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Review article

Calcium-based biomaterials: Unveiling features and expanding applications in osteosarcoma treatment

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A B S T R A C T

Calcium, an indispensable element in bone tissues, plays a crucial role in various cellular processes involved in cancer progression. Its ubiquitous yet spatially distinct distribution in the body presents an opportunity to target calcium homeostasis as a novel strategies for cancer treatment, with specific advantages in osteosarcoma therapy. In this comprehensive review, we retrospect the calcium biology intersected with cancer progression, highlight the unveiling features of calcium-based biomaterials in regulating both bone homeostasis and cancer development. We also provide an overview of recent breakthroughs in cancer therapy that leverage calcium biomaterials, showcasing their potential to serve as versatile, customizable platforms for osteosarcoma treatment and as reservoirs for supporting bone reconstruction.

1. Introduction

Osteosarcoma, the most common primary bone cancer in both children and young adults [1], typically manifests in the distal femur, with typical hallmarks including pain and bone swelling [1]. Approximately 10–20 % of osteosarcoma present with metastasis in the lung, the bone, and occasionally the lymph nodes [2,3]. Particularly, patients with osteosarcoma may experience pathological fractures, which could have an impact on the prognosis [4,5]. The pathological fractures can promote metastasis via tumour microcirculation and introduce tumour contamination into adjacent tissues by forming a local haematoma [5]. Traditionally, osteosarcoma patients were treated with limb salvage surgeries to remove tumours aggressively, followed by multiagent chemotherapy or neoadjuvant therapy to benefit the therapeutic progress. These treatments have increased the 5-year survival rate to approximately 54 % [6]. However, challenges persist in managing metastatic disease and the prevention of recurrence. Patients with metastasis still suffer from a 5-year survival lower than 30 % [7]. Relapse occurs in about 30–40 % patients, leading to a mobility more than 70 % [8,9]. Consequently, it is imperative to develop advanced treatment strategies to address these challenges.

Calcium, as the most abundant element of bone and an essential element for various physiological processes, plays crucial roles in both osteosarcoma and bone biology, influencing secretion, proliferation, mineralization, and gene expression [10]. Moreover, calcium signal is identified as a key regulator of stroma cell behaviour, including non-excitable cells like epithelial cells and endothelial cells, and excitable cells such as neurons [11]. Recent advances in calcium-based biomaterials have achieved impressive outcomes in the therapy of multiple types of cancers. When concentrated at the tumour site, these materials serve as calcium depots and break down the local calcium balance, providing chances to induce calcium overload-related cancer cell death. The substantial impact of calcium on bone homeostasis uniquely positions calcium-based materials for osteosarcoma treatment. Importantly, the design of calcium-based materials holds potential for advancing bone tissue engineering and regeneration.

Given these considerations, there is growing interest in the development of calcium-based biomaterials for manipulating osteosarcoma treatment. This review focuses on the distinctive features of calcium-based biomaterials and and their role in enabling advanced therapeutic approaches in osteosarcoma research (Scheme 1). The review begins by delving into the intricacies of calcium biology to provide a

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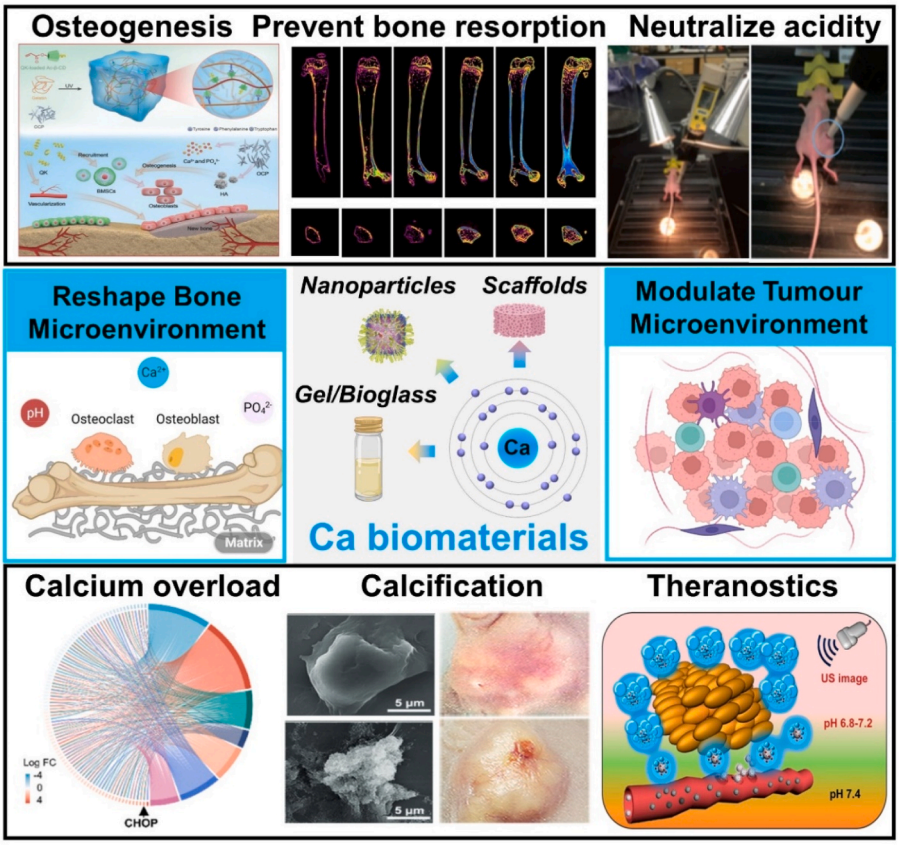

Scheme 1. Calcium-based biomaterials with diverse formats play critical roles in modulate bone and tumour microenvironment with unique features, enabling advanced treatment methods in osteosarcoma. Reprinted with permission [12–18]. (Copyright 2023 American Association for the Advancement of Science; Copyright 2021 American Chemical Society; Copyright 2016 The Royal Society of Chemistry; Copyright 2021 American Chemical Society; Copyright 2022 Elsevier B. V.; Copyright 2016 Wiley-VCH; Copyright 2015 American Chemical Society.) The middle panel of this figure is created with BioRender.com with modifications.

comprehensive understanding of its involvement in cancer processes. It subsequently highlights the specific attributes of calcium biomaterials in cancer treatment. Thereafter, recent investigation in calcium biomaterial-enabled osteosarcoma treatment is summarized in categories. Finally, perspectives on opportunities and challenges are given. In particular, the review seeks to inspire the development of sophisticated treatment strategies based on calcium biomaterials by emphasizing a comprehensive understanding and effective utilization of the crossroads between bone and tumours.

2. Calcium biology in cancer progression

2.1. Calcium distribution and homeostasis

As summarized in Table 1, the distribution of calcium ion (Ca^{2+}) shows large discrepancies between locations under resting conditions. The extracellular milieu maintains a high Ca^{2+} concentration of 1–2 mM, in contrast to the intracellular Ca^{2+} concentration (about 100 nM) [19–21]. With stimulus, Ca^{2+} entry from the extracellular milieu to cytoplasm, triggering a dramatic transfer of Ca^{2+} into mitochondria and endoplasmic reticulum (ER). As detected, the mitochondrial Ca^{2+} can elevate to more than 100 μM in HeLa cells with stimulus [22,23].

Table 1
The Ca^{2+} concentration under resting conditions [19–21].

Location	Ca^{2+} concentration
Extracellular milieu	1–2 mM
Cytoplasm	~100 nM
Nuclei	~100 nM
Mitochondria	~100 nM
Endoplasmic reticulum	0.1–0.5 mM

The Ca^{2+} gradient is reliant on activation of calcium channels, pumps, and exchangers (Fig. 1). Ca^{2+} influx from extracellular milieu to cytoplasm is conducted by a large arsenal of approaches, such as transient receptor potential channels (TRPs) and calcium release-activated calcium channels encoded by *ORAI*. TRPs consist of six subfamilies and show a relatively non-selective permeability to Ca^{2+}, while their activity can be regulated by stimuli [24]. ORAI is an important selective Ca^{2+} influx channel on cell membranes [25]. In excitable cells, purinergic receptors (P2XRs) and voltage-gated calcium channels (Ca_V) provide Ca^{2+} conduction in an ATP-consuming manner. Activation of ORAI1 is associated with ER storage of Ca^{2+} in the process known as "store-operated calcium entry (SOCE)" [26]. During this Ca^{2+} transport, the Ca^{2+} sensor stromal interaction molecule 1 (STIM1) on the ER membrane interacts with nearby ORAI on the plasma membrane through redistribution and transformation, leading to Ca^{2+} depletion from cytosol and storage in the ER [26,27]. Meanwhile, intracellular Ca^{2+} can be transferred to the mitochondria when necessary. The transmembrane mitochondrial calcium uniporter (MCU) complex is responsible for the majority of mitochondrial calcium uptake [28]. Of note, the MCU holds a low Ca^{2+} affinity when compared to those calcium influx proteins on the ER, making mitochondria as an alternative organelle for calcium storage and balance maintenance. As reported, the MCU-depended mitochondrial calcium influx requires a cytosolic calcium concentration of approximately 5–10 μM [29]. Additionally, the nuclear envelope is not a measurable diffusion barrier for Ca^{2+}, allowing direct entry and exit of Ca^{2+} through the nuclear pore complex [30,31].

