# Cellulose nanocrystal (CNC) cationic derivatives induce NLRP3 inflammasome-dependent IL-1β secretion associated with mitochondrial ROS production.

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## Abstract

Crystalline cellulose nanocrystal (CNC) has emerged as a novel material for a wide variety of important applications such as nanofillers, nanocomposites, surface coatings, regenerative medicine and drug and DNA delivery. CNC has a fiber-like structure with sizes in the range of 200-300 nm long and 5-50 nm wide. Despite the great potential applicability of CNC and its derivatives very little is known about their potential immunogenicity. Fiber-like materials have been known for evoking an immune response in particular for activating the NLRP3-inflammasome/IL-1β pathway. In this study we evaluated the capacity of CNC and its cationic derivatives CNC-*g*-poly(AEM)-1, CNC-*g*-poly(AEM)-2, CNC-*g*-poly(AEMA)-1 and CNC-*g*-poly(AEMA)-2 to stimulate NLRP3-inflammasome/IL-1β axis and enhance mitochondrial ROS. Mouse macrophages (J774.A1) were stimulated for 24h with 25, 50 and 100 µg/mL of CNC and its cationic derivatives. IL-1β secretion was analyzed by ELISA, mitochondrial function by JC-1 staining, cytochrome c release, ATP content and total and mitochondrial ROS was assessed by DCF and MitoSox staining, respectively. Mitochondrial ROS and extracellular ATP was significantly increased in cells treated with CNC-*g*-poly(AEMA)-2, which correlates with the strongest effects on IL-1β secretion. Our data also suggest that the increases in mitochondrial ROS and ATP release induced by this compound may be associated with their capability to evoke immune response.

**Keywords:** cellulose nanocrystal, nanomaterial, inflammation, NLRP3 inflammasome, IL-1β, ROS, macrophages.