Nanosurgical Resection of Malignant Brain Tumors: Beyond the Cutting Edge

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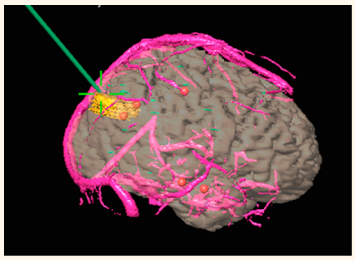
ABSTRACT Advances in surgical procedures and improvements in patient outcomes have resulted from applications of new technologies in the operating room over the past three decades. All surgeons would be excited about the possibilities of improving their resections of tumors for patients with cancer if a new technology were introduced to facilitate this. In this issue of *ACS Nano*, Karabeber *et al.* use a hand-held Raman scanner to probe the completeness of resection of glioblastoma multiforme (GBM), the most malignant brain cancer, in a genetically engineered mouse model. They show that the hand-held scanner could accurately detect gold–silica surface-enhanced Raman scattering nanoparticles embedded within the GBM, resulting in a complete tumor resection. In this Perspective, we review potential applications of nanotechnologies to neurosurgery and describe how new systems, such as the one described in this issue, may be brought closer to the operating room through modifications in nanoparticle size, overcoming the obstacles presented by the blood–brain barrier, and functionalizing nanoparticle conjugates so that they reach their target at highest concentrations possible. Finally, with adaptations of the actual hand-held Raman scanner device itself, one can envision the day when "nanosurgical" procedures will be a part of the surgeon's armamentarium.



It has been recognized for some time that nanomaterials are similar in scale to a multitude of biological systems and processes, thus providing an allure to the utility of nanotechnology for the diagnosis and treatment of human conditions at the molecular level.[1] In terms of medical applications, nanomaterials are used as drug delivery vehicles and as contrast agents for medical imaging and are incorporated in diagnostic devices to detect the extent of human disease.[1]

In the past decade, applications of nanomedicine to the field of surgery have been steadily increasing in number, scope, and design. The term "nanoneurosurgery" was coined initially in 2003 by Dunn and Black to describe the potential of the neurosurgeons to use molecular therapies to complement existing local therapies for the treatment of patients with neurosurgical conditions including malignant brain tumors.[2] There was considerable excitement among neurosurgeons when it was suggested that femtosecond laser systems, nanoneedles, and nanotweezers could be useful technologies to radically change the practice of neurosurgery.[3]

Nanosurgery and Malignant Brain Tumors. Although relatively infrequent in incidence compared to other cancers such as lung, breast, and prostate, brain cancers are notable for their relative resistance to contemporary therapies, their frequent recurrence, and the morbidity and mortality associated with their location and invasive growth patterns. The most common brain cancer that arises within the central nervous system is the glioblastoma multiforme (GBM), which is associated with a 12–15 month survival after diagnosis despite all forms of treatment including neurosurgical resection, focal radiation therapy, and conventional chemotherapy. In this regard, the poor prognosis of patients with GBM is shared with those harboring the other most deadly cancers, including pancreatic, liver, and hepatocellular. The reasons why GBM is so aggressive and difficult to treat is its genotypic and phenotypic heterogeneity, its microscopic infiltration into regions of normal brain, its resistance to focal radiation therapy and chemotherapy, and the presence of the blood–brain barrier (BBB), which precludes access of a wide variety of therapeutic agents to the tumor. There have arguably been neurosurgical advances in treating patients with GBM, including the development of improved neurosurgical

