Self-Folding Hybrid Graphene Skin for 3D Biosensing

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Abstract

Biological samples such as cells have complex three-dimensional (3D) spatio-molecular profiles and often feature soft and irregular surfaces. Conventional biosensors are based largely on 2D and rigid substrates, which have limited contact area with the entirety of the surface of biological samples making it challenging to obtain 3D spatially resolved spectroscopic information, especially in a label-free manner. Here, we report an ultrathin, flexible skinlike biosensing platform that is capable of conformally wrapping a soft or irregularly shaped 3D biological sample such as a cancer cell or a pollen grain, and therefore enables 3D label-free spatially resolved molecular spectroscopy via surface-enhanced Raman spectroscopy (SERS). Our platform features an ultrathin thermally responsive poly(N-isopropylacrylamide)-graphene-nanoparticle hybrid skin that can be triggered to self-fold and wrap around 3D micro-objects in a conformal manner due to its superior flexibility. We highlight the utility of this 3D biosensing platform by spatially mapping the 3D molecular signatures of a variety of microparticles including silica microspheres, spiky pollen grains, and human breast cancer cells.

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Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.8b03461.
The SI includes details of the experimental methods and notes on the Raman and mechanical characteristics of the G-NIPAM-G-Ag skin, versatility of the skin, estimation of the Laplace pressure on water evaporation, estimation of the strain during wrapping on the pollen spike, and Raman spatial mapping of a cancer cell on the skin prior to wrapping (PDF).

The authors declare no competing financial interest.
SERS; graphene; lab-on-a-chip; stimuli-responsive; origami

Motivated by societal needs for diagnosing diseases more efficiently, monitoring biotechnological processes, detecting biochemical warfare agents, and improving food safety, significant efforts in nanotechnology are focused on biosensing. Single cell biosensors can provide detailed information on the composition and activity of individual cells as well as elucidate cellular heterogeneity and statistical variation in multicellular entities such as tissues and tumors. Among the variety of biosensing techniques in development, surface-enhanced Raman spectroscopy (SERS) has unique advantages for label-free, highly sensitive, and selective detection of analytes through the amplification of localized electromagnetic fields on the surface of plasmonic nanomaterials when excited with monochromatic light. SERS measurements are nondestructive and minimally to entirely noninvasive which are advantageous for both in-vitro and invivo measurements.

SERS substrates are developed primarily by depositing or fabricating noble metal nanostructures on rigid and static substrates such as glass and silicon. While these approaches have enabled a range of biochemical studies, there are obvious limitations in the analysis of soft, irregularly shaped and three-dimensional (3D) objects that are ubiquitous in biology. To address these limitations, researchers have explored the creation of flexible SERS substrates such as plasmene nanosheets, metal nanoparticle impregnated paper, polymer membranes and electrospun fibers. These flexible substrates enable better contact with 3D samples and efficient access to the analytes. However, they generally rely on relatively thick substrates, which are unable to form intimate and conformal contact with small, irregularly shaped samples such as a single cell.

Previously, we reported a technique entitled mechanical trap surface-enhanced Raman spectroscopy (MTSERS) wherein a self-folding microgripper with arms composed of a bilayer of SiO/SiO$_2$ (10/15 nm) was coated with gold nanostars. We utilized MTSERS to encapsulate and analyze the structure of microparticles such as Janus beads and live cells. Despite the improved access to the 3D surface, a limitation of this approach is that the arms...
are composed of rigid materials that preclude conformal contact to irregularly shaped objects. Besides, the folding force due to release of residual stress of the hinges and the rigid arms of the grippers can potentially deform the encapsulated cell possibly disturbing its native biomolecular state.\(^{20}\)

Graphene has an atomically thin two-dimensional (2D) monolayer structure with good chemical inertness and biocompatibility. Consequently, it is an attractive substrate for biosensing devices.\(^{21}\) In SERS-based biosensing applications, graphene provides an atomically flat surface, high stability, and a clean vibrational Raman spectrum.\(^{22,23}\) However, graphene shows limited intrinsic Raman enhancement,\(^{25,26}\) stemming solely from the charge transfer between graphene and the analyte. Hence, graphene is often used in combination with plasmonic metal nanostructures for SERS applications.\(^{27}\) Monolayer graphene also has a Föppl–von Kármán number, which is the ratio of the in-plane to out-of-plane stiffness, comparable to that of paper and consequently is an ideal substrate for conformally contacting a 3D object.\(^{28}\) Yet, most of the previously reported graphene-based Raman studies use graphene in a planar geometry with a rigid underlying substrate,\(^{30}\) which does not utilize the excellent flexibility of this 2D material.\(^{31}\)

