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Histological aspects of the "fixed-particle" model of stone-formation: animal studies

Saeed R. Khan

Department of Pathology, Immunology and Laboratory Investigation and Department of Urology,
College of Medicine, University of Florida, Gainesville, Florida

Abstract

Crystallization by itself is not harmful as long as the crystals are not retained in the kidneys and are allowed to pass freely down the renal tubules to be excreted in the urine. A number of theories have been proposed, and studies performed, to determine the mechanisms involved in crystal retention within the kidneys. It has been suggested that urinary transit through the nephron is too fast for crystals to grow large enough to be retained. Thus free particle mechanism alone cannot lead to stone formation, there must be a mechanism for crystal fixation within the kidneys. Animal model studies suggest that crystal retention is possible through both the free and fixed particle mechanisms. Crystal cell interaction leads to pathological changes which promote crystal attachment to either epithelial cells or their basement membrane. Alternatively, crystals aggregate and produce large enough particles to block the tubules particularly at sites where urinary flow is affected because of changes in the luminal diameter of the tubule. Crystal deposits plugging the openings of the ducts of Bellini may be the result of such a phenomenon. Intra-tubular crystals translocating to renal interstitium may produce osteogenic changes in the epithelial or endothelial cells resulting in the formation of the Randall's plaques. Thus, fixation appears to be either through the formation of Randall's plugs, crystal plugs clogging the openings of the ducts of Bellini or sub-epithelial crystal deposits, the Randall's plaques.

Introduction

Urinary supersaturation is necessary for the nucleation and growth of crystals, but it is not enough for the formation of kidney stones [1]. Both stone formers and non-stone formers may produce supersaturated urine and excrete crystals [2] without ever forming kidney stones. The two most common crystalline constituents of kidney stones, calcium phosphate (CaP) and calcium oxalate (CaOx), are frequently seen in the human urine of healthy individuals as well as stone forming patients [3]. These observations have led to the assumption that crystallization by itself is not harmful as long as crystals have a chance to pass freely down the renal tubules and be excreted in the urine. Troubles start when crystals are retained inside the kidney and establish a nidus for stone growth [1]. This review will focus on various aspects of crystal retention based upon the results of experimental animal model studies.

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Kidney Stones

Kidney stones are mineral concretions found in the renal calyces and pelvis, either free or attached to the renal papillae [4]. The attachment is achieved through sub-epithelial plaques, (Randall's plaques) present on the papillary surfaces, or crystal plugs (Randall's plugs), clogging the openings of the ducts of Bellini [4-6]. Randall's plaques are interstitial deposits of apatitic CaP crystals and are seen in stone formers with idiopathic CaOx, brushite, primary hyperparathyroid, small bowel resection, ileostomy stone disease [7]. Randall's plugs on the other hand can be apatite in primary hyperparathyroid stone formers, apatite with CaOx in brushite, obesity bypass, small bowel resection, renal tubular acidosis and apatite with urate in ileostomy stone formers [7]. The Randall's plugs of stone patients with primary hyperoxaluria are made of CaOx [7,8].

Plaques appear to start deep inside the interstitium with the deposition of apatitic CaP in close association with the vasa recta [9,10], collecting ducts [11] and/or loops of Henle (LH) [12,13]. Mineralization continues with the calcification of interstitial components including collagen fibers and membrane bound vesicles [14]. The mineralization front eventually reaches the renal papillary surface and a sub-epithelial Randall's plaque is established. It appears that most plaques stay dormant and hidden under the papillary epithelium without supporting the development of a stone. Some of the plaques however become exposed to the pelvic urine after disruption of the papillary epithelium. In the case of CaOx stone formers, exposure of these plaques to urine metastable with respect to CaOx likely leads to the deposition of CaOx crystals on macromolecule coated apatitic CaP of the plaque [5].

In the presence of high urinary supersaturation with respect to a mineral or excretion of a poorly soluble substance in the urine, crystallization can occur in the renal tubules leading to tubular occlusion or plugging. When it happens in the collecting ducts, plugs extrude out of the ductal openings, examples include, CaOx crystals plugging the ducts of Bellini in the patients with primary hyperoxaluria [8,15] or cystine crystals in the cystine stone formers [15]. These Randall's plugs can not only act as nidi promoting the formation of a stone through an overgrowth [5,16] but can also induce formation of an unattached stone. Since Randall's plugs are exposed to the tubular fluid on the one side and pelvic urine on the other, stone composition depends upon the urinary environment plugs are exposed to. Apatite plugs can be overgrown with brushite on one side and CaOx on the other [17]. An analysis of 142 spontaneously passed papillary stones showed that 19.7% of the stones were most likely formed attached to Randall's plugs of diverse compositions including CaOx, uric acid, monosodium urate and some other unidentifiable substances such as drugs [18].

Drugs such as magnesium trisilicate, ciprofloxacin, sulfa medications, triamterene, indinavir etc. when given in excessive amounts can produce kidney stones [19], most likely through plugging of the terminal collecting ducts. Similarly, poorly soluble dietary components can also crystallize in the renal tubules and produce kidney stones. Adulteration of the baby formula with melamine led to the formation of kidney stones in thousands of infants [20], many requiring hospitalization and some even lost their lives.

Theoretical Considerations

Vermeulen and colleagues proposed that free particles (crystals) traveling in the ducts of Bellini can grow so rapidly that they become trapped because of their size [21].

These crystal plugs act as nidi for the formation of stones attached to the renal papillary tips. This mechanism is called free particle mechanism. Finlayson and Reid calculated the likelihood of such a phenomenon occurring during CaOx stone formation using average normal values for oxalate excretion, rate of crystal growth, tubular dimensions and urinary transit time through the kidneys. They concluded that even if all the oxalate was consumed and crystals grew at the fastest rate possible, crystalline mass would not be sufficiently large to be trapped because of its size, anywhere in the renal tubules or the renal pelvis [1]. They proposed an alternative model, the so-called fixed particle mechanism, in which crystals become attached to the epithelial lining of the tubule at sites of local injury. The attached crystals grow, plug the tubules and become nidi for stone formation.