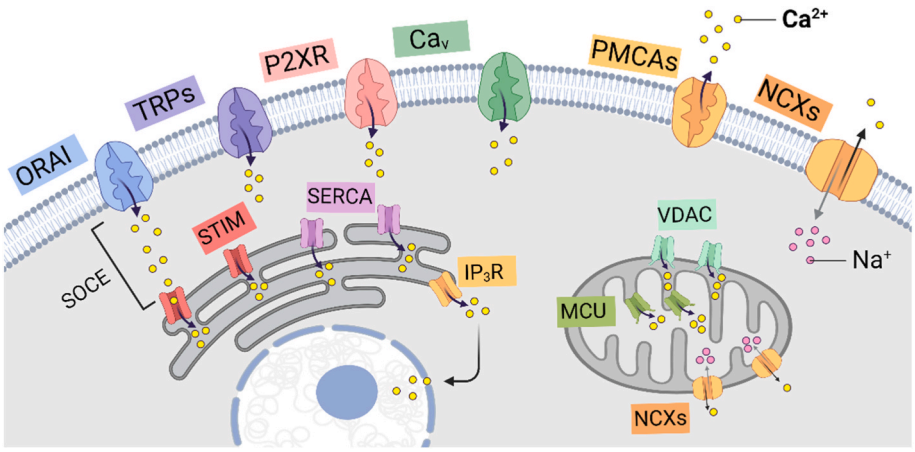

Fig. 1. Typical Ca^{2+} channels, pumps, and exchangers on the cell membrane and organelle membranes. Ca^{2+} concentration discrepancy is controlled by a series channels, pumps, and exchangers. The entry of Ca^{2+} into cells is mediated by the combination of transmembrane proteins such as calcium-release-activated calcium channel protein encoded by the *ORAI* gene (ORAI in the figure), transient receptor potential (TRP) channels, purinergic receptors (such as P2XR), and voltage gated calcium channels (Ca_v). Ca^{2+} efflux to the extracellular milieu is achieved by plasma membrane Ca^{2+} ATPases (PMCAs) or through Na+/Ca^{2+} exchangers (NCXs). The endoplasmic reticulum (ER) stores Ca^{2+} by stromal interaction molecules (STIM) and sarco/endoplasmic Ca^{2+} ATPases (SERCA). The transformed STIM can trap nearby ORAI and contributes the store-operated calcium entry (SOCE). Binding of an inositol triphosphate to its receptor (IP3R) allows Ca^{2+} releases from ER. Translocation of Ca^{2+} into the mitochondrial matrix is mediated by the voltage-dependent anion channel (VDAC) on the outer membrane and the mitochondrial calcium uniporter (MCU) on the inner membrane, while efflux through NCX-mediated ion exchange. Figure created with BioRender.com.

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Ca^{2+} efflux from cells occurs against the calcium gradient, resulting in energy consumption. The major pathways for Ca^{2+} efflux are illustrated in Fig. 1. Plasma membrane Ca^{2+}-transporting ATPases (PMCAs) can be utilized for Ca^{2+} pumping [32]. This process is usually accompanied by other ion influx, for example, the sodium-calcium exchanger (NCX) proteins efflux one Ca^{2+} and influx three Na^{+} simultaneously [33]. The NCXs are found on the plasma, mitochondrial, and ER membranes [34,35]. Meanwhile, the ER expresses Ca^{2+} permeable inositol 1, 4,5-trisphosphate receptor (IP3R) to efflux Ca^{2+} into the cytosol. Taken together, these channels, pumps, and exchangers work in concert to maintain cellular calcium homeostasis.

2.2. Calcium in tumour process regulation

Ca^{2+} regulates many cellular processes as a second messenger in cell signalling. As in Fig. 2A, the intracellular calcium is involved in the regulation of gene expression, energy production, protein folding, and

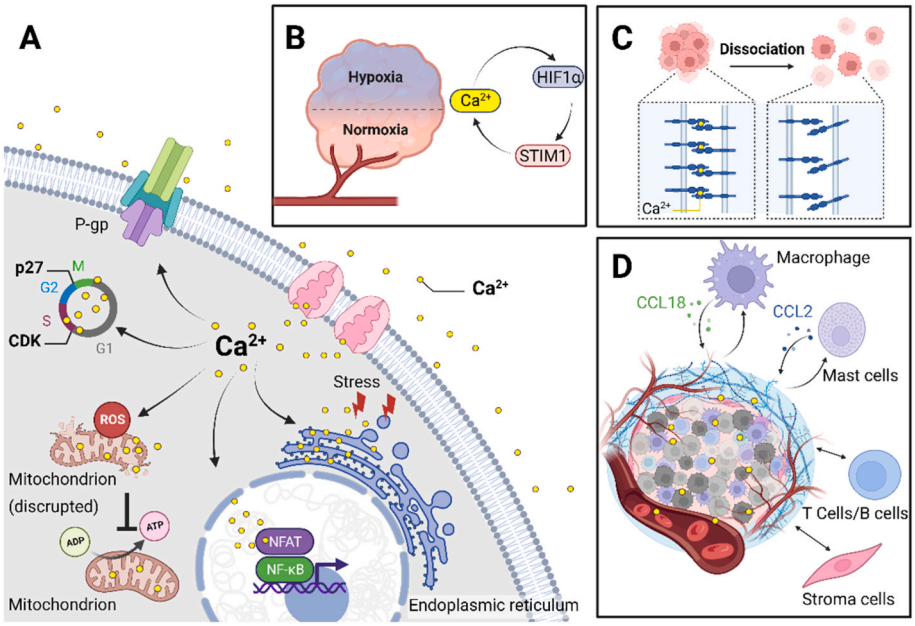

Fig. 2. Intracellular and extracellular calcium regulation on bioprocesses and the intersection with tumour progression.
(A) Ca^{2+} participate in the intracellular bioprocesses. Ca^{2+} regulates gene expression via Ca^{2+}–dependent transcription factors such as nuclear factor of activated T cells (NFAT) and nuclear factor kappa B (NF-κB), affects the checkpoints in mitosis (CDK and p27), and induces overexpression of multidrug resistance 1 (*MDR1*) gene and related proteins such as P-glycoprotein (P-gp). Ca^{2+} plethora causes stress to endoplasmic reticulum and mitochondrial disruption, resulting in impaired protein folding and energy metabolism. **(B)** STIM1-mediated Ca^{2+} influx participates in hypoxia response through HIF1α signalling. **(C)** Solid tumour can be dissociated when the Ca^{2+} dependent connections are smashed via extracellular calcium elimination. **(D)** Calcium signalling in tumour cells may lead to the changes of immune cells and stroma cells in the microenvironment and *vice versa*. Figure created with BioRender.com.

other survival processes. Ca^{2+} binding is essential for the induction of transcription factors such as NFATs and NF-kB, leading to downstream gene expression [36,37]. Cytosolic Ca^{2+} levels are critical for mitosis, as some cell cycle checkpoints are calcium-dependent, including the cell-dependent kinase 2 (CDK2) for G1/S and p27 for G2/M [38]. Specifically, Ca^{2+} has been implicated with multidrug resistance in cancer. Upregulation of TRPC5, an ion channel for Ca^{2+} influx, endows the overexpression of multidrug resistance protein 1 (MDR1) [39]. Thus, calcium channel blockers have shown therapeutic effect in a cohort of multidrug resistant patients [40]. Mitochondrial Ca^{2+} shows intricate intersections. The increase in Ca^{2+} alters the activity of mitochondrial matrix enzymes, thereby increasing ATP production [28]. Meanwhile, high Ca^{2+} levels are linked to reactive oxygen species (ROS) production and mitochondrial damage when in an overload state, resulting in apoptosis and oxidative stress [41]. Ca^{2+} stored in the ER is strongly associated with intracellular signalling in response to multiple signals, promoting cancer cell survival. However, abnormally high Ca^{2+} leads to the unfolded protein in the ER, causing ER stress and dysfunction [42].

Extracellular calcium is related to the cancer cell responses to the environment, including physical conditions and the immune microenvironment. STIM1-mediated Ca^{2+} influx increases the stability of hypoxia-inducible factor-1α, playing a vital role in cancer cell responses to the hypoxic microenvironment (Fig. 2B) [43]. The calcium-dependent cadherin family is the key connexins for cell-cell junctions, and is responsible for the deterioration of dense solid tumours due to the low efficacy of various treatment agents (Fig. 2C). Chelating of Ca^{2+} via disruption of cadherins can be applied to dissociate solid tumours [44,45]. In addition, calcium alter tumourigenesis via crosstalk with the microenvironment (Fig. 2D). Ca^{2+} is involved in tumour immunology due to its role in T cell and B cell biology. Meanwhile, calcium signalling alters the expression of chemokines and their receptors. Activation of L-type calcium channels under hypoxia can elicit the secretion of CCL2 by mast cells at the tumour site [46]. The CCL18 released from tumour-associated macrophages plays a critical role in promoting breast cancer metastasis through its receptor via Ca^{2+} signalling activation [47]. In addition, some non-immune cells may be involved in the crosstalk, such as the cancer-associated fibrosis and mesenchymal stem cells. Taken together, the regulation of intracellular and extracellular calcium levels will influence cancer progression.

