International Core Journal of Engineering Volume 8 Issue 4, 2022
ISSN: 2414-1895 DOI: 10.6919/ICJE.202204_8(4).0094

Preparation and in Vitro Evaluation of Hydrogels Loaded with Doxorubicin

Huihui Zhang, Ting Zhu, Luping Ma, and Lin Qiu*

School of Pharmacy, Changzhou University, Changzhou, Jiangsu 213164, P. R. China

*linqiu@cczu.edu.cn.

Abstract

Cancer treatment has always been a huge challenge for human beings, and it is very important to explore the preparation of safe and effective anti-tumor drugs. Here, we report a polypeptide self-assembly to encapsulate doxorubicin nanosystem DOX@IKFLSVN, and physically characterize it. As a drug carrier, IKFLSVN is continuously connected to the required amino acids in Rink-Amide-MBHA-Resin, prepared by high performance liquid chromatography, and self-assembled to obtain a drug-loaded hydrogel DOX@IKFLSVN, and its in vitro drug release and biocompatibility has been investigated.

Keywords

Doxorubicin; Peptided Hydrogel; In Vitro Evaluation.

1. Introduction

As we all know, cancer has always been one of the main diseases that threated to health and caused death of human bings(1). When it comes to tumor disease, people always scared. Effective and safe monolithic approaches to cancer remain limited. Therefore, the treatment and overcoming of cancer are still the main problems and challenges faced by researchers(2).

The method of Cancer therapy includes chemotherapy, radiotherapy, cell therapy, etc(3). Among them, chemotherapy, as a traditional method, still occupies a pivotal position in the treatment of cancer. However, the commonly used cancer chemotherapy drugs have serious problems such as poor water solubility, large toxic and side effects, and no selective killing of cancer cells(4).

Anti-tumor antibiotics are chemical substances with anti-tumor activity produced by microbial metabolism, which are widely used in clinical practice(5). In recent years, with the understanding of the mechanism of tumor occurrence and development, the research on various anti-tumor antibiotics is also more in-depth(6). These antibiotics have strong anti-tumor activity and high selectivity. Among them, doxorubicin is one of the most broad-spectrum anti-tumor antibiotics, which can kill tumor cells in various growth cycles(7, 8). However, it will inevitably produce extensive biochemical effects on the body, and has a strong cytotoxic effect, thus causing great pain to patients(9).

Therefore, there is a more urgent need to find more effective and safer methods to apply to tumor therapy.

From this perspective, it is crucial to develop safe and efficient delivery vehicles, reduce the toxic and side effects of antitumor drugs, and relieve the suffering of cancer patients(10, 11). Polymer nanomaterials have many unique properties such as increasing water solubility of hydrophobic drugs(12), prolonging drug duration in plasma and tissues, preferential enrichment to tumor sites through enhanced permeability and retention effects, and reducing toxic side effects in humans(13). Therefore, more and more attention has been paid to the development of vectors to deliver anticancer drugs.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202204_8(4).0094

Due to its own characteristics (good biocompatibility, mechanical properties, water permeability and surface properties, etc.), hydrogels have a wide range of applications in the field of biomedicine(14), such as controlled release of drugs and tissue engineering(15). Qiu et al. reported the design of a synthetic peptide that blocks the binding site of the Nck protein and prevents infection of EPEC Caco-2 cells(16). Wang et al. reported pH-switchable antimicrobial hydrogels with nanofiber networks for biofilm eradication and rescue of stalled healing of chronic wounds(17). As an ideal drug-loaded hydrogel: when the desired drug is mixed with the hydrogel, it has certain mechanical properties after injection into the diseased site, binds firmly to the surrounding tissue, and remains stable for a period of time(18, 19) This hydrogel should also have excellent control, it can manipulate the formation of hydrogel in specific parts, precise release of the encapsulated drug, and release range of the drug in the hydrogel, thereby reducing the side effects caused by the drug(20).

