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Bioceramics: from bone substitutes to nanoparticles for drug delivery

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Abstract

Since the second half of the 20th century, bioceramics are used for bone repair and regeneration. Inspired by bones and teeth, and aimed at mimicking their structure and composition, several artificial bioceramics were developed for biomedical applications. And nowadays, in the 21st century, with the increasing prominence of nanoscience and nanotechnology, certain bioceramics are being used to build smart drug delivery systems, among other applications. This minireview will mainly describe both tendencies through the research work carried out by the research team of María Vallet-Regí.

Keywords

biomedical applications; biomaterials; ceramics; Distinguished Women in Chemistry and Chemical Engineering; drug delivery

Introduction

The current presence of biology in outstanding fields of engineering, food and health is denoted by the common use of the term biomaterials. Biomaterials, as such, are materials of interest in the field of biomedical engineering. Their inception, study and assessment combine techniques and know-how from the worlds of science, engineering, biology and medicine. Historically speaking, medicine has evolved from being based on intuition to rely mostly on evidence; in this sense, the current trend of predictive medicine makes heavy use of collected data from clinical trials and pushes forward towards tailored, personalized treatments. The mathematical knowledge required for this purpose is self-evident. Moreover, these last 70 years have also yielded a dramatic evolution in the field of biomaterials, moving from the use of inert materials as living tissue replacements towards the purpose-oriented design of bioactive, biodegradable materials for said replacements. The current third generation of biomaterials is focused on tissue and organ regeneration. Many concepts have changed due to this rapid evolution; the initial focus on replacement shifted towards

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repair, and the current aim is regeneration. While first generation biomaterials were not specifically designed to interact with biological tissue, third generation biomaterials are devised taking into account their subsequent contact with these tissues. Therefore, surface properties of the biomaterial such as topography, surface charge and all aspects of surface chemistry, are extremely important in order to achieve good results when these materials are implanted among living tissue. In this sense, the proper functionalization of the free surfaces of these biomaterials, to facilitate cell adhesion, proliferation and differentiation in optimal conditions, is crucial.

The evolution of ceramics from the 1950s up to the early 21st century has been significant. Inert ceramics began to be used in the 1950s as replacement of damaged parts of the human skeleton. The few ceramics used for this purpose, such as alumina and zirconium, were not specifically designed for biomedical applications.

Nowadays, all bioceramics currently in clinical use are specifically designed to repair and regenerate the human bone. Orthopedics and maxillofacial surgeons resort to several commercial products in supply, providing different types of bioceramics. These commercially available products can be considered 'traditional' bioceramics, i.e. can be used under all applicable regulations and homologations for this kind of prostheses, solving real and specific needs in the clinical field. But there are other promising materials, the so called 'new bioceramics', which are instead at the cutting edge of knowledge; specifically designed for a given function, their real applications may appear in the near future [1, 2]. In Table 1 are depicted the most important bioceramics used for bone repair.

Third generation bioceramics are used as building material for scaffolds supporting cells involved in the regeneration process. From a tissue engineering point of view, these scaffolds must provide mechanical support while being biocompatible, hence not inducing any negative response; their load bearing capability may be temporary. In an ideal scenario, its degradation rate should be similar to the tissue regeneration rate. Additional required features are an interconnected porosity with an optimum pore size distribution, promoting cell and tissue colonization, metabolite transit while offering a high surface area for cell anchoring. These requirements are currently met thanks to new advanced techniques; four dimensional (4D) printing, for instance, is an emerging technology in tissue and organ engineering based in multi-material reprogramming, capable of changing form, function and/or properties as a way of adaptation to changing environments. The printing materials used in tissue and organ regeneration applications must be biocompatible and ready to perform dynamic processes in a physiological environment. Therefore, 4D printing might be a powerful future tool in the biomedical study of functional synthetic organs and tissues; but there are still plenty of scientific and technical requirements to be met [3–7].

Natural ceramics in our body: bones and teeth

All vertebrate species exhibit natural composite materials in their bones and teeth; the inorganic component of said composite is carbonate hydroxyapatite, which is roughly 65 % of the total bone mass, with the remaining mass formed by organic matter and water [8, 9]. The permanently active cells present inside the bones allow considering such materials as

“living biominerals”. Bone formation processes are triggered by the osteoblasts, special cells capable of producing and releasing osteoid, a protein mixture, mainly formed by type I collagen. In a second step, the osteoid is mineralized by controlled deposition of calcium phosphate. Then, the osteoblasts trapped within the mineral phase evolve towards osteocytes which are responsible for the continuous bone formation activity. Simultaneously, a different type of cells, the osteoclasts, performs the bone catabolism and destruction. Bone formation and destruction is a dynamic process which takes place during the development stages of the body, enabling bone growth while preserving its shape and consistency, and ensuring its regeneration in case of fracture. An additional feature of this process is that it constitutes a storage and hauling mechanism for two essential elements, phosphorus and calcium, which are mainly stored in the bones. A similar process takes place in teeth, where the only difference with bone tissue is the presence of an external surface coating, the enamel. The inorganic content of dental enamel is much larger than in bone (up to 90 %), and is formed by large, heavily oriented prismatic crystals. Therefore, the crystallinity and carbonate content of bone and dentine (with similar qualities) are very different from those of enamel. These particular features are directly related to the mechanical qualities of enamel, which is in fact considered the most resistant and tough biological material. However, dental enamel in an adult body does not contain cells, in contrast with bone tissue, and there is no biological enamel regeneration process (any potential deterioration will be irreversible). This aspect evidences the need for enamel-biocompatible materials in the repair of teeth decay [10, 11]. Figure 1 shows the most significant structural features of bone when observed at different magnification degrees.

Artificial ceramics for bone replacement

Ceramic materials used as starting components in bioactive mixtures can be classified in three different groups: calcium phosphates, glasses and glass-ceramics [12]. The purpose of the obtained mixtures, combining two or more components, is to achieve better mechanical response and/or a faster bioactive action. Bone cements, for instance, are produced mixing calcium phosphates with other inorganic salts. Another aim in the study of these ceramics is to design shaping procedures allowing to obtain implants in a given shape or size, with a particular porosity, as a function of the specific final use of the ceramic implant. When the major requirement for a given implant is to achieve fast chemical reaction leading to nanoapatite formation (as precursor of newly formed bone), said implant must be designed as a porous piece, with a certain degree of macropores, in order to enable bone oxygenation and angiogenesis. If such a requirement is not taken into account in the implant design phase, the chemical reaction will be restrained to the external implant surface, if made of bioactive ceramics, or it will not take place at all, if the implant is made of an inert material; in both cases, the implant will achieve the bone replacement requirement, thanks to its solid inner core; but it will not fulfil any bone regenerative function as expected from bioactive ceramics. The lack of regenerative functions in inert ceramics justifies their production in dense and solid forms, as is the case in femoral head implants made of alumina and zirconia [13]. Figure 2 shows the different bioceramics that can be used depending on the function they must play when implanted in the skeletal system.

Other bioceramics such as organic-inorganic hybrids [14–17], template glasses [18–26], star gels [27–29], and silica based ordered mesoporous materials [30–32], were proposed. Their use as bone replacement materials is currently under study.

Ordered mesoporous ceramics were originally designed and studied by the catalysis industry, but thanks to their porosity and composition they have found very promising applications in the biomedical world, due to their drug delivery capabilities and tissue engineering potential. In other words, their relevance in the field of biomedicine stems from two outstanding properties:

- **Surface.** The material is a silica network with silanol groups on the external surface, which are also present in traditional glasses, accepted as bioactive by the scientific community. In this sense, silica-based mesoporous materials are likely to behave similarly to bioglasses, i.e. an apatite-like layer similar to that of natural bone will grow on their surface when immersed in a body fluid. This is the main reason behind the interest of the biomedical world for these materials regarding bone tissue regeneration.
- **Textural properties.** The pores constituting this ordered mesoporosity can be filled with different molecules such as drugs or biologically active species, which in turn can be used as local delivery systems.

Both properties combined triggered the interest in tissue engineering and drug delivery systems.

Figure 3 shows important applications of mesoporous ordered materials in the biomedical field: to obtain scaffolds in bone tissue engineering and as matrixes in drug delivery systems.

Ordered mesoporous ceramics were first reported in the 1990s, when research in new porous solids was trying to find materials with larger pores than zeolites (microporous class materials) in order to improve their potential applications as adsorbents, catalysts and catalysts supports. In a simultaneous fashion, Japanese academic investigators [33] and Mobil Oil Corporation researchers [34, 35] started to employ surfactants as structure directing agents to produce a new type of materials, KSW-n and M41S families of mesostructured materials, respectively. Vallet-Regí et al. determined that these materials opened up new fields of application in drug delivery and bone regeneration through published results in 2001 and 2004, respectively [36, 37]. Since then many studies were performed [30, 38–57].

Bioceramics can be obtained in dense or porous form. Inert ceramics, on the other hand, are obtained through conventional methods started in the 1980s. Traditionally, ceramics are produced under high temperature and pressure. These conditions are not adequate when attempting to produce biomimetic materials, where a small particle size, in the nanometer scale, is paramount. Nowadays, wet route synthesis methods are preferred to obtain bioactive bioceramics, as well as silica mesoporous materials designed as scaffolds for tissue engineering with simultaneous presence of nano-, meso-, and micro-porosities [58–63]. Figure 4 highlights the importance of hierarchical porosity in the bone implants.

It is important to highlight the concept of porosity and its range of order in these materials. Bioceramics with mesopores, that is, pore diameters between 2 and 50 nm, are suitable for applications where drugs or biologically active molecules are loaded, and subsequently released to help in the bone regeneration process.

Macroporous bioceramics, with pore diameters of several microns, are adequate as scaffolds for tissue engineering.

Regarding the particular case of silica mesoporous bioceramics, the controlled delivery capability together with their improved bioactive behavior make them potential candidates to manufacture three dimensional hierarchical ordered porous scaffolds for bone tissue engineering (See Fig. 5).

Drug delivery from silica mesoporous nanoparticles

Among the many different nanoparticles proposed as nanomedicines, probably the most popular are liposomes and lipid-based nanomedicines, protein nanoparticles, polymeric micelles and nanoparticles, polymer-drug conjugates and diverse inorganic nanoparticles [64, 65]. Among the last, mesoporous silica nanoparticles (MSNs) have been deeply investigated as drug delivery nanocarriers because of their physicochemical properties [36, 66–70]. MSNs are very robust, since they are mechanically, thermally and chemically stable. Their great loading capacity within the porous system [71] is due to their outstanding properties for the adsorption of many different types of molecules, such as high surface area (ca. 1000 m²/g), high pore volume (ca. 1 cm³/g) and narrow distribution of tunable pore diameters (2–30 nm). Thanks to those properties, MSNs have been employed as drug delivery systems [14], as nanosystems for diagnosis [72] and nanosystems for gene transfection [73].

