Aesth Plast Surg (2019) 43:243–252
https://doi.org/10.1007/s00266-018-1285-9

ORIGINAL ARTICLE BASIC SCIENCE/EXPERIMENTAL

Recent Advances on Relationship Between Inorganic Phosphate and Pathologic Calcification: Is Calcification After Breast Augmentation with Fat Grafting Correlated with Locally Increased Concentration of Inorganic Phosphate?

Shangshan Li[1] · **Jie Luan**[1]

Received: 6 July 2018 / Accepted: 24 November 2018 / Published online: 14 December 2018

Abstract

Background Pathologic calcification has frequently occurred after breast augmentation with fat grafting as well as other conditions such as breast cancer, trauma, myocardial infarction, arteriosclerosis and even after reduction mammoplasty. Inorganic phosphate, correlated with fat metabolism, is an important factor that induces pathologic calcification such as vascular calcification.

Methods A literature search was conducted using PubMed with the keywords: calcification, inorganic phosphate, fat. Studies related to the process of pathologic calcification, correlation between inorganic phosphate and pathologic calcification, between inorganic phosphate and fat metabolism in pathologic calcification were collected.

Results Various mechanisms were referred to in pathologic calcification among which inorganic phosphate played an important role. Inorganic phosphate could be liberated, under the effect of various enzymes, in the process of fat metabolism. The authors hypothesized that a large-scale necrotizing zone, which could occur in fat grafting with large amounts per cannula, might provide a high-phosphate environment which might contribute to differentiation of surrounding cells such as stem cells or regenerated vessel cells into osteoblast-like cells that induce pathologic calcification.

Conclusion Inorganic phosphate, which was correlated with fat metabolism, played a significant role in pathologic calcification. We firstly hypothesize that calcification after fat grafting may be related to locally increasing concentrations of phosphate in a necrotizing zone. Further research should be conducted to verify this hypothesis.

Level of Evidence V This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors www.springer.com/00266.

Keywords Inorganic phosphate · Pathological calcification · Fat graft

Introduction

Nodules or calcifications frequently occurred after breast augmentation with fat grafting [1–3]. Clinically, studies indicated that multilayer, multipoint and multitunnel with small amounts per cannula injection could reduce fat necrosis and calcifications [4, 5]. However, there was a lack of evidence that illustrated the molecular mechanism of calcification after breast augmentation with fat grafting.

Besides after fat grafting, pathological calcification occurred in various kinds of diseases such as breast cancer, trauma, myocardial infarction, arteriosclerosis and even after reduction mammoplasty [6–10], indicating that there might be some common mechanisms in these pathological calcifications.

Calcification after fat grafting often occurs in the cyst wall located surrounding the necrotizing zones [11] containing new vessel cells [12]. The process of vascularization also occurs in breast cancer, trauma or wound healing followed by breast surgery [13, 14]. Myocardial infarction and arteriosclerosis are vascular diseases. Therefore, the

✉ Jie Luan
luanjieplastic@126.com

[1] Department of Aesthetic and Reconstructive Breast Surgery, Plastic Surgery Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, 33 Badachu Road, Shijingshan District, Beijing 100144, China

authors tried to find out common mechanisms of pathologic calcifications in studies related to vascular calcification.

Mechanisms of vascular calcification were studied worldwide, and various studies indicated that inorganic phosphate (Pi) contributed to the formation of vascular calcification [15, 16]. Pi is also one of the metabolites of phospholipids [17]. Although there was no evidence before, Pi might be produced after fat grafting. Therefore, we searched related studies to find molecular mechanisms and potential relationships between Pi and calcification which could help to prevent calcification after fat grafting.

Materials and Methods

A literature review was conducted using PubMed with the keywords: calcification, inorganic phosphate, fat. Studies related to the process of pathologic calcification, correlation between inorganic phosphate and pathologic calcification, and between inorganic phosphate and fat metabolism were collected.

Results

Process of Pathologic Calcification

The process of pathologic calcification is a combination of the actively regulated process of mineralization similar to bone formation [18, 19] and a passive one that seemed to take place when the abundance of calcification inhibitors were decreased, which was a major step that initiates specific osteogene expression and transdifferentiation into osteoblast-like cells [20]. Similarly, the presence of bioapatite crystals, which are the main components of pathologic calcification, might be achieved by two different growth processes: A pathological crystallization process that occurs in biological niches which is a purely physicochemical process and a matrix-mediated mineralization process in which the extracellular matrix acts as a template for site-directed nanocrystal nucleation [21].

There are molecular and mechanistic similarities between bone formation and vascular calcification (VC), which is one of the common pathologic calcifications [22–24]. The mechanisms of VC have been extensively reviewed [25, 26]. (1) Loss of mineral inhibiting factors: extraosseous biomineralization might be driven not only by upregulation of activating factors but also by downregulation of such inhibiting factors [27]. Ca/P precipitation in the medium occurred over time in the absence of inhibitors even in normal levels of Ca and P [28]. (2) Induction of calcification: as cells matured into osteoblasts or chondrocytes, they expressed osteochondrogenic genes and released a type of membrane-invested microparticle, known as matrix vesicles (MVs), into the extracellular milieu in both non-pathological and pathological conditions [29–32]. These matrix vesicles contained a variety of enzymes and factors, including alkaline phosphatase (ALP) and annexins (Anx) [27, 33, 34] which are critical for growth plate-MV mineral nucleation [35]. (3) Cell death: mineralization detected in vivo and in vitro coincided with an increase in apoptosis [36, 37]. Inhibition of apoptosis resulted in a decrease in mineralization, whereas stimulation of apoptosis increased the degree of calcification [38]. In calcified lesions, thin elastic lamellae were observed by elastin staining, indicating that elastin degradation could occur in the area. Elastin degradation might be involved in the development of vascular calcification [39] through increasing affinity of extracellular matrix (ECMs) for Ca [40], which facilitates growth of hydroxyapatite along the elastic lamellae. (4) Circulating nucleational complexes such as aggregates of calcium phosphate and proteins might initiate ectopic mineralization, and there is evidence that mineralization first occurred in MVs and subsequently spread from vesicles to the extra-vesicular interstices and then into adjacent collagen fibrils [41–43]. Abundant macrophages, associated with regions of MVs [44], perform specialized roles including regulating the inorganic phosphate/pyrophosphate (Pi/PPi) ratio in the intra- and extracellular fluid, managing mineral nucleation, controlling calcium and Pi ion homeostasis, and interacting with the surrounding ECM to direct HA localization and growth [42, 45–47], in initiating matrix mineralization. Extracellular processing resulted in mature three-chained collagen type-1 molecules, which assembled into collagen fibrils [48]. These fibrils then combined to form larger fibers that acted as a natural scaffold for hydroxyapatite crystals to grow between [49]. Bacteria or other agents might also produce such nidi, if present in blood and urine [50, 51]. Ca/P complexes further stimulated macrophages to release MVs capable of mineralization, with increased calcium influx and ALP activity. Ca/P stimulation enriched S100A9 and Anx5 expression in Mac-MVs [44]. Inactivation of the S100A9 gene tended to decrease MVs in plasma. Ca/P-stimulated S100A9−/− mouse peritoneal macrophages released Mac-MVs with lower calcific potential than those isolated from WT mice [44].

