In vitro evaluation of doxorubicin-eluting porous titania microspheres for transcatheter arterial chemoembolization

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ABSTRACT
Preparing drug-eluting beads (DEBs) from radiopaque materials such as titania (TiO$_2$) can meet clinical need for directly visualizing DEBs during drug-eluting bead transcatheter arterial chemoembolization (DEB-TACE). Porous anatase-type TiO$_2$ microspheres with mean volume diameters of approximately 30 µm were obtained when silica nanoparticles (SiNPs) were introduced into the TiO$_2$ matrix by a sol–gel process involving a water-in-oil emulsion, and the SiNPs were then dissolved by subsequent treatment with NaOH solution. Of special note, microspheres prepared using SiNPs of approximately 20 – 25 nm in diameter had a high specific surface area of ~120 m$^2$·g$^{-1}$ and a high doxorubicin (DOX)-adsorption capacity of ~150 mg·ml$^{-1}$, and they gradually released ~10% of the adsorbed DOX within 5 days. The DOX-loaded microspheres were non-cytotoxic, moreover, and exerted anticancer effects on HeLa cells. We propose that the present TiO$_2$ microspheres are potentially useful as novel radiopaque embolic materials for DEB-TACE.

1. Introduction
Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver. HCC is also the sixth most common neoplasm and the third most frequent cause of cancer-related deaths [1]. The four major treatments used for combating HCC are (1) hepatic resection, (2) transcatheter arterial chemoembolization (TACE), (3) percutaneous therapies such as percutaneous ethanol-injection therapy, percutaneous microwave coagulation therapy, and radiofrequency ablation, and (4) chemotherapy. TACE is a specific type of chemoembolization that blocks the hepatic artery to treat liver cancer [2]. In Japan, TACE is widely performed in 32% of patients with unresectable HCC after the initial diagnosis and in 58% of those with recurrent HCC [3]. Gelatin sponge particles and polyvinyl alcohol microspheres have been used as embolic materials in conventional TACE [4,5]. Prior to embolization of the hepatic artery using embolic materials in conventional TACE, a mixed solution of an imaging agent (such as lipiodol) and an anticancer drug (such as doxorubicin or cisplatin) is injected into the arterial supply closest to the tumor.

As an alternative to conventional TACE, TACE using drug-eluting beads (DEB-TACE) has attracted attention [2] for its ability to deliver higher concentrations of anticancer drugs to within the target tumor and to lower the systemic concentration compared with conventional TACE. Typical DEBs comprise sulfonate-modified poly(vinyl alcohol) hydrogel or a copolymer of sodium acrylate and vinyl alcohol. Presently, DC Bead®, HepaSphere™, and Embozene TANDEM™ are approved as embolic materials for DEB-TACE [6–8]. The benefits for survival and tumor response of DEB-TACE over conventional TACE are not yet well defined, although a randomized controlled trial revealed an improved tumor response rate for DEB-TACE compared with conventional TACE [9].

DEBs are radiolucent, since they are polymeric materials, as described above, which makes it impossible to determine their spatial-temporal distribution within target tissues. Hence, a clinical need exists for direct visualization of DEBs to assess the completeness of target tissue embolization (for efficacy) as well as unintentional embolization of non-target tissue (for safety) [10]. Ceramic microspheres with low radiolucency such as titania (TiO$_2$) are candidate radiopaque embolic materials. We reported previously that porous TiO$_2$ microspheres can be prepared by a sol–gel technique [11] and that microspheres can release rhodamine B (a model hydrophilic drug) [12], but the size of the microspheres (~15 µm) was not appropriate for arterial embolization. In this study, we attempted to synthesize porous TiO$_2$ microspheres several dozen micrometers in diameter by modifying the preparation conditions and evaluated the potential of porous TiO$_2$ microspheres as radiopaque DEBs by investigating their doxorubicin (DOX)-loading and releasing ability as well as their cytotoxicity and anticancer efficacy with respect to HeLa tumor cells. Although HeLa cells are not HCC cells...
but, rather, cervical cancer cells, it is possible to evaluate the anticancer efficacy of TiO\textsubscript{2} microsphere samples using HeLa cells in this preliminary study.

