



Investigation of Combined Photodynamic and Plasmonic Photothermal Therapy in a Murine Model of Colorectal Carcinoma



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The aim of the study was to evaluate the effectiveness of combined photodynamic therapy (PDT) and plasmonic photothermal therapy (PPT) in mice with transplanted colorectal cancer.

MATERIALS AND METHODS

Experimental protocol in vitro

Undifferentiated murine colon carcinoma cell line (CT26.WT) was used to induce subcutaneous tumor and cultured in DMEM supplemented with a 10 % FBS, and 1 % penicillin / streptomycin according to ATCC protocol before injection. Animal experiments were carried out using Balb/c and immunodeficient nu/j female mice. Cells were detached from the bottom of culture flask by means of incubation with Trypsin-EDTA for 7 minutes, collected, counted after Trypan Blue staining using Countess™ automated cell counter (Invitrogen) and centrifuged at 800 rcf for 5 minutes. Cell pellet was resuspended in appropriate volume of DMEM. A suspension of 0.25 million CT26.WT cells in 10 μ L serum- and antibiotic-free DMEM was injected subcutaneously into the dorsal side of a left hind limb.

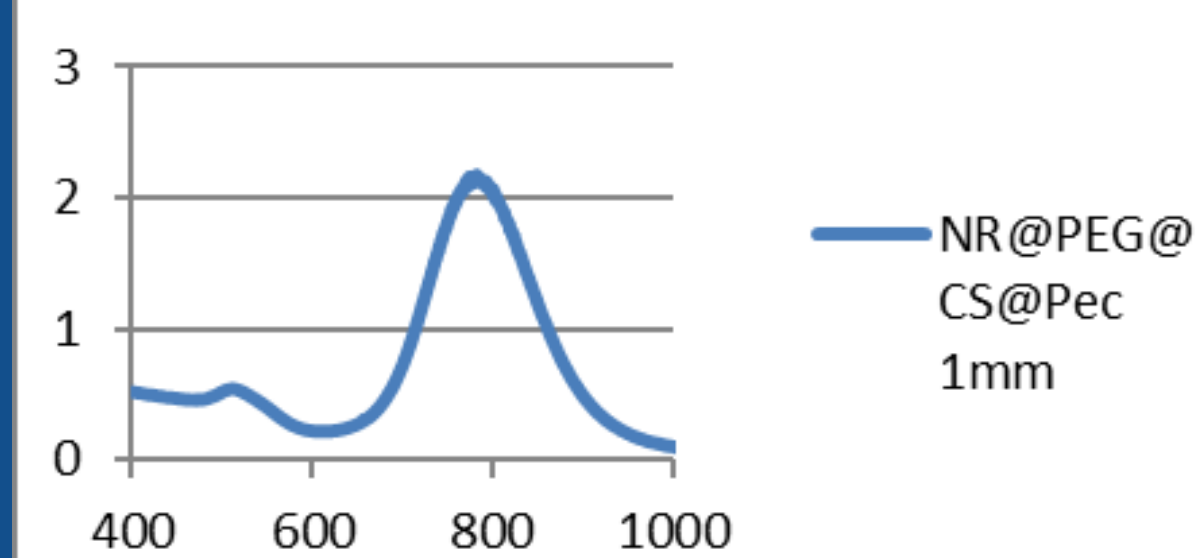
Experimental protocol in vivo

Balb/c mice were intramuscularly injected with 20 μ L of CT-26 colorectal cancer cell suspension, and when the tumor volume reached 50 mm³, therapy was performed. For PPT therapy, gold nanorods (NR780@PEG), coated with chitosan and citrus pectin were used; they were administered intratumorally in a volume of 20 μ L. For PDT, indocyanine green (ICG, Pulsion Medical Inc) diluted in polyethylene glycol (1:100) was used; it was administered intratumorally to mice in a volume of 20 μ L. For combination therapy, NR780@PEG and indocyanine green were administered simultaneously to mice at half doses. After 30 minutes, the tumor was irradiated transcutaneously with a diode IR laser with a wavelength of 808 nm and a power density of 2.3 W/cm² for 5 minutes. The tumor heating temperature was measured using an IRYSYS 4010 (UK) thermal imager. The animals were euthanized, and tumor tissue was collected for histological examination 21 days after therapy.