2. Methods and Experiments

2.1 Materials

Fmoc-protected amino acids and coupling reagents were purchased from GL Biochem, Ltd. Rink amide resin was obtained from Biotage. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reagent), phosphate-buffered saline (PBS buffer), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. Dulbecco's modified eagle medium (DMEM) was obtained from Gibico.IKFLSVN were synthesized in our own laboratory. All experiments were performed with deionized (DI) water (Millipore Milli-Q grade, 18.2 MΩ).

2.2 Preparation of IKFLSVN

It was synthesized with 5 times equivalent amino acid of Rink-Amide-MBHA-Resin resin. First, weigh 5 times equivalents of amino acids, HOBT and HBTU simultaneously and dissolve them in DMF, then add DIEA to make the solution in an alkaline environment to promote the condensation reaction, and then add it to the resin immediately after mixing. Each condensation reaction was carried out under low-speed stirring for 40 min. After the reaction, 20% (v/v) piperidine/DMF solution was used to cleave the Fmoc protecting group at the N-terminal of the amino acid, and the reaction was continued for 30 min. This cycle continues until all amino acids are attached, and then the last amino acid is N-terminal acetylated with acetic anhydride/DMF solution and dried.

A certain amount of the above-mentioned resin is weighed, and TFA cutting solution is added to cut the polypeptide from the branch, which is precipitated with glacial ether, and dried to obtain a crude polypeptide. Add water to dissolve the crude polypeptide and filter, analyze the purity of the polypeptide by reverse-phase high performance liquid chromatography, and purify to obtain a polypeptide solution, which is freeze-dried to obtain IKFLSVN polypeptide solid powder.

2.3 Preparation of Peptided Hydrogel

The obtained IKFLSVN polypeptide powder was dissolved in ionized water at a concentration of 20 mg/mL, and it was completely dissolved by ultrasound. It was allowed to stand for a period of time to form a hydrogel, and deionized water was added to reduce the hydrogel concentration, and its critical gel-forming concentration was investigated.

Weigh a certain amount of doxorubicin hydrochloride and dissolve it in deionized water, so that the concentration of doxorubicin hydrochloride is 1 mg/mL, then weigh 4 mg of IKFLSVN polypeptide powder into the sample bottle, and then add 200 μL of the above-mentioned doxorubicin hydrochloride aqueous solution into the sample bottle. Ultrasound in the vial to completely dissolve the polypeptide powder in the doxorubicin hydrochloride aqueous solution.

2.4 In Vitro Drug Release

Prepare DOX@IKFLSVN polypeptide hydrogel according to the method of 2.2, add 2 mL of pH 5.5 and pH 7.4 phosphate buffer to the vial, take the supernatant at 0, 0.5, 1, 2, 4, 6, 24 hours to test the

ISSN: 2414-1895 DOI: 10.6919/ICJE.202204_8(4).0094

UV absorption at OD 490nm. At the site was taken and put back after sampling, and the in vitro release of DOX was calculated.

2.5 Hemolysis Assay

Take fresh mouse blood into an anticoagulant tube containing EDTA-K2, centrifuge at 1000 rpm for 10 min at 4 °C, remove the plasma, wash red blood cells with PBS(pH 7.4, 10mM), centrifuge at 1000 rpm for 10 min at 4 °C, repeat several times until the supernatant is reached. Clear and transparent, get clean red blood cells. And PBS was used to prepare a red blood cell suspension with a concentration of 20%.

The IKFLSVN protein was configured to 0.625, 1.25, 2.5, 5, and 10 mg/mL in a series of concentrations of 1 mL each in a 1.5 mL EP tube, and the positive control was 1% Triton, and the negative control was PBS solution. Add 20 μL of the prepared red blood cell suspension, incubate in a constant temperature incubator at 37 °C for 2 h, centrifuge at 1000 rpm and 4 °C for 10 min, take pictures, test the UV absorbance at OD 540 nm of the supernatant and calculate Hemolysis rate.