2. Materials and methods

2.1. Preparation of samples

All reagent-grade chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Porous TiO\textsubscript{2} microspheres were prepared using a sol–gel method with a water-in-oil (W/O) emulsion, based on our previous studies [13,14]. The composition and stirring conditions of the oil phase were the same as those in our previous study [14]. The components and mixing conditions of the aqueous phase were also the same as those in our previous study [14], but the methanol content was reduced from 2.7 g to 1.7 g for this study.

Similarly to our previous study [12], silica (SiO\textsubscript{2}) nanoparticle (SiNP) suspensions with varying SiO\textsubscript{2} contents were prepared by adding different amounts of colloidal SiO\textsubscript{2} with SiNPs of different sizes (20 – 25 nm and 40 – 50 nm) (SNOWTEX\textsuperscript{®} XL and SNOWTEX\textsuperscript{®} 50, Nissan Chemical Industries, Ltd., Tokyo, Japan) to ultra-pure H\textsubscript{2}O. The concentrations of the SNOWTEX\textsuperscript{®} XL and SNOWTEX\textsuperscript{®} 50 SiNPs were 40 and 48 wt. %, respectively. The total H\textsubscript{2}O content of the dispersion was fixed at 4.2 g by adjusting the amount of H\textsubscript{2}O added. The SiNP dispersion was mixed with the aqueous phase and then dropped into the oil phase under continuous mixing in a homogenizer to achieve a W/O emulsion. Table 1 shows the sample names, SiNP sizes, and SiO\textsubscript{2}/H\textsubscript{2}O weight ratios in the aqueous phase. The capital letters (STXL or ST50) in the sample names indicate the abbreviated SiNP product names (STXL: SNOWTEX\textsuperscript{®} XL, ST50: SNOWTEX\textsuperscript{®} 50) and the numbers in the sample names (STXL-xx or ST50-xx) indicate the SiO\textsubscript{2} content (wt. %). As a reference, Non-porous TiO\textsubscript{2} microspheres (np-Ti) were prepared as references without adding an SiNP dispersion. Mixing proceeded at 1500 rpm at temperatures of 30°C for 20 min, 40°C for 20 min, and, finally, 55°C for 140 min. After mixing, the W/O emulsions were centrifuged at 3000 rpm for 5 min (CN-1050, AS ONE Corp.), and the supernatant was removed by decantation. Thirty milliliters of pure water were added to the centrifuge tubes, and the samples were washed gently by shaking. This washing process was repeated three times. Finally, the samples were dried at 90°C for 24 h.

2.2. Characterization of samples

Sample morphologies were observed by field-emission scanning electron microscopy (FE-SEM; JEM-7100F, JEOL Ltd., Tokyo, Japan). The crystalline phases of the samples were investigated using a powder X-ray diffractometer (XRD: MiniFlex600HDA, Rigaku Corp., Tokyo, Japan) with a Cu Ka source operating at 40 kV and 15 mA at a scanning rate of 4°·min\textsuperscript{-1}. The structures of the samples were investigated by Fourier transform infrared spectroscopy (FT-IR; FT/IR-6200, JASCO Corp., Tokyo, Japan) by recording the transmission spectra using the potassium bromide (KBr) pellet method. The KBr contents of the tested samples were maintained at approximately 0.125 wt. %. The specific surface area (SSA), total pore volume, and pore size distribution of the samples were measured using a gas adsorption analyzer (Autosorb\textsuperscript{®}-IQ, Quantachrome Corp., Florida, USA). The samples were heated at 200°C for 2 h before measurements were taken to remove adsorbed water. The particle size distributions of the samples were measured with a particle size analyzer (NANO-flex, MicrotracBEL Corp., Osaka, Japan), and the zeta potentials of the samples in pure water were measured using a streaming potential analyzer (Stabino, MicrotracBEL Corp., Osaka, Japan).

