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Research Article

The development of a novel ultrashort antimicrobial peptide nanoparticles with potent antimicrobial effect

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Abstract

Conventional antibiotics are facing significant microbial resistance, which has recently reached previously unnoticed critical levels. As a result of this situation, a large proportion of antimicrobial agents currently used in the clinic have significantly reduced therapeutic potential. Antimicrobial peptides (AMPs) may offer the medical community an alternative strategy to traditional antibiotics in the fight against microbial resistance. Current research efforts are focused on developing technologies that may reduce AMP toxicity while retaining their potent antimicrobial activity and possibly improving their delivery. The ionotropic gelation method was used to encapsulate a novel in-house designed potent ultrashort antimicrobial peptide (USAMP) into chitosan-based nanoparticles (CS-NPs) in this study. WRWRWR -CS-NPs were tested for antibacterial kinetics against two strains of Staphylococcus aureus for four days, and the developed WRWRWR -CS-NPs showed a 3-log decrease in the number of colonies when compared to CS-NP and a 5-log decrease when compared to control bacteria. Loaded WRWRWR into CS-NPs could represent an innovative approach to develop delivery systems based on NPs technology for achieving potent antimicrobial effects against multi-drug resistant and biofilm forming bacteria with negligible systemic toxicity and reduced synthetic costs that are obstructing the clinical development of AMPs generally.

Keywords

antibiotics, peptides, nanoparticles, chitosan, MRSA

Introduction

Bacterial resistance to conventional antibiotics has increased significantly in recent decades, owing to massive overuse and misuse of antibiotics, which was triggered and facilitated by the medical community (Reuter et al. 2016). The growing problem of microbial resistance has been linked to a significant decrease in the number of antibacterial drugs currently being developed and reaching the clinic. Antibiotic resistance has reached critical levels, with reports of the emergence of pan-resistant bacteria, setting the stage for humanity to enter the dreaded post-antibiotic era (Hey and Kesselheim 2017). As a result, there is an urgent need to develop new classes of antimicrobial compounds to limit the problems of microbial resistance. To combat the threat of bacterial and biofilm infections, global research is currently focused on finding novel alternative agents with different mechanisms of action rather than using conventional antibiotics (Carlet et al. 2014). In this regard, antimicrobial peptides (AMPs) have recently been one of the categories that have piqued the interest of researchers as potential alternatives to conventional anti-

biotics (Bochenska et al. 2015). Because of their unique therapeutic efficacy, AMPs are regarded as an excellent alternative. AMPs are amphipathic cationic peptides that are part of the innate host defense of many living eukaryotic organisms, including plants, bacteria, fungi, and yeast. AMPs are small (10 kDa) molecules with a variable length and amino acid sequence (10–50 amino acids) (Brunetti et al. 2016). AMPs have positive charges ranging from (+2 to +9) and contain more than 30% hydrophobic residues with amphipathic properties. When AMPs come into contact with plasma membranes or membrane mimics, they form amphipathic structures that allow them to induce pore formation. Unfortunately, despite the initial enthusiasm for AMPs as a potential antibiotic replacement, many obstacles have hampered their development and clinical use. These limitations are attributed to poor biological fluid stability as a result of inactivation by lipoproteins and anionic albumins. Furthermore, AMPs have poor antimicrobial target selectivity, resulting in undesirable interactions with host macromolecules and high systemic toxicity (Zasloff 2002).

Because of these constraints, the majority of current research efforts are focused on improving AMPs stability and reducing systemic toxicity. In this regard, the encapsulation of antimicrobial peptides by nanocarriers may represent an innovative approach to overcoming some of the issues associated with the limited clinical use of AMPs (Nguyen et al. 2011). Nanotechnology has advanced rapidly in a variety of fields, including infectious diseases and microbiology (Brandelli 2012). When compared to traditional drug-delivery systems, nanotechnology involves structures or materials sized 1 to 100 nanometers that exhibit a powerful and potential leverage in drug-delivery system purposes. Nanoparticles (NPs) are biodegradable materials with nanometric sizes ranging from natural to synthetic polymers and lipids. Because of their high biocompatibility, NPs can contain both hydrophilic and lipophilic drugs. Antimicrobial activity of NPs with loaded drugs may be synergistic. NPs must have specific drug delivery properties in order to be developed for drug delivery (de Jong 2008).

