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# Magnetism for mechanobiology and related biomedical applications

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## **Abstract:**

Magnetic particles dispersed among living cells and subjected to a variable magnetic field can exert mechanical stimulation on the cells, inducing physiological responses. Studies have shown that this low-frequency mechanical stimulation (between 2 and 20Hz) can induce cell death in cancer cells and trigger insulin secretion from pancreatic cells. In the field of neurology, on-going studies are also focused on the influence of magneto-mechanical stimulation against neurodegenerative diseases. A key advantage of this magneto-mechanical approach lies in the ability to adjust mechanical stress on cells remotely, via the applied magnetic field, producing differentiated effects depending on the cell type. This innovative concept opens promising avenues in mechanobiology and related biomedical applications, particularly for treating diseases such as cancer, diabetes, and against neurodegeneration.

Keywords: Magneto-mechanical stimulation, magnetic nanoparticles, magnetic anisotropic particles, magnetic forces, magnetic torques, mechanobiology, cancer, diabetes, neurology, cytoskeleton, mechanosensitive channels

## **Introduction:**

Magnetic nanoparticles (MNPs) are gaining increasing interest in the fields of diagnostics and therapy [1-6]. They are utilized in various medical imaging techniques, particularly as contrast agent in magnetic resonance imaging (MRI), offering enhanced sensitivity and improved spatial resolution compared to traditional contrast agents [7]. They are also used as tracers for tissue or tumor monitoring in Magnetic Particles Imaging (MPI) [8-10]. They enable early detection of diseases such as cancer, cardiovascular diseases, and neurological disorders. Regarding therapies, developments are underway in hyperthermia, a therapeutic approach against certain cancers involving the heating of biocompatible magnetic nanoparticles dispersed among cancer cells under the influence of a high-frequency alternating magnetic field (~100kHz). The resulting heating of the cells close to the particles leads to reduced resistance to chemotherapy when the cell temperature reaches 43°C and cell death when the temperature reaches ~45°C [11-14]. Current research aims to increase the heating efficiency of the particles [15] and improve targeting of cancer cells to avoid affecting healthy tissues. Another application of magnetic nanoparticles in therapy is targeted drug delivery, particularly in the context of chemotherapy [16,17]. The goal is to reduce the side effects of chemotherapy by reducing the dose of injected toxic drug in the bloodstream. This can be achieved using dual-functionalized magnetic nanoparticles designed to recognize cancer cells and to transport the toxic treatment molecules. Once the particles reach their destination, the local release of these toxic molecules can be induced directly at the tumor site through the heating of the magnetic particles under an alternating field [18].

The most commonly used nanoparticles for the aforementioned applications are iron oxide nanoparticles (mostly magnetite,  $\text{Fe}_3\text{O}_4$ ), referred to as SPIONs (Superparamagnetic Iron Oxide Nanoparticles). These particles, ranging in size from a few nanometers to several tens of nanometers, are biocompatible and approved by drug agencies for use in the human body [7].

## **Magneto-mechanical stimulation of cells:**

More recently (since around 2010), other therapeutic approaches against cancer, diabetes, and for neuroregeneration have emerged, based on the mechanical stimulation of cells under the influence of low-frequency vibration (2-20Hz) of magnetic particles dispersed among the cells

[19-29]. A major advantage of magnetism in these approaches is the ability to non-invasively control the intensity of the mechanical stress exerted on cells, by adjusting the characteristics of the applied magnetic field (frequency, amplitude, direction). Tuning the level of mechanical stress allows triggering a range of cellular responses, differentiated based on the nature of the cells [24].

The SPIONs are not the most suitable for this magneto-mechanical stimulation (MMS) of cells due to the fact that, given their nanometric size, they can only generate very low forces in the range of tens of fN using magnetic fields easily accessible in laboratories or hospitals (field range of 10mT to 1T). Figure 1 compares the forces involved in various biological processes with the forces that can be generated by two types of magnetic particles: the SPIONs and anisotropic magnetic vortex microdiscs which were used in earlier studies of MMS of cells [19,20]. The forces involved in biological processes are in the range of picoNewtons (pN) to hundreds of pN [26,27]. The forces generated by superparamagnetic iron oxide nanoparticles (SPIONs) are typically in the range of tens or hundreds of femtonewtons. To achieve higher forces, larger and magnetically anisotropic particles are preferable. Such particles can be actuated by altering the direction of the magnetic field (e.g., using a rotating field), while isotropic particles can only be moved by a magnetic field gradient, which diminishes more rapidly with distance from the field source than the field itself. A practical method to render a magnetic particle anisotropic is to shape it non-spherically, such as in a disc form. Due to magnetostatic considerations, the magnetization of the particle tends to remain within its plane. Consequently, when a magnetic field is applied perpendicular to this plane, the particle will tend to rotate to align its plane with the direction of the field [19,20]. The experiments presented in this article were conducted using disc particles with a diameter of 1.3 microns and a thickness of 60nm made of permalloy alloy ( $\text{Ni}_{80}\text{Fe}_{20}$ ) (see Fig 2a,d,e,f). They are coated with a 10nm layer of gold (Au) to make them biocompatible due to gold's chemically inert nature and easy to functionalize, for example, to render them fluorescent or to target specific cells. The particles are also designed so that their magnetic properties mimic superparamagnetic behavior, meaning they have zero magnetization at zero field to prevent aggregation once dispersed in solution or in tissues, while being easily polarizable under a magnetic field [31]. This is the case for the used NiFe microdiscs (Fig. 2a) that possess a vortex micromagnetic configuration at zero field (as shown by micromagnetic simulations in Fig. 2d and by electron holography in Fig. 2e) and high polarizability under field (See the hysteresis loop of these microdiscs characteristic of

vortex particles -Fig. 2f) [19,20,32]. Other types of particles can also be used, as illustrated in Fig. 2b and 2c such as magnetite particles of much larger sizes than SPIONs.

Those in Fig. 2b are obtained by mechanical milling of magnetite powder (mechano-synthesis) [33]; those in Fig. 2c are produced by chemical synthesis of hematite ( $\text{Fe}_2\text{O}_3$ ) particles, subsequently reduced under hydrogen to magnetite [29]. The advantage of these latter fabrication techniques is the ability to produce these particles in large volume for future biomedical applications, whereas vortex particles, produced by cleanroom technologies (lithography, deposition, lift-off), are only produced in small quantities (a few mg per wafer).

