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Original Article

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ABSTRACT

The development of nanomedicine for gene therapy in the past decade has widely gained interest such as miRNA transfection to cancer cells. Unfortunately, due to its nature character, miRNA is easy to decay by cellular enzymatic process and requires a carrier. As cationic biopolymer, chitosan is widely known as non-viral vector. However, the study of chitosan as miRNA delivery system remains small in number. We purposely to investigate the miRNA loaded chitosan nanoparticles to breast cancer cell lines. To obtain the nanocomplex, we formulate chitosan-antimiR-106b-5p using natrium tripolyphosphate (Na. TPP) through ionic gelation method. We characterized the nanochitosan formula by using gel electrophoresis, nanoquant for encapsulation of entrapment quantification, morphology appearance as viewed by Scanning Electron Microscope (SEM), nanochitosan size analysis; in vitro analysis using MCF-7 and T47D breast cancer cell lines; in-silico prediction of possible gene target; PCR analysis and gel electrophoresis for E2F1/GAPDH expression. We found efficiency entrapment value as respectively 96.7[illegible], particle size analysis 458 ± 11.79 PI 0.65 ± 0.07, spherical morphology as viewed in SEM. No significant difference between the nanochitosan supplemented group and the control group in MCF-7 cells (p=0,067). However, the ratio of E2F1 to GAPDH was significantly lower than the control group after nanochitosan antimiR-106b-5p loaded at concentration 140 nanoMolar (p=0,022) and 35 nanoMolar (p=0,016). Our nanochitosan formula is non-toxic to use in MCF-7 cell line. As the formula was conjugated to synthetic antimiR-106b-5p, the E2F1 expression decreased.

KEYWORDS: chitosan, nanoparticle, miR-106b-5p, breast cancer, E2F1.

INTRODUCTION

miRNA therapy has gained popularity nowadays with many studies focus on evaluating the dysregulated level of this short chain non-coding RNA by correlating them with altered expression of various genes [1]. Cancer is one top list of priority due to the highest morbidities and mortalities reported every year [2]. In 2040, WHO estimates the global burden of cancer will rise approximately by fifty percent from 18,1 million in 2018 to 27,9 million in which 2,1 million cases is caused by female breast cancer [3, 4, 5]. This abnormal level of miRNA intracellular could affect from growth and proliferation, differentiation, apoptotic, cell cycle to tissue development [6, 7, 8]. As well as other gene therapy, miRNA expression could be transfected by using the synthetic oligonucleotides either as a mimic or antagonist [9]. Unfortunately, most gene therapies encounter similar problem especially how to envelope the genes safely accompanied by its release to targeted cells precisely [10].

To answer the challenge regarded the delivery system, scientists also begin to develop various vectors to encapsulate these genes [11]. Nanomedicine in gene therapy applies for specific genes transporting mechanism not only in size but also the biochemical properties packed [12, 13]. One of the main advantages to aim from drugs-based nanoparticles is its ability to enhance the therapeutic level and achieving the desired pharmacological effect especially in malignancy. Drugs toxicity is also part of the challenge widely discussed. To gain precise dosage without affecting normal cells is partly correlated with the delivery system [14 15, 16]. In cancer, nanoparticles that carry drugs to genes could also be designed to reach the cancer cells without affecting the normal cells since its size allowing penetrate the cells more effective [1718, 19]. To ensure its precise ability, it requires safe, less immunogenic and biodegradable carrier. Although viral vector is much still preferable, it still has inconvenient side effects such as immunogenicity, oncogenicity and the DNA size it can uptake [20, 21]. More strategies are developed with better advantages such as cationic polymers [22]. The popularity of cationic biopolymer is shown by better capacity to be modified and ability to package certain genes. Although polyethyleneimine (PEI) has strong gene complexation and high transfection efficiency, studies reported its level of toxicity is higher than chitosan [11, 14].

Chitosan is biopolymer that widely used for medicinal purpose [23]. Chitosan is obtained by alkaline deacetylation of chitin in which could be found from crustaceans of marine arthropods or insects. The nature characteristic of chitosan is its positive charged surface and considered perfect as carrier for gene therapy [21]. Under acid environment, chitosan could form nanoparticles and able to interact with negative charged molecules such as miRNA [24]. Several studies have reported chitosan as nanocarrier for gene therapy. siRNA in PEG-chitosan nanocarrier successfully penetrate the blood brain barrier to reach glioblastoma cell effectively [19, 25]. Using the xenograft model, miR-34a with chitosan encapsulated able to downregulate the metastatic genes as well as MET, Axl, and c-Myc in prostate cancer cell line [26]. Ysrafil (2020) also evaluated the HIF1a expression in ovarian cancer cell line, SKOV3, after miR-155-5p chitosan encapsulated transfection [27].

