CONCLUSIONS

MAGNETIC AND OPTICAL HYPERTHERMIA USING NANOMATERIALS - LIMITATIONS, CHALLENGES AND FUTURE PERSPECTIVES

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Abstract

In this last part of the book, we provide some insights into the current challenges for nanomaterial-based hyperthermia, as well as our reflections on future developments and opportunities in the field.

Keywords

Hyperthermia; nanomaterials; biological applications

1 Introduction

The recent developments in the field of nanomedicine have given rise to a plethora of biomedical applications of nanomaterials. Their size, similar to the one of many biomolecules, their unique properties at the nanoscale, their ability of crossing biological barriers and the possibility of tailoring their surface functionalization enabled the development of nanomaterial-based biosensors, as well as of diagnostic and therapeutic tools (often combined in a single, multifunctional nanomaterial, concept known as theragnosis).

This book provides an overview of the current status of one particular biomedical application of nanomaterials, namely hyperthermia (HT). There are several classes of nanomaterials able to transform the energy absorbed from external sources, such as magnetic fields or light, into heat. Most of the research carried out to date concerns the use of magnetic and noble metal-based plasmonic nanomaterials as HT mediators. Thus, the book focused mainly on these two types of nanomaterials, although some insights into the more recently developed carbon-based nanomaterial hyperthermia were also provided in chapter 11.

In this last part of the book, we would like to highlight the main challenges that in our opinion need to be addressed in order to realize the full potential of hyperthermia as therapeutic modality. We also identified several fields in which we believe future opportunities will arise for hyperthermia applications.

2 Current challenges in nanomaterial-based hyperthermia research

2.1 Synthesis and characterization of nanomaterials for hyperthermia

Much progress has been achieved regarding the design, synthesis, characterization and functionalization of nanomaterials for hyperthermia applications, as discussed in the first four chapters of the book. Synthesizing magnetic and plasmonic nanomaterials with exquisite control over the size, shape and size distribution is undoubtedly a very important first step for their application as hyperthermia agents because it dictates their properties and their ultimate performance. Recent developments also enabled the synthesis of magneto-plasmonic nanoparticles; these hybrid nanomaterials, combining magnetic field and light responsiveness into a single structure could offer more efficient solutions for hyperthermia. Nevertheless, the field is still in its infancy and much research needs to be done before such materials can be used for clinical application, as reviewed by Maenosono and co-workers in chapter 3.

Despite the tremendous advances in the synthesis of nanomaterials suitable for hyperthermia applications, there are still many challenges to be faced. One of the most important points to be addressed concerns the reproducibility of different batches of nanoparticles intended for hyperthermia applications and the scale up of the synthesis. As pinpointed in chapter 13, researchers must also take into account that the manufacturing of nanomaterials under Good Manufacturing Practices (GMP) conditions is a must for the translation to the market/clinic. Although there are successful examples of nanomaterials for hyperthermia already being commercialized (for example, AuroLase® Therapy for lung and prostate cancer) and used in clinical trials, the regulatory path for nanomaterials is still a long and expensive one. Of course, the proper toxicological assessment of nanomaterials for hyperthermia cannot be overlooked, not

only in order to ease their transition from bench to bedside, but also to rule out any possible contribution of the nanomaterial itself to the cellular death/damage observed after HT treatment.

As pointed out in different chapters of the book, a key aspect in magnetic hyperthermia research is related to the thorough characterization of the heating efficiency of magnetic nanoparticles (MNPs) under conditions that are relevant for the clinical setting. It is of course of utmost importance to design and synthesize MNPs with high heating efficiency, as this would enable reducing the dosage in vivo, or using a single administration of MNPs for several hyperthermia treatment cycles. However, in many instances MNPs with exceptional heating properties in aqueous suspensions failed to induce a similar heating in vitro or in vivo, due to several reasons. Often, the heating efficiency (in terms of specific absorption rate, SAR, see chapter 5 for a in-depth discussion on SAR determination) of the MNPs is measured in the laboratory under high frequency and field amplitude conditions; this can be due to magnetic hyperthermia instrumentation limitations, as in many cases the magnetic field parameters are established by the manufacturer and offer little options for modulation by the user. Thus, it is crucial to prepare MNPs that can heat effectively when exposed to rather low magnetic fields (see chapter 1 for a thorough discussion regarding the approved parameters). Likewise, as indicated in various chapters, single domain MNPs typically used for biomedical applications mediate the transformation of the magnetic field energy into heat through Néel and Brown relaxation processes. If the MNPs become internalized by cells and tissues Brownian relaxation is severely diminished or even suppressed due to the high viscosity of the medium and the inability of the MNPs to rotate freely. (Di Corato *et al.*, 2014) Therefore, it is fundamental to assess the heating efficiency of the MNPs in conditions mimicking the cellular environment, such as solvents with high viscosity or gel 3D phantoms, as well as in real in vitro scenarios. While experimental techniques such as SAR and AC susceptibility measurements (see chapter 7) can be used for the characterization of MNPs in the biological environment, in silico numerical simulations can provide powerful tools for the design of MNPs for magnetic hyperthermia applications. (Sanz *et al.*, 2016)