Calcifying nanoparticles (CNP) formed two distinct forms of apatite. The early formed apatite consisted of extremely small flakes, plates and crystals. Under conditions of serum or protein depletion, these apatite crystals coalesced to form larger structures [52]. The size of crystals was important in determining their effects on target cells [53, 54]. Ca/P crystals in the range of 1–2 μm or less in diameter were endocytosed and caused toxic effects in vascular smooth muscle cells (VSMCs) by inducing the

release of proinflammatory cytokines from macrophages, whereas crystals with a diameter greater than 20 μm were mostly inert which was possibly due to lack of cellular uptake of these larger particles [55].

Effect of Inorganic Phosphate on Pathological Calcification

There are two extracellular mineral ions, Pi and Ca, that play critical roles in the regulation of mineralization [56], Ca induced VSMC calcification in vitro and Ca and P are synergistic [57]. In the MVs, the presence of Pi, generated from PHOSPHO1 and PiT1/2 as well as from ATPase and ADPase, accompanied calcium on its way into the mitochondrial matrix, where it formed a precipitate mostly consisting of hydroxyapatite [58–60]. Pi induced the mRNA expression of the M2 without significantly changing the expression of M1 [61].

Pi could induce vascular calcification in a dose-dependent and time-dependent manner [62–64]. The direct addition of Pi had been used successfully in place of β-glycerophosphate as a source of phosphate for physiological and pathological models of mineralization in vitro [36, 65]. Although β-glycerophosphate alone was sufficient to induce in vitro mineralization of osteoblast and chondrocyte cells [65–67], the addition of ascorbic acid or dexamethasone further enhanced mineralization through the upregulation of ALP activity and collagen production [68–72].

Elevated phosphate resulted in loss of smooth muscle markers (SMαactin, SM22α) and elevated expression of osteochondrogenic markers (Runx2/Cbfa1, osterix, ALP, osteopontin) [63, 64, 73, 74].

VSMCs were incubated in different concentrations of Ca and Pi (1, 2, or 4 mM). No calcification was observed when the Ca * Pi molar product was kept below 4 mM^2 in any combination. Calcification was observed when combining 2 mM Ca and 2 mM Pi, or 4 mM Ca and 1 mM Pi, but no calcification was observed when the product was obtained with 1 mM Ca and 4 mM Pi as well as no significant changes in Cbfa1, Bmp2, or SM22α. VSMCs were incubated in the presence of a constant concentration of 1 mM Pi, with increasing concentrations of Ca and calcification increased with the increasing Ca concentration [20]. This study suggested that Ca and Pi were both indispensable in the process of calcification. ALP activity in the VSMCs MVs is regulated by exogenous calcium and β–glycerophosphate. VSMCs might also produce nucleating vesicles as a result of primary osteogenic differentiation or degenerative processes [29, 30].

The homeostasis of Pi depended on the function of sodium–phosphate cotransporter families denominated Na-Pis [75, 76]. Among these Na-Pis, Na-Pi 3 proteins, including PiT-1 and PiT-2, were present in all phyla and they did not share significant homology with the members of the other Na-Pi families [77]. Elevated Pi-induced signaling via ERK1/2 phosphorylation was abrogated in PiT-1-deficient VSMCs. Expression of transport-deficient PiT-1 mutants promoted VSMCs matrix mineralization, but not to the extent observed with PiT-1 WT, suggesting that both Pi uptake-dependent and independent functions of PiT-1 were important for VSMC processes mediating vascular calcification [78].

Besides VSMCs, various kinds of cells had the potential to induce calcification under the condition of high phosphate. Valve interstitial cells (VICs), the main cellular component of the aortic valve [79], were actively involved in the production of extracellular matrix and mineralization [80]. Human VICs expressed PiT-1 and PiT-2. The expression of Pi transporters in VIC culture, on exposure to a mineralizing medium, was increased several folds, with the higher magnitude for PiT-1. Silencing PiT-1 significantly reduced Pi-induced mineralization. The expression of PiT-1 was increased, and the level of Akt-1 was diminished in calcific aortic valve diseased (CAVD) tissues compared to non-mineralized aortic valves [81]. Treatment with Pi caused a reduction in the Akt-1 transcript in VIC culture, whereas treatment with PFA or siRNA against PiT-1 restored the level of Akt-1 which prevented both Pi-induced apoptosis and mineralization [81].

Bone marrow stem cells (BMSCs) had a higher proliferative ability and greater osteogenic differentiation potential, in high-phosphate osteogenic medium, than adipose-derived stem cells (ADSCs) at both the mRNA and protein levels [82].

Treated with high Pi, the amount of precipitated Ca in fixed cells was much higher than that of live cells. This difference was most likely due to the amount of calcification inhibitors, such as PPi, which are produced by live cells and are missing in fixed cells [20].

Mesenchymal stem-like cells (MSLCs) from both calcified and non-calcified human aortic valve specimens were sorted into CD34-negative and positive cells. CD34-negative cells were significantly more sensitive than CD34-positive cells to high inorganic phosphate, calcifying easily in response, which can be reduced by the sodium phosphonoformate, an inhibitor of PiT-1. Significantly, higher numbers of CD34-negative compared with CD34-positive MSLCs were localized in calcified aortic valve specimens obtained from calcified aortic stenosis patients, suggesting that CD34-negative MSLCs are responsible for calcification of the aortic valve [83].

Fibroblast growth factor 23 (Fgf23) was a potent phosphatonin [84–86] which induced increased urinary excretion of phosphate, lowering serum phosphate levels. Fgf23

bound to cell surface receptors with much higher affinity in the presence of a cofactor called klotho [87–95]. Both Fgf23 and klotho ablated mice led to increased accumulation of calcium, phosphate and sodium phosphate cotransfactor activity, developing extensive soft tissue calcification [96]. However, regardless of the dietary phosphate contents, serum phosphorus levels were consistently elevated in Galnt3 knockout mice of which low intact Fgf23 concentration was detected [97]. Calcifications appeared in Galnt3 knockout mice on the high-phosphate diet, whereas high-phosphate diet did not affect serum phosphorus concentration in normal mice [98].

High-phosphate fed (HP) severe uremia (SU) and moderate uremia (MU) mice, achieved by varying the degree of renal ablation, developed extensive arterial medial calcification (AMC), whereas normal phosphate fed (NP) uremic mice did not. AMC in the SU/HP mice was associated with a statistically significant hyperphosphatemia. MU/HP mice were not hyperphosphatemic, but a significant rise in serum Fgf23 and osteopontin (OPN) levels in this group was observed [99].

