ACS APPLIED MATERIALS & INTERFACES

www.acsami.org

Review

Inorganic Biomaterials for Regenerative Medicine

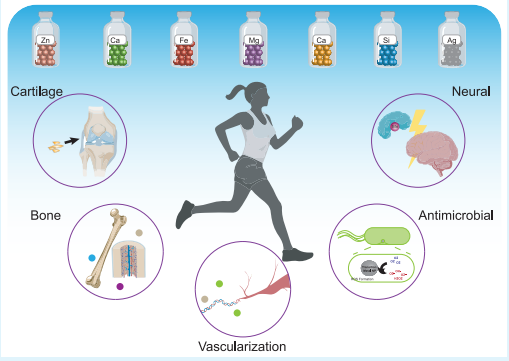
Anna M. Brokesh and Akhilesh K. Gaharwar*

Cite This: *ACS Appl. Mater. Interfaces* 2020, 12, 5319–5344

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Regenerative medicine leverages the innate potential of the human body to efficiently repair and regenerate damaged tissues using engineered biomaterials. By designing responsive biomaterials with the appropriate biophysical and biochemical characteristics, cellular response can be modulated to direct tissue healing. Recently, inorganic biomaterials have been shown to regulate cellular responses including cell−cell and cell−matrix interactions. Moreover, ions released from these mineral-based biomaterials play a vital role in defining cell identity, as well as driving tissue-specific functions. The intrinsic properties of inorganic biomaterials, such as the release of bioactive ions (e.g., Ca, Mg, Sr, Si, B, Fe, Cu, Zn, Cr, Co, Mo, Mn, Au, Ag, V, Eu, and La), can be leveraged to induce phenotypic changes in cells or modulate the immune microenvironment to direct tissue healing and regeneration. Biophysical characteristics of biomaterials, such as topography, charge, size, electrostatic interactions, and stiffness can be modulated by addition of inorganic micro- and nanoparticles to polymeric networks have also been shown to play an important role in their biological response. In this Review, we discuss the recent emergence of inorganic biomaterials to harness the innate regenerative potential of the body. Specifically, we will discuss various biophysical or biochemical effects of inorganic-based materials in directing cellular response for regenerative medicine applications.



KEYWORDS: *ionic dissolution products, ceramics, metals, and composites, bioactivity, tissue healing, tissue engineering, metal ion release*

1. INTRODUCTION

Regenerative medicine approaches harness the innate microenvironment of the body to promote tissue healing via directing immune and progenitor/stem cells to the site of injuries.[1−4] Chemical stimulants, such as biomolecules (proteins, metabolites, and minerals), are used to control and direct cellular functions. For example, vascular endothelial growth factor (VEGF) has been shown to direct the migration of endothelial cells, which is critical in the wound healing process.[5] Additionally, metabolic products that have the ability to direct cellular functions as nutrients are broken down into smaller constituents that are necessary components to various important biochemical pathways.[6] Similarly, ions released from minerals are utilized by the body as signaling molecules by regulating processes such as heartbeat, nerve response, and oxygen transport.[7,8] Minerals also act as building blocks for important tissue systems, such as bone, by providing structural integrity to the skeletal system, and play important roles in homeostasis. Despite the important role of minerals in the human body, limited attention is given to their role in regenerative medicine.

Inorganic biomaterials are attractive for a range of regenerative medicine applications because of their tunable properties (Figure 1A). These properties can be classified as biophysical or biochemical cues that can direct tissue regeneration.[9] Biochemical properties, such as ionic dissolution products or release of therapeutics biomolecules, can direct cellular functions though intracellular signaling. For example, ions such as lithium (Li^+), have been shown to upregulate the wingless INT-1 (WNT) signaling pathway in stem cells and can induce osteogenic differentiation.[10] Additionally, ions comprising minerals are known to play a role in biochemical processes such as regulation of apoptosis in the case of calcium ion (Ca^{2+}),[11] or cofactor activation in the case of magnesium ion (Mg^{2+}).[12] Biomolecules can also be sequestered and released from mineral-based biomaterials to control and direct cellular functions.[13] On the other hand, biophysical properties such as shape, size, surface-to-volume ratio, topography, stiffness, and charge of biomaterials can be modulated by addition of inorganic biomaterials for regenerative medicine.[14] For example, stiff biomaterials have shown to facilitate cells adhesion and promote osteogenic differentiation of stem cells, while soft biomaterials can facilitate chondrogenic differentiation.[15,16] Thus, the biophysical and biochemical attributes of inorganic-based biomaterials dictate their interactions with biological systems, playing a key role in regenerative medicine.

