Review Article

Claudia Petrarca*, Anna Maria Poma, Giulia Vecchiotti, Giovanni Bernardini, Qiao Niu, Anna Giulia Cattaneo, Mario Di Gioacchino, and Enrico Sabbioni

Cobalt magnetic nanoparticles as theranostics: Conceivable or forgettable?

Abstract: Superparamagnetic nanoparticles, exposed to an external variable magnetic field, undergo rapid excitation/relaxation. So-called soft magnets, typically iron-based, rapidly and completely relax when the magnetic field returns to zero. Instead, cobalt-based (CoB) hard magnets retain residual magnetization, a characteristic related with the procedure for nanoparticles (NPs) production. Many researchers are still attracted by the potential of CoB NPs for theranostics as multifaced signal probes for imaging, microrobots, enhanced thermo/radiation therapy, and drug release. Since iron oxide NPs are the only magnetic NPs approved for human use, they are of reference for analyzing the potential of the disregarded CoB NPs. *In vitro* observed toxicity of CoB NPs, largely attributable to cobalt ions and other chemical species released by dissolution, excluded them from further investigations in humans. Nevertheless, experimental evidences documenting the *in vivo* toxicity of engineered CoB NPs remain very few. The surface functionalization adds newer properties and could improve the biocompatibility of NPs, critical for the clinical exploitation. In our opinion, it would be worth to further exploit the potential of finely tunable properties of CoB NPs in *in vivo* systems in order to establish a systematic database of properties and effects suitable for human application.

Keywords: cobalt, magnetism, nanoparticles, safety, synthesis, theranostic, toxicity

1 Introduction

The discovery and exploitation of the superparamagnetism in nanoparticles began with the description of their ferrofluidic behavior [1] which opened the way to the development of nanomaterials with impressive chemical and physical oddities and exponential increase of research on their potential applications in biomedicine. Ferrofluids are colloidal suspensions of very fine (typical size of 10 nm) magnetic particles dispersed in a polar or nonpolar liquid carrier [2]. Application of an external magnetic field to such ferrofluidic materials leads to a very quick increase in magnetization, which is rapidly reduced (or abrogated) when the magnetic field is removed. This behavior is revealed by superparamagnetic iron oxide (FeO)-based nanoprobes that, when excited, acquire a sharp appearance relative to the surrounding environment. For this reason, they attract the interest of researchers as negative contrast enhancing agents for Magnetic Resonance Imaging (MRI) [3,4]. However, these "soft materials" show low residual magnetism (M_r) (Figure 1), one main physical property for this type of medical application.

Moreover, since their early development occurred more than two decades ago, only one carbohydrate-coated iron-based nanoprobe has been approved by FDA for clinical use [5]. Nevertheless, uncoated iron oxide nanoprobes show some degree of toxicity in a therapeutic setting [6]. Furthermore, increased exhaled breath condensate concentrations of lipid, nucleic, and protein oxidation

* **Corresponding author: Claudia Petrarca,** Department of Medicine and Aging Sciences & Center of Advanced Studies and Techologies (CAST) "G. d'Annunzio" University of Chieti-Pescara, Via Luigi Polacchi 11, 66100, Chieti, Italy, e-mail: c.petrarca@unich.it
Anna Maria Poma: Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy
Giulia Vecchiotti: Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy; Center of Advanced Studies and Techologies (CAST) "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy
Giovanni Bernardini, Anna Giulia Cattaneo: Department of Life Science, DBSV, University of Insubria, Via J-H Dunant, 3, 21100, Varese, Italy
Qiao Niu: Shanxi medical University, Institute of Preventive Medicine, Taiyuan, People's Republic of China
Mario Di Gioacchino: Department of Medicine and Aging Sciences & Center of Advanced Studies and Techologies (CAST) "G. d'Annunzio" University of Chieti-Pescara, Via Luigi Polacchi 11, 66100, Chieti, Italy
Enrico Sabbioni: LASA, Department of Physics, Universitá Degli Studi di Milano and INFN-Milano, Via F.lli Cervi 201, Segrate (MI) I-20090, Milan, Italy
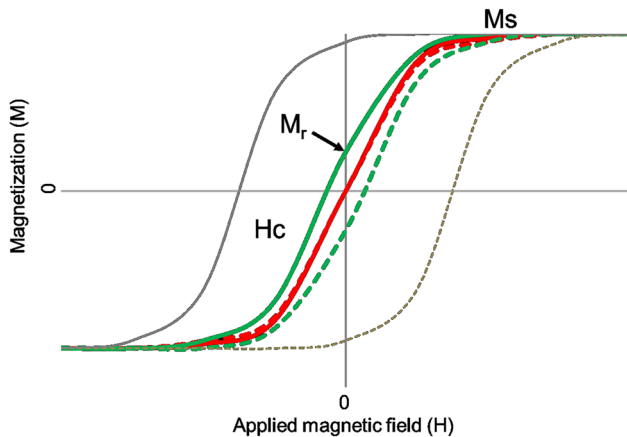
Figure 1: Simulated hysteresis curve for superparamagnetic soft (red) and hard (green) NPs. A similar behavior was recorded comparing cobalt-ferrite and magnetite NPs [19]. The saturation magnetization (M_s), residual magnetization (M_r), and coercivity (Hc) are indicated.

markers were detected in a dose-response correspondence to the environmental monitoring concentrations of NPs, in workers exposed to Fe_2O_3- and Fe_3O_4-NPs compared to control individuals [7]. Besides, cobalt and their oxides, when organized at the nanoscale, show a steep increase of the magnetic order and the other related parameters, so that superparamagnetism appears [8]. Differently from iron-based, CoB NPs behave magnetically as "hard materials" and could be worthily used because they own several specific biochemical and physical properties. For instance, in MRI applications they do not generate confounding signals in the presence of unsaturated hemoglobin, as certain Fe-based NPs do [9]. Other distinctive characteristics of CoB NPs (i.e., large magnetic multiaxial anisotropy, high Curie's temperature, high chemical stability, and mechanical hardness) might purposely be combined with each another to confer to them special "smart" qualities finalized to diagnostic platforms and therapeutic approaches, such as drug release or gene-modifying interventions. Ideally, smart agents should be easy to administer and have low toxicity, short half-life, and fast clearance depending on the diagnostic or therapeutic use. However, also materials with longer half-life should be taken under consideration to allow the follow-up of the evolution of lesions in days and up to months. In addition, magnetic NPs possess juxtaposed superparamagnetic, thermal, photoacoustic, and electric properties [10]. They can also be modified with protective and/or functional (bio)molecules at their surface (preventing dissolution, early phagocytosis, and toxicity), improving the emitted signal (radioactive, bioluminescent, or fluorescent) or target-specific sites (tissues, cells, enzymatic activity, or genome) [11].

