SUPPORTING INFORMATION

SERS and Fluorescence-Active Multimodal Tessellated Scaffolds for Three-Dimensional Bioimaging

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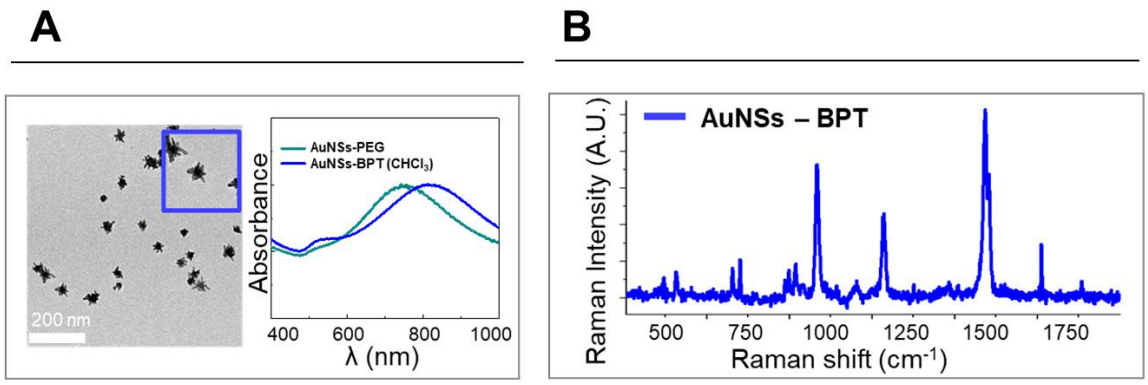

Figure S1. UV-Vis spectra, TEM image (**A**) and characteristic SERS signal (**B**) of AuNS-BPT in $CHCl_3$.

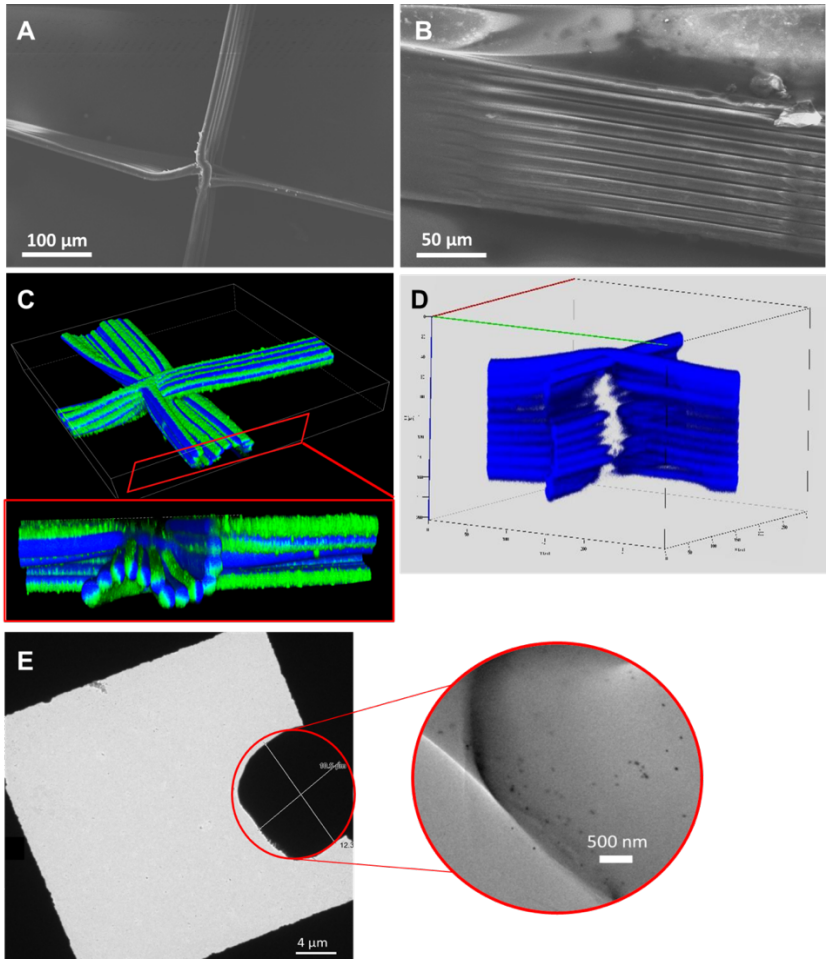

Figure S2. (**A,B**) SEM images of an intersection of the tessellated 500 × 500 µm^2 squares (**A**) and a detailed section where the different layers can be differentiated (**B**). (**C**) Fluorescence image of the square intersection of a bicompartmental polymeric scaffold. In the inset both compartments of each 10 µm fiber are easily differentiated through the green and blue fluorophores. (**D**) Example of scaffold where only one compartment, labeled in blue, was included. (**E**) TEM image of a fiber slice and an inset showing the NPs inside the fiber.