As mentioned above, mesoporous silica exhibits certain outstanding textural features. Their role in loading and release kinetics of biologically active agents will now be reviewed.

Pore diameter, for instance, is a de facto size-selective adsorption parameter, and it also modulates the release rate.

Besides, molecule adsorption is mainly a surface process. Therefore, effective surface area is the governing parameter in molecule adsorption.

When the aim is to confine very large molecules (whether in size or volume, such as proteins), pore volume is the main parameter.

It has been ascertained that release kinetics are mainly determined by the organic functionalization of mesoporous silica walls with different organic groups. Said functionalization may also improve molecule adsorption by promoting host-guest interactions.

When optimized synthesis methods are carried out, silica mesoporous materials can be also obtained in nanoparticle form, opening up new possibilities in medical applications. The synthesis of mesoporous silica nanoparticles (MSNs) follows a modified version of the

Stöber method, using highly diluted conditions in the sol-gel process, obtaining nanoparticles as final product. Surfactants play the role of structure directing agents in this synthesis, as a template; the silica precursors condensate over those templates and the subsequent surfactant removal produces a network of mesoporous cavities.

Most nanoparticles designed for drug delivery have been aimed at treating complex diseases such as cancer. When dealing with the potential treatment of cancer, the use of nanoparticles loaded with therapeutic agents must ensure that these nanoparticles will reach the affected area and will release their agents there. Generally speaking, the drug delivery process to a solid tumor involves five main steps, which are known as the CAPIR cascade: circulation in blood, accumulation and penetration into tumors, cellular internalization, and intracellular release [74].

In order to ensure a long circulation time in the blood stream, several solutions can be used; if the nanoparticle size, for instance, is close to 100 nm, extravasation is avoided; also, surface functionalization can evade interactions with blood components and reticuloendothelial system (RES). In this sense, surface modification of nanoparticles allows to target the tumors; compared with other nanocarriers, MSNs are easily functionalized, because MSNs are able to carry many different grafting reactions using different organic solvents, withstanding relatively high temperatures and multiple organosilanes functionalizing agents. Regarding cellular internalization and intracellular release, MSNs have been observed to penetrate into many different types of cells (Fig. 2), especially when their surface is positively charged [75].

The main challenge nowadays for all types of nanocarriers, not only MSNs, is the effective selection of certain targets within the body. Its importance lies on the absence of discrimination between healthy and cancerous cells in most conventional drugs; hence, a selective nanocarrier would solve this issue by releasing and accumulating these carried drugs in the tumors, instead of in healthy tissues. The targeting mechanisms for these nanoparticles will be reviewed later.

Since the pioneering work on MSNs [36] as drug delivery carriers, there have been many research groups that have investigated this topic [76, 77]. The field of nanomedicine is experiencing a rapid evolution in these last years, with a continuous increase in the number of publications regarding MSNs as stimuli-responsive drug delivery systems, as shown in Fig. 7. There are already several reviews on MSNs for drug delivery in the literature, but in such a dynamic and changing field, regular updates are needed to keep up to date.

Each development in nano-systems with medical applications has entailed new challenges to the design of smart materials capable of responding to new clinical requirements; ceramic nanoparticles play an important role in this context.

A generic desire in medicine is to find the best, or more acceptable, route to administer therapeutic agents from a physiological viewpoint. Nowadays, the dosages prescribed in many cases are excessively high, but they are needed to ensure that the affected area will receive the minimum required dose. Most of the dose administered to the patient acts throughout the whole body, affecting regions where it should not be acting. This is an acute

problem in oncology treatments, where the risk-benefit ratio of chemotherapy complicates the decision making process, due to the cytotoxicity of the drugs to be used. A local and smart drug release would be the answer to these issues.

The potential multifunctionality of many nano or microparticles, such as MSNs, is perhaps their main advantage. Different functions can be simultaneously achieved, such as: Load and subsequent release of different drugs, anchoring of biomolecules such as proteins, vectoring agents or nucleic acids to the external surface of the particle and towards therapeutic targets, anchoring of fluorescent molecules or active complexes for magnetic resonance imaging (MRI) in order to perform optical monitoring, inclusion of magnetic nanoparticles, coating with different materials such as certain polymers or metals such as gold, among other possibilities.

This versatile, smart nanocarriers are very promising candidates to be used in the clinic in the near future to overcome some of the pitfalls of conventional medicine [78].

The surface of MSNs is very easy to chemically modify thanks to the silanol groups present on their surface, allowing to design multifunctional platforms with many different characteristics.

These qualities of MSNs encouraged many research groups to attempt hosting different therapeutic agents in MSNs to be transported to the target tissue [7, 79, 80]. MSNs have been successfully endocytosed by many different mammalian cell lines [81–84] and the in vitro toxicology experiments have shown that MSNs are well tolerated at dosages below 100 $\mu\text{g/mL}$ [85]. It is also worth pointing out that in vivo biocompatibility studies of MSNs on different animal models have shown good tolerance at dosages below 200 mg/kg [86]. Since MSNs for this kind of applications must be administered through intravenous injection to the blood-stream, the hemocompatibility has also been subject of research, achieving positive results [87, 88].

A previous review focused on the state of the art of this first generation of MSNs with Biomedical Applications [89]. However, the aim for the second generation of MSNs should be a successful shift from the preclinical proof of concept to the clinic thanks to positive and consistent results in terms of therapeutic value. In this sense, pharmacokinetic and pharmacodynamics evaluations should clearly state the efficacy and lack of toxicity, together with biodistribution studies before enlisting in clinical trials [90].

Stimuli-responsive MSNs

As already mentioned, the open porosity of MSNs means that it is possible to load therapeutic agents into their network of cavities, but it is also possible to release those agents when in solution, obviously depending on the solvent and the drug itself. Therefore, an efficient closure of the pores is needed to avoid premature release of the load while traveling through the blood vessels, since an unspecific release of the drugs could cause several side effects (Fig. 8).

Different alternatives can be considered to close the pore entrances; the most common is perhaps grafting stimulus sensitive gates to the pore entrances [91] or coating the whole nanoparticle with a cleavable shell that would allow triggering the release when detached. In both cases, the final outcome would be the pore entrance closure that could be opened under the action of a given stimulus. The triggering stimuli can be internal, usually related to a specific parameter of the treated pathology, such as variations in pH, redox potential and enzymes concentration among others; or external, i.e. remotely applied by the clinician, including stimuli such as magnetic fields, ultrasounds, electrical fields or light [92–95]. Previous reviews have focused on stimulus-responsive mesoporous silica, both bulk and nanoparticles, with gate-like assemblies on pore openings [96].

A few examples: magnetic field responsive MSNs

One of the most employed stimuli in nanomedicine is the magnetic field due to their two-fold effect: they can be used to magnetically guide the nanoparticles when using a permanent magnetic field or to locally increase the internal temperature when using an alternating magnetic field [97, 98]. In this example, super-paramagnetic microspheres with an $\text{Fe}_3\text{O}_4@\text{SiO}_2$ core and a mesoporous silica shell were produced with magnetization and large pore volume, as potential candidates to be used as magnetically controlled drug delivery systems [99]. A similar approach was used to obtain spheres with a uniform particle diameter of ca. 270 nm with a core of magnetic $\text{Fe}_3\text{O}_4/\text{Fe}$ and a mesoporous silica shell able to encapsulate Ibuprofen [100]. Regarding their use with MSNs, the most popular strategy consists on incorporating superparamagnetic iron oxide nanoparticles (SPIONs) of ca. 5–10 nm encapsulated within MSNs network during their synthesis [101, 102]. The application of an alternating magnetic field to the system would increase the local temperature, so if the pore entrances were previously closed with a temperature responsive moiety, load release would be triggered. This behavior was achieved encapsulating iron oxide small nanoparticles into the network of MSNs and covering the surface of those MSNs with a thermosensitive polymer, poly(N-isopropylacrylamide), to close the pore entrances to retain the cargo inside avoiding premature release. A polyamine was also added to the thermoresponsive polymer in order to retain proteins within the shell of the nanoparticles (Fig. 9). With this setup, it is possible to release two types of agents simultaneously: the drug encapsulated into the pores (fluorescein was used as model molecule) and the protein retained into the shell (trypsin inhibitor was used as model protein) [103].

As Fig. 9 shows, under an applied alternating magnetic field, the iron oxide nanoparticles increased the local temperature up to a point at which the conformation of the thermoresponsive polymer changed, so the pore entrances were opened and the protein and small molecules were released following different kinetics.

In a different proof of concept of this type of responsive materials, MSNs were functionalized with a single DNA strand and then the cargo was loaded inside the pores [104]. Separately, the complementary DNA sequence was attached to magnetic iron oxide nanoparticles of ca. 5 nm of diameter. Then, both MSNs and DNA-iron oxide nanoparticles were mixed to allow DNA hybridization, as it can be observed in Fig. 10.

The DNA sequence employed was selected due to its melting temperature of 47 °C, so once the system was exposed to an alternating magnetic field, the iron oxide nanoparticles encapsulated into the MSNs network were able to increase the local temperature. This led to the double-stranded DNA melting with the subsequent pore aperture and cargo release. The most remarkable aspect in this proof of concept is the reversibility of DNA linkage: when the magnetic field is switched off and the system cools down to physiological temperature, the DNA strands would hybridize again closing the pores. Later on, applying the field again would reopen the pores, leading to a pulsatile or on-off release mechanism.

Superparamagnetic iron oxide nanocaps have been also employed to close mesoporous silica nanorods: the cell-produced antioxidants trigger the load release under an external magnetic field [105].

Light responsive MSNs

In the last few years, the use of light with different wavelengths (ultraviolet, visible or near-infrared) to trigger load release from MSNs has become very popular. The use of light to trigger the release from MSNs is quite straightforward: it can be easily applied by the clinician, and can be focalized to the targeted tissue; tissue penetrability of light, however, is only of a few centimeters. Of all available wavelengths, UV is the most popular to stimulate load release from MSNs, due to its bond breaking power [106]. In a proof of concept using this technology, MSNs were coated with a protein shell using a photosensitive linker that could be cleaved under light radiation at 366 nm [107]. The shell covering the nanoparticles was also functionalized with transferrin, because it is well known that cancer cells overexpress receptors for that ligand. Thus, once the MSNs are internalized within the tumor cells, UV light would trigger the drug release; this is a suitable treatment approach to light accessible tumors, such as melanomas.

A potential drawback besides its low penetration, however, is the high energy of UV light. The use of visible light is a possible alternative; it is safer and exhibits higher tissue penetrability. In this sense, a proof of concept of a visible light triggered MSNs release system has recently been published [108].