Indeed, CoB NPs are attractive under all those regards and would offer great flexibility of applications in a variety of experimental settings. Regrettably, CoB NPs have been addressed by several experimental findings as more instable and toxic than Fe-based NPs, limiting their further exploitation in living beings and humans as a precautious rule. Otherwise, in the present review we critically reevaluate the current knowledge of the crucial physical (matter) parameters governing the synthesis of superparamagnetic of CoB NPs that could be modified/regulated *ad hoc* to obtain desired outcomes upon interaction with biological systems.

2 The core of superparamagnetic CoB NPs

The magnetic behavior, along with other various outstanding features of metallic NPs, arises from a complex interplay between their chemical nature, lattice symmetry, and shape; furthermore, size appears to be the most relevant parameter, with larger size determining a more disordered surface and longer relaxation time [12] (Figure 2).

As bulk material, cobalt is a ferromagnetic metal. However, when the particle radius is reduced to the nanosize, cobalt behaves as superparamagnetic material, due to the quantum effect [13]. If the radius is small enough, one single NP contains just one magnetic domain that can be finely tuned for theranostic purposes [14]. In turn, the value of the critical radius (r_c) for a controllable single magnetic domain is mainly determined by three physical entities: uniaxial anisotropy (K_u), vacuum permeability (μ_0), and saturation magnetization (M_s) [15], according to:
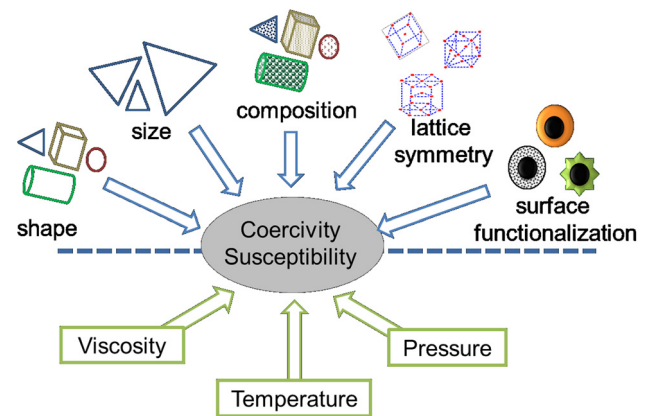

Figure 2: Magnetic properties of NPs are influenced by various factors, either intrinsic (upper part) or environmental (lower part).

Table 1: Physical-chemistry characteristics and related magnetic parameters of CoB NPs

Magnetic CoB NPs		Chemical physical characteristics			Magnetic properties			Ref.
		Size (nm)	Shape	Crystal symmetry	Coercivity (Hc, Oe)	Saturation magnetization (M_s,[a] emu/g)	Residual magnetization (M_r,[a] emu/g)	
Zerovalent	Co@C	–	–	–	123	121	4.1	[140]
	Co	40	–	–	370	137	–	[141]
Oxides	Co_3O_4, prepared at 175°C	11	Spherical	Cubic	–	0.137	–	[23]
	Co_3O_4, prepared at 200°C					0.225		
	Co_3O_4, prepared at 250°C					0.325		
Ferrites	$CoFe_2O_4$	25	Tetragonal	Cubic, fcc	1,209	64.06	25	[89]
	$CoFe_2O_4$@AOT (0.05 g)	25	Tetragonal	Cubic, fcc	1,880	69.62	33	
	$CoFe_2O_4$@AOT (0.1 g)	25	Tetragonal	Cubic, fcc	2,399	71.54	38	
	$CoFe_2O_4$@AOT (0.2 g)	25	Tetragonal	Cubic, fcc	2,415	73.78	39	
	$CoFe_2O_4$@AOT (0.5 g)	25	Tetragonal	Cubic, fcc	2,550	79.05	43	
	$Fe_{45}Co_{55}$	9	Spherical	Cubic	43	111.6	–	[142]
	$Fe_{45}Co_{55}$@graphite	<10	Spherical		70	145		
	$CoFe_2O_4$	21	Hexagonal	Spinel, cubic	16.3	54.9	15.7	[143]
	$CoFe_2O_4$@PVP	24			83.2	60.1	26.7	
	$CoFe_2O_4$@PEG	23			84	63	28	
	$CoFe_2O_4$, co-precipitation	33	Spherical	Spinel, cubic	1492.6	60.85	29.34	[144]
	$CoFe_2O_4$, hydrothermal	14			507.7	56.88	21.44	
	$Fe_{70}Co_{30}$	15				220		[145]
	$CoFe_2O_4$ (annealing: 400°C)	–	–	–	1,725	36	–	[146]
	$CoFe_2O_4$ (annealing: 850°C)				728	63		
	$CoFe_2O_4$ (annealing: 1,000°C)				298	78		
	$CoFe_2O_4$	25–30	Polyhedral	Spinel	5.2	5	–	[147]
	$CoFe_2O_4$@PA90%				5.3	6		
	$CoFe_2O_4$@PA80%				5.3	6		
	$CoFe_2O_4$@PA70%				5.3	7		
	$CoFe_2O_4$@PA60%				5.3	8		
	$CoFe_2O_4$@PA60%				5.4	14		
	$CoFe_2O_4$ (300 K)	6	Spherical	Spinel, cubic		78	52	[19]
	$CoFe_2O_4$ (2.5 K)					99	66	
	$CoFe_2O_4$	10	–	Cubic and hexagonal, mixed	1.656	31	15	[148]
	$Zn_{0.5}Co_{0.5}Fe_2O_4$	3.9	Spherical	–	14.02	29.2	68.85×10^{-3}	[149]
					12.27	23.6	22.56×10^{-3}	

Table 1: Continued

Magnetic CoB NPs	Chemical physical characteristics			Coercivity (Hc, Oe)	Magnetic properties		Ref.
	Size (nm)	Shape	Crystal symmetry		Saturation magnetization (M_s,[a] emu/g)	Residual magnetization (M_r,[a] emu/g)	
Dextran-coated $Zn_{0.5}Co_{0.5}Fe_2O_4$							
$CoFe_2O_4$	36	Spherical	Inverse spinel, cubic	1.1	69	28	[150]
$CoFe_2O_4$@PVA (stirred)	17	–	–	1.013	16	–	[151]
$CoFe_2O_4$@PVA (sonicated)	7			170	7		
$CoFe_2O_4$	9		Single spinel, cubic	234.32	40	–	[152]

Abbreviations: AOT: the surfactant sodium bis(2-ethylhexyl)sulphosuccinat.
[a] emu/g: equals SI unit (A^2/kg).

