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Preparation of biocompatible nanobubbles carrying quercetin and their inhibitory effects on non-small-cell lung carcinoma

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Abstract: Non-small-cell lung carcinoma (NSCLC), as major lung cancer is currently considered as one of the leading causes of mortality and has become a progressively serious global public health burden. However, the conventional drug delivery approaches were unable to efficiently inhibit the proliferation and metastasis of the lung cancer cells. Exploiting Nano bubbles (NBs) as a novel drug delivery system have recently a research hotspot mainly due to their outstanding characteristics such as small size, biosafety and competent drug-transporting ability. Present study aimed to establish a novel biocompatible approach for Nano bubble constructions by the water-in-oil method and loading quercetin inside the obtained micelles. Dextran coating was used for more stability of NBs and the effectiveness of drug delivery to A549 NSCLC and HeLa cells was evaluated. Ultrasound waves were used to stimulate the Nano bubble to release the drug. The approximate size of NBs was 0.149 nm that reach by dynamic light scattering dominantly with spherical shape visualized by TEM. Excellent drug-loading capacity and ultrasound-mediated release of quercetin were confirmed by UV spectrophotometer with the absorption about 1.6. NBs efficiently inhibited the proliferation of NSCLC cells in a concentration-dependent manner as well as the capability to achieve ultrasound enhancement. This experiment showed obtained NBs effectively delivered quercetin into lung cancer cells promoted by ultrasound irradiation. In conclusion, proposed biocompatible quercetin loaded NBs are suitable for ultrasound-targeted drug delivery and are thus a promising strategy for their noninvasive clinical application.

Keywords: Nano-bubble, Quercetin, Non-small cell lung cancer, Drug delivery, Ultrasound

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1 Introduction

Targeted drug delivery systems based on nanotechnology have been extensively developed and studied in the field of Nano-medicine because they are able to maximize the therapeutic effect and minimize adverse side effects. In order to reduce the frequency of doses and reduce the severity of adverse effects due to fluctuations in drug concentrations, more targeted drug delivery is desirable for drugs with a short half-life (≤ 4 hours). Surgery, chemotherapy, radiation therapy and hormone therapy the main common treatment approaches are anti-tumor. However, nonspecific targeting of cancer cells has made these approaches ineffective in a significant number of patients. Nonspecific targeting of malignant cells also necessitates the use of higher doses of the drug to reach the tumor site. Therefore, there are two main barriers to reaching the tumor area with maximum effect. The first is to prevent drug delivery to healthy non-cancerous cells, and the second is to deliver drugs directly to the tumor site. Experimental data has shown that (Yu et al., 2020) G250-TNB combination therapy and targeted ultrasound Nano-bubble degradation can deliver antitumor drugs to local renal cell carcinoma (RCC) areas, increase the concentration of effective local drugs, and increase antitumor effect, so a new method Offers the potential for targeted RCC therapy. Data has shown that (Peng et al., 2019) PTX-AMD070 Nano-bubbles enhance ultrasound imaging in CXCR4 + xenograft tumors and facilitate targeted therapy with targeted ultrasound targeted Nano-bubble degradation. Therefore, this study presents an effective method for integrating molecular ultrasound imaging and targeted treatment of malignant tumors. Studies shown (Khan et al., 2019) Synthesized doxorubicin-containing oxygen nano-bubbles and evaluated the effectiveness of drug delivery to breast cancer MDA-MB-231 and HeLa cells. Increased oxygen levels and ROS production are effective in tumor-derived cell lines. Quercetin has been extensively studied as a chemical prophylactic agent in several cancer models due to its antioxidant, anti-tumor, and anti-inflammatory activity. Data has shown that (Jeong et al., 2009) low doses of quercetin inhibited the proliferation of cancer cells, and this inhibition was due to the cessation of the cell cycle in the G1 stage. Quercetin induces the p21 CDK inhibitor by simultaneously reducing pRb phosphorylation and inhibiting G2/S cell cycle progression by trapping E2F1. Low-dose quercetin caused mild DNA damage and activation of Chk2, which is the main regulator of quercetin expression by p21. In addition, quercetin cyclin B1 and CDK1 reduce essential components of G2/M cell cycle progression and prevent quercetin from inhibiting key NF-Y transcription factor uptake into the cyclin B1 gene promoter. A wide range of molecular mechanisms and genetic alterations are involved in the pathogenesis of ovarian cancer, making it difficult to develop effective therapies. Among the various natural compounds, quercetin has extraordinary anti-cancer and anti-inflammatory properties in vitro and in vitro. Reports revealed that (Vafadar et al., 2020) that quercetin had a cytotoxic effect on ovarian cancer cells. Despite obtaining good results both in vitro and in vivo, studies clinical evaluations of the anti-cancer effects of quercetin, especially in ovarian cancer, have been quantitatively evaluated. Studies revealed that (Hashemzaei et al., 2017) anti-cancer activity of quercetin at 10, 20, 40, 80 and 120 μM in vitro in 9 tumor cell lines showed that quercetin causes apoptosis in all cancer cell lines tested at the concentrations used. Data has been shown that (Srivastava et al., 2016)

