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The *European Journal of Histochemistry* was founded in 1954 by Maffo Vialli and published until 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is published under the auspices of the University of Pavia, Italy.
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table of contents

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BIOMATERIALS, NANOPARTICLES, NANOSTRUCTURES

NANOACTUATION OF THERMOPHILIC ENZYMES BY ALTERNATE MAGNETIC FIELD: POSSIBLE APPLICATIONS IN INDUSTRIAL BIONANOTECHNOLOGY AND IN NANOMEDICINE

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We are combining the capacity of magnetic nanoparticles (NPs) to generate thermal energy under the influence of an alternate magnetic field (AMF) with the characteristics of thermophilic enzymes to obtain NP-enzyme systems[1] possessing optimal activity at high temperatures. We have demonstrated that the catalytic activity of these systems can be modulated in a wireless fashion by applying an AMF. In particular, we have synthetized 10 nm iron oxide NPs that we have functionalized with the thermophilic enzymes α-amylase (AMY) or L-aspartate oxidase (LASPO). Exposing these two NP-enzyme systems (i.e., NP-AMY and NP-LASPO) to an alternate AMF, we have obtained enzymatic activities that are normally reached at 80-90°C without recording a significant raise of the global temperature of the solution in which NP-enzyme systems were suspended.
We are convinced that nanoactuation of thermophilic enzymes by AFM may lead to interesting applications both in industry and in nanomedicine. In industrial applications, enzymatic or even multi-enzymatic processes for *in vitro* synthetic biology could benefit from AFM nanoactuation especially when products, substrates or intermediates are heat-labile. In nanomedicine, NP-enzyme systems might be delivered in the cell cytoplasm or in other cellular compartments and switched on or off with an AMF of appropriate frequency and intensity. This could be exploited to replace missing or non-functional enzymes and regulate them in a remote control fashion. Alternatively, we could avail of these NP-enzyme systems to kill cancer cells[2] or to dissolve atherosclerotic plaques.

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EXPOSURE TO COPPER OXIDE NANOPARTICLES (CuO NPs) MODULATES THE OXIDATIVE STRESS RELATED GENES IN *ARBACIA LIXULA* EMBRYOS

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The development of nanotechnology and the increasing applications of metal oxide nanoparticles (NPs) in a wide range of fields have been rising increasing concerns about their environmental fate and potential toxicity. Although several studies focused on the potential hazards of CuO NPs to aquatic life, their environmental impacts and toxicity mechanisms still have been poorly elucidated.[1,2] Developmental abnormalities such as alterations in larval skeletogenesis and neurotransmission pathways have been reported in sea urchins exposed to copper oxide nanoparticles[3,4,5] but their effects on antioxidant defences have not been investigated.
In the current study, the potential role of oxidative stress in CuO NPs toxicity was evaluated in sea urchin *Arbacia lixula* embryos exposed to three CuO NPs concentrations (0.7, 10, 20 ppb) until the pluteus larval stage (72 hours post-fertilization, hpf).
Quantitative real time PCR revealed a time- and concentration-dependent modulation of oxidative stress-related genes, i.e *Cu/Zn-superoxide dismutase* (*Cu/ZnSod*) and *catalase* (*cat*) together with *metallothionein* (*mt*), here cloned and molecular characterized for the first time. These transcriptional responses strongly support the hypothesis that the toxicity of CuO NPs is related to reactive oxygen species (ROS)-mediated pathway and provide insight into the possible molecular mechanisms underlying copper nanoparticles toxicity in *A. lixula* sea urchins. The obtained results provide new biomarkers for monitoring of aquatic environments while corroborating the suitability of *A. lixula* embryotoxicity assay[6] for future ecotoxicological investigations of impacted marine areas.

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BIOLOGICAL INTERACTIONS AND EFFECTS OF METAL OXIDE NANOCOLLOIDS IN *IN VITRO* AND *IN VIVO* SYSTEMS

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In recent decades, metal oxide nanoparticles (MONPs) have found widespread applications in the biomedical and agricultural fields due to their strong biocidal activity, while their biocompatibility and adverse outcome pathways are still debated. Their effectiveness, based on the high volume surface ratio can be reduced or modified by agglomeration phenomena, which compromise the stability of NPs suspensions. In this matter, the synthesis of MONPs coated with capping agents can help to improve NP stability and avoid agglomeration. Moreover, the application of MONPs in colloidal form is inevitable for better assimilation and functioning of these agents in the bio-systems.
With the aim to investigate the comparative toxicity of colloidal suspensions of CuO and ZnO NPs, coated with different polymers (PEI, PEG or PVA), and to contribute in nanotechnology safety aspects, in this study we used human lung A549 cells and *Xenopus laevis* embryos as *in vitro* model for inhalation toxicity and *in vivo* model for aquatic toxicity respectively.
A549 viability results showed that all coated ZnO NPs and PEI-CuO NPs (>10 µg/mL) were strongly cytotoxic, while PEG-CuO NPs were less effective even at the highest doses. Unexpectedly, the proinflammatory response (IL-8 levels) increased in a dose-dependent manner after treatment with both CuO NPs, regardless of the coating.
The standard Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) evidenced that all coated-ZnO NPs were not embryolethal but able of inducing malformations (mainly abnormal gut coiling and abdominal edema). While the PEG-CuO NPs were the safest, PEI-CuO NPs showed the highest developmental hazard with an LC50 of 7.5 mg/L and a TI of 1.53. Also, the ICP analy-