3. Features of calcium-based biomaterials

Biomaterials such as nano-sized drug delivery platforms and implant scaffolds are widely used in cancer therapy. In principle, nano-sized biomaterials would benefit from at least the following aspects: (1) ameliorating the bioavailability of hydrophobic drugs by increasing their solubility, (2) enabling the simultaneous payload of multiple anticancer agents, (3) protecting against enzymatic degradation, (4) enhancing the accumulation at the tumour site (5) facilitating the phagocytosis to tumour or other target cells, (6) prolonging the circulation and retention time, (7) increasing the penetration ability to barriers such as mucus, tight junction, or gastrointestinal cavity. On the other hand, biomaterial-based scaffolds provide additional technical support to the bone, enabling the bone repair and other orthopaedic treatments. Recently, biomaterials have been designed to exert their intrinsic physical/chemical properties to achieve therapeutic capabilities in cancer, in particular by taking advantage of the special elements. To this end, numerous nano-sized biomaterials have been employed in aspects such as enhancing signal intensity during diagnosis [48,49], inducing photo-triggered hyperthermia [50], generating reactive oxygen species [51], and remodelling the tumour microenvironment by interacting with the accumulated metabolic product/byproducts [52]. Among various elements, calcium holds unique advantages for osteosarcoma treatment compared to other kinds of elements. Calcium-based biomaterials hold a high affinity for the bone tissue, and can be applied directly by the bone tissue. The involvement of calcium-based materials in osteohomeostasis, cancer evasion, and immune regulation also raises opportunities of their synergy with current applied therapeutic agents.

3.1. Effects on osteogenesis

Calcium is an indispensable element in osteogenesis. Indeed, the bone tissue matrix is rich in several calcium-binding proteins (CBPs), leading to the specific affinity and accumulation of calcium at this site [53]. Bone matrix proteins are rich in γ-carboxyglutamic acid (GLA) residues, which provide calcium binding sites (Fig. 3A) [54]. A study of the binding affinity of matrix proteins to hydroxyapatite revealed that calcium is critical in enhancing the binding affinity, suggesting a potential role for calcium in maintaining bone homeostasis [55]. Besides, osteocytes contain abundant intracellular CBPs, such as calmodulin and protein kinase C (PKC) [56,57]. These CBPs are critical for their metabolic processes, leading to a high calcium demand for bone sites. Theoretically, anticancer drugs loaded with calcium biomaterials would be more likely to accumulate at the bone site. And this insight has led to numerous studies of calcium-based carriers for osteosarcoma drugs [58,59]. Moreover, calcium-based biomaterials promote the osteogenic process to keep bone homeostasis, which is unfavourable to cancer growth. As in Fig. 3B, local Ca^{2+} release can enhance osteoinductive activity by interacting with osteoblasts [12].

3.2. Regulation on TAM and osteoclast

Reversing the immune suppression in the tumour microenvironment is the key to successful tumour immunotherapy. T cells, B cells, and macrophages tend to predominate in the tumour microenvironment, albeit the composition of infiltrating lymphocytes is highly heterogeneous between patients [60,61]. T cell activities are critical for the adaptive anticancer immunity. The dynamic regulation of calcium concentration controlled by a plethora of Ca^{2+} channels is tightly linked to the T cell immunity, differentiation, and metabolism, as well summarized in previous review articles [62,63]. In general, the resting cytosolic calcium concentration of T cells is 10 times lower than that of the extracellular spaces [62,64], which favours the rapid and convenient Ca^{2+} influx during T cell bioprocesses. Apart from T cells, macrophages, or tumour-associated macrophages (TAMs) are involved in shaping the cancer immune environment, playing a dual role in tumour immunity. These cells are derived either from infiltrating monocytes or from tissue-resident macrophages originating from the embryo [65]. Activated TAMs can be unofficially classified as M1-like and M2-like. In general, M1-like TAMs are pro-inflammatory and facilitate anticancer immune responses, whereas M2-like TAMs are anti-inflammatory and lead to tumour immunosuppression. In particular, bone tissue is rich in osteoclasts, a type of highly specialized multinucleated macrophage. Osteoclasts are responsible for bone resorption and skeletal remodelling to maintain bone homeostasis under normal conditions, while they may interact with osteosarcoma during the cancer invasion. Osteoclasts are derived from the monocytes lineage, with a requisition of RANKL and M-CSF signalling for differentiation from pre-osteoclasts [66]. As is well known, calcium-based materials are widely applied to treat osteoporosis and other types of bone loss. Recent studies have shown that osteoclast-mediated bone resorption is vital in the osteosarcoma regulation. As in Fig. 4A, osteoclast activation is associated with a “vicious circle’’ of tumourigenesis and bone metastasis. During the tumourigenesis, osteosarcomas manipulate osteoclasts to resorb bone tissue by secreting stimulatory cytokines [67]. Similarly, metastatic breast cancer cells at the bone site also hijack osteoclasts to promote bone degradation and create a metastatic niche [68,69]. Therefore, a wide range of osteoclast inhibitors initially applied to osteoporosis are now being used in osteosarcoma patients in combination with traditional therapeutics.

In macrophage functions, calcium participates in their differentiation, activation, polarization, and crosstalk. Extracellular calcium can be taken up by monocytes/macrophages via micropinocytosis, resulting in

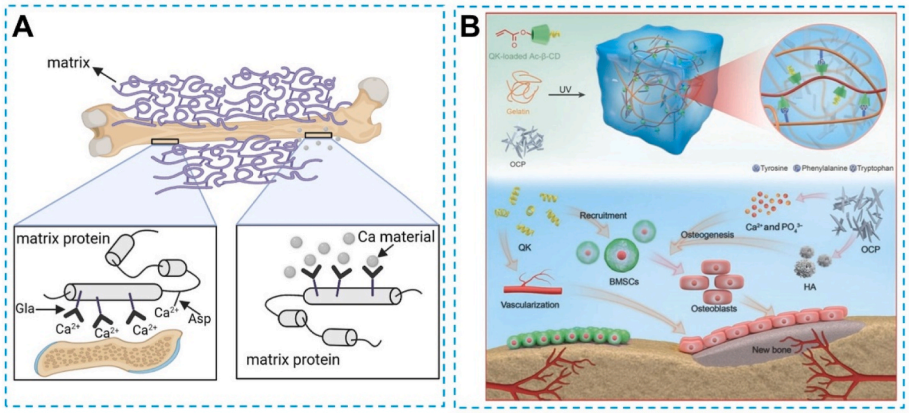

Fig. 3. Effects of calcium-based biomaterials on osteogenesis.
(A) Calcium-based biomaterials bind to the matrix proteins, leading to high affinity to bone site; **(B)** Deposition of materials enhance local Ca^{2+}, leading to enhanced osteoinductive activity [12]. Reprinted with permission. (Copyright 2023 American Association for the Advancement of Science) Fig. 3A created with BioRender.com.

the increased lysosomal activity, inflammasome activation, and inflammasome-related cell death [70,71]. During this process, the elevation of extracellular calcium acts as a danger signal to induce the monocyte differentiation into macrophage-like cells [72]. Calcium plays an essential but divergent role in the macrophage polarization process. Calcium phosphate ceramics with different phase compositions lead to either M1-or M2-like macrophage polarization [73]. This phenomenon may be associated with the distinct Ca^{2+} entry channel, intracellular signalling, and mitochondrial metabolism [74,75]. These findings may shed light on the regulation of M1-like macrophage polarization for osteosarcoma treatment with calcium materials. Calcium involves in the differentiation and apoptosis of osteoclasts. Bone resorption by osteoclasts results in a local "calcium oscillation" that can increase the extracellular Ca^{2+} concentration to 40 mM [76]. This calcium oscillation activates RANKL-induced osteoclastogenesis [77,78]. Simultaneously, a high extracellular calcium concentration also leads to osteoclast apoptosis as a negative feedback [79,80]. In addition, calcium levels also affect stroma cells, such as mesenchymal stem cells (MSCs). Local calcium elevation caused by bone resorption alters the behaviour of MSCs by remodelling their phenotype and thereby contributing to osteogenesis [81,82]. These behaviours implicate a repressive state for the tumour. However, whether calcium is involved in the crosstalk between MSC and osteosarcoma remains to be elucidated. These investigations inspire the development and application of calcium-based materials in bone research.