However, chitosan preparation has various applicable methods. Ionic gelation is the mostly used method since it is cheaper and only require simple stirring. The powder base chitosan must be dissolved under acidic solution and further add a crosslinker for the final formulation [10, 27].

By supplementing the chitosan solution with sodium tripolyphosphate, the chitosan is ready to be conjugated with anionic particles [28, 29]. Therefore, the purpose of this study was to characterize and evaluate the safety of chitosan encapsulation to synthetic antimiR in MCF-7 cell line also to inspect the E2F1 mRNA expression post transfection in p53-mutated cell line, T47D.

MATERIAL AND METHOD

Human MCF-7 and T47D breast cancer cells were obtained from ATCC (Virginia, USA) and maintained in high glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine qualified serum (Massachusetts, USA), 1% Penisilin-Streptomisin (Massachusetts, USA), and 0,5% amphotericin (Massachusetts, USA). antimiR-106b-5p was obtained from Integrated DNA Technologies (Iowa, USA). miRCURY RNA Cell and Plant Kit, Universal cDNA synthesis kit II 8-64 rxns Exiqon was purchased from Woburn, USA. The Infinite® 200 PRO NanoQuant was purchased from Philadelphia, USA. Thermal Cycler (96 Well Capacity) PCR SelectCycler™ II was purchased from California, USA, agarose gel electrophoresis system was purchased from Mupid-One®. Nanoparticles Size Analyzer Horiba Sz-100 was obtained from Japan. Medium Molecular Weight Chitosan was purchased from Sigma Aldrich.

Ch-NP-antimiR-106b-5p preparation

The nanoparticle formulation began by dissolving the medium molecular weight chitosan powder into 1% acetic acid with 24 hours magnetic stirring. The solution later should be pH adjusted to gain acid environment (pH 5.5) by adding 1 M NaOH. Then, to obtain 0.2% chitosan solution, acetate buffer pH 5 was added. The nanocomplex formula later was made by ionic gelation method by mixing 0.2% chitosan with sodium tripolyphosphate (5:1). Incubate for 5 minutes at room temperature. Then, 200 μL of the ready use solution was conjugated with 200 μL antimiR-106b-5p. Incubate for 20 minutes in room temperature.

Efficiency of entrapment

Efficiency of entrapment antimiR-106b-5p chitosan encapsulated was obtained by measuring the free concentration of antimiR106b-5p inside the formula. The Ch-NP-106b-5p was centrifugated for 15 minutes at 13.000 g speed. The supernatant obtained was measured by its absorbance using The Infinite® 200 PRO NanoQuant. Then, absorbance efficiency was generated by comparing the percentage of encapsulated miRNA compared to total miRNA.

$$\frac{Total\ miRNA\ -unencapsulated\ miRNA}{total\ miRNA} x\ 100\%$$

Ch-NP-antimiR-106b-5p morphology measurement

Chitosan antimiR-106b-5p nanocomplex was prepared as 3 μM concentrate by diluting it into nuclease free water. To measure the size is used Nanoparticle Size Analyzer Horiba Sz-100 and to evaluate the morphology is used Scanning Electron Microscope.

Cell viability assay

We observe and analyze the cells tolerability toward the chitosan solution by using cytotoxic tests on MTT assay method. T47D and MCF-7 cells were seeded into 96 well plates with approximately $6x10^3$ cells in each well plate. Be incubated for 24 hours at 37ºC and 5% CO_2. The next day, media was removed from each well and the cells were given 100 μL of nanochitosan solution or high glucose DMEM only as the control group. MTT assay then conducted after 24 hours incubation by pipetting 100 μL 0,5 mg/mL MTT reagent in each well. Incubate it for 4 hours then add 100 μL stopper solution (SDS 10% and 0,01 μL/L HCl) to dissolve the formazan crystals. Incubate again for the next 18 hours in room temperature with the absence of light. The absorbance of each well was measured at a 595 nm wavelength using Micro Plate Reader (Bio-Rad Model 680 XR)

