Transferrin targeted core-shell nanomedicine for combinatorial delivery of doxorubicin and sorafenib against hepatocellular carcinoma

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Abstract

Combinatorial drug delivery is an attractive, but challenging requirement of next generation cancer nanomedicines. Here, we report a transferrin-targeted core-shell nanomedicine formed by encapsulating two clinically used single-agent drugs, doxorubicin and sorafenib against liver cancer. Doxorubicin was loaded in poly(vinyl alcohol) nano-core and sorafenib in albumin nano-shell, both formed by a sequential freeze-thaw/coacervation method. While sorafenib from the nano-shell inhibited aberrant oncogenic signaling involved in cell proliferation, doxorubicin from the nano-core evoked DNA intercalation thereby killing >75% of cancer cells. Upon targeting using transferrin ligands, the nanoparticles showed enhanced cellular uptake and synergistic cytotoxicity in ~92% of cells, particularly in iron-deficient microenvironment. Studies using 3D spheroids of liver tumor indicated efficient penetration of targeted core-shell nanoparticles throughout the tissue causing uniform cell killing. Thus, we show that rationally designed core-shell nanoparticles can effectively combine clinically relevant single-agent drugs for exerting synergistic activity against liver cancer.

From the Clinical Editor: Transferrin-targeted core-shell nanomedicine encapsulating doxorubicin and sorafenib was studied as a drug delivery system against hepatocellular carcinoma, resulting in enhanced and synergistic therapeutic effects, paving the way towards potential future clinical applications of similar techniques.

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Key words: Doxorubicin; Sorafenib; Core-shell nanomedicine; Hepatocellular carcinoma; Transferrin; Poly(vinyl alcohol)

Background

Hepatocellular carcinoma (HCC) is the primary neoplasm of liver with worldwide incidence of ~1 million cases per annum and almost equal death rate. Globally ~170 million people with chronic hepatitis B/C virus (HBV/HCV) infections, are at the risk of developing HCC.1 Sorafenib, a multi-kinase inhibitor with effective antiproliferation potential, is one of the clinically administered monotherapeutic drugs against HCC. It blocks RAF/MEK/ERK, VEGFR and PDGFR signaling pathways together and exerts cytostatic effects.2,3 Another clinically relevant drug administered in the form of chemoembolizing microparticles (DC beads®) is doxorubicin (Dox).4 In clinics, both these drugs, as single agents, have limited therapeutic success. Sorafenib reduces cell proliferation mainly by blocking C-RAF and B-RAF through the inhibition of RAF/MEK/ERK signaling.5 But, most often HCC develops extensive signaling crosstalk and activation of several other compensatory oncogenic pathways, aiding the cells to easily escape from the drug mediated antiproliferative stress.6,7 Moreover, binding of EGFR ligand, amphiregulin is induced after sorafenib therapy, which triggers the activation of ERK and AKT downstream signaling, favoring the proliferation of cells.8,9 In essence, sorafenib provides only cytostatic effect rather than cytotoxicity, and therefore renders only moderate (~6 months) survival advantage for HCC patients.10-11 On the other hand, although Dox is a potent DNA intercalator and topoisomerase inhibitor with effective reactive oxygen species (ROS) releasing capability,
this cytotoxic drug renders limited antitumor activity when administered as single-agent, and therefore being employed mostly in a palliative setting.\textsuperscript{12-14} One of the promising alternative options is to combine the potential cytotoxic activity by Dox with the kinase inhibition by sorafenib such that synergistic antitumor effect may be obtained. This idea recently attracted great attention and in a phase-II clinical trial conducted using sorafenib in combination with Dox, compared to doxorubicin alone, the combination has shown significant improvement in the overall survival of HCC patients (~13.7 months as against 6 months by single agent).\textsuperscript{15} However, a major challenge that remains unresolved is regarding the delivery of both the drugs together towards cancer cells. Free drugs exhibit completely different pharmacokinetic profile and delivering the desired concentration of both the drugs at intracellular region is a challenge. We believe that a nanoformulation that can encapsulate both these drugs within the same carrier may help to solve this problem.

