Mineralization in Health and Mechanism of Kidney Stone Formation

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ABSTRACT : *Mineralization is the process by which minerals are deposited within or outside of the cells. Healthy mineralization occurs in bones, otoconia of ear and teeth, while pathological mineralization can take place in soft tissues. The mechanisms which govern mineralization in health and disease are likely similar. Presence of organic matter is one of the major common factors in both physiological and pathological mineralization processes. The control and regulation of healthy mineralization processes can develop an insight into regulation of pathological mineralization, specifically nephrolithiasis. Crystallisation process in kidney stone formation involves nucleation and growth, epitaxy and aggregation. Identification and study of promoters and inhibitors of nucleation and subsequent growth of crystals, the identification of constituents of crystal matrix and their roles in the pathology of urolithiasis is the prime focus of urolithiasis research. Calcium and phosphate homeostasis ensures that mineralization takes place at the right places and abnormal mineralization is prevented. The purpose of this review is, thus, to understand healthy mineralization in bones, teeth and ears and to use this knowledge for better understanding of mechanism of kidney stone formation.*

KEYWORDS: *Mineralization, Nephrolithiasis, Crystallization, Nucleation ,Epitaxy, Agglomeration*

I. INTRODUCTION

The incidence and prevalence of nephrolithiasis is increasing globally. These increases are seen across sex, race and age. Changes in dietary practices and global warming may influence these trends [1]. Increase in incidence of nephrolithiasis has caused increased morbidity and economic burden across the world. Super saturation of urine with calcium and oxalate is the major cause of pathological mineralization in kidneys leading to nephrolithiasis. Despite major advances in the field of research and technology, the pathogenesis of nephrolithiasis remains poorly understood. Physiological mineralization takes place in bone, cartilage and teeth while ectopic mineralization takes place in soft tissue in response to injury. Physiological mineralization is a highly regulated process and ectopic mineralization was earlier thought to be a passive process, but recent evidences demonstrate that ectopic mineralization is also a regulated process involving imbalance between forces that promote and inhibit precipitation, that occurs in stone formation [2].Recent findings suggest that mechanisms and factors regulating physiological and pathological mineralization may be identical, therefore understanding of the mineralization mechanisms taking place in healthy conditions can give better insights into mineralization during nephrolithiasis. The prevention or cure of nephrolithiasis is possible only by identifying mechanisms operating before stone formation.

II. BONE MINERALIZATION

Human skeleton consists of 206 bones and bone consists of three principal elements- matrix, minerals and cells. The bone cells i.e. osteoblasts (OBs) and osteoclasts (OCs) continuously remodel the bone. OCs dissolve the bone and OBs replace it in a regulated manner. The organic matrix of bone is laid down by osteoblasts, and consists of type I collagen fibers embedded in amorphous ground substrate consisting of sulfated GAGs and various bone proteins. The inorganic matrix is formed by carbonate hydroxyapatite. OBs and OCs develop from osteoprogenitor cells. Some of the osteoblasts terminally differentiate into osteocytes. These osteocytes play central role in cell communication and regulation of bone remodeling.At the site of damage, proapoptotic factors are expressed by dying osteocytes and dying osteocytes also upregulate the expression of Macrophage colony stimulating factor (M-CSF) and RANKL (Receptor activator of NF-κB ligand) [3,4].The bone matrix to be resorbed is thought to be recognized by Arg-Gly-Asp sequence of bone adhesion proteins (osteopontin, bone sialoprotein II and collagen type I) [5] .Adhesion to substrate is mediated by activated integrins by recruitment of adhesion proteins and regulatory proteins [6,7].

The resorbing lacuna is a highly acidic environment and hydroxyapatite is dissolved in this acidic environment making organic matrix available for proteolytic degradation by MMP-9 and cathepsin K and finally the osteoclasts are also destroyed by apoptotic pathways [8,9]. Macrophage like mononuclear cells could also be involved in further collagen degradation and removal of debris created by osteoclasts [10] .Bone formation is performed by the osteoblasts which differentiate at the site of formation from precursor cells derived from the mesenchymal lineage [11]. The master gene Runx-2 turns the mesenchymal stem cells to osteoblastic lineage [12]. Bone formation restores the resorbed matrix by means of deposition of lamellar bone that entraps the more mature osteoblasts in lacunar spaces, where they become osteocytes [13]. Sclerostin is thought to terminate the osteoblastic induction and hence the bone formation by preventing the activation of wntsignal[14,15]. Ectopic mineralization is actively inhibited by various biomolecules including MGP, SPP1, α2- HS glycoprotein, osteoprotegerin, a TNF receptor like molecule ANK and fetuin, a serum glycoprotein that accumulates in bone [16-19].