Genes target expression analysis

T47D cells were seeded about $5x10^4$ each well into 6 well plate. Following for 24 hours incubation, antimiR-106b-5p nanochitosan encapsulated then added to each well with three different concentrations. Incubate again for the next 24 hours. Total RNA was isolated using miRCURY RNA Cell and Plant Kit according to manufacturer protocol. Total RNA concentrations were measured by nanoQuant at 260 to 280 nm wavelength. To gain the reverse transcripted RNA, 10 ng of total RNA was synthesized by using Universal cDNA synthesis kit II 8-64 rxns according to manufacturer protocol. Quantification of E2F1 mRNA (forward: ACTCCTCGCAGATCGTCATCATCT; reverse: GGACGTTGGTGATGTCATAGAT), and GAPDH as housekeeping gene (forward: GGCAAATTCAACGGCACAGT; reverse: AGATGGTGATGGGCTTCCC). The PCR products later run on 2% agarose gel electrophoresis. The bands were quantified using ImageJ software.

Statistical analysis

All the analyzed data were measured as triplicate and outcome value was presented as mean ± standard deviation (SD). We compare the cells viability between the Ch-NP as experiment group with the high glucose DMEM as control group using Independent T-test. Gene target expressions were analyzed with Independent T-test. All presented data were analyzed using SPSS 25 software (SPSS Inc., Chicago, USA) and graphics were performed by GraphPad Prism 8.0. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Efficiency of entrapment

To evaluate the presenting synthetic oligonucleotides encapsulated by the nanochitosan formula, we label both the naked and Ch-NP-antimiR-106b-5p with fluorochrome and run it on 2% agarose gel electrophoresis for 15 minutes. As viewed from the GelDoc, the well from the naked antimiR showed in the background as white greyish area between the dark background while the encapsulated antimiR likely to stay at the base of the well (Fig. 1A).

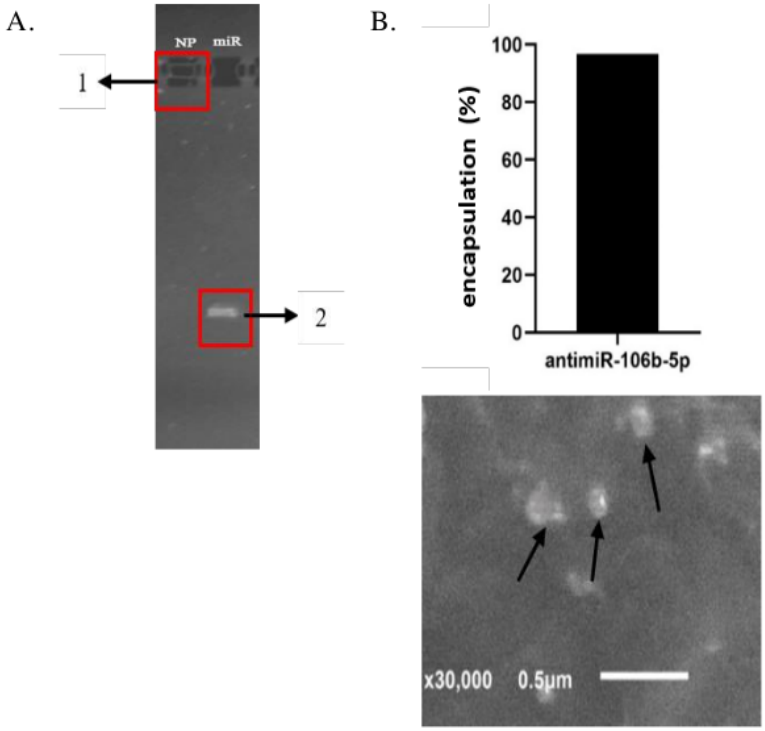

Fig 1: (A) The encapsulated antimir stayed at the base of the well (1) while the illuminating area inside the dark background showing the unencapsulated antimiR (2); (B) Efficiency of entrapment of the Ch-NP-antimiR-106b-5p as measured by NanoQuant was 96.7% and the spherical morphology of nanochitosan as seen by Scanning Electron Microscope (black arrows)

We also quantify the efficiency of entrapment using nanoQuant to measure the percentage of total miRNA encapsulated by the nanochitosan. Using the formula, the value of the efficiency of entrapment was 96.7% (Fig. 1). The obtained value was respectively meant as total miRNA concentrations encapsulated by the chitosan nanoplex through ionic gelation with sodium tripolyphosphate. The spherical morphology of the nanochitosan also has meaningful contribution as viewed from SEM analysis (Fig. 1B).