Here, we leverage the ultrathin, low bending rigidity, biocompatibility, and high stability of graphene to create a flexible, skinlike SERS substrate. The SERS skin is composed of thermally responsive graphene, which has poly(N-isopropylacrylamide) (PNIPAM) brushes grafted on the surface, and plasmonic silver nanocubes (Ag NCs). After patterning the ultrathin SERS skin into well-defined shapes, self-folding is induced by a mild temperature increase, compatible with live cell culture. Importantly, as we show, the ultrathin self-folding SERS skin conformally wraps microobjects with a wide range of mechanical properties and surface irregularities such as rigid microspheres, spiky pollen grains, and soft biological cells. Because of this conformal 3D SERS enhancement, we demonstrate that high fidelity molecular maps can be obtained using confocal Raman microscopy. This conformal 3D spectroscopic approach based on ultrathin skinlike structures offers an unprecedented opportunity for molecular mapping of the 3D surfaces and in particular represents a novel route to bridge the chemical and morphologic domains at the single cell level.

The conceptual scheme and fabrication process flow are illustrated in Figure 1. The approach relies on the conformal wrapping of a 3D object with an ultrathin flexible skinlike SERS substrate with extremely low bending rigidity. The skinlike substrate is capable of conformally coating even a spiky particle such as a pollen grain whose irregular 3D surface can then be mapped using SERS. This 3D skin should be contrasted with a conventional planar rigid SERS substrate whose short-range electromagnetic enhancement allows spectroscopic information to be measured only from analytes in the relatively small contact region between the base of the 3D object and the 2D SERS substrate (Figure 1a). In the absence of the skin, it is very challenging to achieve high-density coverage and uniform distribution of plasmonic metal nanostructures required to obtain a 3D spectroscopic SERS map. Also, without the skin it is virtually impossible to conformally coat the surface of live cells with plasmonic nanoparticles while keeping it viable and preventing internalization. With the hybrid skin, as we show, a high density and uniform coverage of Ag NCs can be obtained and importantly our approach is also compatible with live cell cultures.
In our fabrication process, as illustrated in Figure 1b, we first transferred chemical vapor deposited (CVD) monolayer graphene onto a silicon wafer from the copper foil on which it was grown. We then noncovalently surface functionalized the graphene with an ultrathin polydopamine (PD) intermediate layer, and subsequently grafted thermally responsive PNIPAM brushes; the entire details of which can be found in a previous publication. This surface functionalization results in an ultrathin G-PNIPAM hybrid film of approximately 8–10 nm in thickness, so that it retains a low bending stiffness to facilitate its temperature-induced bending. The thickness of the PNIPAM and PD layer can also be tuned to alter the mechanical and self-folding characteristics of the skin. To enable a pristine, direct contact and strong interactions between graphene and the Ag NCs, we transferred a second layer of CVD graphene atop of the functionalized graphene which separates the Ag NCs from the underlying PNIPAM brushes and provides a cleaner signal (Figure S1). We synthesized plasmonic Ag NCs using a polyol method, screened ligands to obtain uniform coatings of Ag NCs, and discovered that cysteamine-capped Ag NCs have a strong affinity to the graphene surface. The final G-PNIPAM-G-Ag SERS skin was formed by depositing a high density of cysteamine capped Ag NCs with an average size of 20 nm onto the top graphene surface.

We characterized the optical properties of the G-PNIPAM-G-Ag skin and its precursor films using UV–vis spectroscopy (Figure 2a). The pristine CVD graphene is highly transparent with transmittance above 85% across a wide range of wavelengths ranging from 250 to 800 nm. After surface functionalization with temperature responsive PNIPAM brushes, the film remains highly transparent but with slightly reduced transmittance. After coating with a high density of Ag NCs, there are prominent absorbance features at 400 and 600 nm, which correspond with the localized surface plasmon resonance peaks of Ag NCs and their assembly.