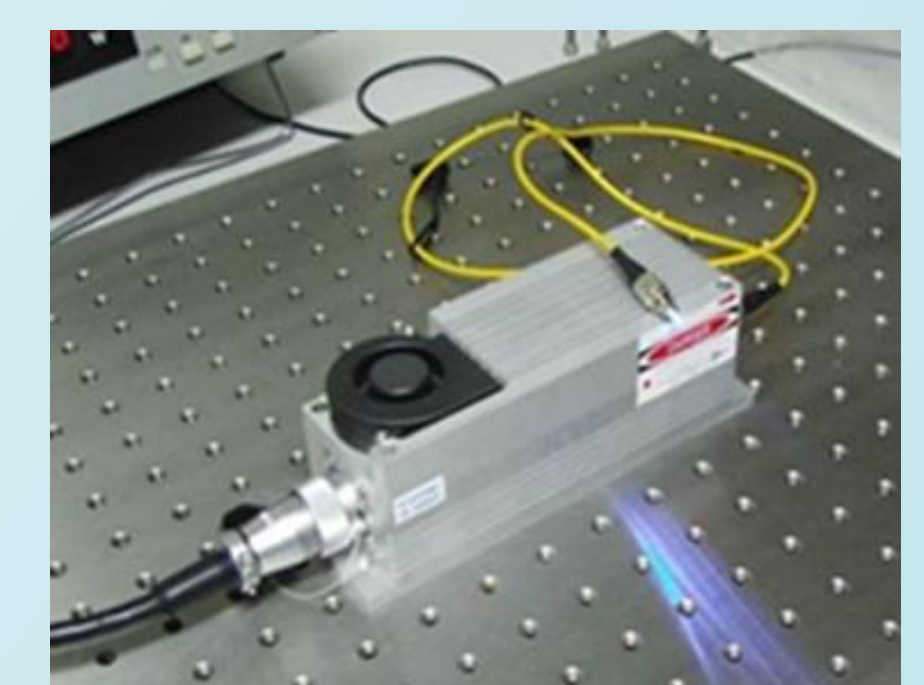
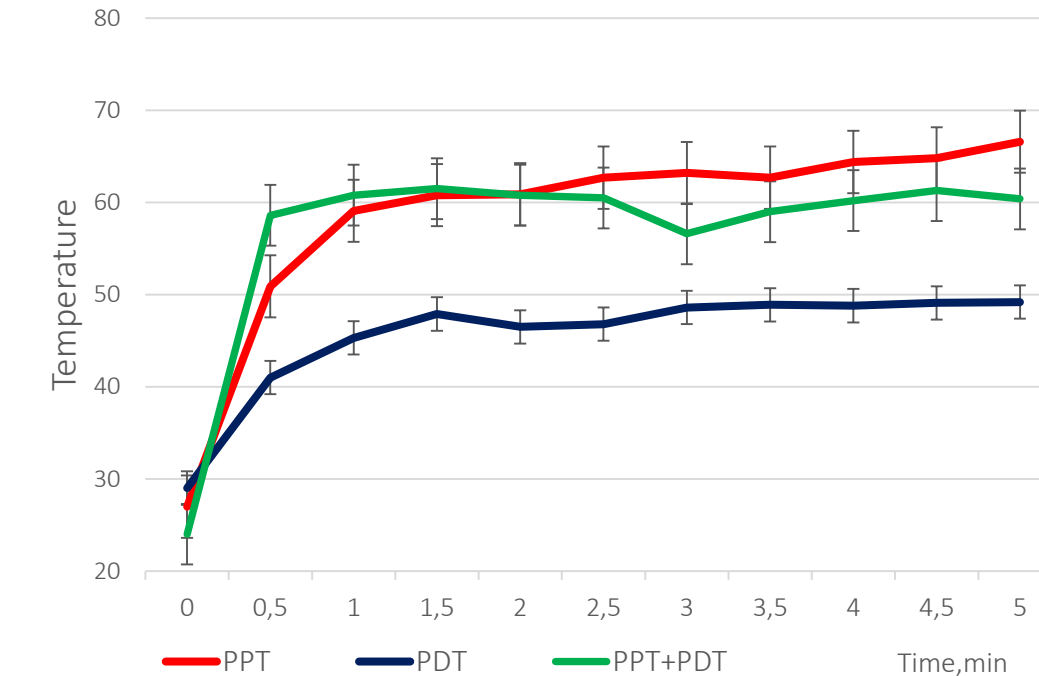
Preparations of gold nanocomposites