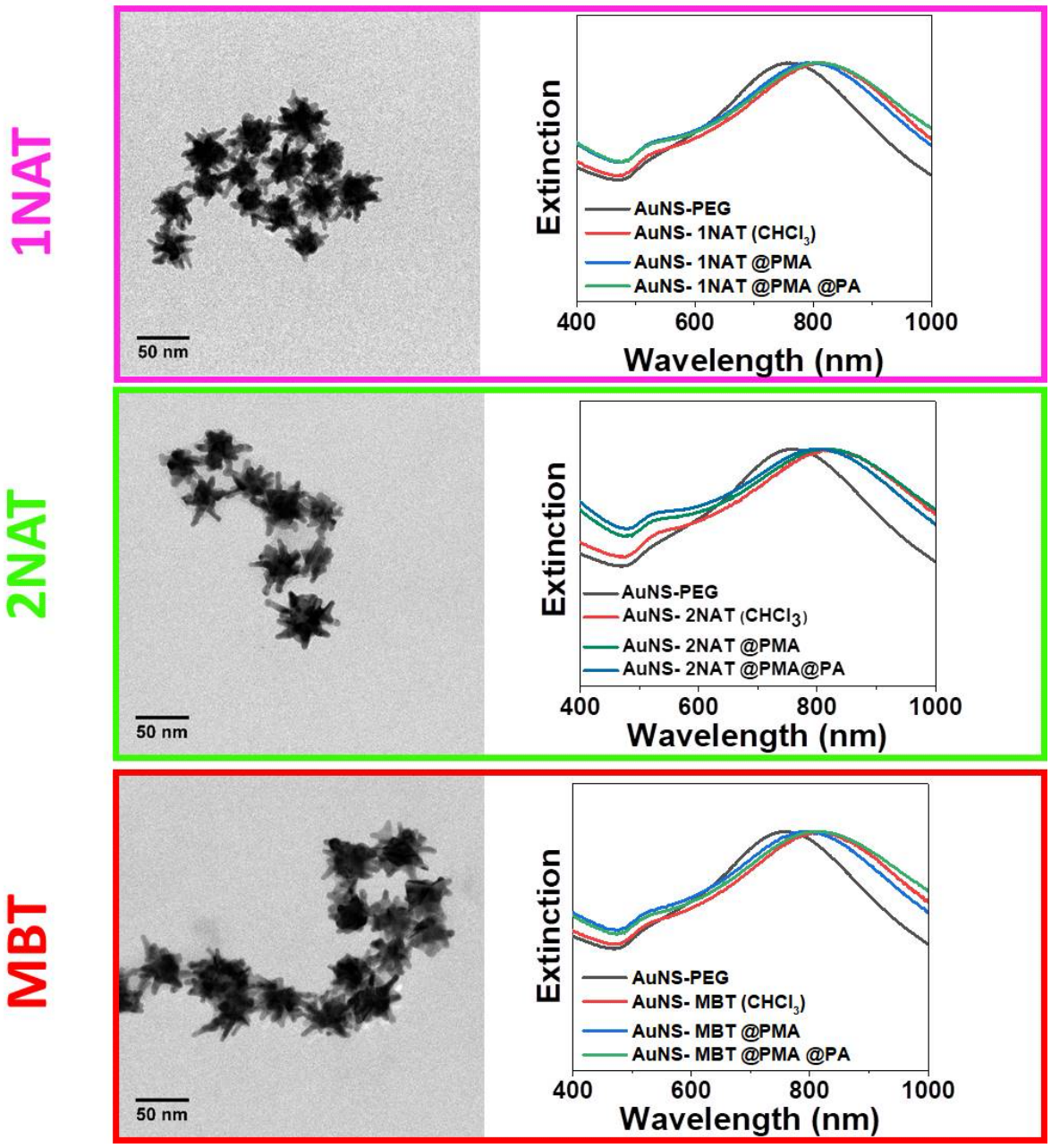

Figure S3: TEM images and UV-Vis spectra of AuNS-1NAT (magenta), AuNS-2NAT (green) and AuNS-MBT (red) during the various functionalization steps.