2.2 In situ temperature monitoring and real-time analysis of cellular effects of hyperthermia

The development of robust and accurate intracellular thermometers for precise mapping of temperature (gradients) inside the cell during HT application is another main challenge in the field, as highlighted in chapter 6. The nanothermometers not only must display high sensitivity and fast response, but they must function under the rather demanding biological environment, meaning different pH conditions, low concentrations, different local chemical environments, etc. Another challenge is that the nanothermometers must be able to determine the local temperature achieved in the close vicinity of the nanoparticle used as heating agent. Combining the heating and temperature measurement functions in a single nanomaterial seems to be the ideal approach, although it increases the complexity of the probe, which could be detrimental for the ultimate clinical application from a regulatory point of view. The analysis of current state-of-the-art reveals a more advanced status of single-probe nanothermometers based on plasmonic nanoparticles when compared to magnetic nanoparticles, due to their insufficient spatial resolution. For magnetic hyperthermia, magnetic particle imaging has emerged as a solution for monitoring the spatial distribution of nanoparticles and could enable non-invasive and real-time temperature

measurements of the region of interest, as discussed in chapter 10 by Rinaldi and co-workers.

A problem often encountered by researchers in the field of hyperthermia is how to assess the real-time cellular response to heat. In the case of optical hyperthermia, coupling a laser source to a fluorescence microscope is relatively straightforward and enables the use of specific fluorescence markers to investigate the real-time cellular effect of heat (for example, analysis of apoptosis and necrosis, membrane permeabilization, changes in membrane fluidity, etc.). For magnetic hyperthermia though, the hyphenation between the fluorescence microscope and the magnetic field applicator is rather challenging due to two main factors. On the one hand, the exposure to the alternating magnetic field can induce the heating and damage of the ferromagnetic components of the microscope (especially the objectives). On the other hand, typical coils used for the fabrication of magnetic hyperthermia equipment are rather large and cannot be adapted to the relatively small space available on the microscope. This issue is at the same time a challenge and an opportunity for magnetic hyperthermia research and some recent advances in the field have been achieved, pointing out that future developments are to be expected (see also Section 2.2). (Connord *et al.*, 2015; Blanco-Andujar *et al.*, 2016; Subramanian *et al.*, 2016)

2.3 Biological effects of heat and standardization of in vitro and in vivo hyperthermia conditions

Two other major challenges that researchers in the field of hyperthermia still face regarding its biological applications are related to the accurate investigation of the biological effect of HT and to the lack of standardization of the HT conditions.

As detailed in chapter 8 of this book, to date there are still many reports dealing only with analyzing the yes/no aspect of cellular death caused by hyperthermia, without an in-depth investigation of the mechanisms of heat-induced cellular death or cell damage. Knowing the exact impact of heat at cellular level is fundamental in the case of cancer therapy, as it is known that cancer cells are more susceptible to heat damage than normal cells, due to the pre-activation of heat shock proteins (HSPs). However, HSPs seem to also mediate the resistance of cancer cells to hyperthermia (thermotolerance), thus displaying a Janus-like behavior. When analyzing cellular effects of nanomaterial-based hyperthermia, researchers must also take into account the (most likely) heterogeneous distribution of the nanoparticles inside tissues, as well as the parameters of the external energy source (potency of laser source and irradiation time in case of optical hyperthermia and frequency and amplitude of the alternating magnetic field and exposure time in case of magnetic hyperthermia). This leads us to the second challenge, related to the current lack of standardization regarding the use of nanomaterials for hyperthermia applications. This affects basically all aspects of hyperthermia, from the synthesis of the nanomaterials, to the measurement of heat generation efficiency (for magnetic hyperthermia being of essence to analyze it also in the biological environment, *vide supra*), administration route, tumor models, duration and frequency of hyperthermia treatment, methodologies to analyze the outcome of the treatment, etc. The comprehensive discussion in chapter 12 by Beola et al. for the case of magnetic hyperthermia shows that there are huge differences in the above-mentioned aspects between different research groups, which renders practically impossible the comparison of the results obtained. We believe that analyzing the exact effects produced by magnetic and optical hyperthermia and trying to standardize the experimental conditions for in vitro and preclinical in vivo research are two aspects that should be

addressed in the near future in order to close the gap between fundamental laboratory research and clinical trials. In this sense, the use of invertebrate animal models could be a powerful tool to obtain a large amount of information whilst reducing animal experimentation, in line with the 3R principle (replacement, reduction and refinement), as highlighted in chapter 9 of this book.