antitumor effects of quercetin in animal models and cancer cell lines showed that quercetin causes cytotoxicity in leukemic cells in a dose-dependent manner. In addition to leukemic cells, quercetin also induces cytotoxicity in Causes breast cancer, however, its effect on normal cells is limited. In addition, quercetin stopped the S phase during the progression of the cell cycle in the cancer cells tested. Experimental data has shown that (Wang et al., 2020) quercetin increases the radio sensitivity of NSCLC cells in a dose- and time-dependent manner. In addition, quercetin treatment increased miR-16-5p expression and decreased WEE1 expression. Different reports revealed that (Zheng et al., 2012) anti-cancer effect and induction of apoptosis by quercetin in human lung cancer cell line A-549 provides further evidence of quercetin inhibiting the growth of human adenocarcinoma A-549 cancer cell line. This effect is associated with the induction of apoptosis in A-549 cells, and the molecular mechanism may be related to decreased expression of the apoptosis regulatory gene bcl-2 and increased expression of the apoptosis regulatory gene box. The results showed that quercetin can cause apoptosis in A549 cells through mitochondrial depolarization by mitochondrial depolarization by causing imbalance in B-cell lymphoma (Bcl2/Bax) and by down-regulation of transducer and transcription activator 3. This study suggests that a quercetin-induced anti-inflammatory pathway in A549 cells contributes significantly to the induction of apoptosis in NSCLC and, therefore, may use a therapeutic application such as inducing potent apoptosis in cancer cells. Research reports revealed that (Dong et al., 2020) the NSCLC HCC827 cell line was controlled with quercetin at different concentrations. Quercetin results in anchorage-independent proliferation and growth of NSCLC cells. At the molecular level, quercetin suppressed Src expression, which subsequently inhibited Fn14/NF-κB signaling. In in vivo methods, quercetin inhibits the growth of solid tumors. After overexpression of Src in NSCLC cells, the anti-quercetin effect of NSCLC was blocked by inducing proliferation and metastasis of NSCLC and activation of Fn14/NF-κB signaling. In addition, Src-induced expression levels cause the growth and potential metastasis of solid tumors in mice.

2 Materials and Methods

2. 1. Nano-bubbles

Epikuron200, Palmitic Acid, SF6 Gas, Dextran, Ultra-pure water, Ethanol 70 percent, Quercetin.

2. 2. Making Nano-bubbles shell

An ethanol solution containing Epikuron® 200 (1%, w/v) and palmitic acid (1%, w/v) was added to perfluoropentan and ultra-pure water under stirring. The solution was saturated with oxygen up to a gas concentration of 35 mg/l. A 2.7% (w/v) dextran solution (Mw dextran = 100,000) was added drop-wise while the mixture was homogenized, using a high-shear homogenizer (Ultraturrax, Germany) for 2 min at 13,000 rpm and continuing the oxygen purge.

2. 3. Quercetin preparation

Quercetin was dissolved in 70% ethanol and stirred for 3 minutes by vertex and add to

made Nano-bubble.

2. 4. Construction of gas core

5% (w/v) SF_6 Gas solution as stabilizing agent.

