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Review Article

Recent Advances in Chitosan-Based Gene Carrier Application and Design

Whinnery C* and Wolff Kirsch

Neurosurgery Center for Research, Loma Linda University, USA

***Corresponding author**

Whinnery C, Neurosurgery Center for Research, Loma Linda University, 11234 Anderson Street, Room A537, Loma Linda, California, ZIP: 92354, United States of America, Tel: 909-558-4000; Ext. 87860; Email: c.whinnery.01@gmail.com

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Abstract

A number of emerging therapies require the safe and effective delivery of engineered genetic molecules to target cells within the body. These molecules include plasmids for gene-based vaccinations and upregulation of specific gene expressions in addition to RNAs for the downregulation of certain gene products. Nanoparticles constructed from the biopolymer chitosan are recognized as potentially ideal non-viral vectors for intravenous transport and delivery of these molecules. The base nanoparticle design demonstrates a number of attractive advantages, such as high biocompatibility, low toxicity, and ease of production. Disadvantages include issues with specific cell targeting, transfection efficiency, and impaired dissociation of genetic materials from nanoparticle polymers. Ongoing research seeks to address these shortcomings and improve the general usability of chitosan nanoparticles. This review highlights and summarizes some of the most recent advancements in chitosan nanoparticle applications as well as innovations in nanoparticle design.

ABBREVIATIONS

NP: Nanoparticle; pDNA: Plasmid DNA; miRNA: microRNA; siRNA: Small interfering RNA; BMP-2: Bone Morphogenic Protein-2; TRBIV: Turbot Reddish Body Iridovirus; MP: Methylprednisolone; HSA: Human Serum Albumin; HBsAG: Hepatitis B surface Antigen; EGFR: Epidermal Growth Factor Receptor; MDR: Multi-Drug Resistance; MDR-1: MDR protein 1

INTRODUCTION

Chitosan is a cationic biopolymer derived through the acetylation of naturally-occurring chitin [1,2]. It is recognized as a potential component in various medical applications due to its multiple attractive properties, including biodegradability, biocompatibility, and a lack of toxicity and immunogenicity [2,3]. Critically, due to their natural positive charge, chitosan polymers easily complex with anionic molecules under mildly acidic conditions without the use of potentially harmful organic solvents [2,4]. This allows chitosan to form stable, nontoxic nanoparticles that incorporate genetic materials such as DNA plasmids (pDNA), microRNAs (miRNAs), and small interfering RNAs (siRNAs) [5-9].

Delivery of such genetic materials to target cells is a critical step for a number of medical applications, including siRNA silencing of genes critical to tumor growth, gene vaccines which supplant antigens with antigen-coding pDNAs, and pDNAs introduced to upregulate specific genes [2,8,10-14]. However, unprotected genetic materials are easily damaged *in vivo* [2, 12, 13]. Chitosan encapsulation is an effective means of protecting plasmids and RNAs during intravenous transit to the target cells and tissues and ehancing their subsequent uptake.

Chitosan-based gene carriers have attracted attention over the years and been examined extensively both *in vitro* and *in vivo* [15-17]. This review concerns itself with some of the most recent developments in nanoparticle application and design.

Recent advances in nanoparticle design

Despite their attractive properties, chitosan nanoparticles do suffer from a number of drawbacks. Multiple studies show that various cell types and tissues are poorly transfected by unmodified chitosan nanoparticles. A number of factors influence this phenomenon, including weak attachment of nanoparticles to the cell surface, impaired uptake of nanoparticles, failure to escape lysosomal degradation, and impaired release of genetic payloads from the nanoparticles, among others [2,8,18-21]. Many potential solutions to these issues have been and continue to be proposed, with the overall goal of enhancing genetic payload delivery to the target cells and tissues. The following are some of the most recent advances in this area.

Nanoparticle modifications to improve targeting, transfection, and payload release

Attachment of polyethylene glycol molecules to the surface of chitosan nanoparticles (PEGylation) is a popular strategy for extending NP circulatory half-lives after intravenous introduction. However, this modification may also prevent proper

uptake of the nanoparticles by tumor cells and risks prolonged overexposure of healthy cells. [8,22]. Surface modification of chitosan nanoparticles with specific ligands is a recognized method of increasing chitosan nanoparticle-to-cell targeting, binding, and transfection efficiency [16]. A recent study by Corbet et al., utilized RGDp as one such ligand in order to target tumor cells. RGDp preferentially binds to alpha-five-beta-three integrins, which are over expressed on several cancer cell types, including the endothelial cells which line tumor blood vessels [8, 23]. These cells would be the primary targets of the nanoparticle.

