University of Rzeszów
Doctoral School

Nanomaterials in biotechnology – Current topics

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(BPI/STE/2023/1/00001/U/00001)

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DOI:10.15584/ste.2025.1

HR EXCELLENCE IN RESEARCH

This publication has been produced as part of project "UR an international PhD student" run by Rzeszów University Doctoral School. The project has been supported by Polish National Agency for Academic Exchange under the STER NAWA program - Internationalization of Doctoral Schools; No BPI/STE/2023/1/00001/U/00001.

Overview

The specialist workshop will be focused on selected current topics related to the use of nanomaterials in different areas of nanobiotechnology:

1- Bioorthogonal chemistry and its applications in nanobiomedicine
2- Nanomaterials in tissue engineering
3- Nanoparticle hyperthermia applications

This specialist workshop will offer students the opportunity to learn about emerging concepts and technologies that are shaping the future of biotechnology and healthcare. It will introduce the concept of bioorthogonality and present selected chemical reactions that are compatible with the biological components of a living being and can therefore be carried out in the presence or within living cells and show how the principles of bioorthogonal chemistry can be applied in nanobiomedicine, for example for selective cell labelling, imaging, and therapeutic strategies. The second part of the workshop will present the design and use of nanomaterials for tissue regeneration, focusing on their physicochemical properties, biocompatibility, and integration into regenerative medicine. Finally, the potential of nanoparticle-mediated hyperthermia for cancer therapy and other therapeutic applications will be discussed.

1. Bioorthogonal chemistry and its applications in nanobiomedicine

This lecture introduces the fundamental concepts of **click chemistry** and **bioorthogonal chemistry**, recognized with the 2022 Nobel Prize in Chemistry for their remarkable advantages, including simplicity, robustness, and near-quantitative yields.

The students will become familiar with the principal bioorthogonal reactions that can be performed within living systems (going from individual cells to whole organisms), transforming them into unique "reaction vessels." Furthermore, an overview of recent advances in nanobiomedicine, made possible by the integration of bioorthogonal chemistry with the distinctive properties of nanomaterials, will be presented.

1.1 Introduction

The term "*click chemistry*," introduced by K. Barry Sharpless in 2001, refers to a class of chemical reactions characterized by exceptional selectivity, rapid kinetics, operational simplicity, high yields, minimal byproduct formation, and overall robustness and efficiency. A useful analogy is the familiar "click" of fastening a seatbelt on an airplane: a simple action that joins two components. Similarly, click reactions are designed to achieve efficient and predictable molecular connections under mild conditions. When these reactions are further adapted to function within the complex environment of living systems, maintaining compatibility with cellular and physiological conditions, the concept evolves into "*bioorthogonal chemistry*," a term denoting chemical transformations that proceed with minimal or no interference with native biological processes.

Despite being relatively new concepts (the first articles on click chemistry and bioorthogonal chemistry were published in early 2000s), their simplicity and effectiveness, as well as their enormous potential in the fields of chemistry, biology, biochemistry, materials science, and nanotechnology, have made click chemistry and bioorthogonal chemistry worthy of the 2022 Nobel Prize in Chemistry, awarded to K. Barry Sharpless, Morten Meldal, and Carolyn Bertozzi. In the words of the chairman of the Nobel Committee for Chemistry, Johan Åqvist, "*This year's Chemistry Prize is about not overcomplicating things, but working with what is easy and simple. Functional molecules can be built even by following a simple path*."

1.2 What is bioorthogonal chemistry?

Bioorthogonal chemistry has its origins in the group led by Carolyn Bertozzi at Stanford University, in response to the growing interest in studying and understanding complex biological and cellular processes in the context of living cells and whole organisms.[1] This task requires the development of strategies that allow biomolecules to be studied and tracked within their native environment, using specific markers (e.g., fluorescent probes). In this context, conventional methods rely on the use of genetically encoded tags, such as green fluorescent protein (GFP), or specific antibodies. However, although antibodies and proteins have enabled advances in our understanding of the roles played by certain proteins in dynamic cellular processes, they are large biomolecules whose use can disrupt the structure and/or function of the protein under study. More importantly, antibodies and proteins cannot be easily used to study other types of biomolecules, such as lipids, nucleic acids, or carbohydrates. Carolyn Bertozzi's group had been studying the complex carbohydrates present in our cell membranes for years. These biomolecules play a very important role in many infectious diseases and cancer and determine each person's blood type. Nevertheless, studying these carbohydrates in their biological environment was not an easy task, due to their heterogeneity, their incompatibility with genetically encoded reporters, and the fact that classic molecular biology tools could not be applied to them (for example, antibodies that recognize these carbohydrates tend to have low affinities).

To overcome this challenge, researchers began to study whether it was possible to chemically modify the biomolecules of interest. This would involve incorporating a chemical group as a probe or reporter into the target biomolecule, using the cell's own biosynthetic and metabolic mechanisms. In this way, in a second stage, the exogenous molecules acting as markers would form selective covalent bonds with the modified target biomolecule (Figure 1.1). The idea of bioorthogonal chemistry was certainly groundbreaking, but it posed a formidable challenge, given all the obstacles encountered at the cellular level. First, a rapid reaction was needed, taking place in an aqueous medium at 37 °C and physiological pH. Moreover, the reagents used had to be harmless to the cell, and the functional groups involved in the reaction could not participate in secondary reactions with other endogenous functional groups, such as amines, thiols, carboxylic acids, etc. Furthermore, not all chemical groups can function as bioorthogonal probes, i.e., they must have the ability to not

interfere (or interfere minimally) with biological functions and, at the same time, have selective reactivity toward their bioorthogonal partner in living systems.

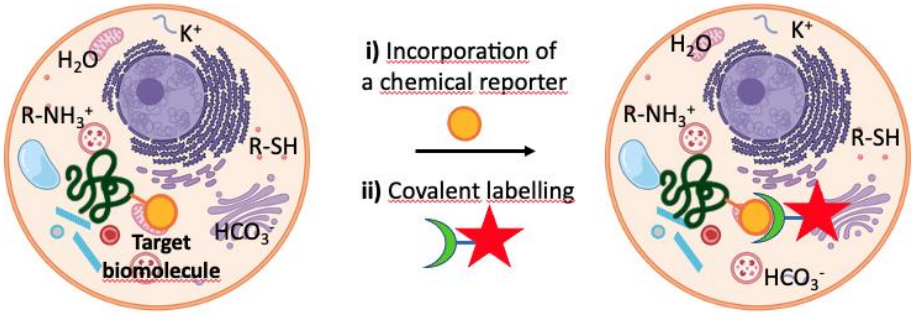

Figure 1.1: Bioorthogonal labelling strategy. In the first step, the chemical reporter (orange circle) is selectively introduced into the target biomolecule (green in the cell). In the second step, the exogenous probe (red star) covalently binds to the chemical reporter through a bioorthogonal reaction. Figure created with Biorender.com.

1.3 Main bioorthogonal reactions

The initial step in a classical bioorthogonal "click" labelling process, as illustrated in Figure 1.1, involves the incorporation of a chemical moiety called **bioorthogonal reporter** into the living system, ideally without inducing significant structural modifications to the target biomolecule. The main chemical groups that meet the requirements for bioorthogonality are ketones, aldehydes, azides, alkynes, and alkenes.[2] Among all these groups, azides have gained immense popularity thanks to their numerous advantages. First, they are truly abiotic groups (absent in virtually all biological systems). Their use therefore provides high selectivity, avoiding possible secondary reactions with nucleophiles abundant in living systems. Second, they are very small triatomic groups, and their incorporation into any type of biomolecule has no significant impact on its structure, properties, and/or functionality. In addition, azides have high metabolic stability under physiological conditions and are only capable of decomposing at temperatures above 120 °C. Finally, azides can act as 1,3 dipoles or as soft electrophiles, both chemical behaviours being very rare in nature.

The main strategy for introducing azide groups into biomolecules is via a process known as metabolic glycoengineering. Modified monosaccharide analogues (called **metabolic precursors**) are taken up by cells, recognized and tolerated by the biosynthetic enzyme machinery, and incorporated into glycans and glycoconjugates (proteins or lipids).[3] This strategy has two fundamental advantages. First, by adjusting the concentration of the

metabolic precursor, a high degree of control over the expression of azide groups in the membrane is achieved (the expression is dose-dependent).[4] Second, it is a universal method, applicable to virtually any cell line, as well as *in vivo* (it has been successfully applied in mice and zebrafish[5]).

The most widely used metabolic precursor is N-azidoacetyl tetraacetylated mannosamine (Ac_4ManNAz), which is deacetylated by intracellular esterases to enter the intracellular enzyme cascade that leads to the expression of glycans on the cell membrane.[6] The tetraacetylated form is preferred to the free form, as this reduces the polarity of the hydroxyl groups of N-acetyl mannosamine and enhances the cellular permeability of the compound.

The three types of bioorthogonal reactions involving azides are the **Staudinger ligation**, the **Cu-catalyzed azide-alkyne cycloaddition** (CuAAC), and the **strain-promoted azide-alkyne cycloaddition** (SPAAC).[2] Staudinger ligation, based on the reaction between azides and triarylphosphines, is in fact the first example of a bioorthogonal "click" reaction in living organisms, described by C. Bertozzi's group in 2000.[7] It involves a modification of the classic Staudinger reaction through the incorporation of an ester group in one of the aryl substituents, thereby conferring stability to the intermediate formed during the reaction and preventing its hydrolysis to give rise to a primary amine (Figure 1.2). In this first example, Bertozzi and her team demonstrated that incubating Jurkat cells (an immortalized T-lymphocyte cell line) with Ac_4ManNAz for the metabolic modification of membrane glycoproteins with azide groups and the subsequent reaction of the azide groups with triarylphosphine resulted in specific labelling of cell membranes. However, this reaction proved to be too slow ($k = 10^{-3}\ M^{-1}\ s^{-1}$) to be efficient *in vivo*; furthermore, it was discovered that phosphines easily underwent oxidation in biological media.[8]

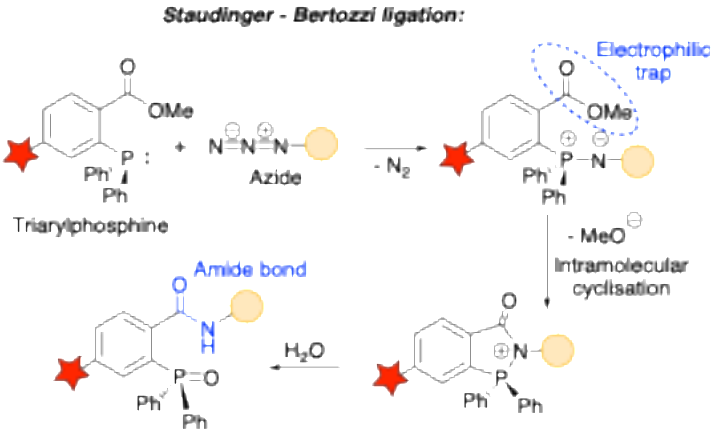

Figure 1.2: Staudinger-Bertozzi ligation.

At around the same time, the groups of K. Barry Sharpless in the USA and Morten Meldal in Denmark independently published a new example of a "click" reaction.[9,10] This is what is now known as the "crown jewel" of click chemistry, the Cu(I)-catalysed azide-alkyne [3+2] cycloaddition (CuAAC). This is yet another example of the modification of an old chemical reaction, the cycloaddition between azides and alkynes to form triazole rings, a reaction described by Rolf Huisgen in the second half of the 20th century. This reaction occurred at high temperatures and pressure, resulting in a mixture of two regioisomers: 1,4- and 1,5-disubstituted triazole adducts (Figure 1.3a). Sharpless and Meldal discovered that the addition of catalytic amounts of copper significantly improved the kinetics of the cycloaddition by approximately seven orders of magnitude, allowing regioselective formation of the 1,4-disubstituted triazole at room or physiological temperature and over a wide pH range (Figure 1.3b). It therefore seemed like an ideal bioorthogonal reaction between two small functional groups, potentially easy to incorporate into biomolecules of interest without altering them. However, the use of copper *in vitro* or *in vivo* can lead to cytotoxic effects resulting from the production of reactive oxygen species (ROS).[11]

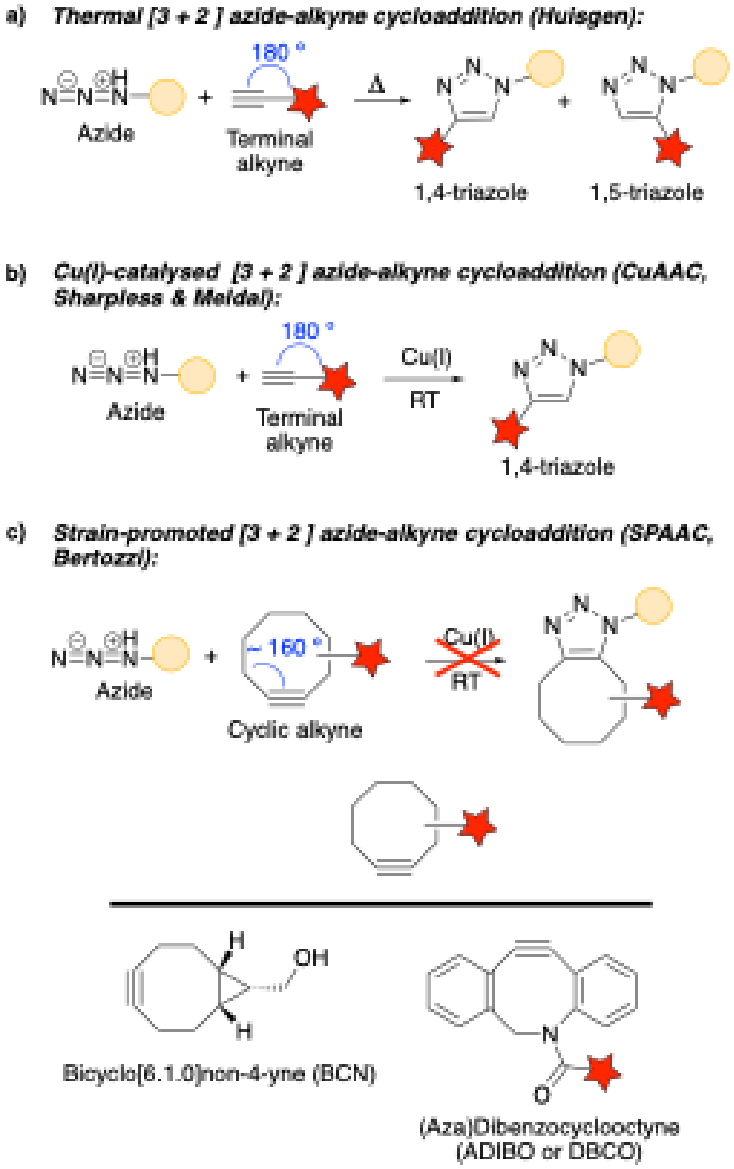

Figure 1.3: Thermal (a) and copper-catalysed (b) [3+2] azide-alkyne cycloaddition, CuAAC, and its bioorthogonal version, SPAAC, (c), including examples of strained alkynes (cyclooctyne derivatives).

This is where Carolyn Bertozzi's brilliance comes into play once again. Inspired by an old study by G. Wittig and A. Krebs describing the "explosive" reaction of cyclooctyne (the smallest stable cyclic alkyne) with phenylazide, Bertozzi developed an alternative [3+2] azide-alkyne cycloaddition that eliminates the need for the copper catalyst by using cyclic or strained alkynes instead of linear alkynes (Figure 1.3c). The triple bond located in an eight-membered ring confers a strain of approximately 18 kcal mol^{-1}, thus favouring a dramatic increase in reaction rate compared to linear alkynes. This new reaction modality was named "strain-promoted azide-alkyne [3+2] cycloaddition" (SPAAC).[12] Afterwards, several research groups focused their efforts on developing various families of strained

alkynes with greater reactivity. This search was motivated by the low reactivity of the first cyclooctynes reported by Bertozzi's group, whose reaction kinetics did not improve over the Staudinger reaction. This allowed kinetics up to 400 times faster to be achieved with biarylazacyclooctine derivatives, BARAC (k = 0.96 M^{-1} s^{-1}, Figure 1.4).[13,14]

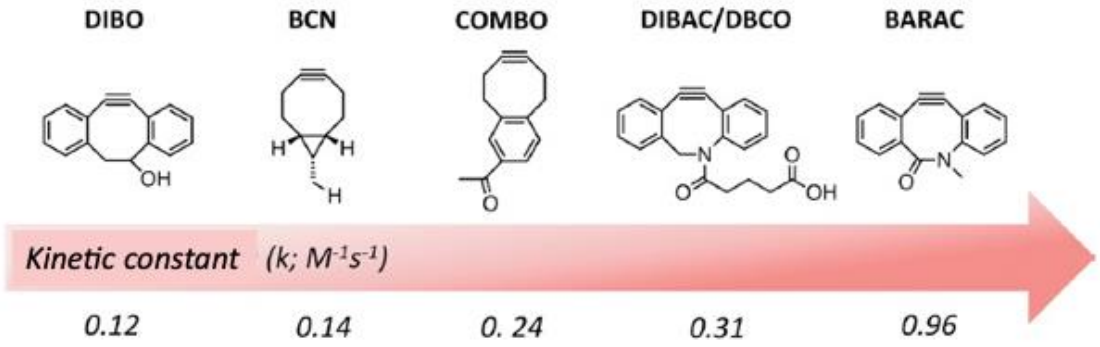

Figure 1.4: Reaction kinetics for different types of cyclooctyne derivatives. DIBO: dibenzocyclooctyne; BCN: bicyclononyne; COMBO: carboxymethylmonobenzocyclooctyne; DIBAC: dibenzoazacyclooctyne (also known as DBCO: dibenzocyclooctyne); BARAC: biarylazacyclooctyne. Adapted with permission from Rigolot, V.; Biot, C.; Lion, C. To View Your Biomolecule, Click inside the Cell. Angewandte Chemie International Edition ***2021****, 60, 23084–23105, Copyright (2021).*

In 2008, the groups led by J. M. Fox and S. A. Hilderbrand described almost simultaneously the **inverse electron demand Diels-Alder reaction** (IEDDA).[15,16] This reaction occurs between an electron-deficient diene, such as 1,2,4,5-tetrazine (Tz), and a dienophile alkene, such as trans-cyclooctene (TCO), norbornene (NB), cyclopropene (CP) or certain cyclooctynes such as bicyclononyne (BCN) or dibenzocyclooctyne (DBCO) (Figure 1.5a). It should be noted that IEDDA represents the fastest bioorthogonal 'click' reaction described to date, with kinetics of up to 10^6 M^{-1} s^{-1} (Figure 1.5b).[17] These kinetics ensure that the reaction can take place efficiently on biological timescales and at low concentrations, similar to those of many biomolecules in living systems.

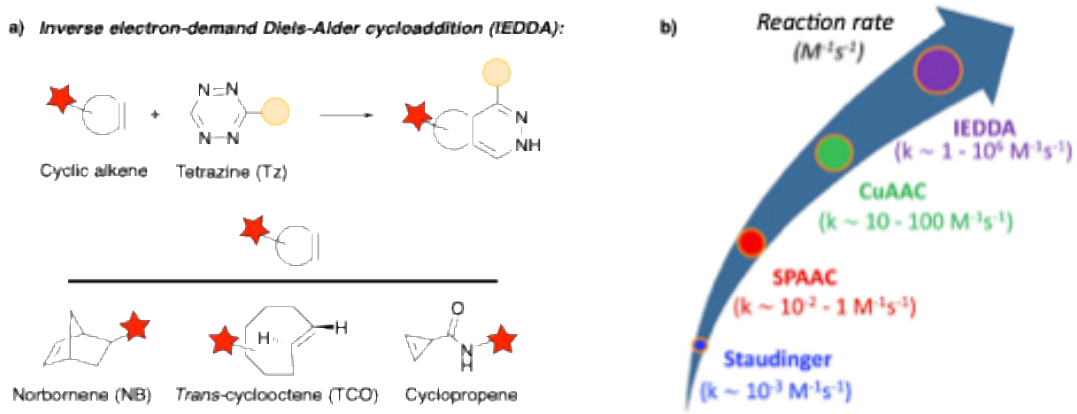

Figure 1.5: a) Inverse electron demand Diels–Alder reaction (IEDDA) and examples of cycloalkenes; b) Comparison of the reaction rates of different types of click reactions.

Finally, other alternative forms of bioorthogonal click chemistry have been described, although their use is less widespread than those mentioned above. This is the case, for example, with so-called 'photo-click' reactions, based on the use of an energy source in the form of photon beams that induce a higher energy state in the reactants involved, from which alternative chemical reaction pathways can effectively proceed.[18] Typical examples of this type of reaction are the ultraviolet (UV)-induced 1,3-dipolar cycloaddition between tetrazoles and alkenes (tetrazole-ene reaction) and the radical addition of thiols to alkenes (thiol-ene reaction).[18]

1.4 Bioorthogonal reactions *in vivo*

The first example of the application of bioorthogonal chemistry *in vivo* was reported in 2004, when Carolyn Bertozzi's group demonstrated that the Staudinger reaction could be carried out not only in cells, but also in extremely complex environments such as living organisms (in this case, mice).[19] However, as mentioned in the previous section, the Staudinger reaction does not have favourable kinetics for the in vivo visualisation of dynamic biological processes. The development of the SPAAC reaction has led to significant advances in this regard, enabling non-invasive imaging of glycans in live zebrafish[5] or *Caenorhabditis elegans*.[20] On the other hand, bioorthogonal chemistry approaches based on the IEDDA reaction has opened a new path for radioimmunotherapy of solid tumours through strategies of pre-targeting antibodies to tumours, followed by radioligand binding

(Figure 1.6).[21] Since its publication in 2010, this strategy has been widely used for diagnostic and therapeutic purposes in cancer.[17,22]

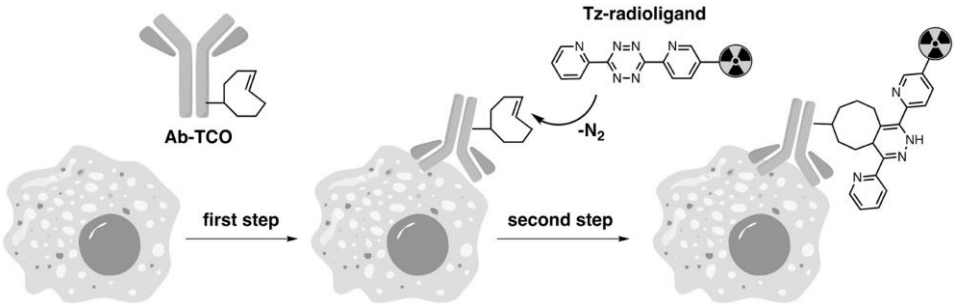

Figure 1.6: Pretargeting strategy based on the IEDDA reaction. TCO: trans-cyclooctene; Tz: tetrazine. Adapted with permission from Rossin, R.; Renart Verkerk, P.; van den Bosch, S. M.; Vulders, R. C. M.; Verel, I.; Lub, J.; Robillard, M. S. In Vivo Chemistry for Pretargeted Tumour Imaging in Live Mice. Angew Chem Int Ed ***2010****, 122, 3447–3450.*

The concept of bioorthogonal chemistry has also allowed scientists to explore the *in-situ* formation of biologically active molecules at their desired site of action,[23] or the selective activation of prodrugs (precursor compounds of drugs, but inactive) in the area to be treated, typically a tumour.[24,25] These experiments have been successfully carried out mainly in mice, although recently the American biotechnology company Shasqi[26] has started a clinical trial representing the first application of bioorthogonal chemistry in humans, using its CAPAC™ ("Click Activated Protodrugs Against Cancer") platform (Figure 1.7).[27] The clinical trial is in phase 2, in which safety and tolerability are being evaluated, as well as the treatment's ability to reduce tumour size in patients with soft tissue sarcoma and head and neck cancer.