2. 5. Dextran preparation

Finally, a 2.7% (w/v) dextran solution was added drop-wise under stirring. The composition of the Nano-bubbles is reported.

3 Results and Discussions

3. 1. Characterization

3. 1. 1. SEM characterization

Use of SEM images to prove the formation of uncoated and dextran coated bubble nanoparticles. Based on images from scanning electron microscopy, the synthesized nanomedicine is morphologically spherical in shape, found in cohesive structures that have a specific regular shape. On the other hand, the size of the synthesized Nano drug based on the reported size based on the size reported from the scanning electron microscope as seen in the image is similar to the sizes obtained based on TEM and DLS analysis.

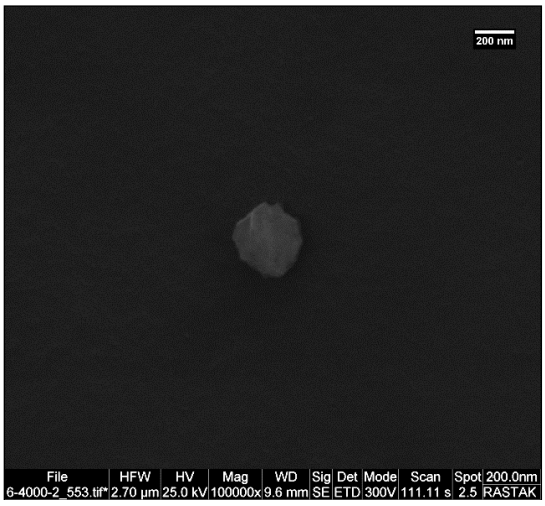

Figure 1: SEM Image.

3. 1. 2. TEM characterization

Use of TEM images for morphology and showing the diameter of drug-containing bubble nanoparticles and dextran coating at two different magnifications. Transmission electron microscopy images with an accelerating voltage of 100 kW bubble nanoparticles that trap the effective herbal drug quercetin and coated by chitosan in two magnifications show the morphological properties of the synthesized nanoparticles as shown in Fig. It is about 325 nm and can be estimated to be spherical in shape.

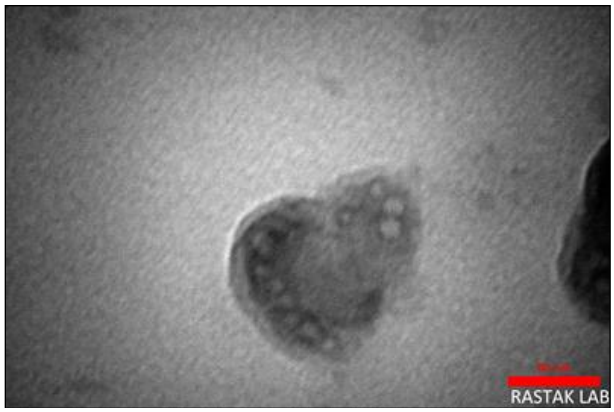

Figure 2: TEM Image.

3. 1. 3. Use of FT-IR to prove the formation of dextran coated bubble nanoparticles

FT-IR analysis was used to prove the coating of bubble nanoparticles.

FT-IR evaluation was performed to study the surface chemistry of nanoparticles as well as to ensure the correct formation of bubble nanoparticles and to establish a covalent bond between the dextran coating and the bubble nanoparticles containing the drug. The figure below shows the graph of FT-IR spectra based on the amount of radiation passing through the bonds in the bubble nanoparticles, dextran, and bubble nanoparticles containing the drug quercetin-dextran. All three spectra have a peak in the wavelength range of 3250-3500 cm-1, which belongs to the hydroxyl groups present in the chemical structure of dextran and the surface of nanoparticles. The specific peaks of the bubble nanoparticles containing the drug are in the wavelength range of 1350, 1045, 1600 and about 1200, which are related to the C-H, C-C, C = C and C-O bonds in the molecular structure of this material, respectively.