Morphology analysis of Ch-NP-antimiR-106b-5p

We conducted particle size analysis of the encapsulated antimiR-106b-5p to evaluate the nanocomplex formation by the chitosan. We obtain the size as respective 458 ± 11.79 with Polydyspersion Index 0.65 ± 0.07 (Table 1). With polydyspersion index less than 0.7, the nanoparticles would likely to distribute uniformly and it would strengthen the ability to reach intracellular.

Table 1. Nanochitosan distribution and size analysis

	Particle size	*Polydyspersion Index* (PI)
Ch-Nps-antimiR-106b-5p	458 ± 11.79	0.65 ± 0.07

Cell viability after nanochitosan transfection

We performed the easiest and effective calorimetric assay (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (methyl thiazolyl tetrazolium; MTT)) to identify the mitochondrial activity from cytochrome b and c from surviving cells. The living cells were shown as purple ring (formazan crystals). This assay was performed to all groups (chitosan supplemented cells and high glucose DMEM only). The absorbance values obtained later analyzed and compared to the control group. We conducted Independent T-test test since our data were normally distributed. As observed by the absorbance value, there was no significant difference neither in the nanochitosan or control group (p=0.067).

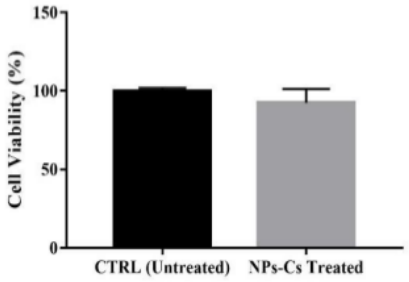

Fig 2: MCF-7 cells after given the chitosan nanoparticles showed no significant cell diminishing.

In-silico identification of miRNA target prediction

Furthermore, we also conduct the bioinformatic analysis to predict the binding location of E2F1 mRNA toward the miR-106b-5p. as predicted by the tools, miR-106b-5p could recognize EF21 at base number 1972-1987 from the chromosome locus with logistic probability 0.74. The near 1 value of logistic probability marked the highest confidence of the binding location.

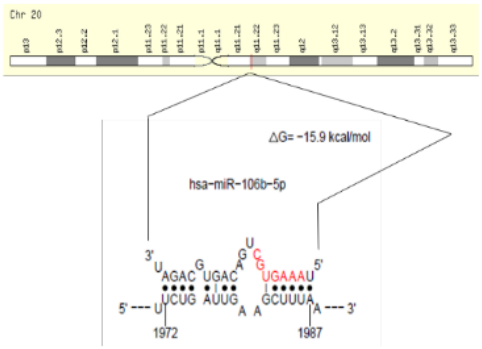

Fig 3: In-silico approach E2F1 gene binding location to miR-106b-5p using STarMirDB combined with GeneCard

E2F1 mRNA expression analysis after Ch-NP-antimiR-106b-5p transfection to T47D cells

We measure the gene target expression by running the extracted RNA to PCR as to predict the nanoparticles ability to deliver the synthetic oligonucleotides intracellular into its functional location on T47D cells. The cells were seeded and incubated into 6 well plate for 24 hours continued with antimiR-chitosan transfection for the next 24 hours. The cDNA from each concentration group (140 nm, 70 nm, 35 nm and control group) were diluted and mixed with the PCR mastermix reagents (GoTaqGreen) then ran to 2% gel agarose electrophoresis. As seen in Fig.5, E2F1/housekeeping gene GAPDH mRNA expression significantly lower in all dosage given compared to the control group treated with free serum DMEM only (Independent T-test; $p<0.05$) in two different concentrations; 140 nm and 35 nm.