The study also examined the efficacy of a multiple siRNA payload designed to simultaneously target multiple metabolic pathways in cancer cells. Tumor heterogeneity and metabolic plasticity allow tumors to survive therapies which block a particular metabolic pathway by switching to another. To that end, expression levels of the MCT1 lactate and ASCT2 glutamine transporter expressions were targeted for downregulation.

Integrin-targeted and non-targeted nanoparticles delivered their siRNA payloads to small lung cancer cells with comparable efficiency *in vitro*. However, cells transfected by non-targeted nanoparticles showed poor downregulation of the target genes. The authors suggest that this is likely due to how the cells incorporate and process integrin-binding versus generalized endocytotic absorptions of non-targeted PEGylated nanoparticles.

In vivo results showed that SiHa tumor xenograft mice which received targeted nanoparticles with mixed siRNA payloads demonstrated a net decrease in tumor growth, whether the nanoparticles were administered through peritumoral injection or intravenous administration. This result was not seen in mice receiving non-targeted nanoparticles through either route. *Ex vivo* analysis confirmed that only targeted nanoparticles accumulated in tumor tissues and successfully suppressed target gene expression. The authors conclude that site-specific RDGp ligand binding and multiple pathway-targeting payloads are modifications of the base nanoparticle design which enhance the use of chitosan nanoparticles in treating multiple cancer types [8].

Gwak et al., report similar results for nanoparticles modified with the glucocorticoid methylprednisolone (MP) in order to target both glucocorticoid and nuclear receptors. *In vivo* administration of nanoparticles to a rat model of spinal cord injury demonstrated that the targeted NPs elicited improved gene expression compared to non-targeted NPs and naked plasmid. Notably, the highest levels of gene expression were seen three days post-injection. The authors suggest this is due to a delay in the disassociation of DNA from the chitosan polymers [24].

One method of enhancing payload release is to incorporate additional negatively-charged components into the nanoparticle to balance the overall charge and weaken the interactions between chitosan polymers and genetic material [2,25-27]. One such component is human serum albumin (HSA). A recent study by Lebre et al. analyzed the effects of incorporating HSA into a chitosan nanoparticle-based hepatitis B gene vaccine introduced through the nasal route. Notably, the group chose to adhere the plasmid to the nanoparticle surface rather than incorporating it into the structure as with the standard design methodology [2].

In vitro work in a human epithelial cell line indicated that nanoparticles with HSA surface modification significantly improved gene expression compared to unmodified control particles. In comparison, nanoparticles which incorporated HSA directly into their structure did not demonstrate significant improvement in expression. This appears to contradict the prediction that HSA incorporation would improve pDNA escape from nanoparticles. The group suggests that, as the pDNA is bound to the nanoparticle surface, the rate of release is not influenced by nanoparticle disintegration alone. It is suggested that HSA incorporation to destabilize the nanoparticle would not have as pronounced an effect under these circumstances. The group further proposes that the observed gene expression enhancement is due to surface HSA improving NP-cell adhesion but did not explore what effect surface-adhered HSA might have on surface-adhered pDNA release. Notably, for either nanoparticle type, plasmid release occurred within hours rather than days. The authors attributed this to the pDNA surface adherence. HSA modification appeared to have no effect on cellular uptake of nanoparticles [2].

In vivo work demonstrated that HSA modified nanoparticles carrying the hepatitis B surface antigen (HBsAg) gene, when introduced through the intranasal route of mice, successfully induced an antigen-specific immune response in multiple mucosal tissues. Naked HbsAg plasmid administered as a control did not elicit a similar effect. The immune response improved when hepatitis B surface antigen was co-administered with the nanoparticle treatment, indicating the potential of a combined treatment [2]. Notably, the study did not examine the efficacy of HSA-modified vs. unmodified nanoparticles *in vivo*. Despite the positive results indicated *in vitro*, the overall advantage of HSA as part of a chitosan-based gene vaccine has yet to be demonstrated.

