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P12-42
Comparative study of the cytotoxicity of hydroxyapatite, tricalcium phosphate and calcium phosphate nanomaterials on Panc-1 and HEK293 Cell Line

S. Cesmeli, C. Oksel Karakus

İzmir Institute of Technology, Department of Bioengineering, Izmir, Turkey

Calcium phosphate-based bioceramic nanoparticles have been actively used in a range of therapeutic applications. Although they are mostly considered as biocompatible materials, the circulation of nanoparticles in the bloodstream raise further questions as to what degree of cellular damage they are capable of causing once carried out to vital organs such as kidney and pancreas. Therefore, there is a clear need to explore potential cellular damage induced by commercially used bioceramic nanoparticles such as hydroxyapatite (HAp), tricalcium phosphate (TCP) and calcium phosphate (CaP).

Cytotoxicity can be determined based on different parameters in cells, such as membrane integrity and mitochondrial damage. In particular, mitochondrial damage is quantified by the increase in the activity of mitochondrial dehydrogenases, resulting from an expansion in the number of viable cells. Mitochondrial dehydrogenases forms formazan dye after the reaction with water-soluble tetrazolium salt (WST-1) reagent. The disruption of the cell membrane results in the translocation of cellular components (i.e., phospholipids, proteins, and enzymes) from inside the cell to the outside. Lactate dehydrogenase (LDH) is a cytosolic enzyme which is released upon cell lysis and can be measured by a reaction in which tetrazolium salt is converted into a red formazan product. The formation of reactive oxygen species (ROS) is another important mechanism which is known to cause damage in the basic building blocks of the cell including DNA, protein and lipids.

In this study, two different cell lines, pancreas cancer (Panc-1) and human embryonic kidney (HEK293), were exposed to varying concentrations of HAp, TCP and CaP nanoparticles. The cytotoxic effects were assessed by WST-1, LDH and dichlorofluorescein diacetate (DCFDA) assays. Prior to toxicity assessments, proliferation experiments for both cell lines were conducted to assess possible interferences with assay components and to determine the optimal number of cells. No interference was observed between bioceramic nanopowders and LDH assay components, indicating that it can be used to evaluate cytotoxicity of nanoparticles. As expected, cell line- and nanoparticle-specific differences were observed in the cytotoxicity of tested materials. In particular, TCP showed relatively higher toxicity compared to Hap and CaP on Panc-1 cell line. None of the tested nanoparticles induced cytotoxicity on HEK293 cell line. Results of ROS assay were in line with cell viability assessments. Taken together, these findings suggest that bioceramic nanoparticles do not induce any significant cytotoxic effect and can be safely used in biomedical applications.

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Evaluation and sub-categorization of ocular irritants using the epiocular tissue model – prediction models for liquids and solids

S. Letasiova[1], L. Hudecova[1], J. Markus[1], Y. Kaluzhny[2], M. Klausner[2]

[1]MatTek In Vitro Life Science Laboratories, s.r.o., Bratislava, Slovakia; [2]MatTek Corporation, Ashland, USA

Assessment of serious eye damage/eye irritation originally involved the use of laboratory animals (OECD TG 405). In 2015, a new test guideline (OECD TG 492) was accepted which enables the use of an in vitro procedure based on reconstructed human cornea-like epithelium (RhCE) to distinguish between chemicals (substances and mixtures) not requiring classification and those that must be labeled for eye irritation or serious eye damage. Chemicals identified as requiring classification for eye irritation/serious eye damage must be further tested to distinguish between eye irritants and those causing serious eye damage. There have been several projects focused on the development of tiered testing strategies for eye irritation assessment which takes in account all drivers of classification. The goal of these projects has been to develop a testing strategy to sub-categorize chemicals which: a) do not require labeling for serious eye damage or eye irritancy (No Category), b) can cause serious eye damage (Category 1 or Cat 1), and c) are eye irritants (Category 2 or Cat 2) [1,2].

In the current project, a set of 13 chemicals (7 liquids and 6 solids) that are listed as proficiency chemicals in draft OECD TG 492B were tested using the RhCE model, EpiOcular. We used a testing strategy developed in CON4EI project and confirmed in ALT4EI project, which combines the most predictive time-points of EpiOcular time-to-toxicity neat and dilution protocols. Liquids and solids were test separately with different methodologies and prediction models. The set of chemicals consisted of 4 Cat 1 chemicals, 5 Cat 2 chemicals and 4 No Cat chemicals. Using the proposed testing strategy, we were able to correctly identify 100% of Cat 1 chemicals (4/4), 100% of Cat 2 chemicals (5/5) and 100% of No Cat chemicals (4/4).

The testing strategy proposed in CON4EI and verified in ALT4EI projects to achieve optimal prediction for all three categories – prediction models for liquids and solids seems to be a very promising tool in an integrated testing strategy (ITS) that can discriminate chemicals to No Cat, Cat 2 and Cat 1.

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