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# Validating metal-organic framework nanoparticles for their nanosafety in diverse biomedical applications

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| **Corresponding Author:** | Silke Meiners<br>Helmholtz Zentrum München<br>Munich, Bavaria GERMANY |
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| Please submit a plain text version of your cover letter here.<br><br>**If you are submitting a revision of your manuscript, please do not overwrite your original cover letter. There is an opportunity for you to provide your responses to the reviewers later; please do not add them here**. | Dear Editor,<br><br>We hereby submit our manuscript, entitled “Validating metal-organic framework nanoparticles for their nanosafety in diverse biomedical applications”, for your consideration for publication in Advanced Healthcare Materials.<br><br>In this study, we comprehensively analysed the nanosafety of different metal-organic framework nanoparticles (MOF NPs) with regard to diverse medical applications such as drug delivery via blood or lung and multifunctional surface coatings of medical implants. For the first time, we tested the biocompatibility of MOF NPs on different effector cells, i.e. cells that directly interact with nanoparticles when these are introduced into the biological system, such as endothelial and lung epithelial and immune cells, primary human gingiva fibroblasts, Schwann and dorsal root ganglion cells. Nanosafety of tested MOF nanoparticles strongly varied with the effector cell types revealing their differential suitability as nanomedical agents for drug delivery and implant coatings. These results thus unambiguously demonstrate the requirement of thorough testing of nanomaterials for their respective nanosafety with respect to their specific medical application and to their interacting primary cell type. Our multidisciplinary approach represents a major advancement in assessing the applicability of MOFs NPs for diagnosis and therapy.<br><br>The field of nanomedicine is a cutting edge field of research that offers new diagnostic and therapeutic options for many diseases. We therefore believe that this work is particularly well suited for being published in Advanced Healthcare Materials and of high interest to its readers.<br><br>We thank you in advance for considering our manuscript for publication and look forward to hearing from you.<br><br>Sincerely,<br><br>Stefan Wuttke and Silke Meiners |
| **Corresponding Author Secondary Information:** | |
| **Corresponding Author's Institution:** | Helmholtz Zentrum München |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Silke Meiners |

| | |
|---|---|
| First Author Secondary Information: | |
| Order of Authors: | Silke Meiners |
| | Stefan Wuttke |
| | Andreas Zimpel |
| | Thomas Bein |
| | Simone Braig |
| | Katharina Stoiber |
| | Angelika Vollmar |
| | Dominik Müller |
| | Kirsten Haastert-Talini |
| | Jörn Schaeske |
| | Maike Stiesch |
| | Gesa Zahn |
| | Alexander Mohmeyer |
| | Peter Behrens |
| | Oliver Eickelberg |
| | Deniz Ali Bölükbas |
| | Silke Meiners |
| Order of Authors Secondary Information: | |
| Abstract: | Metal-organic frameworks (MOFs) are a promising platform for the synthesis of nanoparticles for diverse medical applications. Their fundamental design principles allow for significant control of the framework architecture and pore chemistry, thus enabling directed functionalization for nanomedical applications. However, before applying novel nanomaterials to patients, it is imperative to understand their potential health risks. In this study, we comprehensively analyzed the nanosafety of different MOF nanoparticles for diverse medical applications. We first evaluated the effects of MOFs on endothelial and lung cells, which constitute the first line of defence upon systemic blood-mediated and local lung-specific applications of nanoparticles. Second, we validated these MOFs for multifunctional surface coatings of dental implants using human gingiva fibroblasts. Moreover, we assessed biocompatibility of MOFs for surface coating of nerve guidance tubes using human Schwann cells and rat dorsal root ganglion cultures. The main finding of our study is that the nanosafety and thus principal suitability of our MOF nanoparticles as novel agents for drug delivery and implant coatings strongly varies with the effector cell type. We conclude that it is therefore necessary to carefully evaluate the nanosafety of MOF nanomaterials with respect to their particular medical application and their interacting primary cell types, respectively. |

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# Validating Metal-Organic Framework Nanoparticles for their Nanosafety in Diverse Biomedical Applications

*Stefan Wuttke,*[*a] *Andreas Zimpel,*[a] *Thomas Bein,*[a] *Simone Braig,*[b] *Katharina Stoiber,*[b] *Angelika Vollmar,*[b] *Dominik Müller,*[c] *Kirsten Haastert-Talini,*[c,d] *Jörn Schaeske,*[e] *Meike Stiesch,*[e] *Gesa Zahn,*[f] *Alexander Mohmeyer,*[f] *Peter Behrens,*[f] *Oliver Eickelberg,* [g] *Deniz A. Bölükbas,*[g] *Silke Meiners*[*g]

[a]Department of Chemistry and Center for NanoScience (CeNS), University of Munich (LMU), Munich, Germany.
[b]Department of Pharmacy, University of Munich (LMU), Munich, Germany.
[c]Institute of Neuroanatomy, Hannover Medical School, Hannover, Germany.
[d]Center for Systems Neurosciences (ZSN) Hannover, Hannover, Germany.
[e]Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Germany.
[f]Leibniz University Hannover, Institute for Inorganic Chemistry, Hannover, Germany.
[g]Comprehensive Pneumology Center (CPC), University Hospital, University of Munich (LMU), Member of the German Center for Lung Research (DZL), Munich, Germany.

Keywords: metal-organic frameworks, nanoparticles, nanosafety, drug delivery, nanomedicine

Metal-organic frameworks (MOFs) are a promising platform for the synthesis of nanoparticles for diverse medical applications. Their fundamental design principles allow for significant control of the framework architecture and pore chemistry, thus enabling directed functionalization for nanomedical applications. However, before applying novel nanomaterials to patients, it is imperative to understand their potential health risks. In this study, we comprehensively analyzed the nanosafety of different MOF nanoparticles for diverse medical applications. We first evaluated the effects of MOFs on endothelial and lung cells, which constitute the first line of defence upon systemic blood-mediated and local lung-specific applications of nanoparticles. Second, we validated these MOFs for multifunctional surface coatings of dental implants using human gingiva fibroblasts. Moreover, we assessed biocompatibility of MOFs for surface coating of nerve guidance tubes using human Schwann cells and rat dorsal root ganglion cultures. The main finding of our study is that the nanosafety and thus principal suitability of our MOF nanoparticles as novel agents for drug delivery and

implant coatings strongly varies with the effector cell type. We conclude that it is therefore necessary to carefully evaluate the nanosafety of MOF nanomaterials with respect to their particular medical application and their interacting primary cell types, respectively.

## 1. Introduction

Nanosized materials have been used for various biomedical applications to improve human disease diagnosis and treatment. These nanomedicines can offer various advantages in applications such as their use as imaging agents for early and minimally-invasive diagnosis, increased drug stability and concentration at a local site, minimised drug degradation and clearance, the possibility of specific cell targeting, and the ease of creating inhalable formulations.[1-3] The recent focus of scientific interest in this field has been on the morphology control and surface functionalization of different organic (e.g. polymers, dendrimers or liposomes) and inorganic nanocarriers (e.g., gold nanoparticles, carbon nanotubes, iron oxide nanoparticles or mesoporous silica). In this respect, metal-organic framework (MOF) materials offer the combination of both organic and inorganic design principles and are considered to be a promising new class of nanocarriers.[4-8] Generally, the MOF construct is based on the principle of connecting metal ions or metal-oxo clusters with organic linkers resulting in crystalline and porous materials. The flexibility with which metal clusters and organic linkers can be varied as well as the different possibilities to functionalise MOFs provide a vast number of possibilities for creating functionalized porous MOF nanoparticles (MOF NPs) for specific purposes.[9-11] MOF NPs have already been loaded with different drugs or with gasotransmitter gases, and the *in vitro* and to some degree the *in vivo* efficacy was demonstrated.[12-20] Key parameters for biomedical applications of nanoparticles include their size, morphology, surface properties and chemical composition.[21-23] These properties also determine the potential fields of application for MOFs, ranging from diagnosis

and sensing to therapeutic drug delivery and multifunctional surface modification of medical implants **(Figure 1)**.