Recent advances in chitosan nanoparticle application

A number of diseases exist for which current treatment options are problematic, insufficient, or essentially nonexistent. The attractive properties of chitosan as a gene carrier, with or without additional modifications, make it a subject of interest for groups seeking to develop novel, alternative solutions to address these needs. Many *in vivo* and *in vitro* studies seek to address the efficacy and efficiency of these proposed strategies. The following are highlights from some of the most recent advances in this area of study.

Prostate cancer

In terms of cancer-related death within the United States, prostate cancer is one of the leading killers among men. Metastasis of prostate cancer cells and their establishment within the boney tissues complicate treatment options. The primary issue is the development of resistance to traditional chemotherapeutic agents. [28-33]. One method of addressing this is through the use of tumor suppressive miRNAs. One potential advantage of this method is that a single miRNA species can target multiple

gene products within the tumor and, thus, multiple cellular pathways. This gives suppressive miRNAs a potential advantage over traditional chemotherapeutic agents which operate on a narrower range of targets [33]. Previous studies have identified miR-34a as a promising candidate miRNA for treating metastatic prostate cancer, due to its inhibition of prostate cancer growth in xenografts and its ability to target genes in both tumor cells and the bone. Within later-stage prostate cancers, miR-34 is found to be downregulated [33-37].

A recent study by Gaur et al. investigated whether chitosan nanoparticles carrying miR-34a could successfully inhibit the growth of bone-localized prostate cancer in a mouse model. Nanoparticles were generated with the standard ionic gelation technique, in which chitosan polymers are complexed with negatively charged tripolyphosphate and genetic material to be delivered. Nanoparticles incorporated either miR-34a or negative control miRNAs with a scrambled, non-interfering sequence. All nanoparticles were administered through tail injection.

The study reports that the miR-34a-loaded nanoparticles successfully induced apoptosis and non-canonical autophagy of tumor cells and impeded tumor growth within the bone. The anti-tumor effects were more pronounced than those seen in a subcutaneous model and the authors suggest this is due to the ability of miR-34a to target a gene which affects the bone microenvironment. Despite the apparent success, the study did not investigate the delivery efficiency or biodistribution of the nanoparticles. The authors suggest nonetheless that nanoparticle-based delivery of miR-34a may be an effective means of treating advanced prostate cancer [33].

Overcoming resistance to chemotherapeutic agents in tumors

Non-small cell lung cancer accounts for the majority of lung cancer cases and is often diagnosed only in the advanced stages. The standard first- line treatment regimen of platinum-derived chemotherapeutic agents, such as cisplatin, can be rendered ineffective by the development of resistance in tumors. Increasing the dosage to overcome this issue typically results in a worsening of non-specific side effects, such as neurotoxicity. Recently, Nascimento et al., explored the effectiveness of a combined therapy which utilized siRNA carried by chitosan nanoparticles to sensitize cancer cells to cisplatin. The gene targeted for knockdown, Mad2, is a mitotic protein that is mostly active in cells undergoing active division, such as cancer cells. PEGylated nanoparticles were targeted to epidermal growth factor receptors (EGFR) that are over expressed on the human lung adenocarcinoma line A549.

The study examined the effects of this treatment on mice with subcutaneous xenografts of cisplatin-resistant or non-resistant A549 cells. Treatment with targeted nanoparticles in conjunction with cisplatin showed the greatest inhibition of tumor growth inhibition in both the resistant and non-resistant mice. Nanoparticle-only treatments, both targeted and untargeted, showed a significant but lessened inhibition of both tumor types while cisplatin-only treatment affected only non-resistant tumors. Notably, the combined treatment was effective with only a sub-therapeutic dosage of cisplatin. Though nanoparticle accumulation in the liver and kidney was a concern, histological, enzymatic, and blood serum analysis did not reveal any indication of toxic effects [38].