Kok and Khan reexamined the free or fixed particle mechanism for CaOx stone formation incorporating new data on tubular dimensions, levels of oxalate excretion, supersaturation, crystal growth rates, diurnal variations in urinary flow, as well as the role of crystal aggregation in mass accretion [22]. Their calculations showed that, through aggregation, crystals formed in the glomerular filtrate can become large enough to be trapped in the collecting ducts, particularly those at the end of the long loops of Henle (LH). Aggregation is considered the fastest process by which particles can increase their size in a short span of time [23] and large aggregates have been seen in fresh urine excreted by stone formers [24].

It has been proposed that urinary flow through the renal tubules is laminar [25], in which case, flow velocity should be very small near the epithelium and may even be zero at the epithelial surface. Thus crystals would be travelling at different speeds, moving faster in the middle of the stream and relatively slow near the tubular epithelium. Slow moving crystals would have a chance to grow bigger compared to the fast moving ones. Crystal movement would also be affected by the upward draining of the tubules as well as drag effect of the tubular wall. Robertson took into account these hydrostatic factors and further refined the model [26]. He concluded that crystals forming at the end of the descending limb of the LH and travelling close to the tubular epithelium can become large enough to be trapped in the tubular lumen.

Animal Models

Calcium Oxalate Nephrolithiasis

A variety of animal models using mice, rats, rabbits, pigs and fruit fly have been developed to study various steps involved in the pathogenesis of different kinds of stones. CaOx crystal deposition is almost always induced by the administration of oxalate or its precursors such as ethylene glycol (EG) and hydroxyl-L-proline (HLP). The deposits are mostly aggregates of CaOx monohydrate crystals, occasionally mixed with crystals of CaOx dihydrate. The number and volume of CaOx deposits, their location, and their retention within the kidneys depends upon the quantity of the administered lithogen and urinary levels of oxalate [27].

An acute increase in oxalate, such as through intraperitoneal administration, leads to crystal formation in all segments of the renal tubules [28-30]. Lower levels of urinary oxalate produce smaller crystals which are quickly cleared through urinary excretion [28,30], whereas higher urinary levels of oxalate produce larger crystal deposits. Human kidney stone formers are generally only mildly hyperoxaluria and their stones are generally found connected to renal papillae. Similar conditions in the rats can be induced by administering 0.75% EG [31-33] or 5% HLP [34] through diet with or without ammonium chloride or calcium. In these rats, renal crystals are generally limited to papillary collecting ducts.

Crystals, depending upon intensity of hyperoxaluria, normally deposit at three renal sites, cortico-medullary junctions, papillary tips and renal fornix (Figures 1-2) [35]. These renal sites are unique because of changes in the luminal diameter of renal tubules in different segments of the nephron. At the cortico-medullary junction, the diameter of the tubular lumen decreases from approximately 35 μm in the proximal tubule (PT) to as small as 15 μm in the loops of Henle [35]. Distal tubules and collecting ducts, show acute angles and z-bend at the papillary base [36]. Even though the inner diameter of ducts of Bellini can be as large as 100 μm , their openings into the pelvis at the papillary tips are slit-like with a width of about 28 μm [22]. As a result of these dimensional changes, larger crystal deposits cannot move with the urine and can be seen blocking the tubules at places such as cortico-medullary junction and papillary tips [28,30]. The morphology of renal tubular epithelium also varies considerably in different sections of the nephron. Proximal tubular cells have abundant long microvilli on the luminal side while other segments either have stubby microvilli or smooth surfaces [37]. These features also affect the flow velocity and crystal movement. Smaller crystals appear retained by adherence to the epithelial surface [29] either directly or through microvilli. Crystal deposition also causes epithelial injury and cellular death. Cell death exposes the underlying basement membrane and is followed by epithelial regeneration. Crystals directly bind to the basement membrane [30] with regenerating epithelium growing over it (Figure 3) [38] pushing the crystals into the interstitium and causing crystal retention. The crystals attachment appears to be arranged by integration of the epithelial basement membrane with the organic matrix of the crystals (Figure 3C). Now these crystals are not exposed to the urine and hence to the nurturing calcium and oxalate, thus they will be unable to grow. Moreover the interstitial crystals become exposed to the body's inflammatory response and may eventually disappear [33] indicating the existence of crystal clearing mechanism in the normal kidneys [39]. Dead and dying cells are shed into the lumen where they may promote formation of more crystals, their aggregation and their retention through increased crystalline mass. Eventually cellular degradation products become a part of the organic matrix of growing intratubular nephroliths [30].

Crystals blocking the peripheral collecting ducts or those at the papillary tips or base, produce small protrusions on the papillary surfaces (Figure 1 C, D). Papillary epithelium covering the crystal deposits becomes stretched, develop cracks, and crystals ulcerate through (Figure 2 A-D) to the surface, becoming exposed to the pelvic urine [32,34]. These exposed crystals can act as an encrustation platform for future stone growth (Figure C, D).

Hyperoxaluria and CaOx crystal deposition in the kidneys are also associated with significant changes in the production of macromolecules that modulate crystal aggregation and crystal adherence to the renal epithelium [31,34,40-51].

Cell culture studies have confirmed that exposure to oxalate and CaOx crystals leads to many physiological and pathological changes in the exposed cells from affecting the production of specific mineralization modulating molecules to cell injury, death and promotion of crystal adherence [52-56]. Attachment is mediated by oxalate induced exposure of phosphatidylserine on cell surfaces [57]. A variety of other molecules which are either produced or displayed on cell surfaces during cell proliferation or exposure to oxalate [58-60] may also be involved in crystal attachment and retention within the tubules. CaOx crystals bind to hyaluronan on migrating and proliferating epithelial cells in culture [61]. Urinary macromolecules such as citrate, nuceolin related protein (NRP), glycosaminoglycans, osteopontin (OPN) and bikunin have been shown to be involved in modulating crystal adherence to the epithelial cell surfaces [55,56,61-69]. Normal urine contains macromolecules that inhibit crystal adherence to the renal epithelial cells in culture [70,71]. Increased expression of OPN on renal epithelial surfaces promote crystal adherence [56,72] while free OPN suppresses it [73,74]. Surface bound NRP similarly mediates crystal attachment to the inner medullary collecting duct cells of the renal tubular epithelium [75] through highly acidic region. The secreted acidic fragment of the NRP, however, inhibits crystal attachment.

Direct measurement of interaction force between CaOx monohydrate crystals and Madin-Darby Canine Kidney (MDCK) cells by atomic force microscopy showed positive adhesion force [76]. Prior treatment with oxalate alone increased the adhesion force which is most likely a result of cell membrane damage and exposure of crystal binding sites. The adhesion force was dependent upon ionic concentration, 2.5 times higher in the presence of calcium than magnesium and was high enough to enable crystal binding even in the flowing urine [77].

