Review

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Effect of nanoparticles on the expression and activity of matrix metalloproteinases

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Abstract: Matrix metallopeptidases, commonly known as matrix metalloproteinases (MMPs), are a group of proteolytic enzymes whose main function is the remodeling of the extracellular matrix. Changes in the activity of these enzymes are observed in many pathological states, including cancer metastases. An increasing body of evidence indicates that nanoparticles (NPs) can lead to the deregulation of MMP expression and/or activity both *in vitro* and *in vivo*. In this work, we summarized the current state of knowledge on the impact of NPs on MMPs. The literature analysis showed that the impact of NPs on MMP expression and/or activity is inconclusive. NPs exhibit both stimulating and inhibitory effects, which might be dependent on multiple factors, such as NP size and coating or a cellular model used in the research.

Keywords: enzymes; extracellular matrix; metalloproteinases; nanoparticles.

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1 Introduction

Matrix metallopeptidases, commonly known as matrix metalloproteinases (MMPs), are zinc-dependent proteolytic enzymes whose primary function is the degradation and remodeling of extracellular matrix (ECM) components. ECM is a complex, dynamic structure that conditions the proper tissue architecture. MMPs by digesting ECM proteins eliminate structural barriers and allow cell migration. Moreover, by hydrolyzing extracellularly released proteins, MMPs can change the activity of many signal peptides, such as growth factors, cytokines, and chemokines. MMPs are involved in many physiological processes, such as embryogenesis, reproduction cycle, or wound healing; however, their increased activity is also associated with a number of pathological conditions, such as diabetes, cardiovascular diseases and neurodegenerative and autoimmune disorders. A particular attention is paid to the role of MMPs in the metastasis and progression of malignant tumors. Increased expression and/or activity of MMPs significantly correlate with the ability to metastasize and greater invasiveness in almost all types of human cancers and worse prognosis [1]. Thus, MMPs become a potential target for therapy. Hydroxamate-based MMP inhibitors, such as batimastat, ilomastat, and marimastat, which contain the CONHOH group binding zinc atom at the active site of MMP enzyme, are the first synthetic inhibitors of MMPs allowed for testing on cancer patients. Currently, various other synthetic MMP inhibitors, which differ in chemical structure, activity, and pharmacokinetics, are on the market or in various phases of clinical trials [2, 3]. Despite oncology, MMP inhibitors are used also in other areas of medicine, for example, in diseases related to the central nervous system [4].

Nanotechnology is a rapidly developing field of science, resulting in a variety of applications in industry and everyday life. In medicine, big hope is paid in the use of nanomaterials in the treatment of various diseases. Nanomaterials are foreseen to be used as drug and/or radionuclide carriers but also as agents for direct treatment,

such as thermotherapy and phototherapy, or even nanobots directly killing cancer cells [5]. In contrast, increasing body of evidence suggests that nanoparticles (NPs) might be toxic for mammalian cells or might have other adverse effects, such as the promotion of cancer development and metastasis [6, 7].

In the last few years, the impact of a number of different NPs on selected metalloproteinases has been reported. NPs can modulate the expression and/or activity of MMPs in two ways: either stimulating or inhibiting them. This study is an attempt to summarize the current state of knowledge on the effect of nanomaterials on the expression and activity of MMPs, a key factor in tumor metastasis.

2 General characteristics of MMPs

Although MMPs are multidomain enzymes, all identified MMPs in humans share the same structure that include signal peptide, prodomain, and catalytic domain, which is responsible for the proteolytic activity of the enzyme and contains zinc and calcium ions as a structural or catalytic moiety. Only two MMPs have this simple structure: MMP7 and MMP26. The others comprise the hemopexin-like domain and other domains specific for individual MMPs. Therefore, MMPs show significant variability at the level of tertiary protein structure, which in turn affects their properties. MMPs can be divided into five main subgroups according to their substrate activity and mechanism of action: matrilysins, collagenases, stromelysins, gelatinases, and membrane MMPs; however, some MMPs do not fit into any of these groups and are classified as "others" (Figure 1). Currently, 23 human MMPs are known, but their numbering starts from 1 and ends at 28 but does not include numbers 4, 5, 6, 18, and 22 due to the duplication of names of the same MMP discovered by different research groups at the same time [8, 9].

Matrilysins, also called endometalloproteinases, have the ability to degrade fibronectin, fibrinogen, and collagen type IV. This group contains MMP7 and