Calcium-based materials are beneficial for macrophage regulation as they exert an indispensable element in bone mineralization and show an intrinsic high affinity for bisphosphonates that inhibit bone resorption. Calcium ions and the bisphosphonate agent sodium zoledronate can form metal-organic frameworks (MOF) in a facile approach by exploiting the affinity of phosphates for calcium. As in Fig. 4B, an asymmetric double-layered lipid-coated MOF nanoparticle (CaZol nMOF) was fabricated using a microemulsion method [83]. The CaZol nMOF showed intracellular Ca^{2+} release under acidic endosomal/lysosomal conditions. Recent investigations have shown that calcium-based materials can inhibit osteoclast activity. Bone resorption by osteoclasts can be controlled by phosphate ceramics (CPC) in a steric separation manner (Fig. 4C) [84]. By changing the Ca/P ratio of CPC from 1.4 to 1.67, the relative resorption areas were 17–50 % of that in the control group (Fig. 4D). Employing this advantage, calcium-based materials would benefit the osteosarcoma therapy by inhibiting the osteolysis at the lesion site. As shown in Fig. 4E, a multifunctional V-RZCD was synthesized by incorporating anticancer drug DOX and coating VEGF-modified erythrocyte membrane to a metal-organic framework consisted by calcium ions and zoledronic acid (ZA) [13]. The V-RZCD effectively inhibited Saos-2 osteosarcoma growth in Balb/c nude mice through specifically binding to the vascular endothelial growth factor (VEGF) receptors that are highly expressed on the surface of cell surface. Moreover, the osteolysis was inhibited by ZA frame, with osteogenesis promoted by calcium ions. Similarly, an ammonia-induced calcium phosphate nanostructure showed promising application in inhibiting bone metastasis [85].

3.3. Neutralizing acidity in the microenvironment

Due to the high energy-consuming and hypoxic state, tumour cells more readily employ anaerobic glycolysis instead of the more efficient oxidative phosphorylation to acquire energy [86]. Consequently, the metabolic-derived pyruvate and lactate accumulate at the tumour site, leading to an acidic microenvironment. As in Fig. 5A, the average extracellular pH (pH_e) values of skeletal muscle and bone marrow are 7.35 and 7.15, respectively, whereas that of sarcoma is 6.7 [87,88]. The increased acidity of the environment appears to accelerate tumour progression, including local tumour growth, immune tolerance, and metastasis [89]. Therefore, neutralizing acidity would be beneficial in several cancer therapies.

Most calcium-based materials are alkaline, giving them a good acid neutralizing potential. As in Fig. 5B and C, calcium carbonate with an average size of less than 100 nm (nano-$CaCO_3$) was able to increase the media pH from an average of 7.14–7.25 when incubated with MDA-MB-231 cells for 3 days in a 24-well plate. The neutralization effect of calcium carbonate nanoparticles was also observed in tumour-bearing mouse models. As in Fig. 5D, the intratumoral pH was detected using an invasive pH electrode probe with a diameter of 5 mm to putatively interpret the pH_e. The results showed that the tumour pH increased asymptotically to 7.4 after intravenous administration (Fig. 5E). This neutralization effect further demonstrate the potential therapeutic benefit of tumour growth stasis induction. Apart from calcium carbonate, calcium hydroxide-based material also exhibits similar capabilities. As in Fig. 5F, calcein-functionalized calcium-aluminium layered double hydroxide (CALC) nanosheets were fabricated for the treatment of osteoporosis. The mildly alkaline CALC nanosheets could neutralize the excess H^+ and responsively release calcein into the bone microenvironment, resulting in an effective osteoporosis-immunotherapeutic modality.

3.4. Calcium overload toxicity

Recent progress in the understanding of calcium biology has bridged a new nexus between disruption of calcium homeostasis and

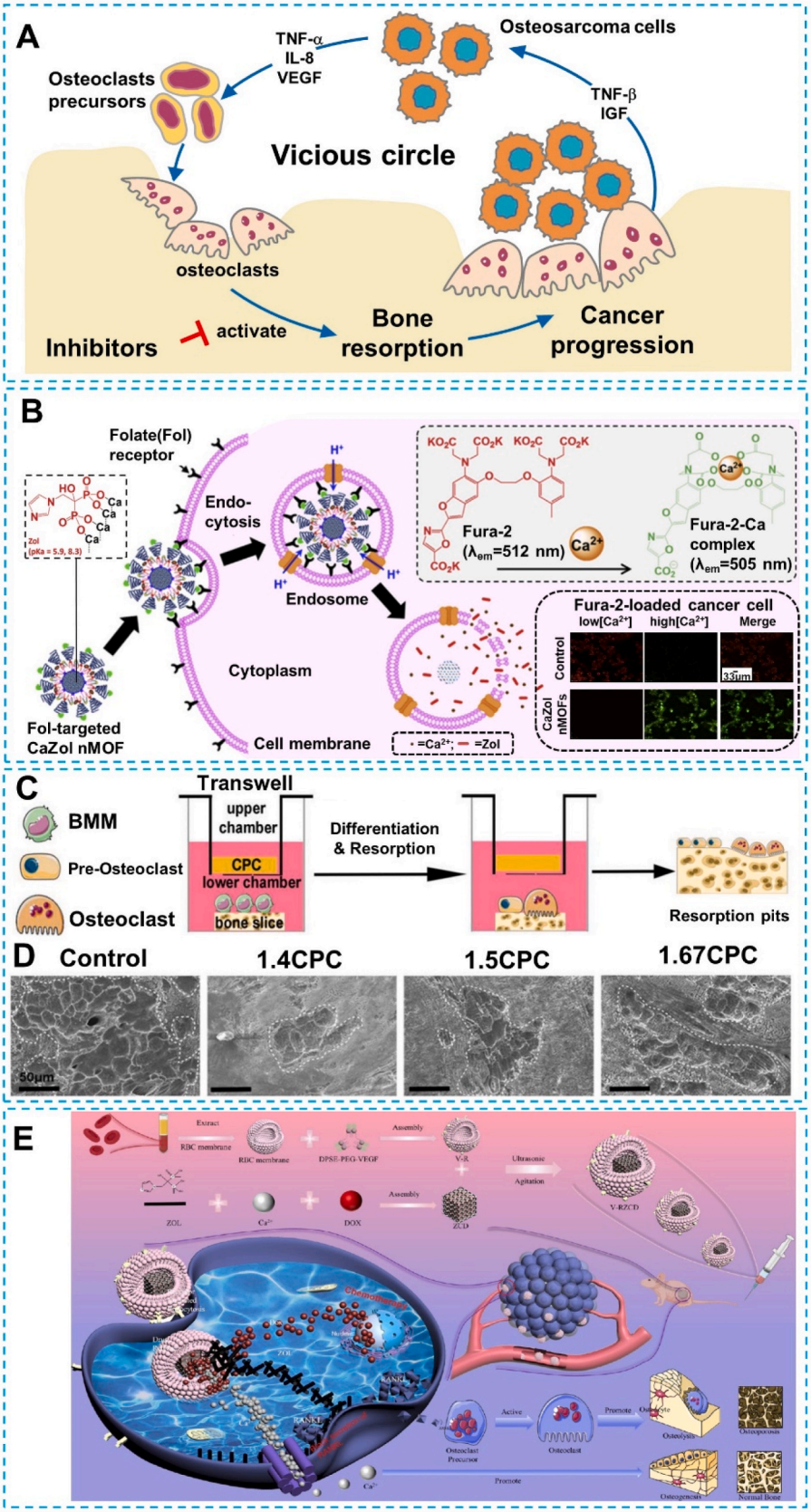

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Fig. 4. Regulation of TAM and osteoclast by calcium-based nanoparticles.
(A) The role of TAM and osteoclast in tumour progression; **(B)** The formation of MOF using calcium ion and bisphosphonate (CaZol nMOF) and its intracellular calcium release [83]; **(C)** Schematic design of a transwell assay to investigate calcium phosphate ceramics (CPC) with different Ca/P ratios on osteoclast-induced bone resorption and **(D)** Resorption pits images of bone slices with corrosion area circled with white dash lines [84]; and **(E)** The anti-osteosarcoma and anti-osteolytic effects of calcium-zoledronate-based multifunctional nanoparticles (V-RZCD) [13]. Reprinted with permission. (Copyright 2016 Elsevier B.V.; Copyright 2021 American Chemical Society).

cytotoxicity, leading to a potential application in calcium-mediated tumour therapy. Indeed, the intracellular calcium transport to mitochondria, ER, and even nuclei to maintain calcium homeostasis. Under high Ca^{2+} conditions, excess Ca^{2+} will accumulate in cells, causing a so called "calcium overload" and leading to a disruption of intracellular calcium homeostasis. Calcium overload in mitochondria and ER can lead to disruption of these organelles, resulting in oxidative stress-related cell damage and irregular fold-related protein dysfunction [92,93]. Calcium overload in nuclei and cytoplasm induce abnormal cell signal transduction, causing the abnormal regulation of downstream processes [94].

