A Tasquinomod-loaded dopamine-modified pH sensitive hydrogel is effective at inhibiting the proliferation of KRAS mutant lung cancer cells

Jun Xu1*, Chuxi Zhang2*, Chun Cheng1,3, Jun Yang1,3, Chenxi Li1,3, Xia Liu4 and Yi Sang1,3

Abstract
Hydrogels can maintain a high local drug concentration during treatments and may be useful to local targeting diseased areas. We propose a pH sensitive hydrogel consisting of poly-vinylpyrrolidone (PVP) and chitosan as a new treatment method for KRAS mutant lung cancer. Addition of dopamine improved the drug loading and release effects of this hydrogel. We demonstrate that Tasquinimod-loading of this dopamine-modified pH sensitive hydrogel is more effective than Tasquinimod alone for inhibiting the proliferation of KRAS mutant lung cancer cells. Combination of conventional drugs with hydrogels may thus provide a new treatment modality for lung cancer.

Keywords
Hydrogels, cancer, Tasquinimod, KRAS mutant lung cancer

Date received: 21 July 2021; revised: 10 December 2021; accepted: 28 December 2021

Introduction
More than 1.5 million people die from lung cancer worldwide each year.1,2 A recent study suggests that a third of the patients with the most prominent subtype of lung adenocarcinoma carry a KRAS mutation.3–6 Current clinical treatment for KRAS mutant lung cancer is based around chemotherapy, but traditional chemotherapy has the several shortcomings, including adverse effects. Moreover, using chemotherapy, a high drug concentration in focal tumor tissues cannot be guaranteed and therefore frequent injections are required to maintain effective drug concentrations.7–10 In order to address these problems and to improve treatment efficiency, we proposed a pH sensitive hydrogel system modified by dopamine and loaded with chemotherapeutic drugs.

Hydrogels are hydrophilic polymers with excellent in vitro and in vivo biocompatibility.11–15 Current applications based on these properties range from cell culture to biological scaffolds and injection drug delivery.16–19 Here, we designed a dopamine-modified pH sensitive hydrogel based on chitosan and polyvinylpyrrolidone (PVP) (Figure 1), which accelerates the release of drugs in the low pH environment of tumor cells.20–23 The adhesion of the material is increased due to the addition of dopamine: the catechol structure of dopamine and the hydrogel material are easily oxidized to a quinone or semiquinone structure when combined, followed by Michael addition or Schiff base reaction

1The First Hospital of Nanchang, Nanchang, Jiangxi, P.R. China
2The First Clinical Medical College of Nanchang University, Nanchang, Jiangxi, P.R. China
3Jiangxi Key Laboratory of Cancer Metastasis and Precision Treatment, Department of Center Laboratory, Nanchang, Jiangxi, P.R. China
4Department of Medical Oncology, Guangzhou First People’s Hospital, School of Medicine, South China University of Technology, Guangzhou, China
*These authors contributed equally to this work and should be considered co-first authors.
Corresponding author:
Yi Sang, The First Hospital of Nanchang, Nanchang, Jiangxi, Nanchang City, Jiangxi Province, 330006, China.
Email: ndsfy001889@ncu.edu.cn

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
after which amino or sulfhydryl groups in the hydrogel appear and adhesion and cohesion are enhanced. Therefore, during treatment, high local drug concentration at the tumor site can be maintained, the drug influx into the systemic circulation is reduced, and side effects of chemotherapy are reduced. But at the same time, the application field of hydrogels is facing some challenges. For example, Christensen LH, Breiting VB and Lloyd AW, Faragher RG, etc. found that the long-term application of hydrogels will have a certain impact on normal cell activities in tissues. Therefore, while taking advantage of the sustained-release drug properties of hydrogels, we shorten the treatment time of hydrogels as soon as possible to reduce adverse effects.

