Extravascular gelation shrinkage-derived internal stress enables tumor starvation therapy with suppressed metastasis and recurrence

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Despite the efficacy of current starvation therapies, they are often associated with some intrinsic drawbacks such as poor persistence, facile tumor metastasis and recurrence. Herein, we establish an extravascular gelation shrinkage-derived internal stress strategy for squeezing and narrowing blood vessels, occluding blood & nutrition supply, reducing vascular density, inducing hypoxia and apoptosis and eventually realizing starvation therapy of malignancies. To this end, a biocompatible composite hydrogel consisting of gold nanorods (GNRs) and thermal-sensitive hydrogel mixture was engineered, wherein GRNs can strengthen the structural property of hydrogel mixture and enable robust gelation shrinkage-induced internal stresses. Systematic experiments demonstrate that this starvation therapy can suppress the growths of PANC-1 pancreatic cancer and 4T1 breast cancer. More significantly, this starvation strategy can suppress tumor metastasis and tumor recurrence via reducing vascular density and blood supply and occluding tumor migration passages, which thus provides a promising avenue to comprehensive cancer therapy.
Progression, recurrence, and metastasis of malignant tumor are three primary challenges that current therapeutic protocols are confronted with. In an attempt to address these challenges, starvation therapy has aroused considerable interest, because it can inhibit tumor via decreasing nutrient supply that is indispensable for tumor growth. Rapid ingredient consumption (i.e., oxygen scavenging, glucose exhaustion, and nutrition intake occlusion) and blood vessel occlusion are two main approaches to give rise to nutrition deficiency and realize starvation therapy. In general, rapid ingredient consumption inevitably occurs to normal cells or tissues and this process is not persistent, since the ever-existing and intact blood supply will continuously provide nutrition. In contrast, blood vessel occlusion strategy capable of permanently occluding blood & nutrition supply is preferred, among which two typical protocols, i.e., intratumoral vascular rupture and intravascular aggregate-induced embolism, are highlighted. Various science communities have witnessed the advance of this blood vessel occlusion-based starvation therapy. However, the two protocols in blood vessel occlusion strategy also confront some intractable challenges, i.e., tumor metastasis, tumor recurrence, and normal blood vessel embolism. More significantly, in the intravascular aggregate-induced embolism protocol, rapid blood flow will wash away the initial nuclei and hamper agglomerate formation, probably disabling vascular occlusion. Fortunately, the in-depth insight into starvation therapy inspires us to pursue new protocols capable of addressing these challenges.

As a versatile biomaterial, hydrogels have garnered considerable attention and they have exhibited significant potential as scaffolds or matrix for drug delivery, cell incubation, and implants. However, as one fundamental structure-determining factor, the ubiquitous internal stress in hydrogel gelation is still regarded as an ominous sign and even as a severe hazard endangering mechanical properties of hydrogels. As a result, most research focused on how to eliminate internal stress rather than exploring its potential value. Actually, from the opposite perspective, internal stresses during gelation also displayed several certain usages, e.g., drug release, micro-/super-lenses and complex three-dimensional (3D) structure.

Enlightened by them, we, in this report, established another starvation therapy protocol, namely extravascular gelation shrinkage-derived internal stress for squeezing blood vessels, cutting off blood and nutrition supply essential for tumor growth and ultimately inhibiting tumor growth. To exemplify it, an organic/inorganic hybrid composite containing PEG-SH-modified gold nanorods (GNR-PEG-SH) and composite hydrogel (chitosan (CS)/mPEG-Mal/pNIPAAm-AAc) is constructed (hydrogel–GNR). With PANc-1 pancreatic tumor and 4T1 breast tumor as models, hydrogel–GNR irradiated by 808 nm laser can produce internal stress during GNR-mediated gelation shrinkage, squeeze and narrow intratumoral and adjoining blood vessels and cut off nutrition supply, consequently inhibiting tumor progression on both PANc-1 and 4T1 solid tumors. Moreover, this starvation therapy can considerably suppress the spontaneous lung metastasis from primary tumor at breast of transgenic mouse model via reducing vascular density, cutting off blood and nutrition supply and occluding migration passages. Furthermore, it also impedes recurrence and metastasis of highly invasive PANc-1 pancreatic cancer after two special physical therapies (i.e., photothermal ablation and ultrasound mechanical destruction), which thus provides another choice for comprehensive cancer therapy. Moreover, this well-established starvation therapy deriving from extravascular gelation shrinkage-derived internal stress can address the two problematic issues that current intravascular aggregate-induced embolism protocols encounter, i.e., initial nuclei leaving caused failure of agglomerate generation and normal blood vessel embolism.

Results

Synthesis and characterization of hydrogel–GNR. GNR-mediated gelation of hydrogel–GNR is shown in Fig. 1a, wherein GNRs can mediate the photothermal transition, trigger gelation and volume shrinkage upon exposure to 808 nm laser irradiation and produce internal stresses. In principle, four pivotal parameters, i.e., low critical solution temperature (LCST), porosity, toughness, and stiffness associated with composition and structure characteristics decide the degree of internal stresses. LCST between 37 °C and 42 °C is appropriate and beneficial for evaluating this starvation therapy via removing the interference of hyperpyrexia-induced apoptosis. Fortunately, pNIPAAm-co-AAc hydrogels serve as the primary framework and determine the LCST of hydrogel–GNR, because the LCST of pNIPAAm-co-AAc can be adjusted via tuning AAc ratio according to the practical demand, that is, more hydrophobic segments will obtain larger LCST. In this regard, the LCST of pNIPAAm-co-AAc can be elevated to 38.7 °C from 32 °C corresponding to pure pNIPAAm (Fig. 1b) when AAc segment percentage in pNIPAAm-co-AAc make ups approximately 20% (Fig. 1c). In particular, the introductions of mPEG-Mal and CS significantly reduce porosity and pore diameter via the physical cross-linking (Fig. 1d)37,38, undoubtedly benefiting the reinforced compactness and internal stress.

Thiol-terminated PEG-modified GNRs (i.e., GNR-PEG-SH) with a length/diameter ratio of 6:1 were employed to mediate the gelation process (Supplementary Fig. 1) due to their typical near-infrared absorption peak at 808 nm (Supplementary Fig. 2)39. GNRs can be conjugated with PEG in the composite hydrogel (CS/mPEG-Mal/pNIPAAm-co-AAc) via the spontaneous reaction (Fig. 1d)39, and their the maximum absorption wavelength fails to be altered during gelation (Supplementary Fig. 3). Depending on their strong photothermal transition at 808 nm (Supplementary Figs 4 and 5), GNRs successfully mediate the sol–gel transition process of hydrogel–GNR (50 ppm) (CS/mPEG-Mal/pNIPAAm-co-AAc/GNRs) (Fig. 1e). Besides attaining photothermal-triggered gelation, GNRs can further reduce porosity and pore size of the ultimate hydrogel–GNR through weaving its 3D spatial network and augmenting cross-linking joints (Fig. 1f)40,41. Therefore, GNRs introduction is expected to contribute to the reinforced mechanical strength and storage modulus of the composite hydrogels, favorably augmenting the gelation shrinkage-derived internal stress. This phenomenon is analogous to previous reports using nanoparticles mingling or composition tuning for enhancing mechanical properties of hydrogels42–44.

Liquid hydrogel–GNR manifests a Newton-fluid property, ensuring its syringeability available for in vivo application (Supplementary Fig. 6). Noticeably, GNRs introduction fails to alter LCST of the ultimate product, since the abrupt inflection points of transmission rate and viscosity remain at 38.7 °C (Fig. 2a and Supplementary Fig. 7). To further comprehended the influence of GNRs on gelation temperature, hydrogel–GNR was subjected to a variable temperature rheological sweep. The crossover between G’ and G’’ that marks gelation formation further corroborates the preferable transition temperature at approximately 39 °C (Supplementary Fig. 8).