Chitosan (CS) is a high molecular weight biodegradable and biocompatible poly cationic polysaccharide (Soppimath et al. 2001). As a result, encapsulating AMPs in CS would create novel nano-therapeutics for the treatment of microbial infections, as well as potentially providing an alternative solution to the limitations that are currently impeding the clinical development of AMPs. Ultrashort Cationic antimicrobial peptides (USAMPs) with fewer than 8 residues are a promising class of AMPs that meet the criteria for novel antimicrobial drug development due to their unrivaled mode of action, which is due to their high diversity in terms of peptide length, amino acid sequence, and structure (Denkbas and Ottenbrite 2006). Furthermore, USAMPs exhibit high antimicrobial activity with negligible or very low toxicity to mammalian host cells. Developing USAMPs is also appealing due to the low production costs, low likelihood of resistance development, and low or negligible hemolytic toxicity associated with these peptides when compared to traditional AMPs. Because of the short sequence of these peptides, the production cost of these molecules is also advantageous. There have been no published studies that demonstrate the incorporation of Ultra-Short Cationic antimicrobial peptides into CS-NPs. The current study aims to assess the ability of CS to encapsulate USAMPs and the efficacy of incorporating USAMPs in chitosan nanoparticles as molecule carriers for maintaining USAMP activity while reducing mammalian cell toxicity.

Materials and methods

Materials

Medium molecular weight Chitosan (Mw 108 kDa) with deacetylation ~92% and sodium tripolyphosphate TPP were employed in all experiments (Sigma–Aldrich. USA).

The antimicrobial peptide employed in the present study is the novel ultrashort cationic antimicrobial peptides (USAMP) with six amino acid WRWRWR. The peptide was designed in house and has no similar to any other peptide deposited in official protein databases. The WRWRWR was obtained from (GL Biochem Ltd., Shanghai, China).

Acetic acid was purchased from Prolabo, France. Micro BCA Assay Kit was purchased from Thermo Scientific, USA. Mueller Hinton Agar was obtained from (Scharlap, S.L, Spain). Phosphate buffer saline (PBS) was purchased from Oxoid, England. Mammalian Vero cell line was purchased from (ATCC, USA). tryptone soy broth (TSB) (EcoBio, Hungary). MTT Formazan was purchased from Santa Cruz biotechnology, USA.

Instruments

Water Bath (Germany) , Elisa Plate Reader (Biotech USA) , Incubator (USA) , Hot plate (USA) , Liquid Chromatography-Mass Spectrometry (LC-MS) (Germany), Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) (Germany) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) (China).

Bacterial strains

The following bacterial strains were obtained from the American type tissue culture collection (ATCC) for the determination and testing of the WRWRWR - CS-NP: The control strain was Staphylococcus aureus (ATCC 29213). Staphylococcus aureus (ATCC BAA-41) was also used in the study, which was clinically isolated in a hospital in New York City in 1994 (MRSA).

Synthesis and purification of peptides

WRWRWR was synthesized using the solid-phase method and Fmoc chemistry, and it was purified using reverse phase high-performance liquid chromatography with an acetonitrile / H2O-TFA gradient. We used arginine (R) to display the positive charge of the peptide and use tryptophan (W) to increase the hydrophobicity of the peptide. The

peptide's identity was confirmed using ESI-MS mass spectrometry (GL Biochem Ltd., Shanghai, China).

