. 2013).

We show that SPIONs exhibit good binding efficiency of Dox, exceptional stability in non–target plasma environment. SPIONs (stable in dispersion for more than 6 h) has the highest release in a slightly acidic environment adequate to the hypoxic or endosomal environment and tumor hypoxic tissue. Cytotoxicity was tested *in vitro* on two types of cells – normal breast and breast cancer. Surface coating and drug immobilization resulted in higher cytotoxicity, due to a synergistic interplay. SPIONs are pronouncedly biocompatible. Our results imply suitability of the use of SPIONs with surface modification for medical purposes.

MATERIAL AND METHODS

Synthesis of SPIONs

KNO_3, KOH and ddd water was added into a screwable vessel. After stirred was added Fe_SO_4 during magnetic stirring. Immediately transfer vessel to a preheated water bath (90 °C) for 2 h.

SPIONs coating with PVP, POES and Chit and noncovalent complexation of Dox

Equal volumes of SPIONs was modified *i*) 10 mg/ml of PVP were mixed and incubated (20 °C, 30 min). After that, the solution of SPIONs was mixed with Dox and incubated (20 °C, 30 min). *ii*) 5 mg/ml of POES were mixed and ultrasonicated. After that, the solution of SPIONs was mixed with Dox and ultrasonicated. *iii*) 2.5 mg/ml of Chit were mixed and ultrasonicated. After that, the solution of SPIONs was mixed with Dox and ultrasonicated. Finally, resulting nanoparticles was separated remove unbound Dox and resuspended in MilliQ water. Loading efficiency (LE) of Dox to SPIONs was analysed by UV–Vis spectroscope Infinite 200 PRO (Tecan, Männedorf, Switzerland) at λ 480 nm.

Scanning electron microscopy (SEM)

SEM analyses were performed using the MIRA 3 electron microscope (Tescan, Brno, Czech Republic) after drying the samples on the grid.

Evaluation of colloidal stability of SPIONs in physiological environments

To demonstrate their colloidal stability SPIONs dispersed in the Ringer´s solution were placed in the stationary rack and kept at 25 °C. The dispersion was photodocumented in annotated time intervals (up to 12 h).

In vitro cumulative drug release kinetic studies

1 ml of SPIONs was dispersed in solutions mimicking various conditions, including plasma (Ringer´s solution), neutral intracellular fluid and acidic environment of endosomes. The temperature was maintained at 37 °C. At fixed time intervals, SPIONs was separated by a magnet and 50 µl of medium was withdrawn and subsequently replaced with fresh medium to maintain the sink conditions. The amount of released Dox was determined by UV–Vis spectroscopy at λ 480 nm. The cumulative release of Dox was calculated as follows:

$$\text{Cumulative release (\%)} = (\text{Dox in the medium}) / (\text{Initial Dox}) \times 100$$

Cell lines and culture conditions

Two human cell lines were used: i) the HBL–100 normal human breast cell line, ii) the MDA–MB–231 –human breast cancer cell line. All cell lines were purchased from Health Protection Agency Culture Collections (Salisbury, UK).

HBL–100 and MDA–MB–231 were cultured in RPMI–1640 with 10% foetal bovine serum (FBS), UKF–NB–4 Iscove's modified Dulbecco medium (IMDM) with 10% FBS. Media were supplemented with penicillin and streptomycin, and the cells were maintained in a humidified incubator Galaxy® 170 R (Eppendorf, Hamburg, Germany). Prior all analyses, cells were counted using Countess II FL (Thermo Fisher Scientific, Waltham, MA, USA).

Estimation of cytotoxicity

The viability was assayed using MTT (3– (4,5–dimethylthiazol–2–yl) –2,5–diphenyltetrazolium bromide) assay. Cell was incubation for 24 h at 37 °C with 5% CO_2 to ensure cell growth. After treatment, 10 µl of MTT [5 mg/ml in phosphate buffered saline (PBS)] was added to the cells and incubated. After that, MTT–containing medium was replaced by 100 µl of dimethyl sulfoxide (DMSO) and, absorbance was determined at 570 nm using Infinite 200 PRO (Tecan, Männedorf, Switzerland).

Descriptive statistics

For the statistical evaluation of the results using paired *t*–test and ANOVA. Unless noted otherwise, the threshold for significance was $p < 0.05$. For analyses Software Statistica 12 (StatSoft, Tulsa, OK, USA) was employed.

RESULTS AND DISCUSSION

Physico–chemical characterization of SPIONs and complexation with Dox

SPIONs were tested for their LEs (loading efficiency) towards Dox. Table 1, 2 and 3 illustrates that the highest LE (red colour) was achieved for SPIONs coated with *i*) 10 mg/ml of PVP *ii*) 5 mg/ml of POES *iii*) 2.5 mg/ml of Chit.

Table 1 Analysis of Dox loading efficiency to SPIONs coated with various amount of PVP.