$$r_c \approx 9 \frac{(AK_u)^{\frac{1}{2}}}{\mu_0 M_s^2}$$

Table 1 reports the experimental values of residual magnetization (M_r) of various different sized CoB NPs with different oxidation state. Zerovalent Co-NPs show the smallest radius (<5 nm), corresponding to the lowest levels of magnetic disorder, conferring better superparamagnetic properties, compared with corresponding Co-oxide NPs [16]. Oxidation reduces the values of saturation magnetization (M_s) of Co-NPs, showing the lowest values [17]. The critical size for good ferroparamagnetic properties approximately spans from 5 nm (Co/FePt alloy NPs) to 20 nm (Fe-oxides) [17], $CoFe_2O_4$ NPs and iron-based NPs having intermediate M_s values [18]. The relatively high energy emitted as heat (thermal energy) allows complete relaxation of soft magnets when the external magnetic field ceases (Figure 3a). Systems with randomly distributed (larger) size or not uniform crystal structure (anisotropic), such as Fe-based NPs, display a "soft magnet" behavior [19]. Instead, CoB NPs behave as "hard magnets," since they are characterized by uniform size and crystal symmetry [20,21] (Figure 3b). Given the dependence of so many and complex physicochemical properties of NPs, the volume-to-specific surface area ratio, rather than the diameter alone, appears to be the most suitable parameter to describe the phenomenon of superparamagnetism. According to the current consensus about the
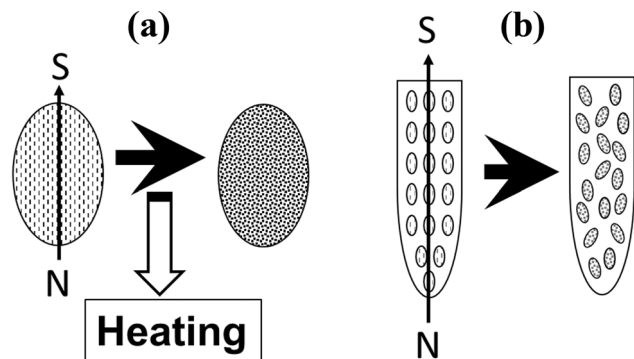

Figure 3: Magnetic relaxation of ferrofluids: stable suspensions of magnetic nanoparticles (MNPs) are ferromagnetic when an external magnetic field applies, and return to their original paramagnetic state, with null residual magnetization, when the external field ceases. Two mechanisms could interact in the magnetic relaxation process: (a) intrinsic rotation of molecules inside the MNPs, which are distributed into a single domain. Chemical nature, volume, and temperature are main regulators of the process that depends on the Nèel relaxation. (b) Extrinsic rotation of MNPs in the colloidal matrix. The driving Brownian rotational motion of single particles depends on shape, temperature, size, and opsonization. More details in ref. [138].

definition of NP, the critical limit value does not exceed $60\,m^2\,cm^{-3}$ [22].

During the demagnetization, heat develops (according to Nèel's relaxation) (Figure 3), which is the main property of multifunctional NPs to be useful for theranostics.

CoB NPs, more prone to behave as hard magnets than their iron-based counterparts, develop higher and more prolonged temperature rise [23]. Beside size and anisotropy, the magnetization of CoB NPs can be influenced by additional characteristics, such as shape [24] and viscosity of the external matrix [25] (Figure 2).

The method used to synthetize the CoB NPs determines their anisotropy degree and, thereof, the critical size, that greatly varies consequently.

3 Synthesis of magnetic CoB NPs

In order to produce CoB NPs with appealing magnetic properties for medical purposes, the synthesis process should be carried on under controlled conditions. Cobalt can crystalize in a multiplicity of phases (with similar energy levels) and oxidizes rapidly at the surface, where the thin layer of oxide would hamper the completion of the synthesis reaction and uniformity of the final product, thus interfering with magnetic properties [26]. The most relevant methods of CoB NPs synthesis are hereby recalled:

(i) Solvothermal method. It is used for the synthesis of superparamagnetic zerovalent metallic cobalt NPs (CoNPs). The process starts with the induction of a single nucleation, under strictly controlled temperature in a reducing or hypoxic environment. Uncontrolled surface modification (aggregation of new nuclei or oxidation) is avoided by constraining the surface of the growing particles with the addition of a surfactant to the reaction mixture; then, a regular lattice of 2 nm spherical NPs with cubic face-centered symmetry and acting as hard magnets are produced by adding hydrazine and triethanolamine [20] (Figure 4a); 6–9.5 nm NPs with epsilon symmetry are synthesized in the presence of oleic acid:trioctylphosphine oxide 5:1 [27,28] (Figure 4b); 5 nm NPs with hexagonal close-packed symmetry are synthetized in oleic acid:diphenylether 1:1 [29] (Figure 4c).

(ii) Single nucleation core. The synthesis of Co-oxide or/ Co-ferrite NPs (CoONPs and CoFeNPs) relies on the same principle of single nucleation core followed by controlled growth of the crystal, as summarized in supplementary files (Figures S1 and S2). The
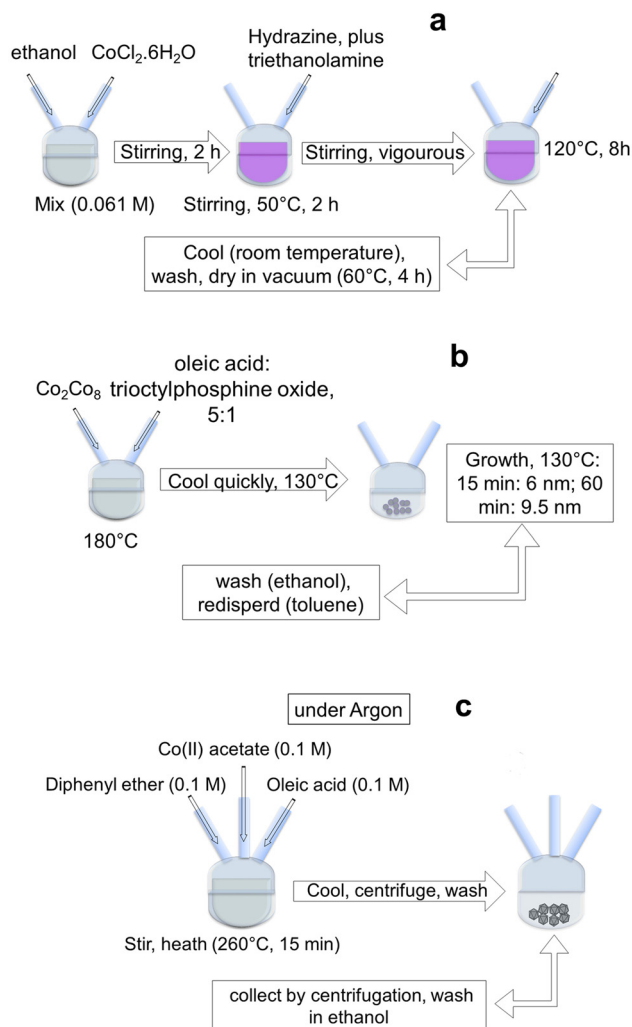

