FABRICATION OF PLGA NANOPARTICLE USING DOUBLE EMULSION SOLVENT EVAPORATION TECHNIQUE AS shRNA YB1 CARRIER

SITI 'AISYAH CHE ARIFFIN, BSc. (HONS)

KULLIYYAH OF ALLIED HEALTH SCIENCES (KAHS), INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA (IIUM), JALAN SULTAN AHMAD SHAH, BANDAR INDERA MAHKOTA, 25200 KUANTAN, PAHANG DARUL MAKMUR, MALAYSIA.

NUR KHAIRUNISA MOHD AFFANDI, BSc. (HONS)

KULLIYYAH OF ALLIED HEALTH SCIENCES (KAHS), INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA (IIUM), JALAN SULTAN AHMAD SHAH, BANDAR INDERA MAHKOTA, 25200 KUANTAN, PAHANG DARUL MAKMUR, MALAYSIA.

AHMAD FAHMI HARUN ISMAIL, Ph.D (CORRESPONDING AUTHOR)

KULLIYYAH OF ALLIED HEALTH SCIENCES (KAHS), INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA (IIUM), JALAN SULTAN AHMAD SHAH, BANDAR INDERA MAHKOTA, 25200 KUANTAN, PAHANG DARUL MAKMUR, MALAYSIA.
ahmadfahmi@iium.edu.my

ABSTRACT

Colorectal cancer was reported to be the second cause of death among cancer patients for over 500,000 mortalities annually (World Health Organization,2018). This may be owed to the lack of a treatment that specifically targets a molecule that comprehensively dysregulate cancer cell growth, survival and metastasis. A multitasking protein namely Y-box-binding protein 1 (YB1) was found to be upregulated and directly contributed in nine of the Hanahan and Weinberg 'Hallmark of cancer'. Silencing this YB1 protein by any means including the use of short-hairpin RNA (shRNA) could prompt positive therapeutic consequences towards colorectal cancer patients. Despite the discovery of the YB1 multifunctional effect two years ago, research evaluating the effects of using nanoparticle delivery with shRNA YB1 encapsulated towards colorectal cancer cells, are limited. Therefore, this *in-vitro* study was conducted to explore the anti-proliferative effects of shRNA YB1 towards colorectal cancer cell lines (HT29) by using double emulsion solvent evaporation technique to fabricate PLGA nanoparticle as the shRNA carrier agent. In this study, the amplification of the shRNA YB1 was done in JM109 *E.coli* before it was extracted and encapsulated into PLGA nanoparticle. The validation of the anti-YB1 plasmid presence was validated through 1% agarose gel electrophoresis. The nanoparticle embedded with shRNA YB1 plasmid was fabricated through double emulsion solvent evaporation method. The resulted size, charge and morphology were observed and recorded. The effect of the YB1 gene silencing towards HT-29 cell proliferation was analyzed using MTT assay. Results revealed the presence of the shRNA YB1 plasmid of approximately 2900 base pair of the shRNA. The size of the shRNA YB1 encapsulated nanoparticle was 345 nm with -26.1 mV charge. As for the HT29 treatment, it was indicated that the usage of shRNA YB1 loaded nanoparticle for 48 hours could reduce the cell viability ($p \leq 0.05$) of the HT29.

KEYWORDS: YB1, PLGA, nanoparticles, double emulsion, colorectal cancer, HT29

INTRODUCTION

Colorectal cancer is defined by the overgrowth of abnormal cells in the upper level of the gastrointestinal body. With 500 000 deaths reported annually, colorectal cancer is described as the second leading cancer death and third most common cancer type in the world (WHO, 2018). Currently, the most sophisticated treatment available for colorectal cancer is chemotherapy which

would induce painful and invasive treatments with visible consequences towards patients, physically and mentally (Valderrama-Treviño et al., 2017). The side effects of this current treatment are also harsh for the patients due to its non-specificity targeting and systemic therapy of the chemotherapeutic agents.