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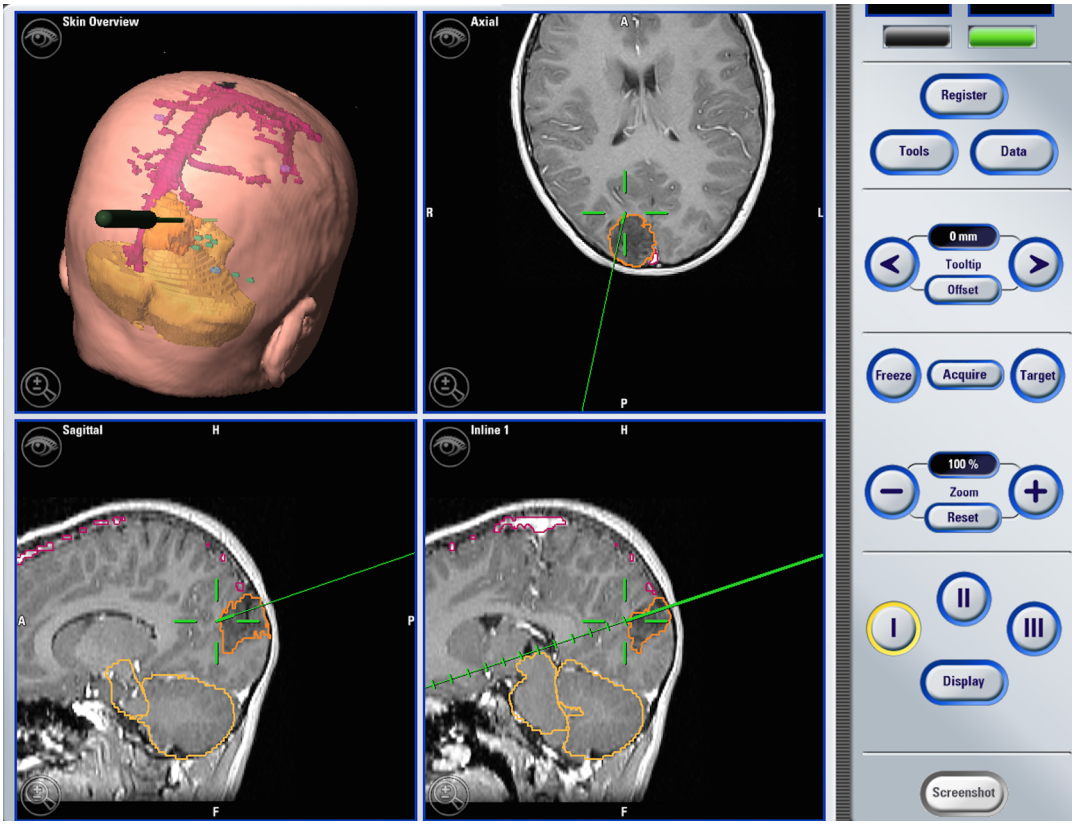

Figure 1. Operative screenshot taken from neuronavigation workstation on a 12-year-old male with a right occipital brain tumor on magnetic resonance imaging (MRI). The tumor is outlined in orange. The green line represents the in-line probe that is used to assess the depth of resection. In this case, the neurosurgeon has resected the brain tumor to its interface with normal brain. Upper left panel depicts the skin overview and in-line probe pointing at the tumor. Advances in neuroimaging, neuronavigation, intraoperative MRI, and continuous neuromonitoring have facilitated the removal of human brain tumors by neurosurgeons while minimizing deleterious effects on adjacent normal brain tissue.

operating systems (Figure 1), implantation of biodegradable polymers and neural stem cells, convection-enhanced delivery (CED), intra-arterial delivery of chemotherapeutics, and the use of immunotherapeutics, to name a few. However, these advances have not translated cogently into improved patient survival. Accordingly, it is clear that new therapies and approaches are warranted to help improve the poor prognosis of patients with this most dreaded and feared cancer.

In this issue of *ACS Nano*, Karabeber *et al.* describe the use of a hand-held Raman scanner to identify surface-enhanced Raman scattering (SERS) nanoparticles that were delivered to a genetically engineered GBM mouse model.[4] Spectral mapping of SERS gold nanoparticle probes with excitation wavelengths in the 700–800 nm near-infrared range has recently been touted as a reliable method for molecular imaging *in vitro* and *in vivo*.[5,6] Karabeber *et al.* show that intravenously delivered gold–silica SERS nanoparticles accurately demarcate the extent of the GBM, and that the GBM could be resected more completely with the hand-held device than with a static Raman microscope. Although somewhat controversial, there is some evidence that the extent of resection in GBM correlates with overall patient survival.[7,8] In this light, the technique described by Karabeber *et al.* holds considerable appeal. Their experimental data suggest that SERS-image-guided resection of GBM is better than the use of an operative microscope and the rival technique that uses 5-aminolevulinic acid (5-ALA)-derived tumor fluorescence.[9,10] The superiority of the hand-held Raman scanner over the static Raman microscope likely relates to the maneuverability of the hand-held device and the ability to overcome overhanging brain tissue that would obstruct the view of the static Raman microscope.[4] In the authors' experience, the hand-held Raman scanner leads to better speed of data acquisition, provides real-time operative guidance, and can be used to interrogate the operative bed at any angle. In comparison to fluorescence-based image-guided resection such as the use of 5-ALA, the unique Raman fingerprint with gold–silica SERS nanoparticles can generate longer lived optical signals because organic dye molecules can photobleach. The fact that some hand-held Raman scanners are already in use in clinical practice is also a point in favor of rapidly moving this technology into the neurosurgical operating room.

