Materials Science and Engineering C 79 (2017) 445–456

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Time-dependent subcellular structure injuries induced by nano-/micron-sized calcium oxalate monohydrate and dihydrate crystals

Xin-Yuan Sun, Kai Yu, Jian-Ming Ouyang *

Department of Chemistry, Jinan University, Guangzhou 510632, China
Institute of Biomineralization and Lithiasis Research, Jinan University, Guangzhou 510632, China

ARTICLE INFO

Article history:
Received 15 February 2017
Received in revised form 6 May 2017
Accepted 13 May 2017
Available online 15 May 2017

Keywords:
Calcium oxalate
Time-dependent
Size effect
Subcellular structure injury

ABSTRACT

Comparative studies were conducted to investigate the time effect of cell injury induced by nano-sized (50 nm) and micron-sized (10 μm) calcium oxalate monohydrate (COM) and dihydrate (COD) crystals in African green monkey renal epithelial (Vero) cells. The effects of nano-/micron-sized COM and COD exposure on Vero cells were investigated by detecting the cell viability, cell morphology, LDH release, reactive oxygen species, mitochondrial membrane potential, cell cycle, and cell apoptosis, as well as the intracellular and extracellular crystal distribution. Nano-/micron-sized COM and COD exposure lead to subcellular organelle injury in varying degrees, but the injury sequence of various organelles differed. The time sequence of organelle injury presenting significant variation was described as follows: cell membrane injury (1 h) < mitochondrial membrane potential decrease (3 h to 6 h) ≈ cell-cycle arrest (3 h to 6 h) < cell apoptosis (12 h). Nano-sized crystals lead organelle injury faster than micron-sized crystals, and COM crystals showed more obvious time-dependent effects than the same-sized COD crystals. This study may provide insights into the damage to renal epithelial cells induced by urinary crystals and the formation mechanism of kidney stones.

1. Introduction

Engineered inorganic and hybrid materials have been widely and increasingly used in different fields such as drug delivery [1,2], therapy [3,4], biosensors [5,6], and environmental remediation [7,8]. Whereas the potential toxicity of exogenous and endogenous particles should be concerned [9], especially for the toxicity of these formed particles during the pathogenic mineralization process in vivo, such as calcium oxalate (CaOx), calcium phosphate and calcium carbonate.

Nephrolithiasis is a common and complex disorder disease caused by the multifactorial components such as geographical location, bacterial infection, low urine volume, and low intake of water, it affects up to 10% of the population at some point during their life [10]. More than 60% of renal stones are CaOx stones [11], the most common of which is calcium oxalate monohydrate (COM) followed by calcium oxalate dihydrate (COD) [12,13].

Humans normally excrete millions of urinary crystals daily, the time for urinary crystals to flow through renal tubular can be calculated according to the tubular diameter and length and the flow rate of urine. It has been suggested that with a transit time across the kidney of 5–10 min, residence time is too short for crystals to grow large enough to be trapped [14,15]. Only after fixing on the surface of renal tubular epithelial cells, the free urinary crystals are capable of growing up and forming stones [16,17]. These fixed crystals on the epithelial surface would effectively reduce the luminal diameter, impede the flow of urine and the passage of any crystals formed upstream, and increase the likelihood that they will adhere to crystals already attached to the tubular walls. With time, the tubule would become blocked with crystals [18]. Thus, adhesion time of fixed crystals must take into account during the formation of kidney stone.

Some researchers have recently emphasized that the adhesion and/or endocytosis of crystals by renal tubular epithelial cells is an important factor of causing the formation of kidney stone [19,20]. Crystals binding to cells occurred within a few minutes of exposure, and the crystals soon became internalized within the cells [21,22]. Crystal adhesion and endocytosis by renal tubular epithelial cells could lead to cellular injury, alterations in cellular structure, compositions, and gene expression, initiation of DNA synthesis, and ultimately cell death [23,24]. In addition, the cell response caused by CaOx crystals in renal tubular fluid was also in a time-dependent manner. For example, Khan et al. [25] observed that exposure to COM crystals leads to time-dependent activation of NADPH oxidase and an increase in the production of Nox4 which was a membrane associated subunit of the NADPH oxidase enzyme. Niimi et al. [26] reported that the expressed superoxide

* Corresponding author at: Institute of Biomineralization and Lithiasis Research, Jinan University, Guangzhou 510632, China.
E-mail address: toyjm@jnu.edu.cn (J.-M. Ouyang).

http://dx.doi.org/10.1016/j.msec.2017.05.081
0928-4931/

dismutase (SOD) and 4-hydroxy-2-nonenal (4-HNE) in renal proximal tubular cell line (NRK-52E) which was co-incubated with COM crystals were all changed in a time-dependent manner. Umekawa et al. [27] measured the expressed MCP-1 mRNA and protein in NRK52E cells, the level of their expression significantly increased following treatments with COM in a time-dependent manner. Moreover, renal tubular epithelial cell injury induced by CaOx crystals in turn increases affinity of crystal-binding, which is a critical process of crystal retention within the kidney [20,28].