Figure 4: Synthesis of zerovalent CoB NPs via solvothermal route. (a) 2 nm MNPs with face-centered cubic symmetry and acting as hard magnets [20]; (b) 6–9.5 nm, epsilon phase [27]; (c) 5 nm, hexagonal close-packed symmetry [139].

precursor could be an inorganic salt or an organic compound, such as cobalt nitrate or cobalt acetate, for cobalt oxide, or salts of iron and cobalt for stoichiometric CoFe NPs. Extremely high temperatures are required for the nucleation and growth of cobalt-ferrite NPs (up to 5,500°C), whereas much lower is needed for cobalt (130–180°C) and cobalt oxide (50–75°C). The composition of the reaction mixture, the reaction time, and temperature are the most important variables to control the final size and the uniformity of the sample [23,30];

(iii) Sol–gel method. It utilizes a stable dispersion of micelles that collides, coalesces, and breaks until a uniform precipitate develops in the presence of cobalt nitrate. The addition of appropriate solvent destroys the micelles and produces a final sample

of monophasic cobalt oxide NPs after calcination at high temperature [31,32]. Uniform CoFe NPs, smaller than those produced in aqueous solutions, can be obtained through a similar procedure [33]. The experimental comparison of the above-described methods, introducing *ad hoc* procedural changes (i.e., molecular ratios in the reaction mixture or lower temperature of calcination), led to the production of NPs of the intended size, shape, nanostructure, crystalline symmetry, and magnetic properties, confirming the outmost relevance of rigorous control of the conditions to get such an accomplishment [8].

(iv) Biosynthetic process. More recently, CoB NPs are being obtained through procedures as technological response to the quest for environment-friendly synthesis of advanced materials. High superparamagnetic Co–(Fe)-based NPs of stoichiometric ($CoFe_2O_4$) and nonstoichiometric composition (i.e., magnetite) with a diameter of 8, 16, and 15 nm, respectively, are produced by the microorganism *Geobacter sulfurreducens* [34]. Another new and proficient system is based on the use of the empty capsid of Cowpea Mosaic Virus as a nanoshell bioreactor which, filled with 10% in volume cobalt chloride, allows to build up magnetic NPs of irregular structure [35]. More recently, *Aspergillus nidulans* was found to be suitable for synthesis of spinel cobalt oxide nanoparticles at an average size of 20.29 nm in spherical shape with sulfur-bearing proteins acting as a capping agent for the synthesized nanoparticles [36]. In general, cobaltous nitrate hexahydrate, cobalt(II) acetate tetrahydrate, cobalt chloride hexahydrate, and cobalt(II) acetyl acetonate were used as precursors for the green synthesis of Co_3O_4 NPs [37].

3.1 Surface modification

Surface coating or covering with a shell generally increases the biocompatibility and the stability of NPs, protecting them from dissolution and oxidation [38] which are massive processes for CoB NPs in aqueous medium. The chemical nature and the amount of the coating can moderately influence the magnetization, as shown in Table 1.

Organic coating of CoB NPs provides a means to reduce dissolution and facilitate further functionalization with targeting molecules [39]. Biehl and coworkers [40] reviewed the rationale for surface functionalization with polyzwitterionic molecules concluding that, since the method of production can modify the performance as

magnetic materials, the functionalized product should be carefully tested in different environments. Gold shell provides a hard protection against dissolution and oxidation which enhances the biocompatibility of CoB NPs. Notably, the shell does not alter *per sé* the superparamagnetic behavior of NPs, but the number of particles embedded inside the shell seems to be important [41,42] (Figure 5a). Smart materials have been developed with more complex coatings, such as multiple layers of polymeric and soft structures added to a hard golden shell, for the release of drugs on demand. For instance, drug-loaded NPs reacting to an oscillating external magnetic field with a spatial distortion provisionally release the drug when the field ceases [43] (Figure 5b). In addition, particles shaped in the form of small caps (nanowontons) behave as sensitive magnetic and photoacoustic probes: in this case, the protective Au-coating improved the bioavailability without altering the richness of the signal [10] (Figure 5c).

More recently, a new material was engineered by cross-linking Co–Fe NPs to a pH-sensitive hydrogel allowing rapid magnetization, possibly given by the average orientation of magnetic polar structures embedded in the gel layer, driven by pH-driven swelling and showing residual weak magnetization after shrinking [44]. Notably, drug-loaded
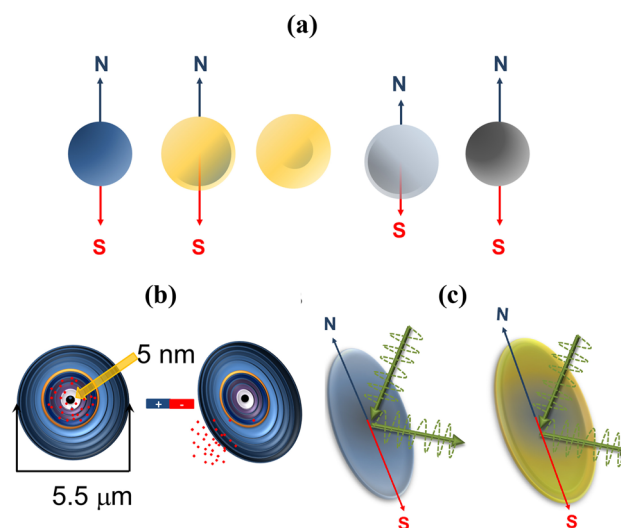

Figure 5: Effect of coating on magnetic properties of cobalt-based NPs. (a) From the left: zerovalent Co, uncoated particle, large particle in a thin golden shell, small particle in a golden shell, Co MNP in a silver shell, Co-oxide MNP. (b) Microcapsules for controlled release of drugs. The inner core, a cobalt-ferrite magnetic nanoparticle sized 5 nm, is embedded in four layers of organic polymers. A thin shell of Au and five organic polymeric layers complete the capsule. If charged with drugs, it reacts to an external magnetic field with distortion of the capsule, permeabilization of layers, and tunable drug release [43]. (c) "Nanowontons," bare (left) or coated with Au (right), display magnetic and acoustic properties [10].

magnetic hydrogels can control the release of drugs in response to a variety of stimuli [45]. An even more tempting coating could be that made of a magnetic material different from that employed in the core. Systems with a ferromagnetic–antiferromagnetic interface show a shift of the magnetization axis, while the juxtaposition of soft and hard magnetic phases generated high-performant, permanent NPs [46,47]. The addition of fluorescent dyes to obtain multiple signals was proved to be efficient in CoB NPs coated with a graphene-like surface of carbon, which did not alter the magnetic properties of the particles and provided a shell easy to be functionalized [48]. Such NPs are a promising probe for *in vitro* assays, with fluorescent signal and recovery on external magnet. Instead, a shell of silica does not preserve the magnetic signal of Co–Fe NPs functionalized with multiple fluorescent dyes [49]. The polymeric system made up with CoO-NPs coated with phosphonomethyl iminodiacetic acid had excellent stability in aqueous medium and, tested *in vitro*, this material showed anticancer activity without adverse effect on normal cells [50].