Calcium Phosphate Nephrolithiasis

Experimental hypercalciuria induced by the administration of vitamin D leads to the deposition of CaP in the renal tubules [78]. Consumption of AIN-76 diet by female rats results in the intratubular deposition of CaP crystals, mostly at the cortico-medullary junction [79] where proximal tubules and loops of Henle meet, somewhat similar to what happens in rats with hyperoxaluria [28]. Increased urinary excretion of calcium in a genetic model of hypercalciuria produces aggregates of free floating CaP crystals in the urinary space [80]. However, sometimes, particularly in renal fornix, crystals were found attached to the papillary surface epithelium.

Targeted disruption of sodium phosphate co-transporter 2a (Npt2a) gene, results in increased urinary excretion of calcium and phosphate [81], and renal deposition of CaP crystals [82]. By 6 months of age, all crystal deposits are interstitial. Deposits consist of aggregates of microspheres of needle shaped or plate like matrix rich crystals of poorly crystalline biological apatite [83]. Apparently crystals start in the tubular lumen and through a loss of tubular structure, secondarily become interstitial (Figure 4). Deletion of the gene encoding

for sodium-hydrogen exchanger factor-1 (NHERF-1) in mice also leads to increased urinary excretion of calcium and phosphate [84] with small interstitial deposits of CaP in the renal papillae. Tamm-Horsfall protein (THP) null mice produced by ablating THP gene in mouse embryonic cells produce CaP crystals in their kidneys. Poorly crystalline and matrix rich interstitial CaP crystals are seen (Figure 5) in widened basement membrane of the collecting ducts and thin limbs of the loop of Henle [85]. Deposits appear nodular when examined using light microscopy. Transmission electron microscopy showed an aggregation of spherical units of various diameter with internal structure reminiscent of a cut tree trunk or onion with concentric rings around a center. Knocking out osteopontin (OPN) gene also leads to production of CaP deposits in renal interstitium in some mice.

Oxamide Nephrolithiasis

Vermeulen studied stone pathogenesis by investigating the formation of oxamide stones in the rats [86,87]. Oxamide is a relatively insoluble diamide of oxalic acid which, when fed to animals forms large crystalline deposits in distal renal tubules, pelvis, ureters and bladder. Oxamide is also uncharged with a small electrical dipole and thus somewhat similar to uric acid. We induced oxamide stone formation in male rats by feeding them oxamide mixed with powdered rat chow [88]. Morphological changes in the kidneys were examined by bright field and polarized light microscopy along with scanning electron microscopy. Oxamide appeared as dendritic crystals organized into spherulitic units. Within 12 hours of being on the diet of 1.2 g oxamide per 100 g of powdered rat chow, rats produced small oxamide deposits in the bladder and renal pelvis. The size, number and distribution of the deposits within the kidneys increased with time. In addition, spherulitic units of oxamide aggregated to form large irregularly shaped or mulberry shaped stones in the ducts of Bellini, ureters and renal pelvis (Figure 6). However no stones or crystals were detected within the renal cortex and proximal tubules during the experimental duration of 144 hours even though stones up to 4 mm in size were seen throughout the urinary tract. The collecting ducts as well as ducts of Bellini appeared dilated. Large stones jammed the ducts of Bellini and plugged their openings into the pelvis (Figure 6). Some of the plugs appeared attached to the tubular epithelium and when fractured to reveal the interior, showed eccentricity. Such observations indicate that deposition might have started by crystal attachment or nucleation on the epithelial surface and grew from there into the lumen. The renal pelvis was also distended but many stones therein appeared free and were easily dislodged. Some of the pelvic deposits also appeared attached to the papillary surface epithelium. Renal tubules that contained crystalline deposits appeared injured, releasing cellular debris into the lumen. Papillary surfaces appeared disfigured and at places were denuded of the surface epithelium.

Melamine Nephrolithiasis

After melamine adulteration of pet food and baby formula was found to be responsible for the death of dogs, cats and children [20,89,90], a number of mice and rat models of melamine nephrolithiasis were developed [91,92]. Melamine nephrolithiasis was induced by the administration of a combination of melamine and cyanuric acid. Crystal deposition and nephrotoxicity were dose and gender dependent [93]. Younger rats and males were more prone to both nephrotoxicity and nephrolithiasis than the females and older rats. Low doses did not produce crystal deposits. Crystal deposition was tubular and more common at

corticomedullary junction and renal papillae. All tubular segments appeared to be involved. Crystals were, however, also seen intracellularly as well as in the glomeruli. Crystal deposition led to dilatation of the tubules and epithelial degeneration through necrosis and apoptosis.

Concluding Remarks

Results of animal model studies demonstrate that increased urinary excretion of sparingly soluble materials such as drugs and participating ions such as calcium, and oxalate, and decreased urinary levels of mineralization inhibitors are essential for crystallization in the kidneys. Renal tubular deposition is dependent upon urinary concentration and supersaturation. Interstitial deposition appears to be less dependent upon supersaturation and more on availability of urinary crystallization modulating macromolecules. As presented in Figure 7, crystal fixation/retention within the kidneys is accomplished by tubular crystals; 1, adhering to renal tubular epithelium and/or their exposed basement membrane; 2, aggregating with other crystals creating a large crystalline mass blocking the tubular lumen; 3, moving into the renal interstitium through transcytosis or total loss of the tubular epithelium; and/or 4, forming in the renal interstitium/epithelial-endothelial basement membrane. What do these results from animal model studies tell us about the pathogenesis of kidney human stones? As discussed earlier, human stones develop attached to renal papillary surfaces through Randall's plaques, the interstitial deposits of poorly crystalline CaP, or to Randall's plugs, the crystal deposits plugging the openings of the ducts of Bellini. Animal models provide a clear indication that tubular plugs are formed in the presence of high supersaturation in the collecting ducts and crystal adherence to their renal epithelium as was suggested by Randall himself [6,94,95]. Animal models have however, provided only limited insight into the formation of sub-epithelial plaques on renal papillary surfaces. Studies of THP and OPN knock out mice suggest that these macromolecules may be involved in the production of at least the interstitial deposits of CaP. Transcytosis of intratubular CaP crystals from the apical to the basal aspect of the epithelium may also lead to the development of interstitial plaques [32,96]. Animal model studies have also shown the existence of a crystal clearing mechanism, with respect to calcium oxalate which provokes an inflammatory response in the kidneys. Why do the apatitic plaques not instigate similar response? Perhaps kidney stone formers have a defective crystal recognition and clearance system, or CaP of the plaques, being poorly crystalline biological apatite, is not recognized as a foreign entity.