Hemolysis(%)=(ODsample-ODnegative control)/(ODpositive control-ODnegative control)*100%.

2.6 In Vitro Cell Cytotoxicity

Colon26 cells were cultured for more than three passages in DMEM high-glucose medium containing 10% fetal bovine serum and 1% penicillin-streptomycin. All the following operations are performed under sterile conditions.

1) Cytotoxicity of IKFLSVN

Weigh a certain amount of polypeptide powder, dissolve it in deionized water, and prepare a series of polypeptide solutions with concentrations of 0.625, 1.25, 2.5, 5, and 10 mg/mL in serum-free medium. The density was placed in a 96-well plate, 0.2 mL/well, so that the cells were dispersed at the bottom of the plate. After incubating in a 37°C, 5% CO2 incubator for 24h, discard the culture medium (n=5), add polypeptide hydrogels with different concentrations, and incubate in the incubator for 24h, add 20μL mtt solution (MTT method) and cells Incubate for 4-6 hours, then aspirate the culture medium, add 150 μL DMSO, shake well, and then measure the absorbance at 570 nm with a microplate reader.

2) In vitro antitumor activity of DOX@IKFLSVN

The in vitro anti-tumor cytotoxicity test of IKFLSVN, DOX@IKFLSVN, and free DOX was carried out according to the method in (1). The concentration of IKFLSVN was 1 mg/mL, and the concentration of DOX was 50 μg/mL. Then aspirate the culture medium, add 150 μL DMSO and shake well, and then measure the absorbance at 490 nm with a microplate reader, and calculate the influence of the samples on the cell development ability in different samples. The cell inhibition rate was calculated using the following formula:

$$\text{Cell inhibition ratio } (\%) = \left(1 - \frac{A}{B}\right) \times 100\%$$

Where A and B are the absorbance values from each sample and negative control, respectively.

3. Result and Discussion

The IKFLSVN polypeptide was synthesized by solid-phase synthesis, and the structure is shown in Figure 1. It can be seen from the liquid phase analysis spectrum before preparation (Figure 2) that only one peak appears in the synthesized product, indicating that there are few types of impurities in the synthesized product. It can be seen from the liquid phase analysis diagram after purification by the liquid preparation instrument that the peaks are basically single, and it can be concluded that the peptide has been purified successfully, and we have obtained pure IKFLSVN peptide.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202204_8(4).0094

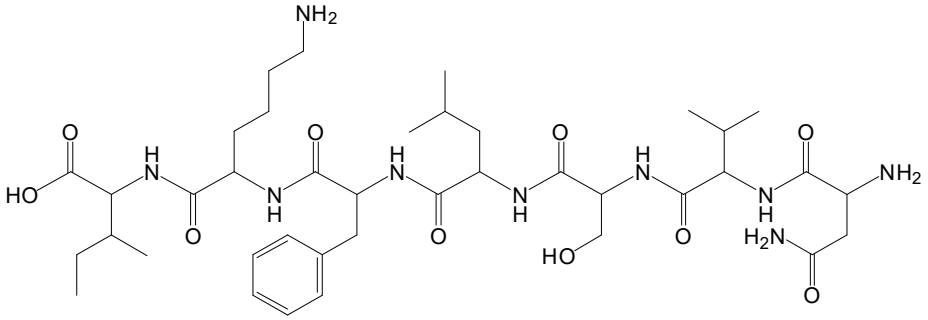

Figure 1. Schematic diagram of IKFLSVN structure.