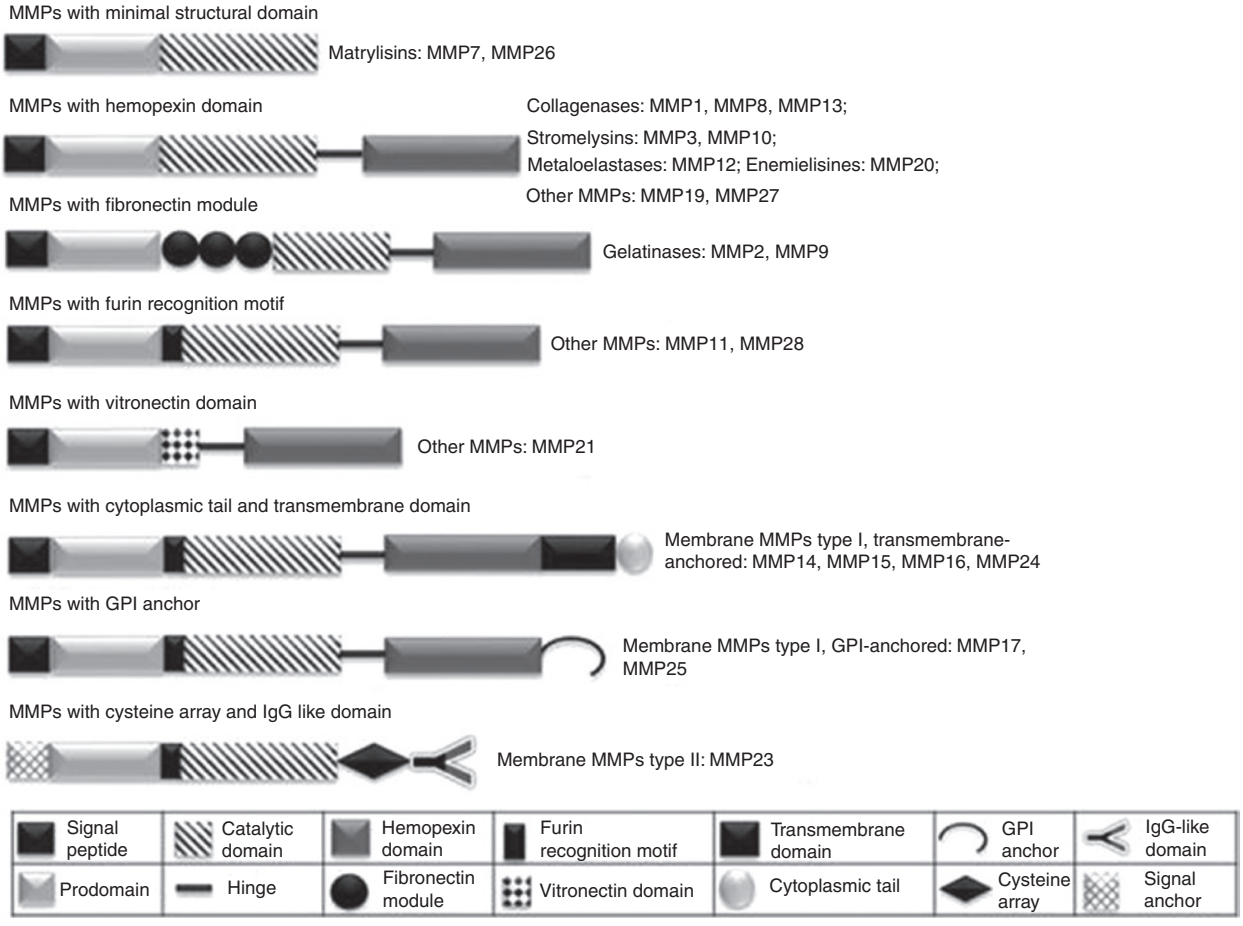

Figure 1: Domain structure of metalloproteinases (own elaboration).

MMP26, characterized by the simplest protein structure. Collagenases, which include MMP1, MMP8, and MMP13, degrade gelatin and practically all collagen subtypes. Their structure is characterized by the presence of hemopexin domain, bonded by a flexible linker with the catalytic domain. MMP10 and MMP3 belong to stromelysins and share substrate specificity, hydrolyzing numerous components of the ECM. MMP3 exhibits higher proteolytic efficiency and is necessary for the activation of several MMP proenzymes. Sometimes, MMP11 is also included to stromelysins due to the similar substrate specificity. However, its structure does not correspond to the other enzymes of this group. The next MMP group are gelatinases, which include MMP2 and MMP9. Structurally, proteins belonging to this group contain the catalytic domain composed of three fibronectin type II modules, which strengthens substrate binding. This structure enables the gelatinases to effectively degrade collagen, gelatin, and elastin, whereas they do not hydrolyze small peptides. Membrane MMPs contain the enzymes that are able to anchor in the cell membrane. They are divided into two subgroups. Type I includes transmembrane-anchored MMP14, MMP15, MMP16, and MMP24 and glycophosphatoinositol-anchored MMP17 and MMP25. An important role of membrane MMPs is the activation of the other MMPs, especially MMP2. Type II contains MMP23 that is characterized by a cysteine array and an IgG-like domain. Interestingly, MMP23 is encoded by two genes located on chromosome 1: *MMP23A* and *MMP23B*, where *MMP23A* is considered a pseudogene. The remaining MMPs are not qualified for any of the groups described above. However, some classifications include two other subgroups: enamelasines and metalloelastases. Elastin-specific MMP12 is the only member of the metalloelastases. The enemelisine group also contains only one protein, MMP20, which hydrolyzes amelogenin in enamel. Both above-described MMPs are similar to collagenases and stromelysins in the chemical structure, in particular to MMP19 and MMP27, respectively. In turn, MMP11 and MMP28 contain a furin recognition motif, whereas MMP21 has a vitronectin domain [8–12].

The final MMP activity depends on the action of several regulatory mechanisms that may interact each other. Most genes encoding MMPs have very low mRNA expression under physiological conditions. Various molecules, such as proinflammatory cytokines and growth factors, may stimulate their expression. The best-known stimulant compounds are interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and vascular endothelial growth factor. Other compounds, such as steroids or retinoids, may reduce the expression of genes encoding MMPs [8–12]. MMP gene expression at the transcription level is also effectively inhibited by transforming growth factor-β. In addition, it has been shown that transcription factors, such as nuclear factor-κB light-chain enhancer of activated B cells [13] and signal transducers and activators of transcription 3 [14], can significantly affect MMP mRNA expression. It also indicated that epigenetic mechanisms play an important role in MMP regulation [15].