The complexity of this matter, describing the changes of magnetic order that follow coating and surface functionalization, as well as size, shape, composition, and methods for preparation, stresses the importance of carefully characterizing newer particles, before considering the exploitation of final products.

4 Cobalt-based NPs as potential theranostic agents

Nowadays, iron-based NPs are the sole approved for clinical use by the international drug authorizing agencies and, therefore, the preferred ones for *in vivo* applications [4,51,52]. Nevertheless, the ability of MNPs to transduce external magnetic field energy into a mechanical or thermal response can be exploited for biomedical applications, with multifaced working hypothesis: *in vivo* imaging, targeted release of drugs, magnetically driven navigation with delivery of thermo/radio/chemotherapy, as well as long-term follow-up of localized lesions, pathogens, and parasites. In spite of this enormous potential, only 14 human studies with magnetic nanoparticles, all iron-based NPs, are retrieved from the Clinical Trials database at this moment [4,53,54] directed to improve imaging of cancer and treatment of cardiovascular, demyelinating, and inflammatory nervous diseases (Figure 6 and Tables S1–S4).

In the meantime, human studies are disallowed for CoB NPs, whose persistent neglect is consequent of their supposed high instability and toxicity for the human organisms, at multiple levels (Figure 6).

Nevertheless, experimental studies are currently carried on to the aim of improving diagnostics and therapeutic tools by implementing CoB NPs (Figure 7) as exposed in the next paragraphs.

4.1 Diagnostic imaging: providing improved and multiple signal

Although Fe-based NPs are well-established and widely accepted since long time as paramagnetic probes for clinical applications, CoB NPs, of comparable paramagnetic properties and even more ductile and appealing as hard magnets, have been relegated to design/develop research studies *in vitro* and in laboratory animals, after toxicity side effects were accidentally described in those systems.

In fact, CoB NPs have been under investigation for the possibility of obtaining multiple signals through, for instance, the functionalization with fluorescent dyes. In addition to the superparamagnetic signal, high sensitivity emission signals were detected making them improved imaging tools [10,49,55]. In a study conducted in rats, the superparamagnetic signal emitted from CoB NPs could be further boosted by the luminescent and radioactive ones by functionalizing the NPs with Ga^{68} and luciferase [56]; in experimental biology, rhodamine-coated fluorescent cobalt–ferrite magnetic NPs have been used to monitor intracellular miR124a during neuronal differentiation of murine P19 cells [56].

4.2 Magnetic driving and navigation of NPs through the circulation

In a *in vivo* rodent model, cells loaded with Co–Fe NPs (enclosed in a silica shell or viral capsid) were able to migrate from the site of injection to distant tissues under the guidance of an externally applied magnetic field and accumulating nearby [57]. However, this cell-based approach might show limits in larger organisms where length of migration and depth of accumulation are increased [58]. When a more targeted experiment was performed, magnetic CoB NPs injected in the tail vein of rats accumulated inside the anterior chamber of the eye, where the external magnetic field was applied. Therefore, the particles could certainly navigate a long way through the vascular bed, but, also in this case, the site of accumulation was superficial and the size of the animal very small [59]. In
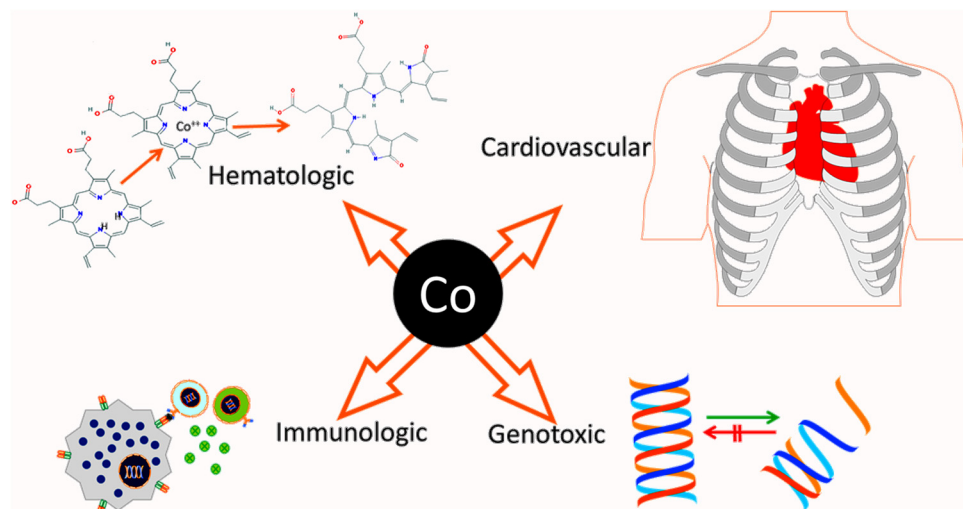
Figure 6: The Cobalt nanoparticles can be toxic at multiple levels, through direct or dissolution-mediated mechanisms. MNPs and dissolved cobalt ions can covalently bound the heme group (hematologic toxicity), stimulate the immunologic system, enhance oxidative damage to the DNA, or injure the cardiovascular system.
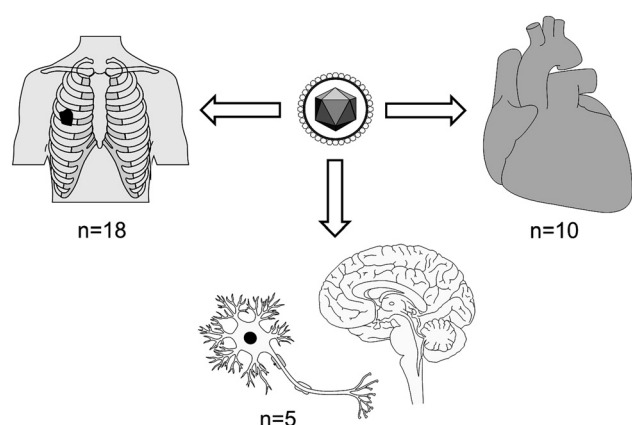

Figure 7: The distribution of studies with MNPs deposited in the Clinical Trials. The most numerous are for enhanced imaging of cancer ($n = 18$), 10 studies are aimed to the treatment and imaging of pathologies in the cardiovascular system, five to the imaging of demyelinating or inflammatory diseases of central nervous system, and five (not shown) to other conditions.

