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In vitro reproduction and ultrastructural detection of the genesis of calcifying cell-derived structures identical to actual hydroxyapatite nucleators in calcific aortic valve stenosis

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Unlike fairly good characterization gained for the major hydroxyapatite nucleators involved in physiological mineralization, i.e. matrix vesicles and apoptotic bodies, no details are available about source and genesis of so called "thick walled cell-derived-products" (CDPs) and "concentrically laminated calcospherulae" (CLCs), which are primary calcium deposition foci in ectopic calcification, including calcific aortic valve stenosis. Here, generation of these structures was successfully reproduced in vitro using an original model simulating either metastatic or dystrophic calcification, subsequent to differential inorganic phosphate supplementations to primary cultures of interstitial cells from bovine aortic valve cusps (AVICs). Superimposed bacterial infectious effects were simulated by additional stimulation with bacterial endotoxin lipopolysaccharide (LPS) and superstimulation with conditioned medium derived from cultures of bovine LPS-stimulated native monocytes/macrophages. At reverted microscope monitoring, AVICs were observed to undergo fragmentation giving rise to irregular debris and/or sporulation-like processes resulting in the formation of barely appreciable punctate bodies, concurrently with specific increase in spectrophotometrically assayed calcium amounts and alkaline phosphatase activity. Ultrastructurally, these cell-derived products showed features comparable to those characterizing CDPs and CLCs in ex vivo samples. The thick wall of CDPs was found to depend on a peculiar AVIC degenerative process culminating with outcropping of multilaminated lipid-containing phthalocyanin-positive layers (PPLs), as revealed by histochemical reactions with Cuprolinic blue. PPLs represented major calcium nucleators, as revealed by co-precipitation of hydroxyapatite needle-like crystals and co-localization of metallic silver deposition after von Kossa staining applied to electron microscopy. In addition, multilaminated PPLs of both degenerating AVICs and CDPs underwent sporulation-like budding and pinching off, so generating a lot of spherical CLC-like bodies, with many being superimposed by near radially oriented hydroxyapatite crystals. In conclusion, the way by which CDPs and CLCs form was ascertained. In addition, in-vitro reproduction of pro-calcific structures identical to those characterizing actual samples from calcified aortic valve cusps suggests the developed in vitro models to be a reliable tool for testing pro-calcific effects exerted by putative etiopathologic agents as well as anti-calcific by therapeutic compounds.

Keywords: in-vitro calcification model, calcific aortic valve stenosis, ectopic calcification