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Magneto-mechanical stimulation of living cells: towards innovative therapies based on mechanobiological effects

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Abstract

Cellular mechano-sensitivity can be exploited to modify cellular functions, opening the doors to the design of innovative therapies. This can be very efficiently achieved through the use of magnetic microparticles dispersed among the cells and actuated in a remotely controlled manner by external variable magnetic fields. Experiments were conducted on glioma cancer cell lines under several conditions: in-vitro 2D in Petri dishes, in-vitro 2D on soft substrates, in-vitro 3D on organoïds/tumoroïds and in-vivo on mice. The possibility to destroy cancer cells in-vitro by magneto-mechanical stimulation was clearly demonstrated. The comparison between the different experimental conditions revealed the key role of the microenvironment on cells physiological reactions to the magneto-mechanical stimulation. It appears that the organoïds/tumoroïds provide a very relevant in-vitro model for the study of these mechanobiological effects before translation to in-vivo studies.

I. Introduction

Mechanobiology represents a vibrant and expanding domain within biology, investigating the interplay between physical forces and biological processes across molecular, cellular, and tissue levels. This discipline seeks to comprehend how cells perceive and react to mechanical stimuli, influencing development, physiology, and disease pathology. Magnetism offers promising avenues in mechanobiology by enabling the remote application of adjustable mechanical stress on cells via the low frequency actuation of magnetic particles dispersed among the cells. This stress can manifest as forces generated by magnetic field gradients or torques resulting from the variable orientation of magnetic fields acting on anisotropic particles. To prevent particle aggregation, the particles are engineered to exhibit superparamagnetic-like behavior. Our investigation involves various particle types, including synthetic antiferromagnetic particles, magnetite particles with antiphase boundaries, and predominantly vortex particles made of a single permalloy disk approximately 1 micron in diameter and tens of nanometers in thickness, coated with gold for biocompatibility (See Fig.1) [1]. Depending on the cellular microenvironment (2D versus 3D, extracellular medium stiffness, in-vitro versus in-vivo conditions) and particle size, the particles may either be internalized by cells through endocytosis or remain in the

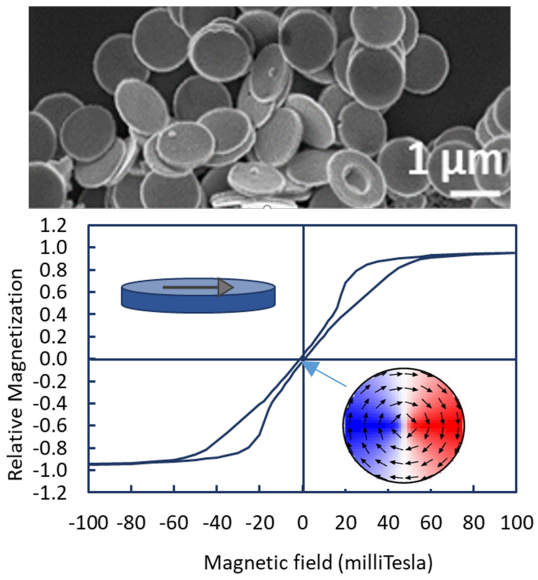

Figure 1: vortex particles, scanning electron microscopy image and hysteresis loop with field applied in-plane.

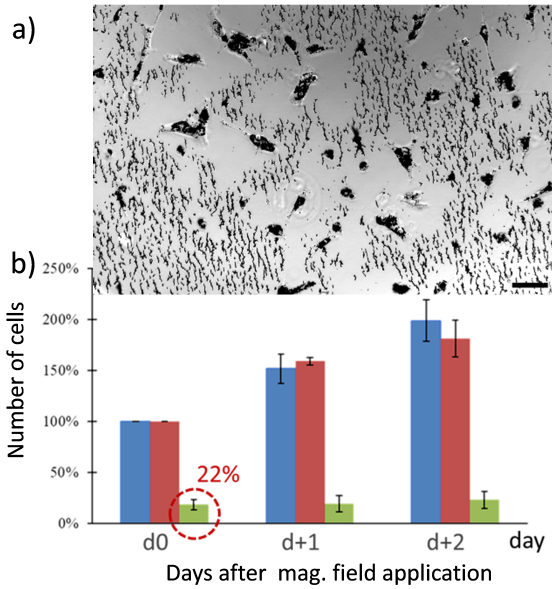

Figure 2: MMS of glioma cells: a) in-vitro experiments showing that the glioma cells spontaneously tend to grab the magnetic microparticles thus clearing up the substrate around them (in black); b) Evolution of glioma cells population following MMS (green bars). About 80% of cells were destroyed after MMS. Besides, proliferation of the still alive cells is stopped for at least 2 days following MMS. Blue and red bars are control experiments: blue: cells alone, red: cells+microdisks+no magnetic field applied.