**Materials and methods**

**Synthesis of materials**

Synthesis of the hydrogel: 0.15 g chitosan, 15 mL secondary water, and 2 mL H+ (3 mol/L) were added to a 5 mL round bottom flask and stirred well. 0.13 g of polyvinylpyrrolidone were added, stir, and vacuumized. 0.2 mL of formaldehyde (37%) were added and the reaction was left at room temperature for 24 h.

Dopamine cross-linking: 0.050 g of dopamine were added to 10 mL of Tris-HCl solution (0.05 mol/L) and stirred to dissolve. 1.0 g of previously synthesized hydrogel was added to the solution, soaked at room temperature for 48 h, filtered, and dried for later use.

Swelling Property of Hydrogel: Equal volume of freeze-dried hydrogels (10 g) were immersed in the PBS (pH 7.45/6) at 37°C. A total of 80 min of testing was carried out, and the hydrogel was taken out and weighed every 10 min until the end of the 80-min experiment (n = 3). The swelling ratio was calculated by the following equation: Swelling ratio (%) = (Wt–W0)/W0 × 100%.

Where Wt represents the weights of swelling hydrogels, and W0 is the initial weights of freeze-dried hydrogels.

Hemolysis rate experiments: 1 mL of prepared red blood cells (Cell density: 4.5 × 10^7/mL) were mixed with 3.67 mL of physiological saline. The solution was centrifuged at 1500 rpm for 15 min. This process was repeated three times to isolate a supernatant free from red blood cells. Four types of hydrogels were mixed with 1 mL of normal saline. After the hydrogel was fully dissolved, add 100 μL of each solution to 1 mL of physiological saline to prepare the test solution. 0.9% NaCl solution (1 mL) was used as a negative control, and deionized water (1 mL) was used as a positive control. Add 100 μL of the prepared red blood cell suspension to each group and incubate for 1 h (37°C). Centrifuge the samples for 15 min (300 rpm) and add 100 μL of each sample to a 96-well plate. The microplate reader measures the result at 540 nm. The rate of hemolysis (%) was calculated as = (As–An)/(Ap–An) × 100%, where Ap represents the OD value of the positive control group, As and An are the OD values of the experimental group and the negative control group respectively.
Cytotoxicity tests: 10 mL human umbilical vein endothelial cells (HUVEC, Cell density: 1 × 10^5/mL) were cultured in RPMI1640 medium and DMEM. All cells were maintained at 37°C in an atmosphere of 5% CO2 and 95% humidity and culture media contained 10% FBS. Cells were incubated for 24 h then seeded into 96-well plates. The same quality of the four types of hydrogels were added to the corresponding medium, sterilize after filtration. Then 1 mg/mL sterile mother liquor was obtained. Add 100 µL of sterile mother liquor to each well and co-cultured with the cells for 1, 3, and 5 days. Finally, a CCK-8 (Focus Bioscience, Shanghai) assay was used to quantify the cell viability, and the optical density (OD) cell viability (%) was measured as OD result/OD control × 100%.

Live-dead cell staining: we used Calcein-AM/PI double staining reagent for live-dead cell staining. First prepare the working solution needed for staining (2 µmol solution A, Calcein-AM; 8 µmol solution B, PI). Take 5 µL 16 mmol solution B stock solution and add it to 10 mL of PBS solution, well mix. Obtain 8 µmol solution B working solution. Add 5 µL 4 mmol solution A stock solution to the 10 mL solution B working solution from the previous step, and make sure to mix well. The obtained mixed working solution (2 µmol solution A, Calcein-AM; 8 µmol solution B, PI) can be directly used for staining cells. Working solution was added to the hydrogel cells of the four experimental groups, incubated for 30 min and then washed with 10 µL PBS. Observe under the microscope (emission wavelength: 490 nm).

Drug-loaded release of materials: pH sensitive and insensitive hydrogels loaded with Tasquinimod (10 µmol) were placed in two pH gradients of 10 mL PBS (5.5/7.35) and allowed to release for 16 days. Every 2 days, 100 µL of the solution from each group were drawn with a pipette, added to a 96-well plate, and 100 µL of PBS were added immediately after extraction to supplement the experimental solution. Results were quantified using a microplate reader (for each experimental group, we set up three identical samples. n = 3).