Chitosan nanoparticles (CS-NPs) and WRWRWR -CS-NPs preparation

The CS-NPs and WRWRWR -CS-NPs were prepared using the simple ionic gelation method described previously, with minor modifications (Yang et al. 2011).To summarize, chitosan was dissolved in 1.75 percent (v/v) acetic acid in a final concentration of about (1 mg/ml), the pH was adjusted to 5 using 1 M sodium hydroxide (NaOH) , and TPP was dissolved in deionized water (1 mg/ml). 500 g of WRWRWR was added to the CS solution for WRWRWR -CS-NP preparation. Nanoparticles were formed after adding 1 mL of TPP aqueous solution dropwise to 5 mL of CS solution. Magnetic stirring was used during the formation, and the mixture was stirred at room temperature for 2 hours. Centrifugation at 2980 g for 2 hours purified the NPs suspension.

Nanoparticles (NPs) characterization

The hydrodynamic radius of NPs was measured using dynamic light scattering (DLS). At 20 degrees Celsius, 1 ml (0.5 mg/ml) of each sample was added to disposable polystyrene cuvettes. The developed NPs' zeta potential (ZP) was measured using a zetasizer ZS (Malvern, UK) at 25 °C in 10 mM phosphate buffer saline PBS, pH 7.4. The samples (0.5 mg/ml) were filtered through a 0.45 m filter unit before being injected into folded capillary cells.

The kinetics of nanoparticles (NPs) release *in vitro* and the evaluation of WRWRWR loading capacity

Following WRWRWR -CS-NPs purification, the amount of WRWRWR found in the supernatant was measured at 562 nm using the Micro BCATM protein assay according to the manufacturer's recommendations (Thermo Fisher, USA).

$$\text{The Encapsulation Efficiency (EE\%)} = \frac{\text{Loaded WRWRWR}}{\text{Total amount (500 µg)}} * 100$$

$$\text{Whereas, Loading content (L\%)} = \frac{\text{Loaded WRWRWR}}{\text{Dry Weight}} * 100$$

Purified WRWRWR -loaded CS-NPs were redispersed in 1 ml of PBS, pH 7.4, and stirred in a shaking incubator at 37 °C. At various time intervals, the sample was centrifuged at 2980 g for 2 hours at 4 °C, and 1 ml of fresh PBS medium was added to replace the collected supernatant. The amount of peptide released from the nanoparticle was determined using the Micro BCA protein assay (Barani et al. 2012).

MTT cell proliferation assay

Yellow tetrazolium (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) MTT is reduced to purple formazan inside the cell by reductase enzymes. As a result, only metabolically active cells can catalyze this reaction and generate the purple formazan crystals. Although these purple crystals are insoluble in water, they can be dissolved in Dimethyl Sulfoxide (DMSO). These crystals' generated color can be measured spectrophotometrically at 550 nm wavelength. Cells were seeded in 5 × 103 cells per well in a flat-bottomed 96-well plate for the MTT assay, and the plates were incubated for 18–24 h at 37 °C supplemented by 5% CO2 for the attached on the bottom of the plates. The following day, different concentrations of WRWRWR -CS-NPs were suspended in RPMI as the dissolving media and added to the cells in the plates (2.5, 5, and 10 mg/ml, loaded with 255, 510, and 1020 g/ml of WRWRWR, respectively). As a control, the untreated medium is used. The plates were incubated for 24 hours at 37 °C with 5% CO2 added. After 24 hours, 20 l of the MTT solution (2.5 mg/ml) was added to each well, and the plates were incubated for 2–5 hours at 37 °C, 5% CO2. The well content was removed after this incubation period (ensure that all the solution in the wells is removed). Each well received 100 l of DMSO, which was thoroughly mixed by pipetting to dissolve the Formazan crystals at the bottom of the wells until a clear purple color was obtained. The plates were then placed on an Elisa Microplate Reader (BioTek, USA) and the absorbance at = 550 nm was measured (Chen and Liang 2013).

Results

Peptide synthesis structure

We use three units of tryptophane and three unites of arginine amion acid to made our hexapetide after that we combined it with 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carboxylic acid to increase its efficacy, the structure of the peptide shown in Fig. 1.