Multiple ectopic calcifications in subcutaneous tissues occurred in a case of human chronic renal failure, which might be related to elevated high serum phosphate [100].

A summary of the effects of inorganic phosphate on pathological calcification is shown in Table 1.

Relationship between inorganic phosphate and fat metabolism in pathologic calcification

In humans, 85% of total body phosphorus is in bone, 14% is intracellular, and only 1% is in extracellular fluid [101].

The most common cause of elevated levels of serum phosphate is reduced urinary excretion with reduced renal function [102]. Disturbance of the serum concentration of phosphate led to the development of hypophosphatemia (< 0.8 mM) or hyperphosphatemia (> 2 mM), compared with the normal concentration range of 1–1.5 mM in humans [103]. In mice, normal serum phosphate levels ranged from 2 to 2.6 mM, whereas animals with surgically induced chronic kidney disease manifest levels of 3.5 mM [28, 104].

Phosphate is mainly an intracellular ion, whereas calcium is mainly an extracellular ion. However, during cell injury or death the ion barrier formed by the cell membrane can be disrupted [105]. Calcium and phosphate ions might then accumulate on the surface of apoptotic bodies through their external phosphatidylserine residues [49].

Serum phosphate levels correlated with cardiovascular calcification, and they were also directly proportional to the low-density lipoprotein (LDL) levels and inversely proportional to the glomerular filtration rate [106]. Lipoprotein-associated phospholipase A2 (Lp-PLA2) was overexpressed in tissues of CAVD. Lp-PLA2 converted oxidized LDL (ox-LDL) into lysophosphatidylcholine (LPC) [107], which was a strong promoter of mineralization through a cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway. Lp-PLA2 was produced locally, within the aortic valve, by macrophages and was transported in the aortic valve by LDL, and enhanced lipid retention/modification [108]. Blood plasma levels of lipoprotein have also been associated with an increased risk of aortic valve stenosis [109]. Fish oils, such as eicosapentaenoic acid (EPA), which inhibited lipid oxidation, also inhibited vascular calcification [110]. Mineralization in VSMCs induced by the free fatty acid, palmitic acid, is blocked by EPA through a mechanism requiring long-chain acylCoA synthetase-3 and nuclear factor-κB [111], suggesting that pro-oxidant lipids positively regulate vascular calcification.

Acidic phospholipids were present at high levels in cells at the mineralizing front in pathologic calcifications [112]. Detergent pretreatment of bioprosthetic heart valves, shown to inhibit mineralization, is likely acted by the detergent-mediated extraction of phospholipids and other proteolipids [113]. Delipidation of bioprosthetic valves significantly reduced their calcific degradation when

Table 1 Summary of effects of inorganic phosphate on pathological calcification

References	Content
Sonou [62] Jono [63]	Pi-induced VC in a dose-dependent and time-dependent manner
Lee [64] Steitz [73] Moe [74]	Elevated Pi resulted in loss of smooth muscle markers and elevated expression of osteochondrogenic markers
Villa-Bellosta [20]	Pi and Ca were both indispensable in the process of calcification
Biber [75] Tenenhouse [77] Chavkin [78]	Homeostasis of Pi depended on Na-Pis, including PiT-1. PiT-1 was important for VSMC processes mediating VC
Mathieu [80]	Mineralization of VICs was induced by high Pi
Zhang [82]	Calcification of BMSCs and ADSCs was induced by high Pi
Nomura [83]	CD34-negative MSLCs are more responsible for calcification of the aortic valve than CD34-positive MSLCs
Ichikawa [98]	Calcifications appeared in the mice with hyperphosphatemia
El-Abbada [99]	Arterial calcification was associated with statistically significant hyperphosphatemia
Zhang [100]	Multiple ectopic calcifications in subcutaneous tissues occurred in a case of human chronic renal failure that might be related to elevated high serum phosphate

Table 2 Summary of relationship between inorganic phosphate and fat metabolism in pathologic calcification

References	Content
Rajamannan [107]	Lipoprotein-associated phospholipase A2 was overexpressed in tissue of CAVD
Kamstrup [109]	Levels of lipoprotein were associated with aortic valve stenosis
Kageyama [111]	Pro-oxidant lipids positively regulate vascular calcification
Bailey [112]	Acidic phospholipids were at high levels in cells at the mineralizing front in pathologic calcification
Shen [114]	Delipidation of bioprosthetic valves significantly reduced their calcific degradation
Martin [17]	Phospholipid was hydrolyzed to produce phosphatidic acid which liberated inorganic phosphate under the effect of ALP
Houston [115] Stewart [116] Roberts [117]	PHOSPHO1, a kind of phosphatase, might be responsible for generating Pi for mineralization through highly specific activities for phosphoethanolamine (PEA) and phosphocholine (PChol)
Roberts [118] Stewart [119]	PEA and PChol played an important role in controlling the first step crystal deposition inside the MVs

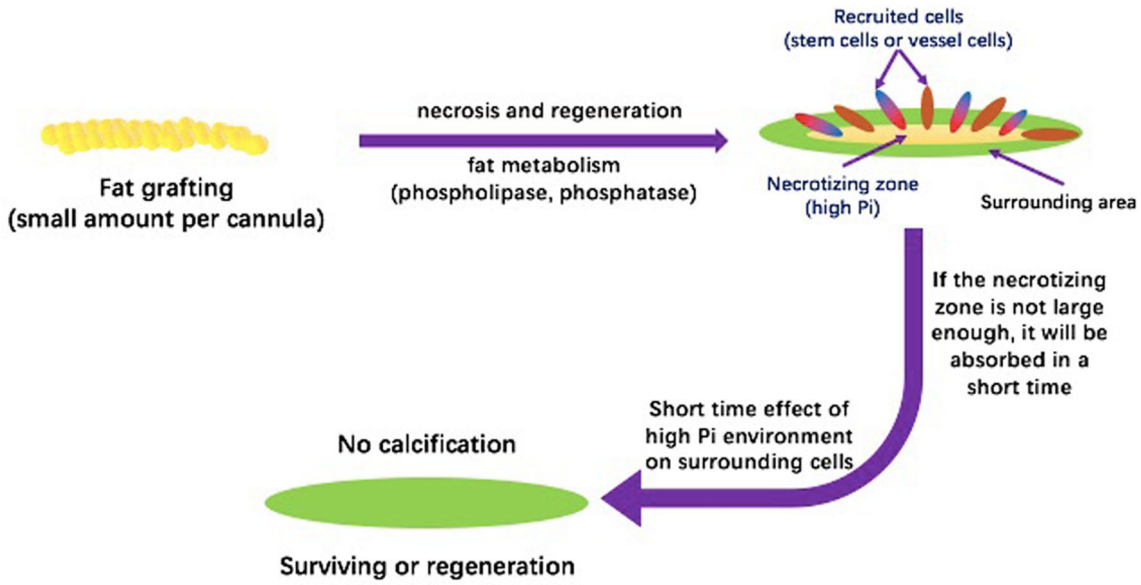

Fig. 1 Hypothesis of no calcification formation after fat grafting with small amount per cannula

implanted in rats [114], which further supported the view above. Phospholipid was hydrolyzed to produce phosphatidic acid which liberated inorganic phosphate under the effect of ALP [17].