	Concentration of PVP (mg/ml)	2.5	5	10
Ultrasonication	10 °C	0	28	17
	20 °C	21	12	21
	40 °C	18	13	19
Incubation 30 min	10 °C	5	14	8
	20 °C	11	13	40
	40 °C	12	11	9

Table 2 Analysis of Dox loading efficiency to SPIONs coated with various amount of POES.

	Concentration of POES (mg/ml)	2.5	5	10
Ultrasonication	10 °C	29	36	21
	20 °C	24	11	18
	40 °C	14	21	17
Incubation 30 min	10 °C	6	15	10
	20 °C	0	13	11
	40 °C	11	8	14

Table 3 Analysis of Dox loading efficiency to SPIONs coated with various amount of Chit.

	Concentration of Chit (mg/ml)	2.5	5	10
Ultrasonication	10 °C		0	0
	20 °C	0	0	5
	40 °C	0	0	7
Incubation 30 min	10 °C	0	0	8
	20 °C	0	0	1
	40 °C	0	0	9

SEM micrographs showed that SPIONs demonstrated relatively uniform oval–to–spherical morphology and were well dispersed (Figure 1A). This was confirmed by incubating modified SPIONs for 12 h. They were found to disperse readily and remained stable in dispersion for more than 12 h (Figure 1B).

In vitro drug release studies

Drug release studies were conducted with various environments, which mimic low pH of endosomes (pH 5), intracellular neutral pH (pH 6.9) and environment of plasma (pH 7.4). Release profiles demonstrate pH–responsive behaviour of SPIONs PVP, SPIONs POES and SPIONs Chit, which the highest released was 58% of complexed Dox during 4 h incubation in endosomal environment in case SPIONs POES (Figure 3).

Figure 1 (A). SEM micrographs of SPIONs (B) Photodocumentation of colloidal stability of synthesized SPIONs, SPIONs PVP, SPIONs POES and SPIONs Chit at start–point (0 h), and 12 h.

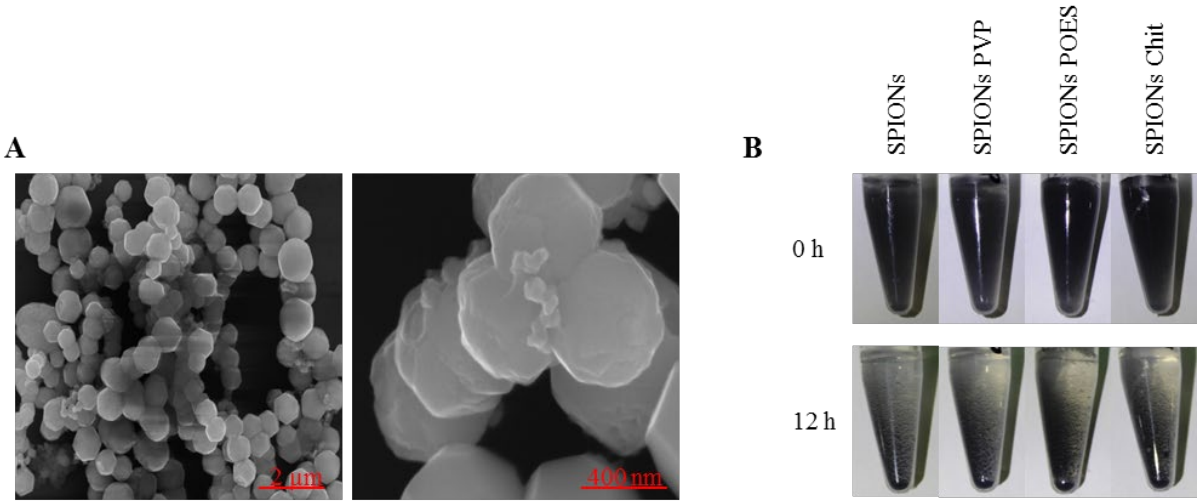


Figure 2 In vitro cumulative release profiles of Dox from SPIONs PVP determined in various physiological pH.

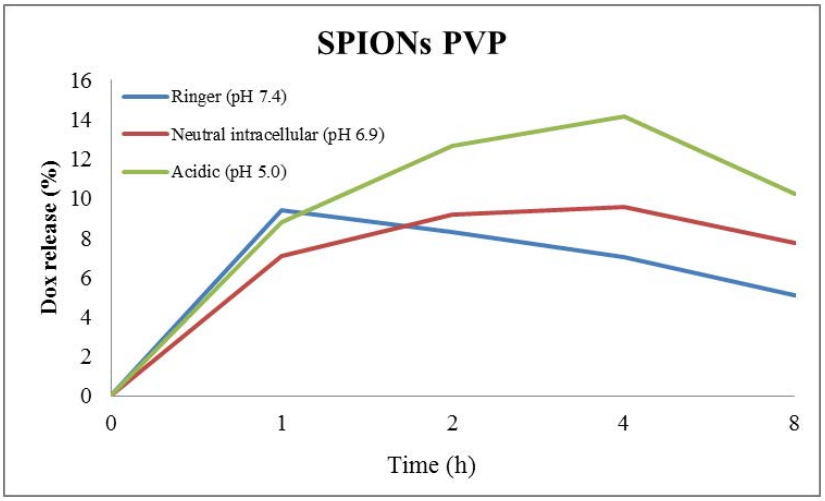


Figure 3 In vitro cumulative release profiles of Dox from SPIONs POES determined in various physiological pH.