2.3. Evaluation of the samples’ DOX loading and release

Two-milligram samples of STXL-10, STXL-20, ST50-10, and ST50-20 were soaked in centrifuge tubes containing 1.5 mL of 0.5 mg·mL\textsuperscript{-1} aqueous DOX solution. The microtubes were rotated at 3 rpm for 0.5 to 4 h at room temperature with a tube rotator (TR-350, AS ONE Corp.). After the desired period, the samples were removed from the DOX solution by centrifugation at 3000 rpm for 3 min (CN-1050, AS ONE Corp.). Then the supernatant was diluted ten-fold, and the DOX concentration in each diluted supernatant was measured using an ultraviolet–visible range spectrometer (V-730BIO, JASCO Corp.). The amount of DOX loaded into the samples was calculated from the DOX concentration measured in the supernatant.
The samples were washed with 1.5 mL of phosphate buffer solution (PBS) at the end of the 4 h experimental period, and the resulting DOX-loaded samples (prepared with STXL-10, STXL-20, ST50-10, and ST50-20) were soaked in 1.5 mL of PBS in microtubes that were then rotated at 36.5°C with a tube rotator at 3 rpm for 15 min to 120 h. After the desired period, each sample was extracted by centrifugation, and the amount of DOX in the supernatant was measured by spectrophotometry. These DOX-loading and -release evaluations were performed for three replicas per sample.

2.4. Evaluation of the in vitro biocompatibility of the samples

The in vitro biocompatibility of the samples was evaluated using Rat fibroblast Rat-1 cells in a procedure similar to that in our previous studies [14,15]. Rat-1 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Wako Pure Chemical Industries Ltd.) with 10% horse serum (Thermo Fisher Scientific, K.K., Tokyo, Japan), 100 units·mL⁻¹ penicillin (Meiji Seika Kaisha, Ltd.), and 100 µg·mL⁻¹ streptomycin (Meiji Seika Kaisha, Ltd.).

Cellular experiments were conducted for the following four groups: a control group (no administration), a pure TiO₂ microsphere group (np-Ti), and two porous TiO₂ microsphere groups (STXL-20 and ST50-20). A cell suspension consisting of 1 × 10⁵ Rat-1 fibroblasts was seeded in each well of a 24-well plate with 0.8 mL medium. Using 24-well cell culture inserts with porous membrane bottoms with 1.0-µm pores (Corning Inc.), test samples (np-Ti, STXL-20, and ST50-20) were added to the wells by deposition on the membrane of the inserts. The sample groups (np-Ti, STXL-20, and ST50-20) were tested at concentrations of 1, 10, and 100 mg/well. Cell proliferation was examined after 1, 3, and 7 days of culture. Total DNA from the cells was extracted using an AllPrep DNA/RNA/Protein Mini Kit (Qiagen GmbH, Hilden, Germany) in accordance with the manufacturer’s protocol [16]. Following extraction, the DNA concentrations were measured by absorbance at 260 nm using a spectrophotometer (e-spect, BM Equipment Co., Ltd., Tokyo, Japan). The values derived from five replicates per sample were presented as the mean DNA content. The results were expressed as the mean ± the standard deviation. Statistical analysis was performed using Tukey’s test, and differences with p-values < 0.05 were considered statistically significant.

2.5. Evaluation of the anticancer efficacy of samples with respect to HeLa cells

STXL-20 samples loaded with different amounts of DOX were evaluated for anticancer efficacy because STXL-20 showed the highest DOX-loading ability among the present samples. First, the samples were autoclaved at 121°C for 20 min. Then, 1.0 mg of each autoclaved sample was soaked in a microtube containing 0.8 mL of aqueous DOX solution at a concentration of 0, 0.01, 0.1, 10, or 100 µg·mL⁻¹. The microtubes were rotated at 3 rpm for 4 h at room temperature. The DOX-loaded samples were then collected from the DOX solutions by centrifugation at 10³ rpm for 1 min (CF15RXII, Hitachi Koki Co., Ltd., Tokyo, Japan). They were soaked in 1.0 mL of pure water in microtubes, gently washed by shaking the microtubes and then collected by centrifugation at 10³ rpm for 1 min.