extracellular matrix. The application of low-frequency (2-20Hz) variable magnetic fields induces mechanical stress on cells, eliciting diverse cellular responses which depend on the stimulation intensity. The stress can disorganize the cell cytoskeleton, alter ionic transduction across the cell membrane leading to the activation of various signaling pathways or even disrupt the cellular membrane. Accordingly, cellular responses may range from contraction accompanied by temporary loss of motility and mitosis, to apoptosis, to necrosis. From a biomedical standpoint, these effects hold significant promise for applications in combating cancer, diabetes, and promoting neuroregeneration, e.g. in cases of spinal cord injury.

II. Magneto-mechanical stimulation of cancer glioma cells

Following a seminal study by Kim et al. [2], we showed in classical in vitro 2D models the capability to induce apoptosis in cancer cells, including glioma and renal carcinoma cells, through the low-frequency vibration (2-20Hz) of magnetic micron-sized disk particles dispersed among the cells (see Fig.2)[3]. This effect is purely mechanical (no significant heating at the used frequency). In vivo experiments on glioblastoma mouse models were conducted by Cheng et al. [4] and ourselves [5]. These experiments yielded contrasted yet promising outcomes: While an enhancement in the survival rate of mice was noted in the former experiment, where particles were injected into the mouse brain concurrently with cancer cells, no such improvement was observed in the latter experiment, where particles were injected into the tumor subsequent to its growth. Unlike the observations in 2D in vitro cell culture models, we demonstrated that the disk microparticles do not penetrate the cells under in-vivo conditions. Nonetheless, they exert a potent anti-invasive effect, as evidenced by reproducible results in in-vitro 3D/tumoroid models, thereby tackling a significant hurdle in glioblastoma treatment.

III. Influence of microenvironment

The comparison of in-vitro experiments and in-vivo experiments revealed a number of salient differences:

- The impact of the magnetic microparticles on the cells proliferation before application of the variable magnetic field strongly depends on the cells microenvironment. This is illustrated in Fig.3 which shows the evolution of number of cells cultured on 2D glass substrate and 2D soft substrate in presence of magnetic particles (MNP) at various MNP concentrations. The substrate stiffness has a clear influence on cells proliferation at high particles concentration.

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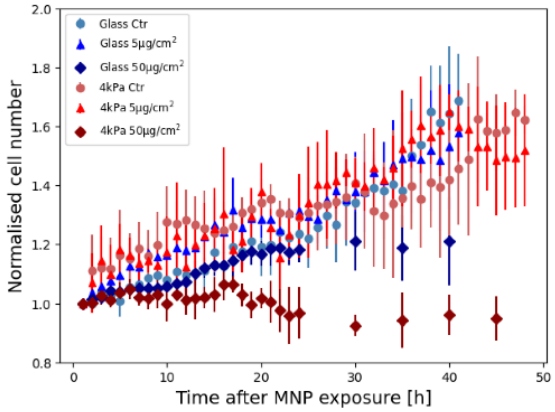

Figure 3: Cellular growth after addition of MNP in culture on glass substrate and on 4kPa hydrogels. Proliferation is altered at MNPs concentration of 50μg/cm^2 with a strong dependence on substrate stiffness.

- Furthermore, while the particles get internalized in 2D in-vitro experiments, they remain in the extracellular matrix in 3D in-vivo mice models. Interestingly, they also remain in the extracellular matrix in 3D tumoroïds model. This points out an important influence of medium stiffness as well as of the steric constrains (2D versus 3D conditions).
- The actuation of the particles and correlatively the mechanical stress transmitted to the cells under given magnetic field conditions depends on the extracellular medium stiffness.
- The dispersion of the particles within the culture medium or the tissue depends also on the microenvironment. At 2D on hard substrate, the cells migrate and particles diffuse much more easily than on soft 2D substrate or at 3D.

IV. Conclusion

Magneto-mechanical stimulation of living cells is a very efficient tool for both fundamental studies in mechanobiology and for altering cells functionalities in view of novel biomedical treatments of cancer but also of diabetes or in neurology. In these studies, the cells microenvironment plays a key role.

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Author's statement

Conflict of interest: Authors state no conflict of interest.

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