Pegylated nanorods (NR780@PEG) were synthesized using a standard procedure. Indocyanine Green (ICG, Pulsion Medical Inc) - 1 mM alcohol solution (95% EtOH) was used, stored at -20 ° C. Chitosan was used as a stock 2% solution in 1% acetic acid. Citrus pectin was used as a 2% aqueous effluent solution. Chitinization and pectinization of the nanorods were carried out according to the following protocol. In two 15 ml centrifuge tubes, 50 μ L of 2% chitosan in 1% CH₃COOH was added to 5 ml of pegylated nanorods (in each tube). The sample was centrifuged at 18,500 $\times$ g for 30 minutes; the sediment was resuspended in 0.5 ml of mQ water and 0.5 ml of 1% citrus pectin (NR777@PEG@CS@Pec) was added. The NR777@PEG@CS@Pec preparation was washed twice by centrifugation at 18,000 $\times$ g for 30 minutes in 2 ml centrifuge tubes, with resuspension in deionized mQ water and sonication in an ultrasonic bath for 1 minute. Chitosan-pectinized particles retain ICG fluorescence after addition, two washes, and incubation in deionized water. The final preparation was dispensed into 4 tubes, each containing 0.5 ml.

NR@PEG@CS@Pec 1mm



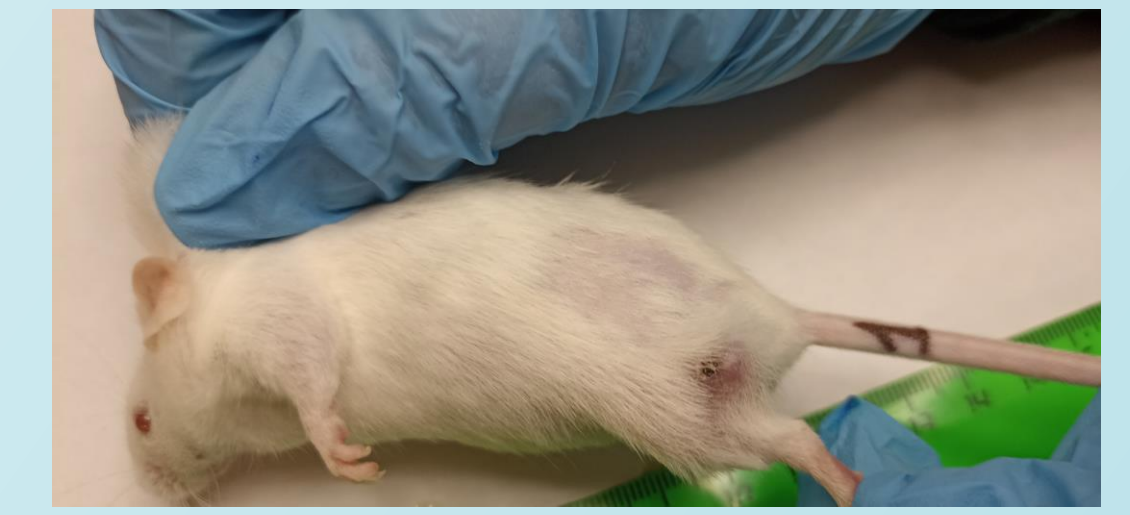
Thermography of tumors



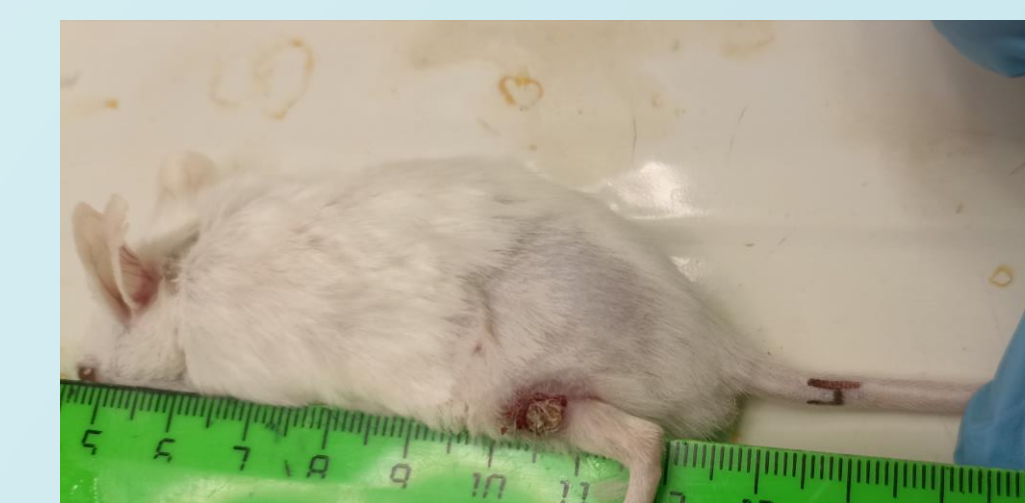
21 days after therapy



Control group



PPT group



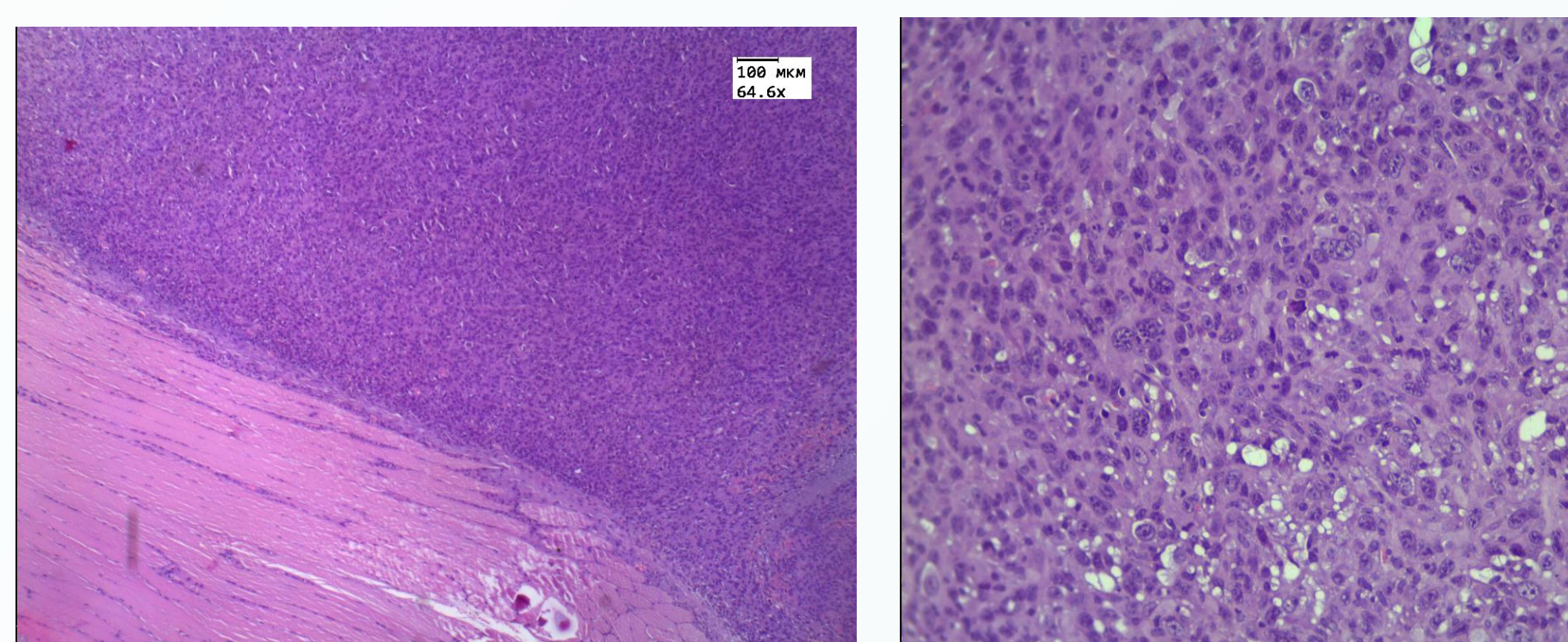
PDT group



PPT+PDT group

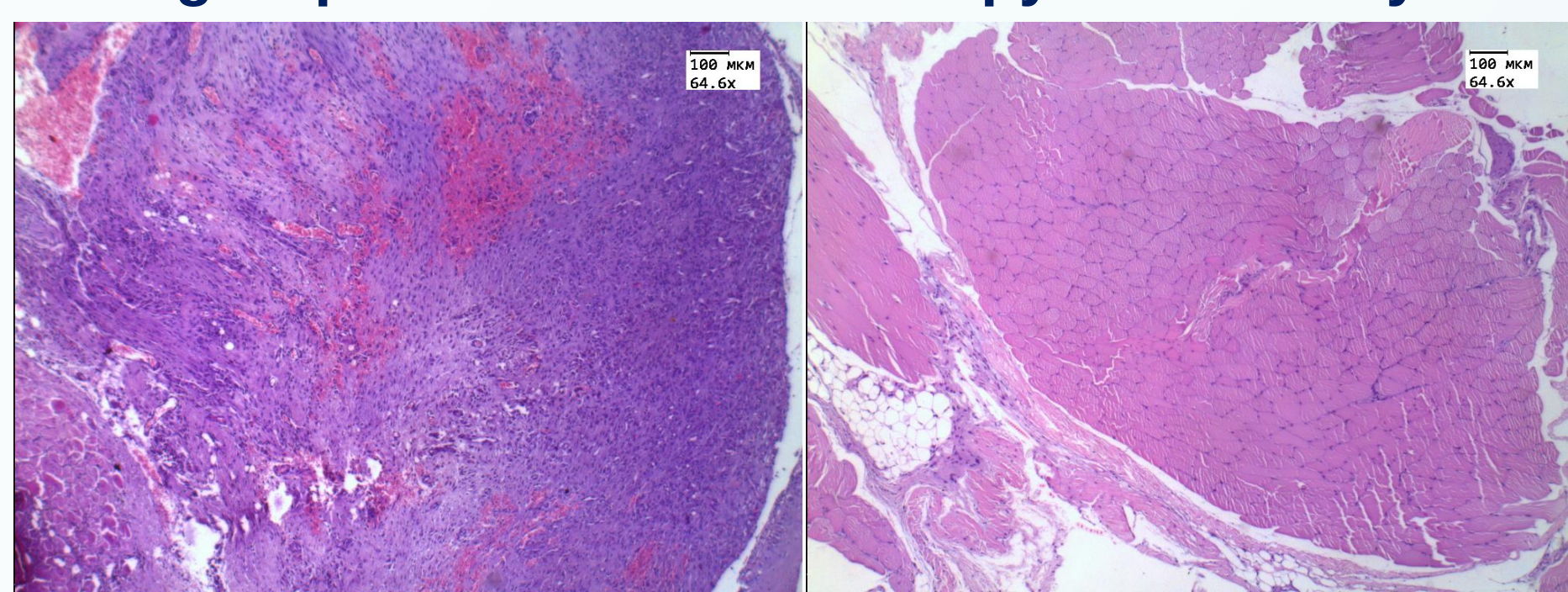
RESULTS

Control group without any treatment after 21 days



The large tumor is clearly demarcated from the muscle tissue. There is no necrosis, but there are areas containing by elongated atypical tumor cells.