Special Issue: Young Investigator Forum

Received: September 30, 2019
Accepted: January 8, 2020
Published: January 28, 2020

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

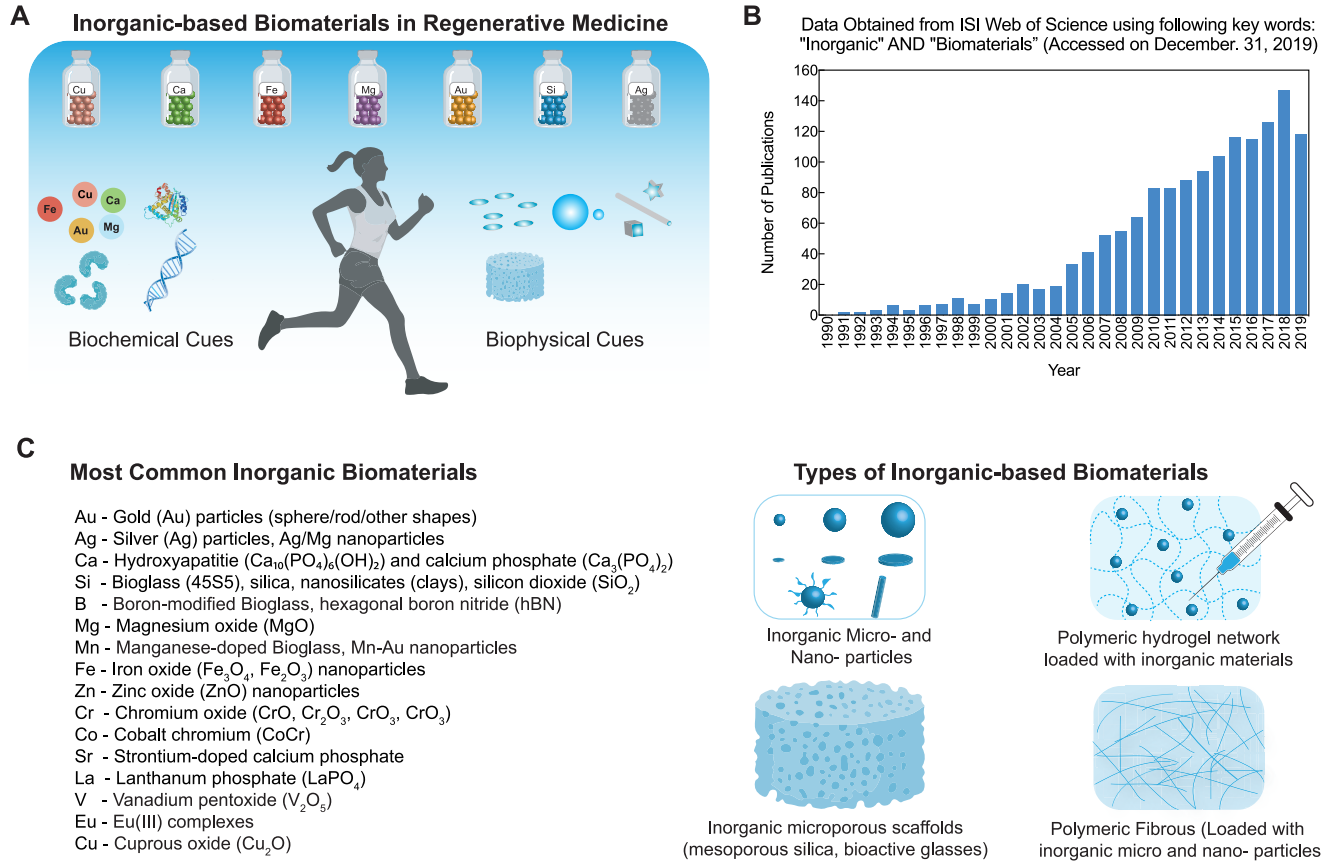


Figure 1. Inorganic biomaterials in regenerative medicine. (A) Inorganic biomaterials provide both biochemical and biophysical signals that stimulate tissue healing and regeneration. (B) Growing trend for "inorganic biomaterials" demonstrated though increase in number of publications for tissue engineering. (C) Some for the most common inorganic nanomaterials investigated for regenerative medicine.