Accordingly, in this work, we have developed a core-shell nanosystem for the targeted delivery of both Dox and sorafenib to effectively impart cytotoxic stress together with kinase inhibition in HCC cells. First, we have \textit{in silico} designed the nanoconstruct and prepared the same by optimizing a wet-chemical method. Poly(vinyl alcohol) (PVA) was used for preparing nano-core and nano-shell was formed by human serum albumin. Both these materials are FDA approved for cancer drug delivery applications.\textsuperscript{16,17} By employing a novel sequential freeze-thaw method followed by ethanol coacervation, PVA–Dox nano-core was prepared initially, and a thin shell of albumin–sorafenib was formed over the nano-core, leading to a PVA–Dox/Alb–sorafenib core-shell nanomedicine. For targeting HCC cells, human serum transferrin was conjugated to the surface of the core-shell nanoconstruct. This strategy resulted in improved nanoparticle uptake and synergistic cytotoxicity in ~92% of HCC cells, tested in both 2D and 3D cultures.

\textbf{Methods}

The detailed methodology of the experiments is provided in supplementary information.
Results

In silico molecular docking

Our earlier study involving in silico investigation of loading sorafenib in albumin indicated that at least 6 molecules of sorafenib can be loaded into single molecule of albumin without significant cross-talk (Figure 1, A). In this study, prior to nanoparticle preparation, we have investigated whether TfR-recognizing amino acid residues of transferrin ligand are affected during its interaction with sorafenib-loaded albumin nano-shell. Docking results indicated that carboxylic (shown as red helix in Figure 1, B) as well as amino terminal lobe of transferrin which are typically recognized by TfR, remains intact when the targeting ligand interacts with albumin-sorafenib nano-shell. Furthermore, transferrin was found to interact with certain specific amino acid residues of albumin that are different from sorafenib binding sites (Figure 1, B), indicating that the interaction of transferrin with the nano-shell does not interfere with the already existing hydrophobic interactions between albumin and sorafenib. The complete list of interacting amino acids and the nature of chemical interactions between sorafenib and albumin shell, and TfR recognizing intact carboxylic and amino lobes of transferrin (C-lobe and N-lobe) are given in the supplementary information (tables S1-S6, and Figure S1, respectively). Figure 1, C shows the in silico model of transferrin-docked albumin–sorafenib nano-shell being recognized by TfR, and Figure 1, D shows simulated model of TfR-targeted albumin–sorafenib nano-shell formed over PVA–Dox nano-core. Thus, the in silico approaches have provided certain indicative information about how a drug/targeting ligand might interact with a nanocarrier/receptor.

Preparation and physicochemical characterization

Following the in silico design, the core-shell nanoparticles were prepared by a two step wet chemical process. Schematic representation of the synthesis is shown in Figure 2, A. At first, Dox loaded polymer nano-core was prepared using freeze-thaw process and a nano-shell of albumin-sorafenib was formed over the nano-core by employing ethanol coacervation. Figure 2, B shows atomic force microscopy (AFM) image of PVA–Dox nano-core indicating size ~90 nm. Figure 2, B inset presents hydrodynamic diameter of the core as determined by dynamic light scattering (DLS), which is in consistence with the AFM measurement. Figure 2, C displays scanning electron microscopy (SEM) image of core-shell particles showing an overall size ~110 nm, and the corresponding DLS graph also confirmed the same. TEM image (Figure 2, C inset) clearly indicated the formation of a unique shell over the polymer nano-core, suggesting the thickness of the shell is ~20 nm. Drug loading and encapsulation efficiency of Dox in PVA nano-core were 3%
and 82%, respectively, and that of sorafenib in albumin nano-shell were 2.4% and 91%, respectively.

FT-IR studies

After characterizing the morphology, Fourier transform infrared spectra (FT-IR) of PVA–Dox nano-core (PD), TfR-targeted albumin–sorafenib nano-shell (AST), and core-shell nanoparticles were individually studied (Figure 2, D). Presence of infrared peaks at 3531 cm\(^{-1}\) and 3328 cm\(^{-1}\) indicated O–H stretching vibration in PVA and Dox.\(^{19}\) The same peaks also overlapped with stretching vibrations in N–H bonds in primary amine groups of Dox.\(^{19}\) Stretching vibration in C = O groups, bending vibration in N–H bonds, and C–C stretching vibration arising from aromatic rings of Dox were indicated by IR peaks at 1732 cm\(^{-1}\), 1583 cm\(^{-1}\), and 1415 cm\(^{-1}\) respectively.\(^{20-22}\) The presence of C = O stretching vibration resulting from secondary alcohol groups of PVA was indicated by peak at 1235 cm\(^{-1}\). In the nano-shell, possibility of forming hydrogen bonds between nitrogen atoms of sorafenib and amino acid residues in albumin was indicated by N–H stretching vibration peak at 3327 cm\(^{-1}\). FT-IR peaks at 1655 cm\(^{-1}\), 1537 cm\(^{-1}\), 1396 cm\(^{-1}\) indicated amide-I, II, and III groups respectively in albumin and transferrin.\(^{23,24}\) The core-shell nanoparticles displayed additive IR peaks of nano-core and nano-shell.