We investigated the performance of the photopatterned G-PNIPAM-G-Ag thin film as a SERS substrate by measuring spectra of a Raman reporter molecule Rhodamine 6G (R6G) deposited on the substrate (Figure 2b). We observed a strong enhancement of the major peaks of R6G, including the ones at 1180, 1310, 1359, 1504, 1571, and 1646 cm\(^{-1}\) which can be assigned to the aromatic ring stretching modes with detection limit down to the nanomolar range. We estimated the Raman enhancement factor (EF) of the hybrid graphene SERS skin for R6G molecule to be on the order of 10\(^8\) (Figure S2), which is comparable with other Ag nanoparticle-based SERS substrates reported in the literature. It is noteworthy that bare graphene also shows a weak SERS enhancement, but it is negligible compared to the G-PNIPAM-G-Ag skin (Figure S3). Also, the Raman spectral peaks from PD and PNIPAM, which were used to functionalize the bottom graphene layer, have negligible intensity as compared to those of analyte molecules such as R6G (Figure S4) and do not interfere with the SERS mapping.

An additional attractive feature is that we can tune both the density and the spatial distribution of the plasmonic nanoparticles on the G-PNIPAM-G-Ag SERS skin. We found that the density of the NCs on the patterned G-PNIPAM-G-Ag SERS skin can be varied from very low to very high density (up to 1800 particles per \(\mu m^2\); Figure 2d–f) by increasing the concentration of the Ag NCs in the solution during its deposition on the graphene skin.
higher density of Ag NCs results in greater SERS enhancement but as discussed later also increases the bending rigidity of the film which is not desirable for self-folding; therefore, we chose to use densities of about 1000 particles per $\mu m^2$ in our 3D SERS studies.

We were also able to create well-defined spatial patterns of Ag NCs on the SERS skin using photolithography prior to the deposition of Ag NCs. Figure 2g–i shows patterns of uniform Ag NCs lines with 12 $\mu m$ width and 8 $\mu m$ spacing on the dumbbell shaped G-PNIPAM-G-Ag skin. When we exposed these patterned substrates to the Raman reporter molecule R6G, only the linear regions patterned with the Ag NCs showed strong Raman signal due to the localized electromagnetic enhancement (Figure 2j). The ability to spatially pattern and control the distribution of nanoparticles on the SERS skin with high precision is important for its integration with other microelectronics and microfluidic devices.

The inclusion of the PNIPAM brushes endows the GPNIPAM-G-Ag SERS skin with a unique temperature responsive self-folding capability. The self-folding process of the dumbbell shaped G-PNIPAM-G-Ag film is shown in Figure 3a in which the two petals fold toward the center when heated in water to 37 °C. The self-folding mechanism is that upon increasing the temperature above the lower critical solution temperature (LCST) of PNIPAM (approximately 33 °C), the densely grafted brush layer undergoes a significant shrinkage in volume (approximately 50%), while the graphene and PD layer does not shrink. The internal strain mismatch induces the folding of the overall structure. The bending stiffness of the GPNIPAM-G-Ag skin increases with increasing Ag NCs density on the surface as confirmed by a theoretical model and nanoindentation experiments (see Section 3 in Supporting Information). The self-folding G-PNIPAM-G-Ag skin can also be patterned to self-fold into other 3D shapes such as a box or flower. Additionally, the Ag NCs can be replaced with other plasmonic nanostructures such as spherical gold nanoparticles (Figures S6 and S7). These results highlight the versatility of the G-PNIPAM-G-Ag skin.

The temperature-induced shape changes and conformal encapsulation capability of the G-PNIPAM-G-Ag skin make it an ideal 3D biosensing platform. As a proof of concept, the dumbbell-shaped G-PNIPAM-G-Ag skin was used to encapsulate various 3D microparticles. Figure 3b–d shows the encapsulation of a silica microparticle with a diameter of 75 $\mu m$; it is evident that after evaporation of water, the GPNIPAM-G-Ag thin film conformally wraps the microparticle surface due to its ultrathin structure and strong capillary forces during drying. High-resolution SEM images (Figure 3d) of the encapsulated silica microparticle show a high density of Ag NCs on the surface, which is critical for SERS enhancement.

For 3D objects analyzed after drying, the surface tension effects during water evaporation play a major role in the conformal wrapping of the hybrid graphene skin. We developed a finite element model (FEM) using the Abaqus/Explicit solver to theoretically estimate the deformation of the hybrid graphene skin during the drying process (see Section 5 in the Supporting Information). If we assume that the water evaporation is a slow and quasi-static process, the system has the force balance condition (Figure 3e) as $\pi R^2 \sigma = -2 \pi R \gamma \cos \theta$, where $R = 125 \mu m$ is the radius of the hybrid graphene skin, $\sigma$ is the Laplace pressure from surface tension, $\gamma = 72 \text{ mN/m}$ is the surface tension of water, and $\theta$ is the contact angle between water and silver nanoparticles, estimated to be in the range of 40° and 79°.