Ultrasound sensitive MSNs

Ultrasound (US) is a very interesting stimulus to be used in nanomedicine thanks to its deep and harmless penetration into living tissues [109–111]. Besides, US is non-invasive and it can be focalized. Our research group developed an US sensitive MSNs drug delivery system, activated with an off the shelf US equipment normally used in rehabilitation clinics [112, 113]. The basis of this system is to decorate MSNs surface with a copolymer made of thermosensitive and US sensitive components to close the pores, avoiding premature load release [114]. When US are applied, a certain part of the copolymer cleaves, changing the hydrophobicity of the copolymer. This leads to a change in copolymer conformation at physiological temperature, opening the MSNs pores and triggering the release of the cargo, as Fig. 11 shows.

Mesoporous silica nanoparticles have been also employed to develop transdermal drug delivery systems that could be simultaneously sensitive to temperature and US stimuli [115]. These MSNs were decorated with a US sensitive polymer following the procedure described above.

Ultrasound has also been tested as guiding and imaging vector for PEGylated MSNs coated with Au nanoparticles and encapsulated in perfluorohexane [116]. In this model, US irradiation effectively triggered drug release while exciting contrast-intensified ultrasound imaging; it also increased ablation efficacy thanks to the US guidance.

pH sensitive MSNs

As already mentioned, internal stimuli are those that take advantage of certain features of the pathology to be treated. pH is one of the most employed to trigger drug release from nanomedicines because pH values are lower in most tumors than in healthy tissues. This difference is due to the high glycolysis rate in cancer cells, which leads to a high production of lactic acid and to a pH decrease. To make the most of these differences in pH, many different approaches have been tested [117, 118], usually based on blocking the pore entrances of MSNs with different moieties grafted to the surface of the nanoparticles through responsive linkers, such as acetal linkers [119], boronate ester [120], ferrocenyl linkers [121], different polymers [122], aromatic amines [123], imine bonds [124], or calcium phosphates that are soluble at acid pH values [125]. Recently, our research group developed a pH responsive MSNs based system to transport and deliver topotecan, a potent cytotoxic agent that commonly degrades at physiological pH, which limits its clinical use [126]. The surface of the topotecan loaded MSNs was decorated with a gelatin sensitive to acid pH, and then Folic Acid moieties were added to the external surface to direct the nanocarriers towards tumor cells that overexpress folic acid receptors.

In this same trend of pH-sensitive MSNs systems, an additional proof of concept has been developed using Self-Immolative Polymers (SIPs) to decorate the external surface of the nanoparticles [127]. These SIPs are made of a linear polymer based on a polyurethane chain with a molecule sensitive to acid pH at one of the ends. Under acid pH, this molecule is cleaved and triggers the disassembly of the polymer from head to tail, yielding the initial monomers, in a process known as self-immolation. MSNs loaded with a model molecule were decorated with a SIP stable at physiological pH values, hence avoiding premature release on healthy tissues. When the system was exposed to acid pH, the polymer was effectively disassembled, opening up the pores and triggering the load release at the acidic environment, as expected.

MSNs loaded with prodrugs

As previously discussed, arguably the main application of nanoparticles for drug delivery is the potential treatment of cancer. In such a medical treatment, however, the transported load is normally a highly cytotoxic drug, and hence it is crucial to avoid premature release before reaching the tumor tissue. This is the main motivation behind stimuli-responsive systems, but it is extremely difficult to design fully safe 'zero release' systems, that is, where

absolutely no release occurs before reaching the target site. An alternative solution could be to load the nanocarriers with cytotoxic agents in an inactive state and, once they arrive at the tumor tissue, activate those agents so the highly toxic compounds would be generated only in the targeted tissue. This approach has been developed employing nanoparticles able to transport a prodrug that could be activated by certain enzymes overexpressed by the tumor tissue [128]. The main limitation for this strategy is the low concentration of activating enzymes in the tumor or even the lack of naturally occurring enzymes necessary to activate certain prodrugs.

In this model, MSNs have been used to devise a system which can generate cytotoxic drugs in situ, within tumor cells, transporting both the non-toxic prodrug, loaded in the pore network, and the enzyme required for the drug activation, grafted to the surface of the MSNs [129]. In this case, the encapsulated prodrug was indol-3-acetic acid and the enzyme grafted at the surface of MSNs was horseradish peroxidase, which is able to transform indol-3-acetic acid into indole-3-carbinol generating cytotoxic compounds such as hydroxyl and reactive oxygen species. The stability of the enzyme was ensured by coating them with a polymeric capsule (Fig. 12).

Selective targeting for MSNs

As previously seen, when considering MSNs for nanomedicine, the importance of localizing nanocarriers into the specific tissues where the therapeutic agent is required is critical. And it is particularly important if nanocarriers are going to be used in cancer therapies, in order to avoid side effects and damage to healthy cells. In fact, it has already been mentioned that cancer is the pathology that is receiving most of the attention from the nanomedicine community, and the reason for that is the possibility of targeting the nanocarriers to specific tissues, both through passive and/or active targeting.

Upon injection of MSNs, or nanoparticles in general, into the blood stream, they tend to accumulate in solid tumors due to the so-called enhanced permeation and retention (EPR) effect or passive targeting [130, 131]. The mechanism responsible is based on the abnormalities present in tumor blood vessels, with wide interendothelial junctions, a great amount of fenestrations and transendothelial pores with dimensions of several 100 nm (Fig. 13). In this scenario, nanoparticles traveling through the bloodstream will probably extravasate through the previously mentioned fenestrations present in tumor vessels and accumulate into the tumor interstitium. Furthermore, those nanoparticles already within the tumor will tend to stay there due to the poor lymphatic drainage provoked by the fast growth of the tumor tissue.

Active targeting, meanwhile, takes advantage of the fact that some tumor cells overexpress certain receptors on their surface. If nanoparticles are functionalized with ligands exhibiting high affinity towards those receptors, the specific retention and uptake of those nanoparticles by cancer cells can be enhanced. This mechanism is particularly interesting because tumor masses are composed of very heterogeneous tissues, with many different types of cells. Therefore nanoparticles must be able to distinguish between tumor cells and other, non tumoral cells that might be present into the neoplastic tissue. Different approaches have been

tested to the decoration of the surface of MSNs with certain ligands able to interact selectively with specific cellular receptors overexpressed in tumor cells. Some examples of targeting ligands grafted to MSNs are transferrin [108, 132, 133], epidermal growth factor [134], folic acid [126, 135–141], methotrexate [142], transactivator of transcription peptides [143–145], interleukin-13 peptide [146], anti-herceptin [147], anti-epidermal growth factor receptor [148], anti-receptor tyrosine-protein kinase [149], metaaminobenzyl guanidine [150], RGD-type peptide [151–155], concanavalin A [156], cyclic RGD [157–159], anti-cell adhesion molecule 1 [160], and vascular endothelial growth factor [161].

When discussing cancer treatment with nanoparticles, an additional problem must be taken into account: the poor penetration of those nanoparticles across the tumor mass. This is due to the presence of collagen in the extracellular matrix of the tumor mass, which increases its density if compared with matrices present in healthy tissues. This high density greatly hinders nanoparticle penetration into the tumor mass. A possible solution for this high density matrix consisted of decorating the surface of MSNs with collagenase, which is a proteolytic enzyme capable of digesting the collagen rich extracellular matrix [162]. This enzyme was protected with pH sensitive polymeric capsules to avoid premature biodegradation before reaching the tumor tissue. Thus, under the typical acidic environments of tumors, those nanocapsules would be decomposed, releasing the collagenase that would digest the extracellular matrix of the tumor, and therefore improving the penetrability of the MSNs deep into the tumor mass.

The combination of high penetration with long travel periods within the blood stream is essential for the success of these nanoparticles in clinical practice, as depicted in Fig. 14.

The road to the future

The forthcoming future advances in biomaterials, both in the fields of prosthetic or replacement devices and as nanoparticles, will require the simultaneous exploration of all these size scales: PICO, NANO, MICRO and MACRO, while molecular and cell biology will provide solutions to clinical problems. In this sense, the porosity of biomaterials should be analyzed at all size scales, in order to fully understand their behavior and to offer new solutions to specific issues. New and future technologies will provide new solutions, and the use of cell-free organs as scaffolds could, with time, be the answer to many problems. We have witnessed an astonishing development of biomaterials in these 70 years, and clearly will not stop in the near future. Thanks to the advances in molecular and cell biology, these last three decades gave rise to intensive efforts in regenerative medicine to promote autonomous regeneration of a damaged organ in the body, something already observed in certain species – such as the salamander but never in humans. Certainly, we are on the right path.

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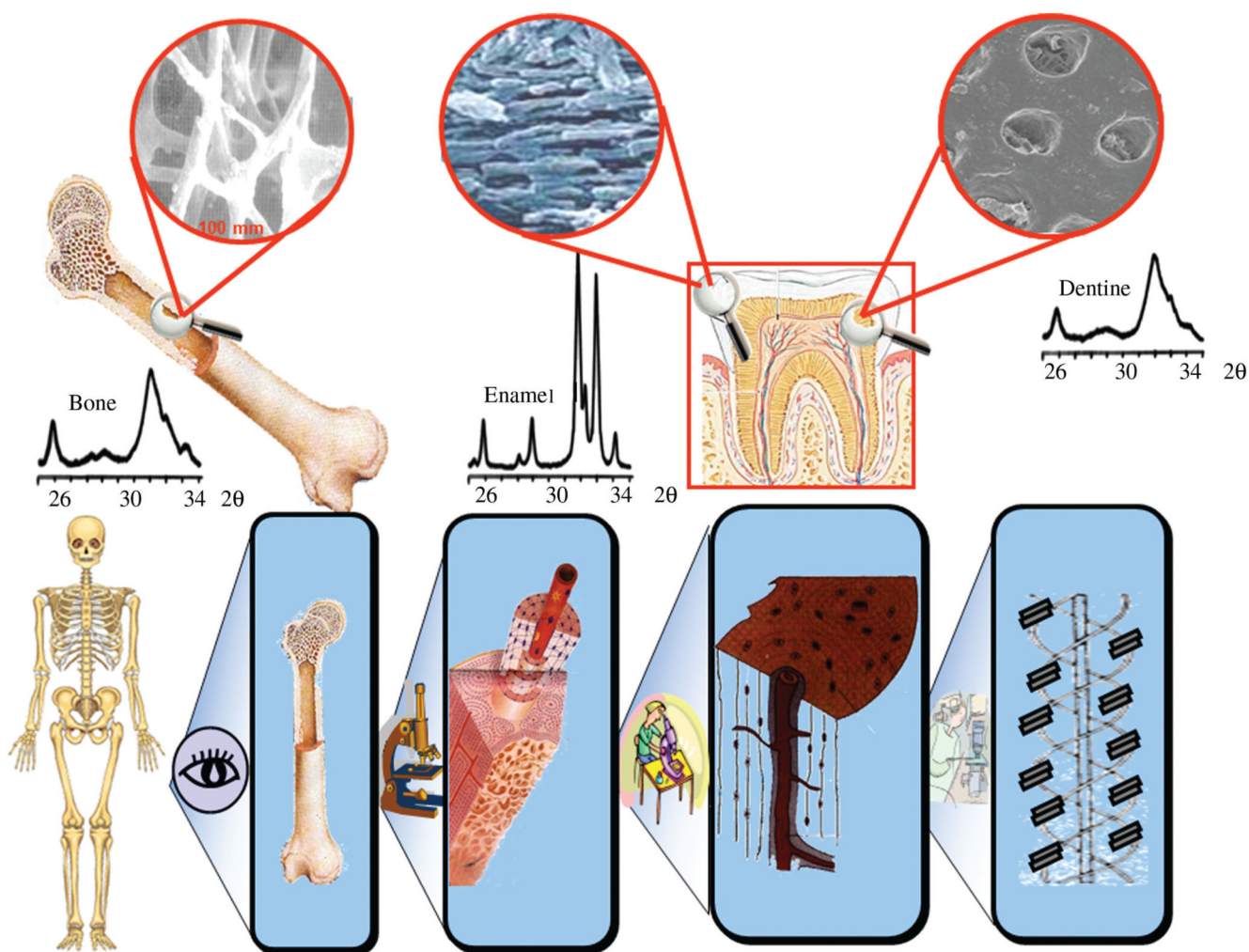