PHOSPHO1, a kind of phosphatase, might be responsible for generating Pi for mineralization through highly specific activities for phosphoethanolamine (PEA) and phosphocholine (PChol) [115–117], which play an important role in controlling the first step of crystal deposition inside the MVs [118, 119]. PEA and PChol are the two most abundant phosphomonoesters in cartilage [120]. PHOSPHO1 also controlled ALP expression in mineralizing cells and was essential for mechanically competent mineralization [121, 122]. PHOSPHO1 was highly expressed in mineralizing chondrocytes [117] and osteoblast-derived MVs [118, 119]. PHOSPHO1 inhibition by MLS-0263839 in mineralizing cells reduced calcification. Combined inhibition of PHOSPHO1 by MLS-0263839 and ALP by MLS-0038949 further reduced calcification [123]. Lansoprazole, an inhibitor of PHOSPHO1 and ALP, also inhibited mineralization [124–126].

A summary of the relationship between inorganic phosphate and fat metabolism in pathologic calcification is shown in Table 2.

Is calcification after autologous breast augmentation with fat grafting correlated with locally increasing concentration of inorganic phosphate?

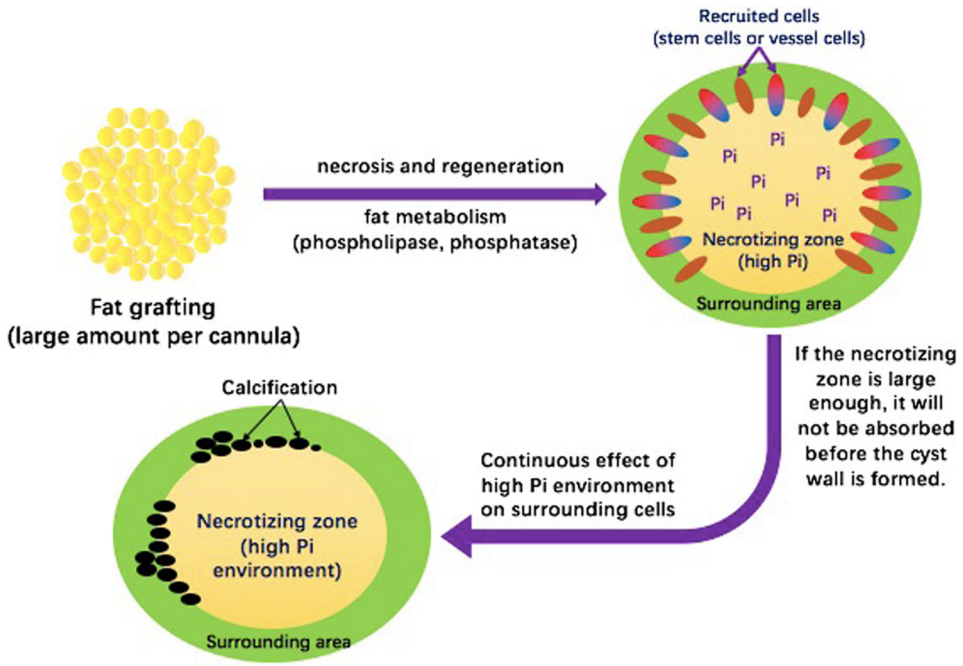

Fig. 2 Hypothesis of calcification formation after fat grafting with large amount per cannula

Nodules or calcifications are common complications after breast augmentation with fat grafting [1–3]. After fat grafting, the innermost zone is a fat necrotizing zone, which might be correlated with oil cyst and calcification [127]. Calcification after fat grafting often occurs in the cyst wall located in the regenerating zones [11] containing stem cells or new vessel cells [12]. We hypothesized that there are similar mechanisms between calcification after breast augmentation with fat grafting and other types of pathologic calcifications such as vascular calcification. Because there is a close relationship between fat metabolism and inorganic phosphate, we hypothesized that during the process of fat necrosis, there are locally increased concentrations of inorganic phosphate, which might provide an environment of mineralization for the recruited cells such as stem cells or regenerated vessel cells surrounding this necrotizing zone. If the range of the necrotizing zone is not large enough, it would be absorbed in a short time. Then the effect of high Pi on the surrounding area was terminated, and no calcification would be formed (Fig. 1). However, if the range of the necrotizing zone is large enough that the process of physical absorption of the necrotic tissue could not be totally completed within a short time, transdifferentiation of the surrounding cells such as stem cells or vessel-related cells into osteoblast-like cells might occur under the continuous effect of high-phosphate environment, which might form cyst walls or calcification in several months (Fig. 2). We thought that most conditions such as breast cancer, trauma, myocardial infarction, arteriosclerosis and reduction mammoplasty that caused pathological calcification would experience the process of necrosis and regeneration which was similar to fat grafting. The authors thought that in the process of necrosis, there would be some products such as phosphate and there were new vessels formed in the process of regeneration. Therefore, our hypothesis could also explain common mechanisms of these pathological calcifications. However, due to the lack of direct evidence, further studies should be conducted to verify this hypothesis and further to verify what kinds of surrounding cells mainly contribute to the formation of calcification under the environment of high phosphate.

Conclusion

Inorganic phosphate, which is correlated with fat metabolism, plays a significant role in pathologic calcification. We firstly hypothesized that calcification after fat grafting might be related to locally increasing concentrations of phosphate in a necrotizing zone, which might provide an environment for the surrounding live cells to transdifferentiate into osteoblast-like cells. Further research should be conducted to verify this hypothesis.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest to disclose.

Ethical Approval All analyses were based on previous published studies, thus ethical approval is unnecessary.

Informed Consent This study was based on previous published studies, thus informed consent was unnecessary.