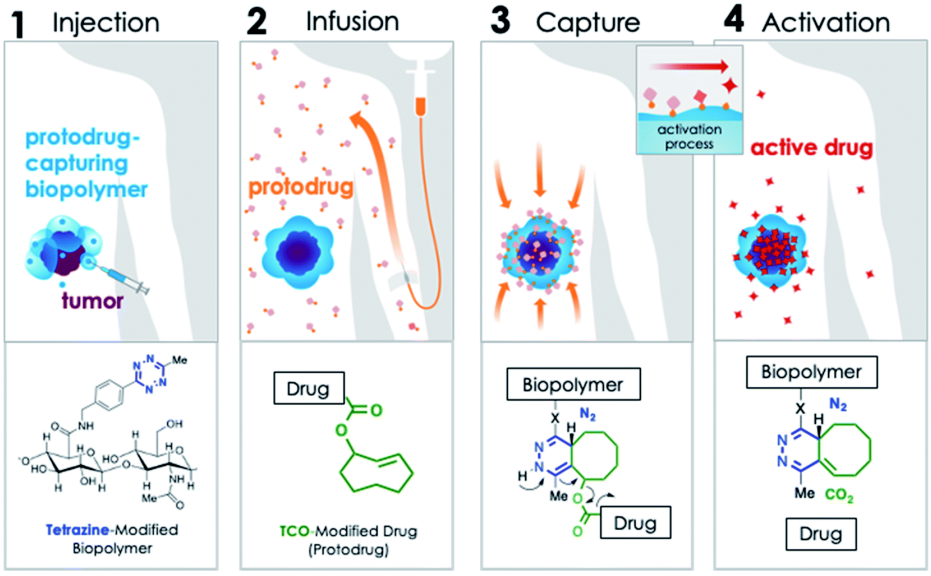

Figure 1.7: Mechanism of CAPAC platform: (1) Tz-modified biopolymer is locally injected at the pathological site. (2) A TCO-modified drug (protodrug) is infused systemically. (3) The protodrug is captured by the biopolymer at the desired site through a rapid covalent reaction between Tz and TCO moieties, followed by (4) chemical rearrangement to release active drug. Reproduced from Wu, K., Yee, N. A., Srinivasan, S., Mahmoodi, A., Zakharian, M., Mejia Oneto, J. M. and Royzen, M., Chem. Sci., ***2021****,12, 1259-1271.*

1.5 Bioorthogonal chemistry and nanomedicine: new applications in diagnosis and therapy

The great potential offered by bioorthogonal chemistry is immense. But over the last two decades, its combination with nanotechnology has led to an exponential growth in the number of publications related to the biomedical applications of this unique approach.[2] This combination allows for new or improved solutions to pressing problems related to diagnosis and therapy, such as sensitivity (in the case of imaging and detection), tumour accumulation of nanoparticles (NPs), optimisation of therapeutic efficacy, etc.

As discussed in the "Introduction to Nanobiomedicine" topic, the origins of nanotechnology date back to 1959, when Richard Feynman postulated the first concepts in nanoscience and nanotechnology in his famous lecture 'There's Plenty of Room at the Bottom'.[28] Feynman, without actually using the term "*nano*", established the implications of manipulating and controlling things on a very small scale. However, it was not until 1974 that the term "*nanotechnology*" was coined by Norio Taniguchi, and its definition established as 'the manipulation of matter with at least one dimension between 1 and 100 nanometres' by the NNI (National Nanotechnology Initiative of the United States), considering the nanometre (nm) as a unit of measurement in the international system representing 10^{-9} metres. The real interest in nanotechnology lies in the fact that, at these dimensions, the properties of materials vary significantly compared to those in their macroscopic state. These new properties emerge because of two main phenomena. The first is the increase in the surface-to-volume ratio, which leads to an increase in the number of atoms on the surface compared to those present in the core of the nanomaterial, thus favouring their reactivity and/or catalytic capacity. The second is the quantum effect, which consists of a modification of the electronic structure, with electrons occupying different energy levels.[29] All of this means that nanomaterials are capable of harbouring very interesting specific properties,

including chemical, electrical, mechanical and optical properties, and that at the same time these can be predefined based on modifications to their shape or size.

On the other hand, considering that most relevant biomolecules and biological structures are found on the nanometric scale, the interaction of nanomaterials with biological entities can increase dramatically in terms of selectivity and efficiency.[30] This has led to the connection between nanomaterials and medicine or biotechnology giving rise to some of the most promising applications of nanotechnology in the 21st century, to the extent that new fields of knowledge such as nanomedicine and bionanotechnology have emerged. In this context, applications such as diagnosis using analytical or imaging techniques, controlled drug delivery, tissue regeneration, and in vivo cell tracking, among others, are being studied. It should be noted that today there is a wide variety of nanomaterials, with gold nanoparticles, polymeric nanoparticles, liposomes, and magnetic nanoparticles being the most widely used in nanobiomedicine.

Certain types of NPs offer significant advantages over conventional methods used to monitor biological processes at the cellular and molecular level, which are based on the use of organic fluorescent probes or fluorescent proteins.[31] For example, the use of different types of NPs in combination with bioorthogonal 'click' chemistry (e.g., quantum dots,[32] nanodiamonds,[33] gold NPs,[34] etc.) has been described for applications in bioimaging, demonstrating improvements in terms of sensitivity, specificity, and temporal stability.

The use of nanotechnology also enables the development of multimodal imaging probes, in which several types of nanoparticles are combined. An example is the platform developed by Lee *et al.*,[35] who prepared polymeric glycol chitosan nanoparticles functionalised with a strained alkyne (BCN) and encapsulating different types of imaging agents (cyanine Cy5. 5 for fluorescence, magnetic iron oxide nanoparticles for magnetic resonance imaging (MRI), and gold nanoparticles for computed tomography imaging, see Figure 1.8). The authors demonstrated the binding of these multimodal nanoparticles using SPAAC to mesenchymal stem cells derived from adipose tissue pretreated with Ac_4ManNAz for the expression of azide groups on the membrane. This strategy allowed the stem cells to be tracked using different imaging techniques for at least 15 days after subcutaneous injection into nude mouse models, whereas without the use of bioorthogonal chemistry, the time window for tracking was reduced to 5 days.

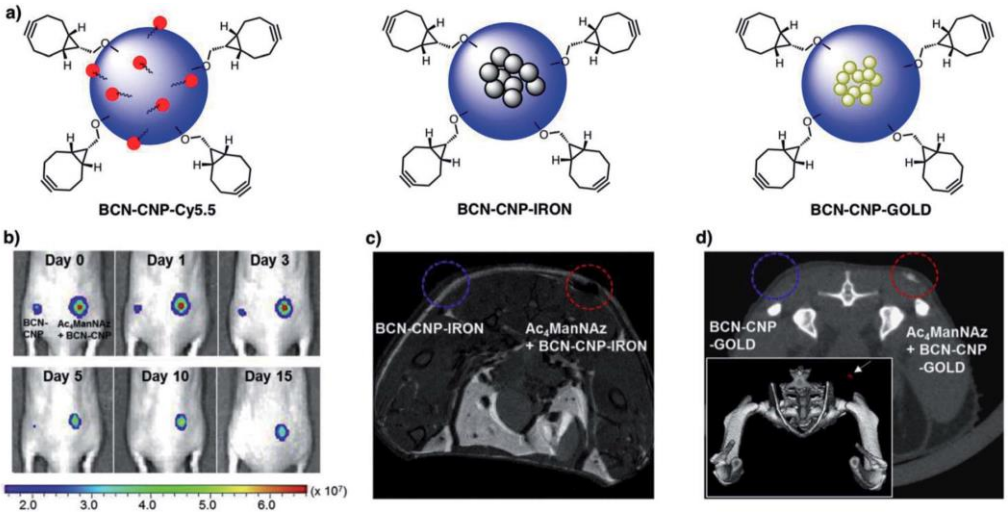

Figure 1.8: BCN-modified glycol chitosan nanoparticles for cell stem labelling and imaging. Top: Schematic structures (a). Bottom: NIRF (b), T2-weighted MRI (c) and micro-CT (d) imaging of mice after transplantation of BCN–CNP-Cy5.5/IRON/GOLD labelled stem cells with and without Ac_4ManNAz pre-treatment. Adapted from Biomaterials, 139, S. Lee et al., "In vivo stem cell tracking with imageable nanoparticles that bind bioorthogonal chemical receptors on the stem cell surface", 12–29, Copyright (2017), with permission from Elsevier.

In the field of diagnostics, the detection of biomolecules remains a challenge, especially when dealing with low concentrations of analytes or complex samples. In this context, the pre-targeting strategy mentioned above can be successfully applied for the development of more sensitive imaging techniques. A relevant example in this field is the strategy developed by Weissleder's group based on the reaction between fluorescent MNPs and antibodies using the IEDDA reaction.[36] This strategy has two variants: the first consists of conjugating the antibody and the MNP before incubation with the cells, while in the second, the cells are initially incubated with the antibody modified with trans-cyclooctene, which acts as a scaffold for multiple covalent bonds of the tetrazine-modified MNPs, thus allowing amplification of the detection signal. This strategy has been used in studies related to the recognition of specific biomarkers in tumour cells[37] and even the detection of pathogenic bacteria.[38]

The use of bioorthogonal click chemistry in combination with NPs has also represented a huge advance in terms of its therapeutic application against cancer through the specific binding of NPs to tumour cells. In this regard, conventional strategies based on the functionalisation of NPs with specific ligands (antibodies, peptides, carbohydrates, etc.) that recognise receptors present on the surface of tumour cells suffer from limitations

related to cell heterogeneity or the limited availability of these receptors. On the other hand, click chemistry in combination with methods capable of introducing artificial receptors into the tumour cell membrane in a controlled manner and independently of the cell type (e.g., through metabolic glycoengineering, as described above) makes it possible to overcome these limitations and better address the vectorisation of NPs to target cells.[2,39] The concept of universal tumour cell labelling using metabolic glycoengineering is described in Figure 1.9.

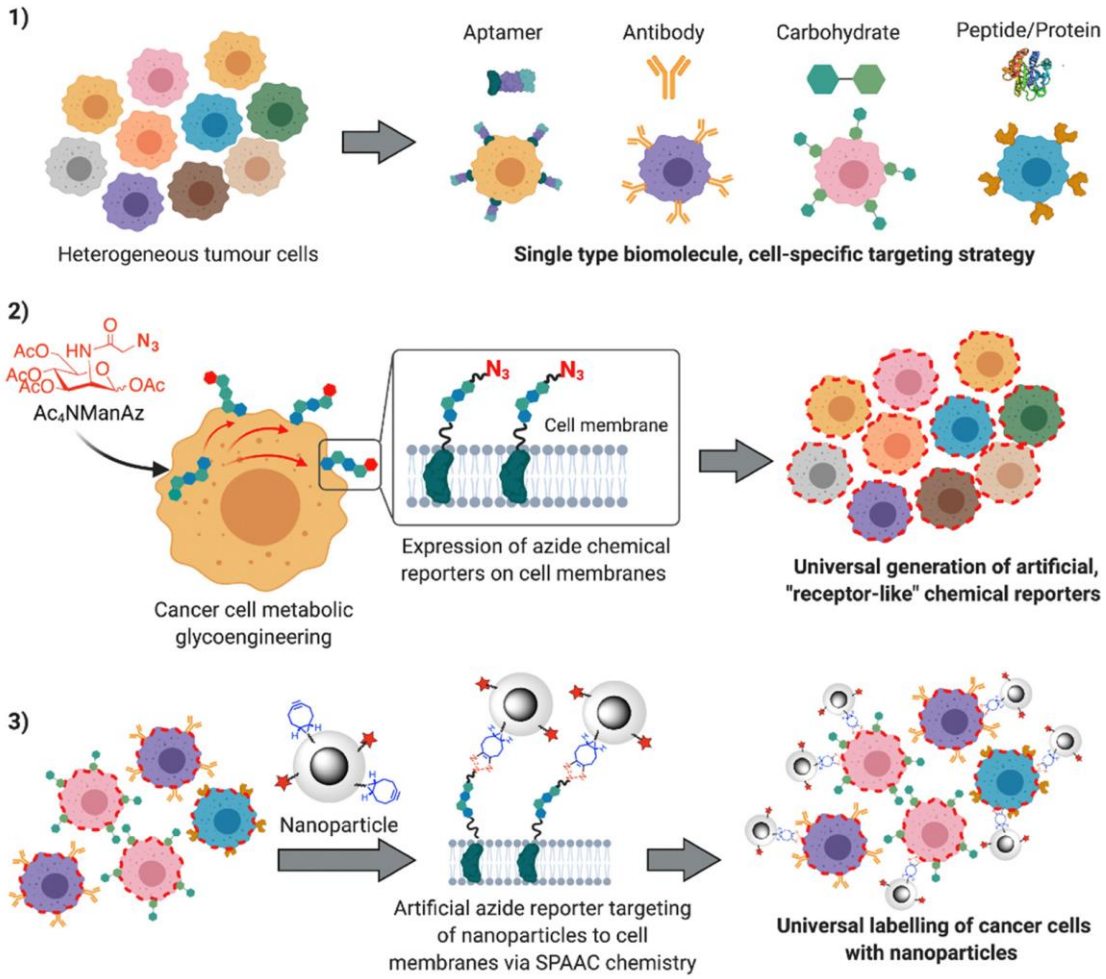

Figure 1.9. Universal labelling of cancer cells using the metabolic glycoengineering approach vs. specific labelling using biological receptors. Reproduced from Idiago-López, J., Moreno-Antolín, E., de la Fuente, J. M. and Fratila, R. M., Nanoscale Adv., 2021, 3, 1261–1292.

The first example of the use of bioorthogonal 'click' chemistry for the specific vectorisation of tumour cells with NPs was described by K. Kim's group in 2012.[40] Using Ac_4ManNAz as a metabolic precursor of azides in the cell membrane, they evaluated the binding of DBCO-functionalised liposomes through the SPAAC reaction, observing preferential binding

compared to the control liposomes without DBCO. Furthermore, this effect was observed in several types of tumour cells: human lung carcinoma (A549), human glioblastoma (U87MG), human nasopharyngeal carcinoma (KB) and human breast adenocarcinoma (MCF7). They also confirmed the potential in vivo application in mice with tumours generated by implantation of A549 cells. To do this, they injected Ac_4ManNAz intratumorally and then administered the functionalised liposomes intravenously. In this way, a preferential accumulation of liposomes in the tumour was observed compared to controls injected with saline buffer instead of Ac_4ManNAz. Furthermore, it was established that the effect was dependent on the concentration of Ac_4ManNAz injected. Since then, this strategy and/or the use of antibodies functionalised with different bioorthogonal groups as click-binding receptors for NPs have been employed on numerous occasions and with different types of NPs, not only for specific vectorisation, but also in combination with photodynamic therapy or controlled drug release applications.[41–43]

Finally, beyond the specific biomedical applications discussed above, due to their great versatility, click chemistry and bioorthogonal chemistry have also been widely used for other purposes, such as the functionalisation of different types of NPs with antibodies, aptamers, peptides, chemotherapeutic drugs or imaging agents.[44] But the possible applications are virtually endless, and the only limit is our imagination. For instance, in our laboratory we are working on a line of research based on the immobilisation of magnetic nanoparticles on cell membranes through bioorthogonal chemistry to study and modulate the fluidity and permeability of the cell membrane and promote intracellular delivery (Figure 1.10). We successfully used this strategy based on bioorthogonal chemistry and nanotechnology for intracellular delivery of cell-impermeant fluorescent probes or for transfection of siRNA into established and dendritic cells.[45,46] Magnetic nanoparticles were immobilised on cell membranes by SPAAC, then cells were exposed to alternating magnetic fields, which caused a localised heating of the membrane, without affecting cell viability (sublethal magnetic hyperthermia, for more details, see section 3 on nanoparticle hyperthermia applications). This localised heating triggered fluidity changes in the plasma membrane, allowing the internalisation of molecules that otherwise cannot cross the membrane.

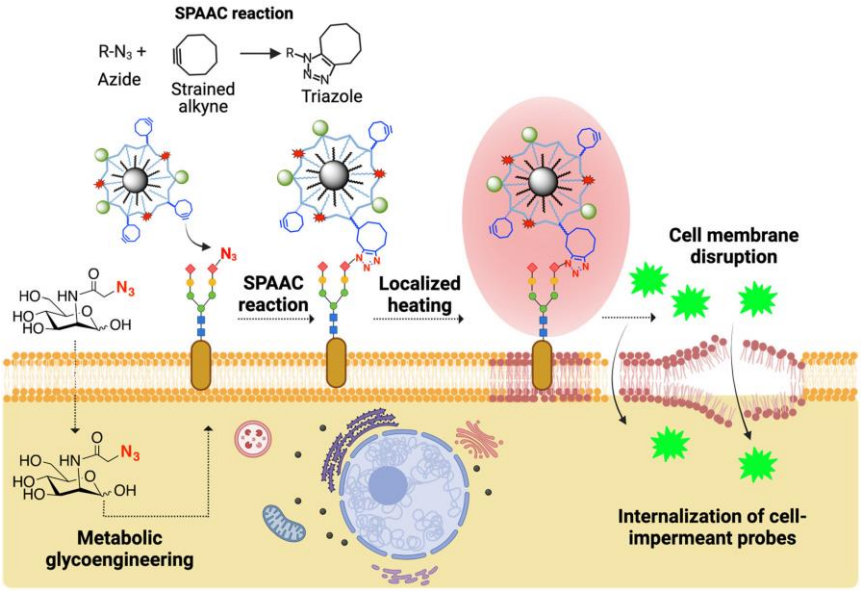

Figure 1.10. Overview of the general concept of MH-mediated intracellular delivery using MNPs immobilized on the cell membrane via SPAAC bioorthogonal chemistry. The MNPs are functionalized with strained alkynes (in blue) and attached to the membrane of cells previously subjected to metabolic glycoengineering to express unnatural azide bioorthogonal reporters (in red). Reproduced from Idiago-López, J., Ferreira, D., Asín, L., Moros, M., Armenia, I., Grazú, V., Fernandes, A. R., de la Fuente, J. M., Baptista, P. V. and Fratila, R. M., Nanoscale, ***2024****, 16, 15176–15195.*

1.6 Conclusions

Bioorthogonal chemistry has initiated a revolution in several fields, including chemistry, biology, and biomedicine. Furthermore, its combination with nanotechnology has led to developments that were unthinkable before its emergence. However, the field of bioorthogonal chemistry is not without limitations, so new developments are likely to occur in the coming years, especially in certain specific areas.

On the one hand, chemists will continue to focus their efforts on developing new bioorthogonal reactions or improving the reaction kinetics of existing ones and/or the stability of the reagents used, especially for their *in vivo* applications. On the other hand, if bioorthogonal chemistry is to be used to visualise small (bio)molecules, such as metabolites or drugs, it is necessary to use very small bioorthogonal probes that cause minimal disturbance to the system under study. We have indicated above that azide is an ideal bioorthogonal probe, but new molecules with characteristics suitable for acting as bioorthogonal probes have also been described, for example cyclopropenes.[47]

There has also been growing interest in the development of bioorthogonal reactions that are orthogonal to each other, thus allowing the tandem use of two or more bioorthogonal reactions. Finally, bioorthogonal chemistry can also be used for the controlled release of molecules, a concept known as 'click-to-release'. This concept uses a bioorthogonal group to mask a functional group, for example an alcohol or an amine, which is released after the reaction of the bioorthogonal group with its respective partner.

Finally, in the field of bioorthogonal chemistry in combination with nanotechnology, the main challenges to be addressed are related to obtaining functional nanoparticles.[23,47] On the one hand, functionalisation with bioorthogonal probes must not compromise the colloidal stability of nanoparticles in aqueous media, which is not trivial if the bioorthogonal probe is hydrophobic. On the other hand, functionalisation must be carried out in such a way that the bioorthogonal group remains accessible for reaction. This can be difficult to achieve if the nanoparticle is also functionalised with some type of larger stabilising agent (e.g., polyethylene glycol) necessary for its use *in vivo*.[47]

1.7 Further recommended reading

Note: the most important articles are highlighted by ***

1) Nat. Chem. Biol., **2005**, 1, 13-21 - Chemistry in living systems; https://doi.org/10.1038/nchembio0605-13 ***

2) Chem. Soc. Rev., **2010**, 39, 1272-1279 - Cu-free click cycloaddition reactions in chemical biology; https://doi.org/10.1039/B901970G

3) Acc. Chem. Res., **2011**, 44, 666-676 - From Mechanism to Mouse - A Tale of Two Bioorthogonal Reactions; https://doi.org/10.1021/ar200148z ***

4) Acc. Chem. Res., **2011**, 44, 805-815 - Bioconjugation with Strained Alkenes and Alkynes; https://doi.org/10.1021/ar200059z

5) Acc. Chem. Res., **2011**, 44, 816-827 - Biomedical Applications of Tetrazine Cycloadditions; https://doi.org/10.1021/ar200037t

6) Acc. Chem. Res. **2018**, 51, 1073-1081 - Constructing New Bioorthogonal Reagents and Reactions; https://doi.org/10.1021/acs.accounts.7b00606

7) Biomaterials, **2017**, 138, 57-68 - A bioorthogonal nanosystem for imaging and *in vivo* tumor inhibition; https://doi.org/10.1016/j.biomaterials.2017.05.036

8) Biomaterials, 148, **2017**, 1-15 - Nano-sized metabolic precursors for heterogeneous tumor-targeting strategy using bioorthogonal click chemistry in vivo; https://doi.org/10.1016/j.biomaterials.2017.09.025

9) Chem. Sci., **2020**, 11, 2155 - Coordinating bioorthogonal reactions with two tumor-microenvironment-responsive nanovehicles for spatiotemporally controlled prodrug activation; https://doi.org/10.1039/C9SC05036A ***

10) Mol. Pharmaceutics, **2017**, 14, 1558-1570 - Artificial Chemical Reporter Targeting Strategy Using Bioorthogonal Click Reaction for Improving Active-Targeting; https://doi.org/10.1021/acs.molpharmaceut.6b01083 ***

11) Nanoscale Adv., **2021**, 3, 1261-1292 - Nanoparticles and bioorthogonal chemistry joining forces for improved biomedical applications; https://doi.org/10.1039/D0NA00873G ***

12) Chem. Rev., **2022**, 122, 340–384 - Click and Bioorthogonal Chemistry: The Future of Active Targeting of Nanoparticles for Nanomedicines?; https://doi.org/10.1021/acs.chemrev.1c00484

13) Nanoscale, **2024**, 16, 15176-15195 - Membrane-localized magnetic hyperthermia promotes intracellular delivery of cell-impermeant probes; https://doi.org/10.1039/D4NR01955E

14) Cancer Immunol. Immunother., **2025**, 74, 292 - Heat up, silence on: *IDO1* gene silencing in THP-1-derived dendritic cells triggered by magnetic hyperthermia; https://doi.org/10.1007/s00262-025-04148-3

15) Biomater. Sci., **2025**,13, 2297-2319 - Harnessing nanoparticles and bioorthogonal chemistries for improving precision of nuclear medicine; https://doi.org/10.1039/D4BM01387E ***

16) Topics in Current Chemistry, **2025**, 383, 12 - Toward Realization of Bioorthogonal Chemistry in the Clinic; https://doi.org/10.1007/s41061-025-00495-y ***

2. Nanomaterials for tissue engineering and regeneration

This lecture offers an overview of the use of nanomaterials for tissue engineering and tissue regeneration. The students will become familiar with the different nanomaterials used for tissue engineering applications and their mechanisms of action. Selected examples of application to different types of tissues will be reviewed and discussed.

2.1 Introduction

Tissue regeneration is one of the most important areas of regenerative medicine, aiming to restore or replace damaged tissues and organs. Traditional methods such as grafts and transplants often face limitations including donor shortages, immune rejection, and poor integration. Nanotechnology, through the development of nanomaterials, can offer novel solutions for tissue engineering, by mimicking the natural extracellular matrix (ECM), enhancing cellular interactions, and enabling controlled delivery of bioactive molecules through smart drug delivery systems.

In tissue engineering, various biomaterial (for example, polymers, ceramics, and inorganic compounds) are used alone or combined with bioactive molecules and cells to trigger differentiation signals and support tissue regeneration at sites of injury or damage. The nanomaterials used in the field of tissue engineering should fulfil the following essential criteria: biodegradability, biocompatibility, biointegration, ease of manufacturing and handling, and cost-effective production.

2.2 Nanomaterials used in tissue engineering

A wide variety of nanomaterials can be used for tissue engineering and regeneration applications (Figure 2.1), including:

- **Nanoparticles**: metallic (e.g., gold, silver, metal oxide), polymeric (e.g., synthetic polymers such as polylactic acid - PLA, polyamide, PLA–glycolic acid copolymer - PLGA), and ceramic (e.g., hydroxyapatite) nanoparticles. These nanoparticles are typically employed for drug delivery, imaging, and enhancing scaffold properties; some NPs also possess antimicrobial properties.