Mechanical and rheological analysis. To demonstrate GNRs conjugation-mediated improvements of mechanical and rheological properties, various dynamic measurements were carried out. Compressive stress–strain inspection indicates that contributed by the significantly augmented weaging and junction point density by GNRs conjugation42, hydrogel–GNR can withstand larger strain than hydrogel alone and display an anti-compression...
behavior, suggesting the increased toughness. More significantly, GNRs chelation substantially increases the failure stress beyond which fracture occurs (Fig. 2b), which suggests that hydrogel–GNR can endure larger stresses. This impressive phenomenon imparts hydrogel–GNR with larger strength and elastic modulus arising from increased mechanical stiffness (Supplementary Fig. 9)36. As a result, the hydrogel–GNR holds considerable potential in resisting blood pressure and squeezing blood vessels of tumor via the gelation shrinkage-derived internal stress. Noticeably, excessive and unchelated GNRs that can impede the cross-linking of components play a negative effect on the mechanical properties of hydrogel–GNR when comparing 50 ppm to 20 ppm. In the following experiments, hydrogel–GNR specifically refers to hydrogel–GNR (20 ppm) unless indicated otherwise.

Afterwards, strain-dependent oscillatory rheology was measured to assess the mechanical stiffness of different samples. Network failure fails to occur until strain is stretched to over 70%, which is in accordance with aforementioned result. Higher yielding point of hydrogel–GNR than hydrogel indicates that the hydrogel–GNR can withstand larger deformation and accommodate larger toughness. Notably, before reaching the yielding point, much larger $G'$ of hydrogel–GNR suggests that GNRs chelation can significantly enhance the mechanical stiffness of hydrogel–GNR by augmenting multivalent joints (Fig. 2c). Furthermore, $G'$ and $G''$ as a function of the angular frequency were determined. The mechanical properties are dominated by $G'$ across the range of frequencies, exhibiting a great anti-shear ability and high strength (Fig. 2d). In particular, GNRs introduction indeed influences the rheological properties of hydrogel–GNR via improving the mechanical stability. Time-dependent rheological result demonstrates shorter gelation time when introducing GNRs (Fig. 2e, f), which is beneficial for rapid solidification when applied in vivo.

**Ex vivo and in vivo vascular narrowing and occlusion test.** Depending on these impressive improvements in mechanical stiffness and reduced porosity and pore size, it is no doubt that such an extravascular gelation shrinkage-induced internal stress will occlude blood & nutrition supply via squeezing blood vessels and resisting blood pressure. To verify it, ex vivo blood vessels will be utilized to explore how this internal stress occludes blood flow. The schematic of experimental apparatus is indicated in Fig. 3a, and red DMEM was first used. More than 2.6 mL DMEM passes through the ex vivo blood vessels before hydrogel–GNR gelation. After gelation, the volume sharply decreases to less than 0.2 mL (Fig. 3b). This intriguing phenomenon robustly supports the hypothesis that gelation shrinkage-derived internal stress can squeeze blood vessels and give rise to vascular embolism. Subsequently, we evaluated the ability of vascular embolism to occlude blood supply that is indispensable for favoring tumor progression. Similarly, due to blood supply occlusion, the volume of gathered blood that passes through the ex vivo blood vessels sharply decreases after gelation (Fig. 3c) and the numbers of red blood cells decreases.

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**Fig. 1** Synthesis and characterizations of hydrogel–GNR. a Schematic on the gelation process of hydrogel–GNR. b Temperature-dependent rheological curves of pNIPAAm without AAc segment, wherein the crossover between $G'$ and $G''$ that represent storage modulus and loss modulus, respectively, is determined as the gelation temperature (that is, LCST). c LCST of pNIPAAm-co-AAc with tunable AAc content (0%, 10%, 20%, and 40%) determined by dynamic oscillatory temperature sweeps, and data are expressed as mean ± standard deviation (SD) ($n = 3$). d Schematic of chelation reaction between GNR-PEG-SH and mPEG-Mal in CS/mPEG-Mal/pNIPAAm-co-AAc. e Digital photos of hydrogel–GNR (50 ppm) before and after 808 nm irradiation, and 20% AAc in pNIPAAm-co-AAc was employed. f Scanning electron microscopic (SEM) images of pNIPAAm-co-AAc (left), composite hydrogel (CS/mPEG-Mal/pNIPAAm-co-AAc) (middle), and hydrogel–GNR (right) with 20 ppm GNRs, wherein 20% AAc in pNIPAAm-co-AAc was employed.
It is found that far lower varied sizes (i.e., 4, 20, and 70 K) as tracers were blood vessels is responsible for the occluded blood supply in the gelation shrinkage-mediated internal stress for squeezing which is beneficial for vascular narrowing. Even after 5 days, the occluded blood supply tests of hydrogel (indicated by arrows), resulting in the successful occlusion of internal stress successfully squeezes and narrows the blood vessel temporarily accompanied by internal stress. Such an extravascular tumor and its adjacent tissues is considerably decreased after on nude mice were explored. Blood supply in blood vessels of tumor and its adjacent tissues is considerably decreased after instant treatment with Laser +hydrogel–GNR (2) (Fig. 4a and Supplementary Movies 3 and 4), suggesting the occurrence of vascular narrowing. Even after 5 days, the occluded blood supply remains unrecovered (Fig. 4a and Supplementary Movie 5), which is beneficial for the persistent starvation therapy.

To figure out whether in vivo vascular narrowing arising from the gelation shrinkage-mediated internal stress for squeezing blood vessels is responsible for the occluded blood supply in tumor, in vivo perfusion studies with FITC-labeled dextrans with varied sizes (i.e., 4, 20, and 70 K) as tracers were first carried out. It is found that far lower fluorescence signal intensity at tumor in treated group (i.e., Laser +hydrogel–GNR) than that in control group (i.e., Laser+GNR) suggests that dextrans are not allowed to enter the tumor due to vascular occlusion (Fig. 4b, c)36. Even though the molecular weight of dextran drops as low as 4 K, these FITC-labeled dextrans in treated group fail to enter tumor. This result sufficiently demonstrates that vascular narrowing in tumors is responsible for the blood flow drop and blood supply occlusion rather than the increased intratumoral pressure.

Moreover, in vivo perfusion studies with clinically used Sonovue™ microbubbles (MBs) as tracers that can be detected under the contrast-enhanced harmonic imaging (CHI) mode was further implemented. Before corresponding treatments in control and treated groups, the 1st injection of Sonovue™ MBs illuminates the tumors in both groups (Fig. 4d, e and Supplementary Movies 6 and 8)36. After corresponding treatments that were carried out after 30–90 min post-1st injection of Sonovue™ MBs for guaranteeing collapse of the 1st injected Sonovue™ MBs, the 2nd injection of Sonovue™ MBs illuminates the tumor in control group, but fails in treated group (Fig. 4d, e and Supplementary Movies 7 and 9). This result further demonstrates that the Laser +hydrogel–GNR treatment in treated group results in vascular narrowing due to the extravascular gelation shrinkage-derived internal stress in tumor.

To directly observe the vascular narrowing phenomenon, laser confocal scanning microscopic (LCSM) images of tumor slices stained by CD31 and CD34 immunofluorescence that characterize new and matured blood vessels, respectively, were captured. Laser +hydrogel–GNR treatment in treated group indeed induces the narrowing of both budding and matured blood vessels in tumor compared with the Laser+GNR treatment in control group (Fig. 4f, g). These intriguing results convincingly demonstrate that extravascular gelation shrinkage-derived internal stress can squeeze and narrow blood vessels in tumor and subsequently result in blood supply occlusion.

Noteworthy, excellent diffusion is a robust guarantee of realizing this special starvation therapy. It is assured that this injectable

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**Fig. 2** Rheological measurements of composite hydrogel and hydrogel–GNR. a Temperature-dependent transmittance of hydrogel and hydrogel–GNR, and the temperature corresponding to the abrupt drop point is designated as the LCST. b Compressive stress–strain curves of hydrogel, hydrogel–GNR (20 ppm), and hydrogel–GNR (50 ppm), respectively. c Strain amplitude sweeps of hydrogel and hydrogel–GNR performed at 39 °C at a constant frequency of 1 rad s⁻¹. d Dynamic oscillatory frequency sweeps of hydrogel and hydrogel–GNR at a fixed strain of 1%. e, f Time-dependent sweep tests of hydrogel (e) and hydrogel–GNR (f). Notes: hydrogel represents CS/mPEG-Mal/pNIPAAm-co-AAc with 20% AAc in pNIPAAm-co-AAc.
CDFI images were captured before and after 808 nm laser irradiation, respectively, wherein white arrows indicate the blood vessels of abdomen artery.