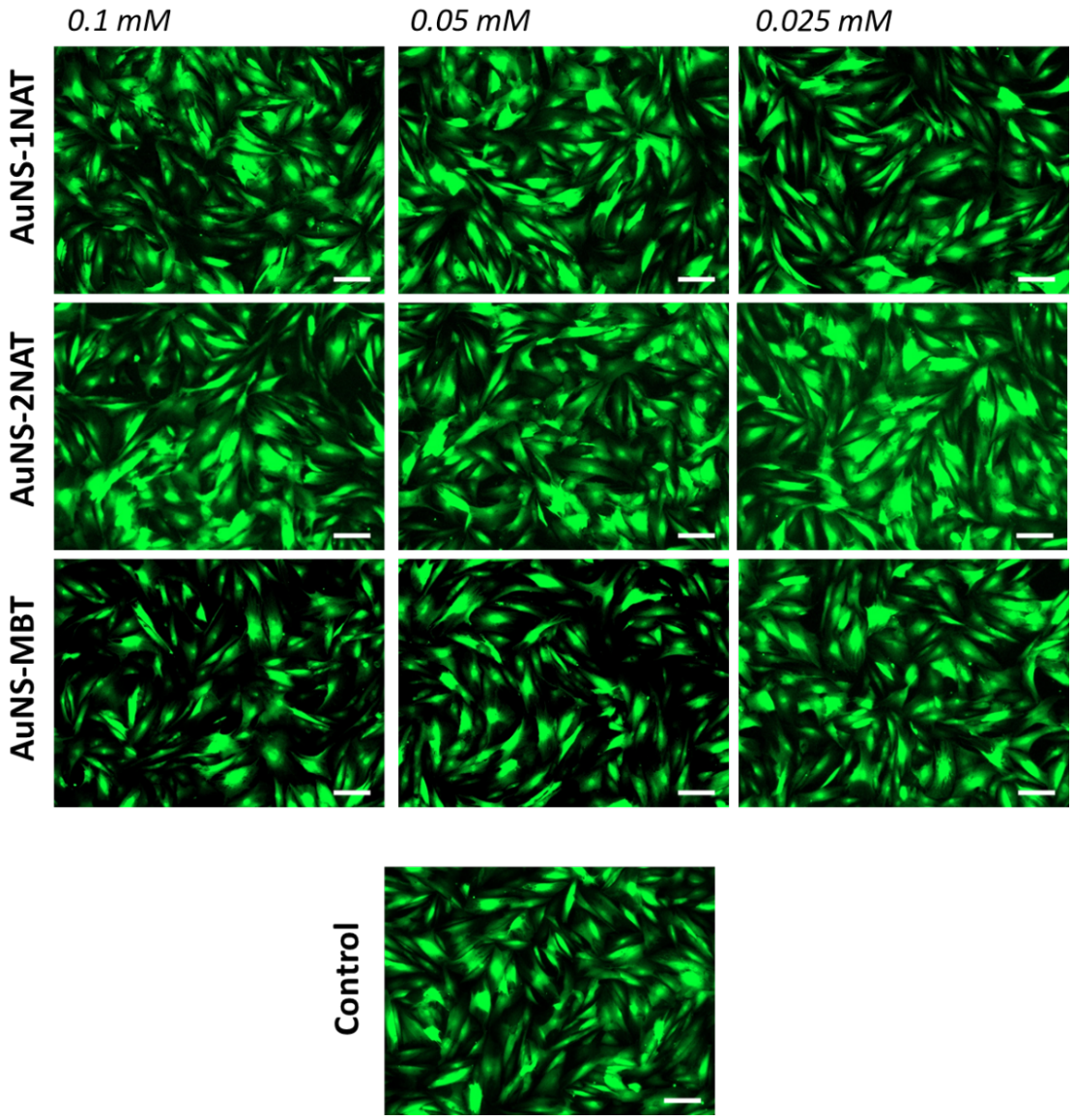

Figure S4. Effect of SERS tags on the overall cell proliferation and viability of HDF cells. GFP-expressing HDF cells (green) display cell number and shape similar to the control sample. PI staining was done to show cytotoxic cells but no red staining was observed. Scale bar: 200 μm.