HeLa cells were cultured in DMEM (Wako Pure Chemical Industries Ltd.) containing 10% fetal bovine serum (Thermo Fisher Scientific, KK), 100 units·mL⁻¹ penicillin (Meiji Seika Kaisha, Ltd.), and 100 µg·mL⁻¹ streptomycin (Meiji Seika Kaisha, Ltd.). The cells were routinely subcultured every third day in a 60.1 cm² culture dish at 37°C in humidified air with 5% CO₂.

A cell suspension consisting of 2 × 10⁴ HeLa cells was seeded in each well of a 24-well plate with 0.8 mL of medium. Into 24-well cell culture inserts with porous membrane bottoms with 1.0-µm pores (Corning Inc.), 1.0 mg of each DOX-loaded STXL-20 sample (prepared using DOX solutions with DOX concentrations of 0, 0.01, 0.1, 10, and 100 µg·mL⁻¹, as described above) was added separately by deposition on the membranes of the inserts (DOX-MS group). As a reference, DOX was added directly to the culture medium to reach DOX concentrations of 0, 0.01, 0.1, 10, and 100 µg·mL⁻¹ (DOX-SOL group). Cell proliferation was examined after 1, 2, and 3 days of culture. Subsequently, HeLa cell viabilities were evaluated using an MTT Cell Proliferation Assay Kit (Trevengen, Inc., Gaithersburg, MD, USA) in accordance with the manufacturer’s protocol. Following extraction, the absorbance at 560 nm was measured using a microplate reader (GloMax®-Multi Detection System, Promega, K.K., Tokyo, Japan). In this study, the absorbance obtained for the DOX-SOL group with a DOX concentration of 0 µg·mL⁻¹ (no administration) was used as a control (A₀°control), and the viability of the HeLa cells was calculated using the following equation, based on the absorbance obtained for each sample (A_sample):

\[
\text{HeLa cell viability} [%] = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

The values were derived from five replicates per sample and presented as the mean viabilities of HeLa cells. The results are expressed as the mean ± the standard deviation. Statistical analysis was performed using Tukey’s test, and differences with p-values < 0.05 were considered statistically significant.

3. Results

3.1. Structure and properties of samples

Figure 1 shows FE-SEM photographs of the samples. Irregularly shaped particles and particles with surface
cracks were partially formed, but microspheres measuring several tens of micrometers in diameter were obtained. Samples STXL-10 and STXL-20 had pores of ~20 nm, and samples ST50-10 and ST50-20 had pores of ~40 nm. The number of pores on the surface of the samples increased with increases in the concentration of SiNP in the starting solution.

Figure 2 shows the particle size distributions of the samples. The sizes of all the samples ranged roughly from 10 to 90 µm, and the mean diameters of np-Ti,

Figure 1. FE-SEM photographs of the samples.
STXL-10, STXL-20, ST50-10, and ST50-20 were 27.9, 33.3, 32.6, 25.1, 28.7 µm, respectively.

Table 2 shows the SSAs and total pore volumes of the samples. Sample np-Ti had a significantly lower SSA and total pore volume than the other samples, and samples STXL-20 and ST50-20 had higher SSAs and total pore volumes than samples STXL-10 and ST50-10, respectively. Samples STXL-10 and STXL-20 also showed higher SSAs than samples ST50-10 and ST50-20, respectively, but there was little difference between the total pore volumes of sample STXL-10 and sample ST50-10 or of sample STXL-20 and sample ST50-20.

3.2. DOX-adsorption capacity and DOX-release profiles of the samples

Figure 5 shows the DOX-adsorption capacity of the samples. Sample STXL-20 showed the highest DOX-adsorption capacity of 0.14 mg·mg⁻¹ after soaking in DOX solution for 4 h. Figure 6 shows the DOX-release profiles of the samples. The present samples released 2–8% of the adsorbed DOX within 5 days. Drug-delivery devices are mainly categorized as either reservoir-type drug-delivery devices or matrix-type drug-delivery devices [21]. The drug-release profile of matrix-type drug delivery devices is determined by the diffusion rate of retained drug molecules. It is known that the drug-release profile of matrix-type [drug-delivery devices follows the Higuchi release equation \( Q = H_k \times t^{1/2} \)], where the cumulative amount of released drugs \( Q \) is proportional to the square root of the soaking time \( t \) [22]. Here, \( H_k \) is the Higuchi constant. As shown in Figure 7, plotting the cumulative amount of DOX released as a function of the square root of the soaking time revealed
Table 2. SSA, total pore volume, and zeta potential for samples.