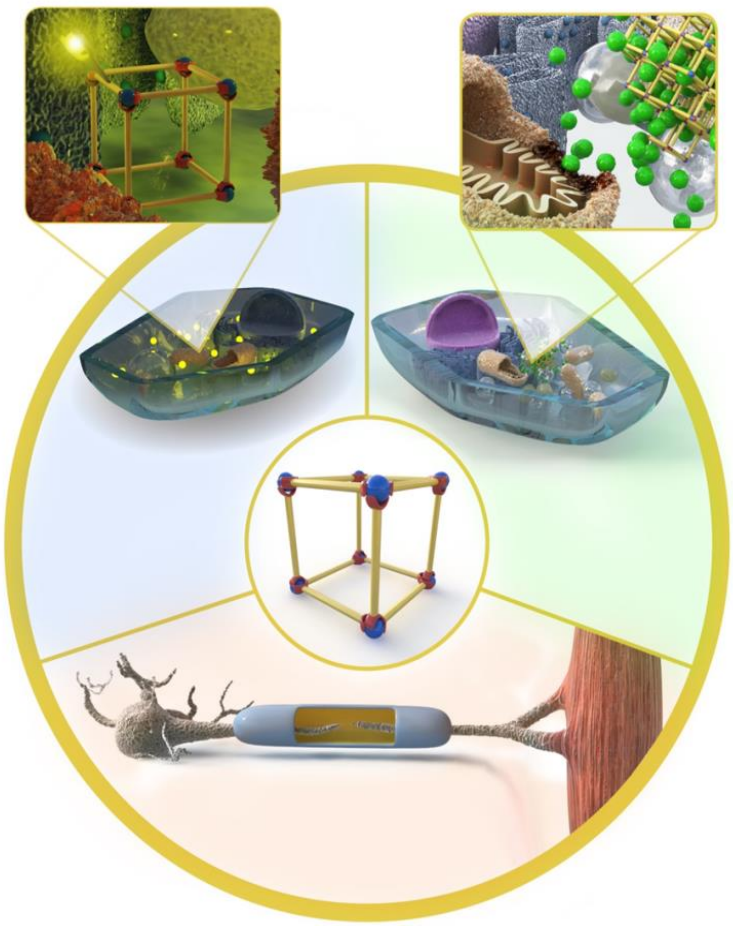

**Figure 1.** Schematic representation of the different possible applications of MOF NPs for diagnosis, therapy and for the creation of smart surfaces.

The same nanomaterials that have been developed for improving diagnosis and therapy, however, may impose health risks to the patient very similar to those known from occupational or environmental particle exposures.[24-26] As such, the application of any novel nanomaterial in the medical context calls for thorough and comprehensive analysis of its cellular biocompatibility and thus nanosafety. In particular, NPs are required to only minimally interfere with the function of their primary effector cells, which are defined as those cells that directly interact with NPs when these are introduced into the biological system. Most surprisingly, so far MOF NPs have not been analyzed for their adverse effects on

primary effector cells, but have only been studied for their *in vitro* toxicity in cancer cells.[10, 27]

Here, we intend to fill this gap by determining and discussing the adverse effects of different MOF NPs for various medical applications ranging from drug delivery to surface coating of medical implants.

We here investigated different types of MOF NPs that have distinct properties. All NPs tested in this study have a spherical morphology in common because studies suggested that this particular shape causes least cytotoxicity.[21, 22] For chemical composition and surface charge diversity we choose a Zr-fumarate (Zr-*fum*) MOF, a Fe-trimesate (MIL-100, MIL standing for Material of Institute Lavoisier) and a Cr-terephthalic MOF (MIL-101). The Zr-fumarate MOF features microporosity of 5-8 Å[28] whereas MIL-100(Fe)[29] and MIL-101(Cr)[30] exhibit mesoporosity. The mesoporosity of MIL-100(Fe) and MIL-101(Cr) allows for the storage of drug molecules inside the nanoparticles. These particles are particularly promising for drug delivery applications.[31] For systemic delivery of any type of functionalized MOF NP by intravenous injections, the endothelium is the first site of particle contact and uptake. It tightly seals the vessel wall to the surrounding tissue and thus maintains blood barrier integrity. Endothelial cells are easily activated upon cell damage by noxious stimuli and particles to express pro-inflammatory surface receptors such as the intercellular adhesion molecule (ICAM). These receptors serve as binding sites to capture patrolling immune cells in the blood for activation of local inflammatory responses. We thus assayed survival, apoptotic cell death and inflammatory activation of human primary endothelial cells in response to treatment with our MOF-NPs.

An additional way of NP delivery is their inhalation via the lung. In fact, inhalation of NPs is a natural route of entry to the body as evidenced by the sometimes detrimental uptake of environmental nanoparticles.[32] Hence, the lung is a unique organ particularly suitable for local drug delivery via inhalation. Its large surface area, thin epithelium layer, and rich blood

supply allow for rapid uptake of inhalatively applied nanoparticles.[3] To assess the biocompatibility of our MOF NPs for inhalative applications into the lung, we investigated the cellular responses of murine alveolar epithelial cells that constitute the main cell type of the air/blood barrier. In addition, we analyzed activation of the main immune cell type of the lung, the alveolar macrophages. These cells are of key importance for clearing particles and toxins from the lung and thus control the initial inflammatory response of the lung to foreign material.

Moreover, we envision the use of MOF NPs for coatings as a new field of application (Figure 1). Medical implants are mainly artificial structures that are widely applied in the clinic to facilitate cellular regeneration of substitute body functions. However, one major adverse effect of implants is that they are colonized by bacteria that trigger subsequent inflammatory reactions and progressive implant and tissue destruction. In this respect, activatable coatings on medical implants would represent a highly innovative approach as shown before.[33-36] To study the general applicability of MOF NPs in this field, we assayed the cytotoxicity of chemically stable MIL-101(Fe), MIL-101(Cr) and Zr-*fum* MOF NPs of different sizes on primary gingiva fibroblasts as effector cells for dental implants.

A different type of medical implants is represented by nerve guidance tubes that are used to bridge transected peripheral nerves in reconstruction surgeries (Figure 1). Currently autologous nerve tissue is used for transplantation to the site of injury. This, however, commonly leads to loss of sensation at the site of harvest.[37] Entubulation strategies with synthetic hollow nerve guidance conduits represent a promising alternative to facilitate peripheral nerve regeneration.[37]. Ideally synthetic nerve guidance tubes provide a perfect regenerative milieu by attracting Schwann cells that locally produce regeneration promoting factors to guide regenerating neurites into the distal nerve segments towards the target tissue (Figure 1).[38] Innovative approaches aim to functionalize biosynthetic nerve guidance channels in order to deliver regeneration promoting molecules.[39-42] Nerve guidance channels

should not only provide guidance but also deliver molecules which could accelerate the speed of peripheral nerve regeneration in order to reach the target tissue before substantial degeneration takes place.[43] To address these issues, we investigated our MOF NPs regarding their biocompatibility with primary adult human Schwann cell cultures as well as in organotypic cultures of rat dorsal root ganglia that contain sensory neurons.

All in all, the aim of our study was to comprehensively investigate the nanosafety and hence the general applicability of different MOF NPs for distinct fields of medical applications. For this purpose, the different experimental setups were designed to be as close as possible to the later applications by the use of the primary effector cells, i.e. endothelial, lung, gingiva and nerve cells, of the respective application field.