In group with combined therapy after 21 days

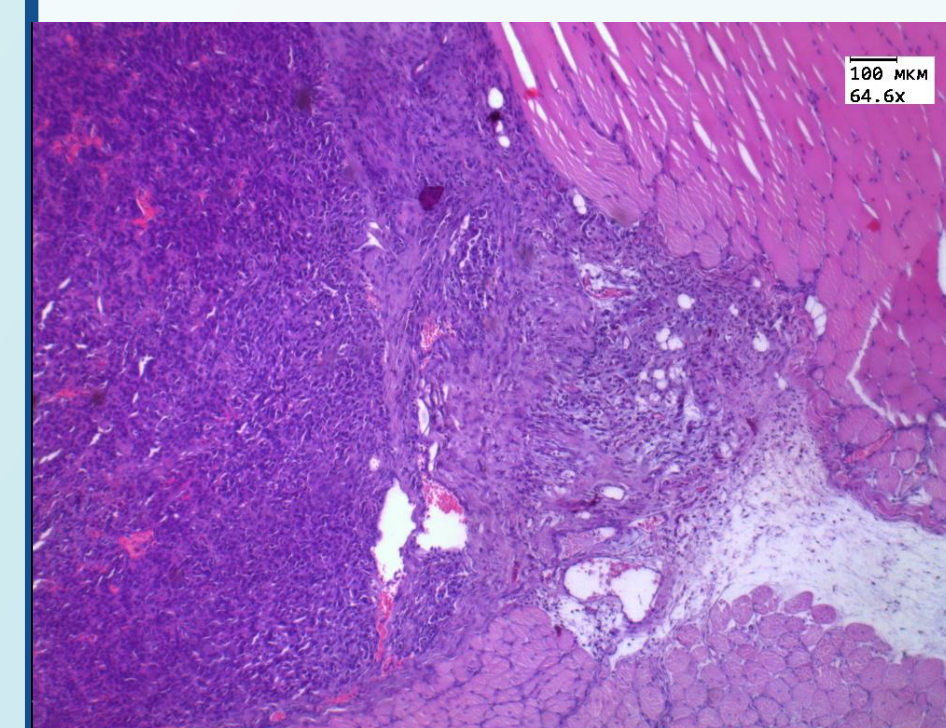


Tumor growth

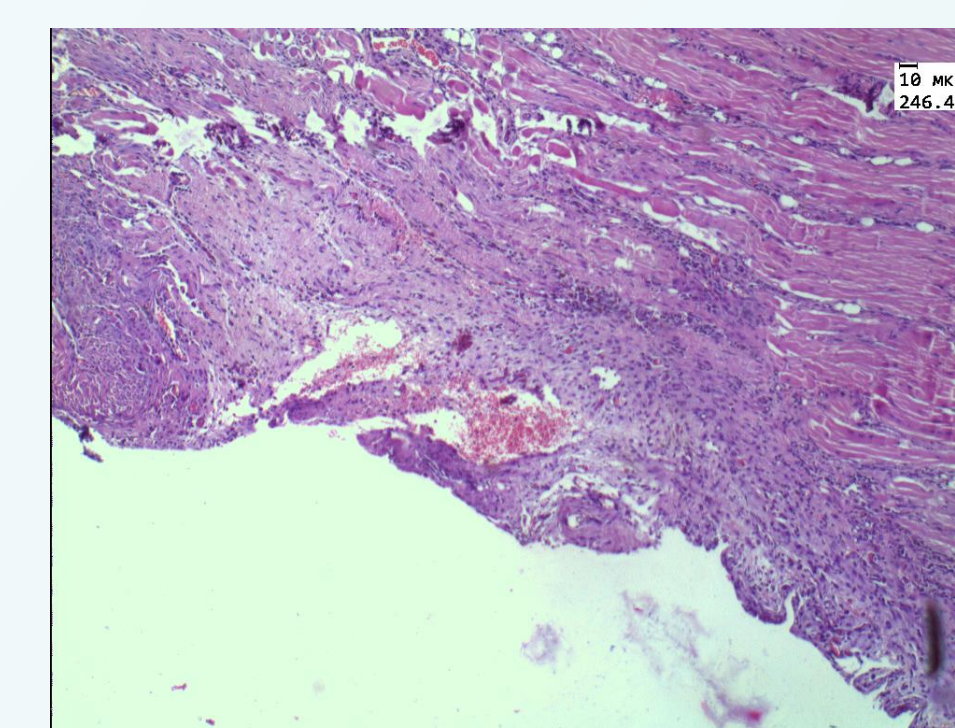
The tumor is absent.

21 days after PPT, one animal had no tumor. In the second mouse the tumor was not visualized; there are fragments of muscle tissue with areas of calcification within them. Necrosis of the epidermis and the formation of a scab was noted, we observed the growth of tumor cells in the skin.