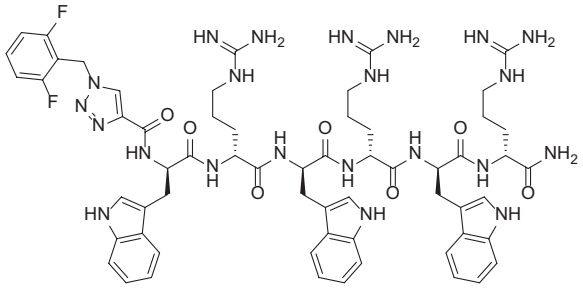

Figure 1. Structure of WRWRWR peptide.

CS-NPs and WRWRWR -CS-NPs Preparation and Characterization

The CS-NPs and WRWRWR -CS-NPs were made using a simple ionic gelation method. The average diameter of the formulated NPs was determined using dynamic light

scattering. The analysis clearly showed that WRWRWR loading increased the average diameter of the NPs. The average diameter of the CS-NP increased from 110.27 0.5 to 118.13 1.01 for the WRWRWR-CS-NP (Table 1). Both CS-NP and WRWRWR -CS-NP had positive Zeta potential values in PBS at pH 7.4 (Table 1), which was most likely due to the cationic nature of CS. The loaded WRWRWR significantly reduced the NP formulation's Zeta potential value.

Table 1. The characterization of CS-NPs and WRWRWR -CS-NPs: surface charge (Zeta potential) and average size diameter.

Formulation	Zeta potential (mV ± SD)	Size (nm ± SD)	PdI[a]
CS-NP	+ 42.3 ± 3.42	110.27 ± 0.5	0.255
WRWRWR **-CS-NP**	+ 33.2 ± 2.6	118.13 ± 1.01	0.272

[a] Polydispersity index of the diameter distribution peak.

WRWRWR loading capacity assessment

The encapsulation efficacy and loading capacity of WRWRWR into CS-NPs were measured at 562 nm using the Micro BCATM protein assay, as shown in Fig. 2 and (Table 2). The reported results for WRWRWR showed an encapsulation efficacy of 53.23 percent and a loading capacity of 10.11 percent, respectively.

Table 2. The characterization of CS-NPs and WRWRWR -CS-NPs: Encapsulation Efficacy (EE), Loading Capacity (L), and formulations yield (Yield).

Formulation	EE (% ± SD)	L (% ± SD)	Yield (% ± SD)
CS-NP	-	-	40.66 ± 1.25
WRWRWR **-CS-NP**	53.23 ± 1.52	10.11 ± 0.2	40.61 ± 0.55

The kinetics of NP release *in vitro*

For 14 days, the WRWRWR release kinetics from WRWRWR -CS-NPs were studied in PBS at pH 7.4. (Fig. 2). The best release model that mathematically fits with this profile, according to the DDsolver software program, is Korsmeyer-Peppas. For 14 days, the system exhibits a slowly released pattern followed by a progressive linear release.

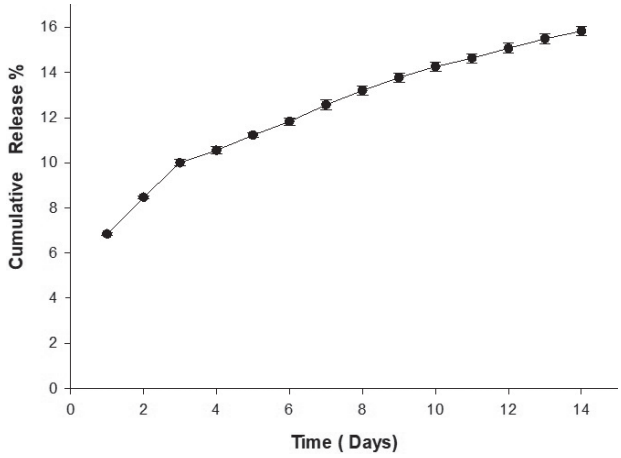

Figure 2. Release kinetics of the WRWRWR from 5 mg/ml WRWRWR -CS-NPs in PBS, pH 7.4.