Fig. 1. Structural features of bones when observed at different magnification degrees. Plots represent XRD data of bone, enamel and dentine.

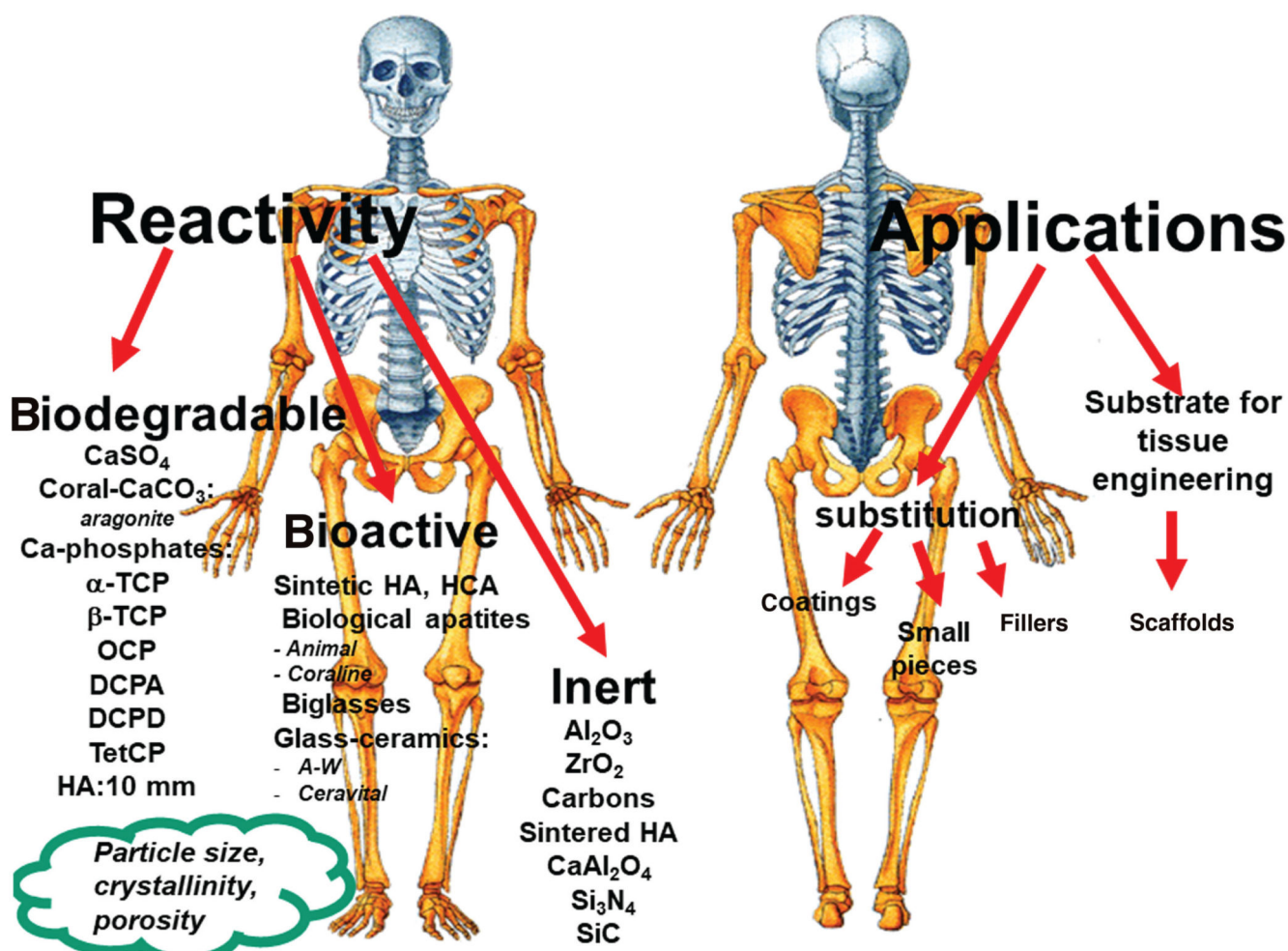


Fig. 2. Different bioceramics are used depending on the function they must play in the skeletal system. (TCP, Tricalcium Phosphate; OCP, Orthocalciumphosphate; DCPA, Dicalcium phosphate anhydrous; DCPD, dicalcium phosphatedihydrate; TetCP, tetracalcium phosphate; HA, Hydroxiapatite; HCA, carbonated hydroxyapatite).

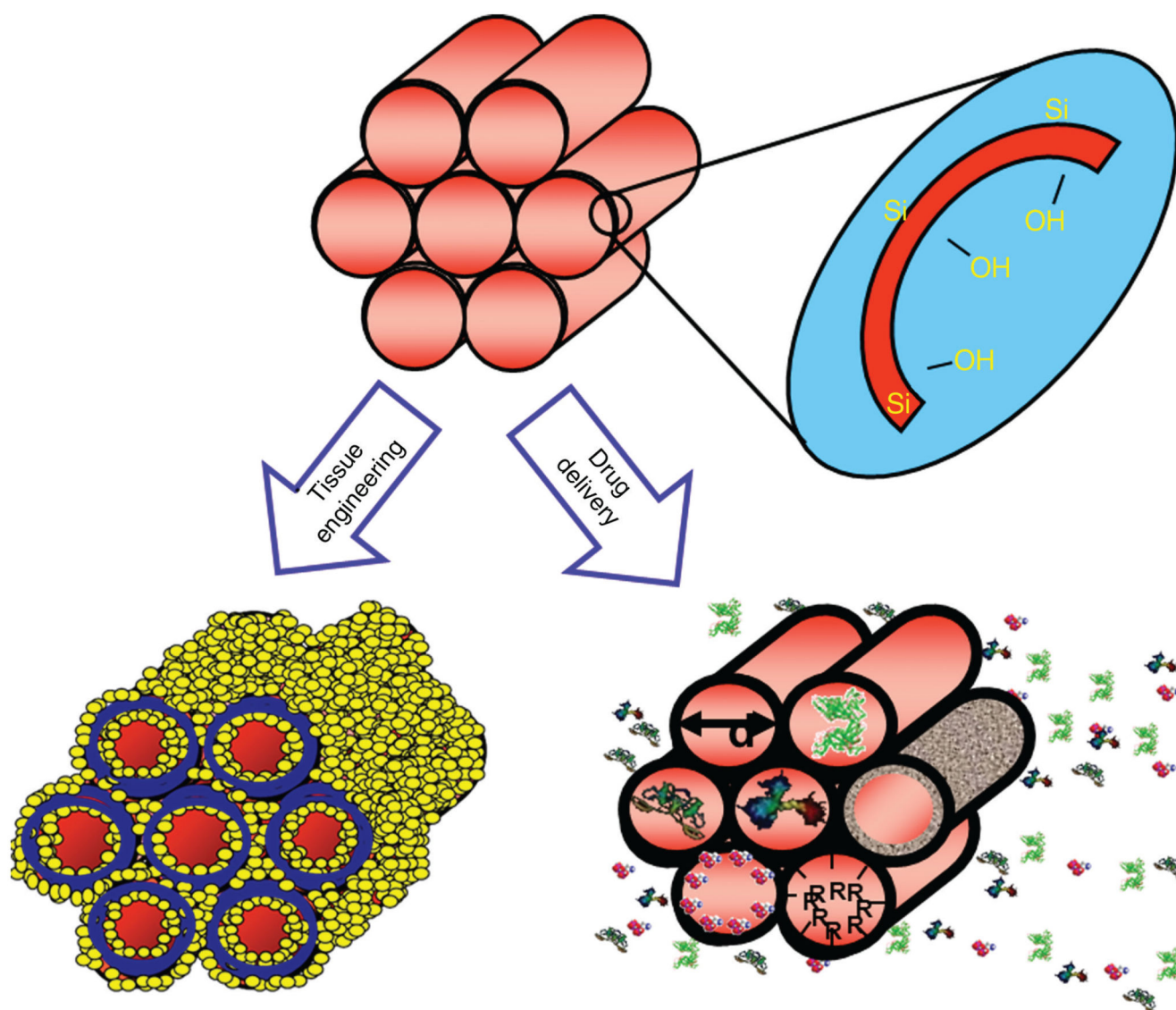


Fig. 3. Mesoporous ordered materials can be used for tissue engineering and drug delivery applications. Yellow spheres represent the hydroxyapatite formation as a consequence of bioactive behavior of the mesoporous material. On the right hand side there are different drugs or biomolecule that can be hosted inside the pores.