Calcium-based biomaterials have been manipulated to induce calcium overload caused toxicity in treated tumour cells. By combining calcium materials with calcium signal regulators, efficient nanodrugs can be designed to induce calcium overload for cancer therapy. Li et al. reported that a biosafety flavone kaempferol-3-O-rutinoside (KAE) can effectively disrupt calcium homeostasis and promote calcium influx, while the nano-sized $CaCO_3$ serves as a Ca^{2+} supplier and an ideal delivery platform to enhance the KAE bioavailability [95]. With cell membrane coating, the formed M@$CaCO_3$@KAE show mitochondrial damage ability, leading to the cytoskeletal collapse and further tumour apoptosis. In addition, the intracellular release of some calcium materials is able to catalyse reactive oxidative species (ROS) and Ca^{2+} in the cytoplasm. CaO_2 loaded with zeolitic imidazolate framework-8 (ZIF-8) and the anticancer drug doxorubicin (DOX) was coated with hyaluronic acid (HA) to form CaO_2@ ZIF-8/DOX@HA nanoparticles [15]. These particles exhibited a rose-like monodisperse spherical structure under TEM observation (Fig. 6A). After internalized by tumour cells, the released CaO_2 can generate H_2O_2 and intracellular Ca^{2+} (Fig. 6B). The excess Ca^{2+} and ROS efficiently disrupt mitochondria to induce apoptosis. The oxidant stress of calcium overload on mitochondria can be further amplified to benefit cancer therapy. Dong et al. fabricated calcium carbonate ($CaCO_3$) based nanoparticles named BSA-TCP/Fe@$CaCO_3$-PEG [96]. The STEM images revealed the core-shell structure of these particles (Fig. 6C). When treated with an ultrasounic field, BSA-TCP/Fe@$CaCO_3$-PEG exhibited triple amplification of tumour oxidative stress by (1) Ca^{2+} overload, (2) release of L-buthionine sulfoximine (BSO) to deplete GSH, and (3) meso-tetra-(4-carboxyphenyl) porphine (TCPP)-mediated sonodynamic therapy (SDT), leading to remarkable cancer treatment efficacy (Fig. 6D). Similarly, Gong et al. showed a facile approach to exfoliate bulk CaH_2 into nano-CaH_2 for synergistic hydrogen-immune cancer therapy [97]. These nano-CaH_2 can dispersion can generate abundant H_2 bubbles in aqueous solution, simultaneously releasing Ca^{2+} and OH^-(Fig. 6E). Intratumoral injection of nano-CaH_2 led to a rapid increase in tumour pH ($\triangle$pH = 0.45, Fig. 6F). Moreover, this nano-CaH_2 benefited anti-CTLA-4 immunotherapy in a synergistic way by (1) providing hydrogen to the TME, (2) calcium overload-induced cancer apoptosis, and (3) neutralizing the acidity in the TME (Fig. 6G). Apart from the effect on mitochondria, calcium material also led to ER dysfunction. GO enrichment analysis of differentially expressed genes (DEGs) revealed that calcium material-treated cells led to changes in ER functions compared to PBS-treated cells, such as DEGs catalogued as "response to ER stress" and "response to unfolded protein" (Fig. 6H) [16]. The GSEA analysis further confirmed significantly activating unfolded proteins in a mouse model treated with the same calcium-based nanoparticle (Fig. 6I) [16]. In this case, the transcription factor CHOP, known for its regulatory functions in response to ER stress, played a central role in the calcium nanoparticle induced apoptosis (Fig. 6J) [16]. Taken together, these bioinformatic analysis showed how calcium overload induces cancer apoptosis via affecting ER. Of note, calcium overload can bring multiple issues to tumour cells simultaneously. In most of the paradigm researches demonstrated in Fig. 6, the material-related toxicity was accompanied by changes in mitochondrial damage, ROS generation, ER stress, and even calcification, which will be discussed in the later section.

3.5. Tumour calcification

As a consequence of disrupt calcium homeostasis, calcium deposition causes calcification. In the clinic, spontaneous calcification has been detected in a wide range of cancers, and has been shown to be a benign prognostic factor [98,99]. In tumour biology, calcification is always associated with the local haemorrhage caused by damaged blood vessels. In addition, tumour cells with disordered calcium metabolism also showed calcification. The usage of calcium-based biomaterials provides external calcium supplementation to tumour cells, which may promote calcification in the tumour site [100,101]. As illustrated in Fig. 7A, a drug-free calcium phosphate (CaP) nanomaterial decorated with folate (FA) was fabricated to induce cancer cell-targeted calcification (CCTC) in a drug-free manner for cancer therapy [17]. After treated with the targeted CaP nanomaterials, significant calcification of HeLa cells overexpressing folate receptors can be visualized under SEM, and the disruption of tumour tissue can be observed by eye when CCTC occurred (Fig. 7B). Zhang et al. reported a highly efficient tumour therapy strategy using sodium hyaluronate-modified calcium peroxide nanoparticles (SH–CaO_2 NPs) [102]. After SH-CaO_2 treatment, the calcified cells can be observed as red using alizarin methods or metallic silver using von Kossa method (Fig. 7C). In mouse models, the SH-CaO_2 led to tumour shrinkage, with evenly distributed metallic silver spots observed under von Kossa staining, indicating the calcification (Fig. 7D). The calcification further led to enhanced signals on micro-CT observation (Fig. 7D). The material-based calcification was used to treat osteosarcoma. Of note, the calcified osteosarcoma may further benefit local bone by providing Ca^{2+} to enhance osteogenesis. As a good paradigm shown in Fig. 7E–F, bioactive glass (BG) containing Cu and Ca was synthesized [103]. Cu species induced Fenton-like reaction-assisted deadly free radicals, whereas Ca species present enhanced calcification for osteogenesis. Taken together, the Ca/Cu-BG nanocomposites performed synergistic osteosarcoma killing and bone generation abilities.

3.6. Calcium material-enabled diagnosis

Computed tomography (CT) scanning is sensitive to mineralized deposits. Therefore, the calcification caused by calcium materials can be utilized in tumour diagnosis by providing high-attenuated CT signals. Indeed, traditionally applied contrast agents for CT imaging are normally based on high atomic number elements such as iodine, gold, or copper, whereas calcium-based imaging agents are rare [104–107]. However, the induced calcification during the treatment can be detected and used as a hallmark for tumour remission. Consequently, calcium material would benefit in a theranostic manner. Liu et al. reported a TME responsive nanocomposite based on CaO_2 for trimodal-enhanced enzyme dynamic cancer therapy and CT imaging [108]. The so-called DCC-HA nanoparticles effectively induce tumour death via generating ROS and depleting GSH (Fig. 8A). Moreover, the excess Ca^{2+} causes tumour calcification to accelerate tumour necrosis, with enhanced CT imaging efficacy (Fig. 8B).