Alternatively, renal tubular epithelial cells or vascular endothelial cells of vasa recta may undergo transformation into osteogenic cells and produce CaP deposits on their basal side [97]. Transformation of vascular smooth muscle cells into osteoblast/chondrocyte phenotype appears to be the first step in vascular calcification in the kidneys [98,99]. The co-existence of idiopathic stone formation and various cardiovascular diseases such as hypertension, myocardial infarction, carotid artery atherosclerosis, coronary heart disease,[9,100-102] leads us to suggest that vascular calcification and RP formation may involve similar molecules and pathways [101,103]. Interestingly, animal models of both CaOx [97] and CaP [104] nephrolithiasis have shown transformation of renal epithelial cells into osteoblast like

cells with increased expression of bone morphogenetic protein, runt-related transcription factor and Osterix.

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References

1. Finlayson B, Reid F. The expectation of free and fixed particles in urinary stone disease. *Invest Urol.* 1978; 15:442–448. [PubMed: 649291]
2. Robertson WG. A method for measuring calcium crystalluria. *Clin Chim Acta.* 1969; 26:105–110. [PubMed: 5356585]
3. Hallson PC, Rose GA. Crystalluria in normal subjects and in stone formers with and without thiazide and cellulose phosphate treatment. *Br J Urol.* 1976; 48:515–524. [PubMed: 13904]
4. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, et al. Kidney stones. *Nat Rev Dis Primers.* 2016; 2:16008. [PubMed: 27188687]
5. Khan SR, Canales BK. Unified theory on the pathogenesis of Randall's plaques and plugs. *Urolithiasis.* 2015; 43(Suppl 1):109–123. [PubMed: 25119506]
6. Randall A. Papillary pathology as a precursor of primary renal calculus. *Journal of Urology.* 1940; 44:580–589.
7. Coe FL, Evan AP, Lingeman JE, Worcester EM. Plaque and deposits in nine human stone diseases. *Urol Res.* 2010; 38:239–247. [PubMed: 20625890]
8. Khan SR, Finlayson B, Hackett R. Renal papillary changes in patient with calcium oxalate lithiasis. *Urology.* 1984; 23:194–199. [PubMed: 6695491]
9. Stoller ML, Meng MV, Abrahams HM, Kane JP. The primary stone event: a new hypothesis involving a vascular etiology. *J Urol.* 2004; 171:1920–1924. [PubMed: 15076312]
10. Taylor ER, Stoller ML. Vascular theory of the formation of Randall plaques. *Urolithiasis.* 2014
11. Haggit RC, Pitcock JA. Renal medullary calcification: a light and electron microscopic study. *The Journal of Urology.* 1971; 106:342–347. [PubMed: 4106437]
12. Weller RO, Nester B, Cooke SAR. Calcification in the human renal papilla: an electron microscope study. *Journal of Pathology.* 1971; 107:211–216.
13. Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SB, et al. Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. *J Clin Invest.* 2003; 111:607–616. [PubMed: 12618515]
14. Khan SR, Rodriguez DE, Gower LB, Monga M. Association of Randall plaque with collagen fibers and membrane vesicles. *J Urol.* 2012; 187:1094–1100. [PubMed: 22266007]
15. Coe FL, Evan AP, Worcester EM, Lingeman JE. Three pathways for human kidney stone formation. *Urol Res.* 2010; 38:147–160. [PubMed: 20411383]
16. Linnes MP, Krambeck AE, Cornell L, Williams JC Jr, Korinek M, et al. Phenotypic characterization of kidney stone formers by endoscopic and histological quantification of intrarenal calcification. *Kidney Int.* 2013; 84:818–825. [PubMed: 23698231]
17. Williams JC Jr, McAteer JA, Evan AP, Lingeman JE. Micro-computed tomography for analysis of urinary calculi. *Urol Res.* 2010; 38:477–484. [PubMed: 20967434]
18. Cifuentes Delatte L, Minon-Cifuentes J, Medina JA. New studies on papillary calculi. *J Urol.* 1987; 137:1024–1029. [PubMed: 3573168]
19. Matlaga BR, Shah OD, Assimos DG. Drug-induced urinary calculi. *Rev Urol.* 2003; 5:227–231. [PubMed: 16985842]