Y-box-binding protein 1 (YB1) is proven to have a major contribution towards all nine Hanahan and Weinberg 'Hallmarks of cancer' including its role in abnormal cell proliferation and metastasis (Maurya et al., 2017). YB1 is overexpressed in many cancer cells, including colorectal cancer and this upregulation is highly correlated with tumor progression, invasion, angiogenesis and metastasis (Zhang et al., 2019). Recent evidence shows that making use of a delivery method that utilizes the function of a short-hairpin RNA (shRNA) that targets to silence a YB1 'bone fide' oncoprotein would be a great help in achieving the purpose of a comprehensive and economical treatment. The silencing therapy should be precise and non-toxic towards the surrounding of the colorectal cancer location. Therefore, as an effort of providing the best least systemic toxic effect of the treatment, a biodegradable polymer named Poly (lactic-co-glycolic acid) (PLGA) is one of the best choices.

PLGA was endorsed by the United States' Food Drugs Administration (U.S FDA) and European Medicines Agency (EMA) for human use (Sharma, Parmar, Kori, & Sandhir, 2016). It gradually degrades into the biocompatible products of lactic and glycolic acid through hydrolysis and is able to release the encapsulated agents slowly over a long period of time. PLGA is a biocompatible, biodegradable and low systemic toxicity polymer that is commonly used in dual emulsion techniques (Colzani et al., 2018). With the said matter in hand, silencing the YB1 oncoprotein using shRNA with the help of PLGA nanoparticle delivery could provide comprehensive positive consequences. The use of nanoparticles would aid in localizing treatment and the usage of FDA approved PLGA would reduce the toxicity of the treatment. Therefore, the aim of this study is to investigate the anti-proliferative effects of the shRNA YB1 using PLGA nanoparticle fabricated by double emulsion solvent evaporation technique towards colorectal cancer (HT29) cell lines.

MATERIALS AND METHODS

Extraction of shRNA YB1 plasmid

ShRNA YB1 transformed *Escherichia coli* was cultured on nutrient agar supplemented with *kanamycin* for 24 hours to yield a single colony. Then the bacteria were further cultured in lysogeny broth supplemented with *kanamycin.* After 8 and 12 hours of bacteria preparation, shRNA YB1 plasmid was extracted by using PureYield™ Plasmid Maxiprep System. The experiment protocol was adapted from Promega Corporation (2009).

Evaluation on pDNA purity and integrity

The extracted shRNA YB1 plasmid was evaluated in terms of purity and pDNA integrity by using Nanodrop 1000 Spectrophotometer and Agarose Gel Electrophoresis respectively.

Fabrication of PLGA nanoparticles

PLGA nanoparticles was fabricated by using double emulsion water-oil-water (WOW) solvent evaporation method adapted from Ismail et al., (2016). 0.1 g of PLGA solid crystal was weighed and dissolved in 2 ml of ethyl acetate to form the oil phase. Then, the first aqueous phase containing 200 µl of distilled water and 6 µl of p53 plasmid DNA was added into the oil phase. The mixture was then homogenized for 2 minutes (30 seconds on, 15 seconds off) and sonicated for 30 seconds (5 seconds on, 3 seconds off) to create the first emulsion. Next, the primary emulsion was added to the second aqueous phase containing 5.5 ml of 4% Polyvinyl Alcohol and 16.5 ml of distilled water. The mixture was homogenized, sonicated with the same parameter as before and stirred for 13 hours to produce a stable structure of nanoparticle.

Next, the nanoparticle solution was centrifuged with parameter of 13000 rpm at 20°C for 15 minutes. The pellet was washed for 2 times with distilled water and finally resuspended with 5 ml of distilled water. Water bath sonicator was used to aid the resuspension process. The same procedure was repeated for the blank nanoparticle formulation without the addition of the p53 plasmid in the first aqueous phase.

Characterization of nanoparticles

The fabricated nanoparticle was characterized in terms of size, zeta potential and morphological surface by using Zeta Sizer, Zeta Potential and Scanning Electron Microscope (SEM) respectively. Prior to SEM observation and examination, the sample was freeze dried and proceeded with sputter coating (gold).

Culture and maintenance of A375 cell lines

HT29 Colorectal Carcinoma Cell lines was cultured and maintained in 10% Complete Growth Medium. The cells were seeded into a 96 well-plate after the cells reached 80% confluency.