Yhee et al., explored the use of a similar scheme in overcoming P-glycoprotein-mediated multi-drug resistance (MDR), in which a multitude of tumor types efflux various chemotherapeutic agents from their cells in order to prevent effective accumulation. Modified but untargeted chitosan nanoparticles were loaded with a siRNA targeting the expression of MDR protein 1 (MDR-1), the membrane transporter primarily responsible for the efflux mechanism. Subcutaneous mouse xenografts were established with an Andriamycin-resistant human breast cancer cell line. The study found that a combined therapy of nanoparticles and low-dose Andriamycin significantly reduced tumor volume compared to the full-dose Andriamycin-only treatment and controls. Importantly, the authors did not observe any considerable negative symptoms in the mice receiving the low-dose Andriamycin in the combined treatment, as opposed to mice receiving the full Andriamycin-only treatment. Western blot analysis showed that the siRNA effectively downregulated the expression of MDR-1, and the authors suggest it is this inhibition that subsequently sensitizes the cells and enables the enhanced effect of Andriamycin [39].

Periodontal disease and other alveolar bone defects

Periodontal disease results in the local degradation of alveolar bone, which leads to possible tooth loss and complicates the use of prosthetics [14,40]. Bone morphogenic protein-2 (BMP-2), an effective inducer of bone formation, is approved by the FDA for use in select bone-augmentative therapies. However, it is currently of limited clinical use due to its rapid diffusion and degradation *in vivo* [14,41]. On-site upregulation of BMP-2 expression is seen as a possible alternative to the introduction of naked BMP-2. In addition, thermosensitive hydrogels, which transition from liquid to solid phase at body temperature, are also designed to enhance the repair of dental defects [14,42-46].

A recent study by Li et al., examined a hybridized approach in which chitosan nanoparticles, loaded with BMP-2 plasmid or unloaded, were incorporated into a chitosan-based thermosensitive hydrogel. Both hydrogels successfully transitioned from liquid to gel *in vivo*. Nanoparticle-incorporating hydrogels demonstrated a reduced solidification time compared to hydrogels without nanoparticles. Rat calvarial defect models with cranial injuries showed improved bone formation when treated with nanoparticle-incorporating and non-incorporated hydrogels when compared untreated controls. Additionally, hydrogels incorporating plasmid-loaded nanoparticles showed the highest levels of new bone formation. Both gel types degraded well *in vivo* and were replaced by newly formed mature bone and connective tissue. Similar results were seen in a beagle periodontal disease model, including a corresponding increase in expression of ALP, an enzyme involved in bone mineralization and osteoblast differentiation [14].

Orally-administered DNA vaccines against diseases in fish aquacultures

DNA vaccines are an attractive method of inducing humoral and cell-mediated immunity due to their ease of production and relative safety, among other advantages. In order to generate an immune response, DNA plasmids coding for specific antigens must be transfected into target cells localized to the nucleus in order to enable translation of the proteins. While viral vectors are the most effective means of transporting the plasmid to the target cells, some safety concerns remain. Chitosan nanoparticles represent a safer alternate vector [2,12,13].

The attraction of non-viral DNA vaccines is not limited to applications in human medicine. Successful aquaculture of turbots (*Scophthalmus maximus*) is in high demand as the fish are regarded as a delicacy. However, turbots are vulnerable to the highly lethal turbot reddish body iridovirus (TRBIV). Commercial breeding efforts require the development of an effective treatment [47,48]. Oral vaccination is considered an especially attractive method due to its ease of administration and preventative nature [48,49]. The use of nanoparticles is suggested as a means for overcoming a number of drawbacks to oral vaccination, such as payload degradation and lack of bioavailability [48,50,51]. To this end, a recent study evaluated the immunization efficacy of an orally administered chitosan nanoparticle incorporating pDNA coding for the major capsid protein of TRBIV. Previous work has demonstrated that the plasmid is effective in generating an anti-TRBIV immune response and protective effect [48].

In vitro experiments showed successful transfection of the plasmid into TK cells. *In vivo* oral treatment of turbots resulted in a 68.1% survival rate at day 30 post-challenge, indicating successful protection against the virus when compared to controls. Detectable levels of anti-TRBIV antibodies were found at day 90 [48].