## 2. Results

### 2.1. Synthesis and Characterization of the MOF nanoparticles

Validation of biocompatibility was performed with different MOFs that provide promising properties for the use as drug nanocarrier as well as for the multifunctional surface coating of implants. Two of them represent a well-known class of mesoporous nanoMOFs (MIL-100 and MIL-101, respectively), which are known to exhibit large spherical pores with diameters ranging from 2.5 to 2.9 nm for MIL-100 and from 3.0 to 3.4 nm for MIL-101 and at the same time featuring chemical stability.[30, 44] These mesopores are accessible for drug molecules through pore windows of approx. 0.5 nm and 0.9 nm for MIL-100 and 1.2, 1.45 and 1.6 nm for MIL-101, respectively. The formation of a supported lipid bilayer around the nanoparticles enhances their biocompatibility and prevents incorporated cargo from premature release.[31] Zr-*fum* MOF NPs are generated from biocompatible building units, i.e., Zr ions and fumarate dianions.[45] A characteristic feature of these MOFs is the fumaric acid linker which is an intermediate in the citric acid cycle and hence a biocompatible molecule. In

addition, these MOFs feature high chemical stability and small pore sizes ranging from 5 to 8 Å[28] and a pore aperture of 3.5 Å. Such stable microporous MOFs as well as the MIL-100(Fe) and MIL-101(Cr) are particularly well suited for external surface functionalization and transport of large molecules such as RNA.[18] The structure and phase purity of the nanoparticles were characterized by powder x-ray diffraction (PXRD; Suppl. Figures S-1, S-5, S-9) and transmission electron microscopy (TEM) with high-resolution images (Suppl. Figures S-2, S-6, S-10). The expected crystallinity was demonstrated. The calculated Brunauer-Emmett-Teller (BET) surface areas extracted from the nitrogen sorption isotherms (Suppl. Figures S-3, S-7, S-11) are in good agreement with reported data.[28, 31] The hydrodynamic diameters of the different nanoparticles determined with dynamic light scattering (DLS) range from about 40 to 250 nm (Suppl. Figures S-4, S-8, S-12; Table S-1). The smaller hydrodynamic diameter of the MIL-100(Fe)@DOPC in comparison with pure MIL-100(Fe) nanoparticles can be explained by the agglomeration behavior of the latter. This tendency to form agglomerates is prevented by a lipid bilayer.[31] Zeta potential measurements were used to determine the effective charge of the nanoparticles and range from 3 to -43 mV for the nanoparticles investigated (Suppl. Table S-1).

### 2.2. Evaluation of nanosafety of MOF nanoparticles designed for drug delivery

For the delivery of mesoporous NPs either by intravenous or inhalative routes, the endothelial and alveolar cell barriers need to be overcome without causing cell damage and activation of inappropriate immune responses. We thus examined the cellular response towards MIL-101(Cr) and MIL-100(Fe) MOF NPs with and without supported lipid bilayers in endothelial cells, alveolar epithelial cells and alveolar macrophages and compared it to the response towards non-lipid-coated control particles.

Human endothelial cells, namely primary human umbilical cord vein cells (HUVEC) and human microvascular endothelial cells (HMEC) were cultured to confluency, exposed for up to 72 hours to DOPC-coated iron or chromium MOFs at a dose range of 25 to 200 µg/ml and assayed for cytotoxic and inflammatory responses compared to non-treated and non-coated particle controls. Staining of HMECs for the cytoskeletal actin protein did not reveal any stress-related rearrangement of actin fibres in response to 24 hours of exposure to MOF nanoparticles (**Figure 2A** and Suppl. Figure S-13). We further analysed for early signs of apoptotic cell death using FACS-based analysis of the DNA content. This technique allows for separation of cells according to their DNA content in the different phases of the cell cycle with dividing S and G2 phase cells containing the doubled amount of DNA compared to cells in the resting G1 phase (Suppl. Figure S-14). Apoptotic cells contain fragmented DNA and can thus be quantified by counting the cells in a sub G1 peak.[46] MIL-101(Cr)@DOPC and also uncoated MIL-101(Cr) did not induce any signs of apoptotic cell death for the full dose range when applied to HMECs for 72 hours. MIL-100(Fe)@DOPC treated HMECs had an increased sub-G1 peak only at the highest particle dose of 200 µg/ml, which did not, however, reach significance **(Figure 2B)**.

This was also observed for the non-coated control particles and indicated that Fe-containing MOFs may induce apoptotic cell death in endothelial cells at higher doses. Expression of the pro-inflammatory surface receptor ICAM1, however, was not affected in HUVECs by any of the particles tested as shown by FACS-based quantification of ICAM1 expression after 24 hours of particle treatment in **Figure 2C**.

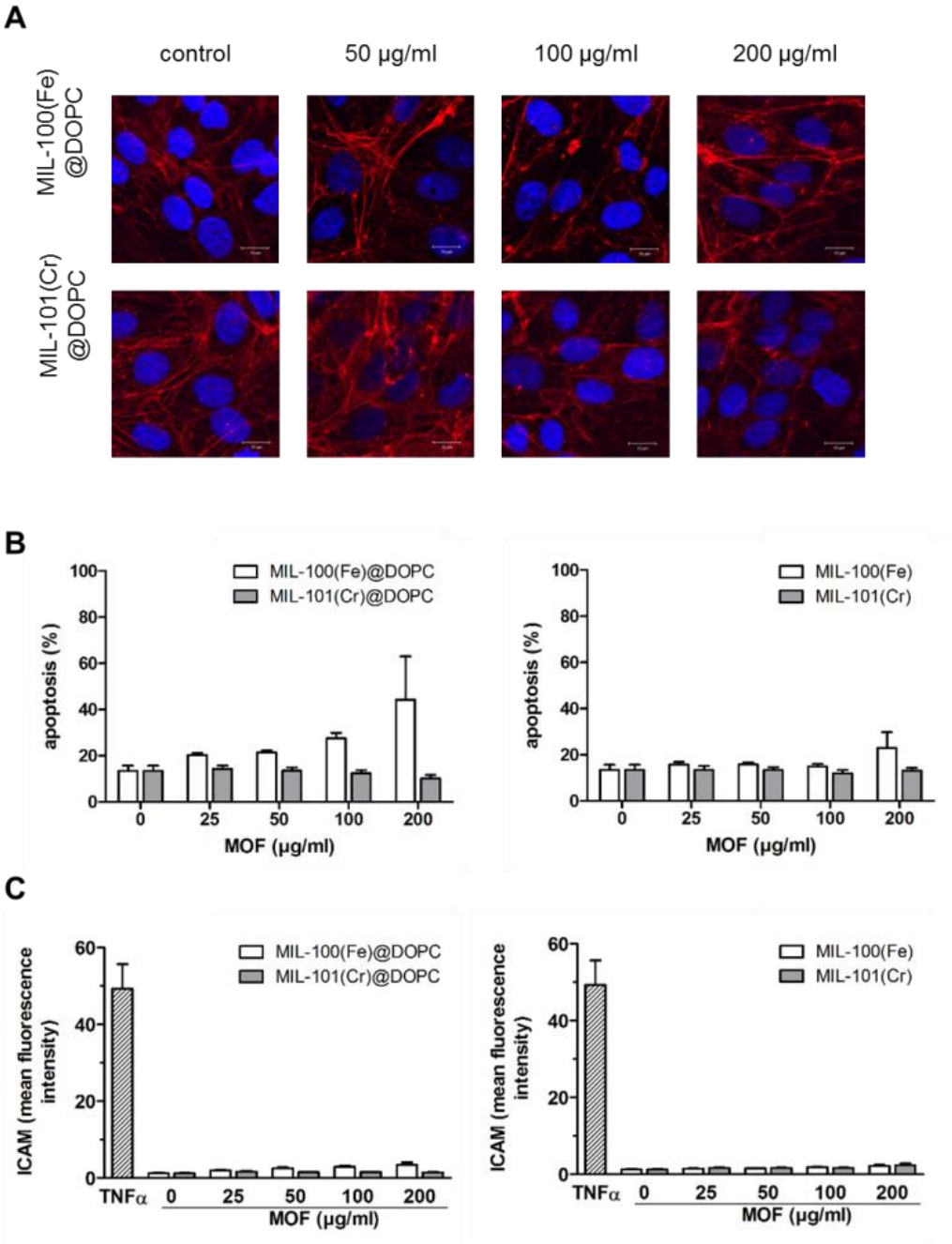


**Figure 1.** Cytotoxic and inflammatory response of human endothelial cells to MIL nanoparticles.

(A) Actin staining of MIL-101(Cr)@DOPC and MIL-100(Fe)@DOPC treated HMEC cells. HMEC cells were treated with the respective DOPC coated Fe- and Cr-MOFs particle doses for 24 h. Cells were fixed, actin and nuclei were stained and analysed by confocal microscopy. (B) Determination of apoptosis rate in HMEC cells after treatment with MIL-101(Cr)@DOPC, MIL-100(Fe)@DOPC, MIL-101(Cr) and MIL-100(Fe). HMEC cells were treated without (0) or with the respective MOF NP concentrations for 72 h, harvested and the percentage of apoptotic cells was measured by FACS analysis. (C) Determination of the inflammatory response in HUVEC cells after treatment with DOPC coated and uncoated Fe- and Cr-MOFs. HUVEC cells were treated without (0) or with the respective nanoparticle concentrations for 24 h, harvested and the level of the inflammatory marker ICAM was determined by FACS analysis.