Assay for bacterial susceptibility

Preparatory experiments were carried out in order to optimize the antibacterial activity of CS-NPs and WRWRWR -CS-NPs over long periods of time. The optimal concentration of TSB in PBS for sustaining bacterial growth for four days has been reported to be 1.25 percent (v/v). Furthermore, it was discovered that 5 mg/ml of RBRBR-CS-NPs was optimal for determining the formula's activity over a 4-day period. The 5 mg/ml of WRWRWR -CS-NPs displayed significant antimicrobial activity against all the studied bacterial strains. The encapsulated WRWRWR -NP was able to inhibit the growth of all the studied bacterial strains employed in this study. The antibacterial activity of WRWRWR -CS-NPs was assessed by measuring the cell viability, which was expressed as $\log_{10}$ CFU/ml for each tested bacterial strain. Then it was compared to that 5 mg/ml of CS-NPs and to the control (Table 3).

Table 3. Antibacterial activity of WRWRWR -CS-NPs as compared to CS-NPs and Control against MRSA *S. aureus* (ATCC 33591), (ATCC 43300), and clinically isolated MRSA *S. aureus* (ATCC BAA-41). (± SD).

ATCC	Log CFU/ml 5 mg/ml WRWRWR -CS-NP	Log CFU/ml 5 mg/ml CS-NP	Log CFU/ml Positive control
29213	2.51 ± 0.045	5.96 ± 0.052	10.79 ± 0.017
BA-41	3.11 ± 0.043	7.06 ± 0.026	9.02 ± 0.012

Assay for cell proliferation using MTT

The developed NP successfully reduced the toxicity of the same amount of loaded WRWRWR against normal cell lines. The results of the cytotoxicity assay revealed that RBRBR has an IC50 value of 187.2 g/ml (Fig. 3). The WRWRWR -CS-NP IC50 was reported to be 6.62 mg/ml.

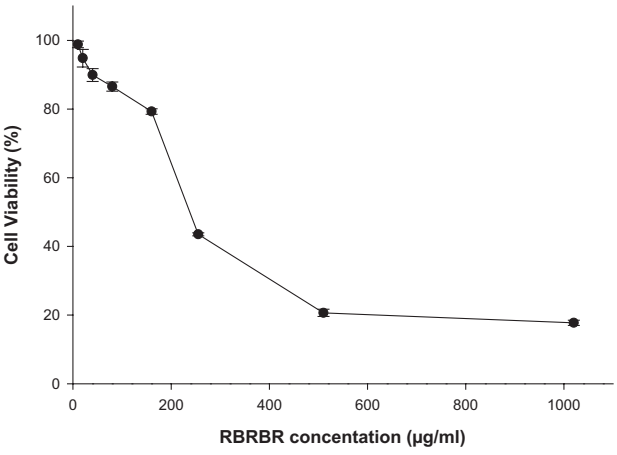

Figure 3. Cytotoxicity of free concentration WRWRWR after treatment with Vero mammalian cell line. The IC_{50} of free WRWRWR reported was 187.2 µg/ml after 24 h incubation.

Discussion

The current work aims to take advantage of NP advancement and USAMPs by encapsulating the in-house

designed novel USAMP named WRWRWR into CS-NPs to determine if the incorporation of WRWRWR into CS-NPs might retain the peptide's antimicrobial activity while reducing its toxicity. When we synthesis the peptide we take into consideration the balance between the hydrophilic and hydrophobic part, so we use arginine (R) to give the peptide the optimum charge (+3) and we use tryptophan to give the hydrophobicity of the peptide , also we comined our peptide with 1-(2, 6-difluorobenzyl)-1H-1, 2, 3-triazole-4-carboxylic acid to increase the antimicrobial activity of the peptide due to the good activity of triazole compound against different type of bacteria.