In the past two decades, a large number of articles have focused on evaluating the potential of inorganic biomaterials for various applications including regenerative medicine (Figure 1B). For example, bioactive glass has previously been used as a scaffolding and substrate material for regeneration of bone tissue due to its bioactive characteristics.[17] Mineral-based micro- and nanoparticles can be easily ingested by cells, so they can provide bioactive cues to cells by release of ionic dissolution products. These micro- and nanoparticles are also combined with various polymers to fabricate scaffolds including fibrous scaffolds, microporous structures, and hydrogels (Figure 1C). These scaffoldings provide physical support for cellular in-growth and tissue integration. Certain mineral-based nanomaterials have also been utilized to bolster the mechanical integrity of hydrogel networks to allow for three-dimensional (3D) printing, which is an emerging approach for fabricating scaffolds for regenerative medicine.

In this Review, we will focus on current state of art in the field of inorganic biomaterials. We will first highlight the unique contributions of biophysical and biochemical properties of inorganic biomaterials in controlling and directing cellular functions. Then, we will focus on the utility of inorganic biomaterials for therapeutic applications. Additionally, the ability of inorganic biomaterials to use both their biophysical and biochemical characteristics to modulate cellular functions will be explored. We will delve into various categories of minerals and mineral-based biomaterials and discuss their applications in regenerative medicine. Finally, we will discuss some of the emerging trends and future applications of inorganic biomaterials.

2. BIOLOGICAL RESPONSE TO MINERAL-BASED BIOMATERIALS

Minerals are nutrients, specifically inorganic compounds comprised of one element or several, that are often essential for the human body to function. Some mineral elements, or dissolution ion products of common minerals, are considered by nutritionists to be macrominerals or essential mineral elements necessary for human biological processes.[18] Elements released from minerals, such as iron, calcium, and potassium, play key roles in maintaining and directing cellular functions. A daily intake of the four necessary macronutrients: vitamins, macrominerals, essential amino acids, and essential fatty acids is required for healthy human function.[19] For example, iron is present in hemoglobin and plays an essential role in maintaining healthy red blood cell function. Iron deficiency has been shown to cause complications, such as anemia, maternal death during pregnancy, and is potentially implicated in decreased cognitive development in children.[20,21] Supplementation alleviates these risks, as humans cannot actively synthesize iron and instead rely on dietary supplementation.[20] Although minerals play an important role in tissue homeostasis, they have not been extensively used to stimulate in situ tissue healing and regeneration.[9]

Inorganic biomaterials (including monolithic as well as composites with polymers) designed for regenerative medicine should respond to cellular signals and interact with

