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SUCCINATE, SILICON DIOXIDE, ALUMINIUM OXIDE, AND SILVER NANO- AND MICROPARTICLE INFLUENCE ON THE MODEL ORGANISM *DROSOPHILA MELANOGASTER*

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Abstract: In the study we subjected for biological testing succinate (5–3000 nm), silica (200–300 nm), aluminium (2500 nm), and silver (500–1000 nm) particles potentially applicable in the production of novel 3D biotextile. The effects of selected particles were evaluated in drosophila model by measuring the parameters potentially to be affected during the development. At the concentrations tested (0.01% and 0.1%), no clear adverse effects on egg to imago viability and locomotor activity, size, and phenotype of enclosed flies were found.

Key words: microparticles, biotextile, drosophila, development

Introduction

Inorganic and organic nano- and microparticles have been studied and applied in various industrial areas (Joudeh and Linke, 2022) – food technology (Younes et al., 2018), agriculture (Aqeel et al., 2022), environmental remediation (Liwarska-Bizukojć and Olejnik, 2020), pharmaceutical biotechnology (da Silva et al., 2023), biomedical application (Abbasi et al., 2023), textile manufacturing (Ļašenko et al., 2022), industrial biotechnology (Laible et al., 2021), and other industrial and biotechnological processes.

Nanoparticles are defined as particles with external dimensions between 1–100 nm (Joudeh and Linke, 2022, Engin et al., 2017), and microparticles are considered as particles in the size range from 100 nm up to 1000 μm (McClements, 2020).

Due to the widespread use of nano- and microparticles and their potential release into the environment, it is important to assess the potentially harmful effects of these particles on human health, as well as the impact on living organisms (Kumah et al., 2023).

The effect of particles on living organisms depends on their composition, size, shape, hydrophobic/hydrophilic properties, charge, stiffness, and presence of functional groups (Sabourian et al 2020, Augustine et al., 2020). Within tissues nanoparticles interact with the extracellular matrix (Engin et al., 2017). Upon contact with the cell surface

nanoparticles, depending on their physicochemical properties, can be internalized through passive transport (diffusion) or active cell transport (endocytosis). It is considered that nanoparticle size 10–60 nm is optimal for internalization process (Sabourian et al., 2020). The main nanoparticle internalization process is clathrin-mediated endocytosis which takes part in all eukaryotic cells. In this process 100–150 nm vesicles are formed. Larger particles 0,5–10 μm are obtained within the cell by phagocytosis, the process performed by professional phagocytic cells (Awashra and Mlynarz, 2023). In animals, phagocytosis is carried out by specific immune cells, and in some invertebrates also by enteric phagocytes. The intestinal phagocytes have not yet been described in the model organism *Drosophila melanogaster* (Hartenstein and Martinez, 2019).

Intracellularly nanoparticles interact with cytoplasmic proteins, nucleus, and other cellular organelles, and cause subsequent cellular responses (Augustine et al., 2020).

In the nucleus, particles, up to 10 nm, enter the passive transport path, while larger particles, up to 50 nm, are internalized by active transport (Sabourian et al., 2020). It is considered that smaller-sized nanoparticles have higher cellular uptake and more pronounced toxic effects (Awashra and Mlynarz, 2023).

Nanoparticles cause adverse effects in biological systems, mainly through the formation of reactive oxygen species (ROS) and the resulting oxidative stress (Augustine et al., 2020, Awashra and Mlynarz, 2023). The effects of nanoparticles on living organisms are being studied both in plants (Gao et al., 2023, Grauda et al., 2015) and animals (Lama et al., 2020) using *in vitro* and *in vivo* systems. Nanoparticle effects depends on whether cells are studied *in vitro* or at organism level (Karkossa et al., 2021). It is convenient to use invertebrate models to assess the impact on the whole organism, as they are subjected to less ethical requirements. (Lama et al., 2020). The fruit fly *Drosophila melanogaster* is a commonly used non-mammalian model organism in biological and medical research, including toxicology. It has well-studied biology and evolutionary conserved basic biochemical and genetic processes are shared with higher eukaryotes (Chifiriuc et al., 2016).

The current study was designed to find out if nano- and microparticles subjected for biological testing could be potentially applicable in the production of novel 3D biotextile with enhanced protecting properties. The effects of selected succinate, silica, aluminium, and silver particles (0.1% and 0.01%) were evaluated by measuring drosophila eclosion rate, and other parameters potentially to be affected during the development.

Materials and methods

Chemicals

Four different types of spherical particles (Ps) were tested. Silver particles (Ag) 500–1000 nm (Enola, Latvia), aluminium oxide particles (Al_2O_3) 2500 nm (Alfa Aesar, USA), silicon dioxide particles (SiO_2) 200–300 nm (Sigma-Aldrich, USA), and amber (succinate) composite particles 5–3000 nm (JLU Technologies, Latvia).