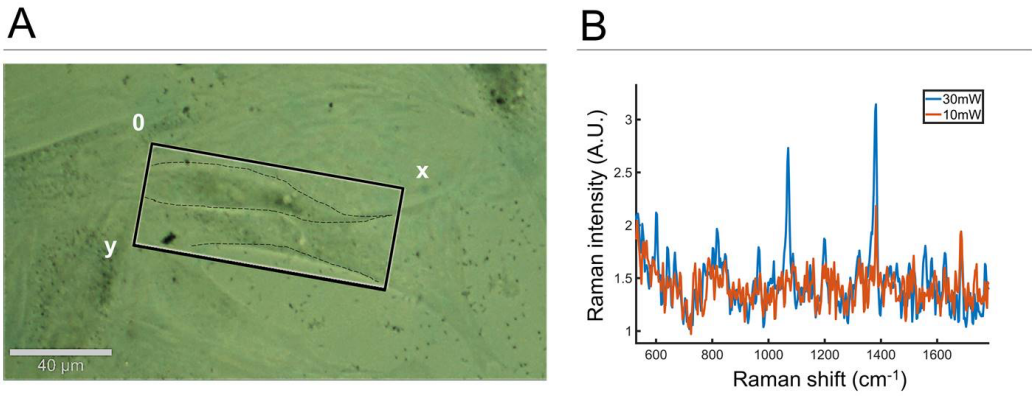

Figure S5. (A) Brightfield image of HDF cells labeled with AuNS-2NAT. Scale bar: 40 µm. **(B)** Average spectra collected from the whole imaging volumes for laser irradiation powers of 10 and 30 mW.

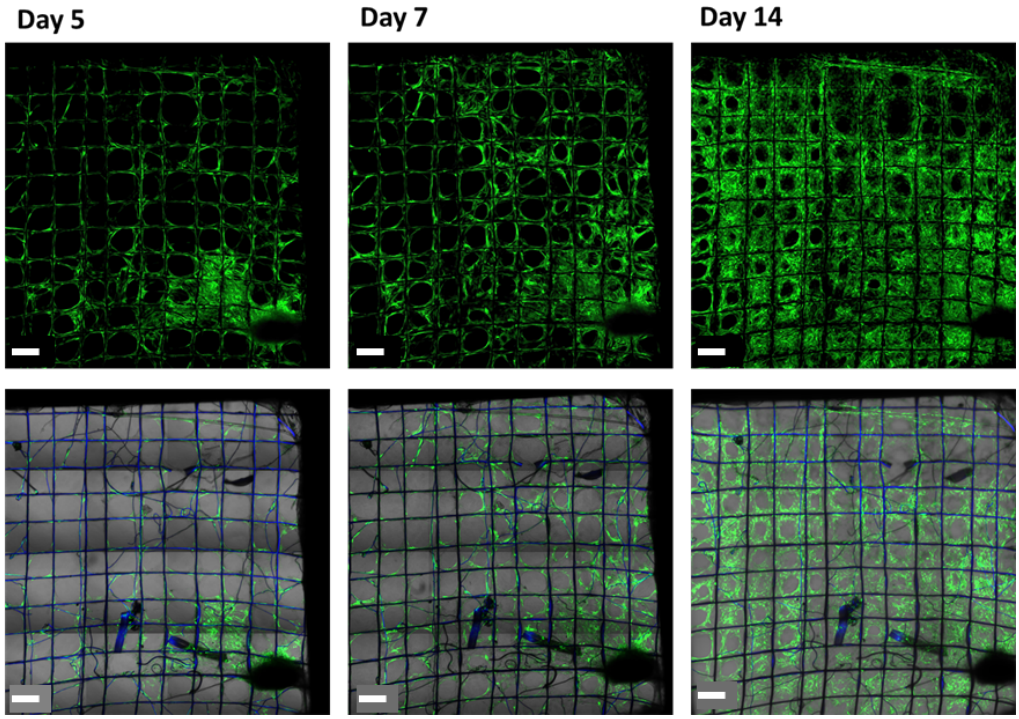

Figure S6. Evolution of HDF cell growth and scaffold structure over a 2-week period. HDF cells were transfected to express GFP (green), the scaffold can be seen in blue. Scale bars: 500 µm.