- **Nanofibers**: electrospun nanofibers made from polymers (e.g., chitosan, collagen, polycaprolactone), mimicking the fibrous nature of the ECM. They support cell adhesion and proliferation and can serve as drug delivery systems; the fabrication process is compatible with the incorporation of nanoparticles.
- **Carbon-based nanomaterials**: graphene, graphene oxide, and carbon nanotubes (CNTs) offer excellent mechanical strength and electrical conductivity and are used especially in neural and cardiac tissue engineering.
- **Nanocomposites**: combine different nanomaterials to create multifunctional scaffolds with improved mechanical, biological, and chemical properties.
- **Nanocrystalline cellulose (NCC)**: represents a renewable and biodegradable material with high mechanical strength and biocompatibility, used in skin, bone, and cartilage regeneration.

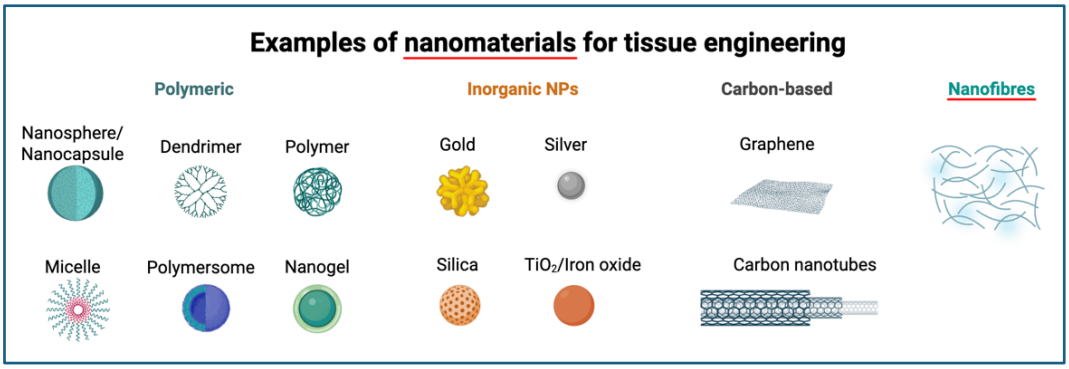

Figure 2.1. Examples of nanomaterials used in tissue engineering. Figure created with Biorender.com

Polymeric nanoparticles (PNPs) are versatile platforms in nanomedicine and tissue engineering due to their unique physicochemical properties and functional adaptability.[48] Their high surface area, tuneable characteristics, low cytotoxicity, and excellent biocompatibility make them ideal candidates for controlled drug delivery systems. Moreover, PNPs can preserve the bioactivity of therapeutic agents by protecting them from enzymatic degradation and thus improving pharmacokinetics and therapeutic efficacy. The performance of PNPs is strongly influenced by their composition, molecular weight, polydispersity, and structural architecture. Common PNP architectures used in tissue engineering include nanospheres, nanogels, polymersomes, micelles, dendrimers, and nanocapsules.

Among **synthetic polymers**, polyethylene glycol (PEG) is widely employed for its biocompatibility, non-immunogenicity, and ability to reduce protein adsorption and renal clearance.[49,50] PEGylation creates a hydration layer around nanoparticles, minimizing nonspecific protein binding, prolonging circulation time, and reducing immune recognition. These properties collectively enhance drug stability and bioavailability. PEG-based modifications have also been extensively applied to improve the pharmacokinetics of nanoformulations. Synthetic polymers such as polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone, and polyanhydrides are also widely used in tissue engineering applications due to their biodegradability and tuneable properties. For instance, PLGA-based systems can encapsulate both hydrophilic and hydrophobic drugs and are extensively applied in tissue engineering.[48] Functionalization strategies, such as incorporating bioactive glass or other polymers, further enhance mechanical strength, hydrophilicity, and biological performance.

Natural polymers, including hyaluronic acid, alginate and chitosan, have been also explored for the delivery of oligonucleotides, DNA, proteins, and small-molecule drugs for tissue engineering and regeneration purposes. Further conjugation with polysaccharides or proteins is often used to improve drug stability and biodistribution. Collagen is another natural polymer that serves as an excellent carrier for drug delivery and tissue engineering applications, including myocardial regeneration and dermal repair in burns and other types of wounds.[48]

Inorganic nanoparticles used in tissue engineering include noble metal (Au, Ag), metal oxide and silica-based NPs. Some of their properties include antibacterial and catalytic activity, mechanical strength, electrical conductivity and responsiveness to external stimuli. These characteristics make them particularly suitable for applications in tissue engineering. Gold nanoparticles (AuNPs) show excellent biocompatibility, ease of synthesis and facile surface modification with thiolated molecules; for these reasons, they have been extensively used in various tissue engineering applications like heart, skin, bone, and neural tissues. They can promote cell differentiation, proliferation, and electrical signal transmission.[51] Silver nanoparticles (AgNPs) are also frequently used in tissue engineering, due to their strong antimicrobial activity.[52] TiO_2 NPs can enhance composite properties by providing bending strength and Young's modulus comparable to those of natural bone,

along with excellent bioactivity, making them widely used in bone tissue engineering as advanced implant materials.[53] Superparamagnetic iron oxide nanoparticles (SPIONs) are widely utilized in biomedicine due to their unique magnetic properties and biocompatibility. These nanoparticles, typically composed of maghemite (γ-Fe_2O_3), magnetite (Fe_3O_4), or ferrites (MFe_2O_4, where M is Mn, Co, Ni, Zn), possess core sizes ranging from 10 to 100 nm. Under an external magnetic field, SPIONs exhibit high magnetisation; however, they do not show residual magnetisation once the field is removed, a phenomenon known as superparamagnetism.[54] This behaviour is critical for preventing particle aggregation, ensuring safety, and enabling precise control in applications such as targeted drug delivery or magnetic resonance imaging (MRI). Moreover, these NPs can generate heat under the application of alternating magnetic fields (process known as magnetic hyperthermia), which allows controlled drug release from thermoresponsive matrices.[55]

Carbon-based nanomaterials can be obtained with different forms and sizes, including nanosheets, nanospheres, nanotubes, nanorods, and nanodots, and are widely applied in multiple fields of biomedicine. In the context of tissue engineering applications carbon nanotubes (CNTs), graphene and graphene oxide (GO) are extensively utilized due to their unique physicochemical properties (high mechanical strength, conductivity, optical responsiveness, etc.). GO consists of a single layer of carbon atoms arranged in a hexagonal lattice and possesses exceptional optical and electronic characteristics, as well as versatility in functionalization.[56] Graphene is also a single layer of sp^2-hybridized carbon atoms arranged in a two-dimensional honeycomb lattice, which imparts a distinctive electronic band structure combining metallic and semiconducting characteristics. Graphene has a high specific surface area, low weight, flexibility, and exceptional mechanical strength due to covalent C-C bonds. This combination of physicochemical properties makes graphene an excellent candidate for interfaces designed to record, modulate, and regenerate delicate neural tissues, including the spinal cord.[57]

Nanocomposites are hybrid materials composed of at least two distinct components, typically combining polymers with fillers or inorganic phases, where at least one component exhibits nanoscale dimensions (≤100 nm). Compared to their individual constituents, nanocomposites usually have superior mechanical properties, enhanced biodegradability, and improved dimensional stability, due to the synergistic integration of organic and

inorganic elements. Their performance can be further tailored by incorporating functional nanomaterials. For instance, as mentioned above, graphene oxide offers exceptional flexibility, biocompatibility, antibacterial activity, high surface area, and facile functionalization, making it highly attractive for tissue engineering. Similarly, hydroxyapatite (HAP) is widely employed in bone tissue engineering due to its osteoconductivity, while gold nanoparticles (AuNPs) can promote apatite formation, further supporting bone regeneration.[48]

The synthesis of the different types of nanomaterials for tissue engineering is extensively covered in different review articles.[58–60] During this workshop, we will discuss several methods for the synthesis of plasmonic (Au and Ag) and magnetic nanoparticles, polymeric nanoparticles, and carbon-based nanomaterials (carbon nanotubes and graphene), together with techniques for their characterization: transmission and scanning electron microscopy (TEM and SEM), X-Ray Diffraction (XRD), Atomic Force Microscopy (AFM), ultraviolet-visible (UV-Vis) and Fourier-Transformed Infrared (FT-IR) spectroscopy, Dynamic Light Scattering (DLS), etc.

Special emphasis will be placed on **electrospinning** for the fabrication of nanofibres, as it represents one of the most employed techniques and offers unique advantages - versatility with a wide range of materials, scalability for continuous production, and ability to create fibres with the desired properties (such as high surface area and tuneable porosity, or structure similar to the extracellular matrix). The method is relatively low-cost, easy to set up, and produces continuous fibres with high mechanical strength.[61] Moreover, it is possible to incorporate nanoparticles, drugs, or bioactive molecules into electrospun fibres, or to create hybrid nanofibers (Figure 2.2).

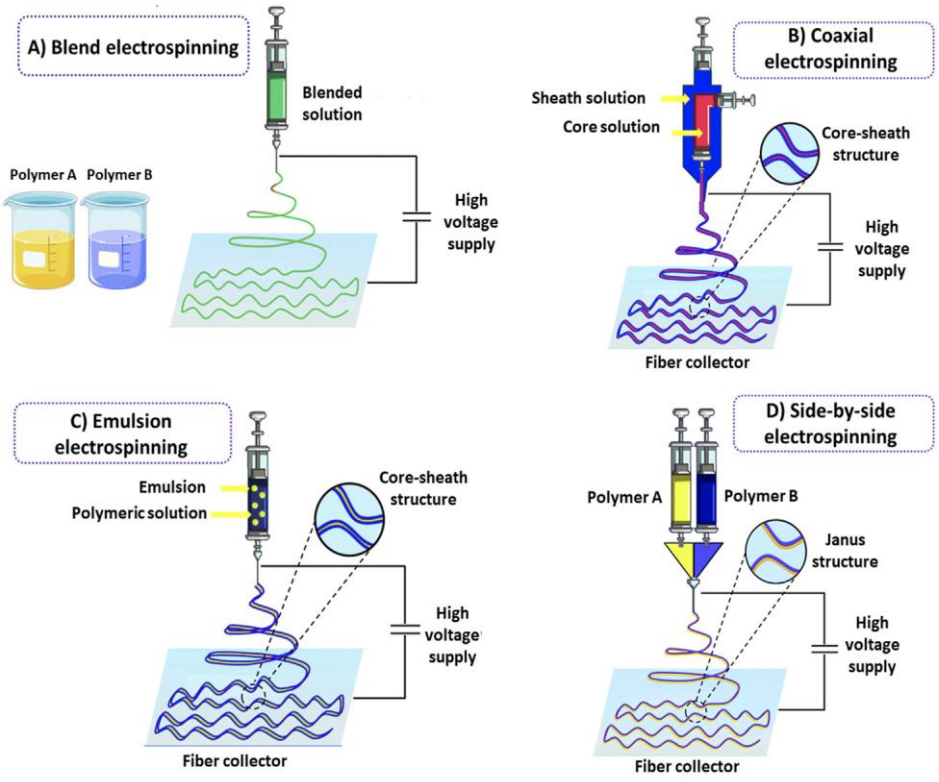

Figure 2.2. Conventional electrospinning methods for hybrid nanofibre fabrication. Reproduced from Abadi B., Goshtasbi N., Bolourian S., Tahsili J., Adeli-Sardou M. and Forootanfar H (2022), Electrospun hybrid nanofibers: Fabrication, characterization, and biomedical applications. Front. Bioeng. Biotechnol. 10:986975, 2022.

In electrospinning, a polymer solution is loaded into a syringe equipped with a metallic needle (a spinneret), and a high voltage is applied between the needle and a grounded collector. The electric field induces charge accumulation on the liquid surface, overcoming surface tension and forming a fine jet that undergoes rapid stretching and thinning as it travels toward the collector. During flight, the solvent evaporates (or the melt solidifies), resulting in the deposition of solid fibres that assemble into a nonwoven mat. In the context of tissue engineering, electrospinning is essential for fabricating 3D scaffolds. Conventional electrospinning employs a charged nozzle containing a polymer solution positioned at a fixed distance from a static collector, resulting in the formation of 2D mats. These mats consist of densely packed nanofibres with surface pores only, limiting scaffold thickness and hindering cell infiltration and nutrient diffusion. To overcome these limitations, researchers have advanced electrospinning techniques to produce 3D nanofibre scaffolds that better replicate the architecture and morphology of the extracellular matrix. The major

advantages of 3D electrospinning include precise control over fibre morphology, size, and scaffold porosity, enabling improved biological performance.

Beyond biochemical signals, the topographical features of electrospun nanofibrous scaffolds are equally critical for creating a microenvironment that supports cell activity during regeneration. By carefully selecting materials and processing parameters, electrospinning offers excellent flexibility in tailoring the shape, size, morphology, and structure of micro- and nanostructures. This capability is particularly advantageous for drug delivery systems, especially for the controlled release of bioactive proteins. Recent trends emphasize the integration of biomimetic scaffolds with growth factors to develop 'smart' bioactive systems, where precise engineering of scaffold morphology, porosity, and composition enables fine-tuning of release profiles.[62]

While electrospinning provides a high surface-to-volume ratio, it lacks the ability to produce well-defined 3D structures with suitable mechanical properties. Conversely, 3D printing enables the fabrication of complex 3D designs with great flexibility but only reaches micron-scale resolution. The combined use of electrospinning and 3D printing can therefore address these shortcomings and enable the fabrication of a new class of materials with superior structural and functional characteristics.

2.3 Applications of nanomaterials in tissue engineering

Nanomaterials have been successfully used to date in different applications for regenerating various tissues, for example: **bone tissue** (nanohydroxyapatite and 2D nanomaterials integrated into 3D-printed scaffolds to enhance osteoconductivity and mechanical strength), **cartilage** (scaffolds based on nanofibers to support chondrocyte growth and ECM production), **neural tissue** (CNTs and graphene-based scaffolds to enable neural cell adhesion and signal transmission), **skin** (nanofibers and NCC-based hydrogels to accelerate wound healing, prevent or combat infections and reduce scarring), **cardiac tissue** (nanomaterials can be used for creating vascular grafts and heart valve replacements). Moreover, bioengineered microneedles can be integrated with nanotherapeutics to enable localized, controlled drug delivery and real-time monitoring of the treatment (microneedle platforms).

Nanomaterials contribute to tissue regeneration through several mechanisms. They can mimic the ECM, by replicating its topography and biochemical and mechanical cues. They can also influence cell behaviour including adhesion, proliferation, differentiation, and migration. Nanoparticles can encapsulate and release growth factors, genes, or drugs in a spatiotemporal manner, (controlled delivery platforms). Finally, conductive nanomaterials facilitate electrical stimulation for neural and cardiac tissue regeneration, creating bioelectronic interfaces.

Bone tissue engineering

In bone tissue engineering, scaffolds are widely employed to provide mechanical support to damaged areas and create an environment that promotes bone regeneration. These scaffolds should therefore have suitable mechanical strength and surface properties that favour cell adhesion, proliferation, and differentiation. Additionally, to ensure optimal performance, materials used for bone tissue engineering should have suitable porosity, osteoconductivity, biocompatibility, and be bioresorbable.

Hydroxyapatite (HAp) is the main natural inorganic component of bone; therefore, HAp-based nanomaterials have been extensively studied for bone regeneration.[63] Often, HAp is combined with other nano- and biomaterials, such as chitosan, PEG, PLGA, collagen, etc.[48] For instance, Liu *et al.*[64] reported a biomimetic nanocomposite nanofibrous scaffold of hydroxyapatite/chitosan (nHAp/CTS) seeded with bone marrow mesenchymal stem cells (BMSCs) and evaluated its potential for bone regeneration. The scaffolds based on nHAp/CTS proved superior in inducing BMSC proliferation when compared to membranous hydroxyapatite/chitosan (mHAp/CTS) and electrospun nanofibrous chitosan (nCTS), both *in vitro* and *in vivo*.

Bioactive glass-ceramic nanoparticles (nBGC) are another class of nanomaterials used in bone tissue engineering due to their superior ability to replicate the inorganic composition of native bone compared to metallic nanoparticles. Singh *et al.* reported bilayered scaffolds fabricated by electrospinning bioactive glass in combination with polyvinyl alcohol (PVA) and silk fibroin (SF), which significantly enhanced the proliferation and osteogenic differentiation of bone marrow-derived mesenchymal stem cells (MSCs).[65]

Carbon-based nanomaterials are also used for bone tissue engineering, especially as scaffolds for the growth, proliferation, regeneration, adhesion and differentiation of bone stem cells, often in the form of hybrid materials. Zhang *et al*.[66] fabricated a nanocomposite with a layer-by-layer (LbL) architecture that closely mimics the hierarchical structure of native bone scaffold, using a cross-linking/hydrothermal/freeze-drying approach. The scaffold was reinforced with nitrogen-doped multiwalled carbon nanotubes (N-MWCNTs), cellulose, and nHA to enhance functional and structural performance. The incorporation of 1 wt% N-MWCNTs into the microporous hybrid scaffold significantly improved mechanical strength while maintaining a rough, porous surface, favourable for cell interaction. The scaffold supported bone mesenchymal stem cell (BMSC) adhesion, proliferation, viability, and mineralization *in vitro*. In vivo, after 12 weeks of implantation, the scaffold successfully repaired defects in the distal femoral condyle of rabbits without eliciting inflammatory responses, as confirmed by micro-CT and histological analyses.

Collagen is the most abundant protein in mammals, and the main component of hard and soft tissues. Collagen is a promising biomaterial for tissue engineering due to its excellent biocompatibility, biodegradability, cell adhesion, low immunogenicity, etc.[67] However, it has low mechanical strength, therefore using collagen alone for bone tissue engineering is not ideal. To enhance its performance, collagen must be modified or combined with a suitable scaffold (Figure 2.2). Currently, collagen is widely employed in hydrogels and scaffolds, providing an excellent matrix for various cell types and enabling 3D cell culture within hydrogel systems. Moreover, such collagen-based matrices can be engineered to contain growth factors, drugs, etc., thus being biologically active, and they can promote cell migration within scaffolds.

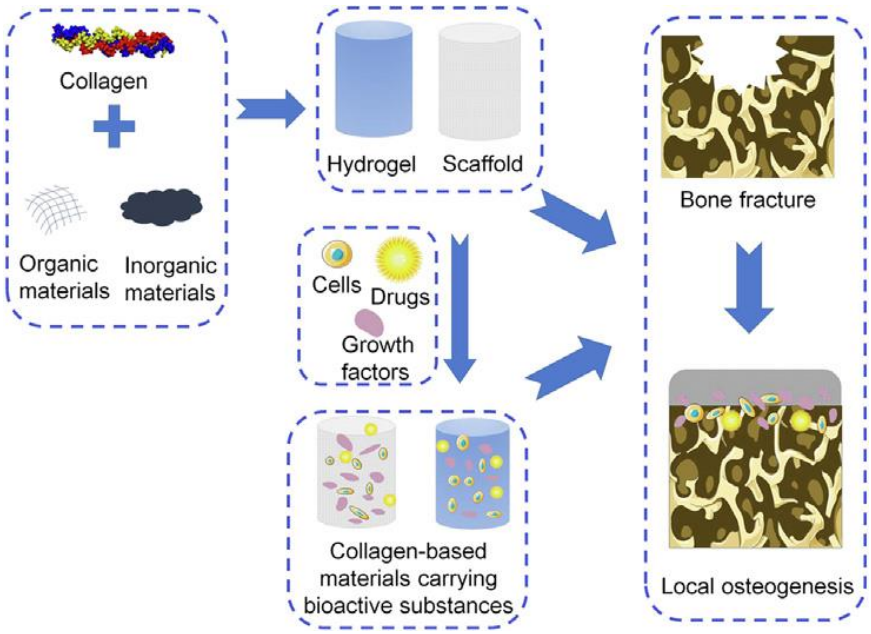

Figure 2.3. Collagen-based biomaterials for bone tissue engineering. Reproduced from Y. Li, Y. Liu, R. Li, H. Bai, Z. Zhu, L. Zhu, C. Zhu, Z. Che, H. Liu, J. Wang, L. Huang, Materials & Design 210 (2021) 110049.

Finally, Cheng *et al.* reported a composite material (based on PVA, SF, and polycaprolactone-PCL) for controlled co-delivery of growth factors through LbL assembly of core-shell nanofibers to enhance bone regeneration in an animal model of bone trauma.[68] In this design, bone morphogenetic protein-2 (BMP-2) was encapsulated within the fibre core, while connective tissue growth factor (CTGF) was immobilized on the fibre surface. This configuration enabled a dual-release profile tailored to bone regeneration: sustained BMP-2 delivery for osteogenesis and rapid CTGF release for early tissue repair. *In vivo* studies demonstrated that this scaffold achieved the largest bone repair area among tested systems, with newly formed bone exhibiting structural and morphological characteristics comparable to native tissue.

Dental tissue engineering

Since the early 21st century, nanomaterials have been used increasingly in dental tissue engineering, due to their unique physicochemical properties and ability to interact at the cellular and molecular levels. Periodontal diseases, which are increasingly prevalent with age, are not only associated with local tissue destruction but also linked to systemic conditions such as cardiovascular disease, diabetes, and rheumatoid arthritis.[48] The progressive impairment of periodontal tissues and their diminished self-repair capacity

highlight the need for advanced therapies capable of restoring both structural integrity and biological function.

Nanomaterials enable improved cell adhesion, controlled drug delivery, and enhanced antimicrobial activity, making them highly suitable for periodontal regeneration. They can be used in several key areas of dental tissue engineering: i) as antibacterial agents to prevent and control oral infections by disrupting biofilm formation and reducing microbial resistance; ii) as nanofillers incorporated into other materials to enhance mechanical strength, wear resistance, and biological compatibility; iii) as advanced coatings for implants, to improve osseointegration and reduce the risk of peri-implantitis; iv) as additives in toothpaste and oral care formulations for improved cleaning, remineralization, and antimicrobial effects.

One of the most common dental problems is periodontitis, an infectious condition marked by the progressive destruction of the tissues that support the teeth. This damage results from a strong inflammatory and immune response triggered by bacteria residing in plaque that adheres to the teeth. Periodontitis is a global health concern, which tends to worsen with age and is linked to both local and systemic health issues. It is also the most prevalent disease in dogs, gaining significance in veterinary medicine as pets live longer lives; interestingly, periodontal disease in humans and dogs shares many common features. Therefore, many research groups have developed constructs for regenerative therapy of periodontal defects, both in dogs and humans.[69–72] Davies and co-workers[73] reported a bilayered PLGA/calcium phosphate scaffold; the internal part of this biomaterial had a topographically complex macro- and nanoporous structure, previously demonstrated to promote osteoconduction, while the outer layer is a thin membrane acting as a barrier to prevent cell invasion. This construct enhanced periodontal regeneration in a canine model to a higher extent than previously reported scaffold. Periodontitis, driven by an exaggerated host immune response to microbial pathogens, lacks sufficient endogenous regulatory T cells (Tregs) to counteract the accelerated alveolar bone loss. Liu *et al.*[74] developed poly(L-lactic acid) (PLLA) nanofibrous spongy microspheres (NF-SMS) as an injectable scaffold designed to support Tregs and recruit endogenous stem/progenitor cells for tissue regeneration. The NF-SMS were engineered with a hyperbranched polymer (HP) polyplex system as a non-viral vector for long-term, sustained delivery of a microRNA (miR-10a)

thought to promote the differentiation of naïve T cells to Tregs. The scaffold also incorporated mesoporous silica nanoparticles (MSNs) to enable rapid release of two growth factors: Interleukin 2 (IL-2) and transforming growth factor beta (TGF-β), which enhance Treg recruitment, proliferation, and differentiation (Figure 2.4). This study identified miR-10a as a key regulator in T cell fate determination and the differentiation of Tregs and demonstrated that miR-10a exerts a significant therapeutic effect in preventing bone loss in a murine model of periodontal disease, primarily through its ability to promote Treg differentiation.