In vitro test of tumor suppressive effect of materials: The experimental components were four groups, including: dopamine modified pH sensitive hydrogel + 2.5 µmol Tasquinimod, dopamine modified pH sensitive hydrogel + 10 µmol Tasquinimod, 5 µmol Tasquinimod, and 10 µmol Tasquinimod. To measure the activity of tumor cells, four groups of hydrogel groups loaded with antitumor drug Tasquinimod were co-cultured with tumor cells in vitro (for each experimental group, we set up three identical samples. n = 3; and the number of cells in each sample: 8 × 10^6).

Cell cycle tests: Cells were seeded in a six-well plate at 1.5 × 10^6 cells per well and allowed to grow to logarithmic growth phase. After 50%–80% adherence, cells were changed to the drug-containing culture medium (three wells per treatment). After incubation for the corresponding time, flow cytometry was conducted with the number of cells analyzed per sample being approximately 10^6.

Cell processing: Cells were treated with trypsin and neutralized with serum-containing medium, centrifuged at 2000 rpm for 5 min, resuspended and washed with pre-cooled PBS. The supernatant was carefully aspirated, leaving about 50 µL.

Cell fixation: Cells were fully resuspended in 1 mL PBS. The cell suspension was gently vortexed while slowly adding 3 mL of pre-cooled absolute ethanol to a final concentration of 75%, incubated at 4°C overnight (18–24 h), and stored at −20°C for 1 month prior to flow cytometry. Fixed cells were washed with pre-cooled PBS twice, centrifuging at 2000 rpm for 5 min, and resuspended in 200 µL PBS, resulting in a final volume of about 400 µL. To reduce cell loss, 1.5 mL centrifugation volumes were used. The bottom of the centrifuge was gently tapped to properly disperse the cells and avoid cell clumps. 20 µg/L RNase, were added to the resuspended cells and cells were incubated in a 37°C water bath for 30 min. 20 µL of propidium iodide (PI) were added to a final concentration of 50 µg/mL and samples were stained for 30 min at 4°C under exclusion of light. For flow cytometry, which was conducted within 24 h of staining, cells were mixed thoroughly and filtered through a 200-mesh filter. Red fluorescence was detected using an excitation wavelength of 488 nm. Light scattering was assessed the same time. The appropriate analysis software was used for cell DNA content analysis and light scattering analysis.

Apoptosis test: Cells were washed twice with cold PBS buffer and resuspended in 1× Binding Buffer to make a suspension of 1 × 10^6 cells/mL. 100 µL of the cell suspension were added to a Falcon test tube, gently mixed, and placed in a dark place at room temperature (20°C–25°C) for 15 min. Cells were washed once with 1× Binding Buffer and the supernatant was removed. 0.5 µg of the SAv-FITC reagent were dissolved in 100 µL of 1× Binding Buffer, added to the tube and gently mixed. 5 µL PI were added and cells were stained at room temperature (20°C–25°C) under exclusion of light for 15 min. 400 µL of 1× Binding Buffer were added to each test tube and apoptosis was quantified on a flow cytometer within 1 h.

Results and discussion

Characterization of materials

We constructed the experimental hydrogel via chemical cross-linking. Digital photographs in Figure 2(a) visualize the brown color of the hydrogel with dopamine, which is obviously different from other transparent and white hydrogels. The hydrogel base is a pure PVP and chitosan hydrogel. The experimental components were four groups, including: dopamine modified pH sensitive hydrogel + 2.5 µmol Tasquinimod, dopamine modified pH sensitive hydrogel + 10 µmol Tasquinimod, 5 µmol Tasquinimod, and 10 µmol Tasquinimod. To measure the activity of tumor cells, four groups of hydrogel groups loaded with antitumor drug Tasquinimod were co-cultured with tumor cells in vitro (for each experimental group, we set up three identical samples. n = 3; and the number of cells in each sample: 8 × 10^6).