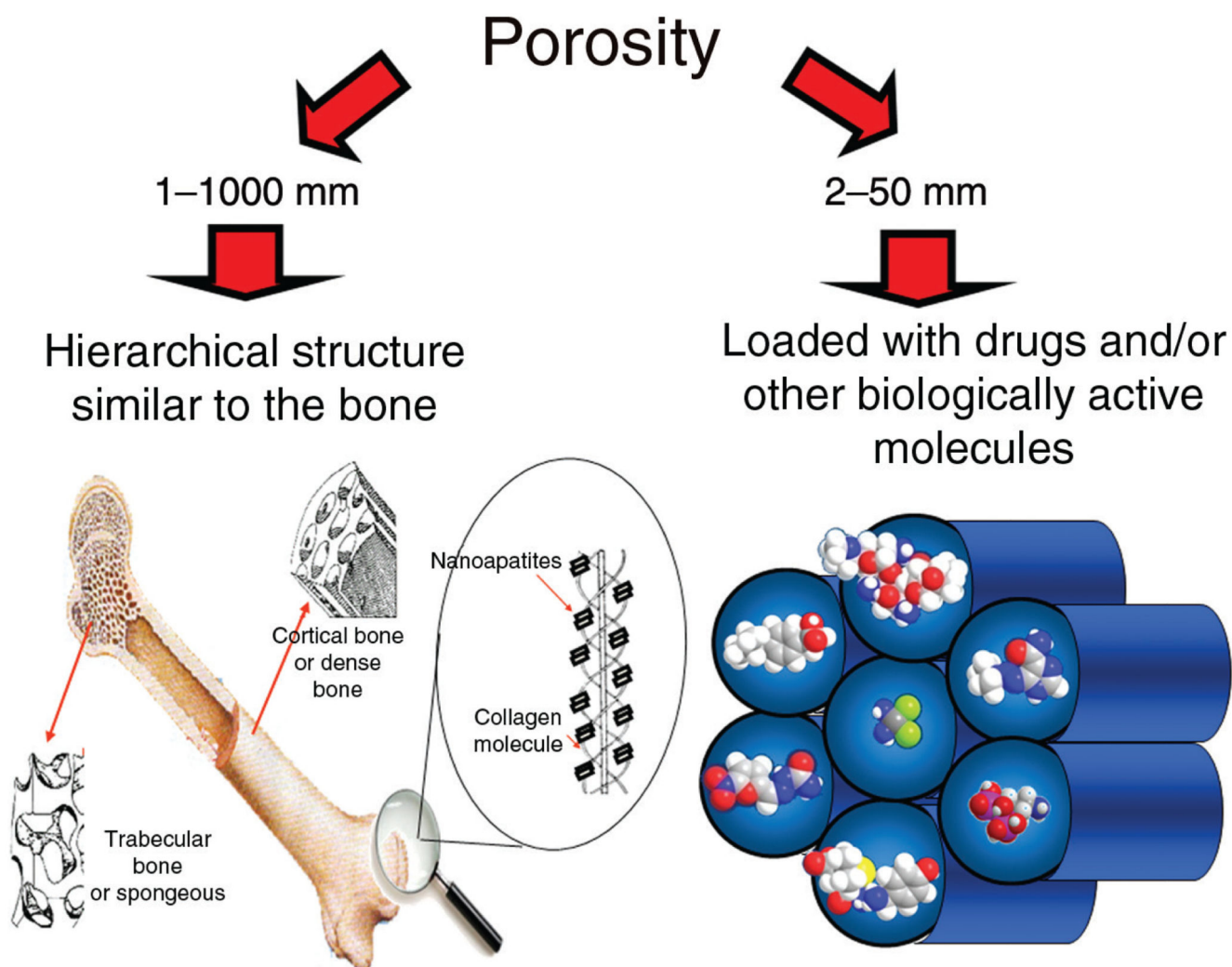


Fig. 4. Pores of different sizes are necessary to play different biological actions.

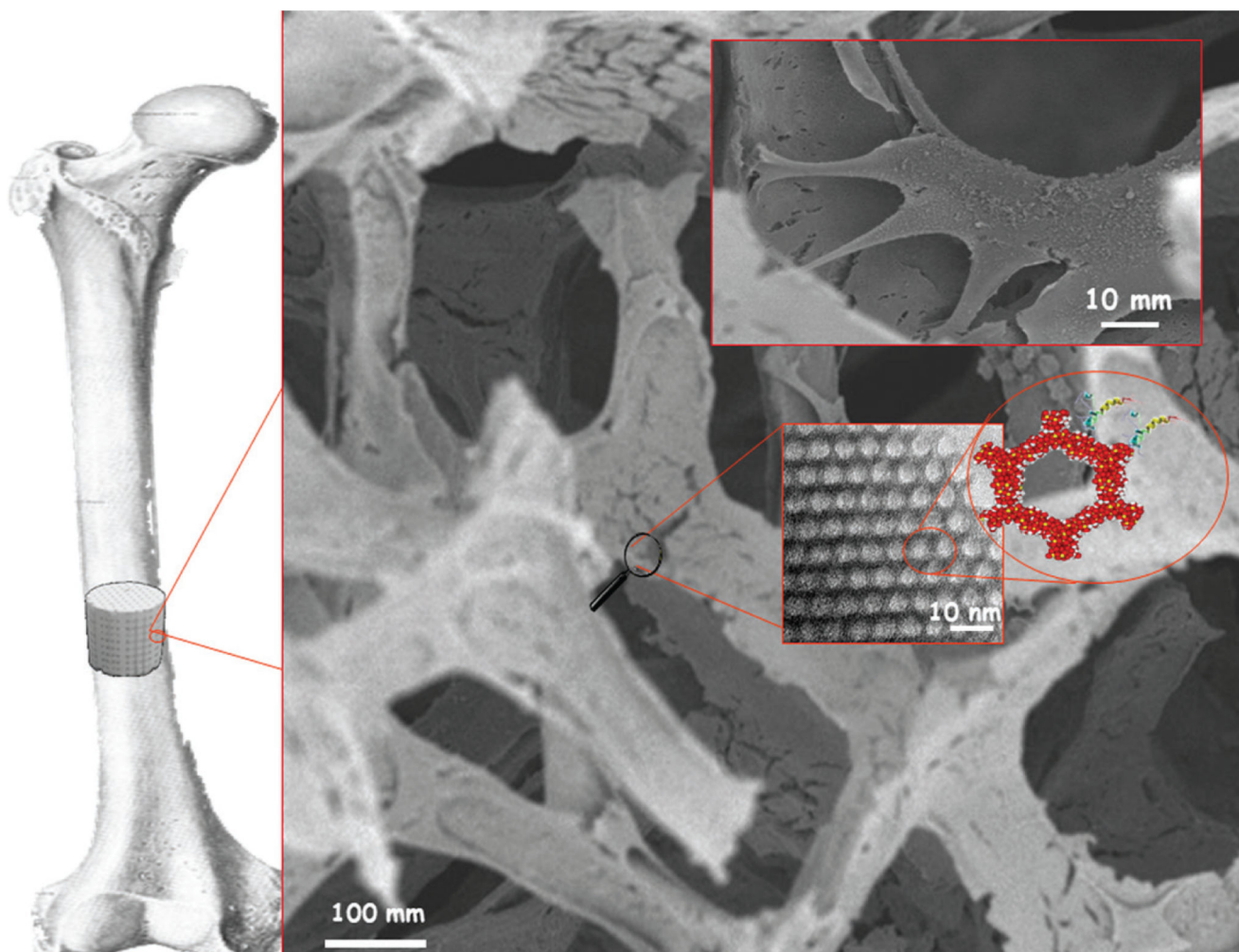


Fig. 5.
The hierarchical porous structure of artificial bioceramics similar to the bone.

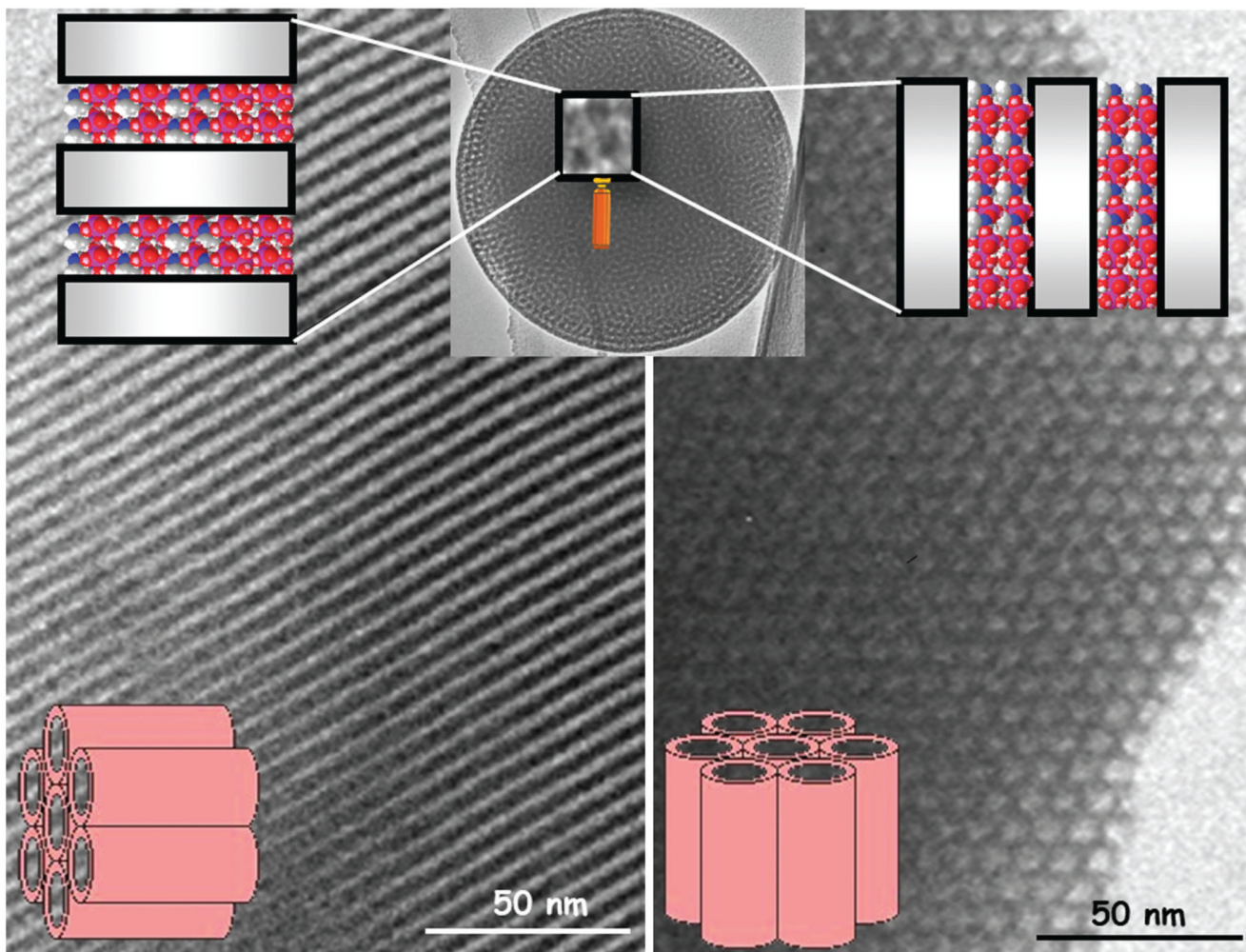


Fig. 6. Transmission Electron Micrographs of mesoporous silica matrices with the possibility to load their pores with drugs.