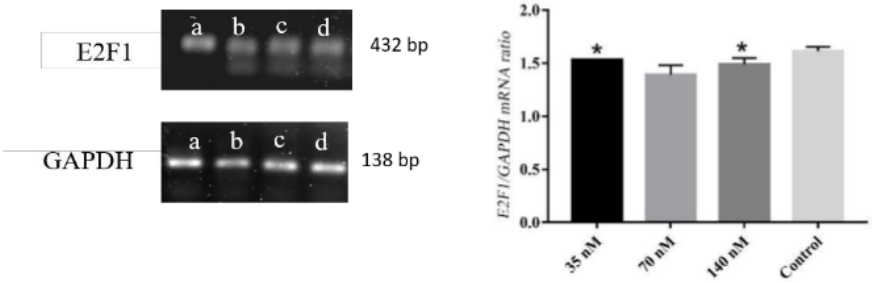

Fig 4: Endogenous E2F1/GAPDH mRNA level after Ch-NP-antimiR-106b-5p transfection given in three different concentrations: 35 nm, 70 nm and 140 nm compared to control group *Independent T-test* p<0.05; * treatment to control significance value; 35 nm (p=0,016), 140 nm (p=0,022). From left to right at concentrations: (a) 35 nm, (b) 70 nm, (c) 140 nm and (d) control group. Data were presented as mean±SD. E2F1: E2F Transcription Factor 1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; RT-PCR: reverse transcription-polymerase chain reaction.

Targeted therapy has been much desirable for the last decades due to its great potential in solving numerous challenges occurred to eradicate certain diseases especially in cancer. The studies of microRNA have gained popularity since it belongs to non-coding RNA in which able to interact with abundant target genes involved in tumorigenesis. It also could affect at the functional protein level [30]. Unfortunately, it is almost impossible to transfecting the miRNA into intracellular and penetrating the membranes safely. The dehydrogenase enzymes could decay it even before reaching the surface also it may lose efficiency due to inability conjugating with certain ligands. In terms to predict the solution, many vectors have been developed to solve this problem and using biopolymer is another strategy to replace the highly immunogenic plasmid viral vector [14].

As drug deliverer, chitosan popularity is well known due to its ability to penetrate toward the tight junction of cell membranes effectively, low immunogenicity, biodegradable, and its polycationic character by nature makes it easy to interact with negative charged nucleic acid [15, 31]. To formulate the chitosan requires certain method and ionic gelation simple complexation is the easiest technique. It is made by conjugating the chitosan with anionic multivalent tripolyphosphate as the crosslinker. The TPP will create strong binding flex with the amine molecules of chitosan to stabilize the matrix. In addition, TPP is less toxic compared to any other chemical crosslinker such as glutaraldehyde [28, 32, 33]. In this study, we aim to formulate the nanochitosan by using the medium molecular weight chitosan under acid environment. Molecular weight may affect the transfection ability and medium weight has notably to induce better bioavailability since it carries more amines and hydroxyl molecules inside. The dissolving solution acidity also enhances the protonation among the amine molecules [33, 34].

Few studies suggested the optimal ratio of chitosan: TPP is about 6 : 1 (Morris *et al*., 2011). Also, other study revealed the effective ratio for chitosan-PEG_{5k} : TPP : miRNA is 30 : 4 : 1 and optimized by fluorescence observation with DyS47 labelled miR-67 mimic. From this ratio, the obtained efficiency of encapsulation is 60% [30]. In this study, we used the nanochitosan formula optimized by Ysrafil *et al*. (2020) with 5:1 ratio of chitosan : TPP. The optimization ratio was tested on SKOV3 cells using cellular uptake measurement by labelling the miRNA with FAM-conjugated label [27].

Furthermore, chitosan nanoparticle exact size could be affected by chitosan concentration. To obtain less than 500 nm nanoparticle size, Katas *et al*. (2013) reduced the concentration about 0.05% [35]. Higher concentration (>0.25%) may induce spontaneous conservation of the formed particles as 1000 nm particles. To avoid the possibility of bigger size, we use the optimized 0.2% chitosan concentration according to Ysrafil study in 2020 [27]. However, the chitosan size particle also depends on the amount of TPP added in ionic gelation method [36]. We found the formula we referred from Ysrafil *et al*. (2020) still showed the less than 500 nm nanoparticle size as to be exact 458 nm.

Nanochitosan transfection optimization has also been shown by antimiR-fluorochrome labelled. The subtle illuminance on naked antimiR implied some of the oligonucleotides retracted under the positive charge given from the electrophoresis. Meanwhile, the encapsulated antimiR stayed at the well base. It indicated the predicted oligonucleotides entrapment by chitosan. The capsulation under polycationic chitosan engages the nucleic acids to form more positive charged particles. As response, the electrostatic energy from agarose gel failed to pull the nanocomplex downward. To analyze the predicted entrapment efficiency, we use the formula to measure the unencapsulated miRNA with nanoQuant. We obtained 96.7% of encapsulation efficiency from the nanocomplex formula. This high percentage may corelate with nearly condensate oligonucleotides by the nanochitosan matrix.