Cell cycle tests: Cells were seeded in a six-well plate at 1.5 × 10^6 cells per well and allowed to grow to logarithmic growth phase. After 50%–80% adherence, cells were changed to the drug-containing culture medium (three wells per treatment). After incubation for the corresponding time, flow cytometry was conducted with the number of cells analyzed per sample being approximately 10^6.

Cell processing: Cells were treated with trypsin and neutralized with serum-containing medium, centrifuged at 2000 rpm for 5 min, resuspended and washed with pre-cooled PBS. The supernatant was carefully aspirated, leaving about 50 µL.

Cell fixation: Cells were fully resuspended in 1 mL PBS. The cell suspension was gently vortexed while slowly adding 3 mL of pre-cooled absolute ethanol to a final concentration of 75%, incubated at 4°C overnight (18–24 h), and stored at −20°C for 1 month prior to flow cytometry. Fixed cells were washed with pre-cooled PBS twice, centrifuging at 2000 rpm for 5 min, and resuspended in 200 µL PBS, resulting in a final volume of about 400 µL. To reduce cell loss, 1.5 mL centrifugation volumes were used. The bottom of the centrifuge was gently tapped to properly disperse the cells and avoid cell clumps. 20 µg/L RNase, were added to the resuspended cells and cells were incubated in a 37°C water bath for 30 min. 20 µL of propidium iodide (PI) were added to a final concentration of 50 µg/mL and samples were stained for 30 min at 4°C under exclusion of light. For flow cytometry, which was conducted within 24 h of staining, cells were mixed thoroughly and filtered through a 200-mesh filter. Red fluorescence was detected using an excitation wavelength of 488 nm. Light scattering was assessed the same time. The appropriate analysis software was used for cell DNA content analysis and light scattering analysis.

Apoptosis test: Cells were washed twice with cold PBS buffer and resuspended in 1× Binding Buffer to make a suspension of 1 × 10^6 cells/mL. 100 µL of the cell suspension were added to a Falcon test tube, gently mixed, and placed in a dark place at room temperature (20°C–25°C) for 15 min. Cells were washed once with 1× Binding Buffer and the supernatant was removed. 0.5 µg of the SAv-FITC reagent were dissolved in 100 µL of 1× Binding Buffer, added to the tube and gently mixed. 5 µL PI were added and cells were stained at room temperature (20°C–25°C) under exclusion of light for 15 min. 400 µL of 1× Binding Buffer were added to each test tube and apoptosis was quantified on a flow cytometer within 1 h.
after addition of dopamine (Figure 2(b) and (c)). This suggests that the addition of dopamine results in faster crosslinking speed and means that the material can quickly set at the tumor growth site, preventing it from shifting to non-treatment sites.

**Acidic pH improves the drug-release capacity of the hydrogel**

A key test for assessing the performance of a hydrogel is quantifying its drug release capacities. We obtained the drug release curve of the material by measuring the OD value of the Tasquinimod using a microplate reader. We tested both neutral and acidic pH environments, the latter mimicking the microenvironment surrounding the tumor to reflect physiological conditions for the use of the material. The release profile was quantified during a 16-day continuous release process. This revealed that the drug release rate is relatively slow at neutral pH levels, with release of approximately 45% of the total loading by day 16. Conversely, in acidic pH levels, the drug release rate is accelerated and the total release is greater, reaching about 70% by day 16 (Figure 2(d)). These data suggest a certain pH sensitivity of the material and highlight that it can release drugs quickly and in large quantities in the tumor microenvironment which tend to have a low pH, but not in tissues with normal pH. We tested the swelling properties of the hydrogel. As shown in Figure 2(e), the swelling ability of hydrogel in acidic environment is worse than that under normal human pH. This shows the effect of acidic conditions on our hydrogels.