| Sample   | SSA [m²·g⁻¹] | Total pore volume [cc·g⁻¹] | Zeta potential [mV] |
|----------|--------------|----------------------------|---------------------|
| np-Ti    | 4.68         | 0.021                      | −35.8               |
| STXL-10  | 97.15        | 0.396                      | −58.7               |
| STXL-20  | 123.94       | 0.577                      | −53.3               |
| ST50-10  | 51.32        | 0.318                      | −56.5               |
| ST50-20  | 69.22        | 0.6                        | −51.9               |

Figure 4. Pore-size distribution of the samples.

Figure 5. Amount of DOX loaded into samples as a function of the soaking time (n = 3, mean ± SD).

Figure 6. DOX-release profiles of the samples (n = 3, mean ± SD).
a linear correlation with a high coefficient of determination (R²) between the cumulative amount of DOX released and the square root of the soaking times.

### 3.3. Cytotoxicity of samples with respect to Rat-1 cells

Figure 8 shows the DNA concentrations after extraction from Rat-1 cells cultured with 1, 10, or 100 mg of each sample. Samples np-Ti and STXL-20 showed no significant differences in DNA concentrations from those of the control group irrespective of the quantity. When 100 mg of sample ST50-20 was used, the DNA concentration after 7 days of culture decreased significantly compared to the control samples and samples with the same amount of np-Ti or STXL-20. The DNA concentration after 7 days of culture with ST50-20 (10 mg) also decreased significantly compared to the control samples and samples with the same quantity of np-Ti. The DNA concentration after 7 days of culture with 1 mg of ST50-20 was significantly lower, moreover, than that for the same amount of np-Ti.

### 3.4. Anticancer effects of samples with respect to HeLa cells

Figure 9 shows the viability of HeLa cells cultured for 1–3 days with DOX-loaded STXL-20 samples or DOX solution controls (DOX concentrations in both cases: 0–100 µg·mL⁻¹). After 3 days of culture, the DOX-SOL groups with DOX concentrations of 1, 10, or 100 µg·mL⁻¹ showed 70% inhibition of cell growth, whereas only the DOX-MS group with a DOX concentration of 100 µg·mL⁻¹ showed this level of cell growth inhibition. Cell viability after 1 day of culture was almost the same for the DOX-SOL group with a DOX concentration of 10 µg·mL⁻¹ as for the DOX-MS group with a DOX concentration of 100 µg·mL⁻¹.

### 4. Discussion

#### 4.1. Structure and properties of samples

Porous microspheres measuring several tens of micrometers in diameter were successfully obtained by the present method, and the number of pores on the surface of the samples could be controlled by changing the concentration of SiNP in the starting solution (Figure 1). The pores of these samples are believed to be formed by dissolution of the SiNP in NaOH solution [12]. Some samples have cracks on their surfaces. The mechanism of surface crack formation is not completely understood, but surface cracks might be formed to reduce the surface tension of the microspheres and retain their lowest energy [23]. Based on the particle size distributions of the samples (Figure 2), the sizes of all the samples ranged from roughly 10 to 90 µm, and the mean diameters of all the samples ranged from 27.9 and 33.3 µm, suggesting that the present samples have diameters applicable to TACE. All the samples were composed mainly of anatase, but sample np-Ti contained rutile in addition to anatase (Figure 3(a)). In samples STXL-10, STXL-20, ST50-10, and ST50-20, which were heated at 600°C in the presence of SiNPs, the SiNPs may have stabilized the TiO₂ particle surfaces to suppress the direct formation of rutile particles and/or may have prevented direct contact between anatase particles and inhibited the phase transformation of anatase to rutile [24,25]. Several IR absorption bands ascribed to Si–O–Si were observed in all the samples.
except sample np-Ti (Figure 3(b)), which suggests that a trace amount of SiNPs remains in these samples even after NaOH treatment. The IR absorption bands at around 1100 cm$^{-1}$ in samples ST50-10 and ST50-20 are more obvious than in samples STXL-10 and STXL-20, indicating that larger amounts of SiNPs remained in samples ST50-10 and ST50-20 than in samples STXL-10 and STXL-20. The broad IR absorption band ascribed to Ti–O and the small IR absorption band ascribed to Si–O–Ti in samples ST50-10 and ST50-20 suggest that partial chemical bonding took place between TiO$_2$ and SiNPs.