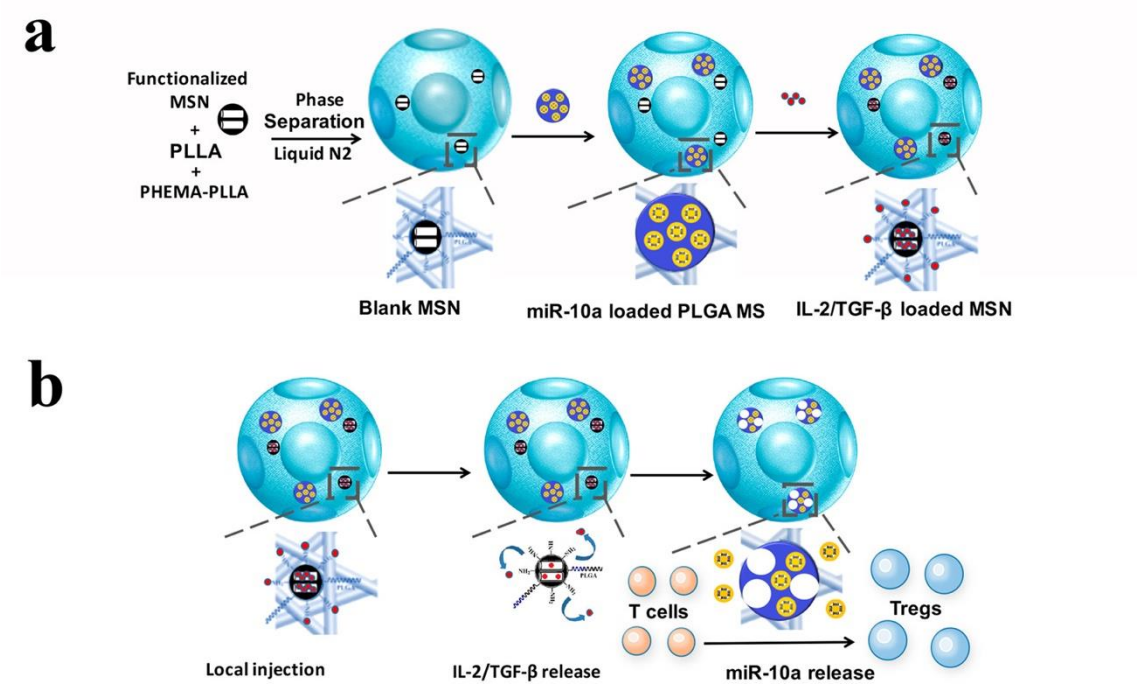

Figure 2.4. Schematic illustration of the fabrication of multifunctionalized NF-SMS. Reproduced from Z. Liu, X. Chen, Z. Zhang, X. Zhang, L. Saunders, Y. Zhou. P. X. Ma, ACS Nano 2018, 12, 10, 9785–9799.

Neural tissue engineering

The nervous system is composed of two primary components: the central nervous system (CNS), which includes the brain and spinal cord, and the peripheral nervous system (PNS), consisting of sensory and motor neurons. Both systems, but in particular CNS, have limited regenerative capacity due to several biological and structural factors, such as an inhibitory environment due to glial scars, limited neurogenesis, presence of myelin-associated inhibitors and a tightly regulated immune response due to the blood-brain barrier, which

limits the infiltration of immune cells that could aid in tissue repair. These factors make CNS and PNS vulnerable to long-term functional impairments following disease or injury.

Neural tissue engineering focuses on developing strategies to repair, regenerate, or replace damaged neural tissues in the CNS and PNS. It combines principles from biomaterials science, stem cell biology, and bioengineering to create scaffolds and delivery systems that support neural cell growth, guide axonal regeneration, and restore functional connectivity. Applications range from treating spinal cord injuries and neurodegenerative diseases to enhancing recovery after stroke or traumatic brain injury.

Spinal cord injury (SCI) can be classified as traumatic (TSCI), typically caused by external forces such as car accidents, or non-traumatic (NTSCI), often resulting from chronic conditions like cancer.[57] Additionally, SCI is categorized using the International Standards for Neurological Classification of Spinal Cord Injury, which incorporates three neurological summary scores developed by the American Spinal Injury Association (ASIA) to assess injury severity. These evaluations determine the lowest intact sensory and motor level (neurological level) and classify the injury as either complete (indicating total loss of sensory and motor function below this level) or incomplete (some sensory and/or motor function is partially preserved). Regardless of its origin, SCI conditions seriously affect the quality of life of the patients and pose an enormous burden on the healthcare systems worldwide. SCI is also one of the most challenging conditions in clinical neuroscience due to the limited regenerative capacity of the CNS, as explained before, and the complex cascade of secondary injury processes, including inflammation, oxidative stress, and glial scar formation. Nanotechnology can provide tools to address these challenges by developing systems for targeted drug delivery, modulation of the hostile post-injury microenvironment, and promotion of neural regeneration. Herein, we discuss some examples related to neural regeneration in SCI; several works highlighting the use of nanomaterials in other neural applications, such as peripheral nerve injury, targeted drug delivery, stimuli-responsive systems and regulation of the injury microenvironment are indicated in the "Further reading" section.

In terms of neural regeneration, carbon-based nanomaterials possess a unique combination of electrical conductivity, mechanical strength, and biocompatibility, being one of the most studied types of nanomaterials. In particular, graphene-based materials (GBMs) were

studied to address key challenges in SCI repair, such as creating a permissive microenvironment for axonal regrowth, reducing inflammation, and restoring neural connectivity. Because of their exceptional electrical conductivity, GBMs facilitate the transmission of bioelectrical signals, promoting neurite outgrowth and synaptic activity.[57] Studies have shown that graphene-based scaffolds combined with electrical stimulation significantly enhance axonal alignment and elongation, mimicking the natural electrophysiological environment of the spinal cord. GBMs are used in flexible and stretchable electronics interfacing the CNS, as described for instance by Lu *et al.*, who demonstrated that flexible 3D porous graphene electrodes could not only sense brain activity patterns with high spatio-temporal resolution but also allowed minimally invasive cortical stimulation (ankle and knee flexion).[75] Girão *et al.* demonstrated that reorganizing a biocompatible nanofibrous systems onto the surface of three-dimensional reduced graphene oxide (rGO) porous networks significantly enhances the formation of interconnected neuronal circuits *in vitro*.[76] Embryonic neural progenitor cells (ENPCs) were seeded onto the composite nanofibers to assess cell viability and identify the most suitable components for integration into the final 3D architecture. These optimized nanofibrous compositions were subsequently employed as physical crosslinkers for rGO sheets, creating unique 3D fibrous porous systems with tuneable structural properties, capable to support viable neuronal circuit formation. On the other hand, planar (monolayer) graphene structures are very appealing for neuroimaging purposes, as they enable the fabrication of transparent and flexible electrodes with superior imaging and electrical stimulation capabilities in comparison to other neural electrodes (e.g., platinum).[77]

Carbon nanotubes (CNTs) have been also used for SCI repair. A key advantage of these materials is their large surface area, which enables extensive and precise interactions with neural cells, triggering essential cellular responses for tissue regeneration.[78] For instance, Usmani *et al*. fabricated 3D implantable scaffolds based on multiwalled carbon nanotubes (MWCNTs) and PEG, which demonstrated good biocompatibility, integration with the spinal cord, and long-term stability *in vivo* after implantation in adult rats with SCI.[79] These scaffolds improved motor function recovery post-SCI by axonal regeneration.

Wound healing

Wound healing is a complex physiological process involving four key phases: haemostasis, inflammation, proliferation, and remodelling/maturation[80] (Figure 2.5). Based on healing time, skin wounds are generally classified as acute or chronic. **Acute wounds** result from a rupture or perforation of the skin layer and typically heal within a short period. In contrast, **chronic wounds** are slow to heal, often associated with underlying conditions such as obesity or diabetes. Angiogenesis plays a critical role in wound repair by forming new blood vessels that supply oxygen, nutrients, and growth factors, thereby accelerating healing. Conversely, impaired or abnormal angiogenesis contributes to chronic wound development, delaying recovery. Therefore, effective wound treatment strategies must address both skin tissue regeneration and vascular restoration.

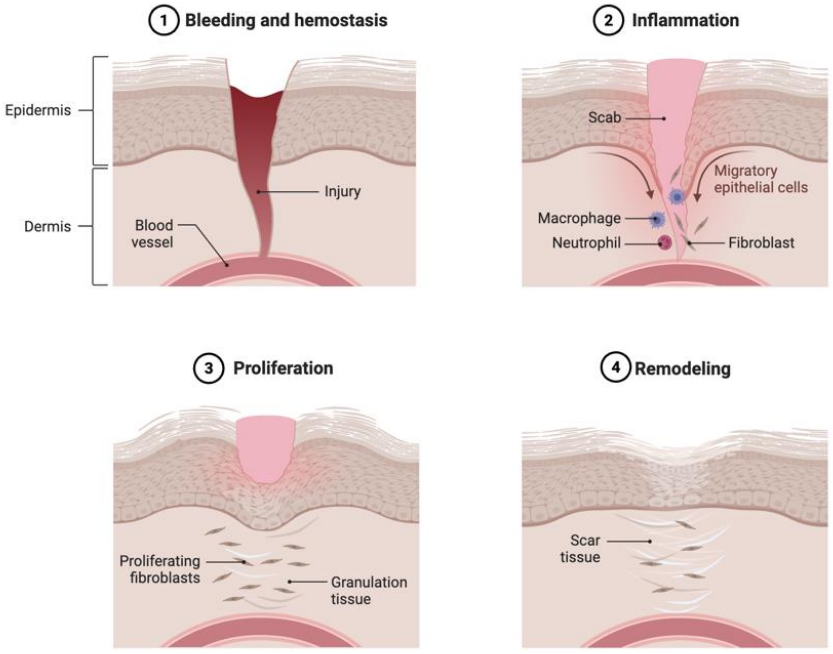

Figure 2.5. Illustration of the wound healing process. Figure created with Biorender.com

Chronic wound management affects millions of patients worldwide and has a high associated cost, putting an immense pressure on the healthcare systems (it has been recently estimated that the global annual cost of chronic wound care products exceeds 22 billion dollars).[81] Nanomaterials can address some of the current challenges associated with wound healing, being used to design smart (stimuli-responsive) drug delivery systems, as antibacterial agents, as promoters of angiogenesis, etc. Moreover, nanomaterials can be tailored to act in different stages of the wound healing process.[80] Below, we discuss some relevant examples for each stage.

Haemostasis starts immediately after injury and involves vasoconstriction, platelet activation and aggregation, and coagulation. Platelets secrete growth factors and cytokines, such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), which play a crucial role in wound healing by recruiting and activating essential immune cells (neutrophils and leukocytes).[80]

Nanomaterials can be used to enhance wound haemostasis by accelerating blood clotting, improving tissue adhesion, and enabling targeted delivery of therapeutic agents. Their small size allows them to interact efficiently with blood components, promoting platelet aggregation and fibrin formation. Additionally, many nanomaterials possess antimicrobial properties, which help prevent infection while supporting the healing process. Their biocompatibility and ability to degrade safely in the body make them ideal for integration into advanced wound dressings. Their porous structure offers a large surface area for blood adsorption and helps create a moist environment that ultimately promotes efficient clot formation. For example, Huang *et al.* developed a biodegradable porous cryogel composed of gelatine (GT) and silver nanoparticles (Ag NPs), offering high water absorption capacity and strong antibacterial and antibiofilm properties to support burn wound healing (burn wounds are highly prone to bacterial infections due to excessive exudate, posing significant challenges in clinical treatment).[82] The GT/Ag NPs cryogel demonstrated a swelling ratio of up to 4000%, enabling efficient exudate absorption and facilitating gas exchange at the wound site. It exhibited strong antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (PA), which are two common pathogens in burn wounds, and effectively disrupted mature biofilms. In a rat model of noncompressible liver haemorrhage, the cryogel outperformed commercial gelatine sponges in haemostatic efficacy. Moreover, in a PA-infected burn wound model, the GT/Ag NPs cryogel significantly enhanced wound contraction, collagen deposition, and angiogenesis, while reducing inflammation more effectively than GT-only cryogels and a commercially available film dressing (Tegaderm™). The cryogel biodegraded within four weeks, minimizing pain associated with dressing removal.

Inflammation begins after blood vessel damage and exudation, causing local oedema. Its primary role is to eliminate pathogens and damaged tissue, setting the stage for proliferation and maturation. This phase typically lasts 2–3 days and is marked by increased

blood flow, redness, heat, and tenderness. Neutrophils and monocytes migrate to the wound, producing reactive oxygen species (ROS) and releasing inflammatory mediators and growth factors. Fibrins and platelets form clots to seal the wound and support tissue regeneration, while lymphatic drainage reduces swelling. Although essential for healing, prolonged inflammation can hinder recovery, making proper wound care and infection control crucial.[80] Nanomaterials are typically used in the inflammation stage due to their inherent antibacterial activity, for delivery of antimicrobial drugs, or as immunomodulators.

Sun *et al*. integrated graphene quantum dots (GQDs, very small - 3-8 nm diameter - spherical graphene-based nanoparticles) with a low dose of a classical disinfectant (hydrogen peroxide, H_2O_2) in an antibacterial band-aid system for wound disinfection *in vivo*.[83] The system was efficient against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. The design of the platform takes advantage of the peroxidase-like activity of GQDs: GQDs catalyse the conversion of H_2O_2, which has limited antibacterial efficacy, into highly reactive hydroxyl radicals (•OH), significantly enhancing its antimicrobial effect. This approach enables effective wound disinfection while avoiding the need for high concentrations of H_2O_2.

The surface properties of nanoparticles can stimulate immune cell recognition and phagocytosis, enhancing inflammation and promoting wound healing. For example, adhesive haemostatic hydrogels based on hyaluronic acid-graft-dopamine and reduced graphene oxide (rGO) with antioxidant and antimicrobial properties have been shown to upregulate CD31, a key protein in endothelial cells that supports neovascularization and immune response.[84] CD31 (also known as platelet endothelial cell adhesion molecule-1 or PECAM-1) facilitates endothelial adhesion and migration, aiding new blood vessel formation, and regulates leukocyte migration to clear pathogens and necrotic tissue. In another example, Niemiec *et al.* described radical scavenging cerium oxide nanoparticles (CNP) conjugated to the anti-inflammatory microRNA (miR)-146a combined with nanosilk to suppress the expression of pro-inflammatory factors IL-6 and IL-8, facilitating diabetic wound healing.[85]

The **proliferation** phase involves the formation of granulation tissue, which fills the wound from its base and edges. This tissue consists of fibroblasts, new blood vessels, stroma, and immune cells. Key processes during this stage include fibroblast proliferation, collagen

synthesis, angiogenesis, wound contraction, and reepithelialisation. Fibroblasts are especially important during this phase of wound healing, producing collagen, elastic fibres, and other matrix components to form scar tissue. Their activity is stimulated by various growth factors essential for wound healing.

Nanomaterials can actively support tissue regeneration, angiogenesis, and cellular activity, enhance fibroblast proliferation and collagen synthesis, and promote angiogenesis by stimulating the expression of growth factors like VEGF and CD31. Additionally, they can be used as carriers for bioactive molecules, enabling controlled and targeted delivery of therapeutic agents that accelerate cell migration and proliferation. Some nanomaterials can mimic the ECM and provide structural support and biochemical cues that guide cell behaviour, further facilitating wound contraction and reepithelialisation. Moreover, as discussed before, several types of nanomaterials show antimicrobial properties, which help maintain a favourable wound microenvironment during the proliferation stage. In many instances, hybrid nanomaterials formulations are used, which combine different properties to achieve synergistic effects. For example, Wang *et al.* used a combination of polyurethane, keratin, and AgNPs to develop a nanofibrous mat for wound dressing. The use of keratin accelerated fibroblast proliferation, while AgNPs showed antibacterial effect against both *E. coli* and *S. aureus* bacteria.[86]

Several types of nanomaterials, such as metallic nanomaterials (including ZnO, CeO_2, and AuNPs), CNTs, graphene-based nanomaterials, bioactive glass, etc., have been shown to have pro-angiogenic effects, promoting neovascularization and improving the blood and nutrient supply to the wound.[87] Lai *et al.* developed a collagen/hyaluronic acid (Col-HA) nanofibrous skin substitute designed for programmable release of four different angiogenic growth factors (VEGF, PDGF, bFGF, EGF).[88] These growth factors were either embedded directly in the fibres or encapsulated in gelatine nanoparticles (GNs). Using a dual-source, dual-power electrospinning technique, Col and HA nanofibres were simultaneously deposited onto a rotating collector, forming a nanofibrous structure closely mimicking the scale and architecture of native ECM (Figure 2.6). Early release of EGF and bFGF promoted epithelialisation and vascular sprouting, while later release of PDGF and VEGF supported blood vessel maturation. The Col/HA/GN membrane enabled sustained growth factor release for up to one month. *In vivo*, in a diabetic rat model, the membrane accelerated wound closure, increased collagen deposition, and improved vessel maturation.

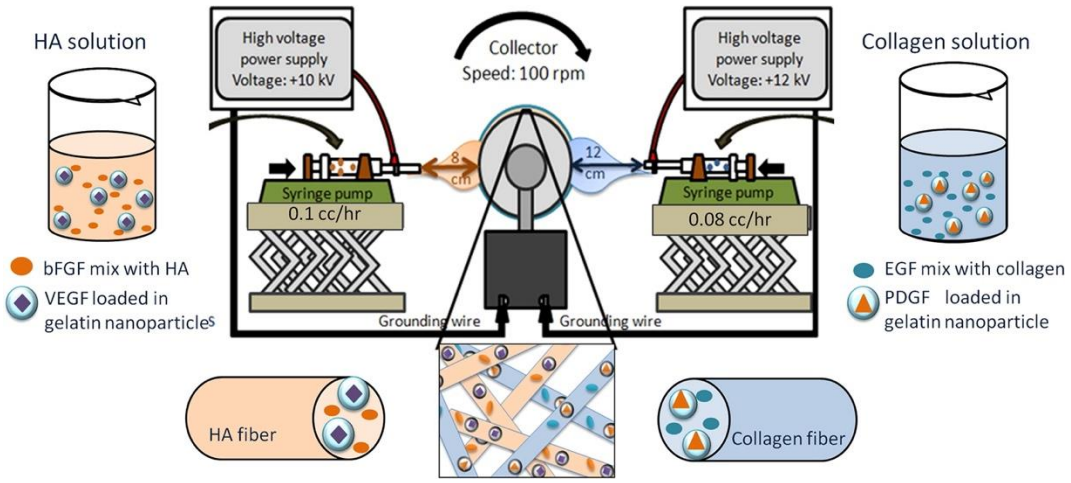

Figure 2.6. Col/HA/GN nanofibre fabrication by electrospinning. The four growth factors were either directly embedded in HA and Col nanofibers or encapsulated in GNs prior to incorporation into nanofibres. Reproduced with permission from H-J. Lai, C-H. Kuan, H-C. Wu, J-C. Tsai, T-M. Chen, D-J. Hsieh, T-W. Wang, Acta Biomaterialia 10 (2014) 4156–4166.

The **maturation** (**remodelling**) phase begins after wound closure and can last from three weeks to a year. During this stage, the wound transitions from repair to remodelling, with structural and functional restoration. Collagen III is gradually replaced by collagen I under the regulation of matrix metalloproteinases (MMPs), enhancing tissue strength, elasticity, and flexibility. As the wound contracts, excess cells are removed by apoptosis (programmed cell death) to prevent excessive scarring. Factors such as infection, poor circulation, age, and nutritional status can impair healing, potentially leading to chronic wounds or ulcers if the process is disrupted.

Similar to the other phases of wound healing, nanomaterials can impact maturation via different mechanisms. They can act as carriers of bioactive factors, including growth factors and signalling molecules that are released into the tissue surrounding the wound. These bioactive factors activate cell signalling pathways, promoting collagen synthesis, cell proliferation, and migration, which accelerate tissue regeneration during maturation. Additionally, due to their high specific surface, nanomaterials can increase the contact area of bioactive molecules with surrounding tissues to promote tissue regeneration. Nanomaterials can also be used to modulate the wound microenvironment, for example by regulating moisture levels or by suppressing the expression of MMPs to avoid collagen degradation and accelerate the wound healing process.

Wang and co-workers fabricated aligned electrospun polycaprolactone-silk fibroin nanofibre membranes loaded with lovastatin (inhibitor of the nuclear translocation of the transcriptional coactivator YAP - Yes-associated protein, which has been shown to promote fibrosis).[89] When these nanofibre membranes were aligned perpendicular to the wound's tension direction, a significant reduction in scar formation (up to 67%) and enhanced skin regeneration were observed *in vivo* in a mouse model. This effect was attributed to their ability to guide early collagen organization, avoiding the excessive deposition of ECM. Additionally, lovastatin-loaded nanofibers inhibited myofibroblast differentiation and migration. Together, the topographical cues and lovastatin synergistically suppressed mechanical signalling and fibrosis progression, further minimizing scarring.

Finally, nanomaterial combinations can be used to address wound healing in its different stages, as demonstrated recently by Shou *et al.*, who designed a platform for mechano-activated cell therapy for diabetic wound healing.[90] The smart bandage platform, enabling wireless magneto-induced dynamic mechanical stimulation (MDMS), included a poly(ethylene glycol) diacrylate (PEGDA)-based hydrogel incorporating an RGD (Arg-Gly-Asp) cell-adhesion motif and thiol-coated magnetic particles (TMP). FDA-approved mechanosensitive dermal fibroblasts and epidermal keratinocytes were encapsulated within the hydrogel to enable magnetically driven mechanical regulation of cellular functions. MDMS enhanced cell proliferation, extracellular matrix deposition, neovascularization, and glucose homeostasis through controlled insulin release, promoting superior wound closure in an *in vivo* diabetic wound mouse model. These effects were mediated by mechanical activation of fibroblasts, which subsequently stimulated co-encapsulated keratinocytes via the Ras/MEK/ERK signalling pathway.

2.4. Conclusions

As discussed above, nanomaterials offer unique properties that make them highly suitable for tissue engineering applications. Their nanoscale dimensions allow scaffolds to mimic the architecture of the native ECM, which is essential for guiding cell adhesion, proliferation, and differentiation. High surface area-to-volume ratios enable efficient incorporation of bioactive molecules such as growth factors, peptides, and drugs, facilitating localized and sustained delivery. Additionally, nanomaterials can be engineered to exhibit tuneable

mechanical properties, matching the stiffness of specific tissues and influencing mechanotransduction pathways. Functional nanomaterials (e.g. magnetic or conductive) enable remote actuation or electrical stimulation, further enhancing tissue maturation and regeneration.

However, several challenges need to be addressed to advance these nanotechnology-based solutions from bench to clinic. Key challenges include large-scale production of nanomaterials, ensuring their long-term biocompatibility, mitigating cytotoxicity, and meeting stringent regulatory standards. For regulatory approval, standardized protocols and comprehensive safety evaluation of the nanomaterials is compulsory. On the other hand, the cost of production and the need for specialized equipment can limit accessibility and commercialization of these materials.

One of the future directions in the field is the development of multifunctional nanomaterials that integrate structural, biochemical, and mechanical cues within a single platform. Smart nanomaterials capable of responding to environmental stimuli such as pH, temperature, or magnetic fields, could enable real-time modulation of cellular behaviour and controlled therapeutic delivery of drugs and bioactive molecules. Another field in which advancements are expected in the coming years is personalized regenerative therapy, in which scaffolds and materials should be developed according to the patient's individual needs. Advances in 3D and 4D bioprinting, combined with smart, stimuli-responsive nanomaterials, are expected to enable the fabrication of functional bioengineered scaffolds and even organs.

For all these future developments, the interdisciplinary collaboration among material scientists, biologists, and clinicians will be critical.