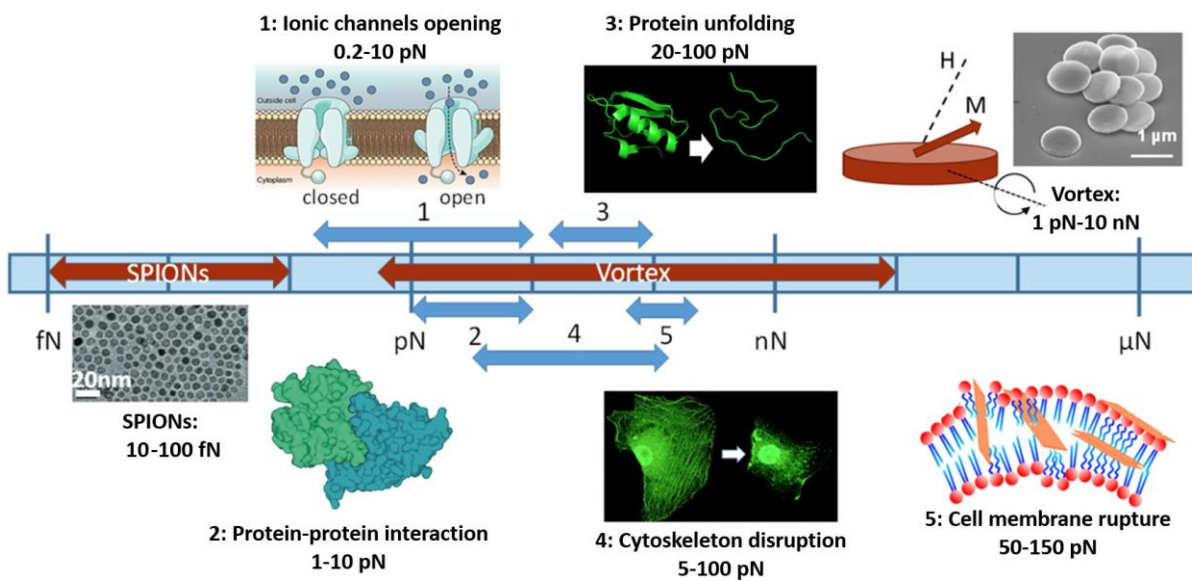


Figure 1 : Comparison of the force ranges involved in different biological phenomena [25] and those that can be created by SPIONs (Superparamagnetic Iron Oxide Nanoparticles) and anisotropic mesoscopic particles, such as magnetic vortex microdiscs. For magneto-mechanical stimulation of cells, mesoscopic anisotropic particles appear more suitable than SPIONs, which generate forces that are too weak due to their nanoscale size.

Particles based on Magnetic shape-memory Heusler alloys such NiMnGa have also been studied for biomedical applications based on caloric or magneto-mechanical effects. It was shown for instance that they can promote the adhesion and proliferation of human fibroblasts without eliciting any cytotoxic effect [34]. Carbon nanotubes filled with Fe nanoparticles have also been used for mechanical nanosurgery of chemoresistant glioblastoma [35]. In all these examples, the magnetic particles are magnetically anisotropic enabling an efficient conversion of the magnetic torque exerted on the particles magnetization into a mechanical torque exerted on the particles' surrounding environment.

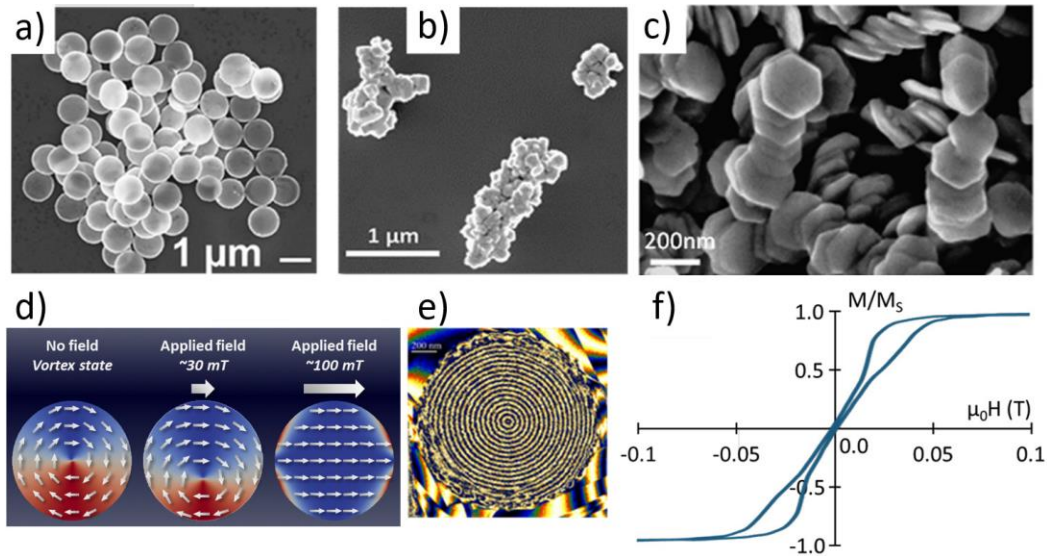
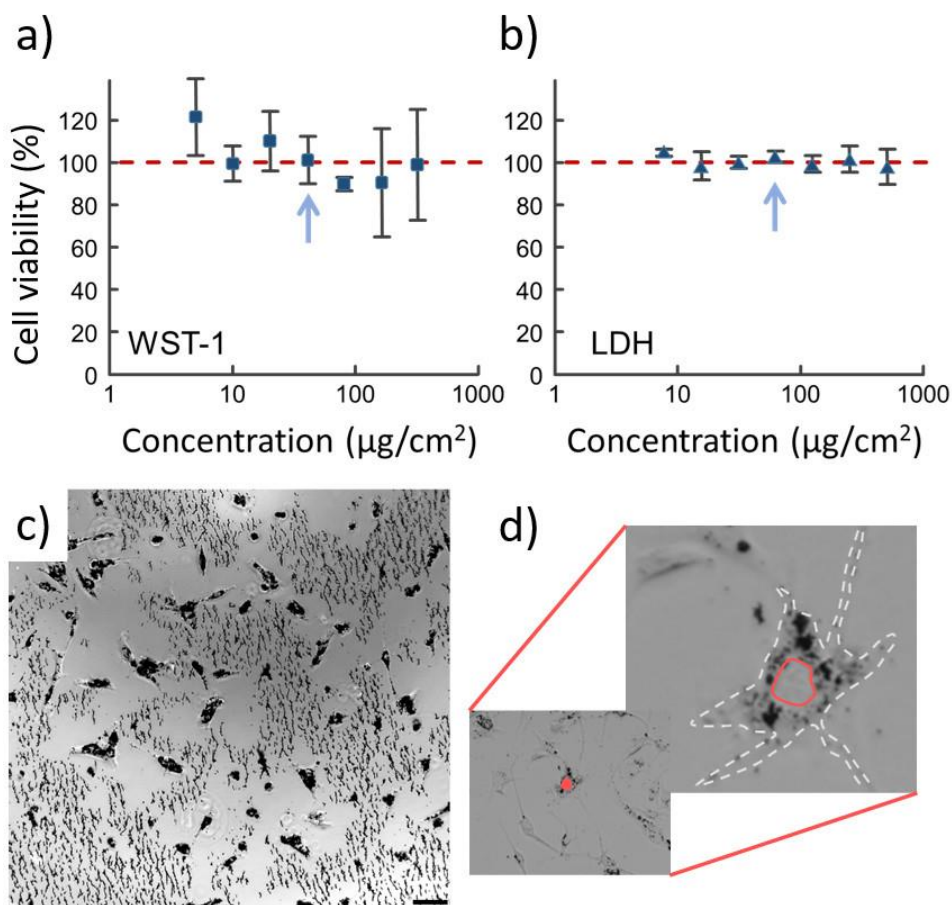