MMPs are secreted from cells in a latent form of zymogens. The presence of prodomain allows maintaining MMPs in an inactive form. The activation of zymogen can be achieved in several ways: (1) by removal of the prodomain by another endoproteinase, (2) by allosteric change of the prodomain conformation, or (3) by change of the bond between the prodomain and zinc ions in the enzyme active center. It has been shown that substances such as plasmin, urea, compounds containing thiol group and the copper atom, and some inorganic substances like cyanide, detergents, and chaotropic agents, can inhibit the proper transition of zymogen to the active enzyme. Active MMPs can also activate another pro-MMPs. The final stage of MMP regulation takes place at the active enzyme level. At this stage, MMPs are primarily regulated by specific endogenous tissue inhibitors of metalloproteinases, which inhibit both active enzymes and the transition of proenzymes into the final form. MMPs are also regulated by nonspecific natural inhibitors, primarily by α2-macroglobulin. Moreover, various extracorporeal factors have the ability to inhibit MMPs. Those compounds include, among others, tetracycline and anthracycline antibiotics and chlorhexidine, a popular antiseptic agent [8–12].

There is also an increasing body of evidence that the activity of MMPs might be also affected by NPs. The chapters below summarize the existing state-of-the-art on the effects of nanomaterials on MMP expression and activity.

3 *In vitro* studies

The majority of *in vitro* studies is focused on commonly used inorganic nanomaterials (Table 1), including NPs of noble metals, such as gold (Au NPs), silver (Ag NPs), and platinum (Pt NPs). Hashimoto et al. [36, 38] studied the impact of Au and Pt NPs coated by polyvinylpyrrolidone (PVP) on murine L929 fibroblasts and RAW264 macrophages. Au and Pt NPs inhibited the activity of Mmp1 of L929 and Mmp8 and Mmp9 of RAW264 cells and did not induce an inflammatory response. These results were further confirmed by the same group in the subsequent

Table 1: Effect of NPs on MMPs expression and/or activity in *in vitro* models.

NPs	Coating	Size	Experimental model	Impact on MMPs	Methodology	References
Stimulatory action						
Ag NPs	None	92.4 ± 45.7 nm	BEAS-2B	↑MMP9	WB (protein)	[16]
Ag NPs	None	8.52 ± 1.82 nm	Caco-2	↑MMP2, ↑MMP9	Gelatin zymography (activity)	[17]
Ag NPs	Citrate, ethylene oxide	5–65 nm (d_{90} = 28 nm), 2–85 nm (d_{90} = 34 nm)	Primary porcine brain capillary endothelial cells	↑Mmp2 (only for ethylene oxide-coated Ag)	Gelatin zymography (activity)	[18]
Ag NPs	None	10 ± 5 nm	Normal human epidermal keratinocytes	↑MMP1	ELISA (protein)	[19]
Ag NPs	None	<100 nm	Rat embryonic cells	↑*Mmp3*, ↑*Mmp9*	DNA microarray (gene), real-time qRT-PCR (gene)	[20]
Ag NPs	None	2 nm	CRL-2014	↑*MMP9*, ↑MMP9	Real-time qRT-PCR (gene), gelatin zymography (activity)	[21]
Ag NPs	None	<150 nm	RAW264.7	↑*Mmp3*, ↑*Mmp11*, ↑*Mmp19*	RT-PCR (gene)	[22]
Ag NPs	None	20 and 70 nm	Human PMN	↑MMP9 (only 20 nm Ag NPs)	WB (protein), gelatin zymography (activity)	[23]
Ag NPs	None	20 and 200 nm	HepG2, A549	↑*MMP10* (only HepG2)	PCR array (gene), real-time qRT-PCR (gene)	[24]
Ag NPs, Au NPs	PVP	65 nm Ag NPs, 50 nm Au NPs	Human osteoarthritic chondrocytes	↑*MMP1*, ↑*MMP3*, ↑*MMP13*	Real-time qRT-PCR (gene)	[25]
Au NPs	Citrate	5, 10, 20, and 40 nm	A549 and 95D	↑*MMP9*, ↑MMP9 (in A549 treated by 5 nm Au NPs and 95D treated with 10 nm Ag NPs)	Real-time qRT-PCR (gene), WB (protein), Luminex technology (protein)	[26]
CeO_2 NPs, TiO_2 NPs, ZnO NPs	CeO_2 NPs and ZnO NPs – COOH, TiO_2 NPs – none	≤10 nm CeO_2 NPs, ≤10 nm TiO_2 NPs, ≤20 nm ZnO NPs	Neutrophils	↑MMP9	WB (protein), gelatin zymography (activity)	[27]
Co NPs	None	20 nm	U937	↑*MMP2*, ↑*MMP9*, ↑MMP2, ↑MMP9	Semi-qRT-PCR (gene), gelatin zymography (activity)	[28]
Co NPs	None	30.6 ± 20.2 nm	Mouse embryonic fibroblasts (wild type)	↑Mmp2, ↑Mmp9	Gelatin zymography (activity)	[29]
MWCNTs	None	13.22 ± 2.94 nm	A549	↑*MMP9*, ↑*MMP12*	Real-time qRT-PCR (gene)	[30]
Ni NPs	None	10–30 nm Ni NPs, 10–60 nm TiO_2 NPs	U937	↑*MMP2*, ↑*MMP9*, ↑MMP2, ↑MMP9	RT-PCR (gene), gelatin zymography (activity)	[31]
SiO_2 NPs	None	100, 150, and 500 nm	Calu-3	↑*MMP9* (only 100 nm)	Real-time qRT-PCR (gene)	[32]