In a next step, we studied the cytotoxic effects of the MOF nanoparticles for lung cells, namely murine alveolar epithelial cells (MLE12) and alveolar macrophages (MH-S). Viability of the cells was quantified after 24 hours of particle exposure using the MTT assay which uses conversion of a stable tetrazolium salt into soluble formazan by metabolically active and thus viable cells. In addition, we measured the amount of lactate dehydrogenase (LDH) in the medium which is released by necrotic cells with disrupted plasma membranes. Viability of MLE12 cells was affected by exposure to higher doses of MIL-101(Cr)@DOPC (200 µg/ml), which corresponded to an increased release of LDH **(Figure 3A)**. Exposure to MIL-100(Fe)@DOPC particles had an even more pronounced effect on metabolic activity and was cytotoxic from doses of 100 µg/ml on (Figure 3A). These cytotoxic effects were even stronger with uncoated particles (Figure 3A) showing that lipid-functionalization improves biocompatibility of both the MIL-100(Fe) and MIL-101(Cr) nanoparticles in lung epithelial cells, respectively. Biocompatibility of Fe-containing MOFs was, however, strikingly different from Cr-MOFs in the alveolar macrophage cell line MH-S. Both DOPC coated and uncoated Fe-MOFs, showed drastically reduced cell viability in MTT and LDH assays **(Figure 3B)**, while Cr-MOFs were well tolerated and only induced cell death at the highest dose of 200 µg/ml (Figure 3B). To investigate the inflammatory response of these particles in the alveolar macrophages, we determined RNA expression levels of well-known pro-inflammatory mediators such as the cytokine interleukin 6 (IL6), tumour necrosis factor α (TNFα), and of the enzyme nitrite oxide synthase 2 (Nos2) which generates high levels of nitric oxide (NO) as part of the phagocytotic response of macrophages towards microorganisms, toxins and particles.[47] In addition, we measured expression of heme oxygenase 1 (HO1) and metallothionein 2 (MT2) that are activated as part of the cellular stress response to metals such as iron.[48, 49] As a positive control for efficient induction of these genes, we stimulated MH-S cells with the lipopolysaccharide (LPS), a bacterial wall component that is a strong and well known trigger for inflammatory gene expression

**(Figure 3C)**.[50] While LPS strongly induced expression of IL6, TNFα and Nos2, we did not observe any obvious inflammatory gene activation for the tested MOF nanoparticles (Figure 3C). In contrast, Fe-containing MOFs induced distinct and dose-dependent upregulation of HO1 and MT2 suggesting pronounced activation of an anti-iron-stress response in alveolar macrophages. Cr-containing MOFs, however, were inert (Figure 3C).

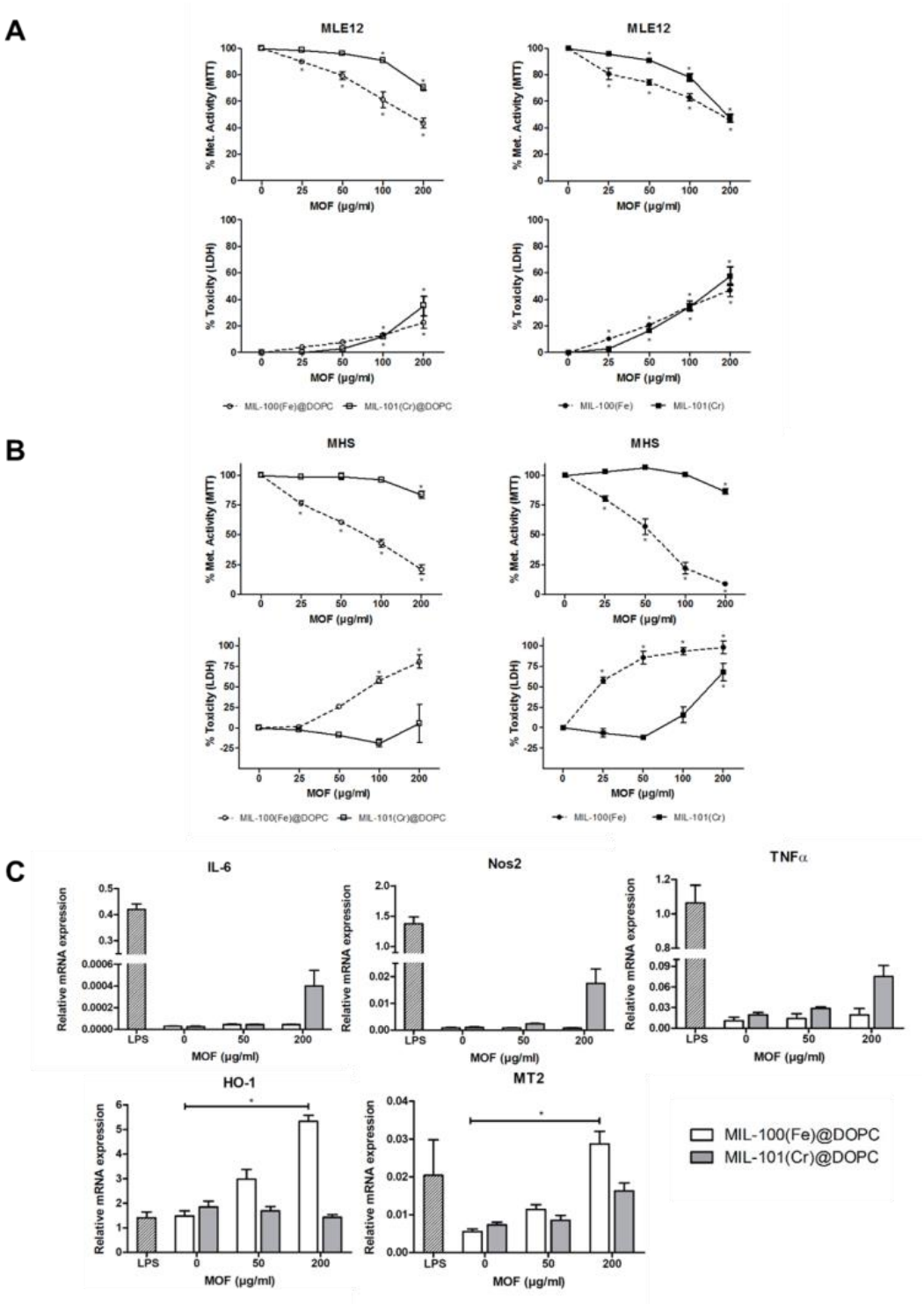

**Figure 2.** Biocompatibility of MIL nanoparticles with the murine alveolar epithelial cell line MLE-12 and the murine alveolar macrophage MH-S cells.
(A) Metabolic activity and toxicity after 24 h of MIL-100(Fe)@DOPC, MIL-101(Cr)@DOPC, MIL-100(Fe), or MIL-101(Cr) exposure to MLE 12 and MH-S cells (B) as analyzed by MTT (upper row) and LDH (lower row) assays, respectively. Untreated cells were set to 100 % survival for the MTT test and 0 % death for the LDH assay. (C) Inflammatory response induced by 4 h exposure to the respective MOFs in MH-S cells as determined by RT-qPCR analysis. 1 µg/mL LPS was used as a positive control to induce pronounced pro-inflammatory gene expression. Values given are mean of three independent experiments ± SEM. * indicates a significant change compared to the respective controls ($p < 0.05$).

Taken together, these data indicate that both Fe and Cr-containing MOFs are well tolerated by endothelial cells whereas the MIL-100(Fe)@DOPC NPs caused some apoptotic cell death at doses of 100 µg/ml. In contrast, alveolar epithelial cells are generally more sensitive and tolerate only lipid-coated Fe and Cr-containing MOFs at lower doses of up to 50 -100 µg/ml, respectively. Alveolar macrophages appear to be particularly sensitive to iron-containing MOF particles, which cause pronounced induction of a cellular stress response. In contrast, Cr-containing MOFs are well tolerated by these immune cells.