2011, a significant step was made in the therapeutic field of tumor targeted with the introduction of magnetic NPs as drug-containing cargos which could be guided by an external magnetic field. For the first time, the principles of microfluidics, robotics, and magnetic driving successfully cooperated to selectively address chemotherapy at a deep tumor site, through the circulatory system in living animals. The Magnetic Resonance Navigation (MRN) technique consisted of directional magnetic driving of a biocompatible fluid of 500 μm polymeric capsules filled with Co–Fe magnetic NPs manoeuvred through the vascular system by an external magnetic field [60].

After that, magnetically navigable agents for chemotherapy were investigated on phantom models mimicking liver arteries [61] and circulation [62]. As expected, the most probable detrimental effect would be embolism; furthermore, the ferromagnetic NPs microrobot navigation, under the control of magnetic force, would induce fluidity microvariations without apparent toxicity of conjugated ferromagnetic NPs as such.

4.3 Magnetic hyperthermia and drug release for tumor ablation

For decades, implants of macroseeds of iron alloys have been safely and efficiently applied for magnetically induced ablative thermotherapy of prostate tumors [63]. In phase I clinical trial on thermotherapy of recurrent prostate cancer, the employed iron oxide magnetic NPs showed no side effects [64], as well as the magnetic iron oxide NPs used for intratumoral thermotherapy were described as safe in glioblastoma patients [65]. However, those approaches could not achieve optimal modulation of the heating and induced massive tissue necrosis and incomplete ablation, in relation to dimension and depth of the tumor. Those disadvantages have been overcome by making biocompatible magnetic NPs which, in clinical trials, were shown to safely accumulate deeply in tumor lesions at the effective dose [66] and, when loaded inside stem cells or polymeric microcarriers, efficiently destroy atherosclerotic plaques [67]. Interestingly, the amount of cobalt in magnetic CoFe NPs determines their heating

response to a variable magnetic field, in experimental conditions [51,68,69]; in this way, it could be tuned to obtain theranostics suitable for diverse clinical applications [19,70] by varying the quantity of Co purposely. At the cellular level, CoB NPs, like others, enter various cell types through endocytosis [71,73] or diffusion across the plasma membrane [74–77] and are retained even when an alternate magnetic field is applied [71]. Remarkably, the endocellular dynamics and fate of CoB NPs are rather complex and are highly relevant to the biological outcome [78] (Figure 8).

Furthermore, the viscosity of the milieu in which magnetic NPs are dispersed affects the physical phenomenon driving heat response and needs to be taken into account. For instance, when dispersed into glycerol whose viscosity is similar to that of the endocellular milieu, the thermal effect of NPs is mainly due to magnetic heating mechanism of Néel relaxation [79]. Hence, the best performing NPs would likely be soft magnetic materials (maghemite, magnetite, and iron–platinum). Anyway, also hard magnets such as CoB NPs, expected to develop heat through the Brown relaxation mechanism (Brownian friction), have shown to be suitable for hyperthermia applications in several, but methodologically disparate, experimental studies [80–82]. These considerations suggest that thermal properties of magnetic nanoparticles might be conveniently modulated by modifying their own viscosity in the endocellular environment.

Dissolution of CoB NPs is a phenomenon known to take place either outside and inside the cell, releasing potentially dangerous ions of cobalt [78]. Instead, Fe-based NPs, under a constant magnetic field, either in cellular [83] or acellular [84] model systems, form large agglomerates and show low dissolution rate. However, until now no study has been addressed to determine whether CoB NPs release ions under similar conditions of magnetization, relaxation, and heat release. Paradoxically, the ion-mediated cytotoxicity of magnetic CoB NPs might even be a looked-for effect for treating tumor cells.

Magnetic CoB NPs have been found to induce thermal necrosis/apoptosis of melanoma [85], human breast [86], and rat gliosarcoma [87] cancer cells *in vitro*. Interestingly, human breast cancer exposed to the magnetic CoB NPs shows higher intracellular reactive oxygen species (ROS), found to mediate Co-ions-induced cytotoxicity [73].

However, these few experimental findings, although suggestive, are not enough to support the medical application of CoB NPs [88]. Nevertheless, it is reasonable to forecast the development of innovative CoB NPs by tuning the chemical–physical properties and their operational
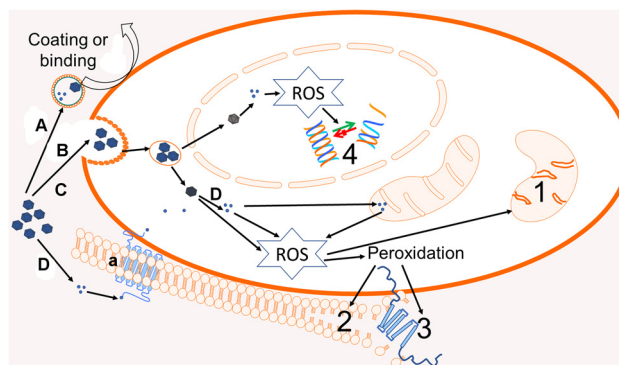

Figure 8: Cobalt oxide MNPs in the extracellular space can (A) interact with albumin or histidine, and be confined to the extracellular environment, or enter the cells through endocytosis (B) or passive diffusion (C). Partial dissolution occurs in both spaces, extra- and endocellular, with release of divalent ions of cobalt (D). The divalent ions of cobalt enter the cells through specific receptors and metalloproteins (a). Inside the cell, both MNPs and ions distribute in the cytoplasm and in the nucleus. Both ions and MNPs enhance the production of ROS and the subsequent oxidative stress, with mitochondrial damage and apoptosis (1), lipid and protein peroxidation, with cell membrane damage (2 and 3), and damage to the DNA (4). Note that zerovalent Cobalt NPs do not cross the cell membrane [77,78].

environment according to the up-to-date findings. This method is useful to overcome problems of toxicity for thermo-ablative cancer treatment, for the future improvement of implantable or injectable devices, for magnetic navigation, heating, and resonance [64].

4.4 Other potential exploitation of CoB NPs in oncology

Despite the existing interdiction to their clinical use that disincentives the exploitation of their full potential, there are various hints that CoB NPs deserve to be further investigated to produce smart NPs usable in theranostics. Their magnetic properties, tunable and regulated by external magnetic fields, can modulate multiple functions, such as imaging, photothermal, and pharmaceutical targeting of lesions, with minimally invasive procedures. The persistence of particles at the site of lesion allows long-term monitoring of relapse. In particular, surface functionalization with binding/reacting molecules [89], graphite [90], or carbon nanotubes [91] does not alter their magnetic behavior and allows efficient loading and temperature-controlled release of antitumor drugs [92]. Furthermore, functionalization of magnetic NPs with radioactive isotopes, currently extensively investigated in preclinical studies, might produce

tools for (potential) simultaneous radiotherapy and hyperthermia and imaging [93].