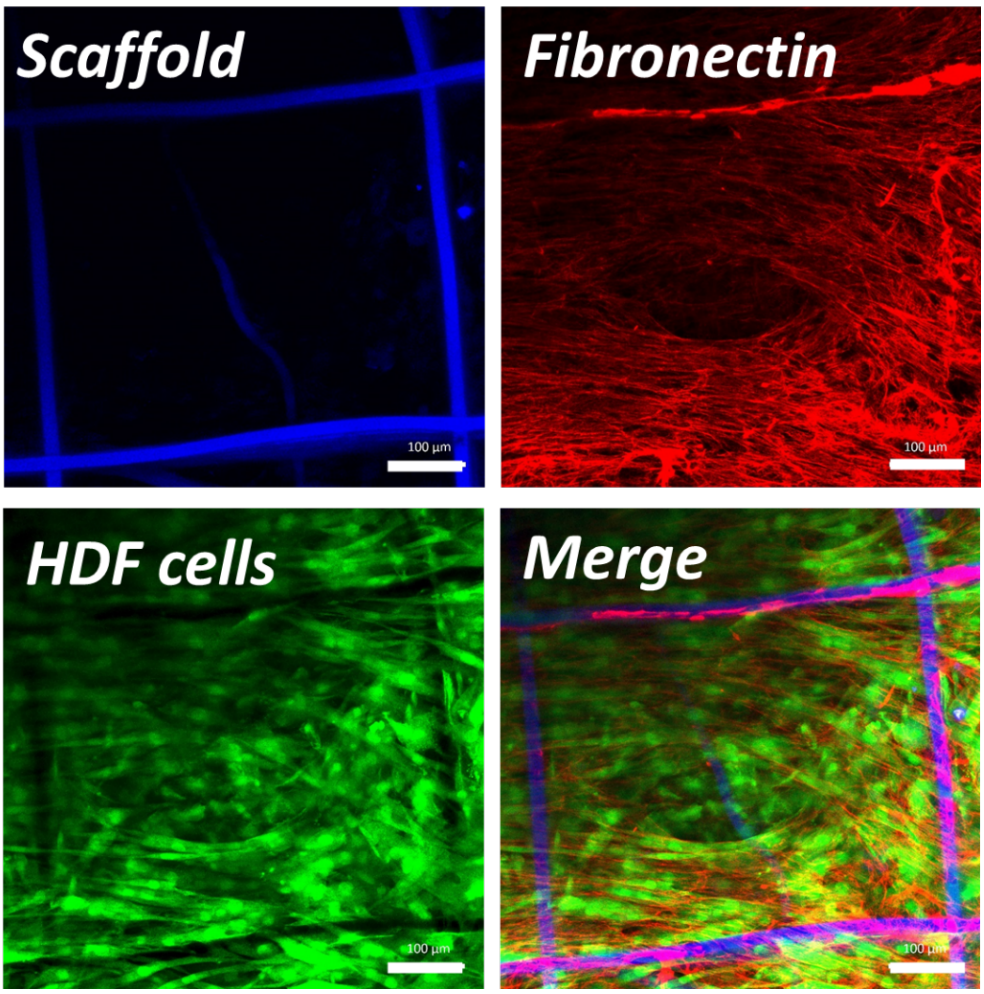

Figure S7. HDF cell network formed in 3D jet printed scaffolds, 14 days after seeding. Maximum intensity projections of Z-stacks (approximately 150 µm thick) showing scaffold (blue), fibronectin (red) and HDF cells (green), and a merged image. Scale bar: 100 µm.

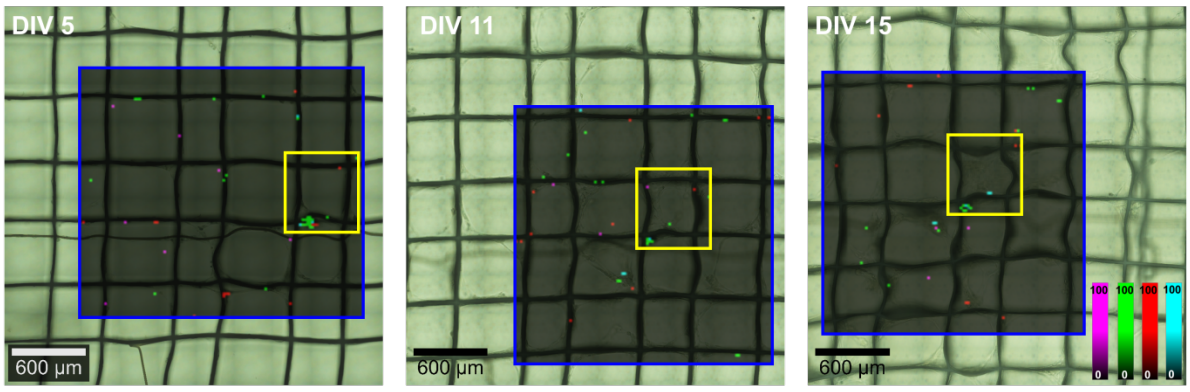

Figure S8. HDF cell growth on the tessellated scaffold over a 2-week period. The cells were labeled with 3 different SERS tags, AuNS-1NAT (in magenta), AuNS-2NAT (in green), and AuNS-MBT (in red). The square shown in blue, overlaid on the brightfield image, was imaged every 3-5 days to monitor the proliferation and migration of HDF cells in the scaffold. The square highlighted in yellow refers to the area imaged in Figure 5 of the main manuscript. The mappings were analyzed with the True Component Analysis tool, capable of correctly identifying the three SERS tags, plus cases in which all three were observed in the same voxel (depicted in cyan).