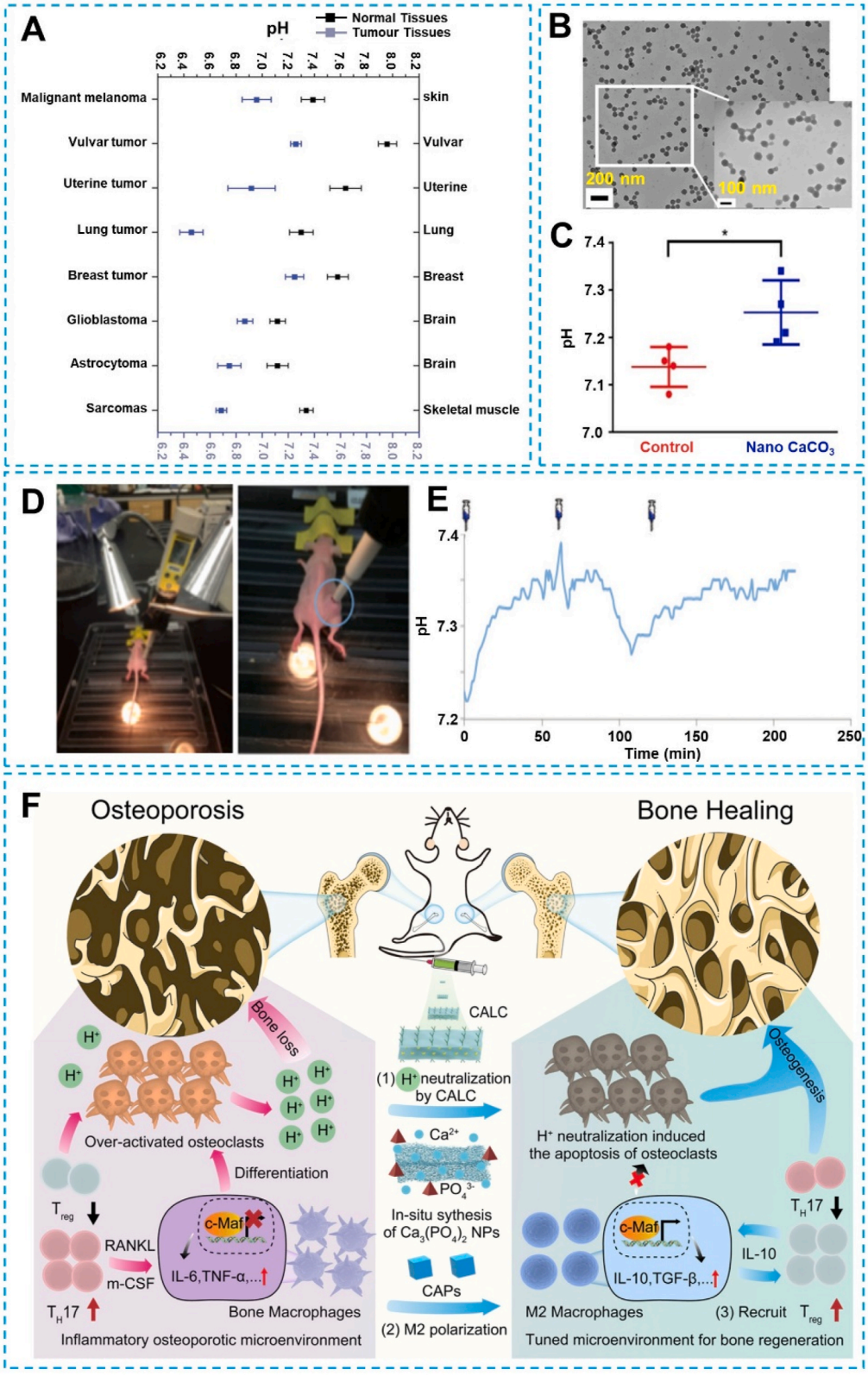

Fig. 5. Calcium-based biomaterials enabled acidity neutralization.
(A) Comparison of pH_e of normal tissue and corresponding tumour site [87]; **(B)** The TEM image of nano sized $CaCO_3$ and **(C)** the capability of Nano $CaCO_3$ on pH_e regulation in tumour cell culture [90]; **(D)** Detection of intratumoral pH in a mouse model, and € intravenous administration of $CaCO_3$ led to pH elevation [14]; **(F)** Scheme of calcium-aluminium layered double hydroxide for acid neutralization and immune regulation [91]. Reprinted with permission.. (Copyright 2018 The Royal Society of Chemistry; Copyright 2021 Springer Nature; Copyright 2016 The Royal Society of Chemistry; Copyright 2022 American Chemical Society.)

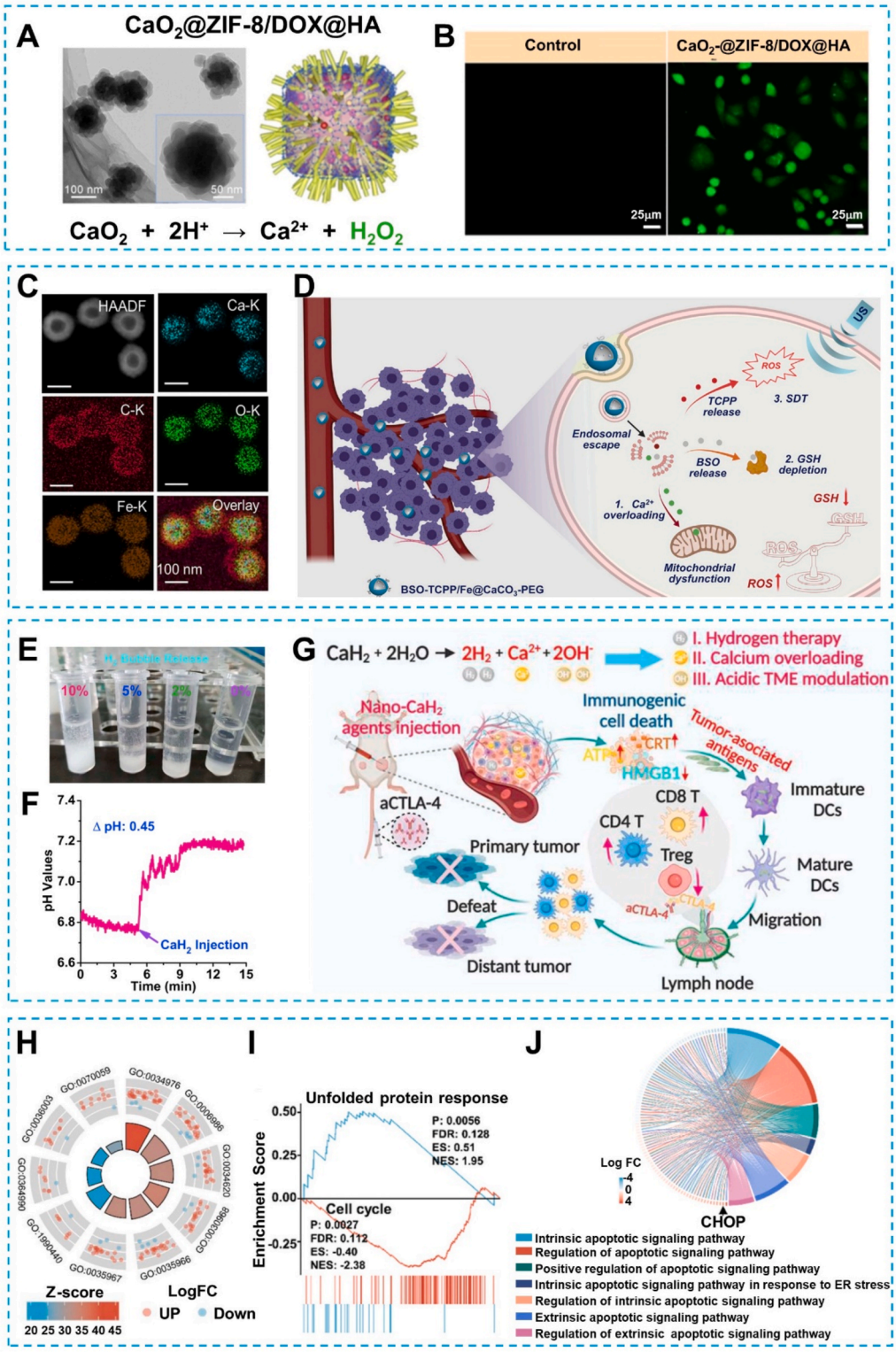

Fig. 6. Inducing calcium overload-related cytotoxicity to treat cancer using calcium-based biomaterials.
(A) The TEM image of CaO_2-zeolitic imidazolate framework-8 (ZIF-8)-doxorubicin (DOX) nanoparticle coated with hyaluronic acid (CaO_2@ ZIF-8/DOX@HA) and **(B)** the induction of oxidative stress by generating intracellular ROS [15]; **(C)** The STEM images showing element distribution in $CaCO_3$ based nanoparticle named BSA-TCP/Fe@$CaCO_3$-PEG and **(D)** the anticancer mechanism by triple amplification of tumour oxidative stress [96]. CaH_2 showed **(E)** bubble generation and **(F)** pH adjustment abilities, leading to **(G)** enhanced anti-CTLA-4 immunotherapy in a synergistic manner [97]; **(H)** GO enrichment analysis indicating calcium-material-related calcium overload causing ER response and intrinsic apoptosis, **(I)** GSEA analysis identified unfolded proteins during this process, and **(J)** Chord diagram of apoptosis related GO pathways suggested CHOP played a critical role of ER stress caused by calcium materials [16]. Reprinted with permission.. (Copyright 2021 American Chemical Society; Copyright 2020 Elsevier B.V.; Copyright 2022 Elsevier B.V.; Copyright 2022 Elsevier B.V.)