References

1. Rubin JP, Coon D, Zuley M et al (2012) Mammographic changes after fat transfer to the breast compared with changes after breast reduction: a blinded study. Plast Reconstr Surg 129:1029–1038. https://doi.org/10.1097/PRS.0b013e31824a2a8e
2. Veber M, Tourasse C, Toussoun G et al (2011) Radiographic findings after breast augmentation by autologous fat transfer. Plast Reconstr Surg 127:1289–1299. https://doi.org/10.1097/PRS.0b013e318205f38f
3. Zocchi ML, Zuliani F (2008) Bicompartmental breast lipostructuring. Aesthetic Plast Surg 32:313–328. https://doi.org/10.1007/s00266-007-9089-3
4. Coleman SR, Saboeiro AP (2015) Primary breast augmentation with fat grafting. Clin Plast Surg 42(301–306):vii. https://doi.org/10.1016/j.cps.2015.03.010
5. Coleman SR (1995) Long-term survival of fat transplants: controlled demonstrations. Aesthetic Plast Surg 19:421–425
6. Ling H, Liu ZB, Xu LH et al (2013) Malignant calcification is an important unfavorable prognostic factor in primary invasive breast cancer. Asia Pac J Clin Oncol 9:139–145. https://doi.org/10.1111/j.1743-7563.2012.01572.x
7. O'Brien EJ, Frank CB, Shrive NG et al (2012) Heterotopic mineralization (ossification or calcification) in tendinopathy or following surgical tendon trauma. Int J Exp Pathol 93:319–331. https://doi.org/10.1111/j.1365-2613.2012.00829.x
8. Delo DM, Guan X, Wang Z et al (2011) Calcification after myocardial infarction is independent of amniotic fluid stem cell injection. Cardiovasc Pathol 20:e69–e78. https://doi.org/10.1016/j.carpath.2010.03.001
9. Kim H, Kang BJ, Kim SH et al (2015) What we should know in mammography after reduction mammoplasty and mastopexy? Breast Cancer 22:391–398. https://doi.org/10.1007/s12282-013-0494-y
10. Tolle M, Reshetnik A, Schuchardt M et al (2015) Arteriosclerosis and vascular calcification: causes, clinical assessment and therapy. Eur J Clin Investig 45:976–985. https://doi.org/10.1111/eci.12493
11. Mineda K, Kuno S, Kato H et al (2014) Chronic inflammation and progressive calcification as a result of fat necrosis: the worst outcome in fat grafting. Plast Reconstr Surg 133:1064–1072. https://doi.org/10.1097/PRS.0000000000000097
12. Pu LL (2016) Mechanisms of fat graft survival. Ann Plast Surg 77(Suppl 1):S84–S86. https://doi.org/10.1097/SAP.0000000000000730
13. Redig AJ, McAllister SS (2013) Breast cancer as a systemic disease: a view of metastasis. J Intern Med 274:113–126. https://doi.org/10.1111/joim.12084
14. Kachgal S, Mace KA, Boudreau NJ (2012) The dual roles of homeobox genes in vascularization and wound healing. Cell Adhes Migr 6:457–470. https://doi.org/10.4161/cam.22164
15. Giachelli CM (2005) Inducers and inhibitors of biomineralization: lessons from pathological calcification. Orthod Craniofac Res 8:229–231. https://doi.org/10.1111/j.1601-6343.2005.00345.x
16. Tabcheh L, Bianchi A, Clement A et al (2014) Phosphate-induced mineralization of tracheal smooth muscle and cartilage cells. Biomed Mater Eng 24:37–45. https://doi.org/10.3233/BME-140972
17. Martin SF, DeBlanc RL, Hergenrother PJ (2000) Determination of the substrate specificity of the phospholipase D from Streptomyces chromofuscus via an inorganic phosphate quantitation assay. Anal Biochem 278:106–110. https://doi.org/10.1006/abio.1999.4420
18. Demer LL (1995) A skeleton in the atherosclerosis closet. Circulation 92:2029–2032
19. Wexler L, Brundage B, Crouse J et al (1996) Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing Group. Circulation 94:1175–1192
20. Villa-Bellosta R, Millan A, Sorribas V (2011) Role of calcium-phosphate deposition in vascular smooth muscle cell calcification. Am J Physiol Cell Physiol 300:C210–C220. https://doi.org/10.1152/ajpcell.00229.2010
21. Cottignoli V, Relucenti M, Agrosi G et al (2015) Biological niches within human calcified aortic valves: towards understanding of the pathological biomineralization process. Biomed Res Int 2015:542687. https://doi.org/10.1155/2015/542687
22. Vattikuti R, Towler DA (2004) Osteogenic regulation of vascular calcification: an early perspective. Am J Physiol Endocrinol Metab 286:E686–E696. https://doi.org/10.1152/ajpendo.00552.2003
23. Demer LL, Tintut Y (2008) Vascular calcification: pathobiology of a multifaceted disease. Circulation 117:2938–2948. https://doi.org/10.1161/CIRCULATIONAHA.107.743161
24. Hruska KA, Mathew S, Saab G (2005) Bone morphogenetic proteins in vascular calcification. Circ Res 97:105–114. https://doi.org/10.1161/01.RES.00000175571.53833.6c
25. Speer MY, Giachelli CM (2004) Regulation of cardiovascular calcification. Cardiovasc Pathol 13:63–70. https://doi.org/10.1016/S1054-8807(03)00130-3
26. Jono S, Shioi A, Ikari Y et al (2006) Vascular calcification in chronic kidney disease. J Bone Miner Metab 24:176–181. https://doi.org/10.1007/s00774-005-0668-6
27. Demer LL, Tintut Y (2014) Inflammatory, metabolic, and genetic mechanisms of vascular calcification. Arterioscler Thromb Vasc Biol 34:715–723. https://doi.org/10.1161/ATVBAHA.113.302070
28. Sage AP, Lu J, Tintut Y et al (2011) Hyperphosphatemia-induced nanocrystals upregulate the expression of bone morphogenetic protein-2 and osteopontin genes in mouse smooth muscle cells in vitro. Kidney Int 79:414–422. https://doi.org/10.1038/ki.2010.390
29. Chen NX, O'Neill KD, Chen X et al (2008) Annexin-mediated matrix vesicle calcification in vascular smooth muscle cells. J Bone Miner Res 23:1798–1805. https://doi.org/10.1359/jbmr.080604
30. Kapustin AN, Davies JD, Reynolds JL et al (2011) Calcium regulates key components of vascular smooth muscle cell-derived matrix vesicles to enhance mineralization. Circ Res 109:e1–12. https://doi.org/10.1161/CIRCRESAHA.110.238808
31. Anderson HC (2003) Matrix vesicles and calcification. Curr Rheumatol Rep 5:222–226
32. Kim KM (1976) Calcification of matrix vesicles in human aortic valve and aortic media. Fed Proc 35:156–162
33. Tintut Y, Patel J, Territo M et al (2002) Monocyte/macrophage regulation of vascular calcification in vitro. Circulation 105:650–655
34. Tintut Y, Patel J, Parhami F et al (2000) Tumor necrosis factor-alpha promotes in vitro calcification of vascular cells via the cAMP pathway. Circulation 102:2636–2642
35. Grskovic I, Kutsch A, Frie C et al (2012) Depletion of annexin A5, annexin A6, and collagen X causes no gross changes in matrix vesicle-mediated mineralization, but lack of collagen X