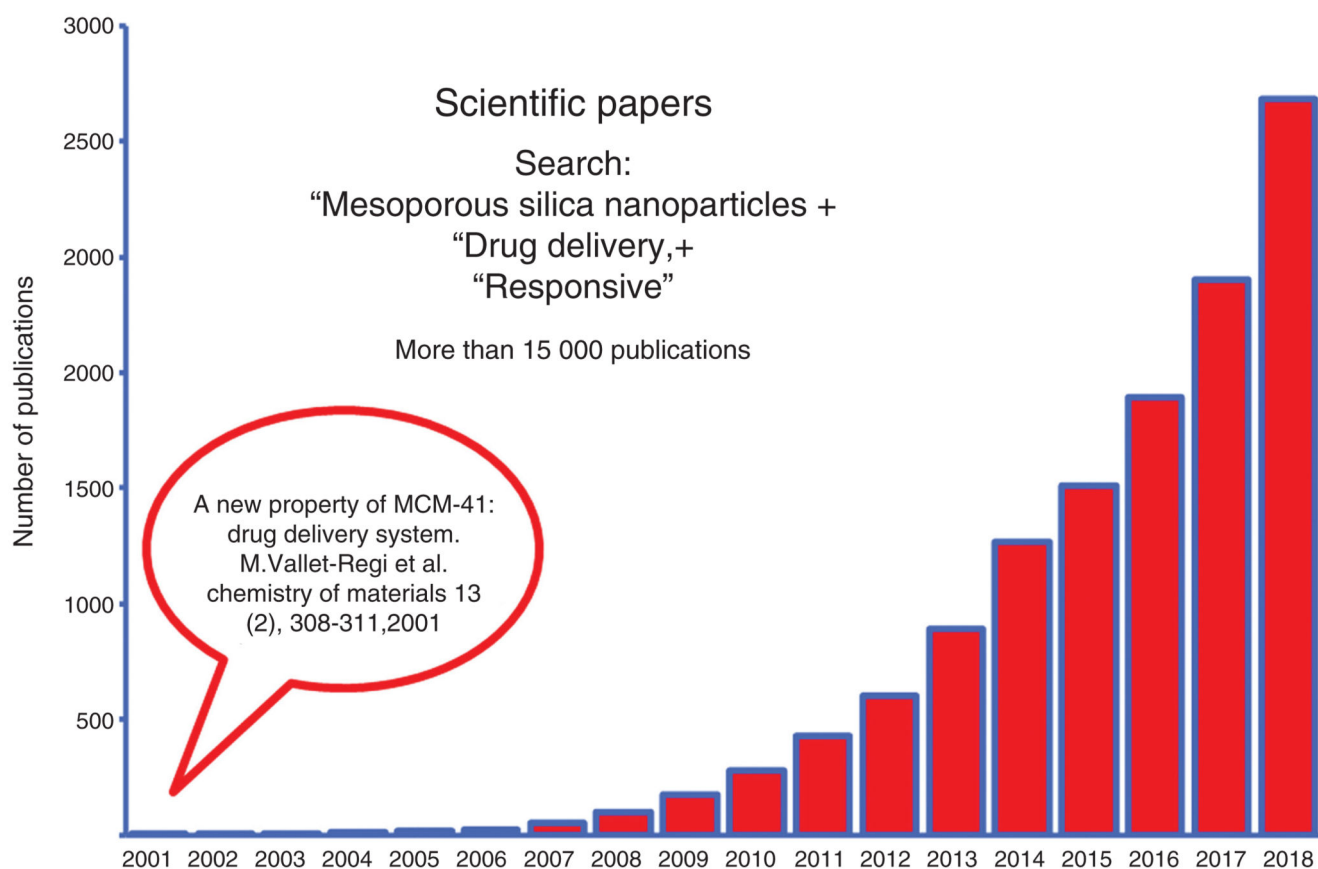


Fig. 7.
Evolution of research articles published on the topic of Responsive-MSNs for drug delivery.
Data from Google Scholar.

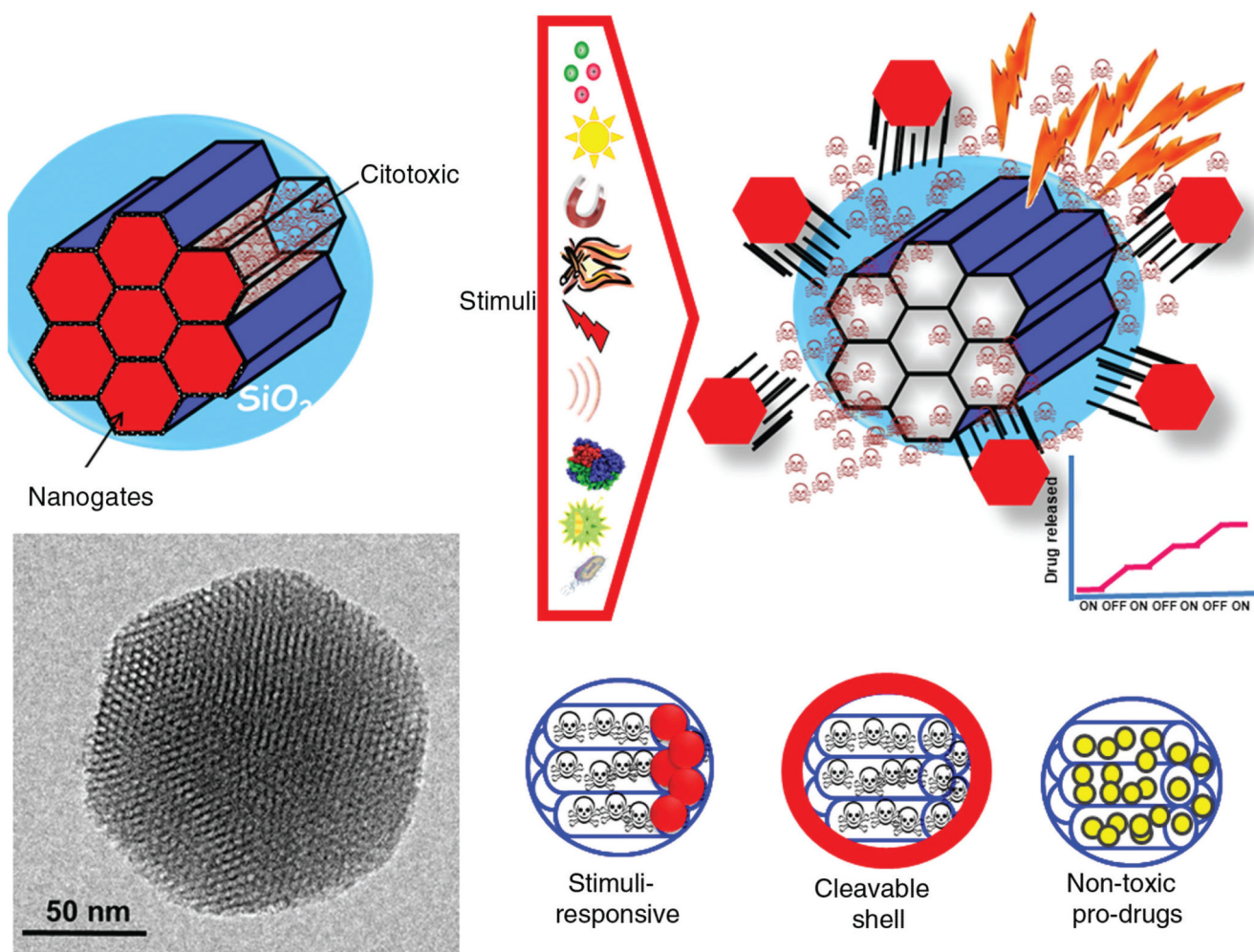


Fig. 8. Schematic representation of stimuli responsive release of MSNs cargo (top), Transmission Electron Microscopy micrograph of MSN (left bottom corner), and the potential solutions to avoid premature release of the cargo (right bottom corner).

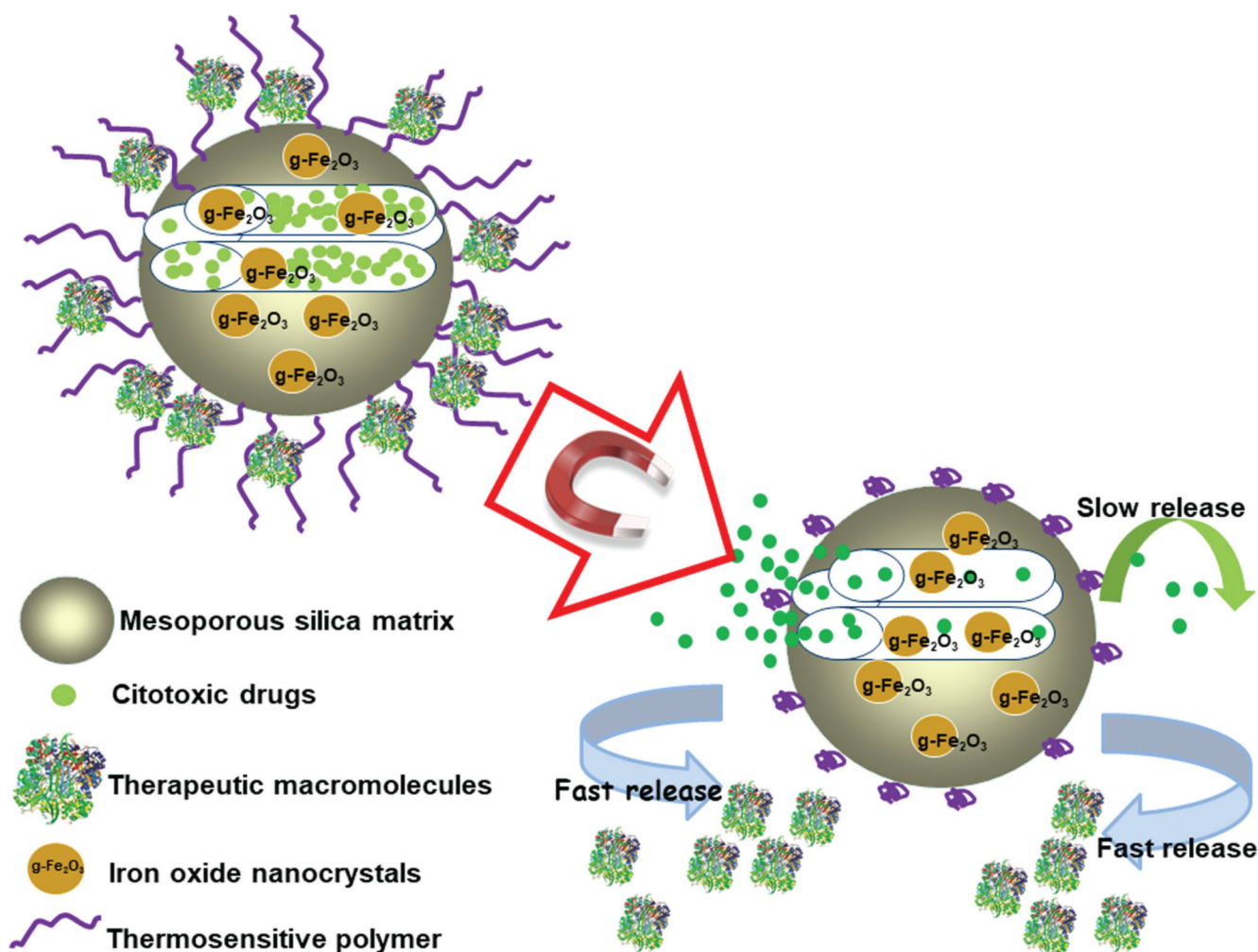


Fig. 9. Schematic representation of release of two types of agents: small molecules encapsulated into the pores and large proteins retained within the shell of nanoparticles triggered by magnetic fields [103].