20. Gabriels G, Lambert M, Smith P, Wiesner L, Hiss D. Melamine contamination in nutritional supplements--Is it an alarm bell for the general consumer, athletes, and 'Weekend Warriors'? *Nutr J.* 2015; 14:69. [PubMed: 26182916]
21. Vermeulen CW, Lyon ES. Mechanisms of genesis and growth of calculi. *Am J Med.* 1968; 45:684–692. [PubMed: 4879831]
22. Kok DJ, Khan SR. Calcium oxalate nephrolithiasis, a free or fixed particle disease. *Kidney Int.* 1994; 46:847–854. [PubMed: 7996806]
23. Finlayson B. The concept of a continuous crystallizer. Its theory and application to in vivo and in vitro urinary tract models. *Invest Urol.* 1972; 9:258–263. [PubMed: 5058765]
24. Robertson WG, Peacock M, Nordin BE. Calcium oxalate crystalluria and urine saturation in recurrent renal stone-formers. *Clin Sci.* 1971; 40:365–374. [PubMed: 5556093]
25. Schulz E, Hengst E, Brundig P, Haerting R, Pirlich W, et al. Disturbed urinary transport in the pelvi-calyceal system in calcium-oxalate stone patients. *Urol Res.* 1987; 15:109–113. [PubMed: 3590428]
26. Robertson WG. Kidney models of calcium oxalate stone formation. *Nephron Physiol.* 2004; 98:p21–30. [PubMed: 15499211]
27. Khan SR. Nephrocalcinosis in animal models with and without stones. *Urol Res.* 2010; 38:429–438. [PubMed: 20658131]
28. Khan SR, Finlayson B, Hackett RL. Histologic study of the early events in oxalate induced intranephronic calculosis. *Invest Urol.* 1979; 17:199–202. [PubMed: 500316]
29. Khan SR, Finlayson B, Hackett RL. Scanning electron microscopy of calcium oxalate crystal formation in experimental nephrolithiasis. *Lab Invest.* 1979; 41:504–510. [PubMed: 513643]
30. Khan SR, Finlayson B, Hackett RL. Experimental calcium oxalate nephrolithiasis in the rat. Role of the renal papilla. *Am J Pathol.* 1982; 107:59–69. [PubMed: 7065125]
31. Khan SR, Johnson JM, Peck AB, Cornelius JG, Glenton PA. Expression of osteopontin in rat kidneys: induction during ethylene glycol induced calcium oxalate nephrolithiasis. *J Urol.* 2002; 168:1173–1181. [PubMed: 12187263]
32. Khan SR. Calcium oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development. *Urol Res.* 1995; 23:71–79. [PubMed: 7676537]
33. Khan SR. Experimental calcium oxalate nephrolithiasis and the formation of human urinary stones. *Scanning Microsc.* 1995; 9:89–100. discussion 100–101. [PubMed: 8553028]
34. Khan SR, Glenton PA, Byer KJ. Modeling of hyperoxaluric calcium oxalate nephrolithiasis: experimental induction of hyperoxaluria by hydroxy-L-proline. *Kidney Int.* 2006; 70:914–923. [PubMed: 16850024]
35. Khan SR, Hackett RL. Retention of calcium oxalate crystals in renal tubules. *Scanning Microsc.* 1991; 5:707–711. discussion 711–702. [PubMed: 1808708]
36. Graves FT. An experimental study of the anatomy of the tubules of the human kidney and its relation to calculus formation. *Br J Urol.* 1982; 54:569–574. [PubMed: 7150907]
37. Verlander JW. Normal ultrastructure of the kidney and lower urinary tract. *Toxicol Pathol.* 1998; 26:1–17. [PubMed: 9502381]
38. Vervaeke BA, Verhulst A, Dauwe SE, De Broe ME, D'Haese PC. An active renal crystal clearance mechanism in rat and man. *Kidney Int.* 2009; 75:41–51. [PubMed: 18784645]
39. Khan SR, Shevock PN, Hackett RL. Acute hyperoxaluria, renal injury and calcium oxalate urolithiasis. *J Urol.* 1992; 147:226–230. [PubMed: 1729537]
40. Grover PK, Dogra SC, Davidson BP, Stapleton AM, Ryall RL. The prothrombin gene is expressed in the rat kidney: Implications for urolithiasis research. *Eur J Biochem.* 2000; 267:61–67. [PubMed: 10601851]
41. Okada A, Yasui T, Hamamoto S, Hirose M, Kubota Y, et al. Genome-wide analysis of genes related to kidney stone formation and elimination in the calcium oxalate nephrolithiasis model mouse: detection of stone-preventive factors and involvement of macrophage activity. *J Bone Miner Res.* 2009; 24:908–924. [PubMed: 19113933]
42. Lu X, Gao B, Yasui T, Li Y, Liu T, et al. Matrix Gla protein is involved in crystal formation in kidney of hyperoxaluric rats. *Kidney Blood Press Res.* 2013; 37:15–23. [PubMed: 23445897]

43. Umekawa T, Hatanaka Y, Kurita T, Khan SR. Effect of angiotensin II receptor blockage on osteopontin expression and calcium oxalate crystal deposition in rat kidneys. *J Am Soc Nephrol.* 2004; 15:635–644. [PubMed: 14978165]
44. Tsuji H, Shimizu N, Nozawa M, Umekawa T, Yoshimura K, et al. Osteopontin knockdown in the kidneys of hyperoxaluric rats leads to reduction in renal calcium oxalate crystal deposition. *Urolithiasis.* 2014; 42:195–202. [PubMed: 24619192]
45. Moriyama MT, Glenton PA, Khan SR. Expression of inter-alpha inhibitor related proteins in kidneys and urine of hyperoxaluric rats. *J Urol.* 2001; 165:1687–1692. [PubMed: 11342956]
46. Khan SR, Glenton PA. Experimentally induced hyperoxaluria in MCP-1 null mice. *Urol Res.* 2011; 39:253–258. [PubMed: 21161647]
47. Khan A, Wang W, Khan SR. Calcium oxalate nephrolithiasis and expression of matrix GLA protein in the kidneys. *World J Urol.* 2014; 32:123–130. [PubMed: 23475213]
48. Iida S, Peck AB, Johnson-Tardieu J, Moriyama M, Glenton PA, et al. Temporal changes in mRNA expression for bikunin in the kidneys of rats during calcium oxalate nephrolithiasis. *J Am Soc Nephrol.* 1999; 10:986–996. [PubMed: 10232684]
49. Gokhale JA, McKee MD, Khan SR. Immunocytochemical localization of Tamm-Horsfall protein in the kidneys of normal and nephrolithic rats. *Urol Res.* 1996; 24:201–209. [PubMed: 8873378]
50. Gokhale JA, Glenton PA, Khan SR. Characterization of Tamm-Horsfall protein in a rat nephrolithiasis model. *J Urol.* 2001; 166:1492–1497. [PubMed: 11547119]
51. Khan SR, Joshi S, Wang W, Peck AB. Regulation of macromolecular modulators of urinary stone formation by reactive oxygen species: transcriptional study in an animal model of hyperoxaluria. *Am J Physiol Renal Physiol.* 2014; 306:F1285–1295. [PubMed: 24598804]
52. Riese RJ, Mandel NS, Wiessner JH, Mandel GS, Becker CG, et al. Cell polarity and calcium oxalate crystal adherence to cultured collecting duct cells. *Am J Physiol.* 1992; 262:F177–184. [PubMed: 1539682]
53. Verkoelen CF, Verhulst A. Proposed mechanisms in renal tubular crystal retention. *Kidney Int.* 2007; 72:13–18. [PubMed: 17429341]
54. Verkoelen CF, van der Boom BG, Houtsmuller AB, Schröder FH, Romijn JC. Increased calcium oxalate monohydrate crystal binding to injured renal tubular epithelial cells in culture. *The American journal of physiology.* 1998; 274:F958–965. [PubMed: 9612335]
55. Yasui T, Fujita K, Tozawa K, Asai K, Soji T, et al. Calcium oxalate crystal attachment to cultured rat kidney epithelial cell, NRK-52E. *Urol Int.* 2001; 67:73–76. [PubMed: 11464121]
56. Yasui T, Fujita K, Asai K, Kohri K. Osteopontin regulates adhesion of calcium oxalate crystals to renal epithelial cells. *Int J Urol.* 2002; 9:100–108. [PubMed: 12028300]
57. Bigelow MW, Wiessner JH, Kleinman JG, Mandel NS. Surface exposure of phosphatidylserine increases calcium oxalate crystal attachment to IMCD cells. *Am J Physiol.* 1997; 272:F55–62. [PubMed: 9039049]
58. Lieske JC, Leonard R, Swift H, Toback FG. Adhesion of calcium oxalate monohydrate crystals to anionic sites on the surface of renal epithelial cells. *The American journal of physiology.* 1996; 270:F192–199. [PubMed: 8769839]
59. Cao LC, Jonassen J, Honeyman TW, Scheid C. Oxalate-induced redistribution of phosphatidylserine in renal epithelial cells: implications for kidney stone disease. *Am J Nephrol.* 2001; 21:69–77. [PubMed: 11275638]
60. Wiessner JH, Hasegawa AT, Hung LY, Mandel GS, Mandel NS. Mechanisms of calcium oxalate crystal attachment to injured renal collecting duct cells. *Kidney Int.* 2001; 59:637–644. [PubMed: 11168945]
61. Verkoelen CF, Van Der Boom BG, Romijn JC. Identification of hyaluronan as a crystal-binding molecule at the surface of migrating and proliferating MDCK cells. *Kidney Int.* 2000; 58:1045–1054. [PubMed: 10972669]
62. Ebisuno S, Nishihata M, Inagaki T, Umehara M, Kohjimoto Y. Bikunin prevents adhesion of calcium oxalate crystal to renal tubular cells in human urine. *J Am Soc Nephrol.* 1999; 10(Suppl 14):436–440.