2.5 Further recommended reading

Note: the most important articles are highlighted by ***

1) ACS Nano, **2018**, 12, 9785−9799 - Nanofibrous Spongy Microspheres To Distinctly Release miRNA and Growth Factors To Enrich Regulatory T Cells and Rescue Periodontal Bone Loss; DOI: 10.1021/acsnano.7b08976

2) ACS Nano, **2019,** 13, 6372–6382 - Controlled Co-delivery of Growth Factors through Layer-by-Layer Assembly of Core-Shell Nanofibers for Improving Bone Regeneration; DOI: 10.1021/acsnano.8b06032

3) Proc. Natl. Acad. Sci., **2020,** 117, 25213 - Functional rewiring across spinal injuries via biomimetic nanofiber scaffolds; doi/10.1073/pnas.2005708117

4) RSC Adv., **2021,** 11, 19041-19058 - Applications of nanomaterials in tissue engineering; DOI: 10.1039/d1ra01849c ***

5) Materials & Design, **2021,** 210, 110049 - Collagen-based biomaterials for bone tissue engineering; https://doi.org/10.1016/j.matdes.2021.110049

6) J. Nanobiotechnol., **2021,** 19, 1 - Nanocomposite scaffolds for accelerating chronic wound healing by enhancing angiogenesis; https://doi.org/10.1186/s12951-020-00755-7

7) ACS Nano, **2022,** 16, 13430–13467 - Is Graphene Shortening the Path toward Spinal Cord Regeneration?; https://doi.org/10.1021/acsnano.2c04756 ***

8) Tissue Eng. Regen. Med., **2022,** 19, 927-960 - An Insight of Nanomaterials in Tissue Engineering from Fabrication to Applications; https://doi.org/10.1007/s13770-022-00459-z

9) Front. Bioeng. Biotechnol., **2023,** 11:1306184 - Graphene-based nanomaterials for peripheral nerve regeneration; doi: 10.3389/fbioe.2023.1306184

10) Front. Bioeng. Biotechnol., **2023,** 11: 1205792 – Engineered biomimetic micro/nano-materials for tissue regeneration; doi: 10.3389/fbioe.2023.1205792

11) Adv. Mater., **2023,** 35, 2304638 - Mechano-Activated Cell Therapy for Accelerated Diabetic Wound Healing; DOI: 10.1002/adma.202304638 ***

12) J. Mater. Chem. B, **2023,** 11, 6225-6248 - Tubular nanomaterials for bone tissue engineering; DOI: 10.1039/d3tb00905j

13) Advanced Drug Delivery Reviews, **2023,** 193, 114670 - Nanomaterials and nanomaterials-based drug delivery to promote cutaneous wound healing; https://doi.org/10.1016/j.addr.2022.114670 ***

14) Chemical Engineering Journal, **2024,** 495, 153640 - Opportunities and challenges of nanomaterials in wound healing: Advances, mechanisms, and perspectives; https://doi.org/10.1016/j.cej.2024.153640 ***

15) Archives of Microbiology, **2024,** 206, 199 - Advancements in wound healing: integrating biomolecules, drug delivery carriers, and targeted therapeutics for enhanced tissue repair; https://doi.org/10.1007/s00203-024-03910-y

16) Nature Reviews Materials, **2024**, 9, 550-566 - Wound management materials and technologies from bench to bedside and beyond; https://doi.org/10.1038/s41578-024-00693-y ***

17) Materials & Design, **2024**, 237, 112617 - Nanosystems-enabled regenerative strategies for spinal cord Injury: Recent advances and future prospects; https://doi.org/10.1016/j.matdes.2023.112617 ***

18) Regenerative Engineering and Translational Medicine, **2025**, Nanomaterials in Regenerative Medicine: Advancing the Future of Tissue Engineering; https://doi.org/10.1007/s40883-025-00416-x

19) Regenerative Biomaterials, **2025**, 12, rbae139 - Electrospinning based biomaterials for biomimetic fabrication, bioactive protein delivery and wound regenerative repair; https://doi.org/10.1093/rb/rbae139

3. Nanomaterials for hyperthermia applications

This lecture introduces the concept of hyperthermia and provides a brief overview of state of the art in nanomaterial-based hyperthermia. Several topics are covered, including the principles of nanoparticle hyperthermia, the design of nanoparticles for hyperthermia applications, the cellular response to heat, and selected examples of hyperthermia applications.

3.1 Introduction

"Hyperthermia" originates from the Greek words *hyper* (meaning "above" or "excessive") and *therme* ("heat") and refers to the elevation of body temperature (systemically or in specific regions) beyond physiological levels, with the purpose of inducing a therapeutic effect. The therapeutic use of heat has been recognized since ancient times.[91–93] References to heat-based treatments appear in traditional Indian medicine (Ayurveda, ca. 3000 BC) and in ancient Egyptian texts such as the Edwin Smith Papyrus (ca. 1700 BC) and the Ebers Papyrus (ca. 1550 BC). Greek and Roman physicians also believed that controlling body temperature could cure most diseases. Parmenides of Elea (ca. 540-480 BC) is known for his quote *"Give me the power to induce fever and I will cure all diseases"*, while Hippocrates (considered the father of medicine) said: *"Those who cannot be cured by medicine can be cured by surgery; those who cannot be cured by surgery can be cured by heat; and those who cannot be cured by heat are to be considered incurable."* The earliest documented use of hyperthermia in cancer treatment appears in *De Medicina* by the Roman physician Celsus (ca. 25 BC-50 AD), who recommended hot baths during the early stages of cancer and was among the first to recognize the increased thermal sensitivity of tumours. During the Middle Age, there were also accounts of tumour regression in patients suffering from smallpox, malaria, or tuberculosis, diseases usually associated with high fever.

The German surgeon Carl D. W. Busch is credited for the first documented case of hyperthermia in cancer treatment. In 1886, he reported the regression of a sarcoma in a 43-year-old patient with erysipelas-induced fever. He noted that tumour growth was inhibited at temperatures exceeding 42 °C, while healthy cells appeared to tolerate the heat. In 1893, American surgeon William D. Coley corroborated these findings and pioneered a method of

inducing fever in cancer patients by injecting pyrogenic toxins directly into tumours (Coley is considered the father of modern hyperthermia and immunotherapy). Toward the end of the 19th century and into the early 20th, various hyperthermia techniques were explored: hot baths, perfusion hyperthermia using the patient's pre-warmed blood, or electrical cauterization. However, these methods often produced inconsistent results and were rather invasive, causing significant discomfort and pain to the patients.

For most part of the 20th century, hyperthermia research focused on understanding the impact of heat at biological level, mostly concerning the different sensitivity of healthy tissues and tumours to heat. After 1970s, the research in the field experienced a boost, very likely related to several key advancements: improved heating technologies, more precise temperature monitoring methods, a deeper understanding of heat-induced biological effects and finally, the emergence of nanotechnology.[94]

3.2 Biological effects of heat

Hyperthermia impacts various cellular functions at multiple levels.[95] Elevated temperatures increase the fluidity and permeability of the cell membrane and can induce cytoskeletal alterations, such as changes in cell shape, membrane blebbing, and shifts in membrane potential. Within the 42-45 °C range, heat has been shown to slow down the synthesis of proteins and nucleic acids. While RNA and protein synthesis typically resume shortly after the thermal stress ceased, DNA synthesis remains impaired. This is largely due to heat-induced protein denaturation and aggregation, which disrupt essential cellular processes, including DNA replication and repair. These effects can explain the synergistic effect of hyperthermia and chemo- or radiotherapy, as heat sensitizes cancer cells to these treatments by compromising their ability to recover from DNA damage. At temperatures below 42 °C, hyperthermia can also increase tumour perfusion and oxygenation, sensitizing cancer cells towards radiotherapy. At temperatures exceeding 42 °C, damage to the tumour vasculature results in reduced blood flow, leading to hypoxic conditions and an increasingly acidic intracellular environment. These microenvironmental alterations are considered to play a significant role in enhancing the radiosensitivity and chemosensitivity of malignant cells.[96]

Heat shock proteins (HSPs) play a critical role in the cellular response to hyperthermia.[97] When cells are exposed to elevated temperatures, protein denaturation and aggregation trigger the activation of heat shock factor 1 (HSF1) and subsequent transcription of HSP genes. These molecular chaperones assist in refolding damaged proteins, prevent aggregation, and stabilize cellular structures, thereby promoting cell survival under thermal stress. In cancer therapy, this protective mechanism can be a double-edged sword: while HSPs are important for the survival of normal cells, they may also promote cancer cell thermotolerance, reducing treatment efficacy. Moreover, certain HSPs, such as HSP70 and HSP90, are involved in oncogenic signalling pathways and immune modulation, making them potential targets for combination therapies that aim to inhibit their function and enhance the cytotoxic effects of hyperthermia. HSP70 assists in protein folding, prevents aggregation of denatured proteins, and facilitates the refolding or degradation of damaged proteins. Under heat stress, HSP70 is rapidly upregulated to protect cells from thermal damage and to maintain cellular homeostasis. In cancer cells, however, HSP70 can promote survival by inhibiting apoptosis and supporting oncogenic signalling, which may reduce the effectiveness of hyperthermia-based therapies. HSP90 is essential for the stability and function of many client proteins, including kinases and hormone receptors involved in cell growth and survival. During heat stress, HSP90 helps maintain the activity of key signalling proteins, including those that are involved in pathways that drive tumour progression. Its overexpression in cancer cells contributes to thermotolerance and resistance to therapy.

3.3 Types of hyperthermia

Clinically, hyperthermia can be applied through three main approaches: local, regional, and whole-body hyperthermia.[91,98] Local and regional techniques are generally used as adjuvant therapies to enhance chemo- and radiotherapy, whereas whole-body hyperthermia may also serve as a standalone treatment for metastatic disease. Heat is typically generated using capacitive, radiative (radio- or microwaves), or infrared-based systems.[99] Local hyperthermia targets small, superficial tumours at approximately 42 °C using external, intraluminal, interstitial, or endocavitary applicators, although penetration depth is limited to a few centimetres. Regional hyperthermia addresses larger or deep-seated tumours via external antenna arrays or regional perfusion with heated fluids, often combined with chemotherapy. Whole-body hyperthermia involves raising systemic temperature to 41-

42 °C for about one hour under anaesthesia. It is achieved through thermal chambers, hot water blankets, or infrared systems, with lower temperatures applied for extended periods when combined with cytotoxic agents.

All these hyperthermia modalities, however, suffer from different drawbacks, such as difficulty to generate heat selectively and uniformly in the desired area (without causing damage to the adjacent tissue) or the lack of tools for precise temperature monitoring.

3.4 Nanomaterial-based hyperthermia

Nanotechnology can address some of the above-mentioned drawbacks of classical hyperthermia. Some types of nanomaterials can convert energy from external sources such as magnetic fields or light into heat. When functionalized with tumour-targeting ligands, these nanomaterials enable selective heat generation within the tumour, minimizing damage to surrounding healthy tissues. Additionally, they can be used for the development of multifunctional theranostic systems integrating therapy and diagnostics in a single nanoplatform.[100] Nanomaterials also offer promising solutions for precise temperature during nanoscale hyperthermia,[101,102] or even the integration of nanoheating and nanothermometry within a single nanoparticle platform. The main two hyperthermia modalities using nanomaterials are magnetic and optical hyperthermia; the basic principles of heat generation in both types of hyperthermia are discussed in the following sections. For both types of hyperthermia (magnetic and optical), the heat generated by nanoparticles or nanomaterials (note that the term "*nanoheater*" is often encountered in the literature to describe nanomaterials in the frame of hyperthermia applications) can be finely tuned to elicit different cellular responses. At temperatures in the range of 41-45 °C, a **sublethal** effect is achieved, with cells undergoing reversible heat injury at different levels (see Section 3.1); if temperatures above 45-46 are reached, necrosis occurs (**lethal** effect).[103]

3.4.1 Magnetic hyperthermia

As already discussed during the classes, magnetic nanoparticles (MNPs), particularly those based on iron oxides such as magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) and ferrites (MFe_2O_4) are widely used for biomedical applications due to their unique magnetic properties, which can be finely tuned by adjusting particle size, shape and composition.

In magnetic hyperthermia, the heating is produced by **energy losses** (Figure 3.1) when the MNPs respond to an external magnetic field that changes its direction (called alternating current magnetic field).[104] If we think about the MNPs as small compass needles, when a magnetic field is applied, these needles try to align with it. But if the field keeps changing direction (which is the case of the magnetic fields used in magnetic hyperthermia, known as alternating magnetic field, AMF), the MNPs keep trying to catch up and re-align. This constant re-alignment takes energy that turns into heat. There are two main types of energy losses responsible for heat generation: **relaxation losses** and **hysteresis losses**.

Bulk magnetic materials are composed of multiple magnetic domains, each containing magnetic moments aligned in a specific direction. As the size of the material decreases, the number of these domains is reduced. If the MNPs are sufficiently small (typically below 25-30 nm in diameter), they become monodomain; in contrast, larger MNPs (up to ~100 nm) generally have several domains and are referred to as multidomain MNPs. Small MNPs typically exhibit **superparamagnetic behaviour**, meaning that at room temperature and in the absence of an external magnetic field, they possess no net magnetic moment. When an external magnetic field is applied, the magnetic moments within the particles align, resulting in a net magnetisation which disappears once the field is removed, as the magnetic moments return to a random orientation. This lack of remanent magnetisation is particularly advantageous for biomedical applications. It helps maintain the colloidal stability of MNPs in biological environments and prevents their aggregation, which is crucial for safe and effective *in vivo* use.

In superparamagnetic, monodomain MNPs, which are commonly used in magnetic hyperthermia, heat production primarily occurs via **two relaxation processes**: Néel relaxation and Brownian relaxation (Figure 3.1).[105,106] **Néel relaxation** involves the internal rotation of magnetic moments within the MNP and is the dominant mechanism when the physical rotation of the entire particle is restricted (this usually applies when MNPs are inside cells or in solid tissues like tumours). This mechanism is especially significant in smaller MNPs (typically under 15 nm in diameter), where it becomes the primary source of heat generation. In **Brownian relaxation**, physical rotation of the entire nanoparticle in the surrounding medium occurs, generating heat; this is the dominant mechanism in larger MNPs in low-viscosity environments. However, MNPs in very viscous media or inside

tumours cannot rotate freely; thus, heat generation by Brownian relaxation is not efficient in these scenarios.

Heat generation by **hysteresis losses** occurs in multidomain MNPs or larger single-domain MNPs with **ferromagnetic behaviour,** meaning that the magnetic moments of atoms align spontaneously in the same direction, even without an external magnetic field. This alignment results in a strong net magnetisation, which can be enhanced in the presence of an external magnetic field; however, unlike superparamagnetic nanoparticles, ferromagnetic MNPs retain some of this magnetisation (called remanence) even after the external field is removed.[107] When these MNPs are exposed to an alternating magnetic field, their magnetic moments continuously try to align with the field, resulting in an energy difference that is released as heat. The heat generated is proportional to the area of the hysteresis curve (Figure 3.2).

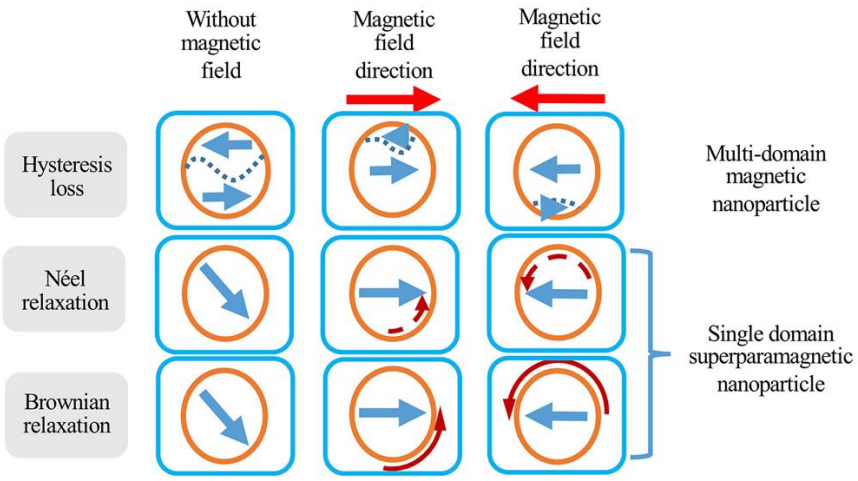

Figure 3.1. Schematic representation of the mechanisms by which MNPs generate heat under an alternating magnetic field. Orange circles: MNPs. Short straight arrows show the magnetic field direction, solid curved arrows represent particle movement, dashed curved arrows indicate changes in magnetic moment direction, and dashed lines mark domain boundaries in multi-domain particles.

Reproduced from Chang D., Lim M., Goos J. A. C. M., Qiao R., Ng Y. Y., Mansfeld F. M., Jackson M., Davis T. P. and Kavallaris M. (2018) Biologically Targeted Magnetic Hyperthermia: Potential and Limitations. Front. Pharmacol. 9:831. doi: 10.3389/fphar.2018.00831.

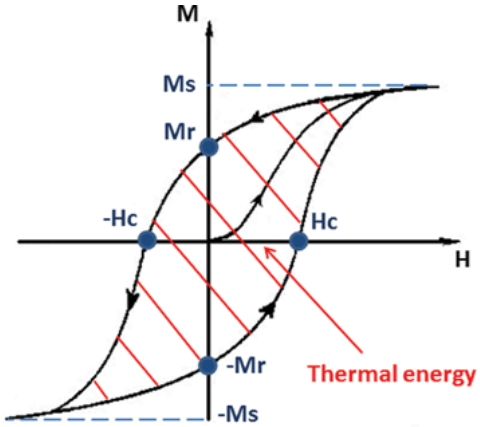

Figure 3.2. Hysteresis loop of a ferromagnetic material. The area of the hysteresis loop represents the energy dissipated as heat during a magnetisation cycle. Reproduced with permission from Hervault, A. and Thanh, N. T. K., Nanoscale, 2014, 6, 11553-11573.

The heating capacity of MNPs is typically measured by calorimetry and is defined as the heating power (P) dissipated per unit of mass of MNPs (m_{MNP}), measured in W/g (this parameter is known as "*Specific Loss Power*", SLP, Equation 1)[108]:

$$SLP = P/m_{MNP} \qquad (1)$$

To compare heating efficiencies measured under different conditions of AMF frequency and field amplitudes, researchers often employ another parameter, called "*Intrinsic Loss Power*", ILP (Equation 2, where H is the amplitude, and f the frequency); however, it should be noted that Equation 2 is only valid for low field amplitudes and frequencies. [104,108]

$$ILP = SLP/H^2 f \qquad (2)$$

Finally, it should be mentioned that for biomedical applications, the product H x f cannot surpass certain values, to avoid overheating of tissues due to Eddy currents; while there is no consensus about the value of the H x f product, several groups proposed values of 2-5 x 10^9 A m^{-1} s^{-1}.[104]

For more information on the design of MNPs for hyperthermia applications, including an in-depth analysis of the effect of synthesis method, size, shape, composition, etc. on the

heating performance of the MNPs, several excellent review articles can be found in the literature.[104,109,110]

3.4.2 Optical hyperthermia

Optical hyperthermia (also called photothermia) uses plasmonic nanomaterials; the name is related to their interaction with light and the localized surface plasmon resonance (LSPR) phenomenon. When plasmonic nanomaterials are excited with light at a certain wavelength, a collective oscillation of the electrons in the conduction band occurs; this oscillation is known as a plasmon.[111] Since the size of the nanomaterials is much smaller than the light wavelength, the oscillation is confined to the nanomaterial surface, hence the name of "localized surface plasmon resonance". Traditional plasmonic nanomaterials include noble metal nanoparticles (gold, silver, platinum) and carbon-based nanomaterials (carbon nanotubes, graphene, graphene oxide, etc.),[112,113] although more recently it was shown that MNPs can also be efficient nanoheaters (either alone or in the form of hybrid nanomaterials called magneto-plasmonic, such as gold-MNP hybrids).[114,115] Importantly, the LSPR wavelength is dictated by the nanomaterial's physicochemical properties (size, shape and aspect ratio, chemical composition). Ideally, for *in vivo* applications, the LSPR band should be in the near infrared (NIR, 700-1100 nm) region of the spectrum, known as the "biological window", in which absorption by biomolecules and water molecules is minimized.[116]

When noble metal nanoparticles are exposed to light (typically a laser) with a wavelength that matches the resonance condition of their surface plasmon oscillations, a significant photothermal effect occurs. This phenomenon is due to the interaction between electromagnetic radiation and the collective oscillation of conduction electrons at the nanoparticle surface (LSPR, as explained above). When the incident light is in resonance with these oscillations, the nanoparticle absorbs energy in a very efficient way, leading to heat generation through a multi-step relaxation process.[117–119] In the first step, named *electronic absorption*, free electrons within the metallic nanoparticle absorb part of the incident laser pulse energy. Next, during *electron-phonon thermalization*, the system reaches internal thermal equilibrium; however, at this stage, no heat is transferred to the surrounding medium. For spherical nanoparticles larger than 5 nm, the electron–phonon relaxation time (τ_t) required to achieve a steady-state temperature distribution inside the

particle is approximately 2 ps. If the laser pulse duration is shorter than τ_t, the generated heat remains confined within the particle. However, in practice τ_t is much shorter than the laser pulse duration, allowing heat to diffuse outward from the nanoparticle into its environment and resulting in an increase in the local temperature of the surrounding medium (*external heat diffusion*).

The heat produced by an individual nanoparticle (Q_{NP}) can be described by Equation 3,[116,118] where C_{abs} is defined as the absorption cross-section area of the nanoparticle and I is the laser power per unit of surface:

$$QNP = C_{abs} \times I \qquad (3)$$

Further details on the photothermal effect and on the design pf nanomaterials for optical hyperthermia can be found in relevant review articles.[120–123]

3.5 Applications of hyperthermia

For many decades, hyperthermia research focused on cancer treatment, both via a direct localized tumour ablation or using the synergy between hyperthermia and chemotherapy and radiotherapy. Magnetic hyperthermia received approval in Europe for clinical use in the treatment of glioblastoma and more recently a pilot clinical trial was conducted to assess its feasibility for the treatment of locally advanced pancreatic ductal adenocarcinoma (PDAC).[124] Photothermal therapy using gold nanoshells also reached clinical trials for the localized photothermal ablation of prostate tumours.[125] Numerous comprehensive reviews on the use of nanomaterial-based oncological hyperthermia as a standalone therapy or in combination with other therapeutic modalities have been published; selected references on magnetic and optical hyperthermia are provided for the interested reader.[94,104,126–130]

In recent years, hyperthermia has been also used to potentiate cancer immunotherapy, as heat can reshape the tumour microenvironment and enhance immune system activity through different mechanisms. Many tumours are immunologically "cold," lacking immune cell infiltration and antigen presentation, which results in an increase resistance to immunotherapy.[131] Hyperthermia can help converting them into "hot" tumors by increasing immune cell infiltration, as heat increases blood flow and vascular permeability, allowing lymphocytes and natural killer (NK) cells to penetrate the tumor. Hyperthermia can also downregulate regulatory T cells and myeloid-derived suppressor cells, which normally

inhibit immune responses, thus reducing immunosuppressive conditions. As described in Section 3.2, heat can trigger stress responses and apoptosis/necrosis in cancer cells, leading to the release of tumor antigens and DAMPs (Damage-Associated Molecular Patterns) that alert the immune system and activate dendritic cells (DCs). HSPs expressed in response to thermal stress act as chaperones for tumor antigens, enhancing antigen presentation and stimulating adaptive immunity.[130] Finally, hyperthermia stimulates pro-inflammatory cytokines (e.g., IL-2, IFN-γ), which boost T-cell activity.[131] For more insights on the combined nanomaterial-based hyperthermia and immunotherapy for the treatment of different types of cancer, please consult the references indicated here.[130–135]

In this class we will focus on some non-traditional applications of sub-lethal hyperthermia, beyond cancer treatment.[136] In recent years, hyperthermia has been used for drug delivery and release,[55,137–139] treatment of cardiovascular diseases[140,141] and bacterial infections,[142,143] remote activation of thermosensitive ion channels,[144–146] or for modulation of cell membrane biophysics.[45,46] Here, we will briefly discuss some of these applications.

Drug release

Magnetic and optical hyperthermia can mediate drug release from thermo-responsive matrices, by altering the matrix structure in different ways.[55,136] If the drug is attached to the matrix through a covalent or non-covalent thermolabile bond, the heat generated by the nanomaterials after applying an AMF or irradiating with a laser can lead to bond breaking and drug release. In the case of thermo-responsive systems based on polymeric or liposomal structures encapsulating magnetic/plasmonic nanomaterial, heat generation leads to a physical transformation of the matrix (e.g., polymer collapse or permeabilization of the liposomal membrane), allowing the drug to be released. A third approach is to encapsulate the drug in porous nanostructures and cap the pores with a thermos-responsive material/bond; heat generation by magnetic or optical hyperthermia would unblock the pores and trigger the release of the drug.