3 Future directions and opportunities for magnetic and optical hyperthermia

3.1 Remote control and modulation of cellular functions using hyperthermia

Although this book is mostly focused on the use of hyperthermia for cancer treatment, nanoscale heating has other potential biological applications, including tissue engineering, regenerative medicine and remote stimulation and control of cellular functions with high spatio-temporal resolution. (Li, Liu and Chen, 2017) During the last decade, several studies demonstrated the use of localized heating produced by magnetic nanoparticles for remote activation of calcium channel ions and triggering of behavioral responses in living animals (Huang *et al.*, 2010) or regulation of glucose homeostasis in mice by means of remote control of gene expression (Stanley *et al.*, 2012, 2015). Local heating induced by plasmonic nanoparticles has been successfully employed for reversible switching of lipid membrane polarity in model and real cell membranes; this enables a tool for increasing the cell membrane permeability and holds great potential for intracellular delivery. (Urban *et al.*, 2016) Laser induced heating using gold nanorods has been also used to promote neuronal growth and to stimulate neuronal electrical activity, (Paviolo *et al.*, 2013; Yong *et al.*, 2014) providing potential solutions for peripheral nervous regeneration therapies. Very recently, the use of plasmonic nanoheating has been extended to "wireless" activation of striated muscle cells using gold nanoshells, pointing out future possible developments in the fields of tissue engineering, regenerative medicine and bionics. (Marino *et al.*, 2017) The remote activation of muscle cells was based on mild laser heating (to ~42ºC) and investigation of the cellular response to the mild heat stimulation conditions revealed that the chronic exposure to thermal stimulation resulted in the upregulation of genes encoding different proteins, among them the well-known heat shock proteins. In our opinion, this so-called "sublethal" hyperthermia will encounter many more applications in the near future and will most likely have a noticeable impact on intracellular drug delivery, transfection, cell membrane biophysics studies and tissue regeneration.

3.2 Development of new instrumentation

As highlighted in Section 1.2, real-time monitoring of cellular response to heat is particularly challenging in magnetic hyperthermia. We believe that much effort will be devoted in the coming years to the developments of new equipment for this purpose, following the examples already reported in the literature. These works successfully addressed the monitoring of cellular processes such as lysosomal membrane permeabilization, reactive oxygen species (ROS) production and cellular membrane damage (Connord *et al.*, 2015) or the investigation of the cellular death pathways (Blanco-Andujar *et al.*, 2016). Despite the technical challenges imposed by the design of such instrumentation, we envisage that this is one of the areas that will provide

significant developments and opportunities in the field of magnetic hyperthermia, especially regarding a better understanding of the cellular effects of heat.

3.3 Hyperthermia and immunotherapy

Since the late 1990s, there has been increasing evidence that hyperthermia can induce antitumor immune response, suggesting the potential of combining hyperthermia and immunotherapy for the treatment of cancer. Briefly, there are several molecular mechanisms responsible for the improved immune response under the application of hyperthermia, including expression of heat shock proteins, activation of natural killer (NKs) and dendritic cells (DCs), and alteration of leucocyte trafficking. (Skitzki, Repasky and Evans, 2009; Frey *et al.*, 2012) More detailed descriptions of the cascade of immune events in response to local tumor heating induced by hyperthermia that ultimately lead to immunomodulation can be found in several reviews (Calderwood, Theriault and Gong, 2005; Skitzki, Repasky and Evans, 2009; Frey *et al.*, 2012; Moy and Tunnell, 2017) Here, we would like to highlight some successful studies involving nanomaterials, including some combinatorial approaches involving nanomaterial-based hyperthermia and immunotherapy that are currently in preclinical or clinical stages of development, thus clearly showcasing the potential of HT in immunomodulation. (Moy and Tunnell, 2017; Vo-Dinh and Inman, 2018) Kobayashi and co-workers demonstrated the first examples of immune modulation induced by magnetic hyperthermia. Magnetoliposome-mediated HT not only led to the destruction of subcutaneous T-9 glioma tissues in which the nanoparticles were injected, but also to the regression of distant tumors, leading to a long lasting acquired immune response. (Yanase *et al.*, 1998) Similarly, the combination therapy using magnetic hyperthermia and immunotherapy was applied for the treatment of malignant melanoma. (Ito *et al.*, 2003) Using interleukin-2 (IL-2), known for enhancing cellular immunity and granulocyte-macrophage colony-stimulating factor (GM-CSF) for the activation of antigen-presenting cells (APCs), the authors showed a nearly complete regression of tumors in mice bearing subcutaneous melanoma. The recent report of Vo-Dinh and co-workers elegantly illustrates the application of immunothermotherapy for the treatment of metastatic cancers in mice bearing primary tumors (directly exposed to HT) and distant tumors (acting as metastatic models). (Liu *et al.*, 2017) The approach, referred to as "Synergistic Immuno Photothermal Nanotherapy (SYMPHONY)", made use of gold nanostar-mediated hyperthermia and immune checkpoint inhibition (targeting the programmed death-ligand PD-L1) and showed not only a direct cancer cell killing effect in the primary tumors, but also an activation of the immune system, as indicated by the regression of distant tumors. Moreover, the combined approach was found to be much more effective than administration of PD-L1 antibodies of hyperthermia alone.

In light of these promising results, and taking into account that several immune checkpoint inhibitors have received FDA approval since 2011 (Hsu, Su and Huang, 2017; Moy and Tunnell, 2017), we are confident that the combination of immunotherapy and hyperthermia will be a very prolific field of research in the coming years.

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