63. Verkoelen CF, van der Boom BG, Kok DJ, Houtsmuller AB, Visser P, et al. Cell type-specific acquired protection from crystal adherence by renal tubule cells in culture. *Kidney Int.* 1999; 55:1426–1433. [PubMed: 10201007]
64. Verkoelen CF, Romijn JC, Cao LC, Boeve ER, De Bruijn WC, et al. Crystal-cell interaction inhibition by polysaccharides. *J Urol.* 1996; 155:749–752. [PubMed: 8558718]
65. Verkoelen CF, van der Boom BG, Kok DJ, Romijn JC. Sialic acid and crystal binding. *Kidney Int.* 2000; 57:1072–1082. [PubMed: 10720959]
66. Yamate T, Kohri K, Umekawa T, Iguchi M, Kurita T. Osteopontin antisense oligonucleotide inhibits adhesion of calcium oxalate crystals in Madin-Darby canine kidney cell. *J Urol.* 1998; 160:1506–1512. [PubMed: 9751404]
67. Wang T, Thurgood LA, Grover PK, Ryall RL. A comparison of the binding of urinary calcium oxalate monohydrate and dihydrate crystals to human kidney cells in urine. *BJU Int.* 2010; 106:1768–1774. [PubMed: 20230382]
68. Thurgood LA, Sorensen ES, Ryall RL. The effect of intracrystalline and surface-bound osteopontin on the attachment of calcium oxalate dihydrate crystals to Madin-Darby canine kidney (MDCK) cells in ultrafiltered human urine. *BJU Int.* 2012; 109:1100–1109. [PubMed: 21883862]
69. Grover PK, Thurgood LA, Wang T, Ryall RL. The effects of intracrystalline and surface-bound proteins on the attachment of calcium oxalate monohydrate crystals to renal cells in undiluted human urine. *BJU Int.* 2010; 105:708–715. [PubMed: 19694711]
70. Kumar V, Pena de la Vega L, Farell G, Lieske JC. Urinary macromolecular inhibition of crystal adhesion to renal epithelial cells is impaired in male stone formers. *Kidney Int.* 2005; 68:1784–1792. [PubMed: 16164655]
71. Grover PK, Thurgood LA, Ryall RL. Effect of urine fractionation on attachment of calcium oxalate crystals to renal epithelial cells: implications for studying renal calculogenesis. *Am J Physiol Renal Physiol.* 2007; 292:F1396–1403. [PubMed: 17267387]
72. Kohri K, Yasui T, Okada A, Hirose M, Hamamoto S, et al. Biomolecular mechanism of urinary stone formation involving osteopontin. *Urol Res.* 2012; 40:623–637. [PubMed: 23124115]
73. Kleinman JG, Alatalo LJ, Beshensky AM, Wesson JA. Acidic polyanion poly(acrylic acid) prevents calcium oxalate crystal deposition. *Kidney Int.* 2008; 74:919–924. [PubMed: 18563053]
74. An Z, Lee S, Oppenheimer H, Wesson JA, Ward MD. Attachment of calcium oxalate monohydrate crystals on patterned surfaces of proteins and lipid bilayers. *J Am Chem Soc.* 2010; 132:13188–13190. [PubMed: 20812679]
75. Sorokina EA, Wesson JA, Kleinman JG. An acidic peptide sequence of nucleolin-related protein can mediate the attachment of calcium oxalate to renal tubule cells. *J Am Soc Nephrol.* 2004; 15:2057–2065. [PubMed: 15284292]
76. Rabinovich YI, Esayanur M, Daosukho S, Byer KJ, El-Shall HE, et al. Adhesion force between calcium oxalate monohydrate crystal and kidney epithelial cells and possible relevance for kidney stone formation. *J Colloid Interface Sci.* 2006; 300:131–140. [PubMed: 16677664]
77. Rabinovich YI, Daosukho S, Byer KJ, El-Shall HE, Khan SR. Direct AFM measurements of adhesion forces between calcium oxalate monohydrate and kidney epithelial cells in the presence of Ca²⁺ and Mg²⁺ ions. *J Colloid Interface Sci.* 2008; 325:594–601. [PubMed: 18619606]
78. Okamoto N, Aruga S, Tomita K, Takeuchi T, Kitamura T. Chronic acid ingestion promotes renal stone formation in rats treated with vitamin D3. *Int J Urol.* 2007; 14:60–66. [PubMed: 17199862]
79. Khan SR, Glenton PA. Deposition of calcium phosphate and calcium oxalate crystals in the kidneys. *J Urol.* 1995; 153:811–817. [PubMed: 7861545]
80. Bushinsky DA, Asplin JR, Grynpas MD, Evan AP, Parker WR, et al. Calcium oxalate stone formation in genetic hypercalciuric stone-forming rats. *Kidney Int.* 2002; 61:975–987. [PubMed: 11849452]
81. Beck L, Karaplis AC, Amizuka N, Hewson AS, Ozawa H, et al. Targeted inactivation of Npt2 in mice leads to severe renal phosphate wasting, hypercalciuria, and skeletal abnormalities. *Proc Natl Acad Sci U S A.* 1998; 95:5372–5377. [PubMed: 9560283]
82. Khan SR, Glenton PA. Calcium oxalate crystal deposition in kidneys of hypercalciuric mice with disrupted type IIa sodium-phosphate cotransporter. *Am J Physiol Renal Physiol.* 2008; 294:F1109–1115. [PubMed: 18337544]