Toward Enhanced Brain Tumor Resections: Beyond the Cutting Edge.

In this issue of *ACS Nano*, Karabeber *et al.* describe the use of a hand-held Raman scanner to identify surface-enhanced Raman scattering (SERS) nanoparticles that were delivered to a genetically engineered GBM mouse model.

As good as the technology described by Karabeber *et al.* appears to be, attention to certain details in nanoparticle design and conjugation may continue to improve their results and lead to a more seamless transition into clinical trials.

(i). Nanoparticle Design: Where Size Matters. Although it is known that nanomaterials in the range of 2−100 nm can impact cellular signaling processes, Jiang *et al.* have demonstrated *in vitro* that there is an optimum range of size, between 40 and 50 nm, where nanoparticles have their greatest cytological effects (Figure 2).[11] Their data suggest that there is a window of opportunity to exploit the size of nanoparticles in given cell systems *in vitro* and *in vivo* to enhance their uptake for maximum desirable effects such as cytotoxicity, apoptosis, or reduced proliferation in the context of cancer therapy. Accordingly, determining the optimum nanoparticle size in the treatment of GBM and other cancers may require additional efforts to test different sized nanoparticles in experimental models before embarking on clinical trials.

(ii). Overcoming the Blood−Brain Barrier. Following intravenous systemic delivery of gold nanoparticles, there is rapid uptake by the spleen and liver in comparison to other organ systems. In the report by Kerabeber *et al.*, inductively coupled plasma mass spectrometry (ICP-MS) was used to quantify SERS nanoparticle uptake by the GBM cells. The authors showed that only about 0.8% of the injected dose of