Results from the 24 hours incubation of MCF-7 cells after given 200 uμ nanochitosan formula showed that no significant cells diminishing compared to high glucose DMEM supplemented cells as control group (Fig.3). It implied that the cell viability remained normal and could grow. Compared to NaCl, unmodified chitosan has lower toxicity value (LD50: 16 g/kg while LD50 for NaCl is 3 g/kg) [14]. As reported by Kean and Thanou (2010), median lethal dose (LD50) and half maximal inhibitory (IC50) for chitosan and its derivates in all cells model is approximately 0.2-2.5 mg/mL [37]. It's much lower compared to any other biopolymer or liposome based vector.

We also assess the effect of antimiR chitosan encapsulation transfection on T47D cells after overnight incubation. Stender *et al*. (2007) reported by using biochemical and bioinformatics analysis to MCF-7 and MDA-MB-231 cells, E2F1 gene is associated with E2 activity during tumor progression [38]. However, since p21 gene as negative control is mostly suppressed by miR-106b-5p activity, the oncogene CIP2A could inhibit the phosphatase of phosphatase-tumor suppressor complex, PP21, and induces the E2F1 overexpression [39]. As shown in Fig 4, the E2F1 relative expression toward the GAPDH as stabile housekeeping gene, the significant value only marked on two different antimiR concentrations, at the lowest and highest concentration, compared to control group. Our in silico assessment showed the E2F1 is one of strong gene target of miR-106b-5p. E2F1 is transcription factor for numerous genes involved for DNA replications [40]. During the cycle cell, cyclin-dependent kinase (CDK) phosphorylated the tumor suppressor protein retinoblastoma protein (pRB) and released E2F1 for various genes could be expressed during DNA synthesis including mitosis [41]. Some studies also suggested the transcription of miR-106b-25 had E2F1 activity involved [42]. T47D cells as part of the luminal A type cell line along with MCF-7 express abundant ERα on the cytosolic membranes and the receptors as suggested by Louie *et al*. (2010), E2F1 as cell cycle transcription gene could be modulated by the ERα together with Sp-1 protein [43]. These findings indicate the plausible reasons of why our E2F1 level only decreased slightly.

CONCLUSION

In summary, we conclude that chitosan nanoparticle could be used as transfecting courier for nucleic acid such as microRNA into the cells without showing toxicity toward the cells. Furthermore, in vitro study revealed that antimiR-106b-5p conjugated with chitosan nanoparticle could induce the expression of E2F1 mRNA expression after transfection.

To verify the strong correlation after treatment, endogenous miR-106b-5p expression should be measured and plotting the values with the mRNA target. Fluorescence-labelled synthetic oligonucleotides (FITC) is also another alternative to evaluate the miRNA-loaded nanochitosan uptake into cells.

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AUTHORS CONTRIBUTIONS

First author is as graduate student, second author as consultant, third author as co-supervisor and fourth author as supervisor.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Kaban K, Salva E, Akbuga J. In Vitro Dose Studies on Chitosan Nanoplexes for microRNA Delivery in Breast Cancer Cells. Nucleic Acid Ther. 2017;27(1):45–55.
2. Sabit H, Cevik E, Tombuloglu H, Farag K, Said OAM, Shaimaa E, *et al*. miRNA Profiling in MCF-7 Breast Cancer Cells : Seeking a New Biomarker. J Biomed Sci. 2019;8(23):1–9.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
4. American Cancer Society. Global Cancer Facts & Figures 4th Edition. 2018; Atlanta : American Cancer Society.
5. World Health Organization. Breast cancer [Internet]. 2018 [cited 2020 Aug 26]. Available from: https://www.who.int/cancer/prevention/diagnosis-screening/breast-cancer/en/
6. Mollaei H, Safaralizadeh R, Rostami Z. MicroRNA replacement therapy in cancer. J Cell Physiol. 2019;(November 2018):1–16.
7. Nigussie Mekuria A, Degaga Abdi A, Mishore KM. MicroRNAs as a Potential Target for Cancer Therapy. J Cancer Sci Ther. 2018;10(06):152–61.
8. Ahmad, J. Hasnain S.E, Siddiqui M. S, Ahamed M, Musarrat J, Al-Khedhairy A. A. MicroRNA in carcinogenesis & cancer diagnostics: A new paradigm. Indian J Med Res [Internet]. 2013;137(4):680–94.
9. Peng B, Chen Y, Leong KW. MicroRNA delivery for regenerative medicine. Adv Drug Deliv Rev [Internet]. 2015;88:108–22.
10. Karimi M, Avci P, Ahi M, Gazori T, Hamblin MR, Naderi-Manesh H. Evaluation of Chitosan-Tripolyphosphate Nanoparticles as a p- shRNA Delivery Vector: Formulation, Optimization and Cellular Uptake Study. J Nanopharmaceutical Drug Deliv. 2013;1(3):1–28.