Instead of above DMEM, data are expressed as mean value ± SD (volume, student’s t test in comparison to Pre-gelation. **P < 0.01 and ***P < 0.001, and the statistical significances were obtained using student’s t test in comparison to Pre-gelation. g) In vivo schematic of extravascular gelation shrinkage-induced internal stress for squeezing blood vessels upon exposure to 808 nm laser. h) CDFI images of abdomen artery of nude mice around which hydrogel–GNR solution was administrated, and in particular, CDFI images were captured before and after 808 nm laser irradiation, respectively, wherein white arrows indicate the blood vessels of abdomen artery.

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**In vivo starvation therapy on PANC-1 xenografted tumor.**

Inspired by above results on in vivo vascular narrowing & embolism and blood & nutrition supply occlusion, exploration using this special starvation therapy on PANC-1 tumor was carried out. Analogous to in vitro results, 808 nm laser is also applicable for triggering intratumoral gelation process, since it can elevate the temperature above 39 °C (Supplementary Fig. 11). In particular, a special administering method represented by Laser + hydrogel–GNR (2) wherein hydrogel–GNR were injected into tumor and its periphery, was employed to simultaneously cut off the blood & nutrition supply in tumor and its adjoining normal tissues (Fig. 5a). The conventional injection method (i.e., intratumoral injection) represented by Laser + hydrogel–GNR (1) plays an inhibitory effect against PANC-1 tumor to some extent (Fig. 5b). As a contrast, the special administering method harvests the largest inhibitory outcome, and the tumor volume approximately levels off to a plateau (Fig. 5b), meaning no increase. Digital photos also indicate negligible tumor growth when treated with Laser + hydrogel–GNR (2) (Supplementary Fig. 12). The therapeutic difference between two groups can be attributed to that the robust internal stress and compact structure of composite hydrogel–GNR in Laser + hydrogel–GNR (2) group can squeeze and narrow blood vessels, induce embolism and occlude blood & nutrition supply in both tumor and its adjacent normal tissues around tumor periphery, while Laser + hydrogel–GNR (1) can merely work in tumor.

Noticeably, another comparison, i.e., pNIPAAm without AAc, was used and a identical injection manner with Laser + hydrogel–GNR (2) was employed. pNIPAAm can spontaneously give rise to gelation due to its low LCST (32 °C) below the body temperature. pNIPAAm gels accommodate loose structure with macropores (Supplementary Fig. 13a) and share poor mechanical strength, elasticity and rheological property (Supplementary Fig. 13b–d). More significantly, re-illumination after experiencing the 2nd injection of SonovueTM MBs (Supplementary Fig. 14) and evident fluorescence signal (Supplementary Fig. 15) at tumor are observed in treated group, akin to control group. This phenomena suggests that pNIPAAm is disabled to resist blood pressures, narrow blood vessels and occlude blood & nutrition supply in tumor consequently failing to delay tumor growth (Fig. 5b). However, it indirectly suggests that the compact structure and robust internal stress in Laser + hydrogel–GNR (2) or Laser + composite (i.e., hydrogel–GNR) features excellent diffusion, since 500 nm silica particles were reported to easily pervade the whole tumor.45 As well, in vivo fluorescence imaging (Fig. 4b) and MR imaging (Supplementary Fig. 10) indicate that the injectable hydrogel–GNR can rapidly diffuse and pervade the whole tumor within 1 min, wherein Cy3-labeled dextran (70 K) and extensively accepted hollow porous Fe3O4 nanoparticles instead of GNRs (Supplementary Fig. 10a,b) served as the fluorescence and T2-weighted MRI tracers, respectively.46
To better understand the underlying principle of this extravascular gelation shrinkage-derived internal stress for starvation therapy in Laser+hydrogel–GNR (2), several pathological examinations including H&E, PCNA and CD31 immunohistochemical assays and CD34&TUNEL immunofluorescence co-staining assay were implemented. Some evident characteristics associated with apoptosis, e.g., chromatin condensation, nucleus disintegration and cell lysis, are observed (Fig. 5d) and

Raw text: Fig. 4 In vivo evaluations on intratumoral vascular narrowing on PANC-1 xenografted pancreatic tumors. a CDFI images of PANC-1 xenografted pancreatic tumors implanted on nude mice that were captured at three time points, i.e., pre-, post- and 5 days post-treatment with Laser+hydrogel–GNR (2). In vivo animal fluorescence images (b) and quantitative signal intensities (c) of PANC-1 xenografted tumor after corresponding treatments in control (i.e., Laser–GNR) and treated (i.e., Laser+hydrogel–GNR (2)) groups and subsequent injections of different FITC-labeled dextrans with varied sizes (i.e., 70, 20, and 4 K), wherein green arrows and dotted ellipses indicate the tumor. Data are expressed as mean ± SD (n = 3). d e Time-dependent ultrasonic images (d) and average acoustic intensities (e) of PANC-1 xenografted tumor before and after corresponding treatments in control and treated groups, wherein the 1st i.v. injection of Sonovue™ microbubbles were carried out, and after 30–90 min, the corresponding treatments in two groups, i.e., control (Laser+GNR) and treated (Laser+hydrogel–GNR (2)), were implemented, followed by the 2nd i.v. injection of Sonovue™ microbubbles; and the ultrasonic images were captured at three time points, i.e., pre-, post-, and 40–120 s post- each i.v. injection of Sonovue™ microbubbles, and red dotted ellipses indicate the tumor. Data are expressed as mean ± SD (n = 3). f g Matured (f) and budding (g) blood vessels of PANC-1 xenografted tumors after corresponding treatments in control and treated groups, and they were stained by FITC-labeled CD34 immunofluorescence (f) and Cy3-labeled CD31 immunofluorescence (g), respectively, scale bar 200 μm. Yellow arrows indicate the intratumoral blood vessels. (h) In vivo animal fluorescence imaging of nude mice-bearing PANC-1 xenografted tumor at three time points, i.e., pre-, 30 s post- and 50 s post-i.t. injection of Cy3-labeled dextran-grafted hydrogels, wherein Cy3-labeled dextran (size: 70 K) instead of GNRs was used. Statistical significances were obtained using unpaired student’s t test, **P < 0.01. Note: Laser+hydrogel–GNR (2) represent the co-injection of hydrogel–GNR in tumor and its periphery and subsequent 808 nm laser irradiation. Hydrogel–GNR (1) are responsible for the successful starvation therapy. After treatment with Laser+hydrogel–GNR (2), the survival time of nude mice is significantly prolonged (Fig. 5c) and no evident weight variation is observed (Supplementary Fig. 16).
Laser + hydrogel – GNR (2) treatment in G4 induces the largest apoptosis (Supplementary Fig. 17a). More significantly, this treatment also acquires the largest inhibitory effects against PANC-1 cell proliferation and CD31-labeled angiogenesis via cutting off nutrition supply due to vascular angiostenosis and occlusion (Fig. 5e, f and Supplementary Fig. 17b, c)\textsuperscript{36}. Moreover, Laser + hydrogel – GNR (2) treatment in G4 brings about the largest apoptotic cells (62%) including tumor cells (82%) and endothelial cells (18%), and simultaneously results in the lowest CD34-labeled vascular density (Fig. 6a–c)\textsuperscript{36}. Thus, this special starvation therapy harvests the most excellent treatment outcome through inducing apoptosis, inhibiting cell proliferation and CD31-labeled angiogenesis and reducing CD34-labeled vascular density.