affects hematopoiesis and the Th1/Th2 response. J Bone Miner Res 27:2399–2412. https://doi.org/10.1002/jbmr.1682
36. Mune S, Shibata M, Hatamura I et al (2009) Mechanism of phosphate-induced calcification in rat aortic tissue culture: possible involvement of Pit-1 and apoptosis. Clin Exp Nephrol 13:571–577. https://doi.org/10.1007/s10157-009-0208-0
37. Duan X, Zhou Y, Teng X et al (2009) Endoplasmic reticulum stress-mediated apoptosis is activated in vascular calcification. Biochem Biophys Res Commun 387:694–699. https://doi.org/10.1016/j.bbrc.2009.07.085
38. Proudfoot D, Skepper JN, Hegyi L et al (2000) Apoptosis regulates human vascular calcification in vitro: evidence for initiation of vascular calcification by apoptotic bodies. Circ Res 87:1055–1062
39. Kumata C, Mizobuchi M, Ogata H et al (2011) Involvement of matrix metalloproteinase-2 in the development of medial layer vascular calcification in uremic rats. Ther Apher Dial 15(Suppl 1):18–22. https://doi.org/10.1111/j.1744-9987.2011.00921.x
40. Rucker RB (1974) Calcium binding to elastin. Adv Exp Med Biol 48:185–209
41. Arsenault AL, Frankland BW, Ottensmeyer FP (1991) Vectorial sequence of mineralization in the turkey leg tendon determined by electron microscopic imaging. Calcif Tissue Int 48:46–55
42. Golub EE (2009) Role of matrix vesicles in biomineralization. Biochem Biophys Acta 1790:1592–1598. https://doi.org/10.1016/j.bbagen.2009.09.006
43. Gufler H, Wagner S, Franke FE (2011) The interior structure of breast microcalcifications assessed with micro computed tomography. Acta Radiol 52:592–596. https://doi.org/10.1258/ar.2011.100489
44. New SE, Goettsch C, Aikawa M et al (2013) Macrophage-derived matrix vesicles: an alternative novel mechanism for microcalcification in atherosclerotic plaques. Circ Res 113:72–77. https://doi.org/10.1161/CIRCRESAHA.113.301036
45. Anderson HC (1995) Molecular biology of matrix vesicles. Clin Orthop Relat Res 314:266–280
46. Golub EE (2011) Biomineralization and matrix vesicles in biology and pathology. Semin Immunopathol 33:409–417. https://doi.org/10.1007/s00281-010-0230-z
47. Xiao Z, Blonder J, Zhou M et al (2009) Proteomic analysis of extracellular matrix and vesicles. J Proteomics 72:34–45. https://doi.org/10.1016/j.jprot.2008.11.011
48. Manolagas SC (2000) Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 21:115–137. https://doi.org/10.1210/edrv.21.2.0395
49. Kirsch T (2006) Determinants of pathological mineralization. Curr Opin Rheumatol 18:174–180. https://doi.org/10.1097/01.bor.0000209431.59226.46
50. Blair B, Fabrizio M (2000) Pharmacology for renal calculi. Expert Opin Pharmacother 1:435–441. https://doi.org/10.1517/14656566.1.3.435
51. Sohshang HL, Singh MA, Singh NG et al (2000) Biochemical and bacteriological study of urinary calculi. J Commun Dis 32:216–221
52. Mathew G, McKay DS, Ciftcioglu N (2008) Do blood-borne calcifying nanoparticles self-propagate? Int J Nanomed 3:265–275
53. Ewence AE, Bootman M, Roderick HL et al (2008) Calcium phosphate crystals induce cell death in human vascular smooth muscle cells: a potential mechanism in atherosclerotic plaque destabilization. Circ Res 103:e28–e34. https://doi.org/10.1161/CIRCRESAHA.108.181305
54. Nadra I, Boccaccini AR, Philippidis P et al (2008) Effect of particle size on hydroxyapatite crystal-induced tumor necrosis factor alpha secretion by macrophages. Atherosclerosis 196:98–105. https://doi.org/10.1016/j.atherosclerosis.2007.02.005
55. Vervaet BA, Verhulst A, Dauwe SE et al (2009) An active renal crystal clearance mechanism in rat and man. Kidney Int 75:41–51. https://doi.org/10.1038/ki.2008.450
56. Huang MS, Sage AP, Lu J et al (2008) Phosphate and pyrophosphate mediate PKA-induced vascular cell calcification. Biochem Biophys Res Commun 374:553–558. https://doi.org/10.1016/j.bbrc.2008.07.062
57. Shanahan CM, Crouthamel MH, Kapustin A et al (2011) Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. Circ Res 109:697–711. https://doi.org/10.1161/CIRCRESAHA.110.234914
58. Kirsch T (2008) Determinants of pathologic mineralization. Crit Rev Eukaryot Gene Expr 18:1–9
59. Greenawalt JW, Rossi CS, Lehninger AL (1964) Effect of active accumulation of calcium and phosphate ions on the structure of rat liver mitochondria. J Cell Biol 23:21–38
60. Weinbach EC, Von Brand T (1967) Formation, isolation and composition of dense granules from mitochondria. Biochem Biophys Acta 148:256–266
61. Villa-Bellosta R, Hamczyk MR, Andres V (2017) Novel phosphate-activated macrophages prevent ectopic calcification by increasing extracellular ATP and pyrophosphate. PLoS ONE 12:e0174998. https://doi.org/10.1371/journal.pone.0174998
62. Sonou T, Ohya M, Yashiro M et al (2015) Mineral composition of phosphate-induced calcification in a rat aortic tissue culture model. J Atheroscler Thromb 22:1197–1206. https://doi.org/10.5551/jat.28647
63. Jono S, McKee MD, Murry CE et al (2000) Phosphate regulation of vascular smooth muscle cell calcification. Circ Res 87:E10–E17
64. Lee K, Kim H, Jeong D (2014) Microtubule stabilization attenuates vascular calcification through the inhibition of osteogenic signaling and matrix vesicle release. Biochem Biophys Res Commun 451:436–441. https://doi.org/10.1016/j.bbrc.2014.08.007
65. Bellows CG, Heersche JN, Aubin JE (1992) Inorganic phosphate added exogenously or released from beta-glycerophosphate initiates mineralization of osteoid nodules in vitro. Bone Miner 17:15–29
66. Tenenbaum HC (1981) Role of organic phosphate in mineralization of bone in vitro. J Dent Res. https://doi.org/10.1177/00220345810600003S0801
67. Chung CH, Golub EE, Forbes E et al (1992) Mechanism of action of beta-glycerophosphate on bone cell mineralization. Calcif Tissue Int 51:305–311
68. Leboy PS, Vaias L, Uschmann B et al (1989) Ascorbic acid induces alkaline phosphatase, type X collagen, and calcium deposition in cultured chick chondrocytes. J Biol Chem 264:17281–17286
69. Wan XC, Liu CP, Li M et al (2008) Staphylococcal enterotoxin C injection in combination with ascorbic acid promotes the differentiation of bone marrow-derived mesenchymal stem cells into osteoblasts in vitro. Biochem Biophys Res Commun 373:488–492. https://doi.org/10.1016/j.bbrc.2008.06.037
70. Maniatopoulos C, Sodek J, Melcher AH (1988) Bone formation in vitro by stromal cells obtained from bone marrow of young adult rats. Cell Tissue Res 254:317–330
71. Coelho MJ, Fernandes MH (2000) Human bone cell cultures in biocompatibility testing. Part II: effect of ascorbic acid, beta-glycerophosphate and dexamethasone on osteoblastic differentiation. Biomaterials 21:1095–1102
72. Mori K, Shioi A, Jono S et al (1999) Dexamethasone enhances In vitro vascular calcification by promoting osteoblastic