In PPT group after 21 days



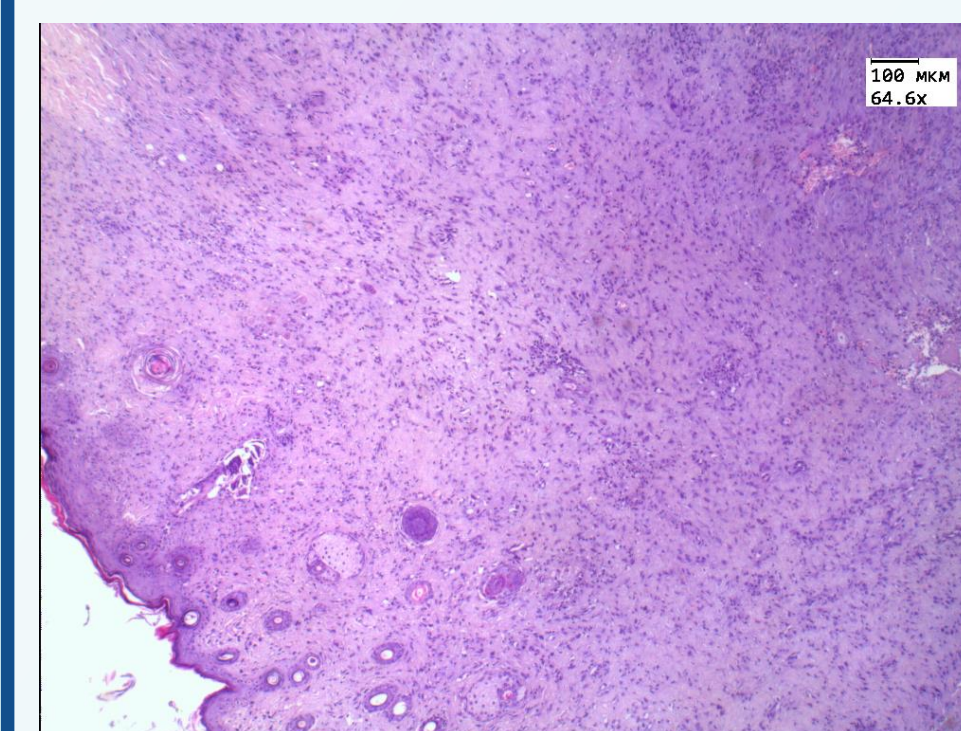
Tumor growth



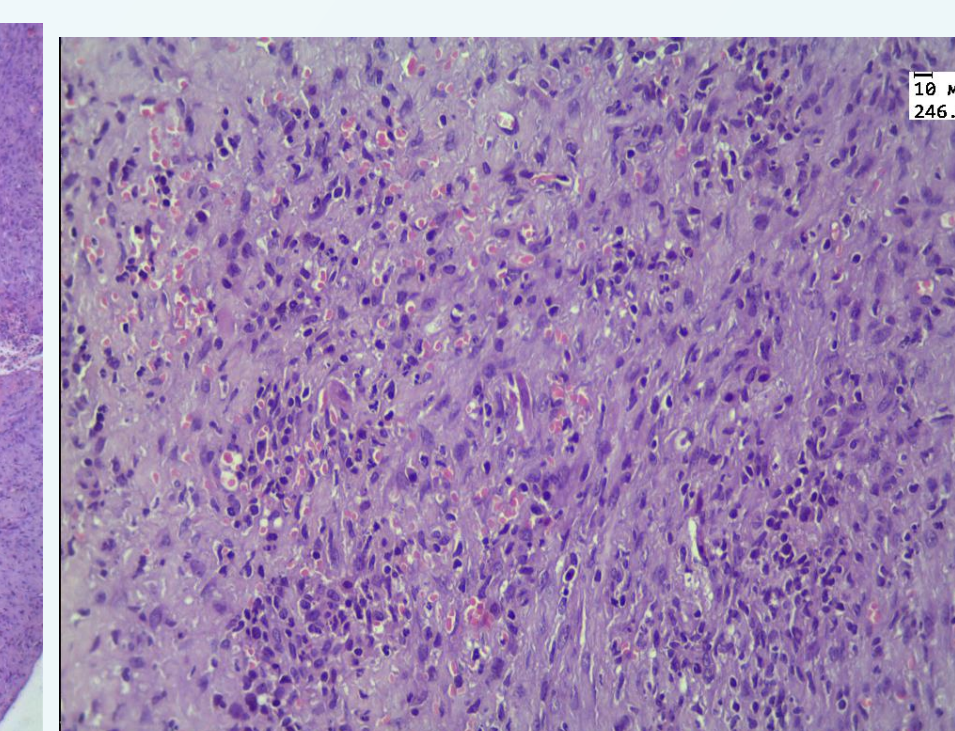
The tumor is absent.

21 days after PPT, one animal had no tumor. In the second mouse, the tumor was clearly demarcated from the muscle tissue. Necroses form small foci covering up to 10% of the section; there are areas with lighter, damaged tumor cells.

In PDT group after 21 days



The tumor is absent.



The growth of tumor cells.

21 days after PDT, one animal had no tumor. In the second mouse, the tumor was not visualized; histological examination revealed skin fragments with pronounced inflammatory infiltration and the growth of tumor cells.

Conclusion

The developed protocol of combined PPT and PDT therapy induces significant hyperthermic effect and marked damage in tumor tissue of transplanted colorectal cancer, but the safety of the proposed therapy requires further study. Chitosan-pectinized gold nanorods retain ICG fluorescence, which facilitate the synergistic mechanism of combined plasmonic photothermal and photodynamic therapy.

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