Furthermore, western blot analysis was carried out to explore the apoptotic mechanism. The persistent blood supply occlusion due to vascular narrowing in this special starvation therapy (i.e., G4) is found to significantly up-regulate hypoxia inducible factor 1α (HIF 1α) and simultaneously up-regulates the pro-apoptotic protein (i.e., P53) (Fig. 5g and Supplementary Fig. 18)\textsuperscript{36}. This
Fig. 5 In vivo evaluation of this starvation therapy in inhibiting PANC-1 pancreatic tumors on nude mice. a Schematic of in vivo experimental process, and hydrogel–GNR was simultaneously injected into both tumor and its periphery where 808 nm laser irradiation was carried out. b, c Time-dependent tumor volume variation (b) and survival rate (c) of PANC-1 tumor-bearing nude mice experiencing different treatments, i.e., control, Laser+GNR, Laser+hydrogel–GNR (1), Laser+hydrogel–GNR (2) and pNIPAAm, wherein (1) and (2) represent tumor injection alone and co-injection of tumor and its periphery, respectively, and pNIPAAm was also injected into both tumor and its periphery. Data are expressed as mean ± SD (*P < 0.05, **P < 0.01 and ***P < 0.001, and the statistical significances were calculated via unpaired Student’s t test in comparison to control. d–f Microscopic images of PANC-1 tumor slices stained by H&E immunohistochemical staining (d), PCNA immunohistochemical staining (e) and CD 31 immunohistochemical staining (f) that were harvested after sacrificing of PANC-1-bearing nude mice treated with aforementioned different treatments (G1-G5) on the 10th day post-treatment. Note: H&E, PCNA and CD31 immunohistochemical assays were employed to evaluate cell structure, cell proliferation, and neovascularization, respectively. g Western blot analysis for analyzing different proteins including Bcl-2, Caspase 3, HSP70, P53, CD34 and CD31 and HIF 1α in PANC-1 tumors receiving aforementioned different treatments (G1-G5) on the 10th day post-treatment. Note: G1-G5 represent control, Laser+GNR, Laser+hydrogel–GNR (1), Laser+hydrogel–GNR (2) and pNIPAAm, respectively.

Fig. 6 In vivo principle exploration of anti-tumor using this special treatment method. a LCSM images of PANC-1 tumor slices co-stained by TUNEL immunofluorescence & CD34 immunofluorescence that were harvested after sacrificing of PANC-1-bearing nude mice treated with aforementioned different treatments (G1-G5) on the 10th day post-treatment, scale bar: 50 μm, and TUNEL & CD34 immunofluorescence co-staining was employed to determine cell apoptosis and vascular density, respectively, wherein FITC (green) and TRITC (red) were used to label TUNEL and CD34, respectively, and nuclei (blue) was stained by DAPI. b, c Semi-quantitative data of apoptotic cells (b) and apoptotic endothelial cells in all apoptotic cells (c) after different treatments with G1-G5 via calculating the ratios of green-labeled cells in whole blue-labeled cells and red and green co-labeled cells in all green-labeled cells according to the TUNEL immunofluorescence staining, respectively. Data are expressed as mean ± SD (n = 3). *P < 0.05, **P < 0.01 and ***P < 0.001, and the statistical significances were obtained using student’s t test in comparison to G1. Note: G1-G5 represent control, Laser+GNR, Laser+hydrogel–GNR (1), Laser+hydrogel–GNR (2) and pNIPAAm, respectively.

result is completely consistent with previous report focusing on intravascular aggregate-mediated blood & nutrition occlusion for starvation therapy46. Noticeably, no expression variation of Bcl-2 pro-apoptotic protein indicates HIF 1α elevation can trigger apoptosis through the pathway, i.e., the stabilization of P5347,48. Intriguingly, the largest down-regulations of CD34 and CD31 proteins in G4 (i.e., Laser+hydrogel–GNR) further demonstrate that this special treatment can induce endothelial cell apoptosis and exert a robust influence on anti-angiogenesis via cutting off blood & nutrition supply. This result is completely consistent with previous TUNEL & CD34 immunofluorescence co-staining and PCNA and CD31 immunofluorescence staining. Therefore, it is not difficult to understand why this starvation therapy harvests the highest apoptotic cells and the strongest inhibitory effects
against cell proliferation, angiogenesis and tumor growth (Figs. 5 and 6), since high expressions of both HIF 1α and P53 are essential for the hypoxia-induced cell death.49–51. In particular, the ranking orders of anti-proliferation, anti-neovascularization, cell apoptosis and expressions of pro-apoptotic proteins and HIF 1α are consistent with that of aforementioned therapeutic outcome in all groups.

Taken all above experimental results together, such a special starvation therapy based on extravascular gelation shrinkage-derived internal stress can squeeze and narrow blood vessels (Fig. 4f, g), occlude blood & nutrition supply (Fig. 4a–e), induce hypoxia (Fig. 5g), starve tumor cells and endothelial cells to death (Figs. 5d–g and 6), reduce vascular density (Fig. 6) and inhibit PANC-1 proliferation and angiogenesis (Fig. 5c, f), which eventually suppresses tumor growth (Fig. 5b). The whole procedure regarding this underlying principle is indicated in Supplementary Fig. 19.

In vivo starvation therapy on 4T1 xenografted tumor. To test the generality of this special starvation therapy, another model, i.e., 4T1 breast cancer implanted on immune-competent BALB/c female mice, was used. Contributed by vascular narrowing and blood & nutrition supply occlusion, the treatment with Laser + hydrogel–GNR (1) or (2) exerts prominently inhibitory effects on tumor growth. In particular, Laser+hydrogel–GNR (2) wherein tumor and its adjoining tissues are treated performs the best in suppressing tumor growth (Fig. 7a, b)36. In contrast, pNIPAAm fails to delay tumor growth due to the poor internal stress and loose structure. These results sufficiently suggest that this special starvation therapy can be extended to other tumor models. During experimental period, no evident weight variation of mice is observed (Fig. 7c)36.

As well, in vivo intratumoral vascular narrowing and blood & nutrition supply occlusion tests were explored on 4T1 xenografted tumor. In vivo perfusion studies with FITC-labeled dextran and SonovueTM MBs as tracers of fluorescence imaging and ultrasound contrast imaging, respectively, were first carried out. Akin to PANC-1 model on nude mice, after treatment with Laser+hydrogel–GNR, dextrans with varied sizes (i.e., 4, 20, 70 K) and the 2nd injected SonovueTM microbubbles fail to enter tumor in comparison to control group (i.e., Laser+GNR) (Fig. 7d–g and Supplementary Movies 10–13)36. These results confirm again that the constricted vessel narrowing arising from the gelation shrinkage internal stress-mediated squeezing against blood vessels in 4T1 tumor and adjacent tissues is responsible for the occluded blood & nutrition supply and suppressed tumor growth.

More significantly, this starvation therapy exerts robust influences on inflammatory microenvironment in tumor. In detail, Laser+hydrogel–GNR (2) in G4 suppresses the secretions of IL1, IL6, IL8, IL10, IL12, CCL22, CCL17, and CCL5 that can promote progression and metastasis of tumor (Fig. 7h) and simultaneously augments the secretions of IL-5, IL-18, IL21, IFN-γ, and TNF-α associated with anti-tumor activity (Supplementary Fig. 20)36. In addition, vascular embolism, reduced vascular density and occluded migration passages in this starvation therapy also hamper tumor cell invasiveness and migration into blood vessels. To demonstrate it, in vitro anti-metastasis experiment was carried out and the apparatus is shown in Fig. 8a. Primary tumors were seeded in culturing zone designated as S0, and S1–S4 are designated as the distant transferring zones. The separation wall between S0 and either one in S1–S4 is made up of hydrogel–GNR. It is clearly found that the number of PANC-1 cells in S0 increases as a function of incubation time, but there are still no evident PANC-1 cells in S1–S4 (Fig. 8b). Quantitatively, as the incubation time proceeds, the cell density continuously rises from 3.2 × 10^5 to 12 × 10^5 per mL after 70 h, while a cell density less than 20 per mL is obtained (Fig. 8c, d)36. These results suggest that hydrogel–GNR after gelation indeed impeded in vitro tumor cell migration and invasion from S0 to S1–S4.