Table 1 (continued)

NPs	Coating	Size	Experimental model	Impact on MMPs	Methodology	References
TiO_2 NPs	None	10 and 15 nm spherical anatase; 25–75 nm spherical anatase with high rutile content; 30–40 nm spherical anatase with low rutile content; 10–40 nm pure rutile with fibrous shape	MRC-5	↑*MMP1*, ↑MMP1 (crystal phase is rutile (pure or mixed with anatase)	Real-time qRT-PCR (gene), WB (protein), immunofluorescence staining (protein), fluorometric assay kit (activity)	[33]
Inhibitory action						
Ag NPs	None	10 ± 5 nm	Normal human epidermal keratinocytes	↓MMP3	ELISA (protein)	[19]
Au NPs	Citrate	5, 10, 20, and 40 nm Au NPs	SGC-7901	↓*MMP9*, ↓MMP9 (effect observed only for 5 nm Au NPs)	Real-time qRT-PCR (gene), Luminex technology (protein)	[34]
Au NPs	None	5, 10, 20, 40, 50, and 60 nm	SW579	↓*MMP2*, ↓*MMP9*, ↓MMP2, ↓MMP9	Real-time qRT-PCR (gene), WB (protein)	[35]
Au NPs, Pt NPs	Citrate, carboxyphenyl (Au NPs), polyacrylate (Pt NPs)	14.7 ± 4.7 nm citrate-Au NPs, 18.7 ± 9.7 nm carboxyphenyl-Au NPs, 3.8 ± 3.0 nm Pt NPs	L929, RAW264	↓Mmp1, ↓Mmp2, ↓Mmp8, ↓Mmp9	Fluorometric assay kits (activity)	[36]
Au NPs, Pt NPs	PVP	36.6 ± 12.4 nm Au NPs, 7.2 ± 6.9 nm Pt NPs	L929	↓Mmp1	Fluorometric assay kits (activity)	[37]
Au NPs, Pt NPs	PVP	36.5 ± 12 nm Au NPs, 7 ± 7 nm Pt NPs	RAW264	↓Mmp8, ↓Mmp9	Fluorometric assay kits (activity)	[38]
Fullerenol	None	Not specified	Mice bone marrow stromal cells	↓*Mmp1*, ↓Mmp1, ↓*Mmp3*, ↓Mmp3, ↓*Mmp13*, ↓Mmp13	Real-time qRT-PCR (gene), immunofluorescence staining (protein)	[39]
$Gd@C_{82}(OH)_{22}$	None	~100 nm	U937, U937/MDA-MB-231 coculture	↓*MMP2*, ↓*MMP9*, ↓MMP2, ↓MMP9	RT-PCR (gene), WB (protein)	[40]
Se NPs	None	80–220 nm	HT-1080	↓MMP2	Gelatin zymography (activity)	[41]
WO_3 nanoplates	None	Orthorhombic and monoclinic WO_3, diameter not specified	HeLa	↓*MMP7*	Real-time qRT-PCR (gene)	[42]
ZnO NPs	Aminopolysiloxane	60 nm ZnO	661W	↓*Mmp9*, ↓Mmp9	Real-time qRT-PCR (gene), ELISA (protein)	[43]

ELISA, enzyme-linked immunosorbent assay; qRT-PCR, quantitative reverse transcription-PCR; WB, Western blotting.

study, where they used citrate or carboxyphenyl-coated Au NPs or polyacrylate-coated Pt NPs. In these experiments, in addition to the previously observed inhibition of Mmp1, Mmp8, and Mmp9 activity, the authors observed a decrease of Mmp2 activity [37]. The MMP inhibitory properties of Au NPs were also confirmed by Wu et al. [34], who described decreased mRNA expression and the protein level of MMP9 in human gastric cancer SGC-7901 cells exposed to 5 nm citrate-coated Au NPs. Larger Au NPs were also tested, but no significant effect on enzyme expression was noticed. The authors also observed an inhibition of growth and cell invasion of SGC-7901 cells, which they interpreted as a result of the decrease of MMP9 expression. In line with this, Au NP treatment decreased *MMP2* and *MMP9* gene expression and the concentration of proteins encoded by them in squamous carcinoma SW579 cells. The effect was observed only for small NP sizes (5 and 10 nm), and larger NPs (20–60 nm) did not affect the tested parameters [35]. In turn, Franková et al. [19] while exploring a wound healing process in normal human epidermal fibroblasts and keratinocytes observed that in keratinocytes 10 nm Ag NPs decreased the level of MMP3 protein but increased the level of MMP1. No changes in MMP2 protein expression was observed in Ag NP-treated fibroblasts. In line with these studies, aminopolysiloxane-coated zinc oxide NPs (ZnO NPs) also decreased Mmp9 expression at both mRNA and protein levels in murine retinal 661W cells [43]. MMP9 was accompanied by suppressed cell proliferation and migration. The inhibitory effect on MMP activity was also observed for less popular particles. Tungsten oxide (WO_3) nanoplates decreased *MMP7* gene expression in HeLa cells [42], whereas selenium NPs (Se NPs) inhibited MMP2 activity in human fibrosarcoma HT-1080 cells [41].