**The hydrogel has no effect on normal cell proliferation**

Biological compatibility testing is particularly important in settings of clinical application. We first tested the hemolysis rate of the materials, which showed that the hemolysis rate of the hydrogel was below 5% in all four experimental groups, suggesting that each group of materials exhibits biological safety (Supplemental Figure S1). Next, we conducted cytotoxicity experiments to further explore the safety of the materials. The four materials were mixed and cultured with human umbilical vein endothelial cells for five days and the cytotoxicity of the materials was assessed. The HUVEC survival rate of all groups was above 90% for all days, highlighting that the material exhibits good biocompatibility (Figure 3(a)). Finally, we performed live-dead cell

---

**Figure 2.** Characterization of hydrogels. (a) Digital pictures of each type of hydrogel. From top to bottom: PVP hydrogel, chitosan hydrogel, PVP + chitosan hydrogel, PVP + chitosan + Tasquinimod hydrogel, PVP + chitosan + dopamine hydrogel. (b) Picture of the PVP + chitosan + dopamine + Tasquinimod hydrogel. (c) Comparison of the formation time of each type of hydrogel, *p < 0.05. **p < 0.01. ***p < 0.001. (d) Release of the drug from the PVP + chitosan + dopamine hydrogel at different pH levels, *p < 0.05. **p < 0.01. ***p < 0.001. (e) Swelling ratio of PVP + chitosan + dopamine + Tasquinimod hydrogel. Data are presented as mean ± SD (n = 3).
staining and found that all four experimental materials showed excellent biocompatibility in human umbilical vein endothelial cells (Figure 3(b)). In summary, these data illustrate the biological safety of materials and support the clinical application of our materials.

The proliferation of A549 cells is effectively inhibited through low-concentration Tasquinimod combined hydrogel

Tasquinimod effectively inhibits the proliferation of nasopharyngeal carcinoma, but there are no reports on its suitability in KRAS mutant lung cancer. We therefore conducted a gradient test for four different drug concentrations of Tasquinimod (0, 2.5, 5, and 10 µmol, and assessed cytotoxicity and viability in treated A549 KRAS mutant lung cancer cells after 5 days. Tasquinimod at 2.5 µmol resulted in an inhibition of tumor viability greater than 30%, with higher concentrations exhibiting even more pronounced inhibitory effects (Figure 4(a); Supplemental Figure S2).

Compared with the traditional one-time injection method, the advantage of hydrogel lies in its sustained release: a superior treatment effect can be achieved by maintaining high drug concentrations in the local treatment environment. We therefore compared hydrogel sustained release to one-time administration in order to explore treatment efficacy. To assess the release properties of the hydrogel, we conducted tests using two gradients of 2.5 and 10 µmol. Using different concentrations of hydrogels and direct administration for treatment of KRAS mutant lung cancer cells in vitro, we found that the hydrogel offers a superior inhibitory effect than one-time administration and exhibits a higher inhibitory peak, which showed a significant inhibitory effect at 2.5 µmol but even more pronounced inhibitory effects with 10 µmol (Figure 4(b)). Moreover, we found that the survival rate of tumor cells in the hydrogel treatment group was lower than in the corresponding control group (Supplemental Figure S3). In addition, we conducted a clone formation experiment to compare the hydrogel drug delivery group with the direct one-time administration group. This revealed that the drug significantly inhibited cell cloning, and the inhibitory effect was more obvious in the hydrogel drug-loaded group (Figure 4(c)–(e)).

The cell cycle of A549 cells is effectively inhibited through low-concentration Tasquinimod combined hydrogel

Finally, we explored the cell cycle stages in each experimental group by flow cytometry. The ability of the cells to divide was weaker in the Tasquinimod group than in the
control group. The cell cycle in the Tasquinimod group was elongated, and the G1 and S phases of the cell were altered (Figure 5(a) and (b)), which may be related to the mode of action of the drug.