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Corresponding to these conditions, we estimate the Laplace pressure $\sigma$ to be in the range of 219.8 and 882.5 Pa. Using the mean density, bending stiffness, thickness of the skin, and loading conditions, we can effectively simulate the deformation process of the hybrid graphene skin on the microparticle, by using a shell model for the skin with the same thickness and bending stiffness and a rigid sphere for the microparticle (Figure 3f,g). We find that the Laplace pressure is large enough to drive the significant deformation of the skin and fully wrap up the microparticle. The deformation forms wrinkles in the radial direction outside the sphere, in agreement with experimental results in Figure 3c. The wrinkles provide another mechanism to release the in-plane deformation and follow the nonzero Gaussian curvature of the sphere surface.\(^{40}\) After wrapping, the van der Waals interaction between the skin and the microparticle will stabilize the closed structure.

The ultrathin skinlike nature of the G-PNIPAM-G-Ag skin allows it to conformally wrap not only regular shaped objects such as the silica microparticle discussed earlier but also irregularly shaped 3D objects. Figure 4a,b shows the structure of an oxeye daisy pollen, which has an irregular and spiky geometry, with an average size of 35 $\mu$m. It is very challenging for conventional rigid biosensing structures and devices to form intimate contact with such spiky microparticles. From Figure 4c,d, we observe that the G-PNIPAM-G-Ag skin conformally wraps the surface of the pollen including its spikes, and characteristic wrinkles of skinlike materials are formed along the edge or bottom of the spikes. We estimate the maximum strain on the hybrid graphene skin when wrapping the pollen spike to be approximately 2.6% (see Section 6 in the Supporting Information), which is much lower that the fracture strain of graphene, which is 0.14 at room temperature.\(^{41}\) This agrees with our observation that no fracture or tearing of the G-PNIPAM-G-Ag skin was seen on wrapping the pollen spikes.

High-resolution SEM images show a high density of Ag NCs coating the 3D surface of the pollen grain due to conformal coating of the skin. The intimate contact between the Ag NCs with the 3D object surface allows high-resolution 3D surface chemical mapping. To demonstrate this feature, the surface of the pollen was adsorbed with R6G molecules prior to its encapsulation with the G-PNIPAM-G-Ag skin. We then used the strong Raman signals of the R6G molecule on the surface of the pollen for spatial mapping using confocal Raman microscopy.

Figure 4e shows the Raman spatial mapping using the 1310 cm\(^{-1}\) R6G peak of the spiky pollen particle at different planes along the Z-axis. We observed the spiky features on the pollen particle in the Z-plane Raman maps with significantly stronger intensity along the peripheral region as compared to the internal area. Also, we were able to reconstruct the irregular 3D surface of the pollen particle by integration of all the 2D Raman maps at a series of focal planes along the Z-axis (Figure 4f). Such 3D nonperturbative chemical mapping cannot be easily achieved by currently available methods.

Cells, tissues, and other biological samples are soft and, as a result, existing rigid SERS substrates are mechanically mismatched (leading to undesirable perturbation of the biospecimen) and do not afford ideal biosensing interfaces. Our flexible self-folding G-PNIPAM-G-Ag SERS skin can be applied for multiplexed 3D spatial mapping of these
biological samples at the single cell level. We demonstrate 3D SERS mapping of cells, by encapsulating MDA-MB-231 breast cancer cells with the dumbbell shaped G-PNIPAM-G-Ag skin. We observed that the cells adhere well on G-PNIPAM-G-Ag (Figure 5a,b) and the cell culture temperature (37 °C) was sufficient to induce the folding of temperature responsive GPNIPAM-G-Ag skin. The breast cancer cells were successfully wrapped within the skin following the temperature induced folding and were viable up to 48 h after encapsulation (Figure S10). The optical microscope and immunofluorescence images of the cell wrapped inside the G-PNIPAM-G-Ag skin are shown in Figure 5c–f, where the green fluorescent antibody (Alexa Fluor 488) and blue fluorescent DAPI stain were used against fibronectin and DNA, respectively. The images indicate that the morphology of wrapped cells and free cells are similar, highlighting low perturbation during the wrapping process.