Figure 2 : Different types of anisotropic particles mimicking superparamagnetism that can be used for magneto-mechanical stimulation of cells: a) Permalloy ( $Ni_{80}Fe_{20}$ ) discs coated with Au [19,20]. b) Micron-sized magnetite ( $Fe_3O_4$ ) particles obtained by ball-milling of magnetite powder [33]. c) Mesoscopic magnetite particles obtained by chemical synthesis exhibiting a magnetic vortex configuration [29]. d) Schematic representation of the micromagnetic configuration evolution of a magnetic vortex under increasing magnetic field. e) Electron holography imaging (credit A. Masseboeuf) of a vortex particle at zero field showing the cylindrical magnetization configuration of the particle (zero magnetization at zero field). f) Hysteresis cycle of vortex particles (magnetization as a function of applied field)

### **In-vitro experiments:**

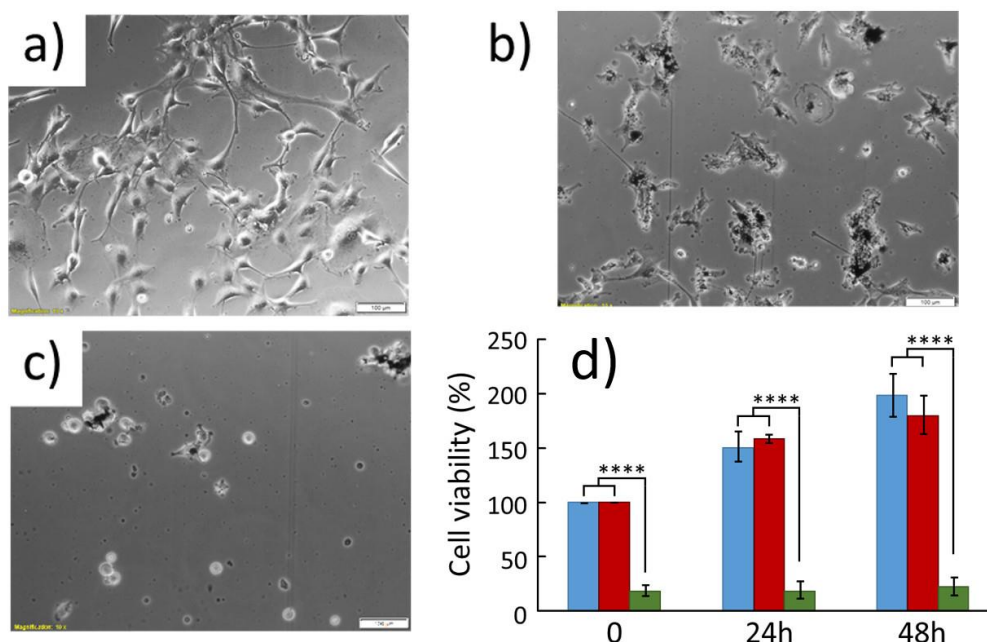
The non-toxicity of the Au/NiFe/Au microdiscs and magnetite particles was verified on U87 glioblastoma cells (brain cancer tumor) through various tests, including the WST (Water Soluble Tetrazolium) test, which assesses the metabolic activity of cells [36], and the LDH (lactate dehydrogenase) test [37], which verifies the integrity of the cell membrane (Fig. 3a and 3b) [20]. Both tests confirmed the non-toxicity of the magnetic particles up to concentrations 50 times higher than those used in magneto-mechanical stimulation (MMS) experiments. Not only are the magnetic particles non-toxic to the cells, but the U87 cells also show a great affinity for these particles, as illustrated in Fig. 3c. In a Petri dish, after 12 hours of cell incubation in the presence of the particles, it became evident that the cells had absorbed all encountered particles within their exploration zone. Furthermore, in these *in-vitro* 2D experiments, within 48 hours, most of the particles had been internalized into the cells despite their micron-sized dimensions (Fig.3d) [20]. The uptake of large particles is typically associated with macrophage

cells, due to their capacity to perform phagocytosis or macropinocytosis. In contrast, non-phagocytic cells are generally thought to rely primarily on clathrin- or caveolin-mediated mechanisms for the internalization of smaller particles [38,39,40]. The process of internalization is complex and influenced by various physicochemical properties of the particles, including size, shape, aspect ratio, and surface charge [41,42]. However, the uptake of particles as large as 3  $\mu\text{m}$  by non-macrophage cells, through multiple pathways, has also been reported [43,44].



*Figure 3 : Non-toxicity of magnetic vortex particles for U87 cancer cells (glioma cells from brain cancer) in the absence of applied magnetic field. a) WST-1 test measuring cell metabolic activity as a function of magnetic particles concentration. The blue arrow indicates the concentration typically used in our experiments. b) LDH test assessing cell membrane integrity. Both toxicity tests demonstrate that Au-coated magnetic particles are non-toxic to cells as long as the magnetic field is not applied. c) U87 cancer cells were incubated for 24 hours with the particles (in black) in cell culture box. It appears that U87 cells exhibit a strong affinity for the magnetic particles and absorb them throughout the area explored by the cells within 24 hours. d) In these in-vitro experiments, within 48 hours, the particles are internalized by the cells.*