Drug release studies

Following FT-IR studies, drug release profile from the core-shell particles was studied using a spectrophotometer. Figure 2, E indicates that both sorafenib and Dox are released in a sustained manner in cell-free aqueous medium (PBS) with ~10% release in the first 24 h. Thereafter, ~50% release was observed for sorafenib in the aqueous medium for 3 weeks. In contrast, although Dox hydrochloride is relatively hydrophilic, controlled release was observed from the PVA core, with ~30% of Dox getting released for 21 days.

Transferrin receptor expression and cellular uptake

In the next step, the expression of TfR in HepG2 cells was studied using flow cytometry. Figure 3, A1 shows unstained cells (control). ~76% of the malignant cells were found to express TfR before iron chelation as noticed from Figure 3, A2. After creating an iron deficient microenvironment by treating the cells with 100 μM DFO, the number of the TfR-expressing cells increased to ~89% (Figure 3, A3). Figure 3, A4 shows that upon addition of iron-loaded transferrin (holotransferrin), the number was reduced to ~42%. Following that, we have studied intracellular uptake of the core-shell nanoparticles in cancer cells. Figure 3, B1 shows that untargeted particles are taken-up by only ~70% of HepG2 cells. Upon conjugating the nanoparticles with transferrin, the uptake was effectively increased to ~83% as shown in Figure 3, B2. After creating iron-deficient microenvironment, the uptake was further increased to ~94% (Figure 3, B3) due to the increase in the number of TfR-expressing cells. Upon addition of iron-loaded transferrin, the uptake was dropped to ~69% due to the saturation of TfR expression in these cells (Figure 3, B4).

Figure 3. (A1–A4) Transferrin receptor (TfR) expression studies in HepG2 cells. (A1) unstained cells (control). The studies were performed before and after enhancing the receptor expression: (A2) before, (A3) after inducing TfR overexpression, and (A4) after blocking the receptor. (B1–B4) Intracellular uptake studies of core-shell nanomedicine in HepG2 cells: (B1) Uptake of untargeted nanomedicine. (B2) Uptake of the targeted nanomedicine before TfR induction, (B3) after inducing TfR expression, and (B4) after blocking the receptor. (For both these studies, TfR expression was induced by pretreating the cells using desferrioxamine, and the receptor blocking was effected by pretreating with iron-loaded transferrin.)
Cytotoxicity studies

After confirming the uptake, cytotoxicity of the core-shell nanoparticles was studied in HepG2 and PLC/PRF/5 cells using alamar blue assay. The study was performed before and after enhancing TfR expression in the cells. As noticed from Figure 4, A, the free drug combination (5 μM sorafenib (S) and 0.5 μM Dox (0.5D)) could produce only ~50% cytotoxicity. Even after creating iron depleted microenvironment by pretreating HepG2 with 100 μM DFO, the cell death imparted by free drug combination did not improve further. Compared to this, untargeted nanoparticles (containing 0.5 μM Dox in nano-core and 5 μM sorafenib in nano-shell) showed improved toxicity, however, only up to ~63%.

In contrast, TfR-targeted core-shell nanoparticles registered ~92% synergistic cell death in the TfR overexpressing HCC cells. Similar trend in cytotoxicity was also observed in mutant p-53 HCC cell, PLC/PRF/5 (supplementary information, Figure S2). Further, the drug-free core-shell nanoparticles did not exert any toxicity towards the cancer cells (supplementary information, Figure S3), suggesting that the observed cytotoxicity is exclusively due to the combined effect of chemodrugs in the nanoparticles.