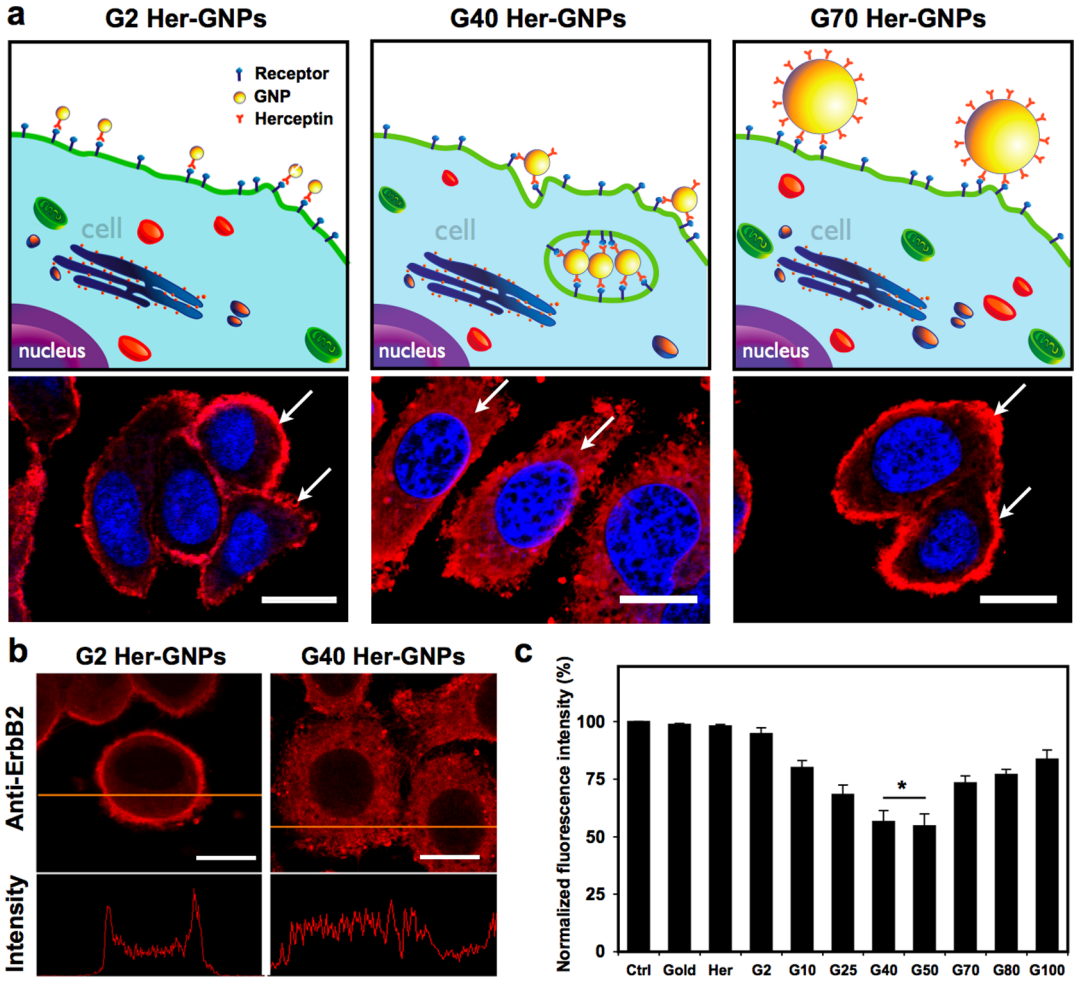

Figure 2. Nanoparticle-mediated cellular response is size-dependent. Downregulation of membrane ErbB2 expression using gold nanoparticles of 2, 40, and 70 nm size (G2, -40, -70 NPs) conjugated with the Herceptin antibody. Illustrations with corresponding fluorescence images of ErbB2 receptor localization after treatment with different sized Her-GNPs. Arrows indicate ErbB2 receptors, and the nucleus is counterstained with DAPI (blue). Internalization of the ErbB2 receptor is optimized in this study with G40 GNPs. Scale bar = 10 μm. Reprinted with permission from ref 11. Copyright 2008 Nature Publishing Group.

SERS particles per gram of tumor tissue was found within the tumor—a relatively small fraction. The clearance by the reticuloendothelial system may be lessened, to some degree, by coating the gold nanoparticles with polyethylene glycol (PEG).[12] Further, it is conceivable that the authors could have achieved a higher percentage concentration of gold–silica SERS nanoparticles within the experimental GBM tumors if they had manipulated the blood–brain barrier prior to intravenous administration of the SERS nanoparticles.

The BBB is a highly selective but permeable cellular substrate comprising brain capillary endothelial cells connected by tight junctions that are separated from astrocytic foot processes by a well-defined basement membrane.[13] For therapeutic agents to cross the BBB, they must use either passive or active transport mechanisms. It has been known for quite some time that small, nonpolar lipophilic agents will readily cross the BBB, whereas polar or water-based compounds will require active transport mechanisms.[14] *In vitro* models of the BBB have been developed for testing the permeability of gold nanoparticle conjugates.[12] Etame *et al.* demonstrated size-dependent permeation of PEG-coated gold nanoparticles whereby the smaller core-size gold nanoparticles coupled to shorter PEG chain length moieties led to optimum penetration of the BBB (Figure 3).[12]

There are conventional, nonfocal strategies to disrupt the BBB, and these include the use of an osmotic agent, such as mannitol, delivered intra-arterially to the brain *via* the carotid arteries, or the use of bradykinin system analogues such as RMP-7, which enhances the permeability of the BBB *via* receptor-mediated mechanisms. However, widespread enhanced permeability of the BBB may have unintended consequences, as the BBB serves to protect the brain from systemic toxins. That is why, in recent times, emphasis has been placed on focal disruption of the BBB to enhance local delivery of therapeutic agents to brain tumors. One such technique to disrupt the BBB focally is the use of transcranial-focused ultrasound (FUS). This strategy employs focused low-frequency ultrasound waves that create nondestructive oscillations of circulating microbubbles. The mechanical energy transmitted by these oscillations alter the ultrastructural features of the BBB, resulting in enhanced focal permeability.[14]

