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Title: Complex cytotoxicity mechanism of bundles formed from self-organised 1-D anodic TiO2 nanotubes layers

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Abstract: The present study reports on a comprehensive investigation of mechanisms of in vitro cytotoxicity of high aspect ratio (HAR) bundles formed from anodic TiO2 nanotube (TNT) layers. Comparative cytotoxicity studies were performed using two types of HAR TNTs (diameter of similar to 110 nm), differing in initial thickness of the nanotubular layer (similar to 35 mu m for TNTs-1 vs. similar to 10 mu m for TNTs-2). Using two types of epithelial cell lines (MDA-MB-231, HEK-293), it was found that nanotoxicity is highly cell-type dependent and plausibly associates with higher membrane fluidity and decreased rigidity of cancer cells enabling penetration of TNTs to the cell membrane towards disruption of membrane integrity and reorganization of cytoskeletal network. Upon penetration, TNTs dysregulated redox homeostasis followed by DNA fragmentation and apoptotic/necrotic cell death. Both TNTs exhibited haemolytic activity and rapidly activated polarization of RAW 264.7 macrophages. Throughout the whole study, TNTs-2 possessing a lower aspect ratio manifested significantly higher cytotoxic effects. Taken together, this is the first report comprehensively investigating the mechanisms underlying the nanotoxicity of bundles formed from self-organised 1-D anodic TNT layers. Except for description of nanotoxicity of industrially-interesting nanomaterials, the delineation of the nanotoxicity paradigm in cancer cells could serve as solid basis for future efforts in rational engineering of TNTs towards selective anticancer nanomedicine.

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