Apoptosis studies

In the next step, we have investigated the mode of cell death using Annexin V/PI apoptosis assay. Figure 4, B shows core-
shell nanoparticles (containing 5 μM sorafenib in albumin shell and 0.5 μM Dox in PVA core) treated cells displaying classical features of apoptosis such as extensive plasma membrane blebbing and separation of cell fragments into green fluorescent apoptotic bodies during the budding process. Red staining in the nucleus indicates that the DNA is also damaged during apoptosis. We have also quantified the apoptotic cells using flow cytometry. Figure 4, C1 shows untreated cells (negative control, NC). Figure 4, C2 and C3 shows that both sorafenib and Dox as individual free drugs could exert only ~38% and ~53% apoptosis, respectively. Even after combining both the drugs (5 μM sorafenib (5S) with 0.5 μM Dox (0.5D)), a maximum of only ~60% apoptosis was registered (Figure 4, C4). In contrast, as separate nanoformulations, the nano-shell (containing 5 μM sorafenib, (5AST)) (Figure 4, D2) and the nano-core (containing 0.5 μM Dox, (0.5PD)) (Figure 4, D3) have shown slight improvement in apoptosis (~39% by shell, and ~61% by core). After combining the two in a core-shell targeted fashion, excellent apoptosis was registered in ~91% cells (Figure 4, D4). Interestingly, pretreating the cells using 100 μM DFO (100DFO) did not affect the viability of TfR overexpressing cells (Figure 4, D1).

Development and characterization of 3D HCC spheroids

After studying the cytotoxicity of the nanoparticles in 2D culture, we have extended the studies to 3D microenvironment by developing HCC spheroids. ~3.75 × 10⁴ cells dispersed in 5 μL volume were used to prepare each individual spheroid.

Figure 5, A1 displays the morphology of alginate-collagen based cell-free spheroids, and Figure 5, A2 shows 3D spheroids embedded with HCC cells after 5 days of culture, where the cancer cells were found to attach and effectively proliferate in the 3D microenvironment. Figure 5, A3 presents the magnified image depicting uniform spreading of liver cancer cells throughout the spheroid (size ~200 μM). After 15 days of culture, enhanced proliferation of cells leading to HCC tissue-like architecture was clearly evident in the 3D system (Figure 5, B1). At this stage, each spheroid contained ~1.8 × 10⁵ cells, as indicated by the metabolic activity measurement using alamar blue assay. Figure 5, B2 shows the consistent increase in resorufin absorbance from the proliferating cells in the 3D environment in a time-dependent manner. Figure 5, C1-C5 shows depth-by-depth confocal imaging of a representative liver tumor spheroid.

Nanoparticle uptake in 3D spheroids

After the successful preparation, we have studied the uptake of core-shell nanoparticles by cells in the 3D spheroid using stereo microscopy. Figure 6, A1-A3 shows the uptake after 24 h, 48 h, and 96 h of incubation, respectively. Clearly, with increase in the incubation period, the color of the spheroid turned from dull to deep orange (color emerged from Dox in the nano-core), indicating that the cancer cells in the 3D culture effectively uptake the core-shell particles in a time-dependent manner.
It may be noticed that the size of the spheroids also gradually increased from ~70 μM to ~200 μM upon prolonged incubation. This is due to the swelling property attributed to the hydrophilic nature of the alginate based 3D culture system. Depth-by-depth imaging of the spheroid by confocal microscopy further confirmed the uptake (96 h) in the 3D environment (Figure 6, B1-B3). Figure 6, B1 displays bright field image of nanoparticles being taken-up by cancer cells. The uptake was confirmed by the fluorescence emission of Dox from nanoparticle internalized cells in the spheroid (Figure 6, B2). Figure 6, B3 presents the merged view of bright field and fluorescent images from anterior (top) to posterior (bottom) side of the spheroid. This confirms the effective uptake of the core-shell particles by almost all the cells in the 3D culture environment.

Cytotoxicity studies in 3D spheroids

After confirming the cellular uptake, cytotoxicity of the core-shell nanoparticles in the spheroids was studied using alamar blue assay. Interestingly, the cytotoxicity in the 3D environment varied considerably compared to that of the 2D culture (Figure 6, C). Targeted core-shell particles (containing 5 μM sorafenib in albumin shell (5AST) and 0.5 μM Dox in PVA core (0.5PD)), which has imparted ~92% cell death in the 2D culture, showed only ~10% cytotoxicity in the 3D spheroid. Even after modulating Dox concentration, the free drug combination (5 μM sorafenib (SS) and 2 μM Dox, (2D)), and equimolar concentration of untargeted nanoparticles (5 μM sorafenib in albumin nano-shell (5AS) and 2 μM Dox in PVA nano-core (2PD)) has registered only ~70% cytotoxicity in the 3D cell culture system. In contrast, HCC spheroids treated with...
**Discussion**