The particles were suspended in an aqueous solution of 0.8% Tween® 80 (polysorbate) with a bath sonicator at 40 kHz for 40 minutes, at a final concentration of 0.4%, and used in the test.

Treatment of the flies

Drosophila melanogaster Canton-S strain was used in all tests. The flies were raised on banana medium (Demerc and Kaufmann, 1996) 8 ml in standard 35 ml plastic vials at 25 ± 1 °C, under 12 : 12 h light : dark cycle (9:00 a.m. to 9:00 p.m.) and 60% relative humidity. If necessary for manipulation, CO_2 anaesthesia was applied for adult flies. Testing suspensions were added to banana medium to final Ps concentrations 0.01% and 0.1% in total setting eight experimental groups containing particles (Ps). To ensure appropriate conditions for development, yeast granules were added by spreading to media surface (Becher et al., 2012).

Viability test

Flies were exposed to Ps throughout the life cycle (Rand et al., 2014). Fertilized eggs were obtained by placing reproductively mature drosophila males and females in egg collection cage with standard medium abundantly covered by yeast. Within eighteen hours eggs at the same pose were washed by saline solution using paint brush and collected in sieve and immediately used in experiments. Fifty eggs per vial were placed in Ps containing media and the control media without Ps. Larvae were allowed to hatch, feed, and undergo a metamorphosis. F1 flies were counted after their eclosion to calculate the egg to imago viability. Data were pooled from two experiments and in total 550 eggs per group were used.

Fertilized eggs produced by up two weeks old F1 flies over eighteen hours period were randomly selected and placed in a Ps-containing media. After the completion of metamorphosis, F2 flies were obtained.

Locomotor activity

Locomotor activity was evaluated by the negative geotaxis – climbing test (Nichlos et al., 2012). Two weeks old F1 males and females were separated by CO_2 anaesthesia one day prior the test and maintained in media without Ps.

Flies were moved into glass cylinder, tapped to the bottom of the cylinder, and allowed to climb. After 30 seconds photo was taken, and climbed hight per each fly was measured with ImageJ software. Test were performed within two experiments, 50–71 males and 40–58 females were tested per group. To avoid the influence of circadian rhythm between experiments, the test was carried out at the same time during the light period.

External morphology

F1 and F2 imagoes were examined under stereomicroscope for external morphological deviations in head, thorax, abdomen, wings, and legs. In total 119–162 F1 flies and 68–168 F2 flies per group were analysed. The presence of visual mutant phenotypes was considered as indicator for a genotoxicity of Ps.

Body size

Body size was rated for F1 male and F1 female flies by thorax length (Lafuente et al., 2018). The measurements were made in two experiments, the mean calculated from 57–89 individuals in each group.

Statistical analysis

All data were analysed using Microsoft Office Excel 365 and SPSS 22 for Windows software. The significance of the differences between control and experimental groups were estimated by one-factor ANOVA followed by the Tukey test for normally distributed data sample sets. For other distribution Mann Whitney U-test was applied.

Results and Discussion

A series of different developmental assays are applied to evaluate the overall effects of toxic substances in drosophila model. The toxic effects can be tested within all stages of drosophila development – the egg, larva, pupa, and imago (Rand et al., 2014).

The approach chosen in the study is to expose drosophila to toxic substance throughout all developmental stages. It is a basic method applied in research on the potential deteriorating effects of nanoparticles, microparticles and other compounds.

Drosophila larval period proceeds up to five days at 25 °C, during the stage extensive feeding and ingestion of testing substances occurs. It is followed by pupal period during which complete metamorphosis occurs. Consequently, toxic substance affects not only survival up to the imago stage, but also anatomical, physiological, biochemical parameters of eclosed adult individuals (Rand et al., 2023).

In the current study flies were exposed to Ps within larval stage and consecutive parameters characterizing the drosophila overall physiological state were examined – egg to imago viability, locomotor activity, body size and external morphology.

The F1 egg to pupa viability of Canton-S in control group was on average 55%. In the aluminium oxide and silver Ps 0.1% groups were observed slight reduction of viability up to 15% (Table 1). Sexual dimorphism of locomotor activity (Figure 1) and body size (Figure 2) was observed in all groups. Male flies have faster movements and smaller body size. No clear reduction of the given parameters was observed in the Ps groups. In all groups of F1 and F2 generation fly external morphology matched the wild type.