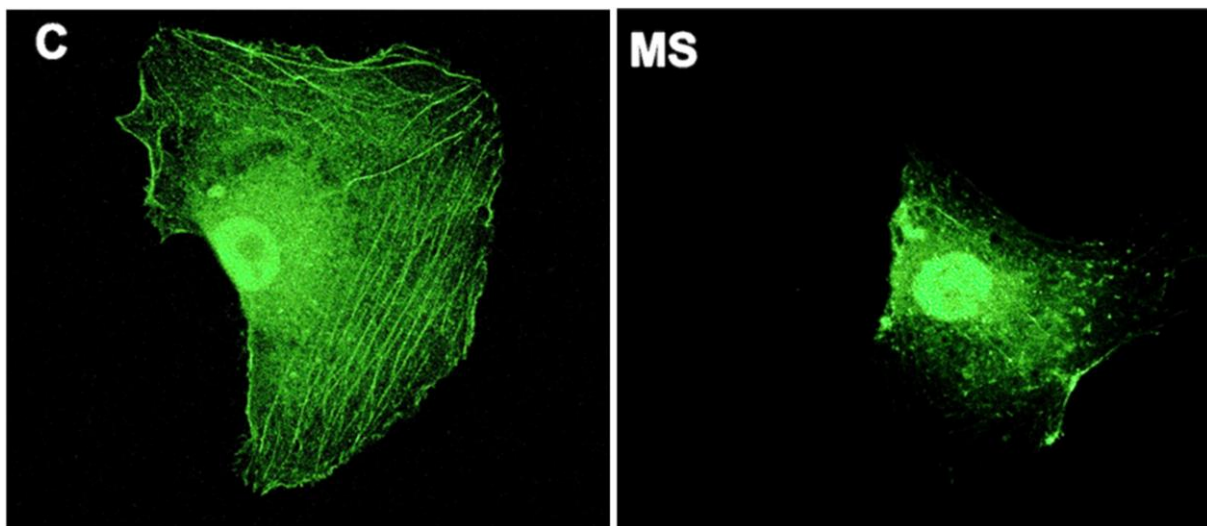
Once the magnetic particles come in close proximity with the cancer cells, or after their internalization, a low-frequency rotating magnetic field (typically between 2 and 20Hz, amplitude 400mT, exposure duration 30-45 minutes in these experiments) is applied to the cell culture using a system of rotating permanent magnets (Halbach cylinder). This vibrates the magnetic particles, which, in turn, mechanically stimulate the cells. The effect of this magnetic stimulation on U87 cancer cells is dramatic, as illustrated in Figure 4. Over 80% of the cancer cells are destroyed by the MMS (Fig. 4d, green bars). Moreover, it appears that the surviving cells become nearly spherical (Fig. 4c), indicating a profound alteration of their cytoskeleton. In addition, the surviving cells cease to divide for at least 2 days after treatment (see green bars in Fig.4d at times 0, 24h, 48h), while non-stimulated cells proliferate normally (Fig. 4a and blue bars in Fig. 4d; Fig. 4b and red bars in Fig. 4d). Similar results were obtained using magnetite particles prepared by mechanical milling, as well as on different types of cancer cells (glioblastoma, kidney cancer, melanoma) [24]



*Figure 4 : In-vitro tests of MMS on U87 glioma cells (brain cancer). a) Control sample: cells alone; b) Control sample: cells + magnetic particles; c) Sample: cells + magnetic particles (100µg/ml) + alternating magnetic field (20Hz, 400mT, 45min); d) Evolution of the number of viable cells, immediately after magneto-mechanical treatment and at 24h, 48h post-treatment: blue bars=cells alone control (a)); red bars=cells + particles (b)); green bars= cells + particles + rotating magnetic field (c)). Note that control cells continue to proliferate (cell count increases at 24h and 48h) while magnetomechanical treatment halts proliferation for at least 48h.*



Next, we demonstrated the strong impact of the MMS on the cell cytoskeleton (Fig.5). The cytoskeleton is composed of fibrous proteins present in the cytoplasm: actin microfilaments (colored green in Fig.5 C), intermediate filaments, and microtubules [45]. Its main role is to provide structure to the cell and to participate in essential cellular processes such as division, motility, intracellular transport, and cellular signaling. MMS of cells leads to a significant disorganization of the cytoskeleton (See Fig.5 MS=after magnetomechanical stimulation, compared to Fig.5 C, which corresponds to the control before stimulation). This cytoskeletal disorganization explains why cells contract, become nearly spherical after mechanical stimulation (Fig.4c), and cease to proliferate for at least a couple of days (Fig.4d).



*Figure 5 : Illustration of the disorganization of the cytoskeleton following MMS of U87 cells (C= non-stimulated control sample, MS= after magneto-mechanical stimulation). The actin fibers clearly visible in the control sample have completely disappeared after stimulation, and the cell has contracted significantly.*

Depending on the intensity of the mechanical stimulation, different effects can be observed. Mild stimulation only causes temporary cell contraction accompanied by a loss of motility and a temporary halt in mitosis (cell division). Stronger stimulation can trigger cell apoptosis [19,20]. Even stronger stimulation can rupture the cell membrane and lead to necrosis [46]. The latter regime is not desirable as it generates cellular debris, which can be sources of inflammation and potential metastasis in the case of cancer cells. The thresholds between these different regimes depend on the nature of the cells. For example, glioma cells are more sensitive to mechanical stimulation than healthy cells because they overexpress PIEZO 1 mechanotransduction channels on their membrane [47]. Under the effect of mechanical

stimulation, these channels open and generate an influx of calcium into the cytoplasm, triggering cell apoptosis. Healthy cells that express fewer of these PIEZO1 channels are less sensitive to mechanical stimulation. This difference should allow for the identification of stimulation conditions that destroy cancer cells without causing lasting damage to healthy cells.

### **In-vivo experiments:**

Based on these very encouraging *in-vitro* results, experiments were conducted *in-vivo* on mice bearing glioblastoma. A first set of experiments consisted in injecting glioma U87 cancer cells in the mice brain, let the tumor grow for 10 days. Subsequently, the magnetic particles were injected into the tumor using a syringe, followed by the application of the rotating magnetic field once a day for 5 days [48]. No inflammatory reactions were observed associated with the particles injection nor any macrophage activity around the particles in contrast to what is usually observed with SPIONs [49]. Following this experimental protocol, however, the survival test results did not show any benefit from MMS (Fig.6a). Subsequent analyses helped to understand the very significant differences between *in-vitro* and *in-vivo* experiments. On one hand, the particles poorly diffused within the tumor volume, so that only the area closest to the particle injection site was treated by mechanical stimulation (Fig.6c). As a result, the tumor continued to grow at its periphery. On the other hand, it was also observed that in these *in-vivo* experiments, the particles were not internalized but remained confined in the extracellular environment, which constitutes a significant difference compared to *in-vitro* experiments (see contrast between Fig.3d and Fig.5b) and may reduce the effectiveness of MMS. This highlights the importance of the microenvironment, particularly its rigidity and heterogeneity, in these experiments [48]. It is likely that smaller particles like those in Fig.2c (hexagonal discs of approximately 200nm) will be more easily internalized *in-vivo* than the 1.3-micron diameter microdiscs used in the experiments of Fig.6.