Urinary supersaturation is the driving force behind crystal formation in the kidneys. In fact, stone formers tend to excrete urine that is more supersaturated of Ca^{2+} and Ox^{2-} than that of non-stone formers [16,29]. The size of initially formed crystallites is closely related to the supersaturation of reaction system. Mersmann et al. [30] stated that the median crystal size is affected by the mean relative supersaturation for inorganic and organic systems with different solubilities, the median crystal size drops with increasing relative supersaturation. Thus, the initial formed urinary crystallites in stone-formers would be smaller than that in healthy controls.

In the previous reports [31,32], the size of crystals varies from a few nanometers to several micrometers in the urine of stone formers and healthy controls. We detected size distribution of urinary crystallite of <1000 nm in the urine samples of 85 healthy persons and 65 lithogenic patients [32]. Most of the crystallites in healthy urine samples were with a narrow particle size distribution from about 20 nm to 400 nm, while the crystallites in lithogenic urines had a broad particle size distribution from 1.1 nm to 1000 nm. In our recent study [33], we have demonstrated that the cytotoxicity of COD crystals toward African green monkey renal epithelial (Vero) cells was size-dependent and increases in the order 50 nm > 100 nm > 600 nm > 3 μm > 10 μm. Besides the size effect of crystals on their cytotoxicity, the crystal phase and exposure time of nano-/micron-sized crystals may also have other unknown effects because the contact between internalized crystals and intracellular different

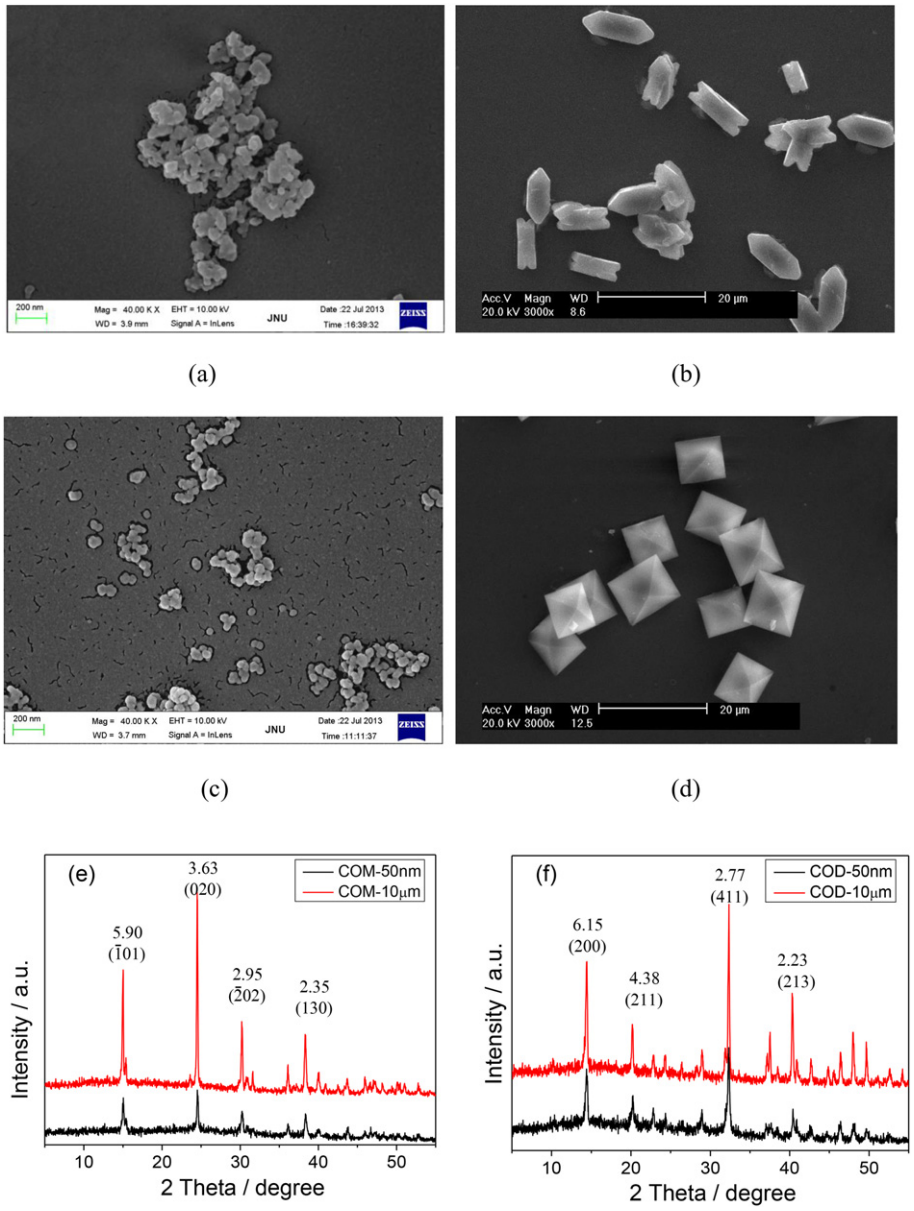

Fig. 1. SEM images and XRD patterns of nano-/micron-sized calcium oxalate crystals. (a) COM-50 nm; (b) COM-10 μm; (c) COD-50 nm; (d) COD-10 μm. Scale bars: (a, c) 200 nm (b, d) 20 μm. XRD patterns of nano-/micron-sized COM crystals (e) and nano-/micron-sized COD crystals (f).