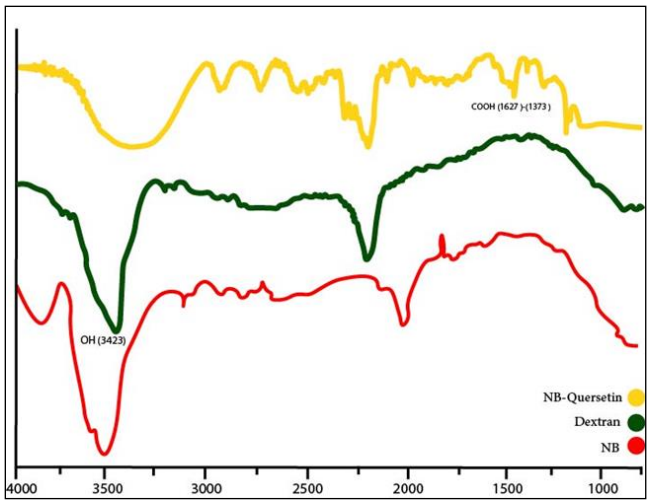

Figure 3: FT-IR

3. 1. 4. Use of zeta potential determination analysis to prove the formation of coated Nano-bubbles

The results of determining the zeta potential of the nanoparticle surface, which is considered as a measure of the stability of nanoparticles in the aqueous environment, are shown

in the pictures. So that before absorbing and trapping the herbal drug quercetin and applying dextran coating, the surface charge of the Nano-bubble changes to -22 and after adding quercetin and applying the dextran coating, the surface charge of the Nano-bubble changes to -24.

RUN 1	RUN2	RUN3
-24.7	-24.6	-24.8

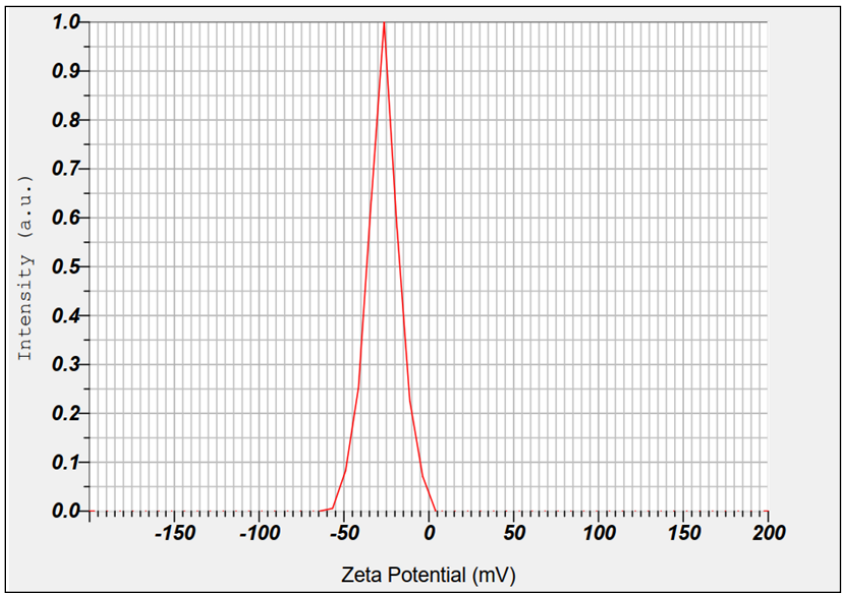

Figure 4: Surface potential of bubble nanoparticles with dextran coating.

3. 1. 5. Proof of size distribution of bubble nanoparticles synthesized using DLS

As shown in the histogram, the hydrodynamic size obtained by DLS analysis in an aqueous medium is larger than the size of the Nano drug based on TEM analysis, which results from the nanoparticle being surrounded by water molecules. And nanoparticles are interconnected because DLS analysis is not capable of separating single nanoparticles and can also consider bonded nanoparticles as a single nanoparticle and report related data based on light scattering as a single nanoparticle size.