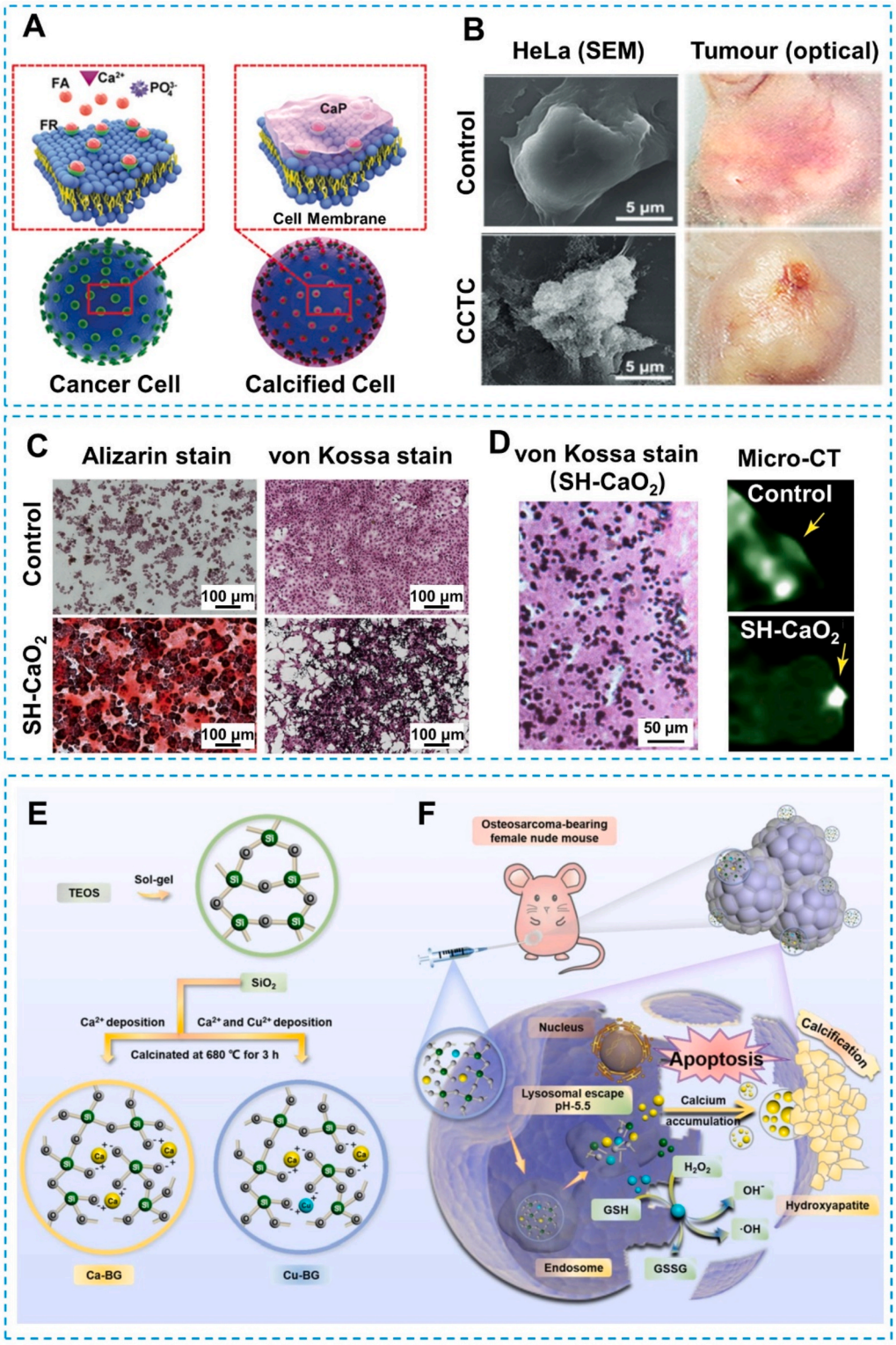

Fig. 7. Calcium material enhanced tumour calcification.
(A) Schematic illustration of cancer cell-targeted calcification (CCTC) caused by calcium phosphate nanomaterial with folate (FA) modification, and **(B)** the images of calcified HeLa cells under SEM or solid tumour [17]; **(C)** Alizarin and von Kossa stain images to observe the calcified cells treated with $SH\text{-}CaO_2$ NPs, and **(D)** the confirmation of tumour calcification using von Kossa stain and micro-CT [102]; **(E)** Scheme of the fabrication of Ca/Cu based bioactive glasses (BG) and **(F)** the mechanism of synergistic anti-osteosarcoma effect of Ca/Cu-BG nanocomposites [103]. Reprinted with permission.. (Copyright 2016 Wiley-VCH; Copyright 2019 Elsevier B.V.; Copyright 2022 Elsevier B.V.)

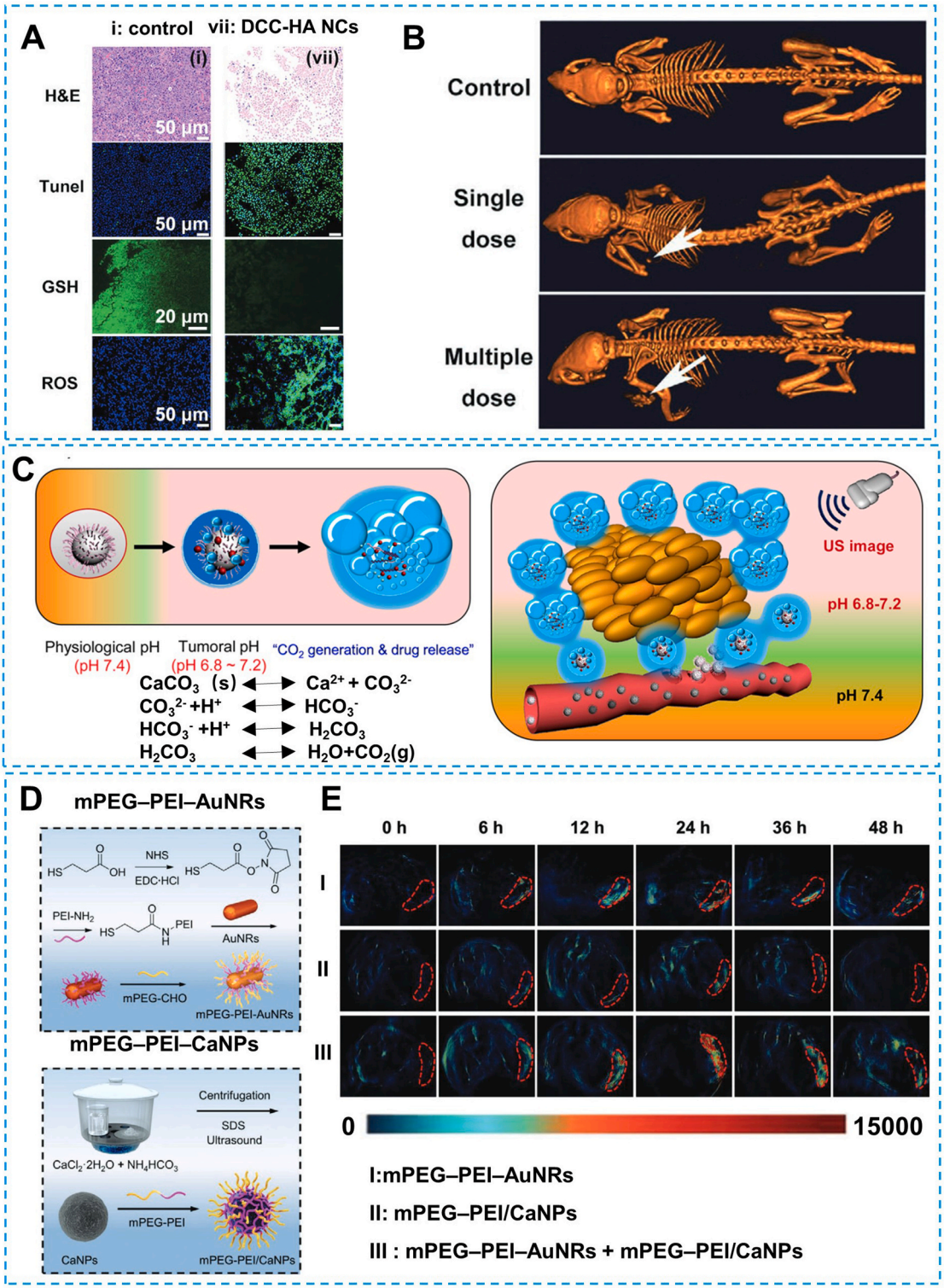

Fig. 8. Calcium material-enabled diagnosis.
(A) Tumour slice photographs with H&E−, TUNEL-, GSH-, and ROS-staining for tumour slices from control mice or mice treated with sodium hyaluronate modified CaO_2 (CaO_2-HA), and **(B)** the reconstructed CT images showing distinct signals appeared at the tumour site with a single-dose or multiple-dose of CaO_2-HA injection [108]; **(C)** Mechanism of $CaCO_3$ microbubble generation at tumour site for ultrasound (US) imaging and cancer therapy [18]; **(D)** The synthesis scheme of mPEG–PEI–AuNRs and mPEG–PEI/CaNPs, and **(E)** the enhanced photoacoustic image (PAI) signals by the combination of the two materials [111]. Reprinted with permission. (Copyright 2021 Wiley-VCH; Copyright 2015 American Chemical Society; Copyright 2021 Wiley-VCH).

Apart from direct CT signal enhancement by calcium ions, some calcium-based materials can participate in ultrasound imaging due to their bubble-generating abilities, for example, calcium carbonate ($CaCO_3$)-based materials [109]. In the TME with pH conditions like 6.8–7.2, $CaCO_3$ nanoparticles can dissolve and produce carbon dioxide (CO_2), thus exhibiting strong echogenic signals that favour ultrasound imaging (Fig. 8C) [18]. Cell membrane-coated $CaCO_3$ can maintain its integrity in the mild acidic TME and generate CO_2 bubbles in the endo/lysosomal conditions (pH 6.5–5.0) [110]. On the one hand, the intracellular CO_2 endowed cavitation and tumour cell necroptosis. On the other hand, the CO_2 at the tumour site is sufficient for echogenic reflectivity under the ultrasound field.