An inhibition of MMP activity and/or expression was also observed for cells treated with carbon-based NPs. Meng et al. [40] reported an inhibitory effect of gadolinium-based metallofullerenol NPs [$Gd@C_{82}(OH)_{22}$] NPs on *MMP2* and *MMP9* mRNA expression and specific protein concentration in U937 cells. Interestingly, in U937 cells grown in coculture with strongly invasive human breast cancer MDA-MB-231 cells, the observed effect was significantly stronger for *MMP9*/MMP9 than for *MMP2*/MMP2. The effect was accompanied by the inhibition of invasiveness of MDA-MB-231 cells. The inhibitory effect of NP treatment on the expression of *Mmp1*, *Mmp3*, and *Mmp13* mRNA and concentration of the relevant proteins was also observed in vertebral bone marrow stromal cells from Swiss Webster mice treated with IL-6, whereas the cytokine induced a significant increase in the expression of all three mentioned MMPs and the pretreatment with fullerenol abolished the effect. Moreover, fullerenol significantly inhibited *Mmp1* and *Mmp13* expression but not *Mmp3* in control, untreated cells. This effect was also observed at the protein level [39].

Contrary to the above-described results, numerous *in vitro* studies have reported that NP treatment stimulates MMP expression or activity. Citrate-coated Au NPs (5 nm) increased *MMP9* expression and MMP9 concentration in A549 cells, whereas 10 nm Au NPs had a similar effect in 95D cells. In both cases, no significant increase was observed for larger particles (20 and 40 nm) [26]. The stimulating effect of NPs was also observed in human osteoarthritic chondrocytes treated with PVP-coated Au and Ag NPs [25]. Both types of NPs stimulated expression of *MMP3*, *MMP1*, and *MMP13* mRNA at the concentration of 160 μM. Interestingly, at a concentration of 250 μM, the expression of the evaluated genes became lower and it remained statistically significant only for *MMP3*. Increased Mmp2 activity was also observed in primary porcine brain capillary endothelial cells treated with ethylene oxide-coated Ag NPs, whereas no effect was noticed for citrate-coated NPs. The effect was associated with an increasing production of reactive oxygen species (ROS) and reactive nitrogen species [18]. In line with this, an increased expression of *Mmp3* and *Mmp9* observed in rat embryonic cells was associated with the inflammatory process, as the expression of inflammatory markers was also elevated [20]. Park et al. [22] showed an increased expression of *Mmp3*, *Mmp11*, and *Mmp19* encoding genes in Ag NP-treated RAW264.7 cells. The observed effect was dependent on NP concentration and it was responsible for other changes, such as oxidative stress and proinflammatory TNF-α protein concentration and mRNA expression growth. Interestingly, Ag NPs were observed only in the cytosol of living cells, which may indicate cytotoxicity according to the Trojan horse mechanism. Inkielewicz-Stepniak et al. [21] showed that the increase in oxidative stress, release of proinflammatory cytokines, and apoptosis level in CRL-2014 cells exposed to Ag NPs was accompanied by an NP concentration-dependent increase in *MMP9* mRNA expression and the final enzyme activity. An increased MMP9 protein concentration and enzyme activity in human neutrophils exposed to 20 nm Ag NPs, but not 70 nm Ag NPs, was accompanied by an increase in the level of prooncogenic cytokines [23]. In line with this, treatment of HepG2 cells with 20 and 200 nm Ag NPs resulted in an increase of *MMP10* mRNA expression. The effect was higher for smaller Ag NPs. Interestingly, the effect was not observed for A549 cells treated in the same way [24].

The long-term effect of NP exposure on the MMP9 protein level was studied on human bronchial epithelial

BEAS-2B cells treated with nontoxic doses of bare Ag NPs. The exposure caused an increase in protein expression accompanied by a significant increase of anchorage-independent agar colony formation and cell migration/invasion. Simultaneously, decrease of epithelial and increase of mesenchymal marker levels were observed [16]. On the contrary, in Caco-2 cells, long-term exposure to low concentrations of Ag NPs did not affect the activity of MMP2 or MMP9 proteins. A statistically significant increase in the ability of cells to grow without adherence to a surface and migration capacity was observed [17].

In vitro stimulating effects on expression or activity of MMPs were also demonstrated for a variety of other inorganic NPs. Armand et al. [33] observed an increase of *MMP1* mRNA expression and MMP1 concentration in human lung fibroblasts (MRC-5 cell line) treated with different forms of titanium oxide NPs (TiO_2 NPs). As not all tested NPs caused the increase of MMP1 activity, the authors suggested that the effect was dependent on a mechanism associated with IL-1β. In line with this, studies by Babin et al. [27] demonstrated an increased concentration and activity of MMP9 protein in neutrophils treated with bare TiO_2 NPs, ZnO NPs, or carboxyl-coated cerium oxide NPs (CeO_2 NPs). The authors linked NP-induced MMP9 stimulation with increased degranulation of neutrophils and induction of the inflammation process. A time- and dose-dependent increase of *MMP2* and *MMP9* mRNA expression was also demonstrated for nickel NP (Ni NP)-treated human myelomonocytic U937 cells, whereas a similar treatment with TiO_2 NPs did not cause any significant effect. Increase in gene expression was accompanied by increased activity of MMP2 and MMP9. The authors suggested that the Ni NP mechanism of action was associated with the deregulation of hypoxia-inducible factor-1α [31]. *MMP9* mRNA expression and protein activity in U937 cells was also raised by a treatment with cobalt NPs (Co NPs) as a consequence of ROS generation [28]. The effects of chronic exposure to Co NPs were studied on wild-type mouse embryonic fibroblasts and its isogenic Ogg1 knockout partner treated for 12 weeks [29]. The exposure induced a significant dose-dependent increase of Mmp2 and Mmp9 activity in both types of cells, which was associated with changes in cell morphology and anchorage-independent cell growth ability.