The significantly lower SSA and total pore volume of sample np-Ti as compared to those of the other samples (Table 2) can be attributed to the fact that sample np-Ti lacked pores on its surface, as shown in Figure 1. It is understood that using higher SiNP concentrations to prepare the samples resulted in higher SSAs and total pore volumes (STXL-20 > STXL-10, and ST50-20 > ST50-10). Smaller SiNPs also showed higher SSAs (STXL-10 > ST50-10, and STXL-20 > ST50-20), but the total pore volume did not increase remarkably with decreases in the SiNP size. We should be careful when comparing STXL-10 with ST50-10 or STXL-20 with ST50-20, because larger numbers of SiNPs remained in samples ST50-10 and ST50-20 than in samples STXL-10 and STXL-20, as shown in Figure 2(b), but this result is also understandable if we assume that the total additive weights (i.e. total additive volumes) of SiNPs were almost the same for sample STXL-10 and sample ST50-10 and for sample STXL-20 and sample ST50-20, and that the SiNPs were uniformly dissolved by NaOH treatment to form pores. The almost total absence of pore size distribution in sample np-Ti (Figure 4) is consistent with the FE-SEM image showing a dense, smooth surface (Figure 1), but the pore sizes in samples STXL-10 and STXL-20 ($\approx$5 nm) and in samples ST50-10 and ST50-20 ($\approx$10 nm) were apparently smaller than those observed by FE-SEM, although the reason is unknown.

All the samples showed negative zeta potentials, but samples STXL-10, STXL-20, ST50-10, and ST50-20 had relatively lower zeta potentials than sample np-Ti (Table 2). Residual SiNPs in these samples might have been responsible for the lower zeta potentials. It was reported that a TiO$_2$/SiO$_2$ composite showed a lower zeta potential in neutral solution than TiO$_2$, because the isoelectric point of SiO$_2$ was lower than that of TiO$_2$ [26]. A similar interpretation can be applied to the present samples.

### 4.2. DOX-adsorption capacity and DOX-release profiles of the samples

Sample STXL-20 showed a DOX-adsorption capacity of 0.14 mg·mg$^{-1}$ (Figure 5). This capacity can be converted to 150 mg·mL$^{-1}$ when we assume that the real density of sample STXL-20 was the same as that of anatase-type TiO$_2$ (3.90 g·cm$^{-3}$), which was higher than the DOX-adsorption capacity of DC Bead® (40 – 80 mg·mL$^{-1}$) [6,27]. The mechanism of DOX adsorption on porous TiO$_2$ microspheres can be interpreted as follows. The present samples showed negative zeta potentials (Table 2), whereas the DOX molecule was positively charged in aqueous solution by protonation of its amine group [6]. DOX molecules may therefore be adsorbed on sample surfaces due to electrostatic interactions. However, DOX has three potential metal-binding sites [28,29]. One of these sites is the nitrogen atom in the sugar moiety, and the other two are the chelating sites of the quinone and phenolic oxygens on either side of the anthracycline aromatic moiety. DOX molecules might also be chemically adsorbed on TiO$_2$ microspheres, therefore, through their potential metal-binding sites. During chemical
adsorption, samples with a higher SSA might adsorb more DOX because only monolayer adsorption is possible. Sample STXL-20 (with a higher SSA) showed higher DOX adsorption than ST50-20, however, although the total pore volume of sample STXL-20 was comparable to that of ST50-20 (Table 2). It should be noted here that sample ST50-20 had a higher DOX-adsorption capacity than sample STXL-10, although sample STXL-10 had a higher SSA than sample ST50-20. This result suggests that the DOX-adsorption capacity was affected not only by the SSA, but also by the total pore volume. As discussed above, DOX molecules might be adsorbed on TiO$_2$ by electrostatic interaction as well as by chemical adsorption; hence, positively charged DOX molecules