differentiation of vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 19:2112–2118
73. Steitz SA, Speer MY, Curinga G et al (2001) Smooth muscle cell phenotypic transition associated with calcification: upregulation of Cbfa1 and downregulation of smooth muscle lineage markers. Circ Res 89:1147–1154
74. Moe SM, Duan D, Doehle BP et al (2003) Uremia induces the osteoblast differentiation factor Cbfa1 in human blood vessels. Kidney Int 63:1003–1011. https://doi.org/10.1046/j.1523-1755.2003.00820.x
75. Biber J, Custer M, Magagnin S et al (1996) Renal Na/Pi-cotransporters. Kidney Int 49:981–985
76. Tenenhouse HS (2007) Phosphate transport: molecular basis, regulation and pathophysiology. J Steroid Biochem Mol Biol 103:572–577. https://doi.org/10.1016/j.jsbmb.2006.12.090
77. Virkki LV, Biber J, Murer H et al (2007) Phosphate transporters: a tale of two solute carrier families. Am J Physiol Renal Physiol 293:F643–F654. https://doi.org/10.1152/ajprenal.00228.2007
78. Chavkin NW, Chia JJ, Crouthamel MH et al (2015) Phosphate uptake-independent signaling functions of the type III sodium-dependent phosphate transporter, PiT-1, in vascular smooth muscle cells. Exp Cell Res 333:39–48. https://doi.org/10.1016/j.yexcr.2015.02.002
79. Cote N, El Husseini D, Pepin A et al (2012) ATP acts as a survival signal and prevents the mineralization of aortic valve. J Mol Cell Cardiol 52:1191–1202. https://doi.org/10.1016/j.yjmcc.2012.02.003
80. Mathieu P, Boulanger MC (2014) Basic mechanisms of calcific aortic valve disease. Can J Cardiol 30:982–993. https://doi.org/10.1016/j.cjca.2014.03.029
81. El Husseini D, Boulanger MC, Fournier D et al (2013) High expression of the Pi-transporter SLC20A1/Pit1 in calcific aortic valve disease promotes mineralization through regulation of Akt-1. PLoS ONE 8:e53393. https://doi.org/10.1371/journal.pone.0053393
82. Zhang W, Zhang X, Wang S et al (2013) Comparison of the use of adipose tissue-derived and bone marrow-derived stem cells for rapid bone regeneration. J Dent Res 92:1136–1141. https://doi.org/10.1177/0022034513507581
83. Nomura A, Seya K, Yu Z et al (2013) CD34-negative mesenchymal stem-like cells may act as the cellular origin of human aortic valve calcification. Biochem Biophys Res Commun 440:780–785. https://doi.org/10.1016/j.bbrc.2013.10.003
84. Consortium, A (2000) Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. Nat Genet 26:345–348. https://doi.org/10.1038/81664
85. Shimada T, Mizutani S, Muto T et al (2001) Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. Proc Natl Acad Sci USA 98:6500–6505. https://doi.org/10.1073/pnas.101545198
86. Bai X, Miao D, Li J et al (2004) Transgenic mice overexpressing human fibroblast growth factor 23 (R176Q) delineate a putative role for parathyroid hormone in renal phosphate wasting disorders. Endocrinology 145:5269–5279. https://doi.org/10.1210/en.2004-0233
87. Stubbs J, Liu S, Quarles LD (2007) Role of fibroblast growth factor 23 in phosphate homeostasis and pathogenesis of disordered mineral metabolism in chronic kidney disease. Semin Dial 20:302–308. https://doi.org/10.1111/j.1525-139X.2007.00308.x
88. Kurosu H, Ogawa Y, Miyoshi M et al (2006) Regulation of fibroblast growth factor-23 signaling by klotho. J Biol Chem 281:6120–6123. https://doi.org/10.1074/jbc.C500457200
89. Goetz R, Beenken A, Ibrahimi OA et al (2007) Molecular insights into the klotho-dependent, endocrine mode of action of fibroblast growth factor 19 subfamily members. Mol Cell Biol 27:3417–3428. https://doi.org/10.1128/MCB.02249-06
90. Goetz R, Nakada Y, Hu MC et al (2010) Isolated C-terminal tail of FGF23 alleviates hypophosphatemia by inhibiting FGF23-FGFR-Klotho complex formation. Proc Natl Acad Sci USA 107:407–412. https://doi.org/10.1073/pnas.0902006107
91. Urakawa I, Yamazaki Y, Shimada T et al (2006) Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 444:770–774. https://doi.org/10.1038/nature05315
92. Nakatani T, Sarraj B, Ohnishi M et al (2009) In vivo genetic evidence for klotho-dependent, fibroblast growth factor 23 (Fgf23) -mediated regulation of systemic phosphate homeostasis. FASEB J 23:433–441. https://doi.org/10.1096/fj.08-114397
93. Razzaque MS (2011) Phosphate toxicity: new insights into an old problem. Clin Sci 120:91–97. https://doi.org/10.1042/CS20100377
94. Razzaque MS (2011) Osteo-renal regulation of systemic phosphate metabolism. IUBMB Life 63:240–247. https://doi.org/10.1002/iub.437
95. Razzaque MS (2009) The FGF23-Klotho axis: endocrine regulation of phosphate homeostasis. Nat Rev Endocrinol 5:611–619. https://doi.org/10.1038/nrendo.2009.196
96. Memon F, El-Abbadi M, Nakatani T et al (2008) Does Fgf23-klotho activity influence vascular and soft tissue calcification through regulating mineral ion metabolism? Kidney Int 74:566–570. https://doi.org/10.1038/ki.2008.218
97. Ichikawa S, Austin AM, Gray AK et al (2011) Dietary phosphate restriction normalizes biochemical and skeletal abnormalities in a murine model of tumoral calcinosis. Endocrinology 152:4504–4513. https://doi.org/10.1210/en.2011-1137
98. Ichikawa S, Gray AK, Padgett LR et al (2014) High dietary phosphate intake induces development of ectopic calcifications in a murine model of familial tumoral calcinosis. J Bone Miner Res 29:2017–2023. https://doi.org/10.1002/jbmr.2242
99. El-Abbadi MM, Pai AS, Leaf EM et al (2009) Phosphate feeding induces arterial medial calcification in uremic mice: role of serum phosphorus, fibroblast growth factor-23, and osteopontin. Kidney Int 75:1297–1307. https://doi.org/10.1038/ki.2009.83
100. Zhang R, Li G, Yang L et al (2016) Multiple ectopic calcifications in subcutaneous tissues with chronic renal failure: a case report. Int J Surg Case Rep 29:113–119. https://doi.org/10.1016/j.ijscr.2016.09.041
101. Lau WL, Festing MH, Giachelli CM (2010) Phosphate and vascular calcification: emerging role of the sodium-dependent phosphate co-transporter PiT-1. Thromb Haemost 104:464–470. https://doi.org/10.1160/TH09-12-0814
102. O'Brien KD, Kuusisto J, Reichenbach DD et al (1995) Osteopontin is expressed in human aortic valvular lesions. Circulation 92:2163–2168
103. Li X, Yang HY, Giachelli CM (2006) Role of the sodium-dependent phosphate cotransporter, Pit-1, in vascular smooth muscle cell calcification. Circ Res 98:905–912. https://doi.org/10.1161/01.RES.0000216409.20863.e7
104. Mathew S, Tustison KS, Sugatani T et al (2008) The mechanism of phosphorus as a cardiovascular risk factor in CKD. J Am Soc Nephrol 19:1092–1105. https://doi.org/10.1681/ASN.2007070760
105. Cox RF, Morgan MP (2013) Microcalcifications in breast cancer: lessons from physiological mineralization. Bone 53:437–450. https://doi.org/10.1016/j.bone.2013.01.013
106. Rajamannan NM (2011) Serum phosphate concentrations: a novel pre-clinical biomarker for cardiovascular calcification. J Am Coll Cardiol 58:298–299. https://doi.org/10.1016/j.jacc.2011.01.047
107. Mahmut A, Boulanger MC, El Husseini D et al (2014) Elevated expression of lipoprotein-associated phospholipase A2 in calcific aortic valve disease: implications for valve mineralization.