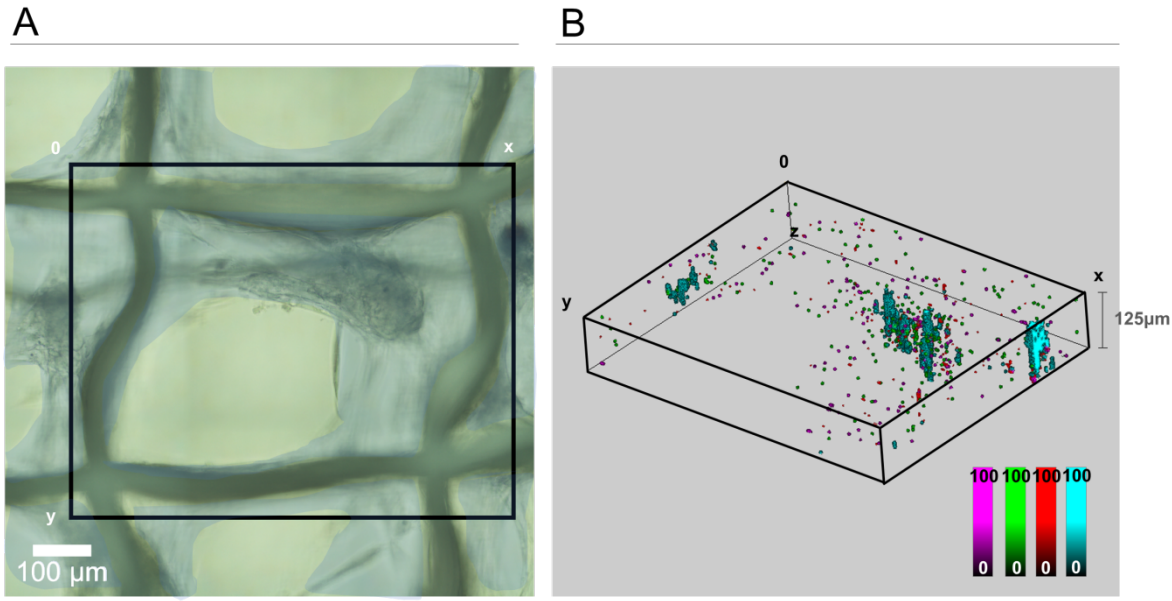

Figure S9. (**A**) Brightfield image of HDF cell distribution around the scaffold fibers. (**B**) Volumetric reconstruction of HDF cell distribution after 25 days growth on a tessellated scaffold. HDF cells were labeled with three different SERS tags, AuNS-1NAT (in magenta), AuNS-2NAT (in green), and AuNS-MBT (in red). The map was analyzed with the True Component Analysis tool to identify the three SERS tags. Where a mixture of all three SERS tags is observed in the same voxel, cyan is used.

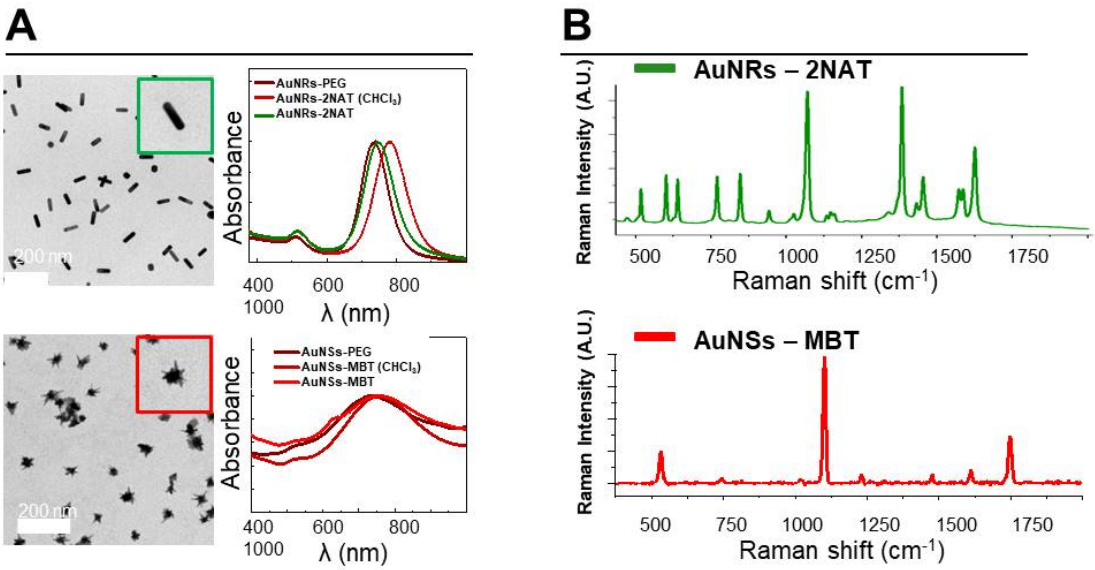

Figure S10. (**A**) UV-Vis spectra and TEM images of AuNRs-2NAT and AuNSs-MBT, pre- and post-coating with PMA and PA. (**B**) Characteristic SERS spectra of AuNR-2NAT and AuNS-MBT in H_2O. A 50× (NA 0.5) long working distance objective was used for SERS measurements.