Cells treatment

After 24-hours incubation, the seeding cells were divided into four group: (i) treated with shRNA YB1 loaded PLGA nanoparticles, (ii) treated with shRNA YB1 plasmid extracts (positive control), (iii) treated with blank nanoparticles (negative control), and (iv) untreated cells. For the treatment group, five concentrations were setup consisting of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml. The treatment was added according to the designed concentrations. The plates were then incubated for 24 and 48 hours at 37 °C, supplemented with 5% CO_2.

Cell viability assay

After 24 and 48 hours of treatment, MTT assay was performed by incubating the treated cells with 0.5 mg/ml of MTT reagent for 4 hours at 37 °C incubator supplemented with 5% CO_2. Formazan crystal in purple colour was produced by the viable cells once the MTT reagent had absorbed into the mitochondria of the cells. After the incubation period, 100µL of DMSO was added into each well and incubated for 1 hour to dissolve the crystal. Then, the absorbance was measured by a microplate reader set at 570 nm of optical density.

Data analysis

All of data collected from the experiment were conducted in triplicate. The mean as well as the standard deviations were calculated and recorded. The collected data were analysed by using IBM SPSS Statistics 25 with One Way ANOVA test. p values <0.05 was considered as statistically significant.

RESULTS

pDNA purity

The purity of the plasmid has been confirmed through two measurements of 260/280 and 260/230 ratio which are 1.83 and 2.12 respectively. While the stock concentration of extracted shRNA YB1 plasmid recorded from the Nanodrop spectrophotometer reading was 445.9 ng/µl.

Nanoparticles characterization

Size and zeta potential

The fabricated nanoparticle using double emulsion solvent evaporation method was analyzed in terms of its charge and Z-average size using Zeta Sizer Analyzer and Zeta Potential as per tabulated in Table 1.

Table 1 Characterization of shRNA YB1 loaded nanoparticles in terms of size and zeta potential.

Properties	**Reading**			
	1	2	3	Average (Mean+SD)
Z-average Size (nm)	456	306	273	345 ± 97
Charge potential (mV)	-27.3	-24.3	-26.0	-26.1 ± 1.5

Morphological surface

In order to validate the characterization of nanoparticles, the nanoparticles were observed by scanning electron microscopy (SEM). It was observed from these micrographs that the nanoparticles of PLGA with shRNA YB1 loaded were uniformly spherical in shape with no formation of pores and perforated outer shell of the fabricated nanoparticles as in Figure 1.

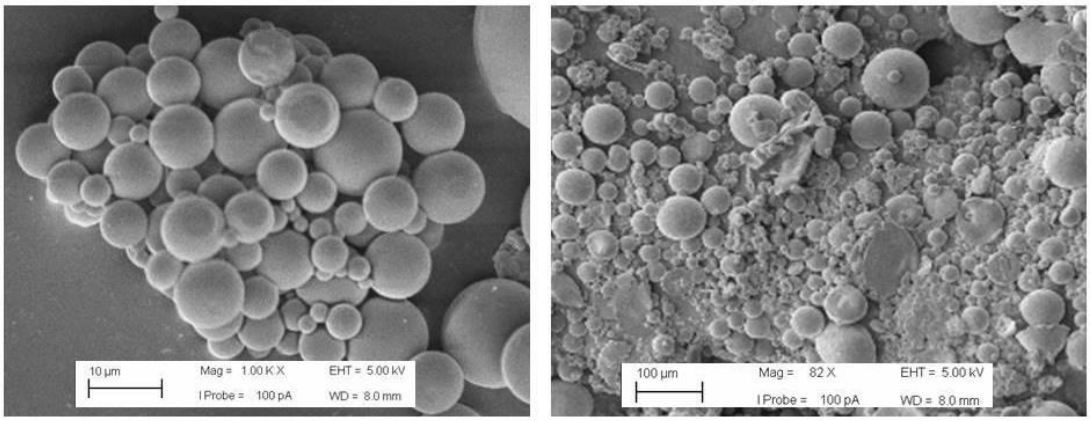

Figure 1 The morphology of shRNA YB1 loaded nanoparticles under magnification of 2.00Kx of Scanning Electron Microscope.