The emergence of photoacoustic imaging (PAI) provides another non-invasive imaging technique with simultaneous high contrast and high spatial resolution. $CaCO_3$-based materials have been reported to enhance the PAI signals by generating microbubbles. As a good paradigm shown in Fig. 8D, polyethylene glycol- and polyethyleneimine were synthesized to modify gold nanorods and $CaCO_3$ nanoparticles, forming mPEG–PEI–AuNRs and mPEG–PEI–CaNPs, respectively [111]. The combination of these two materials exhibited the strongest PA signal in a mouse model (Fig. 8E). Similarly, H2 microbubbles may also be engaged to PAI enhancement. As reported, magnesium nanoparticles have been employed for NIR–II–PAI-guided synergistic burst-like and H_2 cancer therapy [112]. Nevertheless, most studies of microbubble-based tumour imaging were still focused on the application of CO_2.

4. Application of calcium biomaterials for osteosarcoma therapy

The unique advantages of calcium-based materials have inspired their application in osteosarcoma therapy. In general, the reported materials include calcium phosphate (CaP) such as tricalcium phosphate (TCP), amorphous calcium phosphate (aCaP), and hydroxyapatite (HA), calcium carbonate ($CaCO_3$), calcium-based bioactive glasses (Ca-BG), and calcium peroxide (CaO_2). The featured reports are summarized in Table 2.

4.1. CaP

Several types of CaP, such as amorphous CaP, tri-calcium phosphate, and hydroxyapatite, exhibit resemblances to the bone mineral, with featured acidic pH-responsive dissolution properties. These characters make them ideal to load anticancer agents, as mentioned by some typical studies summarized in Table 2. Moreover, the CaP materials can induce osteogenesis in some cases, benefiting osteosarcoma therapy by providing inhibitions to osteoclast activities [114,116]. Indeed, these materials may be shaped as an implant or scaffold instead of nano-sized materials for administration. The bulk implant/scaffold can be embedded in the cavity post surgery, providing extra technical support and chemical nutrient to the bone. Hess et al. tested a porous CaP beads/matrix scaffold in the MG-63 cell line, and the in vitro experiment showed anticancer drugs loaded in the beads scaffold induced much stronger cytotoxicity than those in the matrix [115]. Nevertheless, the in vivo anticancer efficacy of drug-loaded stiff scaffold has not yet been tested.

4.2. Calcium carbonate ($CaCO_3$)

$CaCO_3$-based materials are employed in osteosarcoma therapy mainly due to their pH responsiveness. As in Table 2, the anticancer drugs loaded into these materials can be released in the tumour microenvironment due to the mild acidic pH, and dissolution of these alkaline materials neutralizes the pH in turn [14,118]. Specifically in 143B

Table 2
Featured osteosarcoma therapy using calcium-based materials.

ref	Material-related advantages	Test Model
● ***CaP***		
[113]	i pH-responsive anticancer drug release property	In vitro MG-63 cell culture
[114]	i pH-responsive anticancer drug release property ii Promote osteogenic differentiation in vitro	143B cells inoculated into the left armpit of Balb/c nude mice
[115]	i Chemical and physical resemblance to bone mineral ii Open-porous for drug loading resorbable scaffold for treatment	In vitro MG-63 cell culture
[116]	i Induction of osteogenesis ii Inhibition of osteosarcoma cell growth	In vitro MG-63 cell culture
[117]	i pH-responsive anticancer drug release property ii Mitochondrial-targeted delivery iii Binding with systemically administrated drugs to improve the bioavailability	Subcutaneous 143B tumour model in nude mice
● ***$CaCO_3$***		
[14]	i Tumour site accumulation ii Neutralization of mild acidic pH	Subcutaneous HT-1080 tumour model in nude mice
[118]	i Good biocompatibility and biodegradability ii pH-responsive anticancer drug release property	Subcutaneous K7 tumour model in Balb/c mice
[119]	i pH-responsive anticancer drug release property ii Reduction of bone destruction iii Improvement in biosafety	K7 allograft model and 143B orthotopic model in Balb/c mice
● ***Ca-BG***		
[103]	i Substantial biocompatibility and osteogenesis induction ii ROS induction by calcium overload iii Calcification formation and enhanced CT imaging signals	143B osteosarcoma xenograft model in Balb/c nude mice
[120]	i Rapid anticancer drug release ii Osteogenesis induction	MNNG xenograft model in nude mice and bilateral critical-sized calvarial-defect rat model
● ***CaS/HA***		
[121]	i Only digested by bone resorbing cells such as osteoclasts and cancer cells ii Microporous scaffold-endowed bone formation and disease eradication	Subcutaneous 143B tumour model in nude mice
● ***CaO_2***		
[122]	i Release of H_2O_2 for self-sufficient nanocatalytic cancer therapy ii Ca^{2+} provision to enhance bone regeneration	Subcutaneous MNNG/HOS model in Balb/c nude mice
● ***CaF_2***		
[123]	i Selective toxicity to osteosarcoma cells ii Ca^{2+} induced radiosensitization for irradiation therapy	Orthogonal 143B model in Babl/c nude mice

orthotopic osteosarcoma mouse models, DOX loaded in $CaCO_3$-mineralized polypeptide NPs exerted enhanced antitumour activities with reduced bone resorption than that loaded in $CaCO_3$ -free NPs, resulting in significant increases in the bone volume/total volume (BV/TV) and trabecular numbers (Tb.N) [119].

4.3. Calcium-based bioactive glasses (Ca-BG)

Bioactive glass (BG) exerts numerous merits in bone regenerative applications due to its good osteoconductivity, bioactivity, biocompatibility, biodegradability, and bonding abilities to hard or soft tissues. The commercialized 45S5-BG (Bioglass®) has been widely employed in bone repair [124]. Calcium plays an important role in 45S5-BG, attributing to the binding between bone and 45S5-BG by the formation of an amorphous calcium phosphate layer at the interfaces [125]. Modification of 45S5-BG by functional element doping endows the commercialized BG with unique characters, such as Ga-related selective anti-osteosarcoma abilities [126,127], Fe-related ROS production by Fenton's reaction and hyperthermia treatment [128–130], Ag-related cytotoxicity and antibacterial effects [131], Se-related bone formation bioactivities [132,133], and Mg/Al-related enhancement in stiffness, bioactivity, and biodegradability [134]. Apart from 45S5-BG and its derivatives, other synthesized BGs also exhibited abilities to induce bone regeneration (Table 2). The calcium can further cause ROS generation and calcification-enabled CT signal enhancement, as discussed in the above sections [103]. Notably, BG-based materials are generally fabricated as scaffolds, potentials providing extra technical support to the defective bone site post surgery.

4.4. Others

As in Table 2, other forms of calcium materials have been employed to treat osteosarcoma, including CaO_2, CaS/HA, and CaF_2. In general, the materials were designed/selected due to calcium-ameliorated bone affinity and construction. Taking advantage of their intrinsic properties, these calcium materials performed extra peculiarities. For example, self-sufficient nanocatalysis for osteosarcoma inhibition was achieved by utilizing multifunctional "all-in-one" 3D-printing composite scaffolds [122]. The scaffolds can generate H_2O_2 by the loaded CaO_2, and the H_2O_2 participated in the Fenton-like reaction to produce high-level ROS with the existence of iron oxide in the scaffold. In addition, the CaF_2 matrix was used to enhance the efficacy of adjuvant radiotherapy due to its intrinsic strong interaction ability to X-ray materials [123].

5. Perspective for opportunities and challenges

Despite the remarkable accomplishments in osteosarcoma treatment, pathological fracture treatment is neglected in favour of surgical tumour dissection and post-operative therapies. To this end, calcium-based biomaterials hold great opportunities to make improvement. Calcium-based biomaterials are well-known for their applications in defect bone repair. Recent investigations reveal their intrinsic features for improving cancer therapy and diagnosis.

Meanwhile, some challenges obstacles the investigation and application of calcium-based biomaterials. Firstly, research should tackle the effective concentration range of calcium in regulation. With normal bioactivities and the dynamics of osteohomestasis, most cells are under regular Ca^{2+} oscillations. In vivo application of calcium-based materials may lead to local Ca^{2+} plateaus or at least change the frequency and amplitude of Ca^{2+} oscillations. Therefore, conventional in vitro investigations will provide limited guidance for the in vivo application by mimics under steady Ca^{2+} conditions. Secondly, the investigation of calcium-based biomaterials on osteosarcoma is limited due to the lack of proper animal models. Most of the research demonstrated the anticancer ability and osteogenesis effect using a xenograft osteosarcoma model established by subcutaneous inoculation of human tumour cells to nude mice. These models show drawbacks in (1) the reproduction of bone-osteosarcoma interaction, and (2) the simulation of immune regulation.

6. Conclusion

Due to the high affinity to bone sites and good biocompatibility, calcium-based materials are investigated for a variety of orthopaedic applications. The materials provide abundant Ca^{2+} to alter bone homeostasis, tumour immune microenvironment, and osteosarcoma metabolism, leading to new therapeutic concepts such as calcium overload-induced mitochondria dysfunction and ER stress. Despite some challenges still remain, well-designed calcium-based biomaterials provide great opportunities to integrate cancer inhibition and bone regeneration to meet the clinical demands for osteosarcoma patients with pathological fractures.

Ethics approval and consent to participate

No ethics involved in this work.

Declaration of competing interest

The authors declared there is no confliction of interest.

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