The displayed toxicity of CoB NPs appears not only related to inherent characteristics, but, at various extents, to the cellular targets. Pancreatic cancer cells are less sensitive than ovarian cancer cells [94]; normal lymphocytes and squamous oral cells, but not lymphoma and oral carcinoma cells, are protected from CoB NPs-related toxicity by coating with organic compounds [50]; and melanoma cells magnetic effectively die by heating only when cobalt is incorporated in the core of ferrite NPs [85]. Normal cells can be exempted from unwanted cobalt toxicity related to therapeutic treatment by the functionalization of CoB NPs with antigens or peptides that selectively bind ligands exclusively expressed by tumors cells. In this way, CoB NPs target and accumulate more efficiently at the site of lesion and inside the target cells [50,95]. Analogously, magnetic targeting, intracellular labelling, and selective removal of ovarian cancer cells from the ascitic fluid *in vitro* and *in vivo* are feasible using an external capture magnet [96].

Differently from other metal NPs that need to be PEG-coated to permeate the cell membrane, naked CoB NPs could load a sufficient dose of conjugated doxorubicin drug in the target tumor cell [97].

The conjugation of CoB NPs with porphyrin derivatives produces a powerful, multifunctional therapeutic platform for boron neutron capture, phototherapy, and fluorescence imaging. All these products can accumulate rapidly and in large amounts (80%) in human lung adenocarcinoma A549 cells and their cytoplasmic organelles, resulting to be cytotoxic only when purposely activated with photons or neutrons irradiation, but not in basal conditions [98]. Interestingly, an *in vivo* study with rats showed that recently developed Co-based nanoclusters could accrue in ovarian cancer tumors and effectively elevate intratumoral temperature (following a single intravenous injection), being nontoxic [99].

Hence, several findings gathered on CoB NPs converge towards its uneven intrinsic noxiousness, under certain usage condition. An ordered database on the fundamentals for knowledge, prediction of potential uses, and commercial restrictions of CoB NPs, still lacking, could prompt further research and development of these magnetic NPs for nanomedicine.

5 Mechanisms of CoB NPs toxicity

Weakening of interest and skepticism arouse on CoB NPs' application in nanomedicine for their toxicity observed in animal and human tumor cells *in vitro* [73,100–104] and *in vivo* [95,105,106]. Despite that, it is important to underline that the intrinsic material characteristics of CoB NPs are not the only ones playing a role in cytotoxicity.

These events result by interaction with the milieu (biochemical and physical factors) where NPs have to operate. Keeping this in mind, those concerns might result in overlooking leading to too negatively conditioned technological advancement. Indeed, theoretical reasoning based on the "Ostwald Ripening phenomenon" predicts that high specific surface area of CoB NPs increases the potential for cobalt ions (Co^{2+}) to be released from these NPs. However, Co^{2+} release occurs when CoB NPs are surrounded by aqueous milieu. In that case, spontaneous morphological modifications would occur based on the thermodynamically driven atomic exchange between atoms in solution and atoms in the NPs [107]. Then, in water the extent of the dissolution depends on the size and speciation of Co constituting the NPs. It has been shown that both metallic zerovalent Co^{0}- and Co_3O_4NPs release Co^{2+} ions in biological media. However, the Co^{0} form releases greater amount of Co^{2+}, compared to Co-oxide [77,78,108]. Relevantly for the present dissertation, in the extracellular environment the serum proteins of cell culture media appear to bind the released Co^{2+}, subtracting them to the exchange medium-particle and so facilitating the dissolution of Co-particles. Moreover, CoB NPs can be uptaken by cells and internalized inside the organelles [72,78] where they can dissolve into Co^{2+}, which are massively detected in nuclei and mitochondria [78], otherwise present at ultratrace level under physiological conditions.

It is not clear to which degree the toxicity of CoB NPs results from the released Co^{2+} or to the integer NPs [109]. However, a study with mouse fibroblasts Balb/3T3 found out that Co^{2+} are statistically more cytotoxic, compared to the corresponding nano- and micro-particulated Co. In addition, it was observed that the dissolution rate was size-dependent, being higher for the nanosized compared to the microsized Co-particles. Remarkably, the cytotoxicity increases according to the increase of the total intracellular Co content, rather than Co exposure concentrations [78]. Moreover, micro and nano forms of zerovalent Co-particles activate toxicologically relevant transcriptional pathways implicated in carcinogenesis and inflammation [110]. Noteworthy, for such toxicological experiments high concentrations of Co species were used, in any case far beyond those achivable for therapeutic aims. Therefore, the extent of intracellular Co accrual and the understanding of the size-dependent dissolution of CoB NPs are the crucial points for addressing their

safe use in nanomedicine, by providing protection from dissolution for long-term stability.

Given the described findings on the crucial role of released Co^{2+} in the mechanisms of CoB NPs-induced toxicity, the molecular basis of Co^{2+}-induced toxic effects must be considered. General mechanisms extensively reviewed by experts in this field [111–115] include: (i) formation of ROS in the presence of hydrogen peroxide with generation of hydroxyl radicals (HO˙) via Fenton reaction and irreversible damage by protein, lipid, carbohydrate, and DNA oxidation [114]; (ii) direct interaction, particularly with sulfhydryl groups, and binding to cellular proteins of the redox system (enzymes catalase, heme oxygenase, superoxide dismutase, peroxidase), metabolism (arginase), molecular transport (transferrin, hemoglobin), motility (lymphocyte cytosolic protein), and signalling (phosphodiesterase 3A). This leads to a direct induction of oxidation and loss of biological function [114]. Moreover, Co^{2+} alter Ca^{2+} influx into cells [116], acting as a blocker of inorganic calcium channels, and modify glucose metabolism [117]; (iii) displacement of other essential divalent metal ions in the ion center of metal-activated enzymes (i.e., Zn of alkaline phosphatase [118], Zn^{2+} and Mg^{2+} of cell proteins [78], Fe^{2+} of dioxygenases [119]).

Systemic toxic effects are induced when Co/Co^{2+} ions enter the blood and the lymphatic system and traslocate into different organs. Urinary excretion is the main mechanism of inorganic cobalt clearance from the human body [120], decreasing with time after exposure [111]. Below, we remind some toxicity mechanisms for Co/Co^{2+}-induced systemic effects.

