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Multiple Functionality of Aminoclay Nanoparticles' Based on Wide-spectrum Anti-bactericidal, Algicidal/Microalgal-Flocculation, and Anti-tumor Effects

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Short Communication

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COMMENTARY

Since 1997 [1,2], water-solubilized and positively charged organo-building blocks of aminoclay nanoparticles (ACNPs) have attracted intensive academic and industrial interest for their multifaceted applications [3]. Engineered organic-inorganic ACNPs constituted of high-density amine groups and approximately 10% total amines in aqueous solution, when protonated, acquire a cationic surface property [4]. The ACNPs' unit structure is composed, in its center, of cationic metals Mg, Ca, Zn, Co, Ni, Cu, Al, Fe, and Ce, some of which maintain their respective unique activities [5,6]. On both sides of that structure, a central metal sheet of octahedrally coordinated metal oxide or hydroxide chains in a 3-aminopropyl-functionalized silicate network is overlaid **(Figure 1).** Mass production of ACNPs is feasible from the engineering viewpoint, as the cationic metal species can be reacted with aminosilane precursors in a one-pot sol-gel reaction under ambient conditions [7], this makes possible an ACNP cost reduction to an estimated ~0.5/g, even using high-purity chemicals [8].

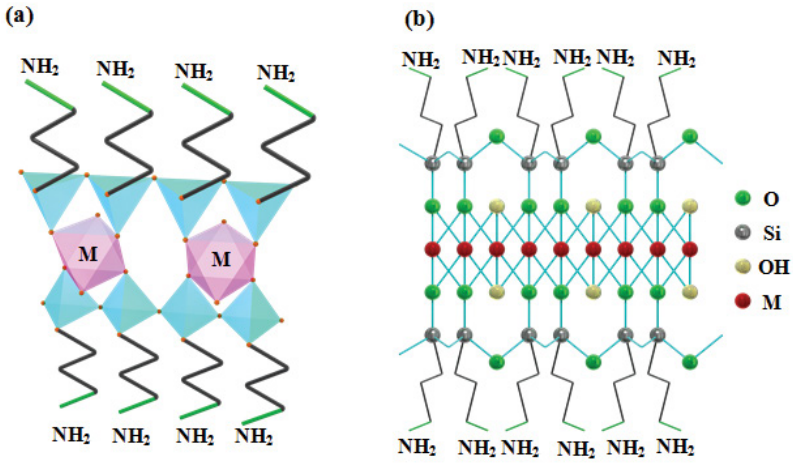

Figure 1. Approximately ideal unit structures of (a) three-dimensional and (b) two-dimensional MAC ($[H_2N(CH_2)3]_8Si_8M_6O_{12}(OH)_4$), as reproduced from the literature [20]. M indicates cationic metal species.

Recently, beyond the preparation of bio-inorganic (nano) composite ACNPs, research interest has been raised in biological fields such as their potential effective anti-bactericidal, algicidal/microalgal-flocculation (i.e. harvesting), and anti-cancer applications. ACNPs' cationic property is derived from the repulsion of protonated amine groups resulting from the delamination of ACNPs in aqueous solution, forming, in the case of Mg-backboned ACNPs, an average hydrodynamic diameter of ~50 nm [9]. In the course of cationic-charged ACNPs' interaction with both gram-negative and -positive bacteria, the cell membranes are partially damaged or disrupted [10]. The most commonly posited mechanism of this process is a strong electrostatic interaction with ACNPs' cell-surface components, not insignificant penetration into internal cells, and consequent release of internal enzymatic products **(Figure 2a)** [11,12]. Thus, this new anti-bacterial ACNPs approach potentially can expand the scope of non-selective inhibition or death for a wide range of bacterial species, thereby achieving an approximately 6 mg/mL minimum inhibition concentration (MIC) value [11]. Alternatively, harmful algal blooms (HAB) in seawater can, at 10 mg/L ACNP agent treatment, be controlled selectively via algal cell lysis, with negligible effects on other organisms **(Figure 2b)** [5,13]. Interestingly as regards the red-tide phenomena, representative algal species such as Raphodophyceae (*Chattonella marina* and *Heterosigma akashiwo*) and Dinophyceae (*Cochlodinium polykrikoides*), having no cell walls, are perfectly disrupted within a few minutes of ACNP accumulation. By contrast, green microalgae species composed of thick-silicate or polysaccharide-complexed cell walls are not disrupted in the presence of ACNPs but rather are flocculated by ACNP bridges among algal cells, and thus offer a superior coagulant functionality **(Figure 2c)** [14,15]. As a result, ACNPs nanomaterial's with ammonium characteristics can be agents of cyanobacteria-bloom inhibition in freshwater [8]. Furthermore, microalgae-transformation studies have shown that ACNP-destabilized algal cell walls admit plasmids into the cell's internal components though a spreading trigger interaction of a mixture of *Chlamydomonas reinhardtii* plasmid, and ACNPs on agar plates along with an additional physical force is required **(Figure 2d)** [16]. This is evidence that ACNPs facilitate the movement of DNA across cellular membranes (including walls) as transfection vectors. For interaction with mammalian cells, the anti-cancer effects of ferric iron atoms at higher concentration of ACNPs (including selective cancer cell death by assistance of target-specific recognition) also have been studied [17]. The generation of hydrogen peroxide (H_2O_2) when cancer cells (even including microalgal cells) propagate can activate ferric iron in ACNPs to produce free radicals, usually •OH free radicals [18]. In fact, it is expected that in the specific ACNP region, cells are damaged with little effect on normal cells. This means that ACNPs at concentrations up to 500 µg/mL can be used as a suitable substrate (matrix) for cytotoxicity-free functionalization or modification purposes **(Figure 2e)** [19]. Syringe-injected dye-labeled ACNPs have been tested in in vivo mice for entrapment, accumulation, and excretion, not incurring any systemic toxicity [5]. Further, systematic in vivo evaluations of such ACNP theranostics in nanomedicine are necessary.