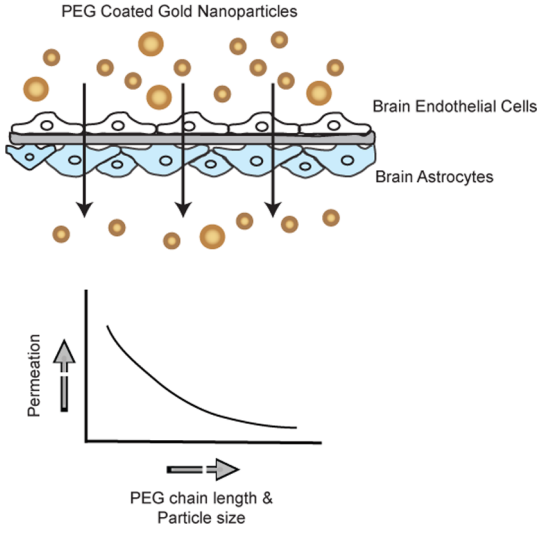

Figure 3. Permeation of the brain microvasculature using an *in vitro* model of the blood–brain barrier. In this model, rat brain endothelial cells are separated from rat astrocytes by a 400 nm microporous membrane. PEGylated gold nanoparticles of various PEG chain length and nanoparticle size are then placed in medium on top of the rat endothelial cells. At fixed time points, the medium below the astrocytes is quantitatively analyzed for gold content by inductively coupled plasma atomic spectrometry. In this model, short PEG length (1000–2000) in combination with small core size exhibits optimum permeation of the BBB. Reprinted with permission from ref 12. Copyright 2011 Elsevier.

> **In recent times, emphasis has been placed on focal disruption of the blood–brain barrier to enhance local delivery of therapeutic agents to brain tumors.**

To enhance the delivery of nanoparticles to the brain, Etame *et al.* used tail-vein-injected microbubbles followed by magnetic-resonance-guided focused ultrasound (MRgFUS) in a rat model to facilitate passage of nanoparticles across the BBB and to enhance the concentration in the cerebral hemisphere (Figure 4).[15] The microbubbles used in this approach are generated as lipid-encased perfluorocarbon gas spheres at 1–5 μm in diameter. Using this technique, the authors showed that they could achieve a 3-fold increase in gold nanoparticles in the MRgFUS-treated cerebral hemisphere *versus* the untreated control hemisphere.

Another potential technique that can be used to circumvent the BBB and increase the concentration of nanoparticles in the brain includes the use of convection-enhanced delivery (CED) in which the nanoparticles are infused directly into the brain using a hydrostatic pressure gradient.[16] This technique has been successfully employed in delivering quantum dots[17] and drug- and antibody-conjugated nanoparticles to experimental brain tumors.[18,19]

(iii). Improving Delivery by Functionalizing SERS Nanoparticle

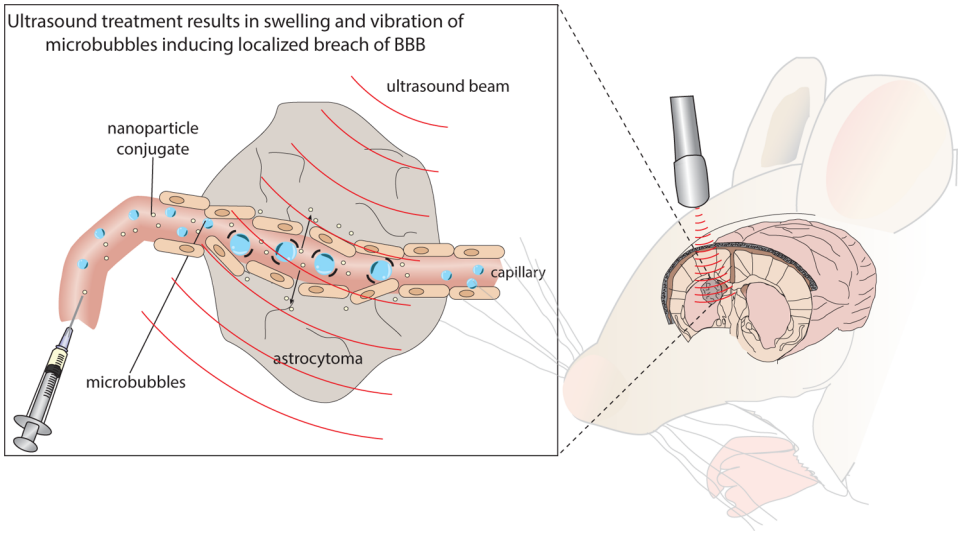

Figure 4. Magnetic resonance guided focused ultrasound disruption of the blood–brain barrier in a rat model. After baseline imaging of the brain, animals received 14 mg/kg of 50 nm PEG–gold nanoparticles by tail vein followed immediately by 0.02 mL/kg lipid-encased perfluorocarbon microbubbles (1–5 μm in diameter) diluted 10:1 in normal saline. As the microbubbles enter the cerebral microvasculature, the delivery of FUS causes them to expand and to collapse, thereby transiently, focally opening the BBB. Opening the BBB enables the gold nanoparticle conjugates to permeate the brain and brain tumor (astrocytoma) specifically at the site of the FUS beam disruption. This can be accomplished without adverse effects such as intracerebral hemorrhage or cerebral injury.