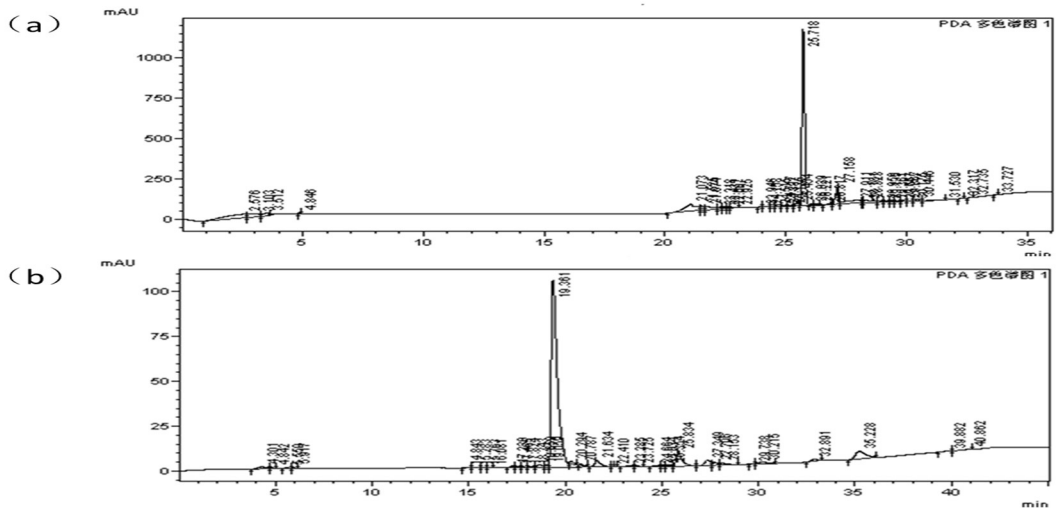

Figure 2. (a) Liquid phase analysis before preparation(b) Preparation and purification of IKFLSVN.

When preparing the polypeptide hydrogel, through the inversion method, it can be seen that the gelation effect is good, and the three-dimensional structure can still be maintained without being damaged when inversion (Figure 3a). As the peptide concentration decreased, the hydrogel started to have a mobile phase at 18 mg/mL (Fig. 3b). Similarly, it can be seen from the inversion method that the doxorubicin hydrochloride-encapsulated polypeptide hydrogel DOX@IKFLSVN can be obtained (Figure 4). At this time, the polypeptide concentration is 20 mg/mL and the doxorubicin hydrochloride concentration is 1 mg/mL. We successfully prepared doxorubicin-loaded peptide hydrogels.

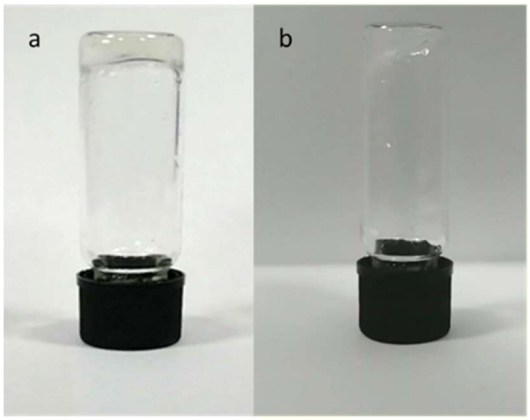

Figure 3. (a) Gelation diagram of IKFLSVN, 20mg/mL (b) Gelation diagram of IKFLSVN, 18mg/mL, the hydrogel starts to flow after inversion.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202204_8(4).0094

Figure 4. DOX@IKFLSVN gel formation diagram.

To quantitatively study the drug release of DOX@IKFLSVN, a 24-h drug release experiment was performed in PBS with different pH values (pH 5.5 and 7.4). It can be seen from the figure 5 that DOX can be quickly released from the gel network, and the lower the pH, the faster the drug release.