In another set of *in-vivo* experiments performed by Cheng et al [50], a positive impact of the MMS on mice survival rate was observed. The difference between these two experiments is that in the latter one, the particles were injected in the mice brain at the same time as the cancer cells so that the particles were dispersed in the whole tumor. In contrast, in the former experiment, the particles were injected once the tumor was formed. This comparison supports the idea that the lack of increase in survival rate in the former experiment was primarily due to a lack of dispersion of the particles in the whole tumor.

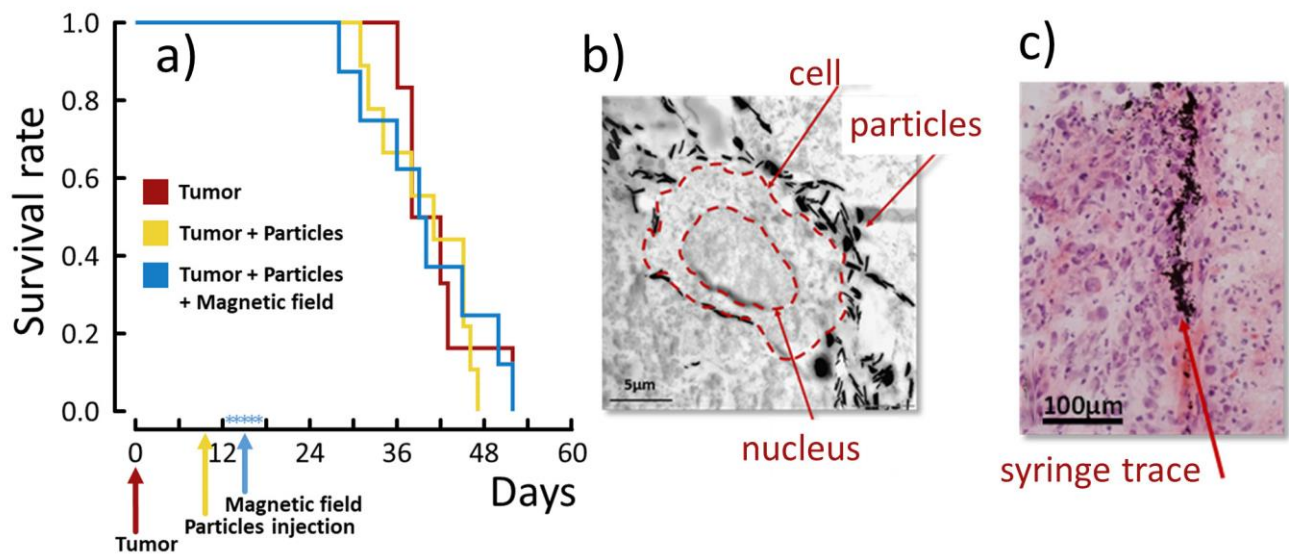


Figure 6 : In-vivo experiments on mice bearing glioblastoma. a) Survival test showing that, with the followed protocol, MMS had no beneficial effect on survival; b) Transmission microscopy image showing that in in-vivo experiments, the particles are not internalized, they remain in the extracellular environment; c) Transmission microscopy image of the particle injection site in the tumor. The particles remain confined near the injection site, which did not allow for the treatment of the entire tumor in these initial in-vivo experiments

The poor particles dispersion in the tumor volume is not necessarily an obstacle to their use in the treatment of glioblastoma. Indeed, when the oncologist performs tumor excision, there is always a risk that aggregates of cancer cells remain in place in the peritumoral area, and these particularly invasive peripheral cells can promote metastases. A possible solution could then consist in applying a biocompatible gel loaded with magnetic particles to the peritumoral zone during tumor excision. The application of the rotating field would then directly treat this peritumoral zone, the usual site of cancer invasion. This approach would circumvent the previously raised issue of poor particles diffusion over the whole tumor volume.

MMS could also be applied synergistically with chemotherapy treatment. The cell contraction resulting from MMS (Fig.4c) implies that surviving cells become more individualized, whereas cancer cells usually interact and collectively develop resistance to chemotherapy treatments [51]. Therefore, chemotherapy effectiveness could be enhanced by synergizing with MMS of cells.

### **3D Spheroids: a more relevant in vitro model to increase the efficacy of in vivo translational research**

The clear differences observed between 2D *in-vitro* experiments in culture plates and *in-vivo* experiments have highlighted the importance of finding *in-vitro* models closer to *in-vivo* conditions. Such a model has been developed in the form of spheroids, which are self-organized cell clusters exhibiting textures similar to biological tissues [52]. These 3D cellular assemblies can be obtained by culturing cells in low-adhesion microtiter plates [53]. Thus, in Corning® Elplasia® microstructured plates, they form within 24 hours, as illustrated in Figure 7 which shows, as example, the formation of spheroids and tumoroids derived from pancreatic cells. These 3D structures constitute a relevant model for drug screening [54] and can even be vascularized [55].

Once the tumoroids are formed, the magnetic particles are dispersed among them. After 24h, they are internalized within the tumoroids. The MMS is then applied as under *in-vivo* conditions. The advantage is that the microenvironment here is much closer to that encountered *in-vivo*.