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

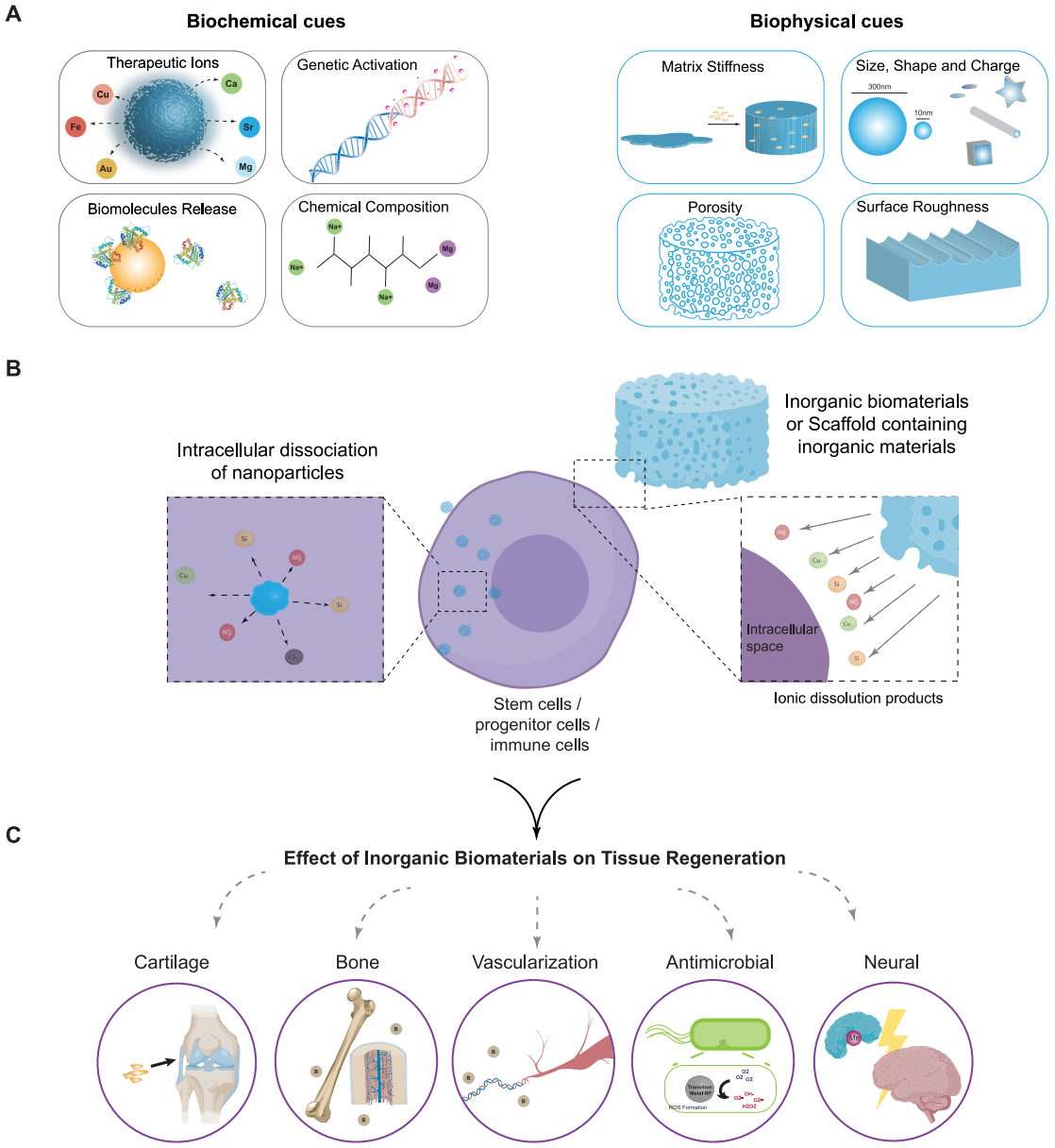


Figure 2. Characteristic of inorganic biomaterials. (A) Biochemical cues include release of therapeutic ions, as well release of biomolecules from inorganic biomaterials, whereas biophysical cues center around the physiochemical characteristics of a biomaterial such as the shape, size or charge. (B) Intracellular and extracellular release of ions from nanoparticles and bulk biomaterials respectively can control and direct cellular functions to stimulate tissue healing and regeneration. Inorganic nanoparticles get internalized by cells and acidic conditions in endosome results in intracellular degradation of nanoparticles into individual ions. Similarly, extracellular releases of therapeutics ions from biomaterials can influence cellular activity. Both these mechanism of ion release is shown to control and direct tissue healing and regeneration. (C) As inorganic biomaterials degrade, the release ions can direct specific cellular processes, such as osteogenesis, chondrogenesis, and adipogenesis. Mineral-based biomaterials also release ions involved in the homeostasis of bodily tissues, such as cartilage, bone, vascular, and neural. Many ions also have antimicrobial activities.

endogenous immune and progenitor/stem cells to stimulate in situ tissue healing and regeneration. Tissue regeneration is a multistep complex process involving various cell types and biologics that function in a well-organized manner. Initial biological response by immune cells dictate the degree of tissue healing and regeneration. Immune response can be classified into a pro-inflammatory or anti-inflammatory response and balance between these responses decides the degree of tissue healing. The type of biomaterials and its biophysical and biochemical cues dictate the overall biological response including tissue healing and regeneration.

