european journal of histochemistry
a journal of functional cytology

ISSN 1121-760X
volume 61/supplement 1
2017

PROCEEDINGS OF THE 63rd CONGRESS OF THE ITALIAN EMBRYOLOGICAL GROUP (GEI)

Rome, 12-15 June 2017
University of Rome "Sapienza"
University of Rome "Tor Vergata"
University of Rome "Roma Tre"

under the auspices of
the University of Pavia, Italy

Published by PAGEPress, Pavia, Italy

Editorial Office:
Via A. Cavagna Sangiuliani 5, 27100 Pavia, Italy
Phone: +39.0382.464340 - Fax: +39.0382.34872
E-mail: info@pagepress.org

Printed quarterly by:
Press Up s.r.l.
via La Spezia, 118/C 00055 - Ladispoli (RM)
Phone and Fax: +39.076.15.27.351

Annual Subscriptions
Europe: Euro 200
All other Countries: $ 250

Subscriptions, cancellations, business correspondence and any enquiries must be sent to PAGEPress Publications, Pavia, Italy.
Cancellations must be received before the end of September to take effect at the end of the same year.

Reg. Tribunale di Pavia n. 289/23.2.1984.

Recognized by the Ministero per i Beni e le Attività Culturali, Italy as a publication of high cultural value.

Associato all'USPI
Unione Stampa Periodica Italiana

european journal of histochemistry
a journal of functional cytology

ISSN 1121-760X
volume 61/supplement 1
2017

ejh

under the auspices of
the University of Pavia, Italy

IN VITRO GENOTOXIC EFFECTS OF TITANIUM DIOXIDE NANOPARTICLES (TIO2 NPS) IN HUMAN SPERM CELLS

M. Santonastaso,[1] F. Mottola,[2] N. Colacurci,[1] C. Iovine,[2] S. Pacifico,[2] M. Ferrara,[2] V. Feola,[2] M. Cammarota,[3] F. Cesaroni,[4] L. Rocco[2]

[1]Dipartimento DBC, Università della Campania "Luigi Vanvitelli"; [2]Dipartimento DiSTABiF, Università della Campania "Luigi Vanvitelli"; [3]Dipartimento Medicina Sperimentale, Università della Campania "Luigi Vanvitelli"; [4]Centro PMA del Cassinate, Italy

E-mail: lucia.rocco@unicampania.it

Among the varieties of engineered nanoparticles (NPs) being used today, titanium dioxide (TiO_2) nanoparticles are one of the most widely used in consumer products, such as plastics, paper, sunscreens, cosmetics, drugs and foods. As regards the massive presence in the environment, the attention of the study has been focused on the genotoxic effects of TiO_2-NPs on human spermatozoa *in vitro*. It is known that in mice TiO_2 NPs are able to cross the blood-testis barrier induced inflammation, cytotoxicity and gene expression changes that lead to impairment of male reproductive system. The study presents new data on the DNA damage in human sperm exposed *in vitro* to two concentrations of n-TiO_2 (1 µg/L and 10 µg/L) for different times (15, 30, 45 and 90 minutes) and the role of ROS as mediators of n-TiO_2 genotoxicity. Primary n-TiO_2 characterization was performed by TEM. The dispersed state of the n-TiO_2 in media was spectrophotometrically determined at 0, 24, 48 and 72 h from the initial exposure, collecting samples of n-TiO_2-enriched culture media. n-TiO_2 concentration value was extrapolated from the calibration curve obtained from n-TiO_2 standard solutions. The genotoxicity have been highlighted by using different experimental approaches (Comet Assay, TUNEL test, DFC Assay, RAPD-PCR). The Comet Assay showed a statistically significant loss of sperm DNA integrity after 30 min of exposure. The results of the TUNEL test showed an increase in sperm DNA fragmentation after 30 minutes of exposure. The RAPD-PCR analysis showed a variation of the polymorphic profiles of the sperm DNA exposed to n-TiO_2 respect to the control sperm DNA. The evidences from DFC Assay showed statistically significant increase of intracellular oxidative stress caused by n-TiO_2. This research provides the first data on the evaluation of the potential genotoxicity of n-TiO_2 on human sperm that occurs through the production of intracellular ROS. The results provide a starting point for investigations on the potential effects that nanomaterials could have on infertility rate.