Likewise, Dubey et al., reported successful oral vaccination of the endangered fringed-lipped peninsula carp via a similar system. Carp immunized with nanoparticle-encapsulated plasmid coding for the recombinant outer membrane protein A (rOmpA) of *Edwardsiella tarda* demonstrated higher antigen-specific antibody levels in the serum than did carp vaccinated with an inactivated whole-cell vaccine. Carp which received no treatment or only blank nanoparticles did not generate any such detectable antibody response. The study reported significant difference in survival rates between the DNA vaccine and inactivated whole-cell vaccinated groups. In addition, *in vitro* tests showed that serum from DNA vaccine-treated carp more effectively inhibited the growth of live *E. tarda* colonies than did serum from inactivated whole cell vaccinated carp. Dubey et al., suggest at least part of this enhanced performance is due to the recognized adjuvant effect of chitosan, which has been shown to enhance both humoral and cell-mediated immune responses. The authors further suggest that this method of treatment would be useful in current efforts to preserve the fringe-lipped peninsula carp via aquaculture [52,53].

CONCLUSION

In conclusion, the use of chitosan nanoparticles as carriers of genetic material appears to be a viable approach for multiple therapies and is attracting significant attention. Though issues remain with design and implementation, new applications and refinements of this technology are emerging on a continual basis. Chitosan nanoparticles show potential and will continue to be investigated.

REFERENCES

1. Illum L. Chitosan and its use as a pharmaceutical excipient. Pharm Res. 1998; 15: 1326-133.
2. Lebre F, Borchard G, Faneca H, Pedroso de Lima MC, Borges O. Intranasal Administration of Novel Chitosan Nanoparticle/DNA Complexes Induces Antibody Response to Hepatitis B Surface Antigen in Mice. Mol Pharm. 2016; 13: 472-482.
3. Baldrick P. The safety of chitosan as a pharmaceutical excipient. Regul Toxicol Pharmacol. 2010; 56: 290-299.
4. Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS. Chitosan as a novel nasal delivery system for vaccines. Adv Drug Deliv Rev. 2001; 51: 81-96.
5. Borchard G. Chitosans for gene delivery. Adv Drug Deliv Rev. 2001; 52: 145-150.
6. Seo SH, Han HD, Noh KH, Kim TW, Son SW. Chitosan hydrogel containing GMCSF and a cancer drug exerts synergistic anti-tumor effects via the induction of CD8+ T cell-mediated anti-tumor imm... Clin Exp Metastasis. 2009; 26: 179-187.
7. Zhang S, Zhao B, Jiang H, Wang B, Ma B. Cationic lipids and polymers mediated vectors for delivery of siRNA. J Control Release. 2007; 123: 1-10.
8. Corbet C, Ragelle H, Pourcelle V, Vanvarenberg K, Marchand-Brynaert J, Preat V, et al. Delivery of siRNA targeting tumor metabolism using non-covalent PEGylated chitosan nanoparticles: Identification of an optimal combination of ligand structure, linker and grafting method. J Control Release. 2016; 223: 53-63.
9. Bugnicourt L, Ladavière C. Interests of chitosan nanoparticles ionically cross-linked with tripolyphosphate for biomedical applications. Progress in Polymer Science. 2016; 60: 1-17.
10. Iorns E, Lord CJ, Turner N, Ashworth A. Utilizing RNA interference to enhance cancer drug discovery. Nat Rev Drug Discov. 2007; 6: 556-568.
11. Sibley CR, Seow Y, Wood MJ. Novel RNA-based strategies for therapeutic gene silencing. Mol Ther. 2010; 18: 466-476.
12. Kutzler MA, Weiner DB. DNA vaccines: ready for prime time? Nat Rev Genet. 2008; 9: 776-788.
13. Huygen K. Plasmid DNA vaccination. Microbes Infect. 2005; 7: 932-938.
14. Li H, Ji Q, Chen X, Sun Y, Xu Q, Deng P, et al. Accelerated bony defect healing based on chitosan thermosensitive hydrogel scaffolds embedded with chitosan nanoparticles for the delivery of BMP2 plasmid DNA. J Biomed Mater Res A. 2016.
15. Roy K, Mao HQ, Huang SK, Leong KW. Oral gene delivery with chitosan--DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nat Med. 1999; 5: 387-389.

16. Bowman K, Leong KW. Chitosan nanoparticles for oral drug and gene delivery. Int J Nanomedicine. 2006; 1: 117-128.

17. Shukla SK, Mishra AK, Arotiba OA, Mamba BB. Chitosan-based nanomaterials: a state-of-the-art review. Int J Biol Macromol. 2013; 59: 46-58.