We performed Raman measurements on the breast cancer cell wrapped inside the hybrid graphene skin and detected strong signals from the lipids and relevant proteins on the cell membrane (Figure 5g). We can attribute the spectral features as follows: 1002 cm\(^{-1}\) (phenylalanine), 1155 cm\(^{-1}\) (carotenoids), 1209 cm\(^{-1}\) (stretching mode in phenylalanine), 1340 cm\(^{-1}\) (CH\(_2\) twist and bend in proteins and lipids), 1447 cm\(^{-1}\) (C–H deformation of lipids), and 1654 cm\(^{-1}\) (amide I).\(^{42,43}\) In contrast, the MDA-MB-231 cells on the bare quartz substrate do not show any measurable Raman peak under the same experimental conditions. Moreover, when a cell was on a flat G-PNIPAM-G-Ag film there was only limited enhancement in the Raman signal as compared with a cell wrapped inside the hybrid skin (Figure 5g). We estimate the Raman enhancement factor of the hybrid graphene SERS skin of the protein peak (1002 cm\(^{-1}\)) and lipid peak (1447 cm\(^{-1}\)) to be 110 and 60, respectively, which is comparable with prior SERS enhancement factors of the cell membrane.\(^{44}\)

The strong Raman signals form the cell membrane also facilitated 3D surface chemical mapping of the cell. For spatial mapping, we used the two major peaks: the 1002 cm\(^{-1}\) protein peak and the 1447 cm\(^{-1}\) lipid peak. As shown in Figure 5h, the Raman mapping of the cell at different focal planes along the z-axis using the protein and lipids peaks allowed us to study the spatial distribution of the biological components and generate a series of 2D confocal chemical maps of the cell surface. We observed that the overall Raman intensity is strongest at the bottom z-plane corresponding to the contact region between the bottom of the cell and the G-PNIPAM-G-Ag skin. We attribute this observation to the fact that a soft biological cell tends to spread out on the substrate (Figure S10) with the largest contact area between the cell membrane and graphene SERS skin at the bottom. Of note, the geometric profile of the cell wrapped inside G-PNIPAM-G-Ag skin was not distorted, unlike the gripping force-induced cellular deformation observed in our previously reported MTSERS platform.\(^{19}\)

Furthermore, we can use the 2D planar image stack to reconstruct a 3D chemical map of the biological cell (Figure 5i), which indicates the spatial distribution of spectral markers over the cell volume. In contrast, for the unwrapped cancer cell (Figure S11), it is possible to achieve spatial mapping with measurable intensity only at the bottom plane which is in contact with the SERS skin. As the focal plane moves away from the bottom plane, the Raman intensity drops off sharply and there is almost no distinguishable Raman mapping.
This result highlights the limited contact area of the cell with 2D planar sensors and the inability to get 3D spatial maps using 2D SERS substrates.