In vivo inhibitory tumor metastasis test. In particular, vascular stenosis, blood occlusion, and reduced vascular density resulting from the extravascular gelation shrinkage of hydrogel–GNR in such an extravascular gelation shrinkage-mediated starvation therapy can blockade the migration passage of tumor cells, which is expected to inhibit in vivo metastasis of late-stage tumor through gel matrix (Zone A in Fig. 8e). In contrast, some late-stage tumors or some physical therapies (e.g., photothermal and ultrasound-based therapy) that can destroy blood vessels in tumor facilitate tumor metastasis (Zone B)11,41. To demonstrate it, a transgenic mouse model that can generate mammary adenocarcinomas after induction by MMTV-PyVmT oncogene was used as the ideal model for evaluating anti-metastasis ability of this special treatment (Supplementary Fig. 23), since secondary metastatic tumors are easily accessible in the lung27–29. The underlying principles of anti-metastasis using this special starvation therapy are displayed in Fig. 8f wherein the Laser + hydrogel–GNR treatment in treated group is expected to cuff off the metastasis pathway via reducing vascular density and occluding blood vessels. In Fig. 8g, no secondary metastatic tumor is observed in lung in treated group, while lots of secondary metastatic tumors in lung emerge in control group. Quantitatively, all mice-bearing mammary adenocarcinomas generate lung metastasis with 9.28 ± 1.60 (mean ± standard deviation) lung nodules in control group. In contrast, only 3 mice (n = 7) generate lung metastasis with 0.57 ± 0.79 lung nodules in treated group (Fig. 8h)36. The effectively suppressed lung metastasis can be attributed to the reduced vascular density and occluded migration passage accompanied in this special starvation therapy. Noticeably, this starvation therapy also delays the growth of primary tumor implanted in the orthotopic breast of the transgenic mice (Supplementary Fig. 23).

As well, to further evaluate the in vivo anti-metastasis using this starvation therapy, ultrasound irradiation uniting microbubbles (MBs) that is designated as US(MBs) serves as a means capable of triggering PANC-1 metastasis according to a previous report11. It is clearly found that the treatment with US(MBs) alone results in evident distant metastasis (Supplementary Fig. 24b) and the metastasis rate reach approx. 85% (Supplementary Fig. 24d). In contrast, no evidently distant metastasis is observed in the experimental group (i.e., Laser+hydrogel–GNR+US(MBs)) (Supplementary Fig. 24a,c). This phenomenon sufficiently demonstrates that the dual functions of hydrogel–GNR, i.e., inhibitory migration has been made in inhibiting tumor metastasis, e.g., nanoparticle endocytosis-mediated anti-metastasis, GNRs photothermal-mediated anti-metastasis54,55, and targeted binding-mediated anti-metastasis56,57. Herein, the well-proven reduced porosity and pore diameter by internal stress-induced shrinkage in hydrogel–GNR are expected to occlude migration pathway of tumor cells and hamper tumor cell invasiveness and migration into blood vessels. To demonstrate it, in vitro anti-metastasis experiment was carried out and the apparatus is shown in Fig. 8a. Primary tumors were seeded in culturing zone designated as S0, and S1–S4 are designated as the distant transferring zones. The separation wall between S0 and either one in S1–S4 is made up of hydrogel–GNR. It is clearly found that the number of PANC-1 cells in S0 increases as a function of incubation time, but there are still no evident PANC-1 cells in S1–S4 (Fig. 8b). Quantitatively, as the incubation time proceeds, the cell density continuously rises from 3.2 × 10^5 to 12 × 10^5 per mL after 70 h, while a cell density less than 20 per mL is obtained (Fig. 8c, d)36. These results suggest that hydrogel–GNR after gelation indeed impeded in vitro tumor cell migration and invasion from S0 to S1–S4.
In vivo inhibitory tumor recurrence. Tumor recurrence after some treatments (e.g., surgery or heat ablation) is another important threat. With cutting off blood & nutrition supply, vascular occlusion stemming from this gelation-derived internal stress is expected to inhibit tumor recurrence after photothermal ablation. To verify it, another special injection approach was employed in the experimental group. In detail, GNRs alone and hydrogel–GNR were accordingly injected into 4T1 tumors implanted on BALB/c mice after experiencing aforementioned different treatments. Error bars are based on SD (n = 6); **P < 0.01 and ***P < 0.001, which were obtained using unpaired Student’s t test in comparison to control group.

In vivo animal inflammatory cytokines obtained by ELISA analysis in 4T1 tumors after aforementioned different treatments (G1–G5) at day 10, and data are expressed as mean ± SD (n = 3). *P < 0.05, **P < 0.01 and ***P < 0.001, which were obtained using student’s t test in comparison to the control group. Note: G1–G5 represent control, Laser+GNR, Laser+hydrogel–GNR (1), Laser+hydrogel–GNR (2) and pNIPAAm, respectively.

in gel matrix and vascular occlusion, cooperatively contribute to the significantly improved anti-metastasis.
laser parameters were instantly carried out for tumor ablation and gelation occurrence (Fig. 9a), respectively. In control group, only GNRs were employed in tumor, followed by 808 nm laser irradiation on the tumor zone of PANC-1 tumor is significantly decreased, which will cut off nutrition supply and inhibit tumor recurrence. As expected, tumor re-growth occurs to PANC-1 tumors in control group due to the presence of residual tumor cells, while no recurrence is observed in the experimental group (Fig. 9c). Statistically, the recurrence rate drastically drops from 80% in control group to 10% in experimental group (Fig. 9d). This result definitely suggests that the occluded blood supply by this special starvation therapy considerably suppresses tumor recurrence after photo-thermal ablation.

**Biocompatibility and biodegradation evaluations.** Systematic experimental evaluations on the biosafety of hydrogel–GNR are necessary, because excellent biocompatibility and biodegradation are the prerequisites of clinical translation. Even though the residual liquid after gelation reaches 50%, the cell viability attains significantly decreased, which will cut off nutrition supply and inhibit tumor recurrence. As expected, tumor re-growth occurs to PANC-1 tumors in control group due to the presence of residual tumor cells, while no recurrence is observed in the experimental group (Fig. 9c). Statistically, the recurrence rate drastically drops from 80% in control group to 10% in experimental group (Fig. 9d). This result definitely suggests that the occluded blood supply by this special starvation therapy considerably suppresses tumor recurrence after photo-thermal ablation.

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accepted biosafety and degradability of pNIPAAM-based hydrogels, evaluating the biodegradability and metabolism of such a composite hydrogel–GNR is necessary. In vitro biodegradation result shows that above 30% hydrogel–GNR gels degrade after 30 days (Supplementary Fig. 29). In vivo level, the classic subcutaneous embedding method was used. Intriguingly, a high biodegradation rate of hydrogel–GNR is observed after 60 days post-embedding and the embedded solid hydrogel–GNR approximately vanishes (Supplementary Fig. 30). As for metabolism, the fate of GNRs that label this composite hydrogel is accessible to indirectly reflect the fates of pNIPAAM-based hydrogels, since the robust covalent bonding between GNRs with hydrogel skeleton makes them fail to shed from hydrogel skeleton during degradation. Over 90% excretions of GNRs through urine and faeces indicate the metabolism pathway of such a composite hydrogel–GNR after degradation (Supplementary Fig. 31).

**Discussion**

In anti-tumor experiments against PANC-1 and 4T1 tumors, regrowth occurred after 10 days post-treatment. The failure of complete tumor eradication was attributed to the decomposition and collapse of gel framework based on the fact that the degradation-arising internal stress recession after long-term incubation (Supplementary Figs 30 and 31) brought about gradual recovery of blood & nutrition supply. To address it, future work can follow two pathways. One pathway is designing robust gel framework that can withstand erosion and keep long-term biostability and robust internal stress with compact structure, which will be beneficial for continuously squeezing and narrowing blood vessels and ocluding blood & nutrition supply. Another is loading anti-drugs or other therapeutic agents for secondary therapy against tumor via extravascular gelation shrinkage-mediated drug release and degradation-mediated drug release.