Hyperthermia-mediated drug release systems have been used for the delivery and controlled release of different types of drugs, including chemotherapeutic agents, antibiotics, anti-inflammatory drugs, etc.[55,136,138] For instance, Cazares-Cortes *et al*. used hybrid nanogels, composed of thermoresponsive polymers and superparamagnetic nanoparticles ("MagNanoGels") for the remote release of doxorubicin (DOX, a well-known

chemotherapeutic).[147] The nanogel based on oligo(ethylene glycol) methyl ether methacrylate (OEGMA) and methacrylic acid (MAA) exhibits a swelling-deswelling transition around 47 °C (which corresponds to the volume phase temperature) at physiological pH (7.5). When exposed to an AMF, the release of DOX increases with respect to the systems not exposed to the AMF, even though no macroscopic heating is detected (Figure 3.3). This enhanced drug release results from local heating induced by the MNPs, causing the polymer network to shrink, as evidenced by the reduction in MagNanoGel size under AMF. Inside cancer cells, DOX-MagNanoGels achieved more efficient DOX internalization compared to free DOX and enabled remote, AMF-triggered intracellular drug release.

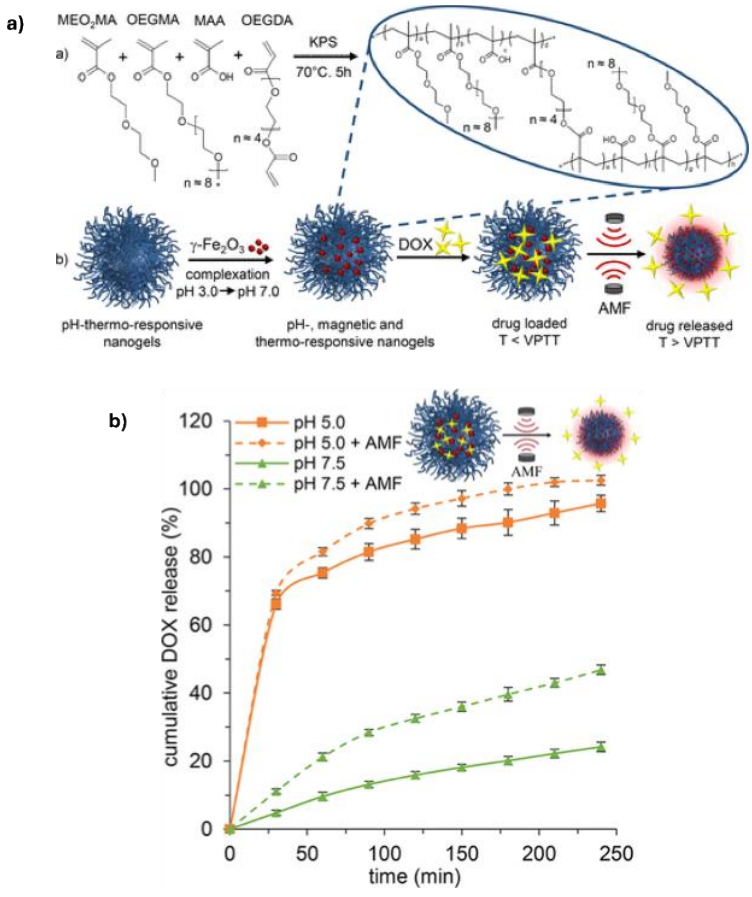

Figure 3.3. a) Scheme of the MagNanoGel system for remotely controlled drug delivery under an AMF. b) Cumulative DOX release profile (%) from the nanogels under different conditions. Reproduced with permission from Cazares-Cortes, E. et al., ACS Appl. Mater. Interfaces 2017, 9, 31, 25775–25788.

Deng *et al.* reported a NIR light-triggered system for the delivery of the antibiotic vancomycin from a polysaccharide hydrogel encapsulating ferric tannate (TA-Fe) nanoparticles and its application to wound disinfection.[148] The hydrogel showed excellent antibacterial properties upon irradiation with a low-intensity NIR laser, which avoids overheating. Navrátil *et al.* described an interesting drug depot system for repeated on-demand release of antibiotics controlled by an AMF.[149] The depot system is composed of core-shell composite microcapsules, with the core composed of micronized drug particles embedded within a low-melting wax hydrophobic matrix and the shell formed by a hydrogel incorporating MNPs. The localized heat generation upon exposure to an AMF leads to an increase in temperature above the melting point of the core material, exposing the drug particles to the surrounding aqueous phase. Drug release could be precisely regulated in an on/off manner by adjusting the sequence and duration of the AMF pulses. This depot system demonstrated a significantly higher loading capacity compared to conventional diffusion-controlled systems containing pre-dissolved drug, as demonstrated by *in vitro* validation using norfloxacin against *E. coli*.

Liposomes are one of the most commonly used nanoparticle systems for drug delivery. Liposomes are spherical vesicles formed from natural or synthetic phospholipids, which self-assemble in aqueous environments to shield their hydrophobic regions, creating a bilayer with a hydrophilic core. Their biocompatibility and ability to encapsulate both hydrophilic and hydrophobic drugs make them highly suitable for drug delivery. Owing to these advantages, liposomes became the first nanostructured drug delivery systems approved by the FDA for clinical use.[150] When combined with nanoheaters, liposomes become thermosensitive and can release their cargo upon laser irradiation or exposure to an AMF. The mechanism of release is based on the changes in the order of the lipid bilayer: when temperatures close to or above the phase transition temperature T_m of the phospholipids are reached, the lipid bilayer suffers a transition from a solid gel phase with low permeability to liquid disordered phase with enhanced permeability (Figure 3.4).[151] At temperatures close to T_m the permeability of the bilayer is the highest, due to the coexistence of both phases.

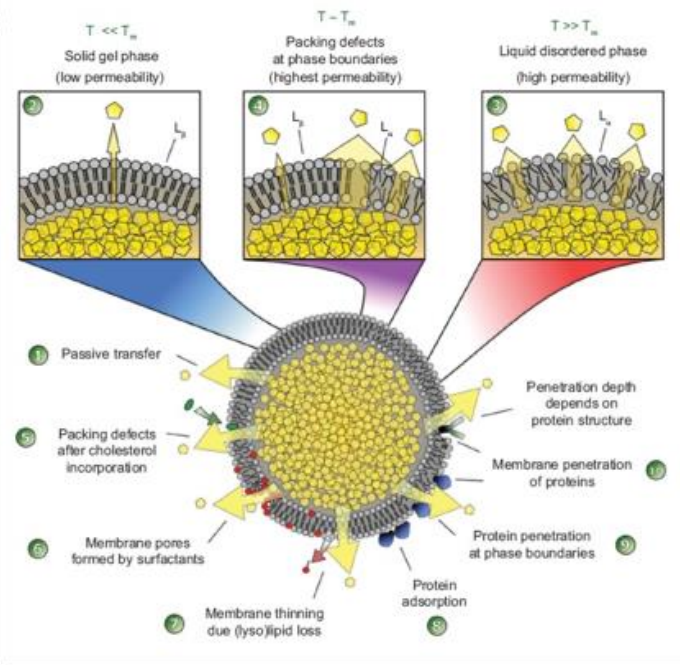

Figure 3.4. Drug release from thermosensitive liposomes. Reproduced from International Journal of Nanomedicine 2014:9 4387–4398.

Liposome composition can be finely tuned to allow release at different temperatures; moreover, liposomes offer flexibility, as the nanoheaters can be placed both inside the lipid bilayer and in the hydrophilic areas of the liposome (attached to the outer membrane or encapsulated inside the hydrophilic core). This allows the design of sequential release systems, such as the one reported by Salvatore *et al.* and based on a liposome/DNA/MNP nanostructured architecture.[152] Hydrophobic Fe_3O_4 MNPs (5 nm diameter) were embedded in the lipid bilayer of the liposome, while hydrophilic Au-MNPs (7 nm Au@Fe_3O_4 NPs obtained by coating the hydrophobic Fe_3O_4 MNPs with a gold layer) were conjugated to the liposome outer membrane via a DNA zipper, see Figure 3.5. Application of a 3.22 kHz AMF for short durations triggered the release of the hydrophilic drug from the aqueous core of magnetoliposomes, while a longer exposure to a 6.22 kHz AMF led to a temperature increase that reached the melting point of the double DNA strand, enabling the release of the zipper therapeutic oligonucleotide.

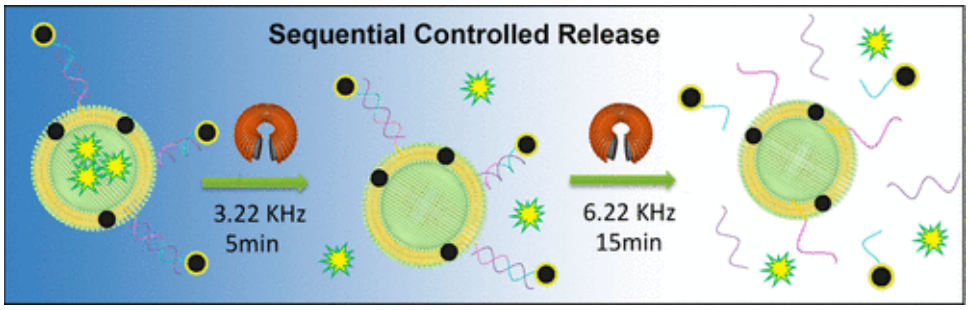

Figure 3.5. Sequential drug release form thermosensitive liposomes. Reproduced with permission from Salvatore A. et al., ACS Nano 2016, 10, 8, 7749–7760.

Remote activation of thermosensitive ion channels

The heat generated by nanoparticles can also be applied for the remote and controlled activation of cellular functions, such as the activation of thermosensitive ion channels. Transient Receptor Potential (TRP) channels are a family of cation channels mediates the sensing of diverse stimuli, including heat, mechanical forces, light, and pain. The TRP vanilloid (TRPV) subfamily forms a tetrameric structure that creates an ion-conducting pore, typically permeable to Ca^{2+}. TRPV1 (primarily expressed in neurons) is a Ca^{2+}-selective channel activated mainly by heat (>42 °C), while TRPV4 (widely distributed across various cell types) is a nonselective cation channel responsive to mechanical stimuli and temperature changes.[153] Huang *et al.* demonstrated one of the earliest uses of localized heating from MNPs for remote cellular stimulation by activating TRPV1 channels with 6 nm manganese ferrite ($MnFe_2O_4$) nanoparticles.[145] The MNPs, coated with streptavidin and tagged with DyLight549, enabled local temperature monitoring via fluorescence changes. Under a radiofrequency magnetic field (40 MHz, 0.67 kA m^{-1}), significant membrane-localized heating (>15 °C in 15 s) occurred without bulk temperature rise. This approach also achieved *in vivo* control of TRPV1 channels, eliciting thermal avoidance behaviour in *C. elegans*. Munshi et al. achieved magnetothermal activation of three distinct brain regions in awake mice, each linked to specific motor behaviours, using 8 nm cobalt ferrite cores with a 2.25 nm manganese ferrite shell and ~5 nm polymer coating for stability.[146] The MNPs were functionalised with neutravidin for targeted binding to biotinylated neuronal membrane proteins. When TRPV1-overexpressing neurons were stimulated under an AMF (570 kHz, 7.5 kA m^{-1}), mice treated with the MNPs exhibited rapid ambulation that persisted for two days. Similar stimulation of the striatum induced rotational movement, while activation of

deeper regions caused motion inhibition, leaving the animals immobile except for head rotation.

Modulation of cell membrane biophysics

The disruption of the cellular membrane via mechanical, electrical, thermal, optical or chemical means is an interesting strategy for direct intracellular delivery, avoiding endosomal entrapment, which is one of the main bottlenecks for efficient drug delivery. Macroscopic heating of cell membranes above physiological temperatures leads to spontaneous disruptions in the plasma membrane that can be used for delivery of small molecules but can induce cell damage and lacks spatiotemporal control of temperature exposure.[154] Nanotechnology offers the possibility of achieving a localised thermal disruption with precise spatiotemporal control of the heat generated. We recently proposed the use of biorthogonal chemistry as universal tool for cell surface engineering with MNPs (see also Section 1.5 and Figure 1.10) and demonstrated that the heat generated by the MNPs anchored on the cell membrane induced a change in its fluidity. We took advantage of this increase in fluidity to promote the internalisation of cell-impermeant small fluorescent molecules and of small interfering RNA (siRNA).[45] Interestingly, the heat generated by the nanoparticles had no detrimental effect on the cell viability, as indicated by different assays evaluating cell morphology, cell cycle, production of ROS and apoptosis-necrosis events. However, cells were able to sense the thermal stimulus, as demonstrated by the increased expression of HSPs. Our localised hyperthermia-promoted transfection method resulted more benign for the cells when compared to standard transfection agents such as Lipofectamine, while attaining a similar transfection efficiency. More recently, we used this approach to deliver siRNA into dendritic cells (DCs), which are antigen-presenting cells with important roles in cancer immunomodulation.[46] Indoleamine 2,3-dioxygenase 1 gene (*IDO1*) is frequently upregulated in various cancers and correlates with poor prognosis by promoting an immunosuppressive tumour microenvironment. Silencing IDO1 in DCs represents a promising gene therapy strategy, given their pivotal role in regulating T-cell activation and function. *IDO1* gene expression was reduced by approximately 70% in THP-1-derived dendritic cells (DCs) following our magnetothermal transfection. The silencing efficiency achieved with MH was again comparable to that obtained using the gold-standard Lipofectamine reagent, while exhibiting markedly lower cytotoxicity. Beyond

gene knockdown, *IDO1* silencing induced significant immunomodulatory effects: mRNA levels of pro-inflammatory cytokines *IL-6*, *TNF-α*, and *IL-12A* were upregulated, whereas the anti-inflammatory cytokine *IL-10* was downregulated. This cytokine profile suggests a shift toward a more immunogenic phenotype, potentially enhancing DC activation and priming for subsequent T-cell-mediated antitumor responses. Therefore, MH-mediated transfection can be an effective strategy for intracellular delivery of gene-silencing agents in hard-to-transfect cells such as DCs and highlight the therapeutic relevance of *IDO1* knockdown in overcoming the immunosuppressive tumour microenvironment.

3.6 Conclusions

Nanomaterial-based magnetic and optical hyperthermia has been extensively studied to date mainly in the frame of cancer treatment, although in recent years new biomedical applications have been reported, such as drug delivery, antibacterial treatments and activation of cellular functions. However, for future developments in the field, several challenges must be addressed.

While significant progress has been made in the design, synthesis, characterization, and functionalization of nanomaterials for hyperthermia applications, including the fabrication of magneto-plasmonic hybrids that combine magnetic and optical responsiveness within a single structure. Despite these achievements, the field still faces challenges related to reproducibility across nanoparticle batches, scale up of the nanomaterial synthesis and production under Good Manufacturing Practices (GMP); all these are essential for clinical translation. It should be noted that regulatory approval for nanomaterials remains a lengthy and costly process.

For magnetic hyperthermia, optimizing heating efficiency under clinically relevant conditions is necessary. High heating performance would allow lower dosages or enable multiple treatment cycles with a single administration. However, nanoparticles that exhibit exceptional heating in aqueous suspensions often fail to replicate this performance *in vitro* or *in vivo*. This discrepancy is partly due to laboratory measurements of SLP under high-frequency, high-field conditions that differ from clinical parameters. Furthermore, magnetic hyperthermia instrumentation often limits user control over field settings. Therefore, designing nanoparticles that heat effectively under low-field conditions approved for clinical use is essential. Another key consideration is the mechanism of heat generation. As

explained in the first part of this class, single-domain magnetic nanoparticles typically convert magnetic energy into heat via Néel and Brownian relaxation. Once internalized by cells, Brownian relaxation is significantly reduced or suppressed due to high intracellular viscosity and restricted particle mobility. Consequently, assessing heating efficiency under conditions that mimic the cellular environment (e.g. viscous media such as glycerol, 3D models) is crucial.

Another challenge is related to the development of robust and accurate intracellular thermometers for precise mapping of temperature gradients during hyperthermia. These so-called "*nanothermometers*" must combine high sensitivity and rapid response with stability under complex biological conditions, (variable pH, heterogeneous chemical environments). Ideally, they should measure temperature in the immediate vicinity of the heating nanoparticle, which has led to interest in multifunctional probes that integrate heating and sensing capabilities. While this approach is conceptually appealing, it adds complexity to the nanomaterial structure and will likely encounter regulatory hurdles for clinical translation. Current state-of-the-art shows that plasmonic nanoparticle-based thermometers offer superior spatial resolution compared to magnetic counterparts, which still face limitations in precise temperature mapping. For magnetic hyperthermia, magnetic particle imaging (MPI) has emerged as a promising solution for non-invasive, real-time monitoring of nanoparticle distribution and potentially temperature estimation.

Another remaining challenge is assessing real-time cellular responses to heat. In optical hyperthermia, coupling a laser source to a fluorescence microscope is relatively straightforward, enabling the use of fluorescent markers to monitor processes such as apoptosis, necrosis, membrane permeabilization, and changes in fluidity. In contrast, integrating fluorescence microscopy with magnetic hyperthermia systems is more technically demanding, as alternating magnetic fields can heat and damage ferromagnetic components of the microscopes, although some commercially available options have been developed in the last years.

As discussed in the previous section, nanomaterial-based hyperthermia also offers opportunities for future developments in areas such as remote modulation of biological functions, the use of sub-lethal hyperthermia as a versatile tool for intracellular drug delivery, transfection, membrane biophysics studies.

3.7 Further reading

Note: the most important articles are highlighted by ***

1) Surg. Oncol. Clin. N. Am., **2011**, 20, 229-235 - The Ongoing History of Thermal Therapy for Cancer; DOI: 10.1016/j.soc.2010.11.001 ***

2) Crit. Rev. Oncol. Hematol., **2002**, 43, 33-56 - The Cellular and Molecular Basis of Hyperthermia. DOI: 10.1016/S1040-8428(01)00179-2

3) Fratila, Raluca Maria; Martínez de la Fuente, J. *Nanomaterials for Magnetic and Optical Hyperthermia Applications*; Elsevier, 2019. ***

4) Chem. Soc. Rev., **2021**, 50, 11614-11667 - Magnetic Nanoparticles and Clusters for Magnetic Hyperthermia: Optimizing Their Heat Performance and Developing Combinatorial Therapies to Tackle Cancer; https://doi.org/10.1039/D1CS00427A ***

5) Advanced Science, **2020**, 7- Smart Gold Nanostructures for Light Mediated Cancer Theranostics: Combining Optical Diagnostics with Photothermal Therapy. https://doi.org/10.1002/advs.201903441

6) Nano Select, **2025**, 6 - Near-Infrared Nanoparticle-Mediated Photothermal Cancer Therapy: A Comprehensive Review of Advances in Monitoring and Controlling Thermal Effects for Effective Cancer Treatment; https://doi.org/10.1002/nano.202400107

7) Nanoscale, **2024**, 16 (33), 15446-15464 - Magnetic Iron Oxide Nanogels for Combined Hyperthermia and Drug Delivery for Cancer Treatment; https://doi.org/10.1039/D4NR02058H

8) Adv. Drug. Deliv. Rev., **2019**, 138, 326-343 - Triggering Antitumoural Drug Release and Gene Expression by Magnetic Hyperthermia; https://doi.org/10.1016/j.addr.2018.10.004 ***

9) Adv. Funct. Mater., **2021**, 31 - Combining Photothermal-Photodynamic Therapy Mediated by Nanomaterials with Immune Checkpoint Blockade for Metastatic Cancer Treatment and Creation of Immune Memory; https://doi.org/10.1002/adfm.202010777 ***

10) Nanoscale **2025** - Magnetic Hyperthermia in Focus: Emerging Non-Cancer Applications of Magnetic Nanoparticles; https://doi.org/10.1039/D5NR03329B ***

References

(1) Prescher, J. A.; Bertozzi, C. R. Chemistry in Living Systems. *Nat Chem Biol* **2005**, *1* (1), 13–21. https://doi.org/10.1038/nchembio0605-13.

(2) Idiago-López, J.; Moreno-Antolín, E.; de la Fuente, J. M.; Fratila, R. M. Nanoparticles and Bioorthogonal Chemistry Joining Forces for Improved Biomedical Applications. *Nanoscale Adv* **2021**, *3* (5), 1261–1292. https://doi.org/10.1039/D0NA00873G.

(3) Du, J.; Meledeo, M. A.; Wang, Z.; Khanna, H. S.; Paruchuri, V. D. P.; Yarema, K. J. Metabolic Glycoengineering: Sialic Acid and Beyond. *Glycobiology* **2009**, *19* (12), 1382–1401. https://doi.org/10.1093/glycob/cwp115.

(4) Ying, L.; Xu, J.; Han, D.; Zhang, Q.; Hong, Z. The Applications of Metabolic Glycoengineering. *Front Cell Dev Biol* **2022**, *10*. https://doi.org/10.3389/fcell.2022.840831.

(5) Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. In Vivo Imaging of Membrane-Associated Glycans in Developing Zebrafish. *Science (1979)* **2008**, *320* (5876), 664–667. https://doi.org/10.1126/science.1155106.In.

(6) Moons, S. J.; Adema, G. J.; Derks, M. T.; Boltje, T. J.; Büll, C. Sialic Acid Glycoengineering Using N-Acetylmannosamine and Sialic Acid Analogs. *Glycobiology* **2019**. https://doi.org/10.1093/glycob/cwz026.

(7) Saxon, E.; Bertozzi, C. R. Cell Surface Engineering by a Modified Staudinger Reaction. *Science (1979)* **2000**, *287* (5460), 2007–2010. https://doi.org/10.1126/science.287.5460.2007.

(8) Chang, P. V.; Prescher, J. A.; Hangauer, M. J.; Bertozzi, C. R. Imaging Cell Surface Glycans with Bioorthogonal Chemical Reporters. *J Am Chem Soc* **2007**, *129* (27), 8400–8401. https://doi.org/10.1021/ja0702380.

(9) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A Stepwise Huisgen Cycloaddition Process Catalyzed by Copper (I): Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem., Int. Ed. Engl.* **2002**, *41* (14), 2596–2599.

(10) Tornøe, C. W.; Christensen, C.; Meldal, M. Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *Journal of Organic Chemistry* **2002**, *67* (9), 3057–3064. https://doi.org/10.1021/jo011148j.

(11) Kennedy, D. C.; McKay, C. S.; Legault, M. C. B.; Danielson, D. C.; Blake, J. A.; Pegoraro, A. F.; Stolow, A.; Mester, Z.; Pezacki, J. P. Cellular Consequences of Copper Complexes Used to Catalyze Bioorthogonal Click Reactions. *J Am Chem Soc* **2011**, *133* (44), 17993–18001. https://doi.org/10.1021/ja2083027.

(12) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. A Strain-Promoted [3 + 2] Azide-Alkyne Cycloaddition for Covalent Modification of Biomolecules in Living Systems. *J Am Chem Soc* **2004**, *126* (46), 15046–15047. https://doi.org/10.1021/ja044996f.

(13) Debets, M. F.; van Hest, J. C. M.; Rutjes, F. P. J. T. Bioorthogonal Labelling of Biomolecules: New Functional Handles and Ligation Methods. *Org Biomol Chem* **2013**, *11* (38), 6439–6455. https://doi.org/10.1039/c3ob41329b.

(14) Rigolot, V.; Biot, C.; Lion, C. To View Your Biomolecule, Click inside the Cell. *Angewandte Chemie International Edition* **2021**, *60* (43), 23084–23105. https://doi.org/10.1002/anie.202101502.

(15) Devaraj, N. K.; Weissleder, R.; Hilderbrand, S. A. Tetrazine-Based Cycloadditions: Application to Pretargeted Live Cell Imaging. *Bioconjug Chem* **2008**, *19* (12), 2297–2299. https://doi.org/10.1021/bc8004446.