11. Arya G, Mankamna Kumari R, Sharma N, Gupta N, Chandra R, Nimesh S. Polymeric nanocarriers for site-specific gene therapy. In: Drug Targeting and Stimuli Sensitive Drug Delivery Systems. 2018. p. 689–714.
12. Wicki A, Witzigmann D, Balasubramanian V, Huwyler J. Nanomedicine in cancer therapy: Challenges, opportunities, and clinical applications. J Control Release [Internet]. 2015;200:138–57.
13. Flühmann B, Ntai I, Borchard G, Simoens S, Mühlebach S. Nanomedicines: The magic bullets reaching their target? Eur J Pharm Sci [Internet]. 2019;128(October 2018):73–80.
14. Cao Y, Tan YF, Wong YS, Liew MWJ, Venkatraman S. Recent advances in chitosan-based carriers for gene delivery. Mar Drugs. 2019;17(6).
15. Martien R, K Irianto ID, Farida V, Purwita Sari D, Farmasi Universitas Gadjah Mada F, Studi Rekayasa Biomedis M, et al. Perkembangan Teknologi Nanopartikel Sebagai Sistem Penghantaran Obat Technology Developments Nanoparticles As Drug Delivery Systems. Maj Farm [Internet]. 2012;8(1):133–44.
16. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, *et al*. Nano based drug delivery systems: Recent developments and future prospects. J Nanobiotechnology [Internet]. 2018;16(1):1–33.
17. Bahrami B, Hojjat-Farsangi M, Mohammadi H, Anvari E, Ghalamfarsa G, Yousefi M, *et al*. Nanoparticles and targeted drug delivery in cancer therapy. Immunol Lett. 2017;190(April):64–83.
18. Xu X, Ho W, Zhang X, Bertrand N, Farokhzad O. Cancer nanomedicine: From targeted delivery to combination therapy. Trends Mol Med [Internet]. 2015;21(4):223–32.
19. Aftab S, Shah A, Nadhman A, Kurbanoglu S, Aysıl Ozkan S, Dionysiou DD, *et al*. Nanomedicine: An effective tool in cancer therapy. Int J Pharm. 2018;540(1–2):132–49.
20. Ishii T, Okahata Y, Sato T. Mechanism of cell transfection with plasmid/chitosan complexes. Biochim Biophys Acta - Biomembr. 2001;1514(1):51–64.
21. Rodriguez A, del A, Angeles M. Non-Viral Delivery Systems in Gene Therapy. Gene Ther - Tools Potential Appl. 2013;3–34.
22. Santos-Carballal B, Aaldering LJ, Ritzefeld M, Pereira S, Sewald N. Physicochemical and biological characterization of chitosan- microRNA nanocomplexes for gene delivery to MCF-7 breast cancer cells. Nat Publ Gr [Internet]. 2015;(July):1–15.
23. Naskar S, Koutsu K, Sharma S. Chitosan-based nanoparticles as drug delivery systems : A review on two decades of research. J Drug Target [Internet]. 2018;
24. Mohammed MA, Syeda JTM, Wasan KM, Wasan EK. An Overview of Chitosan Nanoparticles and Its Application in Non-Parenteral Drug Delivery. Pharmaceutics. 2017;9(53):1–26.
25. Ding Y, Shen SZ, Sun H, Sun K, Liu F, Qi Y, *et al*. Design and construction of polymerized-chitosan coated Fe3O4 magnetic nanoparticles and its application for hydrophobic drug delivery. Mater Sci Eng C [Internet]. 2015;48:487–98.
26. Denizli M, Aslan B, Mangala LS, Jiang D, Rodriguez-aguayo C, Lopez-berestein G, *et al*. Chitosan Nanoparticles for miRNA Delivery. In: RNA Nanostructures: Methods and Protocols, Methods in Molecular Biology,. 2017. p. 219–30.
27. Ysrafil Y, Astuti I, Anwar LS, Martien R, Sumadi FAN, Wardhana T, *et al*. MicroRNA-155-5p Diminishes in Vitro Ovarian Cancer Cell Viability by Targeting HIF1α Expression. Adv Pharm Bull. 2020;10(4):630–7.
28. Rahiemna A., Megafitrah M, Ramadhani P, Mustikawaty A., Martien R. Formulasi