**Conclusions**

The pH sensitive hydrogel developed by us is based on polyvinylpyrrolidone (PVP) and chitosan. Addition of dopamine to the hydrogel provides it with superior gel-forming and supporting properties: due to the addition of dopamine, the cross-linked hydrogel can set faster than the base hydrogel and load a larger amount of drugs without an impact on its pH sensitivity. The research in this report shows that the drug Tasquinimod can inhibit the proliferation of KRAS mutant lung cancer cells, and this effect is elevated when used in combination with the PVP-chitosan-dopamine pH responsive hydrogel system. This novel approach of treating KRAS mutant lung cancer through biological materials provides new avenues for clinical treatment with advantages such as elevated efficiency and fewer side effects.

**Figure 4.** In vitro drug treatment. (a) The effect of drugs under different concentration gradients on the survival rate and inhibition rate of lung cancer cells for five consecutive days. (b) Inhibitory rate and survival rates of lung cancer cells following 2.5 and 10 µmol of the drug under single administration or hydrogel loading administration, *p < 0.05. **p < 0.01. ***p < 0.001. (c and d, e) Clone formation rate and quantitative graphs, *p < 0.05. **p < 0.01. ***p < 0.001. Data are presented as mean ± SD (n = 3).
Authors’ note

The article is original and is consistent with the requirements of this journal. It has been written by the stated authors who are all-aware of its content and approve its submission. It has not been published previously and is not under consideration for publication elsewhere.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Chuxi Zhang https://orcid.org/0000-0002-7480-9908

Supplemental material

Supplemental material for this article is available online.

References

1. Nasim F, Sabath BF and Eapen GA. Lung cancer. Med Clin North Am 2019; 103(3): 463–473.
2. Bade BC and Dela Cruz CS. Lung cancer 2020: epidemiology, etiology, and prevention. Clin Chest Med 2020; 41(1): 1–24.
3. Uras IZ, Moll HP and Casanova E. Targeting KRAS mutant non-small-cell lung cancer: past, present and future. Int J Mol Sci 2020; 21(12): 4325.
4. Wu HZ, Xiao JQ, Xiao SS and Cheng Y. KRAS: a promising therapeutic target for cancer treatment. Curr Top Med Chem 2019; 19(23): 2081–2097.
5. Uprety D and Adjei AA. KRAS: from undruggable to a druggable cancer target. Cancer Treat Rev 2020; 89: 102070.
6. Yang H, Liang SQ, Schmid RA and Peng RW. New horizons in KRAS-mutant lung cancer: Dawn after darkness. Front Oncol 2019; 9: 953.
7. Ferrer I, Zugazagoitia J, Herbertz S, John W, Paz-Ares L and Schmid-Bindert G. KRAS-mutant non-small cell lung cancer: from biology to therapy. Lung Cancer 2018; 124: 53–64.
8. He H, Xu C, Cheng Z, Qian X and Zheng L. Drug combinatorial therapies for the treatment of KRAS mutated lung cancers. Curr Top Med Chem 2019; 19(23): 2128–2142.
9. Nagasaka M, Li Y, Sukari A, Ou SI, Al-Hallak MN and Azmi AS. KRAS G12C game of thrones, which direct KRAS inhibitor will claim the iron throne? Cancer Treat Rev 2020; 84: 101974.
10. Drosten M and Barbacid M. Targeting the MAPK pathway in KRAS-driven tumors. Cancer Cell 2020; 37(4): 543–550.
11. Zhao H, Feng H, Liu J, et al. Dual-functional guanosine-based hydrogel integrating localized delivery and anticancer activities for cancer therapy. Biomaterials 2020; 230: 119598.
12. Rezk AI, Obiweluozor FO, Choukrani G, Park CH and Kim CS. Drug release and kinetic models of anticancer drug (BTZ) from a pH-responsive alginate polydopamine hydrogel: towards cancer chemotherapy. Int J Biol Macromol 2019; 141: 388–400.
13. Mayorova OA, Jolly BCN, Verkhovskii RA, Plastun VO, Sindeeva OA and Douglas TEL. pH-Sensitive dairy-derived hydrogels with a prolonged drug release profile for cancer treatment. Materials 2021; 14(4): 749.
14. Łabowska MB, Cierluk K, Jankowska AM, Kulbacka J, Detyna J and Michalak I. A review on the adaption of alginate-gelatin hydrogels for 3D cultures and bioprinting. Materials 2021; 14(4): 858.