Ionic gelation was used to prepare the formulated WRWRWR -CS-NP, which was then tested for physio-chemical characterization, release kinetics, bacterial susceptibility, and cytotoxicity. Because of the positive charge of CS in acidic media, electrostatic repulsion with the cationic WRWRWR is possible (Almaaytah et al. 2012). To overcome the repulsion, the pH was set to 5 to reduce the positive charge of the CS. The zeta potential was calculated to better understand the interaction of charged molecules and to assess the stability of the nanosystem. Because of the cationic charge of CS, the expected positive value of zeta potential was observed. This value was significantly reduced (p 0.05) by loading WRWRWR into CS-NPs, which was attributed to CS charge conformation and rearrangement (Zhang et al. 2010). Furthermore, WRWRWR -CS-NPs have nanoscale dimensions and a statistically significant increase in average diameter when compared to blank NPs, demonstrating the incorporation of WRWRWR into CS-NP. Surprisingly, the nanosystem exhibits continuous progressive linear release over a long period of time. The novel WRWRWR was loaded into CS-NPs in this study, which provide high-quality features of an improved nanoscale system, such as a stable formula and kinetic release in a linear and controlled pattern (Pollini et al. 2017). The results of this study demonstrated that WRWRWR -CS-NPs was active against both the wild type and the multi-drug resistant clinical isolates of Gram-positive bacteria used in this study. WRWRWR loaded-CS-NPs demonstrated more potent and broad-spectrum antimicrobial effects against all tested bacterial strains, including resistant strains, when compared to empty CS-NPs. The peptide concentration released from the nanocarrier is responsible for the significant reduction in bacteria viability, according to the release pattern. As a result, the developed nanosystem used in this study may be capable of providing a synergistic mode of action in terms of antimicrobial effect that lasted for a continuous 4 days. Furthermore, WRWRWR -CS-NPs significantly inhibits the growth of multidrug resistant and clinical isolates of Gram-positive strains for all bacterial strains used in this study. MTT cell proliferation has achieved a perfect profile in terms of the role of nanosystem in the toxicity management of free WRWRWR. To illustrate, peptide incorporation into the nanocarrier significantly reduced the toxicity of USAMP against either normal cell lines or human erythrocytes. The positive charge and improved binding to microbial membranes can be attributed to the improvement in the toxicity profile and selectivity index in nanosystem formulation (Kolar et al. 2014). The positive charge influences the nanosystem's electrostatic binding to its target cell, facilitating the killing mechanism. Furthermore, increasing the drug's targeting to specific sites would improve efficacy while decreasing systemic toxicity.

Conclusion

The advancement of nanotechnology was used in this study to create an efficient drug delivering system that is an innovative therapeutic model based on CS-NPs loaded with novel USAMP to treat microbial resistance. The requirements characterizations of the nanosystem are excellent. The antimicrobial activity of WRWRWR -loaded CS-NPs in this study indicates that they have potent selective and long-acting activities against a wide range of Gram-positive bacteria, including clinical isolates of resistant strains. The MTT assay results show that WRWRWR loaded into nanocarriers has minimal toxicity against human erythrocytes and normal cell lines while displaying significant selectivity against microbial cells when compared to free WRWRWR. The encapsulation of AMPs in nanoparticles would be a novel and promising approach to AMP delivery.

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References

Almaaytah A, Zhou M, Wang L, Chen T, Walker B, Shaw C (2012) Antimicrobial/cytolytic peptides from the venom of the North African scorpion, *Androctonus amoreuxi*: Biochemical and functional characterization of natural peptides and a single site-substituted analog. Peptides 35(2): 291–299. https://doi.org/10.1016/j.peptides.2012.03.016

Barani H, Montazer M, Samadi N, Toliyat T (2012) In situ synthesis of nano silver/lecithin on wool: Enhancing nanoparticles diffusion. Colloids And Surfaces B: Biointerfaces 92: 9–15. https://doi.org/10.1016/j.colsurfb.2011.10.062