Cell Viability assay

Two sets of data have been set up (i) to compare the mean between concentration of shRNA YB1 loaded nanoparticles and cell viability of HT29 cell lines and (ii) to compare the mean between duration of nanoparticles exposure to the cells and cell viability.

Concentration of nanoparticles vs cell viability

The cell viability was expected to reduce as the concentration of shRNA YB1 loaded nanoparticles increased. The data was found to be not statistically significant with a *p* value of 0.200 and the trend also was inconsistent.

Duration of nanoparticles exposure vs cell viability

Through One Way Anova test analysis, it was found that there was statistically significant relationship between the duration of exposure and cell viability with *p* value of <0.001. Figure 2 shows the trend of the graph which indicated that the longer the duration of exposure the lower the cell viability.

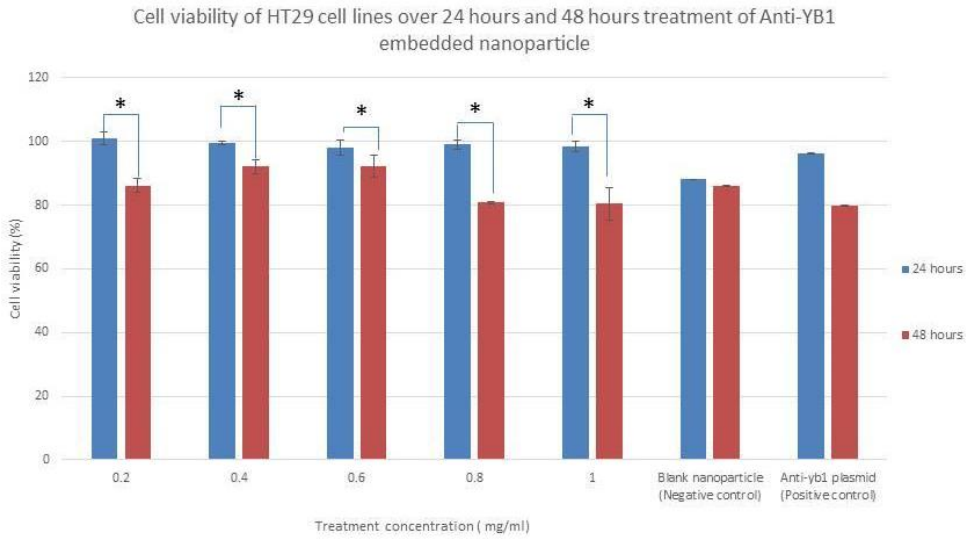

Figure SEQ Figure * ARABIC 2.0 illustrated the range of concentration of shRNA YB1 loaded nanoparticles and their effects on cell viability of HT29 cell lines in 24 and 48 hours. (The asterisk * is to indicate the statistically significant with the *p* value of<0.005)

DISCUSSIONS

pDNA purity

The A260/280 ratio is to determine the contamination of the nucleic acid sample. Pure RNA should have A260/280 ratio somewhere around 1.8 and 2.1 respectively for it to be validated as pure. Referring to the results in Table 1, the ratio showed the plasmid A260/280 ratio was 1.83. This indicated that the plasmid extracted was pure and could exert maximum possible effect of the silencing YB1 protein towards the intended target, colorectal cancer.

The A260/230 ratio shown on the Nanodrop spectrophotometer value is an indicator of the presence of the inorganic contaminants. Samples with A260/230 ratio below than 1.8 were considered to have a significant amount of organic contaminants (Desjardins & Conklin, 2010). Pure samples without any contaminants should show A260/230 ratio with a value near to 2.0. Results of the plasmid extracted, showed 2.13 suggesting that the plasmid extracted was pure and not contaminated, thus the function of the silencing YB1 could be carried out effectively.

Nanoparticles formulation and stability

This fabrication (Double emulsion solvent evaporation) method could yield the size of nanoparticles ranging from 500nm and below (Desjardins & Conklin, 2010). In this current study, nanoparticles in the average size of 345 nm ± 97 was achieved. Approximately 350 nm and below sized nanoparticles was found to be effectively targeting and entering HT29 cell lines and other related gastrointestinal carcinoma cell lines (Kennedy, 2018).