In line with this, McCarthy et al. [32] described a concentration-dependent increase of the expression of *MMP9* gene in human lung submucosal cells (Colu-3) treated with 10 nm amorphous SiO_2 NPs. Interestingly, the effect was not observed for larger NPs (150 and 500 nm). The increase in *MMP9* expression was accompanied by an increase in the level of proinflammatory cytokines and oxidative stress markers.

Although most studies focused on the action of inorganic NPs, a limited number of studies were also conducted on carbon nanotubes. A stimulatory effect of multiwalled carbon nanotubes (MWCNTs) on the expression of *MMP12* encoding gene and a small increase in *MMP9* expression and cell migration were observed in A549 cells [30].

In conclusion, the majority of *in vitro* studies to date have been focused on metal and metal oxide NPs; however, the results are inconclusive, as both stimulatory and inhibitory effects on MMP expression or activity were observed even for the same type of particles. Nonetheless, the results clearly indicate that the effect of NPs is size dependent. It is also clear that the observed effects strongly depend on the cellular context.

4 *In vivo* studies

Similar to the *in vitro* studies described above, *in vivo* studies have also focused mostly on assessing the effect of inorganic metal and metal oxide NPs; however, the majority of *in vivo* studies were carried out on less frequently used NPs, such as Pt and TiO_2 NPs or even rarely used cerium oxide NPs (CeO NPs) or cadmium oxide NPs (CdO NPs) instead of Ag NPs or Au NPs popular in *in vitro* studies.

An increased mRNA expression of *Mmp9*, *Mmp11*, *Mmp13*, *Mmp17*, and *Mmp23* was observed in mouse lung tissues collected on the first day after exposure to a single intratracheal dose of Pt NPs [44]. An initial increase of mRNA expression dropped during the course of the experiment, and in samples collected from animals during the following days (7, 14, and 28 days after exposure), mRNA levels were at the control level. In a similar experimental setup, animals were treated with Fe_3O_4 NPs that caused a time-dependent increase in the expression of *Mmp2*, *Mmp12*, *Mmp19*, and *Mmp23*. The expression of these metalloproteinases increased until day 14 (determinations on days 1, 7, and 14), whereas at day 28 the values were lower than at day 14 [45]. An increased activity of Mmp2 and Mmp9 was also observed in bronchoalveolar lavage fluid (BALF) of mice treated with CdO NPs for 7 consecutive days (3 h/day) on the next day after exposure completion. Interestingly, when measurements were repeated 7 days after exposure, Mmp2 activity returned to the control level, but Mmp9 activity remained slightly elevated. The elevation of MMP activity was accompanied by an increase in the level of inflammatory markers, which suggests a similar mechanism of induction [46].

In line with this, Mmp2 and Mmp9 protein concentration increased in alveolar macrophages isolated from BALF of rats exposed to CeO NPs by a single intratracheal instillation [47]. The increase was observed on the first day after exposure, after which they gradually decreased from day to day; however, even 28 days after exposure, Mmp2 and Mmp9 protein levels remained higher in relation to control. Immunohistochemical analysis demonstrated that MMPs were present in regions exhibiting pulmonary fibrosis [47]. In a similar experimental model, an increasing activity of Mmp2 and Mmp9 enzymes was also observed [48]. TiO_2 NP-induced increase in MMP activity in postnatal exposed mice was described by Ambalavanan et al. [49]. Intranasal instillation was used to treat mice with TiO_2 NPs in two experimental models: as a single dose on the fourth day after birth or as a three-dose regime (on 4th, 7th, and 10th days after birth). In multiple-treated animals, Mmp9 activity in lung homogenates increased as measured on the 14th postnatal day; however, no change of Mmp2 activity was observed. Histopathological examination demonstrated that a single TiO_2 NPs dose led to the infiltration of inflammatory cells. In multiple-treated animals, a strong inflammatory response developed and lung growth stopped. Nevertheless, no significant inhibition of lungs function was observed. No statistically significant effect of TiO_2 NPs and nickel oxide NPs (NiO NPs)

Table 2: Effect of NPs on MMPs expression and/or activity in *in vivo* models.