**Figure 9.** Viability of HeLa cells cultured for 1–3 days in DOX solution (DOX-SOL) and DOX-loaded STXL-20 samples (DOX-MS) ($n = 5$, mean ± SD, Tukey’s test).
may also be trapped in the surface pores of negatively charged porous TiO₂ microspheres by electrostatic attractions.

The present samples released 81.2% of the adsorbed DOX within 5 days (Figure 6), and their release profile was similar to that of DC beads (≥ 10%) [30]. The linear correlation between the cumulative amount of DOX released and the square root of the soaking times (Figure 7) indicates that the release of DOX was diffusion-controlled by the soaking time and that the present porous TiO₂ microspheres can release DOX and act as matrix-type drug-delivery devices.

4.3. Cytotoxicity of samples for Rat-1 cells

Samples np-Ti and STXL-20 were non-cytotoxic because they showed no significant difference in DNA concentration from the control group, irrespective of quantity (Figure 8). Large amounts (≥10 mg) of sample ST50-20 were also cytotoxic, and the cell compatibility of sample ST50-20 was lower than that of np-Ti. The mechanism causing the low cell compatibility of sample ST50-20 is still unknown, but the residual SiNPs in sample ST50-20, based on the FT-IR spectrum (Figure 3), might reduce the cell compatibility. It is interesting to note here that the DNA concentration after 3 days of culture with 100 mg of sample ST50-20 was significantly higher than that in the control group. It was reported that an appropriate amount of silicon (Si) released from akermanite ceramics stimulates L929 fibroblast cell proliferation [31]. We speculate that Si released from sample ST50-20 after 3 days of culture promoted Rat-1 fibroblast cell proliferation, but that the excessive amount of Si released from samples after 7 days of culture inhibited cell proliferation.

4.4. Anticancer effects of samples on HeLa cells

The DOX-SOL groups with DOX concentrations of 1, 10, or 100 µg·mL⁻¹ showed 70% inhibition of HeLa cell growth after 3 days in culture, whereas only the DOX-MS group with a DOX concentration of 100 µg·mL⁻¹ showed the same level of cell growth inhibition (Figure 9). The HeLa cell viability after 1 day in culture for the DOX-SOL group with a DOX concentration of 10 µg·mL⁻¹ was almost the same as that for the DOX-MS group with a DOX concentration of 100 µg·mL⁻¹. As seen in the release profiles of samples shown in Figure 6, sample STXL-20 released approximately 7% of the adsorbed DOX into PBS within 1 day. We speculate that using sample STXL-20 with a nominal DOX concentration of 100 µg·mL⁻¹ released a similar fraction of the adsorbed DOX in the culture medium and, hence, showed cell viability similar to that of the DOX-SOL group with a DOX concentration of 10 µg·mL⁻¹. In contrast, the cell viability of the DOX-MS group with a nominal DOX concentration of 10 µg·mL⁻¹ was higher than that of the DOX-SOL group with a DOX concentration of 1 µg·mL⁻¹. This might be because the fraction of DOX released in the DOX-MS group decreased due to the extremely small amount (0.008 mg) of DOX adsorbed onto the sample.

5. Conclusions

Porous TiO₂ microspheres with an average diameter of approximately 25 – 30 µm were successfully obtained by the present method. The microspheres adsorbed DOX and released DOX gradually into saline. Our results also showed Rat-1 cell compatibility under controlled administration and antitumour activity with respect to HeLa cells. Thus, the present porous TiO₂ microspheres are potential candidate DEBs for TACE.

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Disclosure statement

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