III.   OTOCONIA MINERALIZATION

Otoconia are biominerals present in inner ear required for normal balance and sensation of gravity. The otolith consists of predominant mineral phase of aragonite form of calcium carbonate enmeshed into an organic matrix consisting of complex network of macromolecules with calcium binding functions [20-22]. Otolith growth consists of daily deposition of double deposition of a layer rich in minerals and a layer rich in organic material [23].The otolith calcification is strongly dependent on the chemical composition of endolymphaticfluid asotolith epithelium is not in direct contact with the region of calcification[24,25].The endolymphatic fluid has an alkaline pH and contains all ionic precursors, proteins, collagen and amino acids for otolith formation[22,25,26]. Otoconin-90 comprises 90% of total protein of otoconia and is expressed by non-sensory epithelium of inner ear and other otoconial proteins are otolin ,osteopontin, fetuin-A etc [27-30]. Otoconin-90 is similar in function to that of osteopontin in bone as it influences organic matrix over mineral content and limits crystal sizes. Otoconin-90 reacts with otolin and this complex sequesters calcium ions in ECM for efficient calcification[27]. Otolith matrix protein-1 is required for normal otolith growth and deposition of otolin,andotolin is involved in correct arrangement of the otoliths onto the sensory epithelium of the inner ear and probably in stabilization of the otolith matrix [28].

IV.   DENTAL MINERALIZATION

Hard tissues in tooth are dentin, enamel and cementum produced by odontoblasts, ameloblasts and cementoblasts,respectively.Mesenchymal stem cells form odontoblasts and cementoblasts while epithelial cells give rise to ameloblasts.Ameloblasts,during the secretory stage, differentiate into tall secretory cells. Most of the enamel proteins are secreted into developing enamel matrix and processes of mineralization and protein processing begin. The mineralization continues during the maturation stage and proteases (MMP-20 and KLK-4) remove most of the remaining organic material to free up the space for enamel crystallites to grow[31-35].Amelogenin is the major constituent of enamel matrix that controls mineralization of enamel crystals [36].Amelogenin stabilizes amorphous calcium phosphate while inhibiting precipitation of other calcium phosphates [37].Ameloblastin (AMBN) is the second most abundant **e**xtracellular matrix protein produced by ameloblasts andis found mainly in forming enamel and is associated with regulation of mineral deposition. Another minor protein Enamelin interacts with amelogenin and is critical for mineralization of normal enamel [36,38]. Young Odontoblasts secrete uncalcified matrix called predentin, which is primarily collagenous in structure. The odontoblasts continue to secrete matrix which gets calcified to form dentin. Odontoblasts have processes penetrating predentin and dentin. The non-collagenous components of dentin include dentin specific proteins which are dentin phosphoprotein, dentin matrix protein-1(AG-1) and dentin sialoprotein. DPP may beinvolved in initiation of the first mineral crystals of hydroxyapatite in dentin. Because of its affinity for calcium,DPP may concentrate these ions and participate in formation of beginning apatite crystals [39]. AG1 is a highly negatively charged Molecule and could also be involved in the initiation of HAP formationand/or in regulating crystal growth processes in dentin [40]. DSPmight serve a similar role as a regulator of mineralizationduring dentinogenesis.Osteocalcin and bone sialoproteins alongwith chondroitin sulfate containing proteoglycans are also associated with dentin matrix [41].

Cementoblasts secrete cementum matrix composed of type I collagen and non-collagenous proteins such as osteopontin, bone sialopotein and osteocalcin. Cellular cementum consists primarily of mineral(50%) and type-I collagen with a smaller amount of type-V collagen, similar to bone and dentin whereas part of acellularcementum contains fully mineralized Periodontal ligaments that comprises type-I, type-III, and type-XII collagens [42-46].Within collagenouscementum matrix, inorganic calcium phosphate is deposited as mineral

crystals. Runx 2 and osterix are the important transcription factors and important markers of differentiation ofcementoblasts [45].