The recommended nanoparticle size that would facilitate the transportation and the delivery of the nanoparticle shRNA/drug delivery towards colorectal cancer was recorded as 20-400nm (Cisterna et al., 2016). Therefore, the synthesizing of nanoparticle within the range of 20-400nm would highly contribute towards the development of the targeted therapeutic treatment.

Surface characteristics, surface charge play a key role in protein adsorption, which in turn impacts the pharmacokinetics and biodistribution of nanoparticles. Neutral nanoparticles, on the other hand, as well as those with a slight adverse charge, demonstrate considerably extended half-lives in circulation (Blanco, Shen, & Ferrari, 2015). The surface charge of any nanoparticle intended to deliver any therapeutic action is often recommended to exist as positive charge/cation. This is due to the purpose of delivering the therapeutic action into the negative plasma membrane and to increase the cell permeability using the energy and charge dependent fashion (Lin & Alexander-Katz, 2013).

However, the attractive interaction between the cationic nanoparticle and the negatively charge cell membrane are often observed as toxic due to its rapid internalization (Contini, Schneemilch, Gaisford, & Quirke, 2018) and cationic nanoparticle was found to induce nanoscale disruption/hole through the cell membrane (Chen et al., 2009). Highly cationic nanoparticles also are quickly removed from circulation (Blanco et al., 2015). Therefore, Contini, et.al, (2018) suggested that anionic nanoparticle could produce less toxic effects and could retain the effect therapeutically longer in blood vessels *in-vivo*.

Cell Proliferation

In terms of duration nanoparticle treatment, all concentrations showed significant findings and reduced trend of cell proliferation when comparing between 24 hours and 48 hours treatment. However, subsequent to 48 hours of the treatments towards the colorectal cancer cell lines, no significant trend of reduced cell proliferation among the range treatment concentration was found. The result showed inconsistent trend of the cell proliferation across the concentration of the treatment used. At the concentration of 0.4 and 0.8 mg/mL nanoparticle treatment, the cell proliferation increasing in trend ironically to what it was supposed to be. An effective treatment is expected to show a consistent trend of reduced cell proliferation as the concentration of the nanoparticles treatment used increase. Therefore, few factors could possibly justify the inconsistent trend of the nanoparticles treatment effect.

First, the size of the nanoparticles fabricated by the double solvent emulsion evaporation technique, showed 345nm with a negative charge -26.1 mV. In terms of size, consistent and effective

nanoparticle treatment is expected from nanoparticles sized 10-100nm (Sukhanova et al., 2018). This range of size would allow the nanoparticles to enter the cell and overcome the cell barriers easily. According to Sukhanova et al., (2018), nanoparticles sized smaller than the 5nm would overcome the cell barriers specifically by translocation. For larger nanoparticles especially those larger than 100nm, the transmembrane penetration would possibly be aided by the interaction force, given the charge must be negative and larger than -25mV. However this process would harm the membrane since the membrane pores must be large enough for the entry of the particle. Meanwhile, for the particles of 100 nm size were efficiently adsorbed on the surface of the erythrocyte without causing cell death or morphological shifts in the cells, while particles of 600 nm deformed the membrane and entered the cells, leading to erythrocyte death (hemolysis).

Secondly, the inconsistent trend showed by the treatment might be contributed by the aggregation of the nanoparticles as shown by the Scanning Electron Microscopy images (Figure 1). The inconsistent aggregation of the nanoparticle might contribute towards the inconsistent reading of the cell viability as the treatment is not uniformly distributed even though the volume of the treatment was aligned to be increasing uniformly. Aggregation made the treatment of the nanoparticle unpredictable due to its failure to deliver the plasmid into the cancer cells (Hotze et al., 2010). Some of the factors that may have influenced the aggregation behavior include the poor storage of the nanoparticles, small nanoparticle size polydispersity (10-400nm) and surface charge of nanoparticles not exceeding +30 mv (Mohanraj & Chen, 2006). All of these factors were parallel with the finding of the SEM image thus contributing towards the inconsistent trend of the nanoparticles.

Third, the trend also might be influenced by the bacterial endotoxin contamination of the nanoparticles (Li & Boraschi, 2016). Contamination of the endotoxin could also effect the cell proliferation through lipopolysaccharide toxic effects towards the cell (Li et al., 2017). Along the synthesis and fabrication of the nanoparticles, the nanoparticles produced may have been contaminated by the endotoxin through the equipment used to synthesize the nanoparticle. This factor however could be eliminated through high-temperature sterilization process which was overlooked during the project.