15. Song H, Huang P, Niu J, et al. Injectable polypeptide hydrogel for dual-delivery of antigen and TLR3 agonist to modulate dendritic cells in vivo and enhance potent cytotoxic T-lymphocyte response against melanoma. Biomaterials 2018; 159: 119–129.

16. Lin H, Yin C, Mo A and Hong G. Applications of hydrogel with special physical properties in bone and cartilage regeneration. Materials 2021; 14(1): 235.

17. Nan L, Yang Z, Lyu H, Lau KYY and Shum HC. A microfluidic system for one-chip harvesting of single-cell-laden hydrogels in culture medium. Adv Biosyst 2019; 3(11): e1900076.

18. Oliver-Urrutia C, Rosales Ibañez R, Flores-Merino MV, et al. Lyophilized polyvinylpyrrolidone hydrogel for culture of human oral mucosa stem cells. Materials 2021; 14(1): 227.

19. Li K, Zhu Y, Zhang Q, Shi X, Liang F and Han D. A self-healing hierarchical fiber hydrogel that mimics ECM structure. Materials 2020; 13(22): 5277.

20. Ghosh G, Barman R, Sarkar J and Ghosh S. pH-Responsive bio compatible supramolecular peptide hydrogel. J Phys Chem B 2019; 123(27): 5909–5915.

21. Gao W, Zhang Y, Zhang Q and Zhang L. Nanoparticle-hydrogel: a hybrid biomaterial system for localized drug delivery. Ann Biomed Eng 2016; 44(6): 2049–2061.

22. Wu RS, Lin J, Xing YM, Dai ZL, Wang LW and Zhang XP. pH-Sensitive black phosphorous-incorporated hydrogel as novel implant for cancer treatment. J Pharm Sci 2019; 108(8): 2542–2551.

23. Nimal TR, Baranwal G, Bavya MC, Biswas R and Jayakumar R. Anti-staphylococcal activity of injectable nano tigecycline/chitosan-PRP composite hydrogel using drosophila melanogaster model for infectious wounds. ACS Appl Mater Interfaces 2016; 8(34): 22074–22083.

24. Yang M, Lee SY, Kim S, et al. Selenium and dopamine-crosslinked hyaluronic acid hydrogel for chemophotothermal cancer therapy. J Control Release 2020; 324: 750–764.

25. Hendi A, Umar Hassan M, Elsherif M, et al. Healthcare applications of pH-sensitive hydrogel-based devices: a review. Int J Nanomedicine 2020; 15: 3887–3901.

26. Norouzi M, Nazari B and Miller DW. Injectable hydrogel-based drug delivery systems for local cancer therapy. Drug Discov Today 2016; 21(11): 1835–1849.

27. Sun Z, Song C, Wang C, Hu Y and Wu J. Hydrogel-based controlled drug delivery for cancer treatment: a review. Mol Pharm 2020; 17(2): 373–391.

28. Xue B, Qu Y, Shi K, et al. Advances in the application of injectable thermosensitive hydrogel systems for cancer therapy. J Biomed Nanotechnol 2020; 16(10): 1427–1453.

29. Ciocacu DE, Nicu R and Ciocacu F. Cellulose-based hydrogels as sustained drug-delivery systems. Materials 2020; 13(22): 5270.

30. Christensen LH, Breiting VB, Aasted A, Jørgensen A and Kebuladze I. Long-term effects of polyacrylamide hydrogel on human breast tissue. Plast Reconstr Surg 2003; 111(6): 1883–1890.

31. Lloyd AW, Faragher RG and Denyer SP. Ocular biomaterials and implants. Biomaterials 2001; 22(8): 769–785.

32. Cheng C, Yang J, Li SW, et al. HDAC4 promotes nasopharyngeal carcinoma progression and serves as a therapeutic target. Cell Death Dis 2021; 12(2): 137.