Peaks Summary		
Dia (um)	Vol%	Width
0.149	100	0.0839

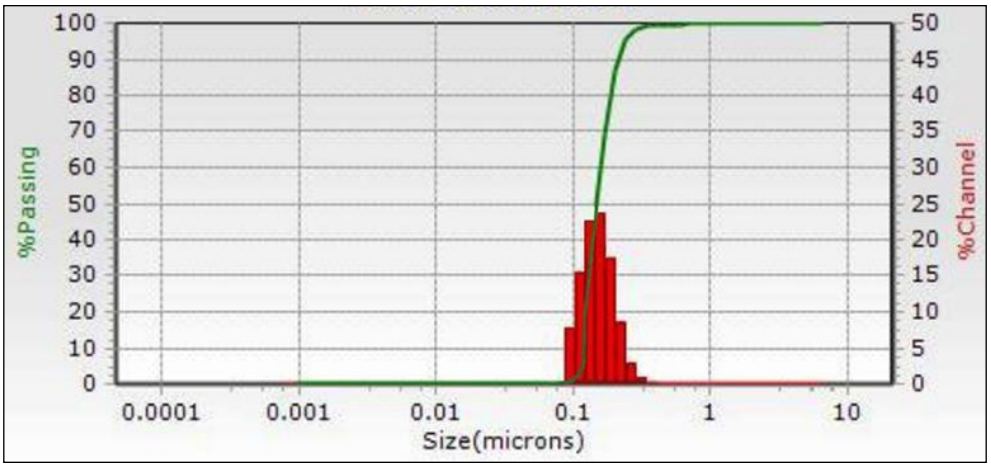

Figure 5: Bubble nanoparticles containing quercetin and dextran coating.

3. 1. 6. Proof of drug uptake and release with the UV

UV test was used to obtain the percentage of drug loading, which is obtained by comparing the percentage of drug loaded to the total drug consumed. Ultraviolet-visible spectra show different concentrations of quercetin. The red diagram of the sample is unknown and the black diagrams are known examples. This analysis shows the amount of useful drug that is loaded on the carrier nanoparticles in different concentrations. At concentrations of 20 to 40 Mm / l, no significant absorption of the drug was observed, but at concentrations of 40 to 500 mL / l, the absorption of the drug was more intense, which can be concluded that no additional drug was loaded in these areas.

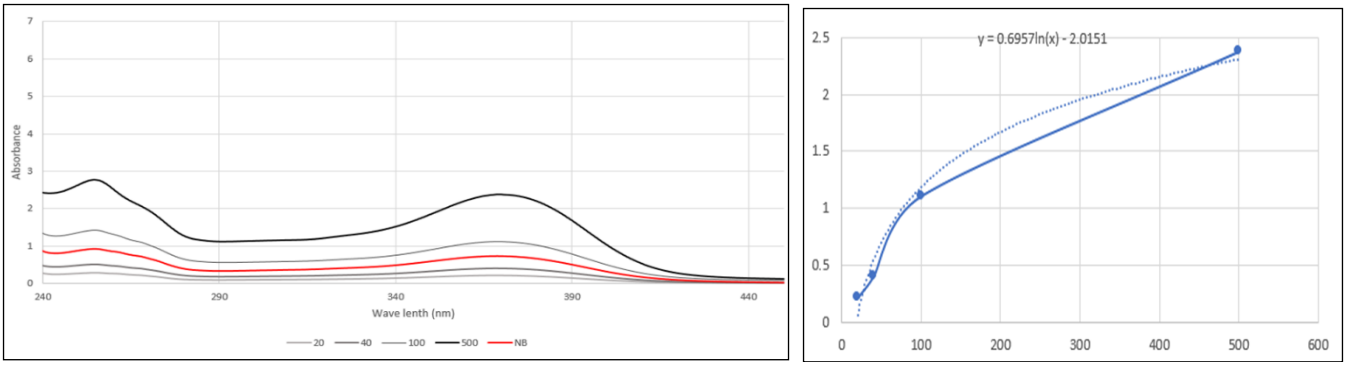

Figure 6: Ultraviolet spectrum of the drug supernatant loaded on the carrier at concentrations of 20 to 500 mM/L.

4 Conclusion

This experiment showed obtained NBs effectively delivered quercetin into lung cancer cells promoted by ultrasound irradiation. In conclusion, proposed biocompatible quercetin loaded NBs are suitable for ultrasound-targeted drug delivery and are thus a promising strategy for their noninvasive clinical application.