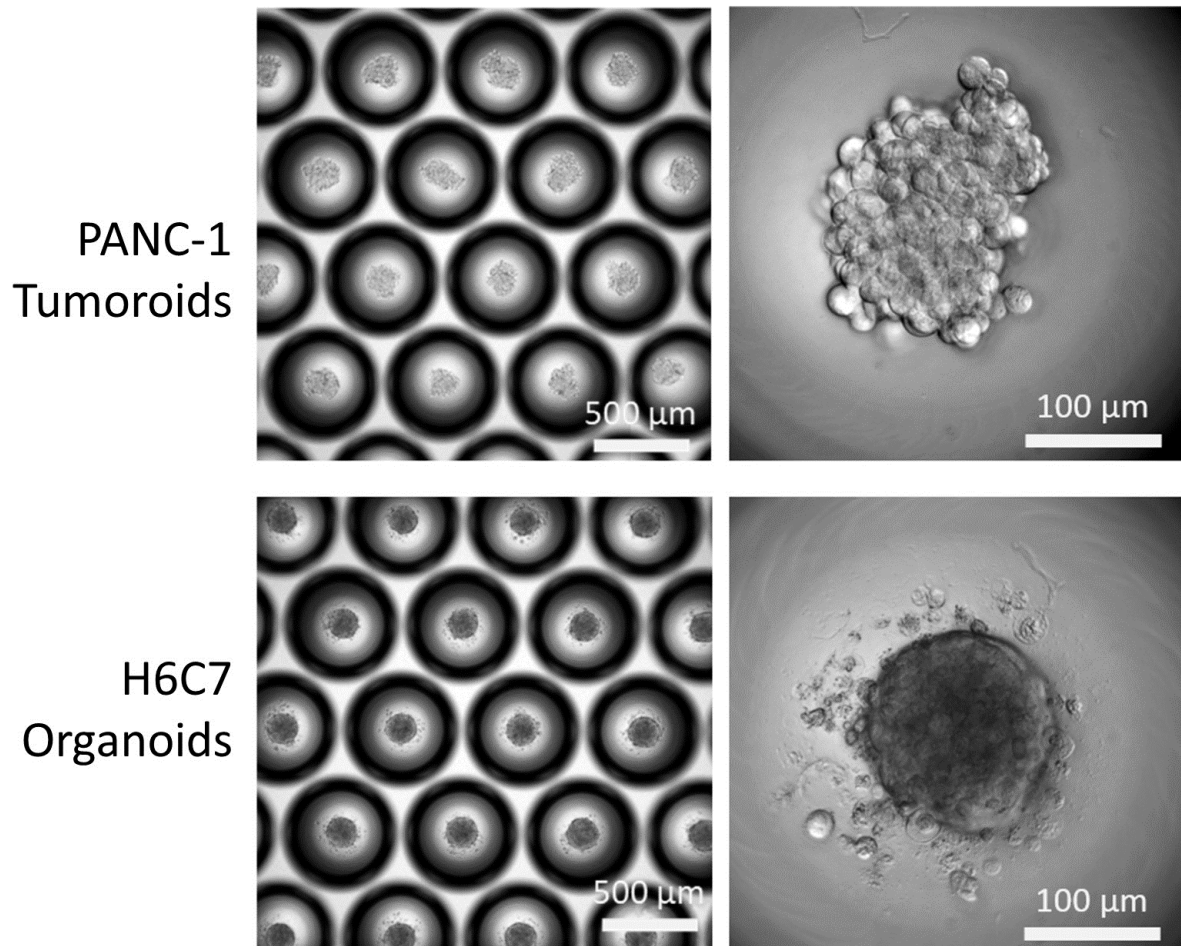
Spheroids have been demonstrated to provide a highly relevant model for the development of novel anticancer strategies [56, 57, 58]. For instance, a critical aspect of these strategies is the ability to deliver therapeutic doses of anticancer drugs to the core of tumors, which is essential for optimizing nanoparticle-based therapies. This is especially challenging due to the disorganized vascular architecture, irregular blood flow, and tissue compression exerted by cancer cells, all of which contribute to reduced drug delivery efficiency [59].

Magnetic drug delivery is one promising technique to achieve therapeutic drug concentrations at tumor sites [60]. This approach utilizes the forces applied to magnetic nanoparticles (MNPs) via an external magnetic field to guide their movement within the body [61], and potentially enhance their perfusion into the tumor mass once localized at the tumor site [62]. However, the efficacy of this method is influenced by the scaling of magnetic forces with the volume of the particles, suggesting that smaller superparamagnetic iron oxide nanoparticles (SPIONs) may not be the optimal choice for this application.

Numerical simulations that account for both the magnetic forces acting on MNPs and the rheological properties of the tumor microenvironment indicate that larger particles, ranging from 200 nm to 300 nm in diameter [63], or even up to 1  $\mu\text{m}$  [64], are more effective than smaller SPIONs in delivering drugs to the core of tumors. In this context, the use of spheroids

as a 3D model provides a valuable tool to more accurately evaluate magnetic drug delivery strategies, particularly with respect to particle dispersion and cellular endocytosis.

A key advantage is that they enable a reliable and meaningful exploration and optimization of many parameters in *in-vitro* experiments prior to translation to *in-vivo* experiments on animal models.



*Figure 7 : Experimental illustration of the formation of pancreatic cancer PANC-1 cell-derived tumoroids and pancreatic healthy H6C7 cell-derived spheroids in ELPLASIA plates.*

Furthermore, recent findings indicate that culturing cells on two-dimensional (2D) soft substrates, which mimic the mechanical properties of the native tissue, helps preserve the original cell phenotype. Such *in-vitro* models can be highly valuable as a preliminary step before transitioning to *in-vivo* experiments. They enable the generation of larger quantities of cells with relevant molecular characteristics, which is often challenging to achieve in three-dimensional (3D) culture systems [65]. This technological advance opens up the possibility of

conducting more extensive biological analyses of the effect of nanoparticles under conditions that are relevant to *in-vivo*. Combined with the analysis of spheroids, these approaches will make it possible to decipher the cell/particle interaction, which can then be optimised for medical application.

The results presented in this paper, in conjunction with an increasing body of literature [24], strongly support the development of a new biomedical field utilizing MMS for therapy. This approach addresses significant biomedical needs, particularly for aggressive cancers that do not respond to conventional therapies, as well as for brain and chronic diseases that pose substantial medico-economic burdens worldwide [66].

For the success of this emerging strategy, it is essential to tailor the magneto-mechanical approach to the physical characteristics of pathological tissues. In addition to cellular and molecular abnormalities, physical abnormalities have been documented in glioblastoma, a paradigmatic deadly form of cancer that exemplifies the need for translation of this innovative therapy to clinical settings. The mechanical properties of the glioblastoma microenvironment are key determinants of invasion, proliferation, and resistance to radio-chemotherapy [67, 68, 69]. These properties contribute to solid stress, which significantly influences tumor aggressiveness [70,71]. Tumor stiffness is highly heterogeneous and can be characterized by magnetic resonance imaging (MRI) and ultrasound [72, 73].

These mechanical abnormalities are also linked to molecular mechanical sensing abnormalities, which likely influence the response to mechanical therapy. For instance, the hyperexpression of PIEZO1 in glioblastoma may explain the heightened sensitivity of glioblastoma cells to MMS compared to healthy tissues [74]. These physical characteristics will impact not only the response but also the diffusion of the effects of MMS. This underscores the need for precise mechanical dosimetry, analogous to that used in radiotherapy, which involves mapping the mechanical properties of the tumor at both the macroscopic and molecular levels to customize mechanical therapy. This new mechanical dosimetry requires rigorous validation using *ex-vivo* 3D models as well as *in-vivo* models of pathologies. The challenge is to develop an innovative and personalized magneto-mechanical theranostic approach, which is urgently needed in clinical practice.

So far, this article has described the effect of MMS within the context of cancer. However, MMS can have other biomedical applications, for instance in the fields of diabetes or neurology.

### MMS stimulating insulin secretion from pancreatic cells:

In the context of type 2 diabetes, it has been shown that MMS of INS1 pancreatic cells can lead to insulin secretion, as illustrated in Fig.8 [75]. Insulin regulates blood glucose levels by facilitating the uptake of glucose into cells for energy production or storage. It maintains balanced blood sugar levels by promoting glycogen storage in the liver and muscle while inhibiting hepatic glucose production. Additionally, insulin influences fat and protein metabolism, promoting fat storage and reducing lipolysis. Through these mechanisms, insulin helps prevent both hyperglycemia and hypoglycemia [76].