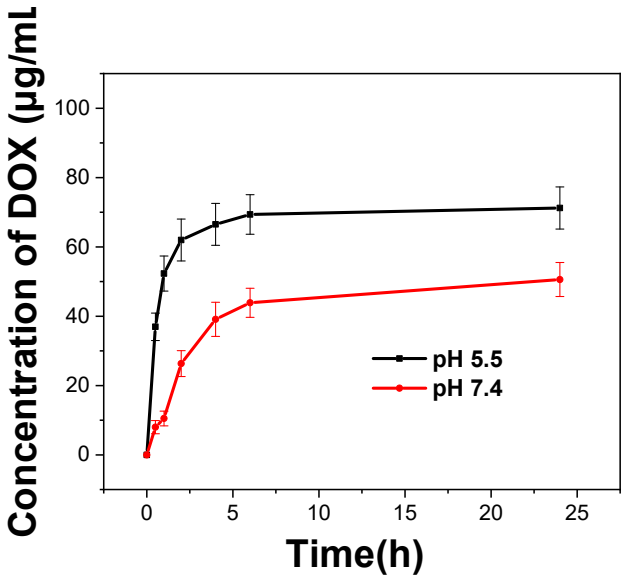

Figure 5. DOX release fromDOX@IKFLSVN at pH 7.4 phosphate buffer, pH 5.5 phosphate buffer, respectively.

Biocompatibility is an important indicator to examine the safety of materials, and hemolysis test is usually used as one of the criteria for evaluating biocompatibility to evaluate the degree of compatibility between materials and blood. When the hemolysis rate is less than 5%, the hemocompatibility of the material is considered to be good. According to the results shown in the figure 6, we calculated the hemolysis rate of a series of concentrations of IKFLSVN. When the concentration of IKFLSVN was as high as 10 mg/mL, the hemolysis rate was still lower than 5%, which proved that we successfully prepared peptide gels with good biosafety.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202204_8(4).0094

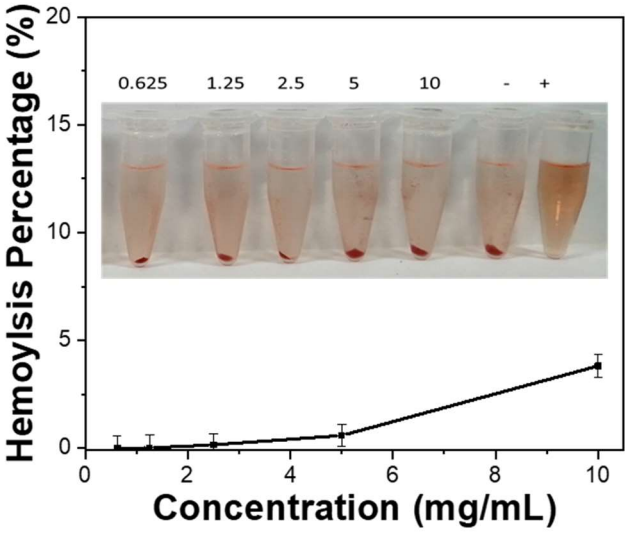

Figure 6. Hemolysis assay of IKFLSVN in PBS. Triton X-100 and PBS were set as positive and negative controls, respectively.

The results of the cytotoxicity test showed that when the polypeptide concentration reached 2.5 mg/mL, the survival rate of Conlon26 was less than 80% (Fig. 7a), and cell death was observed under the microscope (Fig. 7b). When we choose the peptide concentration of 1 mg/mL for in vitro anti-tumor experiments, the inhibition rate of Doxorubicin-coated peptide on Conlon26 is 30% higher than that of pure peptide. At this time, IKFLSVN has no toxicity to CT26 cells, which is comparable to free doxorubicin. The doxorubicin-coated peptide hydrogel exhibited a 10% higher inhibition rate of cells compared with tetrazolium (Figures 7c and d), thus proving that DOX@IKFLSVN has a good antitumor activity.