V.   MINERALIZATION IN NEPHROLITHIASIS

Human body contains a pair of bean shaped kidneys at the rear of abdominal cavity in retroperitoneum. Each kidney comprises an outer cortex and an inner medulla. Kidneys play important functions of excretion of metabolic wastes, maintainence of homeostasis and production of calcitriol, erythropoietin and renin. Nephron is the functional unit of kidneys, which produces urine. Kidneys drain urine via ureter into the urinary bladder. Urine is largely composed of water with urea, chloride, sodium, potassium, creatinine and other ions and compounds. Crystals may be present in the urine of healthy as well as unhealthy subjects. Urolithiasis is the condition in which supersaturated urine tends to crystallize and obstructs urine flow and thus affects kidney function. Kidney stones form when the crystals in urine stick together and grow in size. In most cases these crystals are removed by the flow of urine, but sometimes these crystals stick to the lining of kidneys or urinary tract.The highest risk of CaOx nephrolithiasis was encountered at the end of collecting ducts where crystals formed in nephrons with long loop ofHenle meet and agglomerate [46].Kidney stones differ in shapes, sizes and chemical composition of its constituents. Kidney stones affect up to 5% of the population, with a lifetimerisk of passing a kidney stone of about 8-10% [47].Increased incidenceof kidney stones in the industrialized world is associated withimproved standards of living and is strongly associated withrace or ethnicity and region of residence [48].

A seasonal variation is also seen, with high urinary calcium oxalate saturation inmen during summer and in women during early winter [49].Stonesform twice as often in men as women. The peak age in men is30 years; women have a bimodal age distribution, with peaksat 35 and 55 years. Once a kidney stone forms, the probabilitythat a second stone will form within five to seven years isapproximately 50% [47].Stones in the urinary system result due to increase in urine supersaturation. Urine supersaturation results in subsequent formation of crystalline matter. Crystallisation process can be divided into nucleation, growth, epitaxy and agglomeration. Spontaneous nucleation and agglomeration occur very rapidly whereas the other processes are time consuming.

Nucleation: Nucleation and crystal growth are governed by the differences in energies that are supplied by urinary supersaturation and consumed to build up crystal surface against surface tension. The energy required for spontaneous nucleation is extremely high. It corresponds to at least an 80-fold supersaturation which has never been found in urine or kidney tissue [50].Crystal nucleation is the first step in the formation of stone which can either be homogeneous (precipitation in absence of a foreign body) or heterogeneous (precipitation in presence of a foreign body). Homogeneous nucleation of a salt occurs in unstable zone of supersaturation. Crystalluria and stone formation seem to be the result of hetrogeneous nucleation induced by promoters. Promoters probably present preformed surfaces that reduce the surface energy required for crystallisation.During crystal growth, the free energy of solution continues to decrease as new crystal components are taken from the solution and become part of the crystal structure. Once formed, the crystalline particles can bind to each other in either an oriented or random growth pattern and then grow into a larger particle. Crystallisation processes are modulated by chelators, inhibitors ad promoters, which have a high affinity to stone forming ions. Ion activity is reduced by chelators that trap free ions and form stable complexes. Low molecular weight substances such as magnesium and citrate act as chelators as well as inhibitors. By polymerization or immobilization on surfaces macromolecular substances can convert from inhibitors to promoters [51,52].The pH influences the ionic dissociation of stone forming compounds, chelators and inhibitors.

Epitaxy or aggregation : Following nucleation, the micro crystals can mature by epitaxially mediated crystal growth. Epitaxy is oriented overgrowth of one crystalline material on to a substrate crystalline lattice [53,54].Monoepitaxial growth refers to the adsorption of the molecules or ions one by one on the crystal surface from supersaturated urine and heteroepitaxial growth refers to direct growth of one crystal on a surface of different composition and the surfaces of crystal and substrate [53].

Agglomeration : Agglomeration of crystals is mainly governed by attractive forces of van der Waals, by viscous binding and by electrostatic repulsion [50]. The polyanionic inhibitors of agglomeration that are absorbed on crystal surfaces increase electronegativity and, thus crystal repulsion. However, macromolecular components also have sticky forces that can increase viscous binding and this promotes agglomeration.Tamm Horsfall protein is an important urinary inhibitor of agglomeration [55,56].