### 2.3. Evaluation of nanosafety of MOF nanoparticles designed for implant coatings

While NPs have been primarily used as mobile nanocarriers in medical applications, they can also be used to modify solid surfaces such as dental implants or cellular guidance structures.[40, 42, 51] In order to evaluate the influence of particle size, chemical composition and surface charge we examined Zr-*fum* MOF, MIL-100(Fe) and MIL-101(Cr) MOF NPs (Suppl. Table S-1). In proof-of-concept experiments, we investigated the biological effect of the different MOF NPs on gingival fibroblasts, adult human Schwann cells as well as rat neonatal organotypic dorsal root ganglion (DRG) cultures as effector cell systems for dental implants and nerve guidance tubes, respectively.

Gingival fibroblasts are the primary effector cells that are in close contact with dental implants. Therefore, we tested primary human gingival fibroblasts for their cytotoxic response towards the above mentioned MOF NPs **(Figure 4)**.

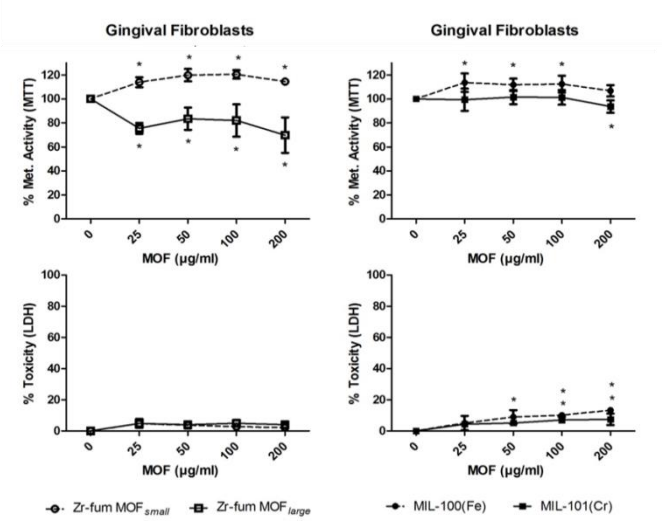


**Figure 3.** Biocompatibility of MOFs with human primary gingival fibroblasts.
Metabolic activity (MTT test) and toxicity (LDH-assay) after 24 h of exposure of gingival fibroblasts to Zr-fum MOFsmall, Zr-fum MOFlarge, MIL-100(Fe) or MIL-101(Cr) particles, respectively. Untreated cells were set to 100 % metabolic activity for the MTT test and to 0 % toxicity for the LDH assay. Values given are mean of three independent experiments ± SEM. * indicates a significant change compared to the respective controls ($p < 0.05$).

Strikingly, none of the tested MOF NPs showed any signs of cytotoxicity on these cells as determined by LDH tests. The metabolic activity just slightly decreased for Zr-*fum* MOF$_{large}$ (Figure 4). Scanning electron microscopy (SEM) analysis did not reveal any obvious morphological signs of cell death after incubation with Zr-*fum* MOF NPs (Suppl Figure S-15A-D).

With regard to NP coating of nerve guidance channels (Figure 1), we used human primary adult Schwann cells as they are the leading supporting cells for peripheral nerve regeneration.[38]

Moreover, Schwann cells are in direct contact with the NP coating on the inner surface of nerve guidance channels (Figure 1). We first analyzed the morphology and metabolic activity

of adult human Schwann cells cultures after 72 h in response to different doses of the MOF NPs **(Figures 5A, 5B)**. We detected a pronounced formation of cell clusters when cultures were treated with doses of 200 µg/ml of the different MOF NPs, except for Zr-*fum* $MOF_{large}$ (Figure 5A). In contrast, lower doses of MOF NPs ranging from 12.5 to 50 µg/ml did not induce any obvious morphological alterations in the growth behavior of adult human Schwann cells (Figure 5A).

This corresponded well to the preserved metabolic activity in the presence of most of the MOF NPs at that dose-range (Figure 5B). Only the MIL-100(Fe) and MIL-101(Cr) nanoparticles induced a significant loss of cell survival at high dosage. The presence of Zr-*fum* $MOF_{small}$ at high dosage also led to slightly reduced metabolic activity (Figure 5B). These data indicate that MOF NPs are generally well tolerated by human adult Schwann cells irrespective of the organic components, metal ion content, and size at lower doses.

In addition to Schwann cells, we monitored the biological response of sensory neurons to the MOF NPs using rat neonatal organotypic dorsal root ganglion (DRG) cultures. The particular feature of these DRGs cultures is that they contain sensory neurons that extend their axons (neurites) into the peripheral space thus mimicking axonal outgrowth to the periphery. Therefore, these cultures provide a unique opportunity to study the response of the main effector cell type for nerve guidance tubes, i.e. neurite outgrowth behaviour of sensory neuronal cells, to novel types of nanomaterial.[40, 52] In this assay, the neurite outgrowth from neonatal rat DRG cultures is quantified by counting the numbers of neurites crossing a circle drawn at 600 µm distance from the centre of each DRG **(Figure 5C)**. Figure 5C shows representative photomicrographs of untreated control DRG and of cultures that have reduced neurite outgrowth upon treatment with the different MOF-NPs added in two concentrations.

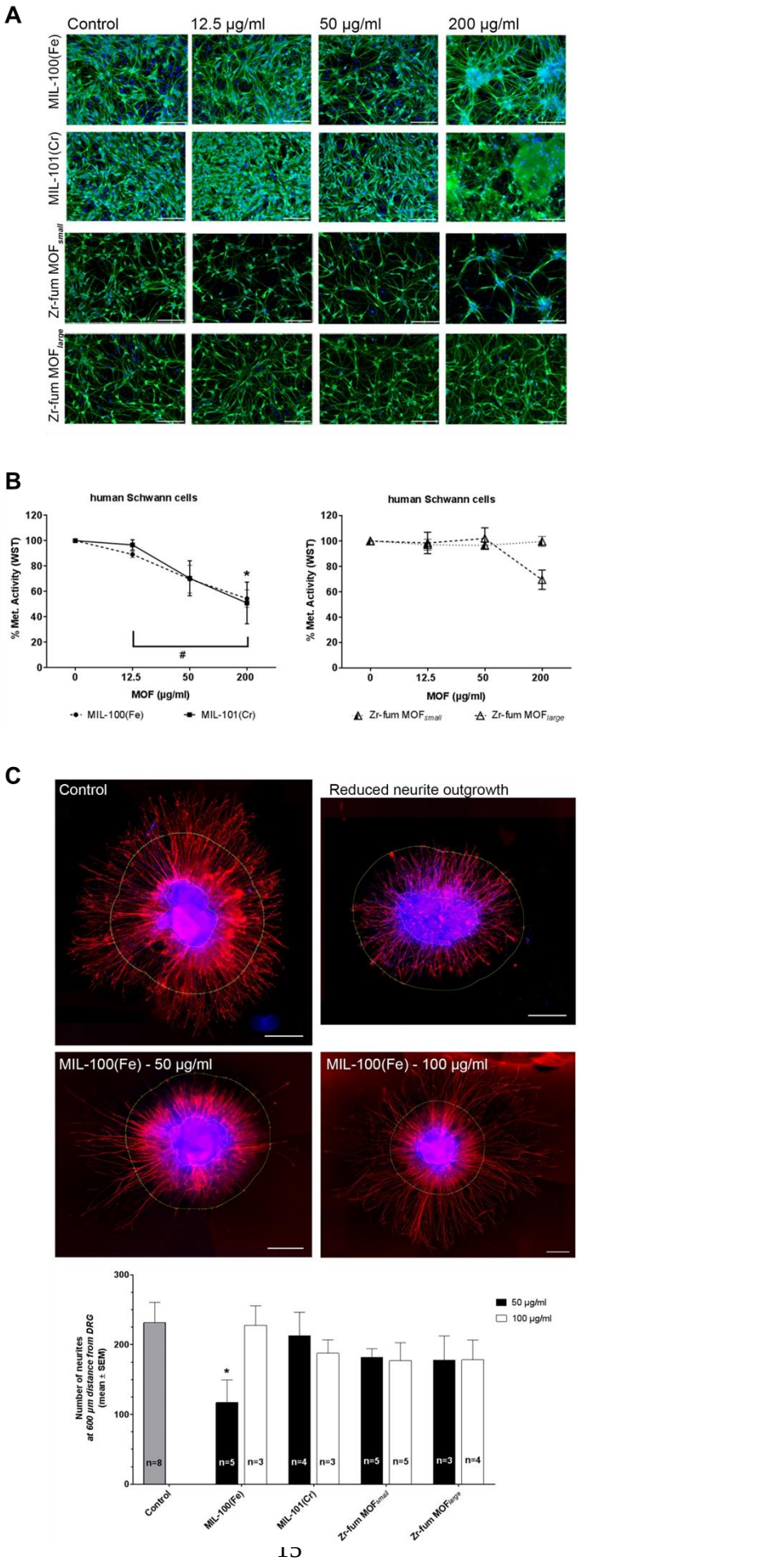
A
Control
12.5 µg/ml
50 µg/ml
200 µg/ml
MIL-100(Fe)
MIL-101(Cr)
Zr-fum MOF small
Zr-fum MOF large
B
human Schwann cells
% Met. Activity (WST)
MOF (µg/ml)
MIL-100(Fe)
MIL-101(Cr)
human Schwann cells
% Met. Activity (WST)
MOF (µg/ml)
Zr-fum MOF small
Zr-fum MOF large
C
Control
Reduced neurite outgrowth
MIL-100(Fe) - 50 µg/ml
MIL-100(Fe) - 100 µg/ml
Number of neurites at 600 µm distance from DRG (mean ± SEM)
50 µg/ml
100 µg/ml
n=8
n=5
n=3
n=4
n=3
n=5
n=5
n=3
n=4
Control
MIL-100(Fe)
MIL-101(Cr)
Zr-fum MOFsmall
Zr-fum MOFlarge