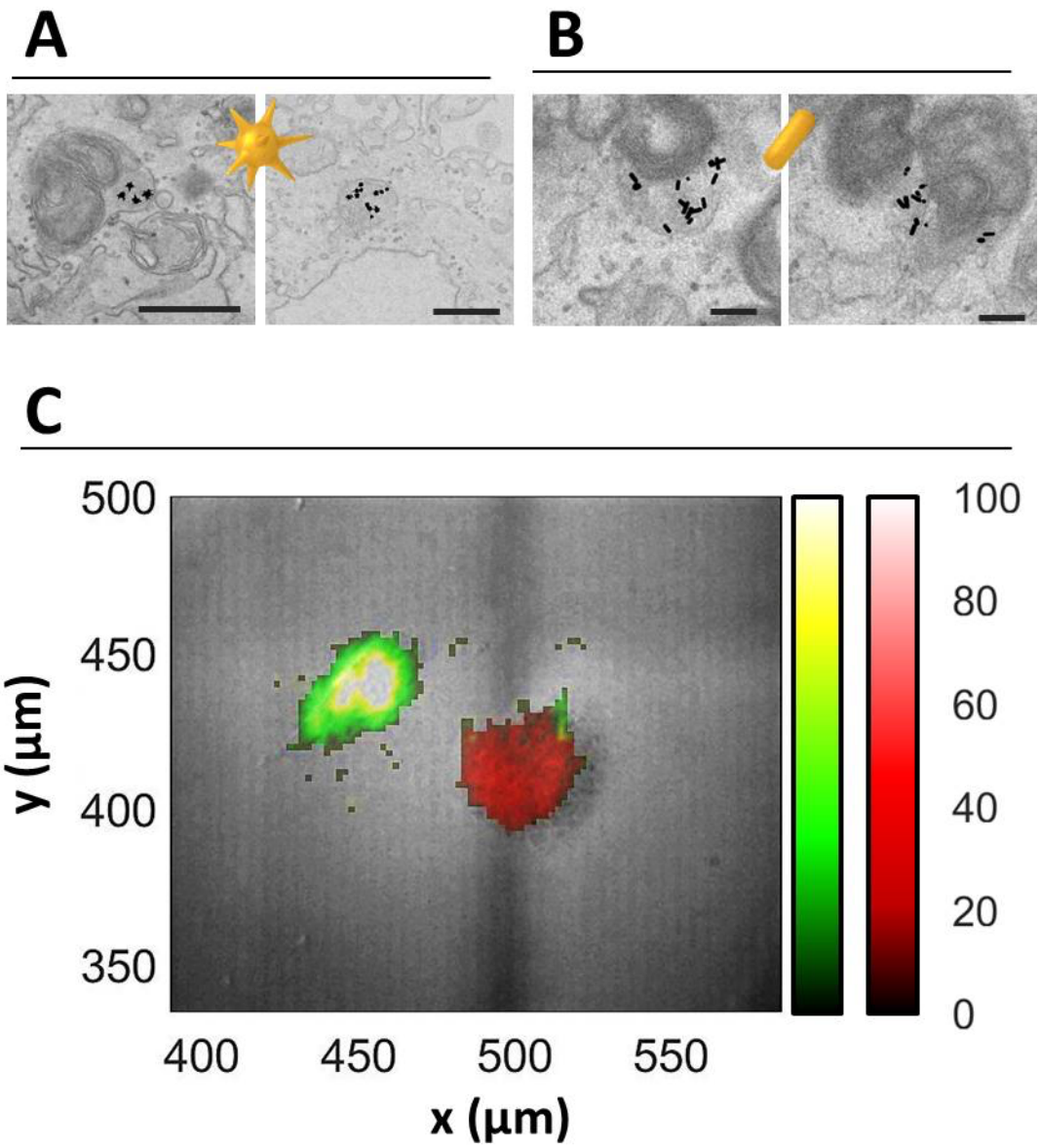

Figure S11. Representative TEM images showing the uptake of AuNS-MBT (**A**) and AuNR-2NAT (**B**) into HDF cells. Scale bar: 500 nm. (**C**) Brightfield optical image overlaid with a false-colored SERS map, showing HDF cells labeled (separately) with AuNR-2NAT (green) and AuNS-MBT (red), and then mixed in a co-culture. The data were treated with Multiple Linear Regression analysis, obtaining the *b-values* representing the intensity of SERS tags in each cell.