As for inhibited recurrence and metastasis, residual tumor cells after surgery or other therapeutic methods probably result in tumor recurrence or metastasis. This starvation strategy can serve as an auxiliary method to address the issues via reducing vascular density, cutting off blood & nutrition supply, occluding the migration passages of tumor cells and starving the residual tumor cells to death before the failure of vascular occlusion (Figs. 8 and 9). Thus, future studies will also focus on designing robust and compact hydrogels with appropriate degradability rate that guarantees continuous work.

Although a preliminary evaluation using this treatment method to inhibit metastasis of late tumors on transgenic mice-bearing breast carcinoma also realizes a suppressed growth for late-stage tumor (Supplementary Fig. 23), systematic evaluations on late-stage tumor treatment are also necessary in future studies to explore the generality of this special starvation therapy. As well, other heat-triggered means (e.g., alternating magnetic field) are expected to replace laser irradiation to trigger extravascular gelation after replacing GNRs with Fe3O4, which can treat tumors in deeper tissues, since magnetic trigger features deeper penetration than light.

In summary, we established a starvation therapy method based on the vascular blockage by extravascular gelation shrinkage-derived internal stress for squeezing blood vessels. An organic–inorganic composite hydrogel (hydrogel–GNR) consisting of GNRs and CS/mPEG-Mal/pNIPAAm-co-AAc with a LCST at 39 °C has been constructed to validate it. Such a hybrid hydrogel can shrinkage upon exposed to 808 nm laser irradiation and produce internal stress to squeeze and narrow blood vessels, ultimately occluding ex vivo and in vivo blood & nutrition supply, which enables in vivo regression of PANC-1 pancreatic cancer on nude mice with prolonged survival rate. Similar results and conclusions were also obtained on 4T1-bearing BALB/c mice, which demonstrate the generality of this starvation therapy. More
significantly, the decreased pore diameter and porosity in hydrogel–GNR can effectively impede cell migration and invasiveness, thus serving as a countermeasure for successfully inhibiting tumor metastasis on transgenic mice-bearing orthotopic breast cancer and nude mice-bearing Panc-1 pancreatic cancer, respectively. As well, it can act as an assisted method to inhibit residual tumor recurrence after photothermal ablation. Such an extravascular starvation therapy holds tremendous potential in addressing the three primary concerns, i.e., tumor progression, tumor recurrence and tumor metastasis.

Methods

Lower critical solution temperature (LCST) study. LCSTs of the pNIPAAm-co-AAc hydrogel with tunable AAc ratios were determined from the intersection where 50% absorption and 50% viscosity were observed. The temperature-dependent transmission of the precursor solution at 450 nm was continuously measured with a spectrophotometer equipped with a peltier temperature control accessory. The temperature-dependent viscosity of the precursor solution was determined at a heating rate of 1.0 °C min \(^{-1}\) using a TA digital viscometer system (Shanghai Niran Intelligent Technology Co., Ltd) equipped with programmed temperature elevation unit. The temperature-dependent measurements regarded room temperature (20 °C) as the starting point. Furthermore, an appropriate AAc content corresponding to an optimal LCST was chosen to investigate the temperature-responsive gelation process of chitosan/pMEG-mal/pNIPAAm-co-AAc composite hydrogel and its combined hydrogel with GNRs (namely hydrogel–GNR), especially referring to the temperature-dependent viscosity and absorption at 450 nm.

In vitro temperature variations exposure to 808 nm laser. 3.5 mL of above-mixed aqueous precursor solution was transferred into a resin cuvette with four transparent sides, which had a diameter of 1 cm and a depth of 4 cm. A thin cover glass was covered on the cuvette to prevent the solution from drying during the experiments. The power density and concentration-dependent photothermal transients were evaluated on an 808-nm continuous wave (CW) laser (Shanghai experiments. The power density and concentration-dependent photothermal mixed aqueous precursor solution was transferred into a resin cuvette with four sides, i.e., control and experimental group. Ellipsoidal hydrogel hydrogel and its combined hydrogel with GNRs (namely hydrogel–GNR), and harvested with 0.25% trypsin-EDTA and rinsed. The cell suspension of cancer cells with diluted 1×10\(^5\) cells per well was incubated for 24 h. Afterwards, the cell culture medium was removed and replaced with PBS for three times, and then 400 μL of MTT (8 mg mL\(^{-1}\) stock) solution was added in each well, and the cells were further incubated for 4 h. Immediately afterwards, the MTT solution was sucked out, and 400 μL of DMSO solution was added, and furthermore, the fluorescence intensity at 480 nm was read on a fluorospectrophotometer (F-2500, Hitachi, Japan) with an excitation wavelength of 480 nm.

Ex vivo blood vessel squeezing and occlusion test. SD rattus norvegicus were supplied by Laboratory Animals Center of Tenth Peoples’ Hospital of Tongji University, and were kept in sterilized cages with supply of filtered air, sterile food, and water. The experiments were performed according to protocols approved by the Laboratory Animal Center of Shanghai Tenth Peoples’ Hospital and were in accordance with the policies of National Ministry of Health. Two aorta vessels with a length of 5 cm and an inner diameter of 1.4 mm were stripped out and rinsed by PBS for use. Their middle sections were immersed into hydrogel–GNR solution and hydrogel–GNR gel, respectively, and one tail end was linked to controllable syringe pump whose rate was set 72 mL h\(^{-1}\) to cause an elevation unit. The temperature-dependent measurements regarded room temperature (20 °C) as the starting point. Furthermore, 10 mL of aqueous hydrogel–GNR solution was added in the culturing medium, and heated to induce gelation. Afterwards, the hydrogel–GNR gel was vertical to squeeze glass slide was cut off and five culture dishes was used in the following experiments. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based cytotoxicity assay was performed to evaluate the cellular response to residual solution of different concentrations. In vitro biosafety evaluation. Murine fibroblast cell (L929 cells, catalogue number: GMS128) was obtained from Shanghai Institutes of Biological Sciences, China Academy of Sciences, and was cultured in high glucose Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin-streptomycin. The cells were cultured at 37 °C in a humidified atmosphere with 5% CO\(_2\), and harvested with 0.25% trypsin-EDTA and rinsed. The cell suspension obtained was used in the following experiments. The 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based cytotoxicity assay was performed to evaluate the cellular response to residual solution of different concentrations. The mixture solution-containing residual precursor solution and DMEM culturing medium with a tunable ratio was added into the 24-well plate (Costar, Corning Incorporated, Corning, NY, USA) containing 8 × 10\(^4\) cells per well and incubated for 24 h. Afterwards, the culture mixture was removed and washed with PBS for three times, and then 400 μL of MTT (8 mg mL\(^{-1}\) stock) solution was added in each well, and the cells were further incubated for 4 h. Immediately afterwards, the MTT solution was sucked out, and 400 μL of DMSO solution was added, and furthermore, the fluorescence intensity at 480 nm was read on a fluorospectrophotometer (F-2500, Hitachi, Japan) with an excitation wavelength of 480 nm.

In vitro inhibitory migration experiment. The special culture dishes available for investigating the inhibitory migration behavior of hydrogel–GNR against human pancreatic cancer cells, i.e., Panc-1 cells (catalogue number: SC5-535, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences) were first designed and manufactured. In detail, normal culturing dishes were washed with ethanol and DI water to remove oil contamination. Subsequently, hydrogel–GNR solution (5 g mL\(^{-1}\), 2 mL) was added and heated to induce gelation. Subsequently, five square glass slides (1.5 cm × 1.5 cm) were placed and adsorbed on the hydrogel–GNR gel via the capillary force, and they were allocated in a cross spread pattern. Furthermore, 10 mL of aqueous hydrogel–GNR solution was added in the culturing medium, and heated to induce gelation. Finally, the hydrogel–GNR gel was vertical to squeeze glass slide was cut off and five culture dishes was used in the following experiments. Panc-1 cell dispersion in DMEM-based culturing medium (density: 2 × 10\(^5\) per mL, volume: 3 mL) was seeded in the middle culturing tank (named as S0), and pure EMEM-based culturing medium (3 mL) was added into other four culturing tanks (named as S1-S4) as control. At certain time points, the number of Panc-1 cell in each culturing tanks recorded.