18. Brown MD, Schätzlein AG, Uchegbu IF. Gene delivery with synthetic (non viral) carriers. Int J Pharm. 2001; 229: 1-2.

19. Vaughan EE, DeGiulio JV, Dean DA. Intracellular trafficking of plasmids for gene therapy: mechanisms of cytoplasmic movement and nuclear import. Curr Gene Ther. 2006; 6: 671-678.

20. Adler AF, Leong KW. Emerging links between surface nanotechnology and endocytosis: impact on nonviral gene delivery. Nano Today. 2010; 5: 553-569.

21. Ragelle H, Colombo S, Pourcelle V, Vanvarenberg K, Vandermeulen G, Bouzin C, et al. Intracellular siRNA delivery dynamics of integrin-targeted, PEGylated chitosan-poly(ethylene imine) hybrid nanoparticles: A mechanistic insight. J Control Release. 2015; 211: 1-9.

22. Li SD, Huang L. Stealth nanoparticles: high density but sheddable PEG is a key for tumor targeting. J Control Release. 2010; 145: 178-178.

23. Danhier F, Le Breton A, Préat V. RGD-based strategies to target alpha(v) beta(3) integrin in cancer therapy and diagnosis. Mol Pharm. 2012; 9: 2961-2973.

24. Gwak SJ, Koo H, Yun Y, Yhee JY, Lee HY, Yoon do H, et al. Multifunctional nanoparticles for gene delivery and spinal cord injury. J Biomed Mater Res A. 2015; 103: 3474-3482.

25. Douglas KL, Piccirillo CA, Tabrizian M. Effects of alginate inclusion on the vector properties of chitosan-based nanoparticles. J Control Release. 2006; 115: 354-356.

26. Peng SF, Yang MJ, Su CJ, Chen HL, Lee PW, Wei MC, et al. Effects of incorporation of poly (gamma-glutamic acid) in chitosan/DNA complex nanoparticles on cellular uptake and transfection efficiency. Biomaterials. 2009; 30: 1797-1808.

27. Liao ZX, Peng SF, Chiu YL, Hsiao CW, Liu HY, Lim WH, et al. Enhancement of efficiency of chitosan-based complexes for gene transfection with poly(γ-glutamic acid) by augmenting their cellular uptake and intr... J Control Release. 2014; 193: 304-315.

28. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014; 64: 9-29.

29. Lee RJ, Smith MR. Targeting MET and vascular endothelial growth factor receptor signaling in castration-resistant prostate cancer. Cancer J. 2013; 19: 90-98.

30. Dayyani F, Gallick GE, Logothetis CJ, Corn PG. Novel therapies for metastatic castrate-resistant prostate cancer. J Natl Cancer Inst. 2011; 103: 1665-1675.

31. Thoreson GR, Gayed BA, Chung PH, Raj GV. Emerging therapies in castration resistant prostate cancer. Can J Urol. 2014; 21: 98-105.

32. Logothetis CJ, Gallick GE, Maity SN, Kim J, Aparicio A, Efstathiou E, et al. Molecular Classification of Prostate Cancer Progression: Foundation for Marker driven-Treatment of Prostate Cancer. Cancer Discov. 2013; 3: 849-846.

33. Gaur S, Wen Y, Song JH, Parikh NU, Mangala LS, Blessing AM, et al. Chitosan nanoparticle-mediated delivery of miRNA-34a decreases prostate tumor growth in the bone and its expression induces non-canonical autophagy. Oncotarget. 2015; 6: 29161-29177.

34. Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat Med. 2011; 17: 211-215.

35. Mudduluru G, Ceppi P, Kumarswamy R, Scagliotti GV, Papotti M, Allgayer H. Regulation of Axl receptor tyrosine kinase expression by miR-34a and miR-199a/b in solid cancer. Oncogene. 2011; 30: 2888-2899.

36. Yamamura S, Saini S, Majid S, Hirata H, Ueno K, Deng G, et al. MicroRNA-34a modulates c-Myc transcriptional complexes to suppress malignancy in human prostate cancer cells. PLoS One. 2012; 7: 29722.