In these experiments [75], the cells were grown on a suspended polymer membrane embedding magnetic particles. The membrane was set into vibrations at frequency between 10 and 40Hz by an alternating magnetic field, thus transmitting a mechanical stress to the INS1 cells.

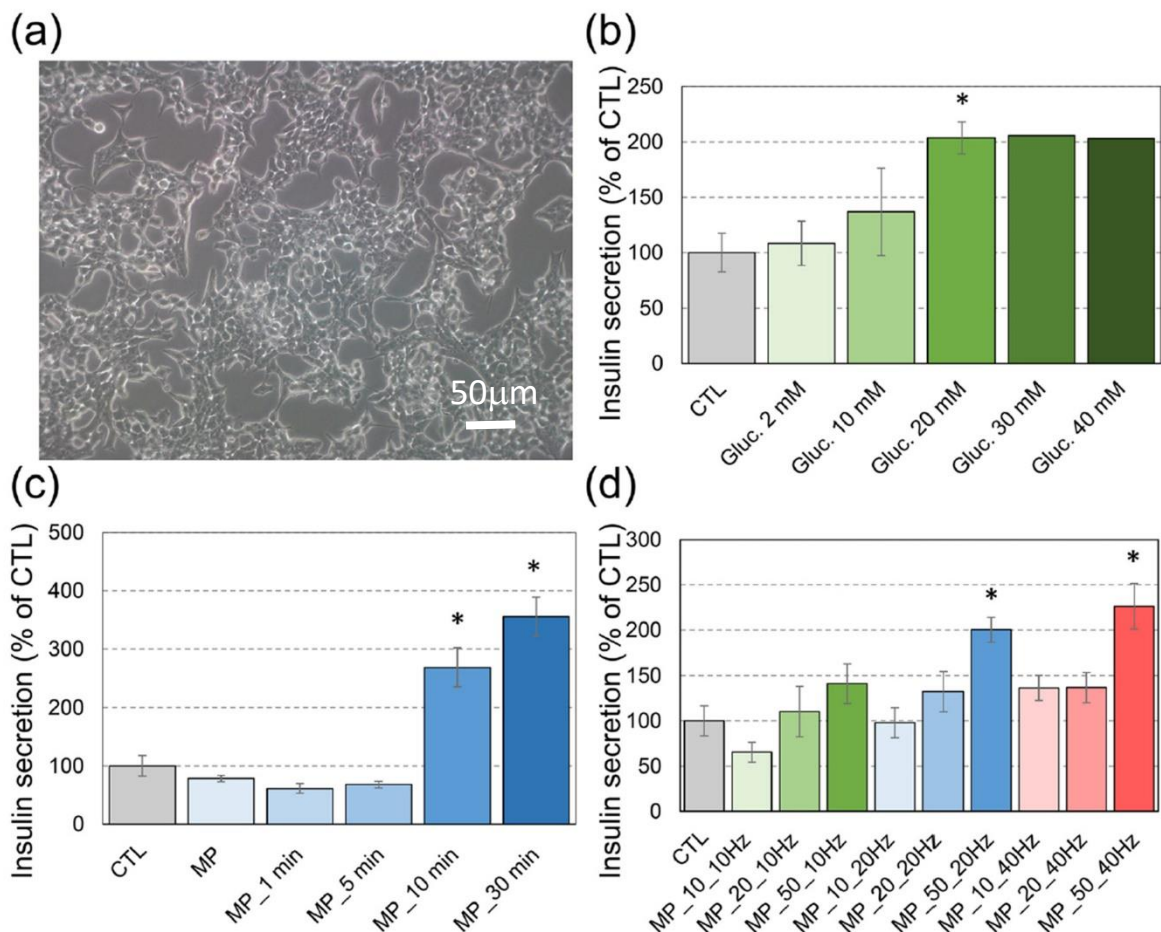


Figure 8 : Illustration of insulin secretion by mechanically stimulated INS1 pancreatic cells through magnetic particles vibration. a) INS1 pancreatic cells; b) Control experiment verifying insulin secretion by INS1 cells in the presence of a glucose solution; c) Observation of insulin secretion by the same INS1 cells, not in the presence of glucose, but under the effect of MMS

*with varying durations of magnetic field exposure from 1 to 30 minutes at 10Hz. A strong insulin secretion is observed for stimulations longer than 10 minutes; d) Insulin secretion depending on the concentration of magnetic particles (10; 20; or 50  $\mu\text{g}/\text{mL}$ ) and depending on the frequency (10; 20; or 40 Hz)*

A control experiments was first performed (Fig.8b) in which the pancreatic cells were exposed to a glucose solution at concentration between 2 and 40mM. A secretion of insulin was then observed which corresponds to the normal reaction of pancreatic cells. In a second experiment, the INS1 cells were submitted to MMS for different durations (Fig.8c) as well as different particle concentrations and oscillating field frequencies (Fig.8d) without any glucose exposure. A very significant secretion of insulin was then observed for MMS longer than 10 minutes [75]. This initial observation is highly stimulating and warrants further investigation to address the following questions: Was the secreted insulin pre-existing in the cells and released upon MMS exposure, or was its production stimulated by the mechanical stress? What are the underlying mechanisms driving MMS-induced insulin secretion? Would the same insulin production occur in INS-1 cells derived from patients with Type 2 diabetes?

The use of magnetic nanoparticles to stimulate insulin secretion from INS-1 pancreatic cells may offer several potential advantages over traditional type 2 diabetes treatments. This approach may allow for precise, on-demand control of insulin release through external magnetic fields, potentially mimicking the natural secretion patterns of a healthy pancreas. By reducing the need for daily insulin injections, it may also improve convenience for patients, offering a less invasive and more targeted method of managing blood glucose levels.

### **MMS in the context of neuronal disorders:**

In the field of neurology, the application of nanomagnetic forces to neuronal cells has garnered significant interest due to its potential to enhance drug delivery by facilitating the opening of the blood-brain barrier. Additionally, this technique holds promise for modulating axonal functions, particularly in the context of neurodegenerative diseases and neuroregenerative therapies. Of particular note is its potential for spinal cord repair, offering an alternative to optogenetic approaches [76]. Indeed, in the field of neuroregeneration, it has been shown that axons elongation and orientation, cytoskeleton dynamics or axonal transport can be induced by attaching magnetic particles to the axons and subjecting them to a magnetic field gradient [77]. The resulting force exerted on the axons allows them to grow in a specific



direction determined by the direction of the magnetic field gradient. This opens up very interesting perspectives for the reconstruction of neuronal connections. This is true both in the peripheral nervous system [78] and the central nervous system [77]. This feature opens up interesting ways to develop treatments for neuronal injuries or neurodegenerative diseases, when neuronal circuits need to be rebuilt. Major evidence have been brought up *in-vitro* using embryonic neuronal cultures. Indeed, Falconeri and colleagues used 100nm diameter magnetic nanoparticles on hippocampal neuronal cultures to show that nanopulling induces microtubules stretch to sustain axon growth and associated local protein translation [77]. However, these results need to be validated in more disease relevant models and *in-vivo*.