**Figure 4.** Biocompatibility of MOFs on adult human Schwann cells and rat organotypic DRG cultures.
(**A**) Representative photomicrographs demonstrating the morphology of adult human Schwann cell cultures treated for 72 h with the different MOFs. The typical Schwann ell morphology is demonstrated by bi- and tripolar cells that are organised in a fish swarm-like way. Negatively affected Schwann cell cultures demonstrate cell clustering. Schwann cells are stained in green (anti-S100 antibody) and the nuclei counterstained in blue (DAPI). Scale bars: 200 µm. (**B)** Line graphs depicting changes in metabolic activity of adult human Schwann cell cultures treated for 72 h with the different MOFs. Values given are mean ± SEM. Significant differences ($p < 0.05$) to control levels (100 %) are marked with *, differences between different doses of MOFs are marked with #. (**C**) Representative photomicrographs demonstrating the neurite outgrowth from organotypic DRG cultures. Neurites have been quantified at a distance of 600 µm from the center of the DRG (green circle). Neurites are stained in red (anti-beta-III-tubulin antibody) and cell nuclei are counterstained in blue (DAPI). Upper left: example of an untreated control culture with unaffected (regular) neurite outgrowth. Upper right: example of a culture with clearly reduced neurite outgrowth (outliner from cultures treated with Zr-fum $MOF_{small}$). Scale bars: 500 µm. The bar graph depicts changes in neurite outgrowth from organotypic DRG cultures treated for 48 h with the different MOFs. Values given are mean ± SEM. Significant difference ($p < 0.05$) with respect to control levels is marked with *.

As an example for a treatment that showed a dose-dependent effect on neurite outgrowth, representative pictures from the MIL-100(Fe) treated cultures are shown: surprisingly, the low concentration of 50 µg/ml MIL-100(Fe) significantly reduced sensory neurite outgrowth while the doubled concentration rescued it to control levels. For Zr-*fum* $MOF_{small}$ and $_{large}$, we did not observe any loss in neurite outgrowth capacity at both doses tested (Figure 5C).

In conclusion, these data demonstrate the principal feasibility of using MIL-100 (Fe) and MIL-101(Cr) as well as Zr-*fum* MOF NPs for coating of dental implants as they were well tolerated by human gingiva fibroblast. For coating of nerve guidance tubes, however, Zr-*fum* $MOF_{large}$ NPs appear to be the most suitable choice as they were best tolerated in both adult human Schwann cells and rat dorsal root ganglia, respectively.

## 3. Discussion

In this study we have comprehensively analysed the nanosafety of different MOF NPs regarding distinct biomedical applications, ranging from systemic blood and local lung-specific drug delivery to coatings of dental implants and nerve guidance tubes **(Table 1)**.

**Table 1.** Nanosafety of the different MOF NPs for the respective application.

| Nanosafety | MIL-100(Fe) | MIL-100(Fe) @ DOPC | MIL-101(Cr) | MIL-101(Cr) @ DOPC | Zr-*fum* $MOF_{small}$ | Zr-*fum* $MOF_{large}$ |
|---|---|---|---|---|---|---|
| **Systemic drug delivery via endothelial barrier (25-200µg/ml)** | ✓ | ✗ | ✓ | ✓ | nd | nd |
| **Local delivery via the lung (25-100 µg/ml)** | ✗ | ✗ | ✗ | ✓ | nd | nd |
| **Coating of dental implants (25-200µg/ml)** | ✓ | nd | ✓ | nd | ✓ | ✓ |
| **Coating of neural guidance tubes (12.5-200µg/ml)** | ✗ | nd | ✗ | nd | ✗ | ✓ |

nd: not determined, ✗: adverse effects, ✓: biocompatible

During systemic nanoparticle-mediated drug delivery via the blood, nanomaterials first get in contact with the endothelium of the vessels. We show that primary human endothelial cells are not affected by the tested MOF NPs, i.e. MIL-100(Fe) and MIL-101(Cr) and their DOPC-coated derivatives up to high doses of 200 µg/ml towards with regard to apoptotic cell death or inflammatory responses. This identifies these MOF NPs to be potentially suitable nanomaterials for systemic delivery via the blood. Although pharmacokinetics, such as trans-endothelial migration, absorption, bio-distribution and elimination of the MOF NPs needs to be investigated in further studies, the fact that these NPs to not destroy the endothelium forms a mandatory prerequisite for potential intravenous (i.v.) application in the future.

Our data on the lung-specific applications of MOFs demonstrate that differences in the composition of the MOF NPs reflect directly on the bio-response of the cells. In general, both lung epithelial and alveolar macrophage cell lines were clearly more sensitive to the lipid-

coated and non-coated MIL-100(Fe) and MIL-101(Cr) NPs compared to the primary human endothelial cells. This might be related to the differential cell culture conditions, as endothelial cells were cultured as a confluent and tight cell monolayer, while the lung cells were grown at subconfluent conditions. However, subconfluent dental fibroblasts were not sensitive to non-coated MIL-100(Fe) and MIL-101(Cr) nanoparticles. Thus, these differential sensitivities most probably reflect the intrinsic differences between the different cell types as also indicated by the differential sensitivity of lung epithelial and lung immune cells to the MOF NPs. Alveolar macrophages showed a striking sensitivity towards iron-containing MOF NPs. MIL-100(Fe)- and MIL-100(Fe)@DOPC-induced toxicity was accompanied by early upregulation of anti-iron stress-response genes. Of note, the iron-containing MOF NPs did not induce upregulation of early inflammatory marker genes such as IL-6, TNFα and Nos2 which are well known mediators of an acute inflammatory response in the lung to foreign material.[53] This is well in line with the previously observed differential response of alveolar macrophages to diverse nanomaterials.[53] Alveolar epithelial cells tolerated these particles well up to doses of 100 µg/ml. In contrast, Cr-containing MOF NPs were well tolerated by both alveolar epithelial cells and alveolar macrophages at doses up to 100 µg/ml. We tentatively attribute this different behavior to the high chemical stability of the Cr-containing MOF NPs. Only high and slightly toxic doses of 200 µg/ml induced inflammatory gene expression in the alveolar macrophages. A second important observation from our study on the pulmonary effector cells is that lipid-coated MOF NPs were better tolerated by alveolar epithelial cells than their non-coated counterparts. This may be due to improved cellular uptake as previously shown by some of us.[31] The lipid layer may also act as a stealth coating, thus preventing certain cellular response mechanisms from being activated. In conclusion, lung epithelial and immune cells are less sensitive to Cr-based MOF NPs and induce no adverse cytotoxic effects at the low and middle dose-range. These particles can therefore be envisioned as biocompatible nanocarriers for inhalative lung-specific drug delivery, whereas

the Fe-based MOF NPs do not seem to be suitable for pulmonary applications. Nanosafety of MOF NPs for surface coating of dental implants was tested in primary human gingiva fibroblasts, which are the effector cells that are in direct contact with nanoparticle-coated grafts. Notably, these cells showed no obvious toxic response towards the tested MOF NPs, i.e. MIL-100(Fe) and MIL-101(Cr) and *Zr-fum* MOF$_{small}$ and $_{large}$. In both assays applied, i.e. measurement of metabolic activity and release of LDH, gingival fibroblasts did not reveal a significant toxic response. Moreover, maintenance of the fibroblastoid morphology of the cells indicated good biocompatibility. The absence of toxicity of the MOF NPs used in this study supports a possible application for coatings of dental implants.