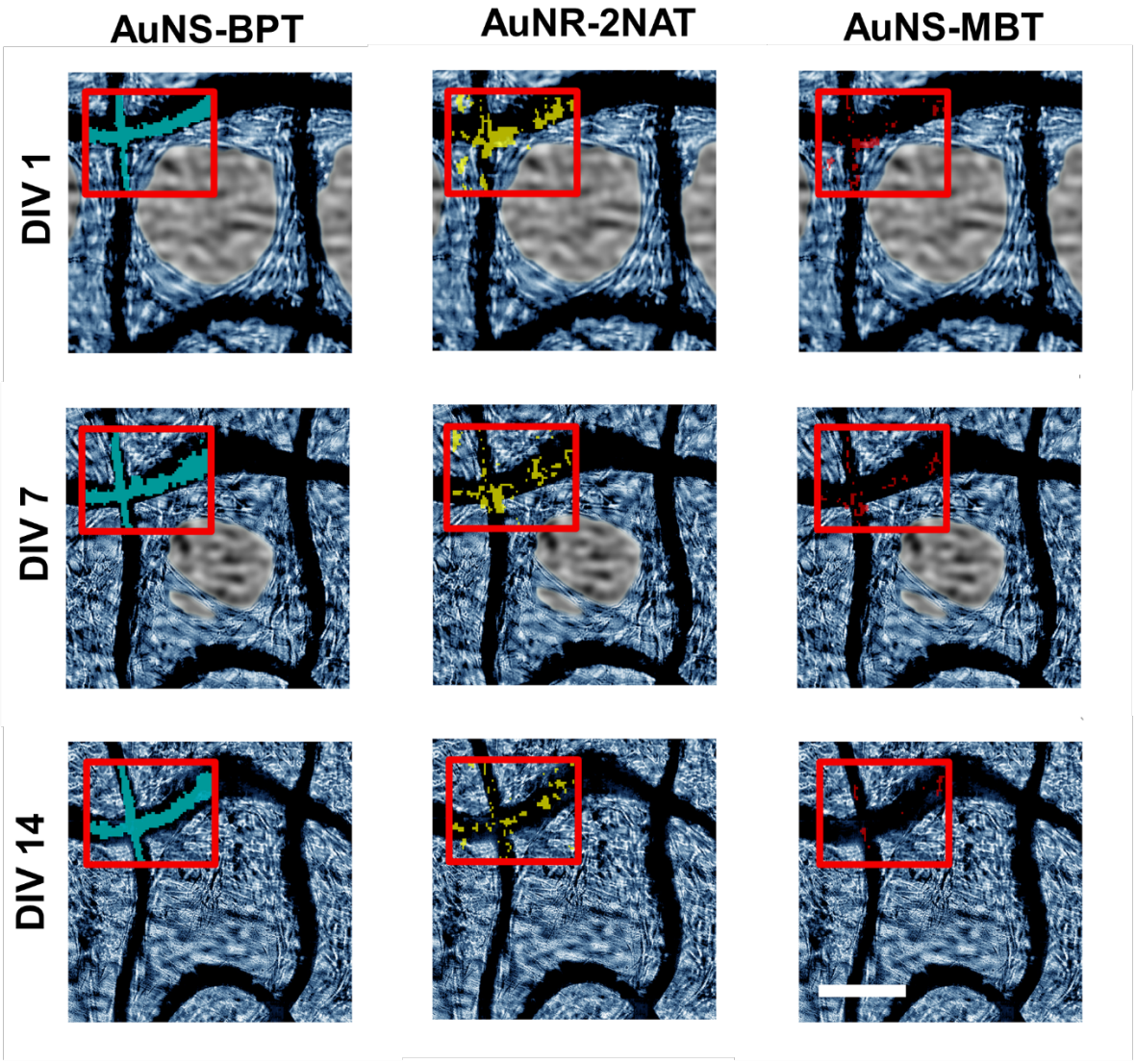

Figure S12. SERS mapping of HDF cell growth over time in a hybrid scaffold. Imaging areas, indicated by red squares, were merged with optical images (HDF cells are false-colored in blue). Colored SERS maps represent the selected reference points (*b values*, see SI methods for more detail) for AuNS-4BPT (cyan), AuNR-2NAT (yellow), and AuNS-MBT (red). A 40× immersion objective was used (N.A. 0.8). SERS imaging was conducted using a 0.8 s integration time with 3.1 mW laser power, The map of one selected area (325 × 225 μm^2) was acquired with a resolution of 5 x 5 μm^2 (XY) and required approximately 1 h and 10 min to be completed. Scale bar: 100 µm.