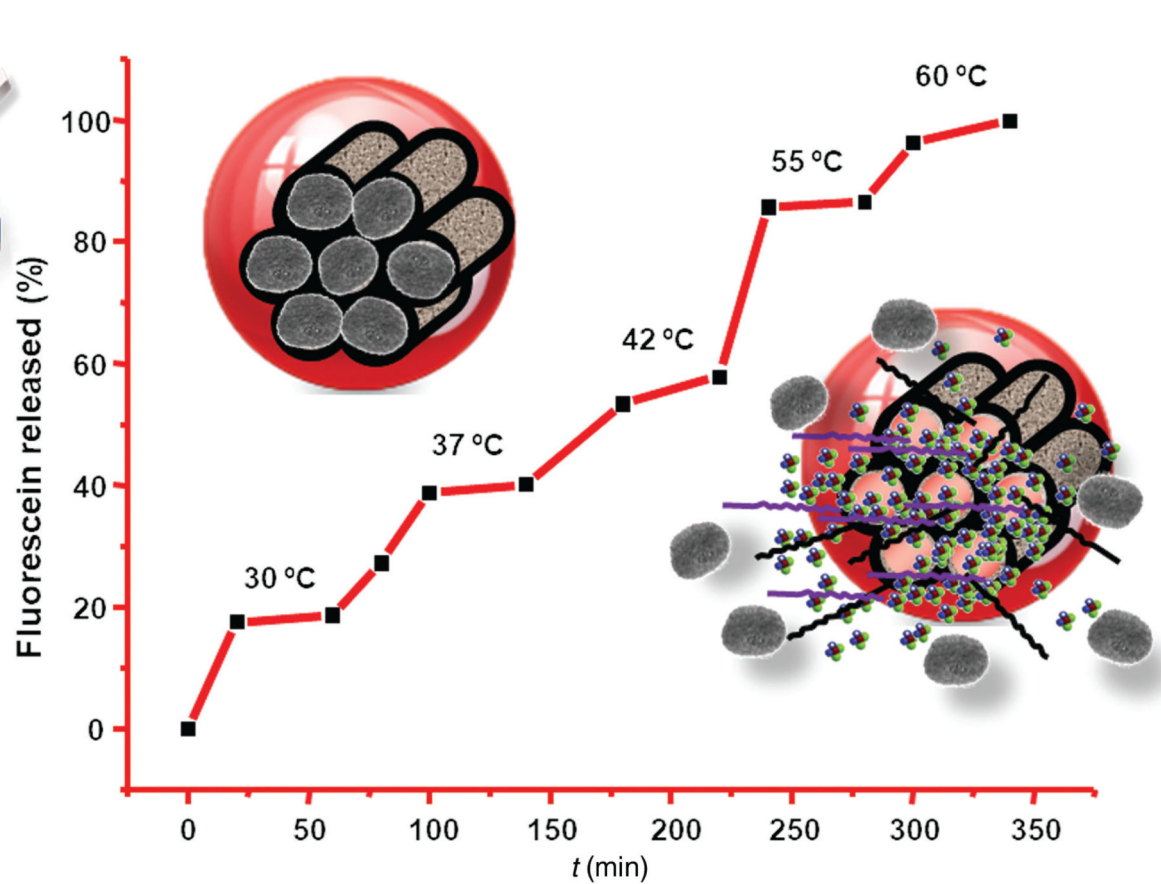


Fig. 10. Schematic representation of pulsatile release from MSNs responsive to magnetic fields [104].

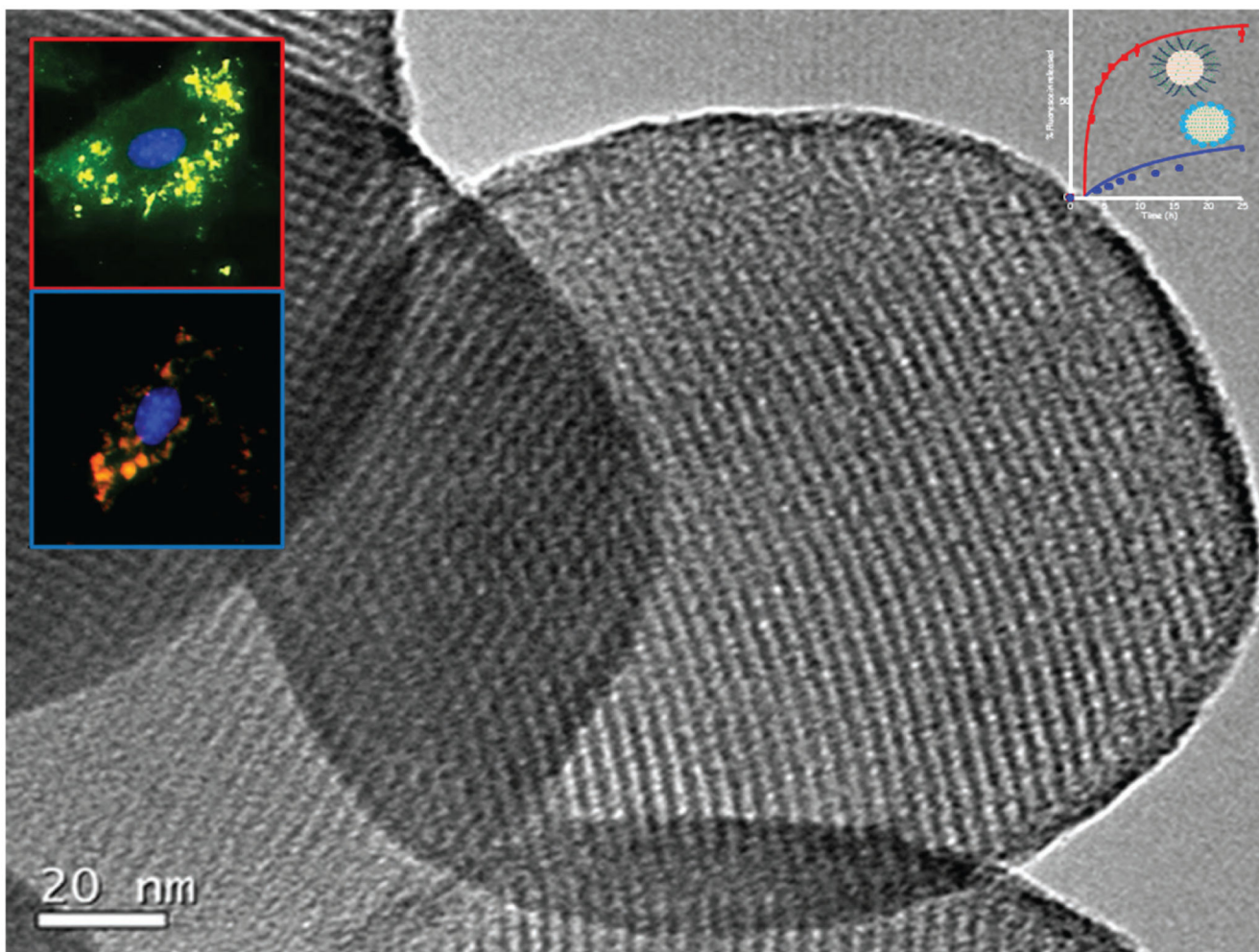


Fig. 11. MSNs internalized into cells (right top corner), TEM micrograph of MSNs decorated with US sensitive polymer (center), and in vial release kinetics from that platform [114].