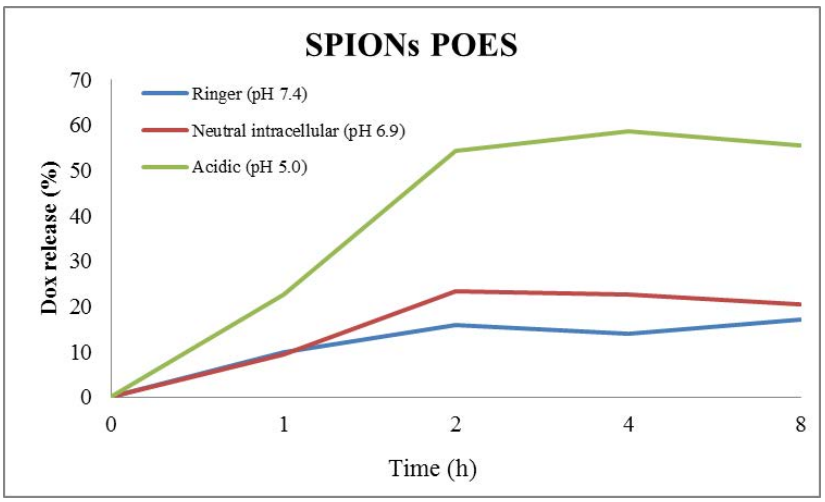
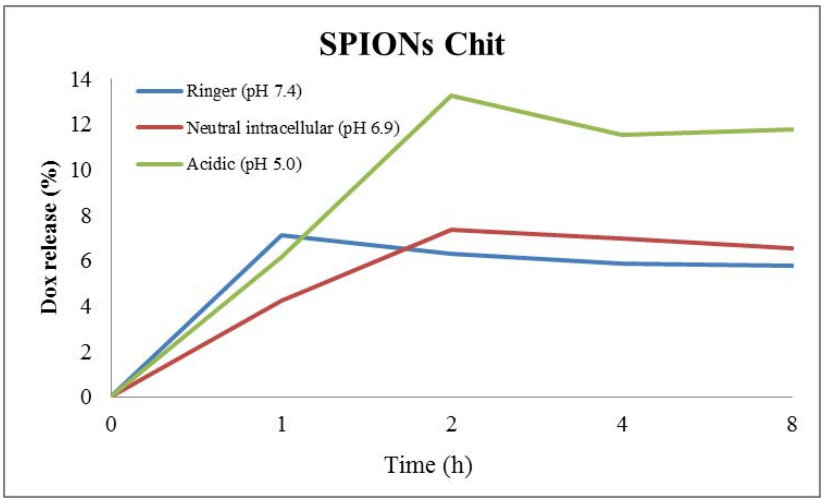


Figure 4 In vitro cumulative release profiles of Dox from SPIONs Chit determined in various physiological pH.



Synergistic cytotoxicity of Dox immobilized on SPIONs

Cytotoxic testing was performed on two different cell lines – breast cancer (MDA–MB–231) and normal breast (HBL–100). Immobilization of Dox resulted in a significant ($p < 0.01$) increase in cytotoxic effects in all tested cells (with the IC_{50} values are summarized in Table 4). Interestingly, non–modified SPIONs did not exerted cytotoxic action in both tested cell lines (n.d. – not detected).

Table 4 Summary of $24IC_{50}$ values obtained from MTT assay for tested cell lines after 24 h treatments. All values are presented as mean ± SD of six biological replicates

	MDA–MB–231	**HBL–100**
	$24IC_{50}$ (μM)	
SPIONs PVP	2	8
SPIONs POES	1	7.1
SPIONs Chit	0.5	1.8
Free Dox	125	89
SPIONs	n.d.	n.d.

CONCLUSION

We designed, prepared and tested cytotoxicity and biocompatibility of various modified SPIONs with exceptional nanomedicine platforms. We also showed that PVP and FDA–approved POES or Chit natural polymer such core–shell nanoparticles which significantly enhances the Dox performance. Our *in vitro* results are restricted and further *in vivo* tests must be carried out in the future, it is obvious that combination of SPIONs and polymers have exhibit potential for nanomedicine. Finally, based on available literature, SPIONs are promising magnetic resonance imaging contrast agents, tissue reparation, or for a drug delivery, hence their use will most likely enable for tracing and imaging.

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