Local inactivation of mineralization inhibitors at specific sites during mineralization or the presence of mineral phase nucleators that promote mineral formation by providing alternate low activation energy pathways at specific sites during mineralization are the two mechanisms which lead to initiation of mineralization under pathological conditions.The factors which influence pathological mineralization are the supersaturation of body fluids with stone forming constituents, role of the organic matrix, and presence of inhibitors or promoters of mineralization in body fluids [57].

Role of calcium, Phosphate and Vitamin D

Calcium is the fifth and Phosphorous the sixth most abundant element in the human body [55]. Calcium phosphate complex has low solubility so it is important agent of mineralization of organic bone matrix. But this low solubility can also cause it to precipitate in blood vessels, joints and kidneys. Therefore body has to create a balance in calcium and phosphate ingestion and their excretion in urine to prevent the deposition of calcium phosphate in wrong places. Maintaining normal range of calcium and phosphate ensures its mineralization in the bone and prevents abnormal calcification of tissues. The functions of calcium include electrical neuromuscular transmission, as an endocrine substance and as a major intracellular signaling molecule. Phosphate is an integral component of many organic substances. Phosphate also modifies protein functions [58]. Calcium homeostasis depends on coordinated handling of calcium mainly in Intestine, Kidneys and Bone. PTH secreted by the parathyroid glands functions to restore normal serum calcium levels by increasing intestinal absorption of calcium, by increasing bone resorption and by increasing renal calcium re-absorption. In the intestine, PTH increases hydroxylation of vitamin D (25(OH)D) to its active form $1,25(OH)_2$ D. This active form of vitamin D increases absorption of calcium via calbindin-D9k and receptors TRPV5 and TRPV6 of apical transient receptor potential vanniloid (TRPV) family [59] .In the kidneys, PTH increases re-absorption of calcium and decreases re-absorption of phosphate. TRPV5 is the rate-limiting step in renal calcium reabsorption and klotho is important to activate TRPV5 [59].PTH increases bone resorption by indirectly stimulating osteoclasts. PTH binds to osteoblasts and the expression of RANKL in osteoblasts is increased and that of osteoprotegerin is decreased. RANKL binds with RANK (its receptor) and RANKL-RANK binding stimulates formation of new osteoclasts. RANKL-RANK binding is inhibited by osteoprotegerin [58].

The regulation of PTH secretion is mediated by calcium sensing receptors (CaSR) present on the calcium sensing cells [60].$1,25(OH)_2$ D stimulates renal reabsorption and intestinal absorption of both calcium and phosphate. Deficiency in $1,25(OH)_2$ D leads to lower serum levels of calcium and phosphate. This leads to stimulation of parathyroid glands to release PTH. $1, 25 (OH)_2$ D affects the bones through its actions on intestine, but some direct effects are also observed. It increases bone mineralization and in excess it mobilizes bone calcium and phosphate.Calcitonin inhibits bone resorption, decreases calcium and phosphate reabsorption in the kidneys and therefore increases calcium and phosphate excretion in urine and regulates $1, 25(OH)_2$ D production.The interplay between PTH, $1, 25(OH)_2$ D and FGF-23 maintains phosphate homeostasis in the body. PTH causes phosphate resorption from bone and decreases its re-absorption in the kidneys by stimulating the activation of 25(OH) D to its active form $1,25 (OH)_2$ D. $1, 25(OH)_2$ D increases absorption of phosphate from intestine. FGF-23 secreted by osteocytes inhibits the production of $1, 25(OH)_2$ D and also inhibits the secretion of PTH. Oral intake of phosphate results in increased secretion of FGF-23 causing increased phosphaturia and decreased production $1, 25(OH)_2$ D [61].

Mechanism of crystal retention in nephrolithiasis

The Free particle theory suggests that under supersaturated conditions, crystals nucleate in the lumen of the nephron. The crystals grow to sufficient size to block the ducts of Bellini. According to the fixed particle theory crystals form under supersaturated conditions and adhere to the walls of kidney to act as nidi for stone growth.In 1937, it was reported by Randall that calcium oxalate stones grow attached to the renal papilla containing interstitial apatite deposits [62].It was also shown that Brushite crystals serve as very effective substrate for nucleation of CaOx. Despite higher supersaturation for CaOxthan Brushite,the initial nucleated crystallite is brushite rather than CaOx.Brushite crystallites are formed in descending limb of loop of henle. As brushite is less thermodynamically stable than HAP,possibly this is the reason for detection of HAP than brushite in Randall's plaques.Randall's plaque are hydroxyapatite deposits that originate in thin loops of Henle. Randall's plaque are found associated with subepithelial plaques suggesting that Randall's plaques overgrow into the subepithelial space and CaOx crystals grow over these subepithelial plaques [63].High urinary calcium, low urine volume and low pH drive formation of plaques in interstitial medullary and papillary region.Due to renal injury, plaque is exposed to supersaturated urine. The renal injury can promote crystal formation by providing substrates for their heterogenous nucleation.Both mature and immature cells surfaces express crystalbinding