J Am Coll Cardiol 63:460–469. https://doi.org/10.1016/j.jacc.2013.05.105
108. Thanassoulis G, Campbell CY, Owens DS et al (2013) Genetic associations with valvular calcification and aortic stenosis. N Engl J Med 368:503–512. https://doi.org/10.1056/NEJMoa1109034
109. Kamstrup PR, Tybjaerg-Hansen A, Nordestgaard BG (2014) Elevated lipoprotein(a) and risk of aortic valve stenosis in the general population. J Am Coll Cardiol 63:470–477. https://doi.org/10.1016/j.jacc.2013.09.038
110. Abedin M, Lim J, Tang TB et al (2006) N-3 fatty acids inhibit vascular calcification via the p38-mitogen-activated protein kinase and peroxisome proliferator-activated receptor-gamma pathways. Circ Res 98:727–729. https://doi.org/10.1161/01.RES.0000216009.68958.e6
111. Kageyama A, Matsui H, Ohta M et al (2013) Palmitic acid induces osteoblastic differentiation in vascular smooth muscle cells through ACSL3 and NF-kappaB, novel targets of eicosapentaenoic acid. PLoS ONE 8:e68197. https://doi.org/10.1371/journal.pone.0068197
112. Bailey MT, Pillarisetti S, Xiao H et al (2003) Role of elastin in pathologic calcification of xenograft heart valves. J Biomed Mater Res, Part A 66:93–102. https://doi.org/10.1002/jbm.a.10543
113. Dewanjee MK, Solis E, Lanker J et al (1986) Effect of diphosphonate binding to collagen upon inhibition of calcification and promotion of spontaneous endothelial cell coverage on tissue valve prostheses. ASAIO Trans 32:24–29
114. Shen M, Kara-Mostefa A, Chen L et al (2001) Effect of ethanol and ether in the prevention of calcification of bioprostheses. Ann Thorac Surg 71:S413–S416
115. Houston B, Seawright E, Jefferies D et al (1999) Identification and cloning of a novel phosphatase expressed at high levels in differentiating growth plate chondrocytes. Biochem Biophys Acta 1448:500–506
116. Stewart AJ, Schmid R, Blindauer CA et al (2003) Comparative modelling of human PHOSPHO1 reveals a new group of phosphatases within the haloacid dehalogenase superfamily. Protein Eng 16:889–895. https://doi.org/10.1093/protein/gzg126
117. Roberts SJ, Stewart AJ, Sadler PJ et al (2004) Human PHOSPHO1 exhibits high specific phosphoethanolamine and phosphocholine phosphatase activities. Biochem J 382:59–65. https://doi.org/10.1042/BJ20040511
118. Roberts S, Narisawa S, Harmey D et al (2007) Functional involvement of PHOSPHO1 in matrix vesicle-mediated skeletal mineralization. J Bone Miner Res 22:617–627. https://doi.org/10.1359/jbmr.070108
119. Stewart AJ, Roberts SJ, Seawright E et al (2006) The presence of PHOSPHO1 in matrix vesicles and its developmental expression prior to skeletal mineralization. Bone 39:1000–1007. https://doi.org/10.1016/j.bone.2006.05.014
120. Kvam BJ, Pollesello P, Vittur F et al (1992) 31P NMR studies of resting zone cartilage from growth plate. Magn Reson Med 25:355–361
121. Yadav MC, Simao AM, Narisawa S et al (2011) Loss of skeletal mineralization by the simultaneous ablation of PHOSPHO1 and alkaline phosphatase function: a unified model of the mechanisms of initiation of skeletal calcification. J Bone Miner Res 26:286–297. https://doi.org/10.1002/jbmr.195
122. Huesa C, Yadav MC, Finnila MA et al (2011) PHOSPHO1 is essential for mechanically competent mineralization and the avoidance of spontaneous fractures. Bone 48:1066–1074. https://doi.org/10.1016/j.bone.2011.01.010
123. Kiffer-Moreira T, Yadav MC, Zhu D et al (2013) Pharmacological inhibition of PHOSPHO1 suppresses vascular smooth muscle cell calcification. J Bone Miner Res 28:81–91. https://doi.org/10.1002/jbmr.1733
124. Gremse DA (2001) Lansoprazole: pharmacokinetics, pharmacodynamics and clinical uses. Expert Opin Pharmacother 2:1663–1670. https://doi.org/10.1517/14656566.2.10.1663
125. Delomenede M, Buchet R, Mebarek S (2009) Lansoprazole is an uncompetitive inhibitor of tissue-nonspecific alkaline phosphatase. Acta Biochim Pol 56:301–305
126. Cox RF, Hernandez-Santana A, Ramdass S et al (2012) Microcalcifications in breast cancer: novel insights into the molecular mechanism and functional consequence of mammary mineralisation. Br J Cancer 106:525–537. https://doi.org/10.1038/bjc.2011.583
127. Kato H, Mineda K, Eto H et al (2014) Degeneration, regeneration, and cicatrization after fat grafting: dynamic total tissue remodeling during the first 3 months. Plast Reconstr Surg 133:303e–313e. https://doi.org/10.1097/PRS.0000000000000066