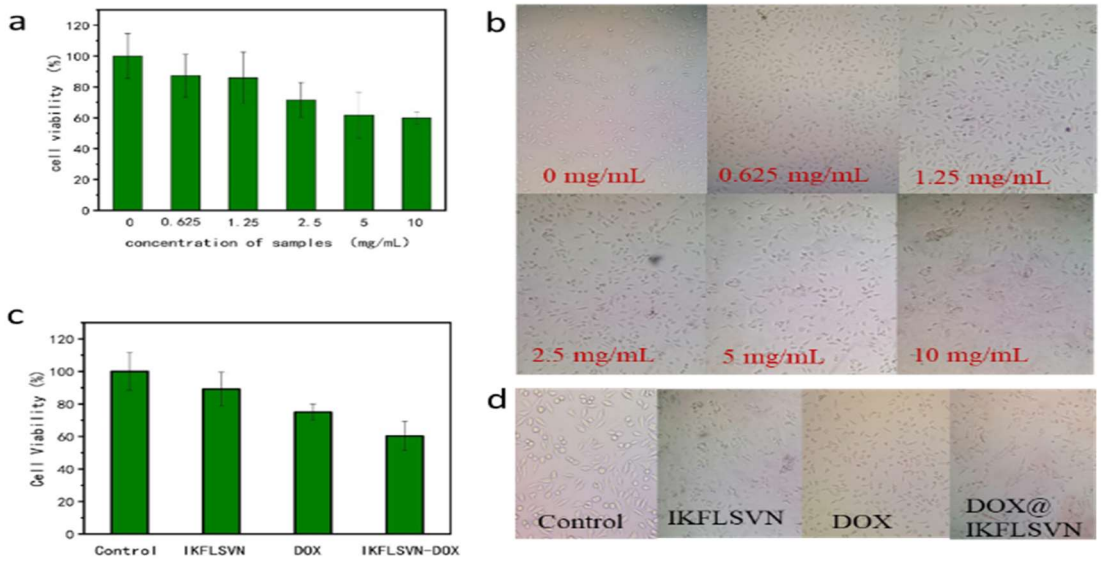

Figure 7. (a) Cytotoxicity of different concentrations of IKFLSVN to Colon 26 (b) Different concentrations of IKFLSVN incubate Colon 26 under the microscope (c) IKFLSVN , DOX@IKFLSVN, the cytotoxicity of free DOX and control to Colon 26 (d) IKFLSVN, DOX@IKFLSVN, free DOX and control incubate Colon 26 cell morphology under microscope.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202204_8(4).0094

4. Conclusion

Doxorubicin can interact with the small molecule polypeptide IKFLSVN to promote the self-assembly of the small molecule polypeptide through the positive-negative charge interaction to form the doxorubicin drug-loaded small molecule hydrogel, which effectively improves the solubility and encapsulation rate of doxorubicin. In vitro drug release experiments showed that the formation of doxorubicin-loaded small molecule hydrogel effectively avoided the sudden release of doxorubicin, and the release rate was related to the pH of the release solution. The lower the pH, the faster the release. Small molecule polypeptides have good biocompatibility, and the in vitro anticancer effect of doxorubicin can be effectively improved by the doxorubicin drug-loaded small molecule polypeptide nanofiber system. MTT experiments showed that compared with free doxorubicin, the doxorubicin-loaded small molecule polypeptide nanofiber system enhanced the cytotoxic effect of doxorubicin on CT26, which provided the possibility for tumor therapy.

References

[1] Song GS, Cheng L, Chao Y, Yang K, Liu Z. Emerging Nanotechnology and Advanced Materials for Cancer Radiation Therapy. Advanced Materials. 2017;29(32).

[2] Joosse SA, Gorges TM, Pantel K. Biology, detection, and clinical implications of circulating tumor cells. Embo Molecular Medicine. 2015;7(1):1-11.

[3] Gobbo OL, Sjaastad K, Radomski MW, Volkov Y, Prina-Mello A. Magnetic Nanoparticles in Cancer Theranostics. Theranostics. 2015;5(11):1249-63.