Regarding a future application of MOF-NPs as coating and nanocarrier for nerve guidance tubes, our data demonstrate the biocompatibility of the MIL-100(Fe) and MIL-101(Cr) as well as *Zr-fum* MOF$_{small}$ and $_{large}$ MOF NPs with adult Schwann cells in the low and middle dose-range. MOF NPs were also generally well tolerated by cultures of dorsal root ganglia and did not notably interfere with the outgrowth of neuronal axons with the prominent exception of Fe-containing MOFs. In particular the low cytotoxic response of adult Schwann cells as well as the inert behavior of sensory neurons towards Zr-*fum* MOF$_{large}$ particles makes those MOF NPs the most promising formulation for surface coating of nerve guidance tubes as suggested previously for polysialic acid and its mimetics.[54] [55] Previously we were able to demonstrate that iron oxide nanoparticles potentially provide a biocompatible tool to delivery of neurotrophic factors in peripheral nerve reconstruction approaches.[40, 56] Our data on the MOF NPs provide now evidence for their potential as delivery system of regeneration promoting peptides within nerve guidance channels.

The most important finding of our comprehensive validation is that there are striking differences in the bio-response of the diverse effector cell types to the distinct MOF-NPs (Table 1). For the MIL-100(Fe) and MIL-101(Cr) particles, differential responsiveness appears to be directly related to intrinsic differences of the cell types as colloidal stability of

these MOFs has been shown to be preserved in different cell culture media for at least 24 hours.[27]

Lipid coated MIL-101(Cr) MOF NPs can be envisioned so far as safe nanoagents for intravenous systemic drug delivery. We have previously shown that the lipid coated MIL-100(Fe) and MIL-101(Cr) MOF NPs are taken up and well tolerated by the T24 bladder carcinoma cell line suggesting that lipid-coated MOF NPs might be feasible nanocarriers for delivery of cytotoxic drugs to tumour cells. In addition, MIL-100(Fe) and MIL-101(Cr) together with *Zr-fum* MOF NPs might be suitable nanoparticles for surface coating of dental grafts. *Zr-fum* MOF$_{large}$ NPs appear to be promising nanomaterial for inner surface modification of nerve guidance tubes. Of note, the lung is particularly sensitive to any nanomaterial but lipid-coated MIL-101(Cr) MOF NPs might be suitable nanoagents for inhalative drug delivery at a low to middle dose ranges. The particular sensitivity of the lung to nanomaterial is well known and constitutes the basis for the hazardous effects of inhalatively taken up environmental NPs.[26] These results unambiguously demonstrate the requirement for thorough testing of nanomaterials for their respective nanosafety in specific biomedical applications as suggested recently.[24, 57]

## 4. Conclusion

MOF chemistry offers a unique platform to create functional NPs for different biomedical applications. However, NPs have been shown to bear potential risks for human health. Therefore, we validated various MOF NPs for specific medical fields of application. To the best of our knowledge, this is the first time that such NPs have been systematically evaluated for their biocompatibility with their primary effector cells. We demonstrate that the tested MOF NPs show differential toxicity and bio-response in different effector cells tested. Thus, this work defines a novel strategy that, in addition to highlighting the potential important risks

of using MOF NPs for specific medical purposes, also demonstrates their differential suitability for applications in drug delivery and for implant coating. Importantly, for the first time we envision the use of MOF NP coatings for dental implants or cellular guidance tubes and show their nanosafety regarding the respective effector cells, such as gingiva fibroblasts and peripheral nerve cells.

Our results thus clearly demonstrate the requirement for thorough testing of nanomaterials regarding their nanosafety in specific biomedical applications as suggested recently, and illustrate the impact of the molecular interface of the MOF NPs for their respective use for systemic drug delivery and surface modification of implants.[24, 57]

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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[1] C. A. Schutz, L. Juillerat-Jeanneret, H. Mueller, I. Lynch, M. Riediker, *Nanomedicine (London, U. K.)* **2013**, *8*, 449.
[2] V. Wagner, A. Dullaart, A.-K. Bock, A. Zweck, *Nat. Biotechnol.* **2006**, *24*, 1211.
[3] S. H. van Rijt, T. Bein, S. Meiners, *Eur. Respir. J.* **2014**, *44*, 765.
[4] H. C. Zhou, J. R. Long, O. M. Yaghi, *Chem. Rev.* **2012**, *112*, 673.
[5] H.-C. J. Zhou, S. Kitagawa, *Chem. Soc. Rev.* **2014**, *43*, 5415.
[6] G. Ferey, *Chem. Soc. Rev.* **2008**, *37*, 191.
[7] H. Furukawa, K. E. Cordova, M. O'Keeffe, O. M. Yaghi, *Science* **2013**, *341*, 974.
[8] S. Kitagawa, R. Kitaura, S. Noro, *Angew. Chem. Int. Ed. Engl.* **2004**, *43*, 2334.