CONCLUSION

The current literature reveals double emulsion solvent evaporation method as a promising method to fabricate effective nanoparticles. However, many researchers have underestimated the potential of the anionic nanoparticle in delivering therapeutic actions into the intended carcinoma cell lines. The gradual shift of research perspectives allows the research to see more aspects of delivering the therapeutic action not merely from the charge attraction. Therefore, support from emerging studies of anionic nanoparticle in targeted therapeutics scope has become wider, acceptable and developing.

As a conclusion, shRNA that targets to silence the YB-1 protein, could be facilitated effectively into the targeted colon carcinoma cell lines using PLGA nanoparticles double emulsion solvent evaporation fabrication method. This promising method was carried out successfully to treat and deliver the therapeutic action despite an average particle size of 300 nm and anionic charge nanoparticle.

The findings from this research aim to provide evidence of the potential effectiveness of double emulsion solvent evaporation method and that the negative charge is capable of delivering the nanoparticle therapeutic action providing few parameters and amendments to be integrated in facilitating the delivery.

ACKNOWLEDGEMENT

The authors are grateful to the Advanced Drug Delivery Lab, Pharmaceutical Department IIUM Research Team and internal research grant with grant number IRAGS18-026-0027.

REFERENCES

Blanco, E., Shen, H., & Ferrari, M. (2015). Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nature Biotechnology*, *33*(9), 941–951. https://doi.org/10.1038/nbt.3330

Chen, J., Hessler, J. A., Putchakayala, K., Panama, B. K., Khan, D. P., Hong, S., … Orr, B. G. (2009). Cationic

Nanoparticles Induce Nanoscale Disruption in Living Cell Plasma Membranes. *The Journal of Physical Chemistry B*, *113*(32), 11179–11185. https://doi.org/10.1021/jp9033936

Cisterna, B. A., Kamaly, N., Choi, W. Il, Tavakkoli, A., Farokhzad, O. C., & Vilos, C. (2016). Targeted nanoparticles for colorectal cancer. *Nanomedicine (London, England)*, *11*(18), 2443–2456. https://doi.org/10.2217/nnm-2016-0194

Colzani, B., Pandolfi, L., Hoti, A., Iovene, P. A., Natalello, A., Avvakumova, S., … Prosperi, D. (2018). Investigation of antitumor activities of trastuzumab delivered by PLGA nanoparticles. *International Journal of Nanomedicine*, *13*, 957–973. https://doi.org/10.2147/IJN.S152742

Contini, C., Schneemilch, M., Gaisford, S., & Quirke, N. (2018). Nanoparticle–membrane interactions. *Journal of Experimental Nanoscience*, *13*(1), 62–81. https://doi.org/10.1080/17458080.2017.1413253

Desjardins, P., & Conklin, D. (2010). NanoDrop microvolume quantitation of nucleic acids. *Journal of Visualized Experiments : JoVE*, (45). https://doi.org/10.3791/2565

Ismail, A. F. H., Mohamed, F., Rosli, L. M. M., Shafri, M. A. M., Haris, M. S., & Adina, A. B. (2016). Spectrophotometric determination of gentamicin loaded PLGA microparticles and method validation via ninhydrin-gentamicin complex as a rapid quantification approach. *Journal of Applied Pharmaceutical Science*, *6*(1), 007–014. https://doi.org/10.7324/JAPS.2016.600102

Kennedy, P. (2018). Targeting of human CD44v6 with engineered nanoparticles for gastrointestinal cancer diagnosis and therapy. Retrieved from https://repositorio-aberto.up.pt/bitstream/10216/115669/2/287403.pdf

Khan, M. I., Adhami, V. M., Lall, R. K., Sechi, M., Joshi, D. C., Haidar, O. M., … Mukhtar, H. (2014). YB-1 expression promotes epithelial-to-mesenchymal transition in prostate cancer that is inhibited by a small molecule fisetin. *Oncotarget*, *5*(9), 2462–2474. https://doi.org/10.18632/oncotarget.1790