Conjugates. The specificity of GBM tumor cell targeting may be improved in the future by surface coating SERS nanoparticles with antibodies that recognize prominent cell surface antigens. In the case of GBM, the epidermal growth factor receptor (EGFR) is frequently mutated and overexpressed.[20] Diaz *et al.* performed surface coating of SERS nanoparticles using an anti-EGFR receptor (Panitumumab).[5] This was followed by MRgFUS delivery to GBM cells in a rat model. These authors were able to show that functionalized SERS nanoparticles measuring 120 nm in size are preferentially taken up by GBM cells *in vivo*, raising the distinct possibility that tumor-specific targeting of SERS nanoparticles may be a useful approach in the future (Figure 5). They also showed that functionalized SERS nanoparticles could be delivered to EGFR-positive tumor cells at the leading edge or invasive front of the GBM. As GBMs most frequently recur at the margin of the interface between tumor cells and normal brain, the reliable and accurate targeting of such invading GBM cells using functionalized SERS nanoparticles could prove to be cytotoxic to these invasive cells and obviate the need to resect the tumor in regions of normal brain, especially when close to critically important neuroanatomical pathways.

Toward Nanoneurosurgery in the Operating Room—Next Steps. Neurosurgeons have worked steadily to improve outcomes in their patients following the resection of brain tumors such as GBM. Advances in neuroimaging, intraoperative neuronavigation, intraoperative use of MRI scanning and ultrasound to assess the extent of resection, and continuous neuromonitoring to avoid injury to nearby functional neuroanatomical pathways have all helped maximize tumor resection while minimizing morbidity in terms of neurological deficits to patients.

It is intuitively appealing for neurosurgeons to consider the intraoperative use of a hand-held device, such as the Raman scanner, to identify regions of residual tumor and to move toward improved resections of GBM. Some of the immediate next steps from an experimental standpoint would include the development of an operative approach to use the Raman scanner in animal models that avoids the use of paraformaldehyde fixed brains and the *ex vivo* approach as described by Karabeber *et al.* Another area of future development no doubt will be to enhance the sensitivity of SERS-particle-tagged tumor cells as, at the present time, the depth of penetration of the device in terms of tumor detection is approximately 5–7 mm. This depth may be insufficient for the detection, capture, and deletion of

The specificity of glioblastoma multiforme tumor cell targeting may be improved in the future by surface coating surface-enhanced Raman scattering nanoparticles with antibodies that recognize prominent cell surface antigens.