(16) Blackman, M. L.; Royzen, M.; Fox, J. M. Tetrazine Ligation: Fast Bioconjugation Based on Inverse-Electron-Demand Diels–Alder Reactivity. *J Am Chem Soc* **2008**, *130* (41), 13518–13519. https://doi.org/10.1021/ja8053805.
(17) Rondon, A.; Degoul, F. Antibody Pretargeting Based on Bioorthogonal Click Chemistry for Cancer Imaging and Targeted Radionuclide Therapy. *Bioconjug Chem* **2020**, *31* (2), 159–173. https://doi.org/10.1021/acs.bioconjchem.9b00761.
(18) Fairbanks, B. D.; Macdougall, L. J.; Mavila, S.; Sinha, J.; Kirkpatrick, B. E.; Anseth, K. S.; Bowman, C. N. Photoclick Chemistry: A Bright Idea. *Chem Rev* **2021**, *121* (12), 6915–6990. https://doi.org/10.1021/acs.chemrev.0c01212.
(19) Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. Chemical Remodelling of Cell Surfaces in Living Animals. *Nature* **2004**, *430* (August), 873–878. https://doi.org/10.1038/nature02817.
(20) Laughlin, S. T.; Bertozzi, C. R. In Vivo Imaging of Caenorhabditis Elegans Glycans. *ACS Chem Biol* **2009**, *4* (12), 1068–1072. https://doi.org/10.1021/cb900254y.
(21) Rossin, R.; Renart Verkerk, P.; van den Bosch, S. M.; Vulders, R. C. M.; Verel, I.; Lub, J.; Robillard, M. S. In Vivo Chemistry for Pretargeted Tumor Imaging in Live Mice. *Angewandte Chemie* **2010**, *122* (19), 3447–3450. https://doi.org/10.1002/ange.200906294.
(22) Patra, M.; Zarschler, K.; Pietzsch, H.-J.; Stephan, H.; Gasser, G. New Insights into the Pretargeting Approach to Image and Treat Tumours. *Chem Soc Rev* **2016**, *45* (23), 6415–6431. https://doi.org/10.1039/C5CS00784D.
(23) Devaraj, N. K. The Future of Bioorthogonal Chemistry. *ACS Cent Sci* **2018**, acscentsci.8b00251. https://doi.org/10.1021/acscentsci.8b00251.
(24) Mejia Oneto, J. M.; Khan, I.; Seebald, L.; Royzen, M. In Vivo Bioorthogonal Chemistry Enables Local Hydrogel and Systemic Pro-Drug To Treat Soft Tissue Sarcoma. *ACS Cent Sci* **2016**, *2* (7), 476–482. https://doi.org/10.1021/acscentsci.6b00150.
(25) Xie, X.; Li, B.; Wang, J.; Zhan, C.; Huang, Y.; Zeng, F.; Wu, S. Tetrazine-Mediated Bioorthogonal System for Prodrug Activation, Photothermal Therapy, and Optoacoustic Imaging. *ACS Appl Mater Interfaces* **2019**, *11* (45), 41875–41888. https://doi.org/10.1021/acsami.9b13374.
(26) *https://www.shasqi.com*.
(27) Wu, K.; Yee, N. A.; Srinivasan, S.; Mahmoodi, A.; Zakharian, M.; Mejia Oneto, J. M.; Royzen, M. Click Activated Protodrugs against Cancer Increase the Therapeutic Potential of Chemotherapy through Local Capture and Activation. *Chem Sci* **2021**, *12* (4), 1259–1271. https://doi.org/10.1039/D0SC06099B.
(28) Richard P. Feynman. There's Plenty of Room at the Bottom. *Eng Sci* **1960**, *23*, 22–35.
(29) Roduner, E. Size Matters: Why Nanomaterials Are Different. *Chem Soc Rev* **2006**, *35* (7), 583. https://doi.org/10.1039/b502142c.
(30) Whitesides, G. M. The "right" Size in Nanobiotechnology. *Nat Biotechnol* **2003**, *21* (10), 1161–1165. https://doi.org/10.1038/nbt872.
(31) Terai, T.; Nagano, T. Fluorescent Probes for Bioimaging Applications. *Curr Opin Chem Biol* **2008**, *12* (5), 515–521. https://doi.org/10.1016/j.cbpa.2008.08.007.
(32) Bernardin, A.; Guyon, L.; Delannoy, P.; Bonnaffe, D.; Texier, I. Copper-Free Click Chemistry for Highly Luminescent Quantum Dot Conjugates : Application to in Vi W o Metabolic Imaging. *Bioconjugate Chem.* **2010**, *21*, 583–588. https://doi.org/10.1021/bc900564w.

(33) Hsieh, F.-J.; Sotoma, S.; Lin, H.-H.; Cheng, C.-Y.; Yu, T.-Y.; Hsieh, C.-L.; Lin, C.-H.; Chang, H.-C. Bioorthogonal Fluorescent Nanodiamonds for Continuous Long-Term Imaging and Tracking of Membrane Proteins. *ACS Appl Mater Interfaces* **2019**, *11* (22), 19774–19781. https://doi.org/10.1021/acsami.9b03640.

(34) Liu, R.; Zhao, J.; Han, G.; Zhao, T.; Zhang, R.; Liu, B.; Liu, Z.; Zhang, C.; Yang, L.; Zhang, Z. Click-Functionalized SERS Nanoprobes with Improved Labeling Efficiency and Capability for Cancer Cell Imaging. *ACS Appl Mater Interfaces* **2017**, *9* (44), 38222–38229. https://doi.org/10.1021/acsami.7b10409.

(35) Lee, S.; Yoon, H. I.; Na, J. H.; Jeon, S.; Lim, S.; Koo, H.; Han, S. S.; Kang, S. W.; Park, S. J.; Moon, S. H.; Park, J. H.; Cho, Y. W.; Kim, B. S.; Kim, S. K.; Lee, T.; Kim, D.; Lee, S.; Pomper, M. G.; Kwon, I. C.; Kim, K. In Vivo Stem Cell Tracking with Imageable Nanoparticles That Bind Bioorthogonal Chemical Receptors on the Stem Cell Surface. *Biomaterials* **2017**, *139*, 12–29. https://doi.org/10.1016/j.biomaterials.2017.05.050.

(36) Haun, J. B.; Devaraj, N. K.; Hilderbrand, S. A.; Lee, H.; Weissleder, R. Bioorthogonal Chemistry Amplifies Nanoparticle Binding and Enhances the Sensitivity of Cell Detection. *Nat Nano* **2010**, *5* (9), 660–665.

(37) Peterson, V. M.; Castro, C. M.; Lee, H.; Weissleder, R. Orthogonal Amplification of Nanoparticles for Improved Diagnostic Sensing. *ACS Nano* **2012**, *6* (4), 3506–3513. https://doi.org/10.1021/nn300536y.

(38) Liong, M.; Fernandez-Suarez, M.; Issadore, D.; Min, C.; Tassa, C.; Reiner, T.; Fortune, S. M.; Toner, M.; Lee, H.; Weissleder, R. Specific Pathogen Detection Using Bioorthogonal Chemistry and Diagnostic Magnetic Resonance. *Bioconjugate Chem.* **2011**, *22* (12), 2390–2394. https://doi.org/10.1021/bc200490r.

(39) Yoon, H. Y.; Shin, M. L.; Shim, M. K.; Lee, S.; Na, J. H.; Koo, H.; Lee, H.; Kim, J. H.; Lee, K. Y.; Kim, K.; Kwon, I. C. Artificial Chemical Reporter Targeting Strategy Using Bioorthogonal Click Reaction for Improving Active-Targeting Efficiency of Tumor. *Mol Pharm* **2017**, *14* (5), 1558–1570. https://doi.org/10.1021/acs.molpharmaceut.6b01083.

(40) Koo, H.; Lee, S.; Na, J. H.; Kim, S. H.; Hahn, S. K.; Choi, K.; Kwon, I. C.; Jeong, S. Y.; Kim, K. Bioorthogonal Copper-Free Click Chemistry in Vivo for Tumor-Targeted Delivery of Nanoparticles. *Angewandte Chemie - International Edition* **2012**, *51* (47), 11836–11840. https://doi.org/10.1002/anie.201206703.

(41) Lee, S.; Koo, H.; Na, J. H.; Han, S. J.; Min, H. S.; Lee, S. J.; Kim, S. K.; Yun, S. H.; Jeong, S. Y.; Kwon, I. C.; Choi, K.; Kim, K. Chemical Tumor-Targeting of Nanoparticles Based on Metabolic Glycoengineering and Click Chemistry. *ACS Nano* **2014**, *8* (3), 2048–2063.

(42) Hapuarachchige, S.; Kato, Y.; Artemov, D. Bioorthogonal Two-Component Drug Delivery in HER2(+) Breast Cancer Mouse Models. *Sci Rep* **2016**, *6* (1), 24298. https://doi.org/10.1038/srep24298.

(43) Lee, S. H.; Park, O. K.; Kim, J.; Shin, K.; Pack, C. G.; Kim, K.; Ko, G.; Lee, N.; Kwon, S.-H.; Hyeon, T. Deep Tumor Penetration of Drug-Loaded Nanoparticles by Click Reaction-Assisted Immune Cell Targeting Strategy. *J Am Chem Soc* **2019**, *141* (35), 13829–13840. https://doi.org/10.1021/jacs.9b04621.

(44) Taiariol, L.; Chaix, C.; Farre, C.; Moreau, E. Click and Bioorthogonal Chemistry: The Future of Active Targeting of Nanoparticles for Nanomedicines? *Chem Rev* **2021**, acs.chemrev.1c00484. https://doi.org/10.1021/acs.chemrev.1c00484.

(45) Idiago-López, J.; Ferreira, D.; Asín, L.; Moros, M.; Armenia, I.; Grazú, V.; Fernandes, A. R.; de la Fuente, J. M.; Baptista, P. V.; Fratila, R. M. Membrane-Localized Magnetic Hyperthermia Promotes Intracellular Delivery of Cell-Impermeant Probes. *Nanoscale* **2024**, *16* (32), 15176–15195. https://doi.org/10.1039/D4NR01955E.

(46) Ferreira, D.; Asín, L.; Idiago-López, J.; Grazú, V.; de la Fuente, J. M.; Fratila, R. M.; Baptista, P. V.; Fernandes, A. R. Heat up, Silence on: IDO1 Gene Silencing in THP-1-Derived Dendritic Cells Triggered by Magnetic Hyperthermia. *Cancer Immunology, Immunotherapy* **2025**, *74* (9), 292. https://doi.org/10.1007/s00262-025-04148-3.

(47) Row, R. D.; Prescher, J. A. Constructing New Bioorthogonal Reagents and Reactions. *Acc Chem Res* **2018**, *51* (5), 1073–1081. https://doi.org/10.1021/acs.accounts.7b00606.

(48) Zheng, X.; Zhang, P.; Fu, Z.; Meng, S.; Dai, L.; Yang, H. Applications of Nanomaterials in Tissue Engineering. *RSC Adv* **2021**, *11* (31), 19041–19058. https://doi.org/10.1039/D1RA01849C.

(49) Davis, F. F. The Origin of Pegnology. *Adv Drug Deliv Rev* **2002**, *54* (4), 457–458. https://doi.org/10.1016/S0169-409X(02)00021-2.

(50) Jokerst, J. V; Lobovkina, T.; Zare, R. N.; Gambhir, S. S. Nanoparticle PEGylation for Imaging and Therapy. *Nanomedicine* **2011**, *6* (4), 715–728. https://doi.org/10.2217/nnm.11.19.

(51) Saravanan, M.; Yadalam, P. K.; Anegundi, R. V.; Ramadoss, R.; Krishnamurthi, I.; Arumuganainar, D. Gold Nanoparticles in Tissue Engineering and Regeneration. In *Gold Nanoparticles for Drug Delivery*; Elsevier, 2024; pp 331–352. https://doi.org/10.1016/B978-0-443-19061-2.00011-0.

(52) Meher, A.; Tandi, A.; Moharana, S.; Chakroborty, S.; Mohapatra, S. S.; Mondal, A.; Dey, S.; Chandra, P. Silver Nanoparticle for Biomedical Applications: A Review. *Hybrid Advances* **2024**, *6*, 100184. https://doi.org/10.1016/j.hybadv.2024.100184.

(53) Nosrati, H.; Heydari, M. Titanium Dioxide Nanoparticles: A Promising Candidate for Wound Healing Applications. *Burns Trauma* **2025**, *13*. https://doi.org/10.1093/burnst/tkae069.

(54) Guzman-Rocha, D. A.; Aranda-Herrera, B.; Acosta Torres, L. S.; Arenas-Arrocena, Ma. C.; Garcia-Contreras, R. Modified Scaffolds With Magnetic Nanoparticles in Periodontal Tissue Regeneration. *Nano Select* **2025**, *6* (1). https://doi.org/10.1002/nano.202400077.

(55) Moros, M.; Idiago-López, J.; Asín, L.; Moreno-Antolín, E.; Beola, L.; Grazú, V.; Fratila, R. M.; Gutiérrez, L.; de la Fuente, J. M. Triggering Antitumoural Drug Release and Gene Expression by Magnetic Hyperthermia. *Adv Drug Deliv Rev* **2019**, *138*, 326–343. https://doi.org/10.1016/j.addr.2018.10.004.

(56) Wang, Q.; Zhang, Y.; Ma, Y.; Wang, M.; Pan, G. Nano-Crosslinked Dynamic Hydrogels for Biomedical Applications. *Mater Today Bio* **2023**, *20*, 100640. https://doi.org/10.1016/j.mtbio.2023.100640.

(57) Girão, A. F.; Serrano, M. C.; Completo, A.; Marques, P. A. A. P. Is Graphene Shortening the Path toward Spinal Cord Regeneration? *ACS Nano* **2022**, *16* (9), 13430–13467. https://doi.org/10.1021/acsnano.2c04756.

(58) *Nanomaterials in Tissue Engineering Fabrication and Applications*; Gaharwar, A. K., Sant, S., Hancock, M. J., Hacking, S. A., Eds.

(59) Han, F.; Meng, Q.; Xie, E.; Li, K.; Hu, J.; Chen, Q.; Li, J.; Han, F. Engineered Biomimetic Micro/Nano-Materials for Tissue Regeneration. *Front Bioeng Biotechnol* **2023**, *11*. https://doi.org/10.3389/fbioe.2023.1205792.

(60) Sharma, R.; Kumar, S.; Bhawna; Gupta, A.; Dheer, N.; Jain, P.; Singh, P.; Kumar, V. An Insight of Nanomaterials in Tissue Engineering from Fabrication to Applications. *Tissue Eng Regen Med* **2022**, *19* (5), 927–960. https://doi.org/10.1007/s13770-022-00459-z.

(61) Abadi, B.; Goshtasbi, N.; Bolourian, S.; Tahsili, J.; Adeli-Sardou, M.; Forootanfar, H. Electrospun Hybrid Nanofibers: Fabrication, Characterization, and Biomedical Applications. *Front Bioeng Biotechnol* **2022**, *10*. https://doi.org/10.3389/fbioe.2022.986975.

(62) Dai, X.; Nie, W.; Shen, H.; Machens, H.-G.; Böker, K.; Taheri, S.; Lehmann, W.; Shen, Y.; Schilling, A. F. Electrospinning Based Biomaterials for Biomimetic Fabrication, Bioactive Protein Delivery and Wound Regenerative Repair. *Regen Biomater* **2025**, *12*. https://doi.org/10.1093/rb/rbae139.

(63) Hoveidaei, A. H.; Sadat-Shojai, M.; Mosalamiaghili, S.; Salarikia, S. R.; Roghani-shahraki, H.; Ghaderpanah, R.; Ersi, M. H.; Conway, J. D. Nano-Hydroxyapatite Structures for Bone Regenerative Medicine: Cell-Material Interaction. *Bone* **2024**, *179*, 116956. https://doi.org/10.1016/j.bone.2023.116956.

(64) Liu, H.; Peng, H.; Wu, Y.; Zhang, C.; Cai, Y.; Xu, G.; Li, Q.; Chen, X.; Ji, J.; Zhang, Y.; OuYang, H. W. The Promotion of Bone Regeneration by Nanofibrous Hydroxyapatite/Chitosan Scaffolds by Effects on Integrin-BMP/Smad Signaling Pathway in BMSCs. *Biomaterials* **2013**, *34* (18), 4404–4417. https://doi.org/10.1016/j.biomaterials.2013.02.048.

(65) Singh, B. N.; Pramanik, K. Development of Novel Silk Fibroin/Polyvinyl Alcohol/Sol–Gel Bioactive Glass Composite Matrix by Modified Layer by Layer Electrospinning Method for Bone Tissue Construct Generation. *Biofabrication* **2017**, *9* (1), 015028. https://doi.org/10.1088/1758-5090/aa644f.

(66) Liang, X.; Duan, P.; Gao, J.; Guo, R.; Qu, Z.; Li, X.; He, Y.; Yao, H.; Ding, J. Bilayered PLGA/PLGA-HAp Composite Scaffold for Osteochondral Tissue Engineering and Tissue Regeneration. *ACS Biomater Sci Eng* **2018**, *4* (10), 3506–3521. https://doi.org/10.1021/acsbiomaterials.8b00552.

(67) Li, Y.; Liu, Y.; Li, R.; Bai, H.; Zhu, Z.; Zhu, L.; Zhu, C.; Che, Z.; Liu, H.; Wang, J.; Huang, L. Collagen-Based Biomaterials for Bone Tissue Engineering. *Mater Des* **2021**, *210*, 110049. https://doi.org/10.1016/j.matdes.2021.110049.

(68) Cheng, G.; Yin, C.; Tu, H.; Jiang, S.; Wang, Q.; Zhou, X.; Xing, X.; Xie, C.; Shi, X.; Du, Y.; Deng, H.; Li, Z. Controlled Co-Delivery of Growth Factors through Layer-by-Layer Assembly of Core–Shell Nanofibers for Improving Bone Regeneration. *ACS Nano* **2019**, *13* (6), 6372–6382. https://doi.org/10.1021/acsnano.8b06032.

(69) Owen, G. Rh.; Jackson, J.; Chehroudi, B.; Burt, H.; Brunette, D. M. A PLGA Membrane Controlling Cell Behaviour for Promoting Tissue Regeneration. *Biomaterials* **2005**, *26* (35), 7447–7456. https://doi.org/10.1016/j.biomaterials.2005.05.055.

(70) de Vicente, J. C.; Recio, O.; Martín-Villa, L.; Junquera, L. M.; López-Arranz, J. S. Histomorphometric Evaluation of Guided Bone Regeneration around Implants with SLA Surface: An Experimental Study in Beagle Dogs. *Int J Oral Maxillofac Surg* **2006**, *35* (11), 1047–1053. https://doi.org/10.1016/j.ijom.2006.06.008.

(71) Roriz, V. M.; Souza, S. L. S.; Taba, M.; Palioto, D. B.; Grisi, M. F. M. Treatment of Class III Furcation Defects With Expanded Polytetrafluoroethylene Membrane Associated or Not With Anorganic Bone Matrix/Synthetic Cell-Binding Peptide: A Histologic and

Histomorphometric Study in Dogs. *J Periodontol* **2006**, *77* (3), 490–497. https://doi.org/10.1902/jop.2006.050081.

(72) Kim, E. J.; Yoon, S. J.; Yeo, G.-D.; Pai, C.-M.; Kang, I.-K. Preparation of Biodegradable PLA/PLGA Membranes with PGA Mesh and Their Application for Periodontal Guided Tissue Regeneration. *Biomedical Materials* **2009**, *4* (5), 055001. https://doi.org/10.1088/1748-6041/4/5/055001.

(73) Carlo Reis, E. C.; Borges, A. P. B.; Araújo, M. V. F.; Mendes, V. C.; Guan, L.; Davies, J. E. Periodontal Regeneration Using a Bilayered PLGA/Calcium Phosphate Construct. *Biomaterials* **2011**, *32* (35), 9244–9253. https://doi.org/10.1016/j.biomaterials.2011.08.040.

(74) Liu, Z.; Chen, X.; Zhang, Z.; Zhang, X.; Saunders, L.; Zhou, Y.; Ma, P. X. Nanofibrous Spongy Microspheres To Distinctly Release MiRNA and Growth Factors To Enrich Regulatory T Cells and Rescue Periodontal Bone Loss. *ACS Nano* **2018**, *12* (10), 9785–9799. https://doi.org/10.1021/acsnano.7b08976.

(75) Lu, Y.; Lyu, H.; Richardson, A. G.; Lucas, T. H.; Kuzum, D. Flexible Neural Electrode Array Based-on Porous Graphene for Cortical Microstimulation and Sensing. *Sci Rep* **2016**, *6* (1), 33526. https://doi.org/10.1038/srep33526.

(76) Girão, A. F.; Sousa, J.; Domínguez-Bajo, A.; González-Mayorga, A.; Bdikin, I.; Pujades-Otero, E.; Casañ-Pastor, N.; Hortigüela, M. J.; Otero-Irurueta, G.; Completo, A.; Serrano, M. C.; Marques, P. A. A. P. 3D Reduced Graphene Oxide Scaffolds with a Combinatorial Fibrous-Porous Architecture for Neural Tissue Engineering. *ACS Appl Mater Interfaces* **2020**, *12* (35), 38962–38975. https://doi.org/10.1021/acsami.0c10599.

(77) Park, D.-W.; Ness, J. P.; Brodnick, S. K.; Esquibel, C.; Novello, J.; Atry, F.; Baek, D.-H.; Kim, H.; Bong, J.; Swanson, K. I.; Suminski, A. J.; Otto, K. J.; Pashaie, R.; Williams, J. C.; Ma, Z. Electrical Neural Stimulation and Simultaneous *in Vivo* Monitoring with Transparent Graphene Electrode Arrays Implanted in GCaMP6f Mice. *ACS Nano* **2018**, *12* (1), 148–157. https://doi.org/10.1021/acsnano.7b04321.

(78) Jia, Z.; Li, W. Nanosystems-Enabled Regenerative Strategies for Spinal Cord Injury: Recent Advances and Future Prospects. *Mater Des* **2024**, *237*, 112617. https://doi.org/10.1016/j.matdes.2023.112617.

(79) Usmani, S.; Franceschi Biagioni, A.; Medelin, M.; Scaini, D.; Casani, R.; Aurand, E. R.; Padro, D.; Egimendia, A.; Ramos Cabrer, P.; Scarselli, M.; De Crescenzi, M.; Prato, M.; Ballerini, L. Functional Rewiring across Spinal Injuries via Biomimetic Nanofiber Scaffolds. *Proceedings of the National Academy of Sciences* **2020**, *117* (41), 25212–25218. https://doi.org/10.1073/pnas.2005708117.

(80) Fu, W.; Sun, S.; Cheng, Y.; Ma, J.; Hu, Y.; Yang, Z.; Yao, H.; Zhang, Z. Opportunities and Challenges of Nanomaterials in Wound Healing: Advances, Mechanisms, and Perspectives. *Chemical Engineering Journal* **2024**, *495*, 153640. https://doi.org/10.1016/j.cej.2024.153640.

(81) Liu, T.; Lu, Y.; Zhan, R.; Qian, W.; Luo, G. Nanomaterials and Nanomaterials-Based Drug Delivery to Promote Cutaneous Wound Healing. *Adv Drug Deliv Rev* **2023**, *193*, 114670. https://doi.org/10.1016/j.addr.2022.114670.

(82) Huang, Y.; Bai, L.; Yang, Y.; Yin, Z.; Guo, B. Biodegradable Gelatin/Silver Nanoparticle Composite Cryogel with Excellent Antibacterial and Antibiofilm Activity and Hemostasis for Pseudomonas Aeruginosa-Infected Burn Wound Healing. *J Colloid Interface Sci* **2022**, *608*, 2278–2289. https://doi.org/10.1016/j.jcis.2021.10.131.

