Biomechanical sensing of in vivo magnetic nanoparticle hyperthermia treated melanoma tumor using magnetomotive optical coherence elastography

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Table S1. Stiffness changes after magnetic nanoparticle hyperthermia (MH) treatments

| Case | Local cellularity (%) | N\textsuperscript{†} | $\Delta T_{\text{Max}}$ (°C) | $f_0^\gamma$ change (%) | Statistical significance |
|------|----------------------|----------------------|-----------------------------|------------------------|-------------------------|
|      |                      | After D1 | After D2 | After D1 | After D2 | B/D1 | B/D2 | D1/D2 |
| 0 kA/m (Control) | 3 | -2.55±0.67 | -1.73±0.21 | -6.0±10.3 | -13.3±22.1 | NS | NS | NS |
| 61.2 kA/m, 5 min/dose | 4 | 9.98±3.40 | 9.30±1.82 | -37.4±22.0 | -36.8±17.5 | ***** | ***** | ***** |
| 61.2 kA/m, 9 min/dose | 3 | 5.75±4.45 | 8.50±3.68 | 39.8±32.1 | 48.5±66.7 | ***** | *** | NS |
| 79.7 (Low) | 1 | 8.3 | -24.6±9.1 | (n = 18) | **** |
| 86.7 (High) | 1 | 14.9 | 14.6±16.8 | (n = 14) | NS |

Abbreviations: MNPs, magnetic nanoparticles; MM-OCE, magnetomotive optical coherence elastography; $N$, number of tumor samples; $n$, number of MM-OCE measurements; $B$, before treatment; $D1$, 1\textsuperscript{st} MH dose treatment, $D2$, 2\textsuperscript{nd} MH dose treatment. Statistical significance/insignificance are denoted with asterisks and hyphens, respectively. ******, *****, and *** denote a $p$-value less than $10^{-5}$, $10^{-4}$, and $10^{-3}$; “NS” denotes $p$-value $> 0.01$.

\textsuperscript{†}Two samples are excluded from analysis – one without successful delivery of MNPs to the tumor core; the other with MNPs located in a highly heterogeneous tumor microenvironment (Figure S2).

\textsuperscript{‡}Temperature measurement performed only on 2 out of 3 mice.

\textsuperscript{§}Only 3 out of 4 mice were MH-treated for the 2\textsuperscript{nd} time.
Figure S1. Correlation between picrosirius red (PR) color-based cellularity metrics and cell density (nuclei count per unit area) quantified based on Masson’s trichrome (MT) and DAPI. A-C. Representative images of low (I) and high (II) cellularity tumor sections stained with PR (A), MT (B), and DAPI (C). D. PR-based cellularity correlates well with the cell density quantified based on MT histology (Pearson’s $r = 0.789$) (I). The cell density of the high cellularity group ($5.2 \pm 1.0 \times 10^3$ counts/mm$^2$) was higher than that of the low cellularity group ($1.4 \pm 0.1 \times 10^3$ counts/mm$^2$) (two-sample t-test $p = 0.014$) (II). The nuclei were stained as dark purple in MT histology, B (I, II), whose representative nucleus (red) and MNP (blue) segmentation was shown in E (I, II), respectively. F. PR-based cellularity also correlates well with cell density quantified based on DAPI histology (Pearson’s $r = 0.786$) (I). The cell density of the high cellularity group ($4.8 \pm 1.0 \times 10^3$ counts/mm$^2$) was higher than that of the low cellularity group ($2.8 \pm 0.5 \times 10^3$ counts/mm$^2$) (two-sample t-test $p = 0.013$) (II). The correlation analyses (D, F) were conducted on 8 AMF-treated tumors (11 in total, excluding one that showed absence of MNPs at tumor core, one that had MNPs trapped in a highly heterogeneous environment, Figure S2, and one severely necrotic tumor with limited histological sections, Figure 2B).
Figure S2. Results from an MNP-injected melanoma tumor with MNPs present both at the tumor center and in the epidermis. A. Images of Masson’s trichrome (MT, upper panel) and picrosirius red (PR, bottom panel)-stained slides visualize the complex environment around the MNPs (blue asterisks) with either high or low local cellularity at the tumor site or immediately adjacent to collagen structures. B. Enlarged area from the PR-stained slide in (A) imaged with both brightfield (left panel) and polarized (right panel) light. Blue asterisks indicate MNPs. C. Optical coherence tomography (OCT, grey-scale) and the corresponding magnetomotive optical coherence tomography (MM-OCT, green and red) images obtained from two representative locations (Loc), where significant MM-OCT signals were observed at both sites, but D. the corresponding magnetomotive optical coherence elastography (MM-OCE) results exhibited noticeable differences, potentially suggesting sensitivity of MM-OCE to the local tumor environment near MNPs. E. The low rise of temperature after magnetic hyperthermia (MH) treatments.