5.1 Hematological effects

Cobalt therapy in the treatment of anemia induces polycythemia and hypothyroidism [121]. Co^{2+} inhibit the synthesis of heme *in vivo* by acting on the biosynthesis of 5-delta-amino levulinate and its conversion into heme, resulting in the generation of cobalt protoporphyrin [122]. Heme oxidation in many tissues is also stimulated. In addition, cobalt acts to increase erythropoietin release from damaged renal cells, which stimulates the production of red blood cells [123].

5.2 Thyrotoxic effects

Cobalt impaired thyroid activity and goiter formation [124]. The inhibition of iodine uptake and tyrosine iodinase activity by cobalt prevent the incorporation of iodine into thyroxine [114], being considered the mechanistic basis of the observed hypothyroidism induced by cobalt therapy [125].

5.3 Myocardial effects

Cobalt-induced cardiomyopathy was observed in beer drinkers [126]. Mitochondrial changes are representative of disturbance in energy production/utilization. The irreversible chelation of alpha-lipoic acid –SH groups in the citric acid cycle by Co^{2+} under hypoxic conditions is considered the mechanistic basis for the pathogenesis of Co-induced cardiotoxicity [127].

5.4 Neurotoxic effects

High levels of cobalt released from metal prosthesis can induce neurotoxic effects involving several neurologically related organ systems (i.e., auditory, ocular, central, and peripheral nervous systems) [128]. Such effects of cobalt are mediated through depletion of neurotransmitters such as dopamine, noradrenaline, and serotonin and presynaptic blockage of calcium channels [128].

5.5 Immunological effects

Metallic cobalt, or in the form of water-soluble ionized salts, is allergenic causing immediate type I as well as delayed type IV hypersensitivity reactions [113], rhinitis, and asthma. IgA and IgE antibodies specific to cobalt have been observed in humans [129]. Cobalt ions can act as hapten binding to serum proteins to form hapten-like complexes to produce immunogenic products that can account for allergic reactions [130]. T-lymphocytes regulate cobalt sensitivity, while cobalt reduces the proliferation *in vitro* of B and T lymphocytes and the release of IL-2, IL-6, and INF-γ [74]. The release of Co-ions is associated with proinflammatory reaction involving monocyte and lymphocyte reactions [131].

5.6 Carcinogenicity and genotoxicity

Cobalt sulfate and other soluble cobalt(II) salts are classified as "possibly carcinogenic to humans" (Group 2B),

while the mixture dust cobalt/tungsten carbide (Co/WC) as "probably carcinogenic to humans" (group 2A) [132]. Soluble cobalt induces mutagenic effects in mammalian cells *in vitro* by a direct damage to DNA by a Fenton-like mechanism; or indirectly by a mechanism involving inhibition of repair of DNA damage, particularly concerning the incision and polymerization steps, through interaction with zinc-finger DNA repair proteins, considered the most relevant mechanism for metal carcinogenicity than binding to DNA [133]. Two mechanisms are involved in the Co-particles-induced mutagenic effects: production of ROS by both Fenton and non-Fenton (i.e., in the absence of H_2O_2) mechanisms, resulting in DNA damage, and release of Co^{2+} which inhibit DNA repair processes [132].

These above-reported Co^{2+}-related biochemical mechanisms, general or specific for Co^{2+}-induced systemic effects, are relevant also for Co-particles species. In fact, the toxic reaction that produces ROS leads in parallel to the oxidation of metallic cobalt and hence to the formation of ionic species. However, the described cobalt-related systemic effects are in general uncommon because of a high toxicity threshold.

Systemic Co toxicity manifests with a variable presentation of neurological, cardiovascular, and endocrine symptoms, depending on the systemic Co levels (blood/urine). These systemic effects, called "arthroprosthetic cobaltism" syndrome, were initially observed in metal-on-metal implant recipient subjects, accompanying the presence, in the periprosthetic tissue, of electron dense nanosized debris inside the macrophages [113,134,135].

6 Conclusions

The theoretical basis for further research on this nanosubstance that might reasonably be voided of toxic features and, therefore suitable in theranostics, is exposed.

Cobalt-based NPs, which behave as hard magnets, could be considered the possible counterpart of the soft magnets composed of iron oxide MNPs. The residual magnetization is higher in MNPs with a core of cobalt oxide or cobalt–ferrite, in comparison with zerovalent cobalt, while selected variants in the procedure for production, such as the temperature or the relative content of iron in cobalt–ferrite MNPs, can tune the saturation magnetization. The crystal symmetry of the final product mainly supports this effect. The surface functionalization with hard or soft shells protects from dissolution and could also change the magnetic properties.

The alternative option of using CoB NPs to overcome some limits of the iron-based soft magnets requires appropriate precautions. It is mandatory for the surface functionalization or shell of CoB NPs to reduce the feared cobalt toxicity since cobalt particles applied in theranostics medicine could result in exposure to ions that may potentially reach the threshold of high toxicity. In addition, cobalt nanotoxicity must be further investigated from a mechanistic point of view. In this context, the aspect of speciation will have to be the core of future studies in this area. In any case, the intracellular distribution of Co^{2+} released from CoB NPs entering the cells plays a fundamental role in determining the toxic effects of Co-particles. Thus, drawing any conclusions about the differential toxicity of particles relative to ions should be done with great care, as uptake effect relationships must be considered [78,136].

At the present, there is a lack of tiered strategy for the characterization of CoB NPs, which reduces the potential of these tools. Indeed, a range of CoB NPs nanomaterials may offer a powerful platform for biological implementation in theranostics, as improved probes and catalysts. In nanomedicine, the supposed toxicity of Co dictates the choice of iron oxide MNPs, considered more biocompatible. Consequenlty, CoB NPs are poorly exploited in this field [137], with a great waste of potentiality. Only the diagnostic utilizations improve, without any advance in therapy: neither trials nor experimental studies have been performed focusing on thermotherapy with CoB MNPs in medicine, clinical or veterinary, or on other possible therapeutic applications. Nevertheless, under this point of view, CoB MNPs offer several advantages in comparison with those of iron oxide. Their higher residual magnetization allows a more persistent signal; the magnetic heating is more tuneable and, therefore, more compatible with the necessary avoidance of local necrosis. These NPs that do not produce interfering signals with the iron bound to heme could be used as radiation isotope against tumor growth.

In our opinion, it is time to revise the band of these tools in medicine. Even the evaluation of the risk for toxicity could be obsolete, considering newer ways to prevent the dissolution of toxic species of Co. For example, a golden shell may be able to make CoNPs biocompatible, preventing dissolution and modulating magnetism and magnetic heating. Phase I clinical trials could be set up and conclusively determine the fate of these promising, but until now neglected, MNPs, following a severe control of all steps of production, complete characterization of the product, and tired test for toxicity, according to the current approved protocols.

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