NPs applied	Coating	Size	Experimental model	Impact on MMPs	Methodology	References
Stimulatory action						
CdO_2 NPs	None	15.3 ± 0.1 nm	Mice	↑Mmp2, ↑Mmp9	Gelatin zymography (activity)	[46]
CeO_2 NPs	None	6.25–17.5 nm	Rats	↑Mmp2, ↑Mmp9	ELISA (protein), immunohistochemistry staining (protein)	[47]
CeO_2 NPs,	None	17.3 nm	Rats	↑Mmp2, ↑Mmp9	ELISA (protein), gelatin zymography (activity)	[48]
Fe_3O_4 NPs	None	5.3 ± 3.6 nm	Mice	↑*Mmp2*, ↑*Mmp12*, ↑*Mmp19*, ↑*Mmp23*	RT-PCR (gene)	[45]
NiO NPs	None	20 nm	Rats	↑*Mmp9*	Real-time qRT-PCR (gene)	[51]
Pt NPs	PVP	20.0 ± 11.4 nm	Mice	↑*Mmp9*, ↑*Mmp11*, ↑*Mmp13*, ↑*Mmp17*, ↑*Mmp23*	RT-PCR (gene)	[44]
TiO_2 NPs	None	6 nm	Mice	↑Mmp9	Gelatin zymography (activity)	[49]
No effect						
NiO NPs, TiO_2 NPs	None	139 ± 12 nm (NiO NPs), 51 ± 9 nm (TiO_2 NPs)	Rats	*Mmp2*	RT-PCR (gene)	[50]
Inhibitory action						
Au NPs	None	4–26 nm (average particle size 21.3 nm)	Rats	↓Mmp2	Gelatin zymography (activity)	[52]
$Gd@C_{82}(OH)_{22}$	None	~22 nm	Mice	↓*Mmp2*, ↓*Mmp9*, ↓MMP2, ↓MMP9	Real-time qRT-PCR (gene), ELISA (protein), immunohistochemistry staining (protein), fluorometric assay kit (activity)	[55]
$Gd@C_{82}(OH)_{22}$	None	~100 nm	Mice	↓*Mmp2*, ↓*Mmp9*, ↓Mmp2, ↓Mmp9	RT-PCR (gene), WB (protein)	[40]
MWCNTs	None and PA coated	Not specified	CAM	↓Mmp3	WB (protein)	[56]
Pt NPs	None	20–40 nm	Rats	↓*Mmp9*	Real-time qRT-PCR (gene)	[53]
Se-substituted hydroxyapatite NPs	None	183 ± 6 nm	Mice	↓Mmp9	Immunohistochemistry staining (protein), WB (protein)	[54]

on the expression of *Mmp2* gene in BALF of rats exposed to NPs by inhalation was also shown by Morimoto et al. [50]. In turn, Chang et al. [51] showed increased mRNA expression of *Mmp9* in BALF derived from the lungs of rats intratracheally exposed to NiO NPs.

On the contrary, Opris et al. demonstrated a decreased Mmp2 activity in the liver of diabetic rats administered with Au NPs [52]. In line with this, Medhat et al. noted a decrease in the expression of *Mmp9* encoding gene in the liver tissue of rats treated with Pt NPs, in which oxidative stress was previously induced with diethylonitrosamine [53]. A significantly reduced Mmp9 protein concentration in tumor tissue after treatment with Se-substituted hydroxyapatite was also reported in mice injected with human HCCLM9 cells that led to the development of hepatocellular carcinoma. Although an inhibition of tumor growth was not observed, overall, the survival rate of animals was increased [54]. Inhibitory activity against selected MMPs was also demonstrated for gadolinium metallofullerenol [$Gd@C_{82}(OH)_{22}$] NPs. Kang et al. showed that, in nude mice with pancreatic cancer, $Gd@C_{82}(OH)_{22}$ NPs significantly reduced *Mmp2* and *Mmp9* mRNA expression as well as protein concentration and the activity of Mmp2 and Mmp9 accompanied by the inhibition of the growth of cancer cells [55]. $Gd@C_{82}(OH)_{22}$ NPs significantly reduced also *MMP2* and *MMP9* mRNA expression and corresponding protein concentration accompanied by reduced tumor growth and metastatic potential in mice inoculated with human breast cancer MDA-MB-231 and MCF-7 cells [40]. In line with this, significantly down-regulated Mmp3 protein expression was also demonstrated in chicken chorioallantoic membrane exposed to MWCNTs, which was accompanied by the inhibition of the formation of branches and maturation of endothelial cells, which indicates their antiangiogenesis properties [56].

In summary, the results of *in vivo* studies revealed that NPs induced both the increase and decrease of MMP expression or activity (Table 2). A small amount of studies and a lack of coherence of the results make drawing specific conclusions difficult. Apparently, the effect of NP exposure on MMP expression or activity depends on the type of cell line or tissue used and the type of NPs. In addition, *in vivo* studies to date have focused on a different set of NPs than *in vitro* studies.

5 Mechanism of action of NPs

A mechanism of NP action on MMP activity is still obscure due to the incomplete experimental design of planned studies. A majority of studies describing changes in MMP activity did not attempt to explain whether the observed effect was a result of direct interaction with enzyme or the change occurred due to altered gene expression and/or protein concentrations [17, 18, 29, 36–38, 41, 46, 49, 52]. Nevertheless, Hashimoto et al. hypothesized that a negatively charged PVP coating of NPs binds to Zn ions in the MMP active center and inhibited the enzyme activity [37, 38]. A similar problem occurred in studies that focused only on the MMP protein concentration without elaborating the changes in gene expression and/or final enzyme activity [16, 19, 47, 54, 56].