In vitro degradation of hydrogel–GNR. Configuration of SBF solution was carried out according to previous report. Typically, First, 7.996 g of NaCl, 0.350 g of NaHCO\(_3\), 0.224 g of KCl, 0.228 g of K\(_2\)HPO\(_4\), 1.3 cm s\(^{-1}\). The tensile stress–strain curves were carried out at a fixed strain of 1% with an angular frequency range of 0.01 rad s\(^{-1}\). The stress and strain between each point were recorded at the elastic modulus with at least parallel tests for each hydrogel. Noticeably, every test was repeated for three times.

Mechanical measurement. The tensile stress–strain measurements of pNIPAAm, composite hydrogel and composite hydrogel–GNR were performed on gels using a tensile/compressive tester (FR-108B, Faru Co., China). The cylindrical gel samples with 13 mm in diameter and 7 mm in thickness were set on the lower plate and stretched by the upper plate which was connected to a load cell (500 N). The compressive tests of the gels were obtained at the speed of 1 mm min\(^{-1}\). The tensile stress (σ) was approximately calculated as

\[
σ = F_{load}/πd^2t, \quad \text{where} \quad d = \text{diameter}, \quad t = \text{thickness}.
\]
biochemicalindiceswereobtainedtomeasurethebloodquality,whilethenormal
tissueswerestainedbyHematoxylinandEosin(H&E)toevaluatethetissue
bioavailabilityofthishydrogel–GNR.

Invivovalcularocclusiontestonbloodvesselsinabdomen.Kunmingmice
withanaveragebodyweightofabout20gweresuppliedbyLaboratoryAnimals
Center ofTenth Peoples’ Hospital of Tongji University, and the experiments
were performed according to protocols approved by the Laboratory Animal
Center of Shanghai Tenth Peoples’ Hospital and were in accordance with the policies
of National Ministry of Health. 2 mL of hydrogel–GNR (20 ppm) was
intraperitoneally (i.p.) injected into the periphery of vein blood vessels in abdomen under the gui-
dance of CD1 mouse with a body weight of about 20 g were supplied by
Laboratory Animals Center of Tenth Peoples’ Hospital of Tongji University, and
were kept in sterilized cages with supply of filtered air, sterile food, and water.

During establishing the solid tumor model, 0.2 mL cell suspension (2 × 10^6 cells)
in DMEM medium without serum/phenol red was injected subcutaneously into the
back of nude mice using a 27-gauge needle. Tumors were allowed to grow to 100 ±
20 mm^2 (mean ± standard deviation) prior to any experiment (designated as
experimental day 1). Mice were monitored for general well-being, weight, tumor
volume and survival rate.

Similar procedures are available for establishing 4T1 breast xenograft tumor
wherein nude mice and PAN-C1 cells in PAN-C1 model establishment were
replaced by immune-competent Balb/c mice (female) and 4T1 cells, respectively.
4T1 cell line (catalogue number: SCSP-535) was purchased from Shanghai
Institutes of Biological Sciences, Chinese Academy of Sciences. All in vivo
experiments were performed according to protocols approved by the Laboratory
Animal Center of Shanghai Tenth Peoples’ Hospital and were in accordance with the
policies of National Ministry of Health.

In vivophotothermaltemperaturemeasurements. 200 μL of hydrogel–GNR
solution in PBS (5 g per 100 mL) was i.t. injected into the PAN-C1 tumor
implanted on nude mice (n = 3). Subsequently, 808 nm laser irradiation at a power
density of 0.15 W/cm^2 was carried out during, within which the time-dependent
temperature variation was visualized and recorded on the IC camera (FLIR
Scorpio3-Series) to determine the optimal irradiation duration.

In vivostarvationtherapyagainstPANC-1and4T1tumors.Fiveexperiments
were randomly chosen before any further experiments (n = 6), i.e., control group
(designated as G1) that only saline was i.t. injected into tumor; Laser–iGNR group
designated as G2) wherein 808 nm laser irradiation was applied after i.t. injecting
GNRs into tumor; Laser–hydrogel–GNR (1) group (designated as G3) wherein
808 nm laser irradiation was applied after i.t. injecting hydrogel into tumor alone.
Laser–hydrogel–GNR (2) group (designated as G4) wherein 808 nm laser irradiation
was applied after i.t. injecting hydrogel–GNRs into both tumor and its
surrounding periphery, and pNIPAAM group (designated as G5) wherein only
pNIPAAM was i.t. injected into tumor and its periphery that spontaneously trigger
complete dissolution and pNIPAAm-co-AAc can withstand erosion and no Au atoms in living body.

Detailedinformationofallusedantibodies.The names, suppliers and catalog
numbers of employed antibodies for western blotting, immunohistochemistry, immuno
chemochemistry, flow cytometry and ELISA are given in the follow part and they
are listed as antigen first, followed by supplier, catalog number and clone.

Indirect intratumoral vascular occlusion. Two groups were randomly chosen
before and after the corresponding treatment in Laser–i-hydrogel–GNR (2), and on
5th day post-treatment, the images and movie of blood velocity variation in tumor
tissues was further recorded.

On the 10th day, some mice in the five groups (n = 3) were sacrificed and their
tumors were taken out for various pathological examinations including
western blot, CD34 & TUNEL immunofluorescence co-staining, hematoxylin and eosin
(H&E), CD34 and proliferating cell nuclear antigen (PCNA) immunohistochemistry were carried out, and the observed via laser
confocal scanning microscopy (LCSM) on Nikon A1R/A1. The semi-quantitative
number and percentage of apoptotic cells in total, apoptotic tumor cells and apoptotic endothelial cells labeled by CD34 can be obtained via counting the
the number of harvested LCSM images in CD34 & TUNEL immunofluorescence co-staining.

All uncropped western blot scans have been provided in the Source Data file.

Moreover, significantly stronger signal was observed in tumor tissue samples after treatment with enzyme-linked
innonimmuneassayassay(ELISA).

In vivo metabolism test. Nude mice bearing PANC-1 tumor (n = 3) was
employed, and GNR metabolism was used to evaluate the degradation and
secretion of this composite hydrogel, since robust chemical chelation between GNR
and pNIPAAm-co-AAc can withstand erosion and no Au atoms in living body.
After treatment with G4 (i.e., Laser–hydrogel–GNR (2)), nude mice were kept
in special metabolic cage, and at each certain time point, faeces and urine were
collected to be dissolved by nitrohydrochloric acid under heating at 50°C. After
centrifugation, the undissolved residues were discarded via filtration by 2000
nm filtering membrane, and the dissolved solution was normalized to a constant
volume (10 mL) by adding ultrapure water. Ultimately, Au concentration in the
normalized solution was determined by inductively coupled plasma optical emis
sion spectrometry (ICP-OES) on Agilent Technologies 725, and the secreted
Au amount was obtained. Ultimately, the time-dependent accumulatively secreted
percentage of Au-labeled hydrogel–GNR can be obtained via calculating the ratio
of accumulative Au mass to initially added Au mass.