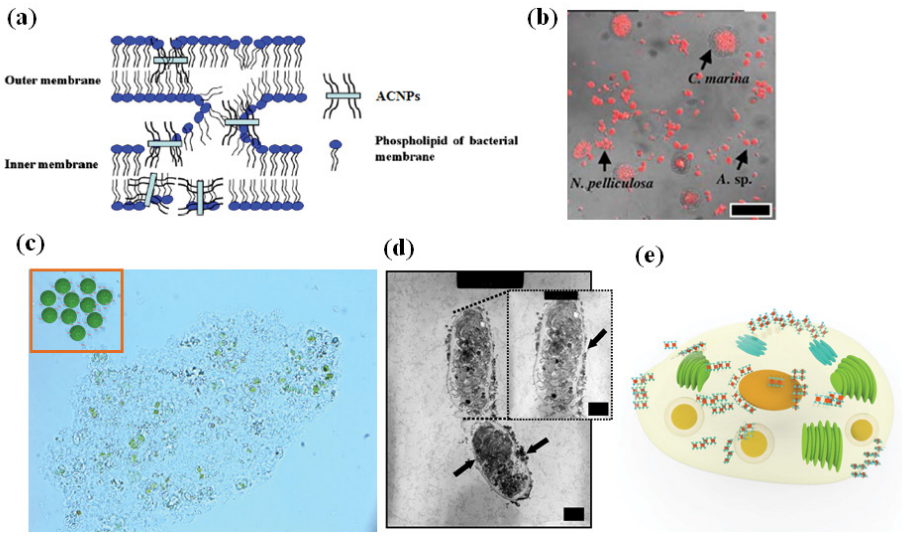

Figure 2. Schematic representation of (a) bacterial-cell disruption, (b) confocal microscopy image of selective Chatonella marina lysis, (c) optical microscopy image of flocculated (i.e., harvested) green microalgae where inset shows enlarged flocculated cells, (d) cross-sectioned transmission electron microscopy (TEM) image of pit and hole formation (marked by arrows) in microalgal cell walls by ACNP coating, and (e) schematic representation of ACNPs accumulation on surface, penetration into cytosol and access to nucleus of mammalian cells, as reproduced from the literature [1,2,6,11,14].

Despite ACNPs' amazing features, obstacles to their application remain. First, in antibacterial applications, although neither photocatalytic cell damage nor toxic release of ions by such metal (oxide) can be considered in the case of ACNP usage, bacteria after ACNP treatment can survive later due to the primary amine-related killing mechanism. Second, the cost of ACNPs' utilization in commercialized microalgae-based biorefinement remains prohibitively high. Thus, our current focus is the development of an economical ACNP-based microalgal biorefinement process [20]. Finally, with regard to medical applications such as theranostics, further clinical trials for enhancement of therapeutic efficiency and advanced selective targeting techniques are necessary. With these challenges met and overcome, engineered ACNPs will make various ground-breaking contributions in their anti-bacterial, algicidal/microalgal-flocculation and anti-tumor applications.

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