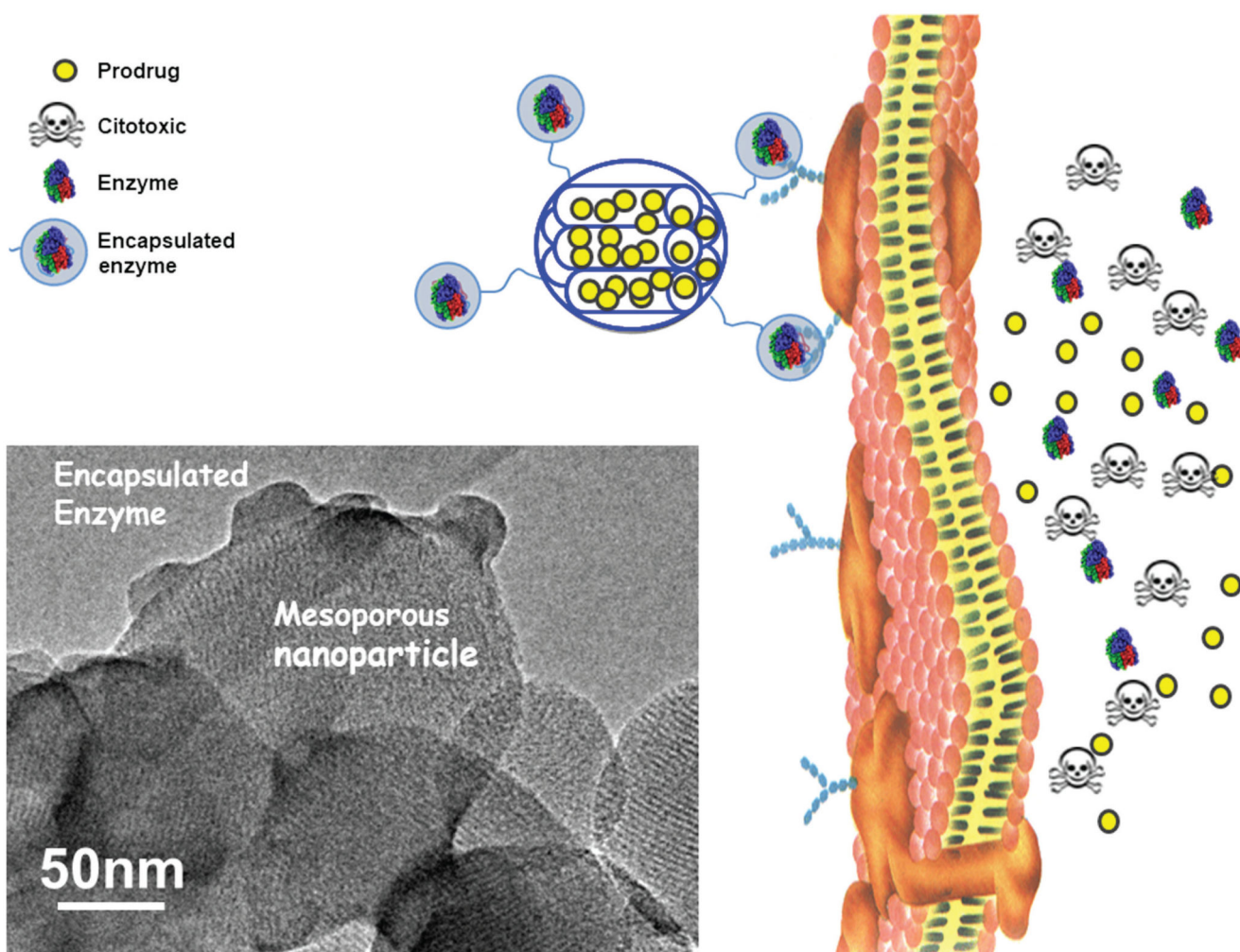


Fig. 12. TEM micrograph of MSNs with the encapsulated enzyme on their surface (left) and schematic representation of cell penetration [129].

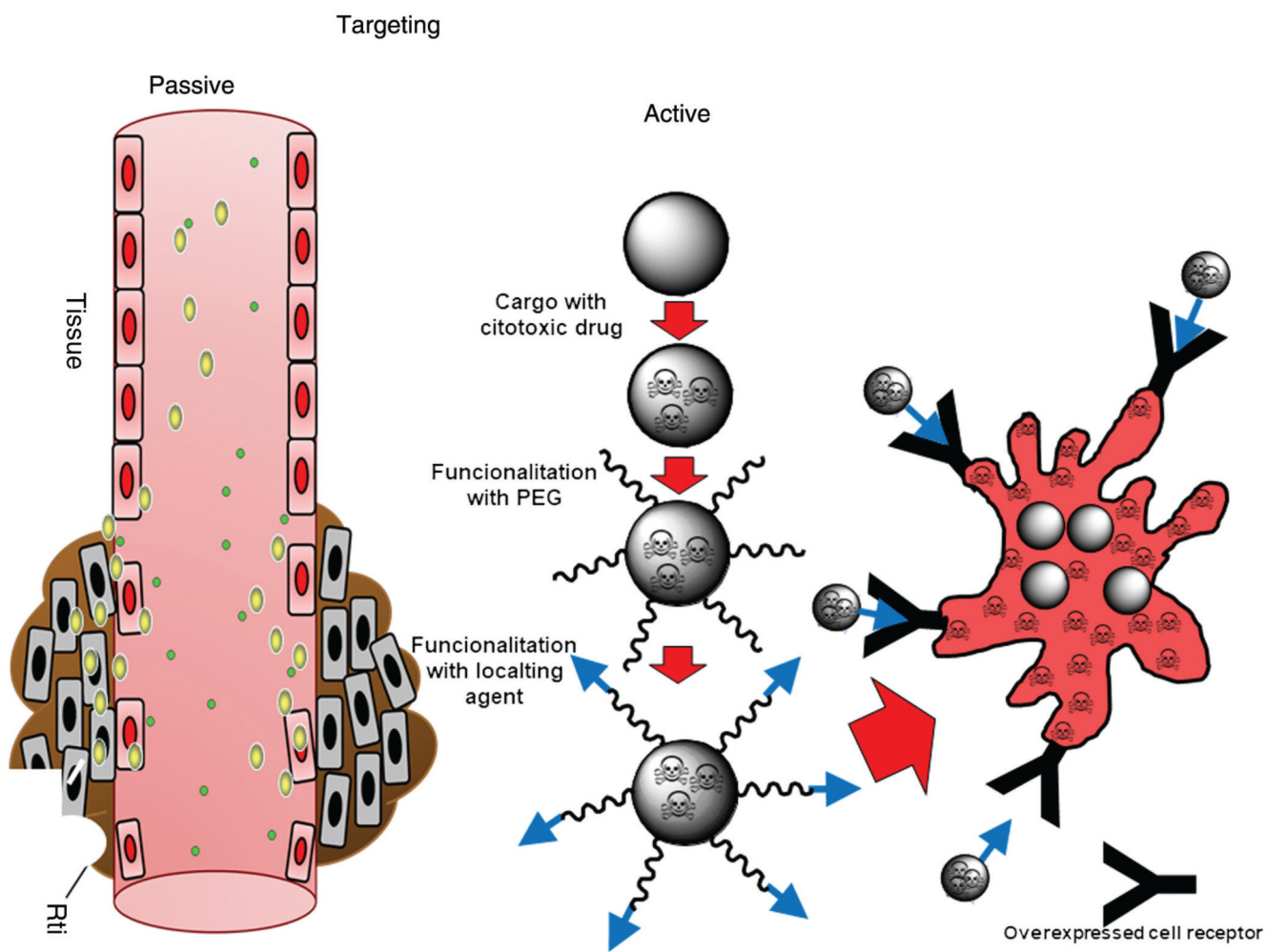


Fig. 13.
Passive and active targeting.

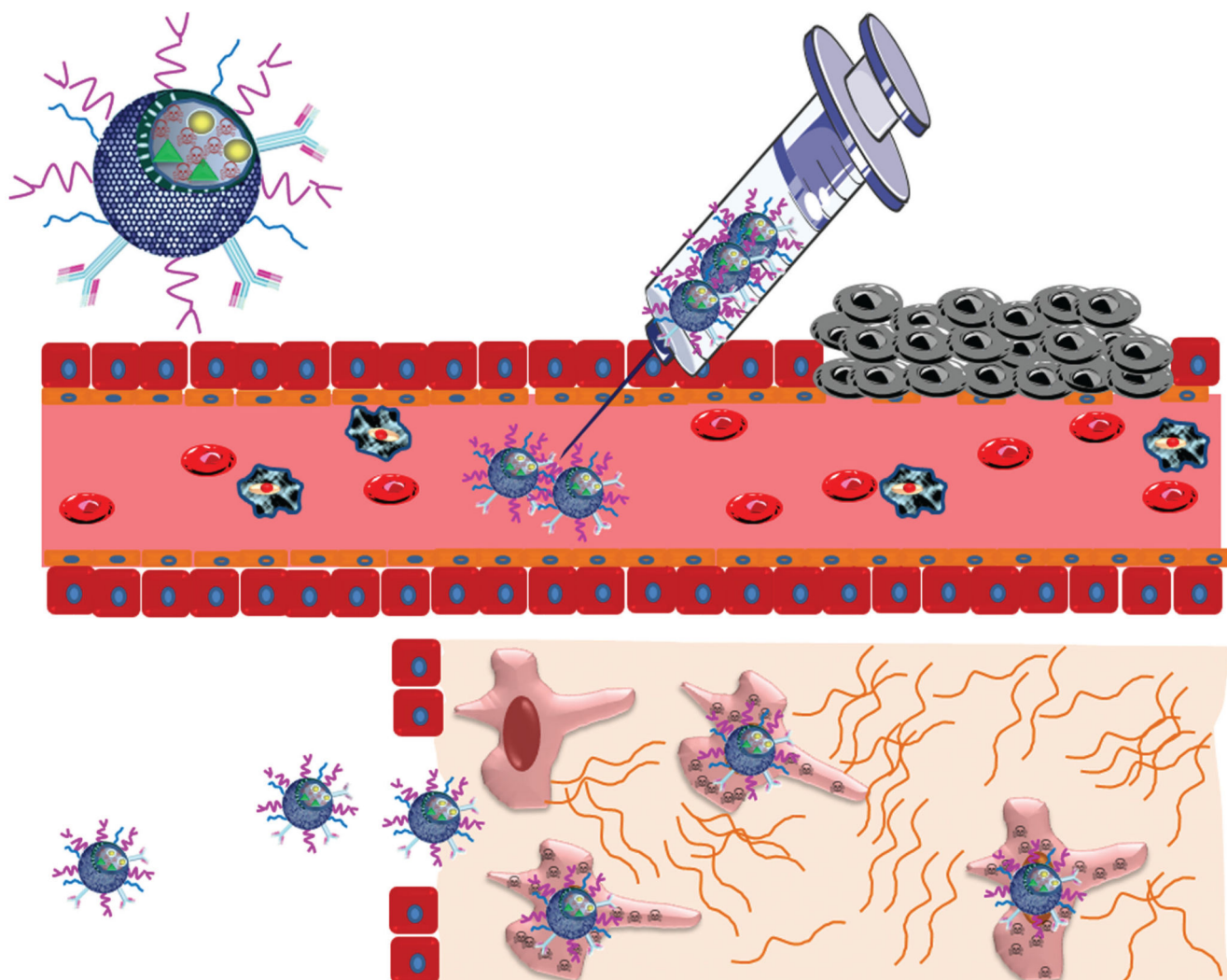


Fig. 14. Schematic representation of stealth nanoparticles loaded with drugs and injected on the blood vessels (top) and tumor penetration of nanoparticles with targeting abilities (bottom).

Table 1
The three generations of bioceramics used for bone repair.

	1 st generation	2 nd generation		3 rd generation
Ceramics as bone repair materials				
Type of bioceramic	Bioinert non-absorbable	Biodegradable resorbable	Bioactive Surface reactive a surface reactive	Scaffolds of biologically active molecules
In vivo reactivity	Isolated by a non-adherent fibrous capsule	Dissolved after a specific time	Tightly bonded to living tissues through	Stimulating living tissues regeneration
Examples	Alumina: Al ₂ O ₃ Zirconia: ZrO ₂ Carbons, mainly pyrolytic and as fibers in composites	Calcium phosphates Calcium sulfate Calcium phosphates and sulfates + ZnO, Al ₂ O ₃ , FeO ₃ Coralline CaCO ₃	Hydroxyapatite (HA), pure and substituted Hydroxycarbonate apatite (HCA) Glasses: by melting and sol-gel Glass ceramics: A/W glass-ceramics® and Ceravital®	Bioglass®: in particulate form Porous bioactive and biodegradable ceramics Advanced bioceramics: mesoporous materials, organic-inorganic hybrids