Nonetheless, a considerable part of research showed that the most likely mechanism of NP action is the deregulation of the expression of genes encoding the appropriate MMPs at the transcriptional (mRNA) level [20, 22, 24, 25, 30, 32, 42, 44, 45, 51, 53]. Several studies revealed altered gene expression confirmed at the protein level [26, 33–35, 39, 40, 43, 55] or final enzyme activity without measuring the protein level [21, 28, 31] (Figure 2).

Only Armand et al. [33] and Kang et al. [55] determined the expression of MMPs encoding genes, relevant protein level, and their enzyme activity. Armand et al. indicated that the stimulatory effect of NPs is the effect of proinflammatory IL-1β, whose increased level of mRNA expression and protein concentration was measured in NP-treated cells. Moreover, a large part of the described studies indicate that the stimulating effect of

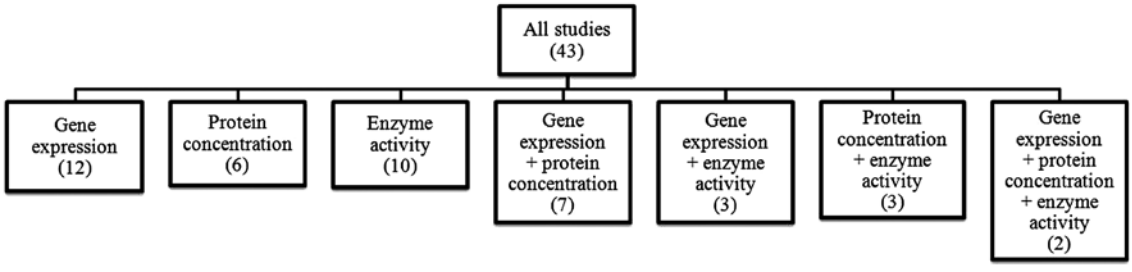

Figure 2: Distribution of cited articles according to methodology used to evaluate NPs effects on MMPs.

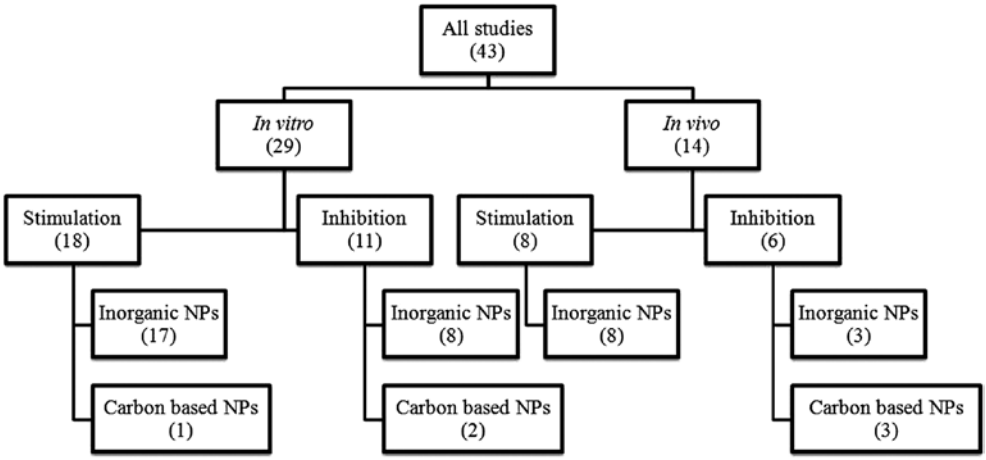

Figure 3: Distribution of cited articles according to the type of studies, observed effect and NPs type used.

NPs is a result of the induction of inflammation [20–23, 27, 32, 33] accompanied by an increase in the level of a number of proinflammatory proteins. The increased level of MMPs also seems to be associated with excessive ROS production [18, 21, 22, 28, 32].

Nonetheless, a mechanistic approach to explain the effect of NPs on MMPs was presented by Kang et al. [55]. They suggested that $Gd@C_{82}(OH)_{22}$ NPs inhibits MMP9 proteolysis, mainly via an exocite interaction, whereas the catalytic enzyme site based on zinc ions plays a minimal role. Molecular dynamics simulations revealed that $Gd@C_{82}(OH)_{22}$ NPs specifically binds near the ligand specificity loop S1 and the binding is controlled by nonspecific interactions (electrostatic, hydrophobic, hydrogen) [55]. A slightly different mechanism of action has been described for MMP2. For MMP2, $Gd@C_{82}(OH)_{22}$ NPs could block either the Zn^{2+} catalytic site directly or the S1′ loop indirectly. The initial adsorption of $Gd@C_{82}(OH)_{22}$ on MMP2 is determined by surface electrostatics and then its further location of the most beneficial binding sites [57].

6 Summary

The review of the available literature revealed that various NPs affect the expression of selected MMPs in both *in vitro* and *in vivo* conditions. However, the reported results are not conclusive. *In vitro* and *in vivo* studies demonstrated that inorganic NPs of metals and their oxides exhibited both stimulatory and inhibitory effects. The induced effect depended on many factors, for example, NP size and coating or a cellular model used in the research. Nevertheless, a larger number of studies indicated the stimulating effect of inorganic NPs on MMP expression or activity. Although a number of studies focused on nonmetallic NPs, such as carbon-based NPs, which is markedly lower (Figure 3), it seems that carbon-based NPs might have an inhibitory effect on MMP expression and/or activity. As the effect of NPs on MMP expression and activity might be dependent on multiple factors, including NP multimodality and cellular context, more systematic studies both *in vivo* and *in vitro* are necessary.

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