nanopartikel kitosan-PGV- Dengan Metode Ionic Gelasi. J Saintifika Gadjah Mada [Internet]. 2011;3(2011):17–22.
29. Adhyatmika A, Martien R, Ismail H. Preparasi Nanopartikel Senyawa Pentagamavunon-0 Menggunakan Matriks Polimer Kitosan Rantai Sedang dan Pengait Silang Natrium Tripolifosfat Melalui Mekanisme Gelasi Ionik Sebagai Kandidat Obat Antiinflamasi. Maj Farm. 2018;13(2):65.
30. Nguyen MA, Wyatt H, Susser L, Geoffrion M, Rasheed A, Duchez AC, *et al*. Delivery of MicroRNAs by Chitosan Nanoparticles to Functionally Alter Macrophage Cholesterol Efflux in Vitro and in Vivo. ACS Nano. 2019;13(6):6491–505.
31. Ragelle H, Vanvarenberg K, Vandermeulen G, Preat V. Chitosan Nanoparticles for SiRNA Delivery In Vitro. In: Methods in molecular biology (Clifton, NJ). 2016. p. 143-150.
32. Katas H, Alpar HO. Development and characterisation of chitosan nanoparticles for siRNA delivery. J Control Release. 2006;115(2):216–25.
33. Villegas-Peralta Y, López-Cervantes J, Madera Santana TJ, Sánchez-Duarte RG, Sánchez-Machado DI, Martínez-Macías M del R, *et al*. Impact of the molecular weight on the size of chitosan nanoparticles: characterization and its solid-state application. Polym Bull [Internet]. 2020;(0123456789).
34. Yang HC, Hon MH. The effect of the molecular weight of chitosan nanoparticles and its application on drug delivery. Microchem J [Internet]. 2009;92(1):87–91.
35. Katas H, Raja MAG, Lam KL. Development of chitosan nanoparticles as a stable drug delivery system for protein/siRNA. Int J Biomater. 2013;2013.
36. Morris GA, Castile J, Smith A, Adams GG, Harding SE. The effect of prolonged storage at different temperatures on the particle size distribution of tripolyphosphate (TPP)-chitosan nanoparticles. Carbohydr Polym [Internet]. 2011;84(4):1430–4.
37. Kean T, Thanou M. Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev [Internet]. 2010;62(1):3–11.
38. Stender JD, Frasor J, Komm B, Chang KCN, Kraus WL, Katzenellenbogen BS. Estrogen-regulated gene networks in human breast cancer cells: Involvement of E2F1 in the regulation of cell proliferation. Mol Endocrinol. 2007;21(9):2112–23.
39. Laine A, Westermarck J. Molecular Pathways : Harnessing E2F1 Regulation for Prosenescence Therapy in p53-Defective Cancer Cells. Clin Cancer Res. 2014;20(14):3644–51.
40. Petrocca F, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, *et al*. E2F1-Regulated MicroRNAs Impair TGFβ-Dependent Cell-Cycle Arrest and Apoptosis in Gastric Cancer. Cancer Cell. 2008;13(3):272–86.
41. Denechaud PD, Fajas L, Giralt A. E2F1, a novel regulator of metabolism. Front Endocrinol (Lausanne). 2017;8(NOV):1–8.
42. Wei CY, Tan QX, Zhu X, Qin QH, Zhu FB, Mo QG, et al. Expression of CDKN1A/p21 and TGFBR2 in breast cancer and their prognostic significance. Int J Clin Exp Pathol. 2015;8(11):14619–29.
43. Louie MC, McClellan A, Siewit C, Kawabata L. Estrogen receptor regulates E2F1 expression to mediate tamoxifen resistance. Mol Cancer Res. 2010;8(3):343–52.

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