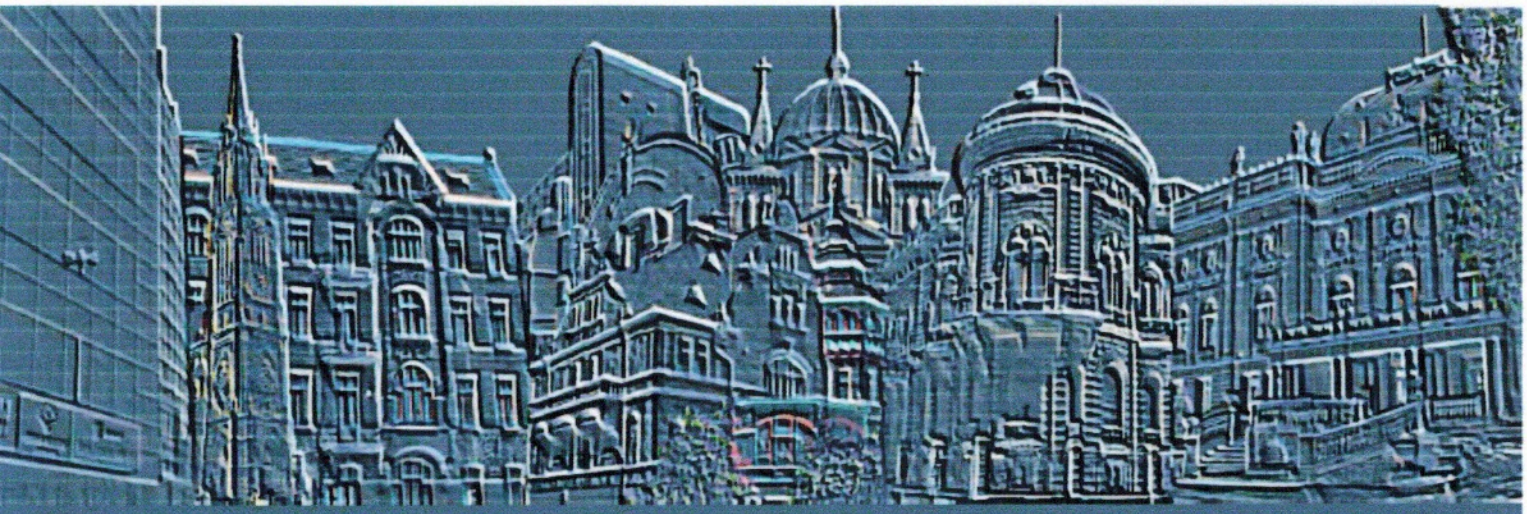

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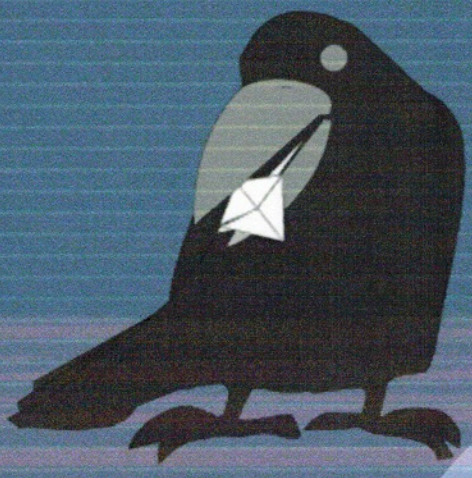

ABSTRACT BOOK

Editors:
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Lodz University of Technology
Department of Semiconductor and Optoelectronic Devices
211/215 Wolczanska Street, 90-924 Lodz
POLAND

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DO COPPER NANOPARTICLES STIMULATE BLOOD VESSEL DEVELOPMENT?