In vivo circulating test. Two groups were randomly chosen before experiments, i.e., Laser–iGNR group (i.e., control group, designated as G2)
wherein 808 nm laser irradiation was applied after i.t. injecting GNRs into both tumor, and Laser+hydrogel–GNR (2) group (i.e., treated group, designated as G4) whereby, imaging was applied after i.t. injecting hydrogel–GNR into both tumor and its surrounding periphery. First, fluorescence imaging was used to evaluate the intratumoral vascular occlusion arising from the squeezing and narrowing of intratumoral blood vessels caused by this extravascular gelation shrinkage-induced internal stress. In detail, after corresponding treatment in each group, the in vivo mouse-bearing PANC-1 tumor or BALB/c mice in each group (n = 3) was chosen. Before corresponding treatment in each group, the 1st i.v. injection of clinically used Sonovue™ microbubbles (0.2 mL) into nude mice or BALB/c mice was carried out in both groups via tail vein, and then the ultrasonic images and movies under both CHI and B fundamental imaging (BFI) modes was instantly captured on GE LOGIQ E9. After 30 min-90 min, the corresponding treatments in both groups, i.e., treated (i.e., Laser+hydrogel–GNR or G4) and control (i.e., Laser+GNR or G2) groups, were carried out. Afterwards, the 2nd i.v. injection of Sonovue™ microbubbles into nude mice or BALB/c mice was implemented in both groups via tail vein, and then the ultrasonic images and movies under CHI mode was instantly captured again. As well, the occlusion test of another group, i.e., nPNAAPM Group (G5), was also evaluated on nude mouse-bearing PANC-1 tumor using in vivo fluorescence imaging to evaluate the diffusion of hydrogel (Sonovue™ microbubbles as tracers) in both groups via tail vein, and then the ultrasonic images and movies under CHI mode was instantly captured again. As well, the occlusion test of another group, i.e., nPNAAPM Group (G5), was also evaluated on nude mouse-bearing PANC-1 tumor using in vivo fluorescence imaging to evaluate the diffusion of hydrogel (Sonovue™ microbubbles as tracers) in both groups via tail vein, and then the ultrasonic images and movies under CHI mode was instantly captured again.

Ultimately, nude mouse-beariing PANC-1 xenografted tumor (n = 3) was used to evaluate the diffusion of composite hydrogel, wherein Cy3-labeled dextran (size: 70 K) was first used instead of GNRs to label the composite hydrogel, and was i.t. injected into PANC-1 tumor, and in vivo animal fluorescence imaging was carried out on IVIS Lumina II with Andor iKon camera at three time points, pre, 30 s post and 50 s post-i.t. injection of hydrogel–dextran into PANC-1 tumor. Afterwards, T2-weighted MRI under the T2-TSE-Tra sequence on SIEMENS MAGNETOM Vario syngo MR B17 was carried out instead of in vivo animal fluorescence imaging to evaluate the diffusion of hydrogel-GNR, and hollow porous Fe3O4 nanoparticles act as the T2 contrast tracer. Before and after treatment with injection of contrast agents, T2-weighted MR images were captured using the following parameters: TR = 1740 ms, TE1 = 4.36 ms, TE2 = 11.90 ms, TE3 = 19.44 ms, TE4 = 26.98 ms, TE5 = 54.32 ms, BW = 260 Hz, Thickness = 2 mm, Slices = 20, FOV = 200×200 mm. As well, after 1 day post-corresponding treatments in both groups including Laser+GNR group (G2, designated as control group) and Laser+hydrogel–GNR (2) (G4, designated treated group), nude mouse-beariing PANC-1 tumors in each group (n = 3) was instantly chosen and sacrificed and tumors were excised. Afterwards, Cy3 labeled CD31 and FITC-labeled CD34 immunofluorescence stainings of tumor tissue slices were instantly carried out in two groups for LCSM observation on Leica TCS SP5.

In vivo test on inhibiting tumor skin–skin metastasis. Two groups with 6 mice-bearing PANC-1 tumor in each group were set, and they were control and experimental group, respectively. In control group (i.e., US(GB)), microbubbles were first injected into the PANC-1 tumor and its surrounding periphery, and then generated the gelation process via laser irradiation, which was identical to the treatment in aforementioned Laser+hydrogel–GNR (2). Subsequently, the treatment that is marriage of intravenous MBs injection and US irradiation was carried out. Laser irradiation with a power density of 0.15 W cm−2 was instantly applied after injecting hydrogel–GNR, and the duration time was 3 min in sum with pulse duration of 30 s and interval time of 10 s. The concentration of hydrogel–GNR (20 ppm) was 5 g per 100 mL, and the injection volume was 200 μL. The duration time that was carried out on a portable ultrasound apparatus (Chattanooga, USA) at the power of 1 W (100% duty cycle) was also pulsed with four cycles, and each cycle contained a 30 s irradiation duration and an interval time of 30 s between two irradiations, and the ultrasound irradiation under the same conditions was repeated for 5 days at the site of the primary tumor. The growth of lung metastatic tumors in the primary tumor was monitored in a real-time manner, and the metastasis site of PANC-1 tumors was recorded after 10 days.

In vivo inhibition test of tumor recurrence. Photothermal resection was used to investigate the tumor recurrence. Two groups with 10 nude mouse-beariing PANC-1 tumor in each group were set, and they were designated as control group and experimental group, respectively. In the experimental group, hydrogel–GNR first injected into the periphery of PANC-1 tumor, and laser (1) was applied to induce the gelation and make gelate the PANC-1 tumor. Before and after injecting PANC-1 tumor is expected to incompletely ablate tumor in the presence of laser (2) irradiation and generate residual tumor tissues available for recurrence, which is one typical model of tumor recurrence. In treated group, the pre-treatment with injection of hydrogel–GNR into the normal tissues surrounding PANC-1 tumor and laser (1) irradiation is anticipated to trigger gelation process of such a composite hydrogel, narrow periphery blood vessels surrounding tumor and occlude blood and nutrition supply towards residual tumor tissues and ultimately suppress tumor recurrence. Herein, laser (1) irradiation with a power density of 0.15 W cm−2 was instantly implemented after injecting hydrogel–GNR, and the duration time was 3 min in sum with a pulse duration of 30 s and an interval time of 10 s, and the concentration and volume of hydrogel–GNR (3%) were 5 g per 100 mL and 500 μL. The parameters of laser (2) irradiation were power density of 0.30 W cm−2 for continuous 3 min, and the dose and volume of GNRs were 10 nM and 200 μL. The tumor recurrence in two groups was monitored after 5 days.

In vivo test on inhibiting breast-to-lung metastasis. A transgenic mouse model that can generate orthotopic mammary adenocarcinomas after induction by MMTV-PyVmt oncogene has been accepted and used as the ideal model for evaluating anti-metastasis basing on this special treatment in this revised manuscript, as the majority of the tumor-bearing transgenic mice develop secondary metastatic tumors in the lung, as evidenced by many references. The transgenic mice with an average body weight of about 20 g were supplied by Laboratory Animals Center of Tenth Peoples’ Hospital of Tongji University that purchased from Shanghai Hanyin biotech. Co.LTD, and the experiments were performed according to protocols approved by the Laboratory Animal Center of Shanghai Minhang Hospital and were in accordance with the policies of National Ministry of Health. Transgenic mice-bearing orthotopic breast tumor were randomly divided into two groups (n = 7), i.e., control (G1) and treated (i.e., G4 or Laser+hydrogel–GNR (2)) groups. After corresponding treatment in each group, transgenic mice were raised, and at the end day of experiments (day 28), the mice in both groups were sacrificed and lungs were excised and stained by triphenyltetrazolium chloride (TTC) according to the instruction. Eye-naked observation was carried out to count the number of tumor transferred from the primary tumor in subcutaneous breast pad.

Statistical analysis. All the experiments were performed in triplicate. The obtained data were expressed as the mean ± standard deviation (SD) and the statistical significance between two groups was analyzed by unpaired Student’s t test through SPSS 19.0 (SPSS, Chicago, IL, USA). Single, double and triple asterisks represent P ≤ 0.05, 0.01, and 0.001, respectively, wherein *P < 0.05 was considered statistically significant and ***P < 0.01 was extremely significant.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All relevant data are available from the corresponding authors upon reasonable request. The source data including Figs. 1c, 3c–f, 4c, 5bcd, 6bcd, 7bcd,e and i and 8c and Supplementary Figs 9, 16, 17, 18, 20, 22, 25, 26, 27, 29, 30b and 31 are provided as a Source Data file, and they also have been uploaded in a generalist repository named Open Science Framework with an identifier (https://doi.org/10.17605/OSF.IO/WQP7A).

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Author contributions
K.Z. conceived and designed the project. K.Z., Y.F., Y.H., H.Y., X.G., Y.P., B.Z., W.Y., and H.L. performed in vitro experiments, and K.Z., D.D., Y.F., Y.H., W.R., C.L., and L.S. performed in vivo experiments. K.Z. wrote the manuscript, and K.Z., Y.C., and H.X. supervised the project. All authors discussed the results and commented on the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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