EFFECTS OF NANOPARTICLE TREATMENTS ON THE DEVELOPMENT OF THE WATER FROG, *PELOPHYLAX* SINKL *ESCULENTUS*

G. Scillitani,[1,] G. Bernardini,[2] R. Rossi,[3] M. Mastrodonato[1]

[1]Department of Biology, Laboratory of Histology and Comparative Anatomy and [3]Department of Emergency and Organ Transplants, University of Bari "Aldo Moro", Bari; [2]Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy

E-mail: giovanni.scillitani@uniba.it

The increasing use of nanoparticles (NP) in industrial and medical applications renders necessary to understand how they affect both biological and environmental systems. Therefore, we tested the effects of three NP (cobalt, nickel, iron) on developing embryos of the water frog *Pelophylax* sinkl. *esculentus*. This frog is commonly found in Italian wetlands and is regarded as a good biomarker. Samples came from an artificial tank in the University Botanic Garden in Bari. Embryos at the developmental stage 10 (earliest involution of blastopore dorsal lip) were treated with iron, nickel or cobalt NP from IoLiTec (Heilbronn, Germany). A control group and three treatments per NP at increasing concentrations (LC50/2, LC50, and 2xLC50, according to literature) were considered, for a total of ten groups. Each group included about 20 individuals. Groups were monitored for the following ten days. A low mortality was observed and it was similar throughout the groups. Total length (LT) and eye diameter (LO) were significantly higher in the control (mean LT in control = 7.8 mm, mean LT in treatments = 6.3 mm; mean LO in control = 0.3 mm, mean LO in treatments = 0.2 mm). Significant differences in proportions were observed between controls and treatments in developmental stages (30% of controls reached stage 23 and 66% stage 21, lower values were observed in treatments). Malformations were observed in about 30% of controls, whereas in treatments they reached 60%. The most common malformations observed were abnormally large ventral mass and bent body axe, followed by abnormal development of the head and eye malformations. Investigations of integument at light, SEM and TEM microscopy revealed that in the epidermis of the treatments muciparous cells were hypertrophic, ciliated cells showed a higher number of cilia in respect to controls and ionocytes presented several swelled mitochondria, indicating a stressed condition. Besides, treatments were infested by the chytrid fungus *Batrachochytrium dendrobatidis*, as indicated by the several zoospores and zoosporangia observed in the epidermis. It is concluded that NP treatments are responsible of reduced growth, developmental delay and malformations, increase of secretion and oxidative stress in the cells of the integument, as well as and reduced resistance to fungal infections.

THE ROLE OF PEROXISOMES DURING ADULT NEUROGENESIS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

C. Scopa,[1,2] A. Fracassi,[1] R. Scardigli,[2,3] S. Moreno[1]

[1]Department of Science-LIME, University "Roma Tre", Rome; [2]Laboratory of Neurotrophic Factors and Neurodegenerative Diseases, European Brain Research Institute (EBRI), Rome; [3]CNR-Institute of Translational Pharmacology, Rome, Italy

E-mail: c.scopa@ebri.it

In adult mammals, neurogenic niches include the subventricular zone (SVZ) of lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus,[1] Neurogenesis has been reported to be altered in neurodegenerative disorders, particularly Alzheimer's disease (AD).[2] Our group described peroxisomal changes in the brain of a transgenic mouse AD model (Tg2576), at the onset of disease.[3-5] On the other hand, Peroxisome Proliferator Activated Receptors (PPARs) have been suggested to play an important role in neural stem cell (NSCs) fate determination.[6,7] To address the role of peroxisomes during adult neurogenesis in early AD, we investigated the expression of specific markers in neurogenic niches both *in vivo* and *in vitro*. Immunohistochemical analysis of SVZ and SGZ in 3-month-old mice reveals stronger staining for PMP70 - a major peroxisomal membrane protein - and PPAR in Tg2576, as compared to WT. Accordingly, western blotting analyses of neurospheres lysates from 1.5-month-old Tg2576 demonstrate significantly higher levels of both markers than in WT. Also, confocal microscopy of differentiated neurospheres shows enhanced immunoreactivity in Tg2576 cells, as compared to their WT counterparts. Interestingly, while PMP70 appears equally expressed in neurons and astrocytes, PPAR preferentially colocalizes with GFAP,