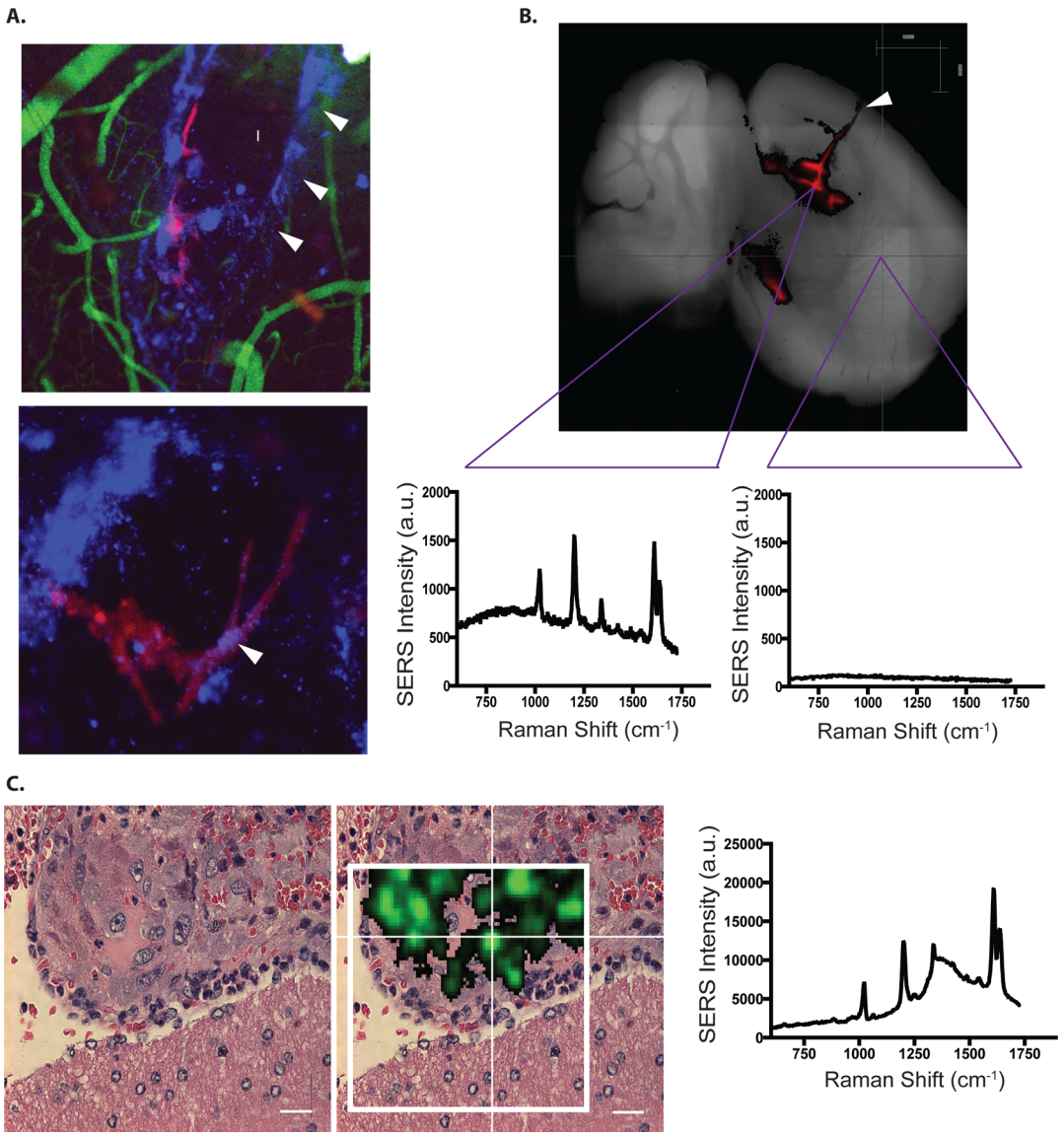

Figure 5. Optical tracking of glioblastoma multiforme cells using near-infrared SERS-functionalized gold nanoparticles. In this example, U87 GBM cells were incubated with anti-EGFR-SERS440 nanoparticles for 24 h before implantation into the right frontal lobe of NOD/SCID male mice through a cranial window. One day after tumor cell injection, the U87 GBM cells were imaged in anesthetized live mice through the cranial window using a two-photon laser confocal microscope. The mice were then euthanized and their brains fixed in 3.7% paraformaldehyde. Brain slices through the nascent GBM tumors were submerged in PBS for *ex vivo* fluorescent and Raman spectral imaging. In this image, U87 GBM cells marked by the anti-EGFR-SERS440 tag can be visualized separately from the surrounding brain parenchyma. Raman spectrum from the site defined by the crosshairs is demonstrated. Mapped region indicated by white box outline. Scale bars = 20 μm. Reprinted with permission from ref 5. Copyright 2014 Elsevier.

GBM cells that are beyond the enhancing tumor margin and are residing in regions of normal or near-normal brain.

Future development of the handheld Raman scanner may include the addition of a component to the device that can not only detect SERS nanoparticle positive GBM cells but also remove these tumor cells simultaneously using, for example, an ultrasonic aspirator or laser. It is conceivable that minimally invasive strategies, such as robotics, could be built into the Raman scanner device to automate the process whereby the tumor margin is inspected circumferentially and methodically to avoid human error.

Finally, it will be important for investigators to continue to explore optimization of SERS nanoparticle size, composition, and surface coating while reducing uptake to the reticuloendothelial system in order for these technologies to have their maximal effects and to reach clinical trials.

Conflict of Interest: The authors declare no competing financial interest.

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