[9] A. Carne, C. Carbonell, I. Imaz, D. Maspoch, *Chem. Soc. Rev.* **2011**, *40*, 291.
[10] P. Horcajada, R. Gref, T. Baati, P. K. Allan, G. Maurin, P. Couvreur, G. Férey, R. E. Morris, C. Serre, *Chem. Rev.* **2012**, *112*, 1232.
[11] S. Furukawa, J. Reboul, S. Diring, K. Sumida, S. Kitagawa, *Chem. Soc. Rev.* **2014**, *43*, 5700.
[12] V. Agostoni, P. Horcajada, M. Noiray, M. Malanga, A. Aykaç, L. Jicsinszky, A. Vargas-Berenguel, N. Semiramoth, S. Daoud-Mahammed, V. Nicolas, C. Martineau, F. Taulelle, J. Vigneron, A. Etcheberry, C. Serre, R. Gref, *Sci. Rep.* **2015**, 5: 7925.
[13] P. Horcajada, T. Chalati, C. Serre, B. Gillet, C. Sebrie, T. Baati, J. F. Eubank, D. Heurtaux, P. Clayette, C. Kreuz, J.-S. Chang, Y. K. Hwang, V. Marsaud, P.-N. Bories, L. Cynober, S. Gil, G. Ferey, P. Couvreur, R. Gref, *Nat. Mater.* **2010**, *9*, 172.
[14] K. Khaletskaya, J. Reboul, M. Meilikhov, M. Nakahama, S. Diring, M. Tsujimoto, S. Isoda, F. Kim, K.-i. Kamei, R. A. Fischer, S. Kitagawa, S. Furukawa, *J. Am. Chem. Soc.* **2013**, *135*, 10998.
[15] S. Diring, D. O. Wang, C. Kim, M. Kondo, Y. Chen, S. Kitagawa, K.-i. Kamei, S. Furukawa, *Nat. Commun.* **2013**, 4: 2684
[16] A. C. McKinlay, P. K. Allan, C. L. Renouf, M. J. Duncan, P. S. Wheatley, S. J. Warrender, D. Dawson, S. E. Ashbrook, B. Gil, B. Marszalek, T. Düren, J. J. Williams, C. Charrier, D. K. Mercer, S. J. Teat, R. E. Morris, *APL Mater.* **2014**, *2*, 124108.
[17] J. W. Brown, B. L. Henderson, M. D. Kiesz, A. C. Whalley, W. Morris, S. Grunder, H. Deng, H. Furukawa, J. I. Zink, J. F. Stoddart, O. M. Yaghi, *Chem. Sci.* **2013**, *4*, 2858.
[18] C. He, K. Lu, D. Liu, W. Lin, *J. Am. Chem. Soc.* **2014**, *136*, 5181.
[19] T. Baati, L. Njim, F. Neffati, A. Kerkeni, M. Bouttemi, R. Gref, M. F. Najjar, A. Zakhama, P. Couvreur, C. Serre, P. Horcajada, *Chem. Sci.* **2013**, *4*, 1597.
[20] A. Carné-Sánchez, I. Imaz, M. Cano-Sarabia, D. Maspoch, *Nat. Chem.* **2013**, *5*, 203.
[21] A. Nel, T. Xia, L. Madler, N. Li, *Science* **2006**, *311*, 622.
[22] P. Rivera-Gil, D. Jimenez De Aberasturi, V. Wulf, B. Pelaz, P. Del Pino, Y. Zhao, J. M. De La Fuente, I. Ruiz De Larramendi, T. Rojo, X.-J. Liang, W. J. Parak, *Acc. Chem. Res.* **2013**, *46*, 743.
[23] C. Y. Tay, M. I. Setyawati, J. Xie, W. J. Parak, D. T. Leong, *Adv. Funct. Mater.* **2014**, *24*, 5936.
[24] A. E. Nel, W. J. Parak, W. C. Chan, T. Xia, M. C. Hersam, C. J. Brinker, J. I. Zink, K. E. Pinkerton, D. R. Baer, P. S. Weiss, *ACS Nano* **2015**, *9*, 5627.
[25] H. F. Krug, *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 12304.
[26] G. Oberdorster, E. Oberdorster, J. Oberdorster, *Environ. Health Perspect.* **2005**, *113*, 823.
[27] R. Grall, T. Hidalgo, J. Delic, A. Garcia-Marquez, S. Chevillard, P. Horcajada, *J. Mater. Chem. B* **2015**, *3*, 8279.
[28] G. Zahn, H. A. Schulze, J. Lippke, S. König, U. Sazama, M. Fröba, P. Behrens, *Microporous Mesoporous Mater.* **2015**, *203*, 186.
[29] P. Horcajada, S. Surble, C. Serre, D.-Y. Hong, Y.-K. Seo, J.-S. Chang, J.-M. Greneche, I. Margiolaki, G. Ferey, *Chem. Commun.* **2007**, 2820.
[30] G. Férey, C. Mellot-Draznieks, C. Serre, F. Millange, J. Dutour, S. Surblé, I. Margiolaki, *Science* **2005**, *309*, 2040.
[31] S. Wuttke, S. Braig, T. Preiß, A. Zimpel, J. Sicklinger, C. Bellomo, J. O. Radler, A. M. Vollmar, T. Bein, *Chem. Commun.* **2015**, *51*, 15752.
[32] A. Peters, H. E. Wichmann, T. Tuch, J. Heinrich, J. Heyder, *Am. J. Respir. Crit. Care Med.* **1997**, *155*, 1376.
[33] N. Ehlert, P. P. Mueller, M. Stieve, T. Lenarz, P. Behrens, *Chem. Soc. Rev.* **2013**, *42*, 3847.
[34] N. Ehlert, M. Badar, A. Christel, S. J. Lohmeier, T. Luessenhop, M. Stieve, T. Lenarz, P. P. Mueller, P. Behrens, *J. Mater. Chem.* **2011**, *21*, 752.

[35] N. Ehlert, A. Hoffmann, T. Luessenhop, G. Gross, P. P. Mueller, M. Stieve, T. Lenarz, P. Behrens, *Acta biomater.* **2011**, *7*, 1772.
[36] R. Lensing, A. Bleich, A. Smoczek, S. Glage, N. Ehlert, T. Luessenhop, P. Behrens, P. P. Muller, M. Kietzmann, M. Stieve, *Acta biomater.* **2013**, *9*, 4815.
[37] R. Deumens, A. Bozkurt, M. F. Meek, M. A. Marcus, E. A. Joosten, J. Weis, G. A. Brook, *Progress in neurobiology* **2010**, *92*, 245.
[38] H. Millesi, *Acta neurochir. Supplement* **2007**, *100*, 37.
[39] C. Meyer, S. Wrobel, S. Raimondo, S. Rochkind, C. Heimann, A. Shahar, O. Ziv-Polat, S. Geuna, C. Grothe, K. Haastert-Talini, *Cell transplant.* **2016**, *25*, 159.
[40] M. Morano, S. Wrobel, F. Fregnan, O. Ziv-Polat, A. Shahar, A. Ratzka, C. Grothe, S. Geuna, K. Haastert-Talini, *Int. J. Nanomed.* **2014**, *9*, 5289.
[41] C. Meyer, L. Stenberg, F. Gonzalez-Perez, S. Wrobel, G. Ronchi, E. Udina, S. Suganuma, S. Geuna, X. Navarro, L. B. Dahlin, C. Grothe, K. Haastert-Talini, *Biomaterials* **2016**, *76*, 33.
[42] X. Gu, F. Ding, D. F. Williams, *Biomaterials* **2014**, *35*, 6143.
[43] W. Daly, L. Yao, D. Zeugolis, A. Windebank, A. Pandit, *J. R. Soc., Interface* **2012**, *9*, 202.
[44] D. Cunha, M. Ben Yahia, S. Hall, S. R. Miller, H. Chevreau, E. Elkaïm, G. Maurin, P. Horcajada, C. Serre, *Chem. Mater.* **2013**, *25*, 2767.
[45] G. Wißmann, A. Schaate, S. Lilienthal, I. Bremer, A. M. Schneider, P. Behrens, *Microporous Mesoporous Mater.* **2012**, *152*, 64.
[46] I. Nicoletti, G. Migliorati, M. C. Pagliacci, F. Grignani, C. Riccardi, *J. Immunol. Methods* **1991**, *139*, 271.
[47] T. Lawrence, G. Natoli, *Nat. Rev. Immunol.* **2011**, *11*, 750.
[48] S. W. Ryter, A. M. Choi, *Antioxid. Redox Signaling* **2002**, *4*, 625.
[49] M. A. Lynes, J. Hidalgo, Y. Manso, L. Devisscher, D. Laukens, D. A. Lawrence, *Cell stress chaperones* **2014**, *19*, 605.
[50] S. Gordon, F. O. Martinez, *Immunity* **2010**, *32*, 593.
[51] N. J. Wood, H. F. Jenkinson, S. A. Davis, S. Mann, D. J. O'Sullivan, M. E. Barbour, *J. Mater. Sci.: Mater. Med.* **2015**, *26*, 201.
[52] S. Wrobel, S. C. Serra, S. Ribeiro-Samy, N. Sousa, C. Heimann, C. Barwig, C. Grothe, A. J. Salgado, K. Haastert-Talini, *Tissue Eng., Part A* **2014**, *20*, 2339.
[53] A. Beyerle, A. Braun, A. Banerjee, N. Ercal, O. Eickelberg, T. H. Kissel, T. Stoeger, *Biomaterials* **2011**, *32*, 8694.
[54] J. Bushman, B. Mishra, M. Ezra, S. Gul, C. Schulze, S. Chaudhury, D. Ripoll, A. Wallqvist, J. Kohn, M. Schachner, G. Loers, *Neuropharmacology* **2014**, *79*, 456.
[55] K. Haastert-Talini, J. Schaper-Rinkel, R. Schmitte, R. Bastian, M. Muhlenhoff, D. Schwarzer, G. Draeger, Y. Su, T. Scheper, R. Gerardy-Schahn, C. Grothe, *Tissue Eng., Part A* **2010**, *16*, 3085.
[56] O. Ziv-Polat, A. Shahar, I. Levy, H. Skaat, S. Neuman, F. Fregnan, S. Geuna, C. Grothe, K. Haastert-Talini, S. Margel, *BioMed Res. Int.*, **2014**.
[57] D. A. Bolukbas, S. Meiners, *Nanomedicine (London, U. K.)* **2015**, *10*, 3203.

Stefan Wuttke,* Andreas Zimpel, Thomas Bein, Simone Braig, Katharina Stoiber, Angelika Vollmar, Dominik Müller, Kirsten Haastert-Talini, Jörn Schaeske, Meike Stiesch, Gesa Zahn, Alexander Mohmeyer, Peter Behrens, Oliver Eickelberg, Deniz A. Bölükbas, Silke Meiners*

**Validating Metal-Organic Framework Nanoparticles for their Nanosafety in Diverse Biomedical Applications**

ToC figure