83. Khan SR, Canales BK. Ultrastructural investigation of crystal deposits in Npt2a knockout mice: are they similar to human Randall's plaques? *J Urol.* 2011; 186:1107–1113. [PubMed: 21784483]
84. Weinman EJ, Mohanlal V, Stoycheff N, Wang F, Steplock D, et al. Longitudinal study of urinary excretion of phosphate, calcium, and uric acid in mutant NHERF-1 null mice. *Am J Physiol Renal Physiol.* 2006; 290:F838–843. [PubMed: 16249272]
85. Liu Y, Mo L, Goldfarb DS, Evan AP, Liang F, et al. Progressive renal papillary calcification and ureteral stone formation in mice deficient for Tamm-Horsfall protein. *Am J Physiol Renal Physiol.* 2010; 299:F469–478. [PubMed: 20591941]
86. Borden TA, Vermeulen CW. The renal papilla in calculogenesis of oxamide stones. *Invest Urol.* 1966; 4:125–132. [PubMed: 5951299]
87. Gill WB, Vermeulen CW. Oxamide Crystalluria and Urolithiasis, Rat and in Vitro Observations. *Invest Urol.* 1964; 1:339–349. [PubMed: 14113091]
88. Khan SR, Hackett RL, Finlayson B, Konicek JR. Light and scanning electron microscopic studies of oxamide urolithiasis in rats. *Scan Electron Microsc.* 1981:155–162. [PubMed: 7330576]
89. Cianciolo RE, Bischoff K, Ebel JG, Van Winkle TJ, Goldstein RE, et al. Clinicopathologic, histologic, and toxicologic findings in 70 cats inadvertently exposed to pet food contaminated with melamine and cyanuric acid. *J Am Vet Med Assoc.* 2008; 233:729–737. [PubMed: 18764706]
90. Bischoff K, Rumbleha WK. Pet food recalls and pet food contaminants in small animals. *Vet Clin North Am Small Anim Pract.* 2012; 42:237–250. v. [PubMed: 22381176]
91. Kobayashi T, Okada A, Fujii Y, Niimi K, Hamamoto S, et al. The mechanism of renal stone formation and renal failure induced by administration of melamine and cyanuric acid. *Urol Res.* 2010; 38:117–125. [PubMed: 20179916]
92. Lu X, Gao B, Wang Y, Liu Z, Yasui T, et al. Renal tubular epithelial cell injury, apoptosis and inflammation are involved in melamine-related kidney stone formation. *Urol Res.* 2012; 40:717–723. [PubMed: 23053219]
93. Lu X, Wang J, Cao X, Li M, Xiao C, et al. Gender and urinary pH affect melamine-associated kidney stone formation risk. *Urol Ann.* 2011; 3:71–74. [PubMed: 21747595]
94. Randall A. The etiology of primary renal calculus. *International Abstract of Surgery.* 1940; 71:209–240.
95. Randall A. The Origin and Growth of Renal Calculi. *Ann Surg.* 1937; 105:1009–1027. [PubMed: 17856988]
96. Kumar V, Farell G, Yu S, Harrington S, Fitzpatrick L, et al. Cell biology of pathologic renal calcification: contribution of crystal transcytosis, cell-mediated calcification, and nanoparticles. *J Invest Med.* 2006; 54:412–424.
97. Joshi S, Clapp WL, Wang W, Khan SR. Osteogenic changes in kidneys of hyperoxaluric rats. *Biochim Biophys Acta.* 2015; 1852:2000–2012. [PubMed: 26122267]
98. Shanahan CM. Vascular calcification. *Curr Opin Nephrol Hypertens.* 2005; 14:361–367. [PubMed: 15931005]
99. Moe SM, Chen NX. Mechanisms of vascular calcification in chronic kidney disease. *J Am Soc Nephrol.* 2008; 19:213–216. [PubMed: 18094365]
100. Rule AD, Roger VL, Melton LJ 3rd, Bergstralh EJ, Li X, et al. Kidney stones associate with increased risk for myocardial infarction. *J Am Soc Nephrol.* 2010; 21:1641–1644. [PubMed: 20616170]
101. Khan SR. Is oxidative stress, a link between nephrolithiasis and obesity, hypertension, diabetes, chronic kidney disease, metabolic syndrome? *Urol Res.* 2012; 40:95–112. [PubMed: 22213019]
102. Ando R, Nagaya T, Suzuki S, Takahashi H, Kawai M, et al. Kidney stone formation is positively associated with conventional risk factors for coronary heart disease in Japanese men. *J Urol.* 2013; 189:1340–1346. [PubMed: 23159273]
103. Bagga HS, Chi T, Miller J, Stoller ML. New insights into the pathogenesis of renal calculi. *Urol Clin North Am.* 2013; 40:1–12. [PubMed: 23177630]
104. Jia Z, Wang S, Tang J, He D, Cui L, et al. Does crystal deposition in genetic hypercalciuric rat kidney tissue share similarities with bone formation? *Urology.* 2014; 83:509. e507-514.

Figure 1.

Experimentally induced hyperoxaluria and CaOx nephrolithiasis in rats. Hyperoxaluria was induced by administration of ethylene glycol [31]. Kidney were sectioned and were examined using a scanning electron microscope. **A.** Renal cortex showing CaOx monohydrate crystals (*) protruding from the renal parenchyma. A number of renal tubules (T) are clearly visible. **B.** A large deposit of the CaOx monohydrate crystals (*) has completely blocked a renal tubule and impaired the interstitium. Some tubules (T) appear unobstructed. **C.** A large CaOx stone is protruding out of the kidney (K) at the renal papillary base. **D.** Higher magnification of the stone in the C. Stone consists of large CaOx dihydrate (*) crystals as well as comparatively smaller CaOx monohydrate crystals (Ox).