37. Krzeszinski JY, Wei W, Huynh H, Jin Z, Wang X, Chang TC, et al. miR-34a blocks osteoporosis and bone metastasis by inhibiting osteoclastogenesis and Tgif2. Nature. 2014; 512: 431-435.

38. Nascimento AV, Singh A, Bousbaa H, Ferreira D, Sarmento B, Amiji MM. Overcoming cisplatin resistance in non-small cell lung cancer with Mad2 silencing siRNA delivered systemically using EGFR-targeted chitosan nanopar... Acta Biomater. 2017; 47: 71-80.

39. Yhee JY, Song S, Lee SJ, Park SG, Kim KS, Kim MG, et al. Cancer-targeted MDR-1 siRNA delivery using self-cross-linked glycol chitosan nanoparticles to overcome drug resistance. J Control Release. 2015; 198: 1-9.

40. von Arx T, Hardt N, Wallkamm B. The TIME technique: a new method for localized alveolar ridge augmentation prior to placement of dental implants. Int J Oral Maxillofac Implants. 1996; 11: 387-394.

41. Wang EA, Rosen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, et al. Recombinant human bone morphogenetic protein induces bone formation. Proc Natl Acad Sci U S A. 1990; 87: 2220-4.

42. Quirynen M, Teughels W, van Steenberghe D. Microbial shifts after subgingival debridement and formation of bacterial resistance when combined with local or systemic antimicrobials. Oral Dis. 2003; 9: 30-37.

43. Roberts MC. Antibiotic toxicity, interactions and resistance development. Periodontol 2000. 2002; 28: 280-297.

44. van Winkelhoff AJ, Herrera Gonzales D, Winkel EG, Dellemijn-Kippuw N, Vandenbroucke-Grauls CM, Sanz M. Antimicrobial resistance in the subgingival microflora in patients with adult periodontitis. A comparison between The Netherlands and Spain. J Clin Periodontol. 2000; 27: 79-86.

45. Southard GL, Godowski KC. Subgingival controlled release of antimicrobial agents in the treatment of periodontal disease. Int J Antimicrob Agents. 1998; 9: 239-253.

46. Sculean A, Nikolidakis D, Schwarz F. Regeneration of periodontal tissues: combinations of barrier membranes and grafting materials - biological foundation and preclinical evidence: a systematic review. J Clin Periodontol. 2008; 35: 106-116.

47. Shi CY, Wang YG, Yang SL, Huang J, Wang QY. The first report of an iridovirus-like agent infection in farmed turbot, Scophthalmus maximus, in China. Aquaculture. 2004; 236: 11-25.

48. Zheng F, Liu H, Sun X, Zhang Y, Zhang B, Teng Z, et al. Development of oral DNA vaccine based on chitosan nanoparticles for the immunization against reddish body iridovirus in turbots (*Scophthalmus maximus*). Aquaculture. 2016; 452: 263-267.

49. María Ángeles Esteban. An overview of the immunological defenses in fish skin. ISRN Immunology. 2012.

50. Otten GR, Schaefer M, Doe B, Liu H, Srivastava I, Megede Jz, et al. Enhanced potency of plasmid DNA microparticle human immunodeficiency virus vaccines in rhesus macaques by using a priming-boosting regimen with rec... J Virol. 2005; 79: 8189-8200.

51. Epstein JE, Charoenvit Y, Kester KE, Wang R, Newcomer R, Fitzpatrick S, et al. Safety, tolerability, and antibody responses in humans after sequential immunization with a PfCSP DNA vaccine followed by the recombinant protein vaccine RTS,S/AS02A. Vaccine. 2004; 22: 1592-603.

52. Dubey S, Avadhani K, Mutalik S, Sivadasan S, Maiti B, Girisha S, et al. Edwardsiella tarda OmpA Encapsulated in Chitosan Nanoparticles Shows Superior Protection over Inactivated Whole Cell Vaccine in Orally Vaccinated Fringed-Lipped Peninsula Carp (*Labeo fimbriatus*). Vaccines. 2016; 4: 40.

53. Zaharoff DA, Rogers CJ, Hance KW, Schlom J, Greiner JW. Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination. Vaccine. 2007; 25: 2085-2094.

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