Li, Y., & Boraschi, D. (2016). Endotoxin contamination: a key element in the interpretation of nanosafety studies. *Nanomedicine*, *11*(3), 269–287. https://doi.org/10.2217/nnm.15.196

Li, Y., Shi, Z., Radauer-Preiml, I., Andosch, A., Casals, E., Luetz-Meindl, U., … Boraschi, D. (2017). Bacterial endotoxin (lipopolysaccharide) binds to the surface of gold nanoparticles, interferes with biocorona formation and induces human monocyte inflammatory activation. *Nanotoxicology*, *11*(9–10), 1157–1175. https://doi.org/10.1080/17435390.2017.1401142

Lin, J., & Alexander-Katz, A. (2013). Cell Membranes Open "Doors" for Cationic Nanoparticles/Biomolecules: Insights into Uptake Kinetics. *ACS Nano*, *7*(12), 10799–10808. https://doi.org/10.1021/nn4040553

Maurya, P. K., Mishra, A., Yadav, B. S., Singh, S., Kumar, P., Chaudhary, A., … Mani, D. A. (2017). Role of Y Box Protein-1 in cancer: As potential biomarker and novel therapeutic target. *Journal of Cancer*, *8*(10), 1900–1907. https://doi.org/10.7150/jca.17689

Mohanraj, V., & Chen, Y. (2006). Nanoparticles- A Review. *Tropical Journal of Pharmaceutical Research*, *5*(1), 561–573.

Promega Corporation. (2009). PureYield ™ Plasmid Maxiprep System.

Sharma, S., Parmar, A., Kori, S., & Sandhir, R. (2016). PLGA-based nanoparticles: A new paradigm in biomedical applications. *TrAC Trends in Analytical Chemistry*, *80*, 30–40. https://doi.org/10.1016/J.TRAC.2015.06.014

Sukhanova, A., Bozrova, S., Sokolov, P., Berestovoy, M., Karaulov, A., & Nabiev, I. (2018). Dependence of Nanoparticle Toxicity on Their Physical and Chemical Properties. *Nanoscale Research Letters*, *13*(1), 44. https://doi.org/10.1186/s11671-018-2457-x

Valderrama-Treviño, A. I., Barrera-Mera, B., Ceballos-Villalva, J. C., & Montalvo-Javé, E. E. (2017). Hepatic Metastasis from Colorectal Cancer. *Euroasian Journal of Hepato-Gastroenterology*, *7*(2), 166–175.

https://doi.org/10.5005/jp-journals-10018-1241

Zhang, W., Du, M., Wang, T., Chen, W., Wu, J., Li, Q., … Yin, L. (2019). Long non-coding RNA LINC01133 mediates nasopharyngeal carcinoma tumorigenesis by binding to YBX1. *American Journal of Cancer Research*, *9*(4), 779–790. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/31106003

Blanco, E., Shen, H., & Ferrari, M. (2015). Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nature Biotechnology*, *33*(9), 941–951. https://doi.org/10.1038/nbt.3330

Chen, J., Hessler, J. A., Putchakayala, K., Panama, B. K., Khan, D. P., Hong, S., … Orr, B. G. (2009). Cationic Nanoparticles Induce Nanoscale Disruption in Living Cell Plasma Membranes. *The Journal of Physical Chemistry B*, *113*(32), 11179–11185. https://doi.org/10.1021/jp9033936

Cisterna, B. A., Kamaly, N., Choi, W. Il, Tavakkoli, A., Farokhzad, O. C., & Vilos, C. (2016). Targeted nanoparticles for colorectal cancer. *Nanomedicine (London, England)*, *11*(18), 2443–2456. https://doi.org/10.2217/nnm-2016-0194

Colzani, B., Pandolfi, L., Hoti, A., Iovene, P. A., Natalello, A., Avvakumova, S., … Prosperi, D. (2018). Investigation of antitumor activities of trastuzumab delivered by PLGA nanoparticles. *International Journal of Nanomedicine*, *13*, 957–973. https://doi.org/10.2147/IJN.S152742

Contini, C., Schneemilch, M., Gaisford, S., & Quirke, N. (2018). Nanoparticle–membrane interactions. *Journal of Experimental Nanoscience*, *13*(1), 62–81. https://doi.org/10.1080/17458080.2017.1413253