(83) Sun, H.; Gao, N.; Dong, K.; Ren, J.; Qu, X. Graphene Quantum Dots-Band-Aids Used for Wound Disinfection. *ACS Nano* **2014**, *8* (6), 6202–6210. https://doi.org/10.1021/nn501640q.
(84) Liang, Y.; Zhao, X.; Hu, T.; Chen, B.; Yin, Z.; Ma, P. X.; Guo, B. Adhesive Hemostatic Conducting Injectable Composite Hydrogels with Sustained Drug Release and Photothermal Antibacterial Activity to Promote Full-Thickness Skin Regeneration During Wound Healing. *Small* **2019**, *15* (12). https://doi.org/10.1002/smll.201900046.
(85) Niemiec, S. M.; Louiselle, A. E.; Hilton, S. A.; Dewberry, L. C.; Zhang, L.; Azeltine, M.; Xu, J.; Singh, S.; Sakthivel, T. S.; Seal, S.; Liechty, K. W.; Zgheib, C. Nanosilk Increases the Strength of Diabetic Skin and Delivers CNP-MiR146a to Improve Wound Healing. *Front Immunol* **2020**, *11*. https://doi.org/10.3389/fimmu.2020.590285.
(86) Wang, Y.; Li, P.; Xiang, P.; Lu, J.; Yuan, J.; Shen, J. Electrospun Polyurethane/Keratin/AgNP Biocomposite Mats for Biocompatible and Antibacterial Wound Dressings. *J Mater Chem B* **2016**, *4* (4), 635–648. https://doi.org/10.1039/C5TB02358K.
(87) Nosrati, H.; Aramideh Khouy, R.; Nosrati, A.; Khodaei, M.; Banitalebi-Dehkordi, M.; Ashrafi-Dehkordi, K.; Sanami, S.; Alizadeh, Z. Nanocomposite Scaffolds for Accelerating Chronic Wound Healing by Enhancing Angiogenesis. *J Nanobiotechnology* **2021**, *19* (1), 1. https://doi.org/10.1186/s12951-020-00755-7.
(88) Lai, H.-J.; Kuan, C.-H.; Wu, H.-C.; Tsai, J.-C.; Chen, T.-M.; Hsieh, D.-J.; Wang, T.-W. Tailored Design of Electrospun Composite Nanofibers with Staged Release of Multiple Angiogenic Growth Factors for Chronic Wound Healing. *Acta Biomater* **2014**, *10* (10), 4156–4166. https://doi.org/10.1016/j.actbio.2014.05.001.
(89) Chen, Z.; Xiao, L.; Hu, C.; Shen, Z.; Zhou, E.; Zhang, S.; Wang, Y. Aligned Lovastatin-Loaded Electrospun Nanofibers Regulate Collagen Organization and Reduce Scar Formation. *Acta Biomater* **2023**, *164*, 240–252. https://doi.org/10.1016/j.actbio.2023.04.015.
(90) Shou, Y.; Le, Z.; Cheng, H. S.; Liu, Q.; Ng, Y. Z.; Becker, D. L.; Li, X.; Liu, L.; Xue, C.; Yeo, N. J. Y.; Tan, R.; Low, J.; Kumar, A. R. K.; Wu, K. Z.; Li, H.; Cheung, C.; Lim, C. T.; Tan, N. S.; Chen, Y.; Liu, Z.; Tay, A. Mechano-Activated Cell Therapy for Accelerated Diabetic Wound Healing. *Advanced Materials* **2023**, *35* (47). https://doi.org/10.1002/adma.202304638.
(91) van der Zee, J. Heating the Patient: A Promising Approach? *Annals of Oncology* **2002**, *13* (8), 1173–1184. https://doi.org/10.1093/annonc/mdf280.
(92) Glazer, E. S.; Curley, S. A. The Ongoing History of Thermal Therapy for Cancer. *Surg Oncol Clin N Am* **2011**, *20* (2), 229–235. https://doi.org/10.1016/j.soc.2010.11.001.
(93) Dou, Y.; Hynynen, K.; Allen, C. To Heat or Not to Heat: Challenges with Clinical Translation of Thermosensitive Liposomes. *Journal of Controlled Release*. Elsevier B.V. 2017, pp 63–73. https://doi.org/10.1016/j.jconrel.2017.01.025.
(94) Fratila, R. Maria.; Martínez de la Fuente, J. *Nanomaterials for Magnetic and Optical Hyperthermia Applications*; Elsevier, 2019.
(95) Hildebrandt, B.; Wust, P.; Ahlers, O.; Dieing, A.; Sreenivasa, G.; Kerner, T.; Felix, R.; Riess, H. The Cellular and Molecular Basis of Hyperthermia. *Crit Rev Oncol Hematol* **2002**, *43* (1), 33–56.
(96) Mallory, M.; Gogineni, E.; Jones, G. C.; Greer, L.; Simone, C. B. Therapeutic Hyperthermia: The Old, the New, and the Upcoming. *Crit Rev Oncol Hematol* **2016**, *97*, 56–64. https://doi.org/10.1016/j.critrevonc.2015.08.003.

(97) Li, G. C.; Mivechi, N. F.; Weitzel, G. Heat Shock Proteins, Thermotolerance, and Their Relevance to Clinical Hyperthermia. *International Journal of Hyperthermia* **1995**, *11* (4), 459–488. https://doi.org/10.3109/02656739509022483.
(98) Wust, P.; Hildebrandt, B.; Sreenivasa, G.; Rau, B.; Gellermann, J.; Riess, H.; Felix, R.; Schlag, P. Hyperthermia in Combined Treatment of Cancer. *Lancet Oncol* **2002**, *3* (8), 487–497. https://doi.org/10.1016/S1470-2045(02)00818-5.
(99) Peeken, J. C.; Vaupel, P.; Combs, S. E. Integrating Hyperthermia into Modern Radiation Oncology: What Evidence Is Necessary? *Front Oncol* **2017**, *7* (June). https://doi.org/10.3389/fonc.2017.00132.
(100) Datta, N. R.; Krishnan, S.; Speiser, D. E.; Neufeld, E.; Kuster, N.; Bodis, S.; Hofmann, H. Magnetic Nanoparticle-Induced Hyperthermia with Appropriate Payloads: Paul Ehrlich's "Magic (Nano)Bullet" for Cancer Theranostics? *Cancer Treat Rev* **2016**, *50*, 217–227. https://doi.org/10.1016/j.ctrv.2016.09.016.
(101) Lee, J.; Kotov, N. A. Thermometer Design at the Nanoscale. *Nano Today* **2007**, *2* (1), 48–51. https://doi.org/10.1016/S1748-0132(07)70019-1.
(102) Brites, C. D. S.; Lima, P. P.; Silva, N. J. O.; Millán, A.; Amaral, V. S.; Palacio, F.; Carlos, L. D. Thermometry at the Nanoscale. *Nanoscale* **2012**, *4* (16), 4799. https://doi.org/10.1039/c2nr30663h.
(103) Chu, K. F.; Dupuy, D. E. Thermal Ablation of Tumours: Biological Mechanisms and Advances in Therapy. *Nat Rev Cancer* **2014**, *14* (3), 199–208. https://doi.org/10.1038/nrc3672.
(104) Gavilán, H.; Avugadda, S. K.; Fernández-Cabada, T.; Soni, N.; Cassani, M.; Mai, B. T.; Chantrell, R.; Pellegrino, T. Magnetic Nanoparticles and Clusters for Magnetic Hyperthermia: Optimizing Their Heat Performance and Developing Combinatorial Therapies to Tackle Cancer. *Chem Soc Rev* **2021**, *50* (20), 11614–11667. https://doi.org/10.1039/D1CS00427A.
(105) Chang, D.; Lim, M.; Goos, J. A. C. M.; Qiao, R.; Ng, Y. Y.; Mansfeld, F. M.; Jackson, M.; Davis, T. P.; Kavallaris, M. Biologically Targeted Magnetic Hyperthermia: Potential and Limitations. *Front Pharmacol* **2018**, *9*. https://doi.org/10.3389/fphar.2018.00831.
(106) Asín, L.; Stepien, G.; Moros, M.; Fratila, R. M.; de la Fuente, J. M. Magnetic Nanoparticles for Cancer Treatment Using Magnetic Hyperthermia. In *Clinical Applications of Magnetic Nanoparticles*; Thanh, N. T. K., Ed.; CRC Press, 2018; p 305.
(107) Hervault, A.; Thanh, N. T. K. Magnetic Nanoparticle-Based Therapeutic Agents for Thermo-Chemotherapy Treatment of Cancer. *Nanoscale* **2014**, *6* (20), 11553–11573. https://doi.org/10.1039/c4nr03482a.
(108) Wells, J.; Ortega, D.; Steinhoff, U.; Dutz, S.; Garaio, E.; Sandre, O.; Natividad, E.; Cruz, M. M.; Brero, F.; Southern, P.; Pankhurst, Q. A.; Spassov, S. Challenges and Recommendations for Magnetic Hyperthermia Characterization Measurements. *International Journal of Hyperthermia* **2021**, *38* (1), 447–460. https://doi.org/10.1080/02656736.2021.1892837.
(109) Noh, S.; Moon, S. H.; Shin, T.-H.; Lim, Y.; Cheon, J. Recent Advances of Magneto-Thermal Capabilities of Nanoparticles: From Design Principles to Biomedical Applications. *Nano Today* **2017**, *13*, 61–76. https://doi.org/10.1016/j.nantod.2017.02.006.
(110) Crăciunescu, I.; Palade, P.; Iacob, N.; Ispas, G. M.; Stanciu, A. E.; Kuncser, V.; Turcu, R. P. High-Performance Functionalized Magnetic Nanoparticles with Tailored Sizes and

Shapes for Localized Hyperthermia Applications. *The Journal of Physical Chemistry C* **2021**, *125* (20), 11132–11146. https://doi.org/10.1021/acs.jpcc.1c01053.
(111) Mayer, K. M.; Hafner, J. H. Localized Surface Plasmon Resonance Sensors. *Chem Rev* **2011**, *111* (6), 3828–3857. https://doi.org/10.1021/cr100313v.
(112) Tabish, T. A.; Dey, P.; Mosca, S.; Salimi, M.; Palombo, F.; Matousek, P.; Stone, N. Smart Gold Nanostructures for Light Mediated Cancer Theranostics: Combining Optical Diagnostics with Photothermal Therapy. *Advanced Science* **2020**, *7* (15). https://doi.org/10.1002/advs.201903441.
(113) Ukhtary, M. S.; Saito, R. Surface Plasmons in Graphene and Carbon Nanotubes. *Carbon N Y* **2020**, *167*, 455–474. https://doi.org/10.1016/j.carbon.2020.05.019.
(114) Van de Walle, A.; Figuerola, A.; Espinosa, A.; Abou-Hassan, A.; Estrader, M.; Wilhelm, C. Emergence of Magnetic Nanoparticles in Photothermal and Ferroptotic Therapies. *Mater Horiz* **2023**, *10* (11), 4757–4775. https://doi.org/10.1039/D3MH00831B.
(115) Roca, A. G.; Lopez-Barbera, J. F.; Lafuente, A.; Özel, F.; Fantechi, E.; Muro-Cruces, J.; Hémadi, M.; Sepulveda, B.; Nogues, J. Iron Oxide Nanoparticles (Fe3O4, g-Fe2O3 and FeO) as Photothermal Heat Mediators in the First, Second and Third Biological Windows. *Phys Rep* **2023**, *1043*, 1–35. https://doi.org/10.1016/j.physrep.2023.10.003.
(116) Qin, Z.; Bischof, J. C. Thermophysical and Biological Responses of Gold Nanoparticle Laser Heating. *Chem Soc Rev* **2012**, *41* (3), 1191–1217. https://doi.org/10.1039/C1CS15184C.
(117) Govorov, A. O.; Richardson, H. H. Generating Heat with Metal Nanoparticles. *Nano Today* **2007**, *2* (1), 30–38. https://doi.org/10.1016/S1748-0132(07)70017-8.
(118) Baffou, G.; Quidant, R. Thermo-Plasmonics: Using Metallic Nanostructures as Nano-Sources of Heat. *Laser Photon Rev* **2013**, *7* (2), 171–187. https://doi.org/10.1002/lpor.201200003.
(119) Beik, J.; Abed, Z.; Ghoreishi, F. S.; Hosseini-Nami, S.; Mehrzadi, S.; Shakeri-Zadeh, A.; Kamrava, S. K. Nanotechnology in Hyperthermia Cancer Therapy: From Fundamental Principles to Advanced Applications. *Journal of Controlled Release* **2016**, *235*, 205–221. https://doi.org/10.1016/j.jconrel.2016.05.062.
(120) de la Encarnación, C.; Jimenez de Aberasturi, D.; Liz-Marzán, L. M. Multifunctional Plasmonic-Magnetic Nanoparticles for Bioimaging and Hyperthermia. *Adv Drug Deliv Rev* **2022**, *189*, 114484. https://doi.org/10.1016/j.addr.2022.114484.
(121) Shen, S.; Qiu, J.; Huo, D.; Xia, Y. Nanomaterial-Enabled Photothermal Heating and Its Use for Cancer Therapy via Localized Hyperthermia. *Small* **2024**, *20* (7). https://doi.org/10.1002/smll.202305426.
(122) Badir, A.; Refki, S.; Sekkat, Z. Utilizing Gold Nanoparticles in Plasmonic Photothermal Therapy for Cancer Treatment. *Heliyon* **2025**, *11* (4), e42738. https://doi.org/10.1016/j.heliyon.2025.e42738.
(123) Oudjedi, F.; Kirk, A. G. Near-Infrared Nanoparticle-Mediated Photothermal Cancer Therapy: A Comprehensive Review of Advances in Monitoring and Controlling Thermal Effects for Effective Cancer Treatment. *Nano Select* **2025**, *6* (3). https://doi.org/10.1002/nano.202400107.
(124) Rubia-Rodríguez, I.; Santana-Otero, A.; Spassov, S.; Tombácz, E.; Johansson, C.; De La Presa, P.; Teran, F. J.; Morales, M. del P.; Veintemillas-Verdaguer, S.; Thanh, N. T. K.; Besenhard, M. O.; Wilhelm, C.; Gazeau, F.; Harmer, Q.; Mayes, E.; Manshian, B. B.; Soenen, S. J.; Gu, Y.; Millán, Á.; Efthimiadou, E. K.; Gaudet, J.; Goodwill, P.; Mansfield, J.; Steinhoff, U.; Wells, J.; Wiekhorst, F.; Ortega, D. Whither Magnetic

Hyperthermia? A Tentative Roadmap. *Materials* **2021**, *14* (4), 706. https://doi.org/10.3390/ma14040706.

(125) Rastinehad, A. R.; Anastos, H.; Wajswol, E.; Winoker, J. S.; Sfakianos, J. P.; Doppalapudi, S. K.; Carrick, M. R.; Knauer, C. J.; Taouli, B.; Lewis, S. C.; Tewari, A. K.; Schwartz, J. A.; Canfield, S. E.; George, A. K.; West, J. L.; Halas, N. J. Gold Nanoshell-Localized Photothermal Ablation of Prostate Tumors in a Clinical Pilot Device Study. *Proceedings of the National Academy of Sciences* **2019**, *116* (37), 18590–18596. https://doi.org/10.1073/pnas.1906929116.

(126) Bravo, M.; Fortuni, B.; Mulvaney, P.; Hofkens, J.; Uji-i, H.; Rocha, S.; Hutchison, J. A. Nanoparticle-Mediated Thermal Cancer Therapies: Strategies to Improve Clinical Translatability. *Journal of Controlled Release* **2024**, *372*, 751–777. https://doi.org/10.1016/j.jconrel.2024.06.055.

(127) Silva, F. A. L. S.; Chang, H.; Incorvia, J. A. C.; Oliveira, M. J.; Sarmento, B.; Santos, S. G.; Magalhães, F. D.; Pinto, A. M. 2D Nanomaterials and Their Drug Conjugates for Phototherapy and Magnetic Hyperthermia Therapy of Cancer and Infections. *Small* **2024**, *20* (13). https://doi.org/10.1002/smll.202306137.

(128) Rana, P.; Garima; Devi, S.; Kaur, G.; Singh, S. K.; Mittal, N. Magnetic Hyperthermia-Based Therapies for Cancer Targeting: Current Progress and Future Perspectives. *Medical Oncology* **2025**, *42* (10), 453. https://doi.org/10.1007/s12032-025-03020-9.

(129) Chauhan, A.; Saini, A.; Sharma, D. The Evolution of Integrated Magnetic Hyperthermia and Chemodynamic Therapy for Combating Cancer: A Comprehensive Viewpoint. *Nanoscale Adv* **2025**, *7* (16), 4820–4836. https://doi.org/10.1039/D4NA01004C.

(130) Xiong, L.; Liang, B.; Yu, K. Magnetic Hyperthermia in Oncology: Nanomaterials-Driven Combinatorial Strategies for Synergistic Therapeutic Gains. *Mater Today Bio* **2025**, *33*, 102070. https://doi.org/10.1016/j.mtbio.2025.102070.

(131) Abreu, M. M.; Chocron, A. F.; Smadja, D. M. From Cold to Hot: Mechanisms of Hyperthermia in Modulating Tumor Immunology for Enhanced Immunotherapy. *Front Immunol* **2025**, *16*. https://doi.org/10.3389/fimmu.2025.1487296.

(132) Cheng, H.; Tsao, H.; Chiang, C.; Chen, S. Advances in Magnetic Nanoparticle-Mediated Cancer Immune-Theranostics. *Adv Healthc Mater* **2021**, *10* (1). https://doi.org/10.1002/adhm.202001451.

(133) Liu, C.; Yan, B.; Wen, N.; Li, W.; Wang, J.; Li, H.; Wang, S.; Shi, K.; Liu, X. Engineering Energy-Responsive Magnetic Nanomaterials to Improve the Efficacy of Dendritic Cell-Based Immunotherapy. *Adv Ther (Weinh)* **2023**, *6* (1). https://doi.org/10.1002/adtp.202200234.

(134) Chang, M.; Hou, Z.; Wang, M.; Li, C.; Lin, J. Recent Advances in Hyperthermia Therapy-Based Synergistic Immunotherapy. *Advanced Materials* **2021**, *33* (4). https://doi.org/10.1002/adma.202004788.

(135) Lima-Sousa, R.; Melo, B. L.; Alves, C. G.; Moreira, A. F.; Mendonça, A. G.; Correia, I. J.; de Melo-Diogo, D. Combining Photothermal-Photodynamic Therapy Mediated by Nanomaterials with Immune Checkpoint Blockade for Metastatic Cancer Treatment and Creation of Immune Memory. *Adv Funct Mater* **2021**, *31* (29). https://doi.org/10.1002/adfm.202010777.

(136) Gavilán, H.; Gallo-Cordova, A.; Rábade Chediak, M. L.; Páez Rodíguez, A.; Morales, M. del P.; Gutierrez, L. Magnetic Hyperthermia in Focus: Emerging Non-Cancer

Applications of Magnetic Nanoparticles. *Nanoscale* **2025**. https://doi.org/10.1039/D5NR03329B.
(137) Nikazar, S.; Barani, M.; Rahdar, A.; Zoghi, M.; Kyzas, G. Z. Photo- and Magnetothermally Responsive Nanomaterials for Therapy, Controlled Drug Delivery and Imaging Applications. *ChemistrySelect* **2020**, *5* (40), 12590–12609. https://doi.org/10.1002/slct.202002978.
(138) Patri, S.; Thanh, N. T. K.; Kamaly, N. Magnetic Iron Oxide Nanogels for Combined Hyperthermia and Drug Delivery for Cancer Treatment. *Nanoscale* **2024**, *16* (33), 15446–15464. https://doi.org/10.1039/D4NR02058H.
(139) Seynhaeve, A. L. B.; Amin, M.; Haemmerich, D.; van Rhoon, G. C.; ten Hagen, T. L. M. Hyperthermia and Smart Drug Delivery Systems for Solid Tumor Therapy. *Adv Drug Deliv Rev* **2020**, *163–164*, 125–144. https://doi.org/10.1016/j.addr.2020.02.004.
(140) Liu, J.; Guo, X.; Zhao, Z.; Li, B.; Qin, J.; Peng, Z.; He, G.; Brett, D. J. L.; Wang, R.; Lu, X. Fe3S4 Nanoparticles for Arterial Inflammation Therapy: Integration of Magnetic Hyperthermia and Photothermal Treatment. *Appl Mater Today* **2020**, *18*, 100457. https://doi.org/10.1016/j.apmt.2019.100457.
(141) Dai, T.; He, W.; Yao, C.; Ma, X.; Ren, W.; Mai, Y.; Wu, A. Applications of Inorganic Nanoparticles in the Diagnosis and Therapy of Atherosclerosis. *Biomater Sci* **2020**, *8* (14), 3784–3799. https://doi.org/10.1039/D0BM00196A.
(142) Qi, X.; Xiang, Y.; Cai, E.; Ge, X.; Chen, X.; Zhang, W.; Li, Z.; Shen, J. Inorganic–Organic Hybrid Nanomaterials for Photothermal Antibacterial Therapy. *Coord Chem Rev* **2023**, *496*, 215426. https://doi.org/10.1016/j.ccr.2023.215426.
(143) Irmak Šalandová, M.; Fratila-Apachitei, L. E.; Apachitei, I.; Zadpoor, A. A. Alternating Magnetic Field and Induction Heating as Antibacterial Therapy in Implant-Associated Infections. *International Journal of Hyperthermia* **2025**, *42* (1). https://doi.org/10.1080/02656736.2025.2563301.
(144) Munshi, R.; Qadri, S. M.; Pralle, A. Transient Magnetothermal Neuronal Silencing Using the Chloride Channel Anoctamin 1 (TMEM16A). *Front Neurosci* **2018**, *12*. https://doi.org/10.3389/fnins.2018.00560.
(145) Huang, H.; Delikanli, S.; Zeng, H.; Ferkey, D. M.; Pralle, A. Remote Control of Ion Channels and Neurons through Magnetic-Field Heating of Nanoparticles. *Nat Nanotechnol* **2010**, *5* (8), 602–606. https://doi.org/10.1038/nnano.2010.125.
(146) Munshi, R.; Qadri, S. M.; Zhang, Q.; Rubio, I. C.; del Pino, P.; Pralle, A. Magnetothermal Genetic Deep Brain Stimulation of Motor Behaviors in Awake, Freely Moving Mice. *Elife* **2017**, *6*. https://doi.org/10.7554/eLife.27069.
(147) Cazares-Cortes, E.; Espinosa, A.; Guigner, J.-M.; Michel, A.; Griffete, N.; Wilhelm, C.; Ménager, C. Doxorubicin Intracellular Remote Release from Biocompatible Oligo(Ethylene Glycol) Methyl Ether Methacrylate-Based Magnetic Nanogels Triggered by Magnetic Hyperthermia. *ACS Appl Mater Interfaces* **2017**, *9* (31), 25775–25788. https://doi.org/10.1021/acsami.7b06553.
(148) Deng, H.; Sun, J.; Yu, Z.; Guo, Z.; Xu, C. Low-Intensity near-Infrared Light-Triggered Spatiotemporal Antibiotics Release and Hyperthermia by Natural Polysaccharide-Based Hybrid Hydrogel for Synergistic Wound Disinfection. *Materials Science and Engineering: C* **2021**, *118*, 111530. https://doi.org/10.1016/j.msec.2020.111530.
(149) Navrátil, O.; Lizoňová, D.; Slonková, K.; Mašková, L.; Zadražil, A.; Sedmidubský, D.; Štěpánek, F. Antibiotic Depot System with Radiofrequency Controlled Drug Release.

Colloids Surf B Biointerfaces **2022**, *217*, 112618. https://doi.org/10.1016/j.colsurfb.2022.112618.

(150) Franco, M. S.; Gomes, E. R.; Roque, M. C.; Oliveira, M. C. Triggered Drug Release From Liposomes: Exploiting the Outer and Inner Tumor Environment. *Front Oncol* **2021**, *11*. https://doi.org/10.3389/fonc.2021.623760.

(151) Hossann, M.; Kneidl, B.; Peller, M.; Lindner, L.; Winter, G. Thermosensitive Liposomal Drug Delivery Systems: State of the Art Review. *Int J Nanomedicine* **2014**, 4387. https://doi.org/10.2147/IJN.S49297.

(152) Salvatore, A.; Montis, C.; Berti, D.; Baglioni, P. Multifunctional Magnetoliposomes for Sequential Controlled Release. *ACS Nano* **2016**, *10* (8), 7749–7760. https://doi.org/10.1021/acsnano.6b03194.

(153) Del Sol-Fernández, S.; Martínez-Vicente, P.; Gomollón-Zueco, P.; Castro-Hinojosa, C.; Gutiérrez, L.; Fratila, R. M.; Moros, M. Magnetogenetics: Remote Activation of Cellular Functions Triggered by Magnetic Switches. *Nanoscale* **2022**, *14* (6), 2091–2118. https://doi.org/10.1039/D1NR06303K.

(154) Morshedi Rad, D.; Alsadat Rad, M.; Razavi Bazaz, S.; Kashaninejad, N.; Jin, D.; Ebrahimi Warkiani, M. A Comprehensive Review on Intracellular Delivery. *Advanced Materials* **2021**, *33* (13), 2005363. https://doi.org/10.1002/adma.202005363.