For diseases affecting the central nervous systems such as Alzheimer's or Parkinson's diseases, magnetic techniques not requiring the injection of magnetic nanoparticles are preferred such as transcranial magnetic stimulation (TMS). These methods are being explored for their potential to enhance brain plasticity, modulate neurotransmitters release, and improve cognitive and motor functions. TMS uses pulsed magnetic fields to induce electric currents in specific brain regions, potentially restoring normal activity in areas affected by neurodegenerative conditions [79, 80].

Another development of magnetic nanoparticles is their use in conjunction of magnetic resonance imaging (MRI) as a new contrast agent. Indeed, the use of SPION shows increased MRI imaging efficiency to detect Alzheimer's disease related cellular features, namely A $\beta$  plaques. In this context, SPION are functionalized using A $\beta$ <sub>1-42</sub> peptide [81] to specifically target A $\beta$  plaques.

An alternative approach for treating neurological disorder is based on the use of magnetoelectric (ME) nanoparticles [82, 83, 84, 85]. They are capable of converting magnetic fields into electrical signals, useful for neural stimulation. These nanoparticles are made from multiferroic or core-shell materials of composition of BaTiO<sub>3</sub>-CoFe<sub>2</sub>O<sub>4</sub> [83, 84, 85], which exhibit both magnetic and electric properties. Their shape and size influence how they interact with magnetic and electric fields, and their small size (~20 nm-30 nm) [82, 83] allows them to cross biological barriers in particular the blood-brain barrier. When injected into the brain, they can be actuated by external magnetic fields—either static (DC) or alternating (AC)—to generate localized electric fields. These fields can depolarize neurons, enabling remote, non-invasive brain stimulation. This approach can offer some control over neural activity and has potential for innovative treatments of neurological disorders such as Parkinson's disease, epilepsy, and depression, using deep brain stimulation or neuromodulation therapies.

### **Clearance or long-term impact of magnetic particles following injection in the body:**

Upon intravenous injection, the majority of magnetic nanoparticles (MNPs) are rapidly eliminated from the bloodstream. Larger particles, particularly those exceeding a few tens of nm, are primarily cleared through the liver and spleen via macrophage-mediated phagocytosis [86, 87]. In contrast, smaller nanoparticles (typically under 6-8 nm) are often filtered out and excreted via the renal system [88]. The clearance rates of these nanoparticles are influenced by their size and surface modifications, with smaller particles generally exhibiting more rapid clearance, whereas larger particles tend to have extended circulation times [89].

Notably, in certain contexts—such as the administration of vortex micro-disk particles into the brains of mice—macrophage activity was not detected, allowing these particles to persist in the tissue for extended periods [48]. This observation raises an intriguing question: can we retain such particles indefinitely within the body, functioning similarly to micro-prostheses that can be activated as needed? The potential retention of magnetic microparticles within the body presents a complex array of implications, which can be both beneficial and detrimental, depending on the specific application and properties of the nanoparticles. For instance, these particles may be advantageous in the context of repetitive applications of magnetic-mediated strategies, such as in the treatment of glioblastoma by MMS or targeted drug delivery, where they could serve as reservoirs for therapeutic agents.

Nevertheless, the long-term retention of magnetic nanoparticles in the body raises several significant challenges and risks. The foremost concern is the long-term biocompatibility of the nanoparticles. While they may not provoke immediate adverse effects, chronic exposure could lead to immune responses, resulting in complications such as chronic inflammation, fibrosis, or activation of the immune system [90, 91]. Specifically, the reticuloendothelial system may continuously attempt to clear or degrade retained particles, potentially causing inflammation in organs such as the liver or spleen [92].

Moreover, many magnetic nanoparticles, particularly those that may degrade over time, risk releasing ions such as iron or nickel, which can exhibit toxicity at certain concentrations [93]. The likelihood of such toxicity escalates with prolonged retention, especially if the nanoparticles are not fully inert or if they lack appropriate biocompatible coatings [93].

In summary, while the prospect of retaining magnetic nanoparticles in the body for therapeutic applications is compelling, it necessitates thorough investigation into their biocompatibility, long-term effects, and potential toxicity to ensure safe and effective utilization in medical contexts.

### **Conclusion:**

In conclusion, MMS of cells represents a burgeoning field with significant potential for both fundamental mechanobiology studies and groundbreaking biomedical applications. The ability to externally manipulate mechanical stress on cells offers a unique advantage, enabling researchers to exploit differential responses between diseased and healthy cells. This approach has shown particular promise in addressing challenges within oncology, diabetes, and neurology.

The versatility of MMS lies in its precision and controllability, which allows for the fine-tuning of mechanical forces to target specific cellular behaviors. This method can be employed to study cellular mechanotransduction pathways, elucidate the role of mechanical cues in disease progression, and ultimately develop targeted therapies that leverage these mechanistic insights. The potential to customize treatments based on individual cellular responses marks a significant step towards personalized medicine.

This interdisciplinary research domain necessitates robust collaboration between physicists, biologists, and clinicians. Physicists bring expertise in the design and optimization of magnetic fields and mechanical devices, while biologists contribute a deep understanding of cellular responses and mechanisms. Both contribute to the understanding of the mechano-biological phenomena at play. Clinicians provide critical insights into the practical applications and therapeutic potential of these technologies, ensuring that laboratory findings are effectively translated into clinical practices.

Despite the promising advances, several challenges remain. There is a need for comprehensive studies to validate the efficacy and safety of magneto-mechanical therapies across different cell types and disease models. Furthermore, the development of sophisticated tools and techniques to precisely measure and control mechanical forces at the cellular and subcellular levels will be crucial for advancing this field.

Future research should focus on integrating MMS with other therapeutic modalities, such as chemotherapy and immunotherapy, to enhance treatment efficacy and overcome resistance

mechanisms. Additionally, expanding the understanding of the molecular and biophysical mechanisms underlying cellular responses to mechanical stimulation will be pivotal in refining and optimizing these therapies.

Overall, MMS offers a transformative approach to disease treatment and mechanobiological research. By harnessing the interplay between physical forces and cellular behavior, this field holds the promise of innovative treatments and enhanced understanding of complex biological processes. Continued interdisciplinary efforts will be essential in realizing the full potential of this promising technology, paving the way for novel therapeutic strategies and improved patient outcomes.

#### **Data availability :**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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