[4] Gonzalez-Fierro A, Duenas-Gonzalez A. Drug repurposing for cancer therapy, easier said than done. Seminars in Cancer Biology. 2021;68:123-31.

[5] Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, et al. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Research. 2011;39:W339-W46.

[6] Dadsetan M, Liu Z, Pumberger M, Giraldo CV, Ruesink T, Lu LC, et al. A stimuli-responsive hydrogel for doxorubicin delivery. Biomaterials. 2010;31(31):8051-62.

[7] An H, Yang Y, Zhou ZW, Bo YY, Wang Y, He YN, et al. Pectin-based injectable and biodegradable self-healing hydrogels for enhanced synergistic anticancer therapy. Acta Biomaterialia. 2021;131:149-61.

[8] Mei LX, Xu KM, Zhai ZR, He SY, Zhu TT, Zhong WY. Doxorubicin-reinforced supramolecular hydrogels of RGD-derived peptide conjugates for pH-responsive drug delivery. Organic & Biomolecular Chemistry. 2019;17(15):3853-60.

[9] Pugazhendhi A, Edison T, Velmurugan BK, Jacob JA, Karuppusamy I. Toxicity of Doxorubicin (Dox) to different experimental organ systems. Life Sciences. 2018;200:26-30.

[10] Gaharwar AK, Peppas NA, Khademhosseini A. Nanocomposite Hydrogels for Biomedical Applications. Biotechnology and Bioengineering. 2014;111(3):441-53.

[11] Yang KR, Han Q, Chen BP, Zheng YH, Zhang KS, Li Q, et al. Antimicrobial hydrogels: promising materials for medical application. International Journal of Nanomedicine. 2018;13:2217-63.

[12] Lai YX, Wang LJ, Liu Y, Yang GJ, Tang CL, Deng Y, et al. Immunosensors Based on Nanomaterials for Detection of Tumor Markers. Journal of Biomedical Nanotechnology. 2018;14(1):44-65.

[13] Li Z, Tan SR, Li S, Shen Q, Wang KH. Cancer drug delivery in the nano era: An overview and perspectives (Review). Oncology Reports. 2017;38(2):611-24.

[14] Norouzi M, Nazari B, Miller DW. Injectable hydrogel-based drug delivery systems for local cancer therapy. Drug Discovery Today. 2016;21(11):1835-49.

[15] Li JL, Xing RR, Bai S, Yan XH. Recent advances of self-assembling peptide-based hydrogels for biomedical applications. Soft Matter. 2019;15(8):1704-15.

[16] Qiu J, Nie Y, Zhao Y, Zhang Y, Li L, Wang R, et al. Safeguarding intestine cells against enteropathogenic Escherichia coli by intracellular protein reaction, a preventive antibacterial mechanism. Proc Natl Acad Sci U S A. 2020;117(10):5260-8.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202204_8(4).0094

[17] Wang J, Chen XY, Zhao Y, Yang Y, Wang W, Wu C, et al. pH-Switchable Antimicrobial Nanofiber Networks of Hydrogel Eradicate Biofilm and Rescue Stalled Healing in Chronic Wounds. ACS Nano. 2019;13(10):11686-97.

[18] Li J, Mo LT, Lu CH, Fu T, Yang HH, Tan WH. Functional nucleic acid-based hydrogels for bioanalytical and biomedical applications. Chemical Society Reviews. 2016;45(5):1410-31.

[19] Liu XY, Sun XB, Liang GL. Peptide-based supramolecular hydrogels for bioimaging applications. Biomaterials Science. 2021;9(2):315-27.

[20] Qi Y, Min H, Mujeeb A, Zhang Y, Han X, Zhao X, et al. Injectable Hexapeptide Hydrogel for Localized Chemotherapy Prevents Breast Cancer Recurrence. ACS Appl Mater Interfaces. 2018;10(8):6972-81.