Figure 2. Renal papilla of a rat with experimentally induced hyperoxaluria and CaOx nephrolithiasis. Hyperoxaluria was induced by administration of ethylene glycol [31]. **A.** Papillary tip (P) showing nodular CaOx stone S. **B.** Higher magnification showing a nodular stone (S) protruding out of the renal papilla. **C.** Close up of the stone surface. Papillary surface Epithelium (E) has stretched and cracked open exposing the subepithelial CaOx crystals (Ox). **D.** Higher magnification showing monoclinic CaOx monohydrate crystals (Ox) mixed with cellular degradation products and fibrillar organic matrix.

Figure 3.

Light and transmission electron microscopic analysis of hyperoxaluric kidneys with CaOx crystal deposits. Acute hyperoxaluria was induced in rats by intraperitoneal injection of sodium oxalate [30]. **A.** Longitudinal section through the papillary tip. Papillary surface epithelium (E) is torn off during processing exposing a layer of subepithelial CaOx monohydrate crystals (arrows). Crystals are arranged like pearls of a necklace as a result of their attachment to the tubular basement membrane. Urinary space is marked by a star. **B.** Transmission electron microscopic image showing CaOx crystals in the tubular lumen. Crystals are lost during processing leaving behind crystal ghosts (CG). The crystal ghosts are surrounded by epithelial cells marked by white arrows. Dark arrows point to the intact basement membrane of the the tubular epithelium. Bar=12.5 μ m **C.** Crystal ghost (CG) is surrounded by the epithelial cells as shown by white arrows and is attached to basement membrane depicted by dark arrows. Star indicates tubular lumen. Bar=10 μ m

Figure 4.

Transmission electron microscopy of renal tubule of a Npt2a knock out mice (modified from J Urol 186:1107-13, 2011) [82,83]. **A.** Renal tubular lumen is completely occluded by calcium phosphate deposit (black star). Tubular epithelial cells appear normal with intact luminal membrane (arrows). Bar=10 μ m. **B.** Entire tubule is replaced by the calcium phosphate deposit which appears as a minestone with characteristic concentric laminations and scalloped surface. Black arrows mark the outer limit of the stones. White arrows point to the other normal appearing tubule with intact epithelia. Bar=5 μ m

Figure 5.

Light and transmission electron microscopy of renal tubule of THP knock out mice (modified from Am J Physiol Renal Physiol 299: 469-478, 2010) [85]. **A.** Kidney section stained with von Kossa that detects calcium phosphate shows interstitial deposits (arrows). **B.** Transmission electron microscopic image showing interstitial zone (arrows) between two tubules. Bar=5µm **C.D.** Higher magnification images showing membrane bound vesicles and concentrically laminated spherical entities (black arrows) reminiscent of deposits in the Randall's plaques [4,8,14]. C, Bar=2µm; D, Bar=1µm

Figure 6.

Scanning electron microscopic analysis of rat kidneys with oxamide nephrolithiasis modified from Scan Microsc 3:155-162, 1981 [88]. **A.** Fractured surface of a papillary tip showing collecting ducts (CD) with and without oxamide deposits (O). The collecting duct with the oxamide deposits is blocked and enlarged. **B.** Tip of the renal papilla showing oxamide deposits (O) completely blocking the opening of duct of Bellini. Opening is enlarged and lined (arrows) by the papillary epithelium (PE). Crystals are retained inside the tubule because the deposit has grown large enough to block the tubular opening into the pelvis. **C.** Tip of the papilla showing 2 empty collecting ducts (CD) and one filled with a protruding oxamide (O) deposit. **D.** Surface of a fractured oxamide (O) deposit present in the opening of the duct of Bellini (CD)). Fractured surface shows eccentricity, concentric laminations (arrows), and site of attachment (bold white arrow) suggestive of a unidirectional growth.

Figure 7.

Diagrammatic representation of fixed particle mechanism based upon results of animal model studies, particularly of CaOx nephrolithiasis. **A** and **B** represent epithelial lining of the terminal collecting ducts, **A** side representing exposure to milder hyperoxaluria and fewer CaOx crystals; **B** side representing excessive hyperoxaluria and abundant crystals. **C** represents papillary surface epithelium/urothelium while **D** represents the vascular endothelium. The first step is the increased concentration of calcium ($\text{Ca}^{\uparrow}$) and oxalate ($\text{Ox}^{\uparrow}$) ions in the urine and its low crystallization inhibitory potential represented here by low citrate ($\text{Cit}^{\downarrow}$). This leads to the formation of CaOx crystals (X). Fewer, smaller, crystals move through the tubules and excreted out with the urine as crystalluria particles. Crystals coming in contact with the tubular epithelial cells are either endocytosed on the apical side (1) and exocytosed into the renal interstitium or cause damage to the renal epithelial cells leading to their death and ejection (2) exposing the tubular epithelial basement membrane (3). Many crystals either attach to the damaged epithelium or the exposed basement membrane (4). Some cross into the interstitium. Interstitial crystals become surrounded by the macrophages (5) and may eventually be disintegrated. Other intratubular crystals aggregate with each other and cellular degradation products (6) eventually growing large enough to obstruct the duct of Bellini (7) and produce Randall's plug (DP), which is now continuously bathed in the pelvic urine on the one side and tubular urine on the other. Interstitial crystals may lead to localized inflammation and deposition of collagen fibers (L). Interstitial crystals may also induce osteogenic changes in the tubular epithelial and/or vascular endothelial cells (D) producing matrix vesicles (M) and biological calcium phosphate (P). These initial deposits promote further mineralization of the collagen fibers which eventually reaches the papillary surface epithelium producing sub-epithelial deposits

of biological apatite, the Randall's plaque (RP). Loss of the papillary surface epithelium exposes the Randall's plaque to the pelvic urine. Exposure of the Randall's plaques and plugs to the pelvic urine metastable with respect to the CaOx promotes its deposition (Yellow Stars) on the plaques (RP) and plugs (DP) and formation of CaOx kidney stones attached to papillary surfaces.