Table 1. F1 generation egg to imago viability in Ps containing media

Group	*N*	egg to imago viability %		
		Mean	±	*SD*
Control	11	54.9	±	5.54
Succinate 0.01%	11	46.5	±	10.12
Succinate 0.1%	11	55.5	±	8.54
SiO_2 0.01%	11	51.1	±	9.61
SiO_2 0.1%	11	48.7	±	7.55
Al_2O_3 0.01%	11	52.4	±	5.85
Al_2O_3 0.1%	10	42.0	±	12.65*
Ag 0.01%	11	49.8	±	9.05
Ag 0.1%	11	44.0	±	10.39

The data are expressed as mean ± standard deviation. The significance of the differences between means of *Ps* exposed groups and control was determined by the ANOVA Tukey test, $*P < 0.05$ (SPSS 22).

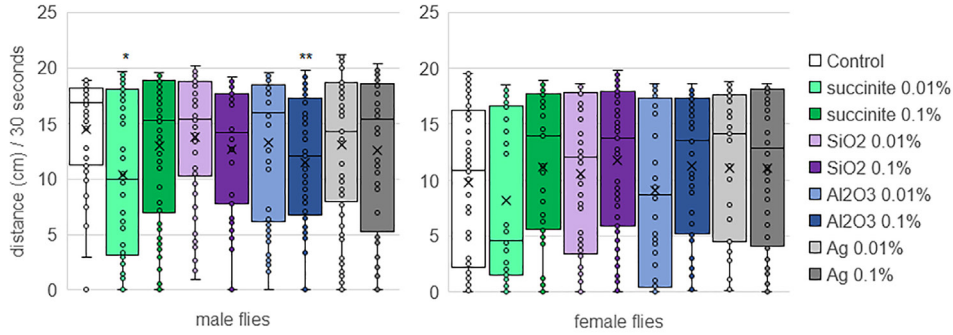

Figure 1. Locomotor activity of F1 imagoes developed in Ps-containing media. The data are expressed as boxplots. The significance of the differences between Ps exposed groups and control was determined by the Mann-Whitney U-test (SPSS 22), **P* ‹ 0.05; ***P* ‹ 0.001.

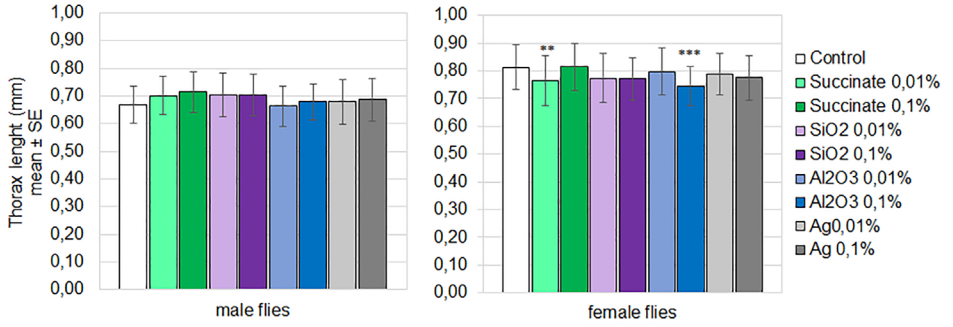

Figure 2. Body size of F1 imagoes developed in Ps-containing media. The data are expressed as mean ± standard deviation. The significance of the differences between means of Ps-exposed groups and control was determined by the ANOVA Tukey test (SPSS 22), *P ‹ 0.05; ***P* ‹ 0.001.

Unlike succinate, Al_2O_3, SiO_2 and Ag nano- and microparticles have been widely applied in different industries, and therefore research data on their toxicity are available. In the drosophila model toxic effects of aluminium oxide, and silver nanoparticles in diameter up to 100 nm have been detected. Al_2O_3 particles (< 50 nm), along phenotypic alterations, induced decrease of locomotor activity of eclosed flies (Anand et al., 2019), similarly, Ag particles (20–100 nm) caused developmental delay, reduced fly size and impaired locomotion (Singh et al., 2021).

In our research Al_2O_3 and Ag Ps in dimeter above 500 nm were tested, and no significant alterations in development, locomotor activity, and phenotype were observed. Non-compliance between our findings and previously published data could be due to the size of tested particles. Larger particles are considered less toxic to the cells (Awashra and Mlynarz, 2023).

The silicon dioxide Ps (> 100 nm) did not exert deteriorating effects on examined parameters, and it is in accordance with previous published data by Peropadre and coauthors in 2023.

Since the ability to induce oxidative stress has been demonstrated for given composition nanoparticles (Mirshafa et al., 2018, Singh et al., 2021, Peropadre et al., 2023), further characterization of Ps included in 3D biotextiles is required, paying attention to biochemical and molecular markers that participate in oxidative stress regulation processes.

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