In conclusion, we have developed an ultrathin 3D self-folding flexible skinlike SERS substrate and demonstrated its utility in mapping the surface chemistry of both regular and irregularly shaped soft and rigid biological samples. This platform successfully tackles several challenges that have impeded the ability to capture and perform in situ label-free chemical analysis of single live cells in 3D. First, the ultrathin and compliant skin allows conformal and intimate contact all over the 3D object while minimizing sample perturbation and significantly improving the information that can be obtained during biosensing. Second, the self-folding and wrapping is triggered by biologically compatible conditions and does not necessitate the use of tethers or wires; hence, it allows facile integration with existing microfluidic systems. Third, the skin is highly versatile and can be patterned into different shapes and sizes to encapsulate and analyze a range of objects. Fourth, the surface of the skin can also be equipped with other nanostructures or functional elements and devices, which can further expand its application to other electrical or electro-chemical biosensing modalities. Finally, the ability to rapidly and selectively profile the surfaces of cells in 3D in conjunction with chemometric tools would permit the study of fundamental biological processes such as cell surface glycosylation. Such analyses are challenging to perform with existing techniques due to limitations in resolution, sensitivity, and/or required experimental conditions. Consequently, we anticipate that this novel skin-encapsulation and spectroscopic platform will be utilized to probe 3D spatiotemporal activity with single cell resolution of broad relevance to cell biology, drug discovery, and biomedical engineering.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Schematic illustrations of the self-folding hybrid graphene skin for 3D biosensing. (a) Conceptual comparison of SERS analysis of a 3D irregularly shaped microobject using a conventional, rigid 2D SERS substrate (left) and the hybrid graphene skin (right). The self-folding skin can conformally wrap 3D object facilitating 3D spatially resolved SERS measurements. (b) Schematics illustrating the fabrication process of the hybrid G-PNIPAM-G-Ag film with thermal responsive properties. The fabrication (left to right) involves surface functionalization of PNIPAM brushes followed by transfer of a second layer of graphene and deposition of Ag NCs for SERS enhancement. (c) Schematics illustrating that the skinlike GPNIPAM-G-Ag film can be photolithographically patterned into well-defined 2D shapes, such as a dumbbell, and then induced to self-fold into a closed 3D geometry and encapsulate 3D micro-objects by a mild increase in temperature.
Figure 2.
Characterization of the G-PNIPAM-G-Ag skin. (a) UV–vis spectra of the pristine graphene, G-PNIPAM, G-PNIPAM-G-Ag film, and an aqueous solution of Ag NCs. (b) Raman spectra of different concentrations of R6G deposited on the surface of the patterned G-PNIPAM-G-Ag skin. (c) SEM images of the dumbbell-shaped G-PNIPAM-G-Ag skin with (d) 750, (e) 1000, and (f) 1800 Ag NCs per $\mu m^2$. Panels d–f correspond to the same spatial region as the dotted yellow square in panel c. (g–i) SEM images of the dumbbell shaped G-PNIPAM-G-Ag skin with photolithographically defined line patterns of Ag NCs on the surface. Panels h and i are progressively zoomed-in regions corresponding to the dotted yellow squares in panels g and h, respectively. (j) Raman spatial mapping of the G-PNIPAM-G-Ag skin with patterned Ag NCs lines using the R6G peak at 1310 cm$^{-1}$. Panel j corresponds to the same spatial region as h.
Figure 3.
Self-folding and wrapping of a microparticle with the G-PNIPAM-G-Ag skin. (a) Optical microscopy image of the dumbbell shaped GPNIPAM-G-Ag skin in the flat state (left panel), during (center panel), and after (right panel) thermally triggered self-folding. (b) Optical image showing the wrapping of a spherical silica microparticle using a dumbbell shaped G-PNIPAM-G-Ag skin. (c) Optical and (d) SEM image showing that the G-PNIPAM-G-Ag conformally wraps around the microparticle after drying with characteristic skinlike wrinkles. The inset shows a higher-magnification image of the region indicated by the red dotted square and indicates that the surface of the microparticle has a high density of Ag NCs. (e) Schematic of a model illustrating the effect of the surface tension between the hybrid graphene skin and the particle during drying. $\sigma$ and $\theta$ indicate the Laplace pressure and contact angle. (f,g) FEM results showing the deformed graphene skin on the silica microparticle after water evaporation, with two different conditions: (f) $\sigma = 219.8$ Pa for $\theta = 79^\circ$, and (g) $\sigma = 882.8$ Pa for $\theta = 40^\circ$. The images show both the top view and section view of the shell model for the skin, colored by principle in-plane strain within the neutral layer ($\varepsilon = 0$ cyan, $\varepsilon = 0.02$ green).
Figure 4.
The 3D SERS spatial mapping of a spiky pollen wrapped with the G-PNIPAM-G-Ag skin. (a,b) SEM image of a pristine pollen grain at different magnification. (c,d) SEM image of a pollen grain wrapped inside the G-PNIPAM-G-Ag skin, which shows characteristic skinlike wrinkles and high-density nanoparticles on the surface. (e) The 2D SERS mapping of the pollen at a series of focal planes along the Z-axis. (f) Reconstructed 3D surface Raman spectroscopic spatial map of the pollen showing characteristic spiky features.
Figure 5.
Wrapping of live breast cancer cells inside the G-PNIPAM-G-Ag skin and 3D SERS spatial mapping. (a) Bright-field and (b) fluorescent live/dead assay (calcein AM and ethidium homodimer-1) images of the cells cultured on the surface of the 2D patterned G-PNIPAM-G-Ag skin. The dotted white circles in panel d correspond to the positions of the patterned G-PNIPAM-G-Ag dumbbells. (c) Bright field and (d) the corresponding immunofluorescence image (green, Alexa Fluor 488; blue, DAPI) of the cancer cells wrapped by the folded G-PNIPAM-G-Ag skin. The higher-magnification images of a single
cell in the dotted yellow square regions of panel c and d are shown in (f,g), respectively. (h) Raman spatial maps of the wrapped cell at different focal planes along the z-axis. The green color corresponds to the lipid peak intensity at 1447 cm$^{-1}$ and red color indicates the protein peak intensity at 1002 cm$^{-1}$. (i) The reconstructed 3D Raman spatial map of the lipid peak at 1447 cm$^{-1}$ for a single cell wrapped by the G-PNIPAM-G-Ag skin.