Hepatocellular carcinoma is an aggressive disease that is typically diagnosed late and has median survival of ~6 months for majority of patients and ~20 months for <5% of patients having localized lesions. Although surgical resection is the standard of care, systemic chemotherapy is the mainstay therapy, most of the patients are not eligible for surgery because of the extent of tumor spread and underlying liver dysfunction. In these patients, systemic chemotheraphy is the standard of care. A multikinase inhibitor, sorafenib, and doxorubicin (Dox) in the form of implantable microbeads (DC beads®), are the two FDA-approved formulations used in the clinics. However, as single agents, these drugs give only limited therapeutic outcome. Recent studies have indicated that multifunctional nanoparticles can play an integrated role for effectively combating various cancers. In this work, we have hypothesized that inhibiting the critical kinase signaling by sorafenib together with DNA intercalation by Dox may yield synergistic antitumor effects in liver cancer. Accordingly, we have developed a HCC-targeted core-shell nanoparticle that is capable of delivering both sorafenib and doxorubicin together. In order to optimally design the nanoconstruct, prior to wet chemical realization, in silico docking simulations were employed. The simulation results indicated strong hydrophobic interactions between sorafenib and albumin which was substantiated by the excellent drug encapsulation efficiency up to 91%. Probably, these interactions lead to the effective binding affinity of sorafenib (~99.5%) with albumin that had been observed in vivo.

Drug content analysis indicated that ~9 molecules of sorafenib was formed by alcohol coacervation. Thereafter, transferrin ligand was conjugated to the albumin shell for providing HCC-specific targeting capability to the core-shell system. The prepared nanoparticles displayed excellent aqueous dispersibility and showed no aggregation when stored at room temperature for 15 days. FT-IR results indicated intact functional groups of Dox and sorafenib after the formation of core-shell structure. Thus, we have successfully achieved the loading of a relatively hydrophilic (Dox) and hydrophobic (sorafenib) drug together within a unified core-shell construct of total size ~110 nm without direct drug–drug interactions.

Drug release studies indicated sustained release of sorafenib and Dox in the aqueous medium for 3 weeks. In case of sorafenib, the controlled release might be attributed to the strong hydrophobic interactions with albumin, as predicted earlier. However, compared to Dox, a relatively enhanced release was observed for sorafenib. Probably, the protein nano-shell (~20 nm) degrades at a faster rate and the release of sorafenib is also governed by diffusion coupled with physical degradation. In contrast, PVA was physically cross-linked during the preparation of the nano-core. Further, the existence of hydrogen bonds between PVA and Dox, and the presence of albumin shell over the nano-core might have contributed to the controlled release of hydrophilic Dox.

In cell studies, ~89% of the malignant liver cells showed overexpression of TIR after creating iron-depleted microenvironment. This indicates that HCC cells utilize iron for the cell metabolism and its enhanced uptake is facilitated by the overexpressed TIR, and reasoned the potential of using iron loaded transferrin for targeted delivery of the nanoparticles towards liver cancer cells.

Flow cytometry data on nanoparticle uptake substantiated this observation, where the targeted nanoparticles were internalized by 94% of TIR overexpressing cells. This confirmed that sorafenib binding with albumin did not affect the TIR-mediated endocytosis of the targeted nanoparticle, as predicted in silico. Transferrin-mediated endocytosis was further confirmed by the TIR blocking experiment where the nanoparticle uptake was reduced to ~69% upon supplementing iron-loaded transferrin to the malignant cells. Since the uptake was quantified by detecting...
the fluorescence of Dox in the nanocore, as a control, we have also studied the uptake of free Dox uptake using flow cytometry (supplementary information, Figure S5). In that, we could observe that forward scattering (FSC) alone was increased, while side scattering (SSC) remains unaffected. However, in nanoparticle treated cells, SSC was also significantly increased, which showed that cellular internal granularity is modulated, indicating nanoparticle uptake. Some observed FSC in the nanoparticle treated cells may be due to the released Dox from the core-shell particles after the cellular uptake.