ANDRÉ CHWALIBOG[1], MONIKA ŁUKASIEWICZ[2], ABDULLAH SCOTT[1], KATARZYNA MITURA[3], JAN NIEMIEC[2], KRISHNA PRASAD VADALASETTY[1], EWA SAWOSZ[4]

[1] *University of Copenhagen, Department of Veterinary and Animals Sciences, Copenhagen, Denmark*
[2] *Warsaw University of Life Sciences, Department of Poultry Breeding, Warsaw, Poland*
[3] *Koszalin University of Technology, Department of Biomedical Engineering, Koszalin, Poland*
[4] *Warsaw University of Life Sciences, Department of Animal Nutrition and Biotechnology, Warsaw, Poland*
corresponding author: ewa_sawosz@sggw.pl

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Abstract

Chicken embryo model was used to evaluate effect of Cu nanoparticles (NanoCu) on angiogenesis. Fertilised eggs were injected with NanoCu, $CuSO_4$ and with placebo or not injected. The development of embryos and blood network, as well as gene expression related to vessel development was evaluated. The study indicated that NanoCu have pro-angiogenic properties at the systemic and molecular levels.

1. Introduction

Copper is a key microelement required by animals and humans. It plays an important role as a cofactor of diverse enzymes and it is essential in the development of blood vessels, stimulating angiogenesis, vasculogenesis and endothelial cell migration. However, the ions released from Cu salts can be toxic. We hypothesized that NanoCu might affect blood vessel development without causing toxic effects. Thus, the objective of the study was to evaluate the influence of NanoCu, in comparison to $CuSO_4$, on angiogenesis, using a chicken embryo model.

2. Material and methods

Fertilised chicken eggs were divided into four groups (4 x 100 eggs): not injected (control), injected with phosphate buffered saline (placebo), injected with solutions of $CuSO_4$ or NanoCu (50 ppm). The injections took place on the first day of incubation. The eggs were incubated for 20 days. The network of blood vessels in the chorioallantoic membrane (CAM) was evaluated at day 9 and 12. At day 20, the embryos were weighed and the samples of organs and breast muscles were taken for pending analyses. Gene expression at the mRNA level was measured using the quantitative polymerase chain reaction method.

3. Results and discussion

The *in ovo* administration of $CuSO_4$ and NanoCu did not influence the body and organ weights at day 20. All embryos developed normally and no genetic or other defects were detected. Both forms of Cu promoted the development of vessels, however, the number of branches and length of vessels was greater in the NanoCu group compared to the control and the $CuSO_4$ group (Table 1).

Table I. The number of branches of blood vessels examined on implants (with diameter 1 cm) incubated on the chicken embryo chorioallantoic membrane, after *in ovo* treatment with $CuSO_4$ and nanoparticles of Cu (NanoCu)

	Control	$CuSO_4$	NanoCu	SEM
Number of branches	7.9^{a}	12.6^{b}	17.3^{c}	0.43
Length of vessels mm	7.3^{a}	11.5^{b}	13.2^{b}	0.97

a, b, c - within rows means bearing different superscripts differ significantly at $P<0.001$.

The results indicate that NanoCu have pro-angiogenic properties at the systemic level, to a greater degree than $CuSO_4$ salt. Gene expression in pectoral muscles at the mRNA level, at day 20 of embryogenesis, was affected by Cu administration. NanoCu treatment almost doubled the expression of the vascular endothelial growth factor (*VEGF-A)* compared to the control. The fibroblast growth factor (*FGF2)* expression was about five-folder higher in the $CuSO_4$ group and seven-fold higher in the NanoCu group than in the control. VEGF-A is regulated by hypoxia, oxidative stress, growth factors and cytokines. FGF2 plays a crucial role in VEGF-dependent vasculogenesis, it is also highly involved in cell proliferation a consequently a strong inducer of the proliferating cell nuclear antigen (PCNA) - a marker of cell division. This was reflected in a significant increased *PCNA* expression after NanoCu treatment.

4. Conclusions

The *in ovo* administration of Cu as $CuSO_4$ salt or NanoCu had no negative effects on embryo development. NanoCu increased vascularization of implants maintained on CAM to a greater extent than $CuSO_4$. The pro-angiogenic properties of NanoCu were confirmed on the molecular level. The results suggest that NanoCu can be considered as a potential agent in vascular therapies.

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