Acknowledgments

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Conflict of interests

The authors state that there are no conflicts of interests regarding the publication of this article.

References

Dong, Y., Yang, J., Yang, L., & Li, P. (2020). Quercetin inhibits the proliferation and metastasis of human non-small cell lung cancer cell line: the key role of Src-mediated fibroblast growth factor-inducible 14 (Fn14)/nuclear factor kappa B (NF-κB) pathway. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, *26*, e920537-1. doi: 10.12659/MSM.920537

Hashemzaei, M., Delarami Far, A., Yari, A., Heravi, R. E., Tabrizian, K., Taghdisi, S. M., Sadegh, S. E., Tsarouhas, K., Kouretas, D., Tzanakakis, G., Nikitovic, D., Anisimov, N. Y., Spandidos, D. A., Tsatsakis, A. M., & Rezaee, R. (2017). Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. *Oncology Reports*, *38*(2), 819-828. https://doi.org/10.3892/or.2017.5766

Jeong, J. H., An, J. Y., Kwon, Y. T., Rhee, J. G., & Lee, Y. J. (2009). Effects of low dose quercetin: Cancer cell-specific inhibition of cell cycle progression. *Journal of Cellular Biochemistry*, *106*(1), 73-82. https://doi.org/10.1002/jcb.21977

Khan, M. S., Hwang, J., Lee, K., Choi, Y., Seo, Y., Jeon, H., Hong, J. W., & Choi, J. (2019). Anti-tumor drug-loaded oxygen nanobubbles for the degradation of HIF-1α and the upregulation of reactive oxygen species in tumor cells. *Cancers*, *11*(10), 1464. https://doi.org/10.3390/cancers11101464

Peng, Y., Zhu, L., Wang, L., Liu, Y., Fang, K., Lan, M., Shen, D., Liu, D., Yu, Z., & Guo, Y. (2019). Preparation of nanobubbles modified with a small-molecule CXCR4 antagonist for targeted drug delivery to tumors and enhanced ultrasound molecular imaging. *International Journal of Nanomedicine*, *14*, 9139. doi: 10.2147/IJN.S210478

Srivastava, S., Somasagara, R. R., Hegde, M., Nishana, M., Tadi, S. K., Srivastava, M., Choudhary, B., & Raghavan, S. C. (2016). Quercetin, a natural flavonoid interacts with

DNA, arrests cell cycle and causes tumor regression by activating mitochondrial pathway of apoptosis. *Scientific Reports*, *6*(1), 1-13. https://doi.org/10.1038/srep24049

Vafadar, A., Shabaninejad, Z., Movahedpour, A., Fallahi, F., Taghavipour, M., Ghasemi, Y., Akbari, M., Shafiee, A., Hajighadimi, S., Moradizarmehri, S., Razi, E., Savardashtaki, A., & Mirzaei, H. (2020). Quercetin and cancer: new insights into its therapeutic effects on ovarian cancer cells. *Cell & Bioscience*, *10*(1), 1-17. https://doi.org/10.1186/s13578-020-00397-0

Wang, Q., Chen, Y., Lu, H., Wang, H., Feng, H., Xu, J., & Zhang, B. (2020). Quercetin radiosensitizes non-small cell lung cancer cells through the regulation of miR-16-5p/WEE1 axis. *IUBMB Life*, *72*(5), 1012-1022. https://doi.org/10.1002/iub.2242

Yu, Z., Wang, Y., Xu, D., Zhu, L., Hu, M., Liu, Q., Lan, W., Jiang, J., & Wang, L. (2020). G250 antigen-targeting drug-loaded nanobubbles combined with ultrasound targeted nanobubble destruction: a potential novel treatment for renal cell carcinoma. *International Journal of Nanomedicine*, *15*, 81. doi: 10.2147/IJN.S230879

Zheng, S. Y., Li, Y., Jiang, D., Zhao, J., & Ge, J. F. (2012). Anticancer effect and apoptosis induction by quercetin in the human lung cancer cell line A-549. *Molecular Medicine Reports*, *5*(3), 822-826. https://doi.org/10.3892/mmr.2011.726