After studying the uptake, optimum concentrations of sorafenib and doxorubicin for exerting synergistic cytotoxicity have been chosen based on the results from dose-response cytotoxicity experiments (supplementary information, Figure S6-S7). At first, we have screened different concentrations of free sorafenib and free Dox individually, and the IC50 for each of these drugs was noted. Following that, the cytotoxicity of free drug combination (sorafenib + Dox), nano-core, nano-shell, and core-shell nanoparticles were individually studied. It may be noted that even after creating iron depleted microenvironment where TIR expression was enhanced, the cytotoxicity imparted by the free sorafenib (5 μM) and Dox (0.5 μM) combination did not improve further (~53%) (Figure 4, A). In contrast, core-shell nanoparticles containing both these drugs exerted ~92% synergistic toxicity in both HepG2 and PLC/PRF/5 cells upon targeting using transferrin ligand. This clearly indicated the advantage of delivering both the drugs together to intracellular regions in a cell-targeted fashion. Further, even though the drug release studies in cell-free condition indicated only limited release of Dox and sorafenib (~20% and ~30%, respectively) for 96 h, a significant cell death was observed for the same time by the targeted nanoparticles. We believe that, after the cellular uptake, higher concentration of drugs might have been released intracellularly due to enzymatic degradation of albumin shell. Annexin-PI assay performed in the nanoparticle treated cells using flow cytometry indicated that the cell death was mediated by apoptosis. The cells in distinct populations appear like a gradation because the cells experienced similar pattern of cell death, mostly by late-stage apoptosis, as confirmed by confocal microscopy. Therefore, both annexin and propidium iodide (PI) were bound to most of these late-apoptotic cells, except that there was a gradation in the binding pattern due to the variation in the number of exposed phosphatidylserine molecules and penetration of PI in the damaged nucleus between the different cell populations.

After studying the cytotoxicity in 2D culture system, we have extended the studies to a 3D microenvironment by developing HCC spheroids where cell–cell and cell–matrix interactions are considerably higher and the functions of individual cells closely mimic tissue-like characteristics, thereby bridging the gap between 2D culture and in vivo systems.41

The active proliferation of HCC cells in the spheroids was clearly indicated by the consistent increase in resorufin absorption in a time-dependent manner. Our optimized alginate-collagen 3D spheroids displayed a total size of ~200 μm wherein the malignant cells in similar 3D culture system have been shown to retain tissue-level physiological properties such as albumin synthesis, elsewhere.42

Uptake studies performed in the 3D culture system have displayed excellent penetration and internalization of targeted nanoparticles all throughout the HCC spheroids, as evident from the change of body color of spheroids from dull to bright orange upon increase in incubation time. The uptake was further confirmed by optical section imaging using confocal microscopy, where bright red fluorescence emitted from the Dox in nano-core was clearly observed from every section of the 3D spheroid. This is an important data suggesting that the targeted nanoparticles are able to penetrate through the 3D tissue-like architecture and is endocytosed by each individual cell in the spheroid.

Interestingly, the cytotoxicity assay performed in the 3D spheroids displayed a relatively limited cell death compared to that of the 2D culture. This was expected, because, the cell–cell and cell–matrix interactions are considerably enhanced in the 3D environment, and the individual cancer cells mimic tissue-like physiological properties, thereby limiting the sensitivity of chemodrugs.43 We have overcome this challenge by modulating the concentration of Dox in PVA nano-core to 2 μM, resulting in ~93% synergistic cytotoxicity in the 3D culture.

The cytotoxicity results were further validated by live–dead staining under confocal microscopy. Before treatment, we could observe mere bright green fluorescence emitted from the metabolically-active cancer cells in the spheroids due to the selective internalization of calcine, without any necrotic cell core. Maybe spheroids of size ~200 μM permit the penetration of oxygen and nutrients, and therefore do not necessarily possess a visible necrotic core, as reported elsewhere.44 Upon treatment with the targeted core-shell nanoparticles, dose dependant toxicity was observed within the spheroid. Under optimized dose of 5 μM sorafenib in albumin shell and 2 μM Dox in PVA core, almost complete cell death was indicated by the bright red fluorescence due to the selective uptake of ethidium homodimer by the dead cells in the spheroid.

In conclusion, we have shown that by simultaneously inhibiting aberrantly active oncogenic kinase and imparting cytotoxic stress using a TIR-targeted nanomedicine containing two FDA approved drugs, doxorubicin and sorafenib, promising synergistic toxicity can be achieved against hepatocellular carcinoma cells.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nano.2014.05.003.

References


17. 8-dimethoxy-1,2,3,4-tetrahydrophthalene, the key intermediate in the synthesis of anthracycline antibiotics. Tetrahedron-Asymmetry 2001;12:3155-61.