Desjardins, P., & Conklin, D. (2010). NanoDrop microvolume quantitation of nucleic acids. *Journal of Visualized Experiments : JoVE*, (45). https://doi.org/10.3791/2565

Ismail, A. F. H., Mohamed, F., Rosli, L. M. M., Shafri, M. A. M., Haris, M. S., & Adina, A. B. (2016). Spectrophotometric determination of gentamicin loaded PLGA microparticles and method validation via ninhydrin-gentamicin complex as a rapid quantification approach. *Journal of Applied Pharmaceutical Science*, *6*(1), 007–014. https://doi.org/10.7324/JAPS.2016.600102

Kennedy, P. (2018). Targeting of human CD44v6 with engineered nanoparticles for gastrointestinal cancer diagnosis and therapy. Retrieved from https://repositorio-aberto.up.pt/bitstream/10216/115669/2/287403.pdf

Khan, M. I., Adhami, V. M., Lall, R. K., Sechi, M., Joshi, D. C., Haidar, O. M., … Mukhtar, H. (2014). YB-1 expression promotes epithelial-to-mesenchymal transition in prostate cancer that is inhibited by a small molecule fisetin. *Oncotarget*, *5*(9), 2462–2474. https://doi.org/10.18632/oncotarget.1790

Li, Y., & Boraschi, D. (2016). Endotoxin contamination: a key element in the interpretation of nanosafety studies. *Nanomedicine*, *11*(3), 269–287. https://doi.org/10.2217/nnm.15.196

Li, Y., Shi, Z., Radauer-Preiml, I., Andosch, A., Casals, E., Luetz-Meindl, U., … Boraschi, D. (2017). Bacterial endotoxin (lipopolysaccharide) binds to the surface of gold nanoparticles, interferes with biocorona formation and induces human monocyte inflammatory activation. *Nanotoxicology*, *11*(9–10), 1157–1175. https://doi.org/10.1080/17435390.2017.1401142

Lin, J., & Alexander-Katz, A. (2013). Cell Membranes Open "Doors" for Cationic Nanoparticles/Biomolecules: Insights into Uptake Kinetics. *ACS Nano*, *7*(12), 10799–10808. https://doi.org/10.1021/nn4040553

Maurya, P. K., Mishra, A., Yadav, B. S., Singh, S., Kumar, P., Chaudhary, A., … Mani, D. A. (2017). Role of Y Box Protein-1 in cancer: As potential biomarker and novel therapeutic target. *Journal of Cancer*, *8*(10), 1900–1907. https://doi.org/10.7150/jca.17689

Mohanraj, V., & Chen, Y. (2006). Nanoparticles- A Review. *Tropical Journal of Pharmaceutical Research*, *5*(1), 561–573.

Promega Corporation. (2009). PureYield ™ Plasmid Maxiprep System.

Sharma, S., Parmar, A., Kori, S., & Sandhir, R. (2016). PLGA-based nanoparticles: A new paradigm in

biomedical applications. *TrAC Trends in Analytical Chemistry*, *80*, 30–40. https://doi.org/10.1016/J.TRAC.2015.06.014

Sukhanova, A., Bozrova, S., Sokolov, P., Berestovoy, M., Karaulov, A., & Nabiev, I. (2018). Dependence of Nanoparticle Toxicity on Their Physical and Chemical Properties. *Nanoscale Research Letters*, *13*(1), 44. https://doi.org/10.1186/s11671-018-2457-x

Valderrama-Treviño, A. I., Barrera-Mera, B., Ceballos-Villalva, J. C., & Montalvo-Javé, E. E. (2017). Hepatic Metastasis from Colorectal Cancer. *Euroasian Journal of Hepato-Gastroenterology*, *7*(2), 166–175. https://doi.org/10.5005/jp-journals-10018-1241

Zhang, W., Du, M., Wang, T., Chen, W., Wu, J., Li, Q., … Yin, L. (2019). Long non-coding RNA LINC01133 mediates nasopharyngeal carcinoma tumorigenesis by binding to YBX1. *American Journal of Cancer Research*, *9*(4), 779–790. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/31106003