molecules such as Phosphatidylserine, CD44, osteopontin, hyaluronanbut, while they are available on surfaces of immature cells, in mature cells these moleculesbecome available only after injury [64-67].

Thus, epithelial damage promotes crystal adherence to the renal epithelium [68].Free hyaluronan can act as crystal binding molecule at later segments of distal and collecting tubules [69].Altered exposure of Annexin II on surface of renal tubular cells could promote retention of crystals [70].Alteration of number or composition of sialic acid containing glycoconjugates by cell injury,drugs or genetic factors may cause retention of crystals within the nephron [71].Intrarenal prostaglandins may defend against stone formation by protecting collecting duct cells from adhesion of crystals.[72]Exposure of renal epithelial cells to CaOx as well as CaP crystals induces the production of monocyte chemoattractant protein-1 and ED-1 positive monocytes and macrophages [73].The development of inflammation and migration of macrophages to plaque site leads to ulceration of subepithelial plaque to the renal papillary surface leading to nidus for stone formation. Almost all the modulators of crystal nucleation, growth and retention are produced by the kidneys and excreted in urine. On exposure to oxalate ions and/orCaOx crystals there is increased gene expression and production of these modulators such as OPN. ROS are produced in kidneys due to exposure of renal epithelial cells to the oxalate ions and/or CaOx crystals. NADPH oxidase is implicated in the ROS generation on exposure to CaOx or oxalate ions [74].Excessive exposure can lead to production of more ROS thus inducing antioxidant defense in the kidneys and causing oxidative stress resulting in renal injury [75].After the loss of renal epithelial integrity, subepithelial plaque is exposed and is covered by a layer of urine proteins such as THP and OPN. Inclusion of intracrystalline proteins into biominerals is a mechanism to impart fine control over the process of biomineralization[76].Within this layer of proteins additional apatite crystals precipitate due to supersaturation for calcium phosphate species. Additional proteins adsorb over apatite forming another layer and repeated crystallization and coatings to form the ribbon morphology [77].

Intracrystalline proteins destabilize the crystalline structure by increased lattice strain and reduced crystallite size.It is proposed that channels created and filled by the intercrystalline proteins are invaded by proteases present in organic matrix of kidney stones or in the kidney or in urine and thus help in dissolution of crystals retained within the kidney [78].At some point, crystallization driven by supersaturation overcomes the moderating effect of proteins and crystals extend outwards into the urinary space and begin to form a stone of successive layers of apatite and finally CaOx itself [77].The proposed model of layered spherulitic growth suggests that alternating organic and polycrystalline layers appear by continuous crystallization which is inhibited by precipitation of organic material and the neighboring crystalline layers are connected by the means of fine crystalline channels remaining in the organic layers[79].An increase in urinary supersaturation along with deficiency of crystallization inhibitors leads to stone formation. After oxalate exposure, surviving cells are able to adapt to ambient baseline level of oxalate and proliferate normally without further toxicity with increased expression of HSP-70 [80].The tubular epithelium dedifferentiates for retention of relatively small crystals and it also translocates these crystals to interstitium where they can subsequently be dissolved[81].It was also postulated that osteopontin could be present in the stone matrix to inhibit mineralization under conditions of supersaturation. But under conditions of supersaturation or due to environmental factors,inhibitory effect of such proteins could be overcome and this could instigate formation of a metastable amorphous phase which could worsen the condition [82].

VI.   CONCLUSION

The advancement in nephrolithiasis research should be focused on study of healthy biomineralisation systems. The healthy biominerals are synthesized in a strictly regulated fashion under the control of modulators and inhibitors. Mechanism of their strict regulation can lead to better understanding of the pathogenesis of nephrolithiasis.

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