Bochenska O, Rapala-Kozik M, Wolak N, Kamysz W, Grzywacz D, Aoki W, Ueda M, Kozik A (2015) Inactivation of human kininogen-derived antimicrobial peptides by secreted aspartic proteases produced by the pathogenic yeast *Candida albicans*. Biological Chemistry 396(12): 1369–1375. https://doi.org/10.1515/hsz-2015-0167

Brandelli A (2012) Nanostructures as promising tools for delivery of antimicrobial peptides. Mini-Reviews in Medicinal Chemistry 12(8): 731–741. https://doi.org/10.2174/138955712801264774

Brunetti J, Roscia G, Lampronti I, Gambari R, Quercini L, Falciani C, Bracci L, Pini A (2016) Immunomodulatory and anti-inflammatory activity in vitro and in vivo of a novel antimicrobial candidate. Journal of Biological Chemistry 291(49): 25742–25748. https://doi.org/10.1074/jbc.M116.750257

Carlet J, Pulcini C, Piddock L (2014) Antibiotic resistance: a geopolitical issue. Clinical Microbiology and Infection 20(10): 949–953. https://doi.org/10.1111/1469-0691.12767

Chen L, Liang J (2013) Peptide fibrils with altered stability, activity, and cell selectivity. Biomacromolecules 14(7): 2326–2331. https://doi.org/10.1021/bm400618m

de Jong (2008) Drug delivery and nanoparticles: Applications and hazards. International Journal of Nanomedicine 3(2): 133–149. https://doi.org/10.2147/IJN.S596

Denkbas E, Ottenbrite R (2006) Perspectives on: Chitosan drug delivery systems based on their geometries. Journal of Bioactive and Compatible Polymers 21(4): 351–368. https://doi.org/10.1177/0883911506066930

Hey S, Kesselheim A (2017) Reprioritizing research activity for the post-antibiotic era: Ethical, legal, and social considerations. Hastings Center Report 47(2): 16–20. https://doi.org/10.1002/hast.685

Nguyen L, Haney E, Vogel H (2011) The expanding scope of antimicrobial peptide structures and their modes of action. Trends in Biotechnology 29(9): 464–472. https://doi.org/10.1016/j.tibtech.2011.05.001

Kolar SS, Luca V, Baidouri H, Mannino G, McDermott AM, Mangoni ML (2014) Esculentin-1A(1-21)NH2: A frog skin-derived peptide for microbial keratitis. Cellular and Molecular Life Sciences 72(3): 617–627. https://doi.org/10.1007/s00018-014-1694-0

Pollini S, Brunetti J, Sennati S, Rossolini G M, Bracci L, Pini A, Falciani C (2017) Synergistic activity profile of an antimicrobial peptide against multidrug-resistant and extensively drug-resistant strains of gram-negative bacterial pathogens. Journal of Peptide Science 23(4): 329–333. https://doi.org/10.1002/psc.2978

Reuter K, Steinbach A, Helms V (2016) Interfering with bacterial quorum sensing. Perspectives in Medicinal Chemistry 8: 1–15. https://doi.org/10.4137/PMC.S13209

Soppimath K, Aminabhavi T, Kulkarni A, Rudzinski W (2001) Biodegradable polymeric nanoparticles as drug delivery devices. Journal Of Controlled Release 70(1–2): 1–20. https://doi.org/10.1016/S0168-3659(00)00339-4

Yang S, Lin F, Tsai H, Lin C, Chin H, Wong J, Shieh M (2011) Alginate-folic acid-modified chitosan nanoparticles for photodynamic detection of intestinal neoplasms. Biomaterials 32(8): 2174–2182. https://doi.org/10.1016/j.biomaterials.2010.11.039

Zasloff M (2002) Antimicrobial peptides of multicellular organisms. Nature 415(6870): 389–395. https://doi.org/10.1038/415389a

Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S (2010) DDSolver: An add-in program for modeling and comparison of drug dissolution profiles. The AAPS Journal 12(3): 263–271. https://doi.org/10.1208/s12248-010-9185-1