Biophysical cues are defined as physical characteristics of biomaterials, such as topography, charge, size, electrostatic interactions, and material stiffness that guide biological processes (Figure 2A). For example, the surface charge of a nanomaterial has the ability to dictate and control adsorption of proteins, a phenomena also known as protein corona formation.[22] The type of protein corona and charge density plays a vital role in defining cellular interactions, such as cellular internalization. Aside from their charged nature, nanomaterials with the same surface area with decreased size provide large curvatures, restricting the cellular uptake.[23] In addition the size of biomaterials has a significant influence on cellular ingestion. For example, smaller nanomaterials are readily internalized via cadherin-mediated endocytosis compared to micron-size biomaterials.[24] By combining mineral

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

Table 1. Summary of Cell Modulatory Capabilities for Elements/Biomaterials[a]

element	valence	effect	ref
calcium (Ca)	+2	calcium signaling pathways, bone/lattice structure	46, 47, 50, 61, 305
strontium (Sr)	+2	upregulates bone formation, causes osteoclast apoptosis	66, 68−71
magnesium (Mg)	+2	enzyme cofactor: hexokinase, glucose-6-phosphatase, DNA polymerase	12, 41
silicon (Si)	+4, −4	collagen fibril alignment	114−116
		material component increasing biodegradation	
boron (B)	−3	upregulate osteoblast specific genes (Col1)	132, 138, 140
		upregulate ERK, FAK, increase vascularization	
iron (Fe)	+2, +3	enzymatic cofactor, hemoglobin component, stent structural component	153, 158, 161
copper (Cu)	+2	mitochondrial enzymatic cofactor	168, 178, 180, 182, 183
		upregulate HIF-1a, increase vascularization	
		part of standard neural function, promotes neural differentiation	
zinc (Zn)	+2	enzymatic cofactor, transcription cofactor, zinc-finger binding domains	27, 170, 185, 193, 196
		stent material component improving biodegradation	
		component of neural function and signaling	
chromium (Cr)	+3, +6	increases ROS which upregulates (HIF)-1a, involved in vascularization pathways but also potentially damaging at high concentrations or high oxidative state (+6)	205, 211
cobalt (Co)	+2, +3	mimics hypoxia and increases ROS which upregulate VEGF and FGF	91, 163, 216, 222
		stimulate VEGF to enhance osteoblast proliferation	
manganese (Mn)	+2, +3, +4, +6, +7	affect glycosylation of proteoglycans (part of cell adhesion), important to BMD	231, 232, 234, 236, 306
		neuronal differentiation	
molybdenum (Mo)	+3, +6	enzymatic cofactor	219, 263, 267, 269
		structural component in implants	
		imaging capabilities due to SPR	
gold (Au)	+1, +3	ROS generation, osteogenesis activated by stimulation of RUNX2 via p38/MAPK cascade	246, 250, 307
silver (Ag)	+1	antimicrobial (antiadhesion), ROS generation	308, 309
vanadium (V)	+5, +6	accumulates in bone increasing BMD and potentially replaces P in hydroxyapatite	275, 276, 280, 281, 283, 286, 310−312
		potentially increases angiogenesis, thought to be due to the production of ROS	
		antifouling films	
europium (Eu)	+3	production of ROS increases vascularization and potentially aids in osteogenesis	292, 295
lanthanum (La)	+3	activation of osteogenesis, though the exact mechanism is unclear	296, 297

[a]Some of these elements may provide useful in the field of therapeutics and have the potential have useful applications being incorporated in nanomaterials to modulate cellular fate. It may be relevant to observe different type of minerals in combination, or utilize their potential cytotoxic effects as a therapeutic device for cancer treatment. In any case, it is valuable to explore all options when developing new nanomaterials, as any bit of information could be the next link to solving tough biological problems.

particles with a polymeric network, a range of scaffolds including hydrogels and fibrous networks can be fabricated. Through the addition of different amounts of micro- and nano-sized inorganic particles to polymeric networks, biophysical properties such as matrix stiffness and roughness can be altered, which can directly influence cellular morphology. Specifically, with an increase in matrix stiffness, osteogenic differentiation in stem cells can be induced.[15,25] Overall, the biophysical properties of mineral-based biomaterials can provide specific cellular cues, such as directing protein adsorption, internalization, and cell morphology, suggesting that mineral-based biomaterials can be utilized to control stem cell differentiation and ultimately direct tissue regeneration.

Biochemical cues are ions, cofactors, cytokines, and signaling molecules which are used to activate specific genes or pathways to direct cellular responses. For example, cellular ingestion of mineral-based nanomaterials can allow release of bioactive ions within the cytosol sometimes due to lower pH (i.e., 5.5 in the endosome) (Figure 2B). Ion release from inorganic biomaterials is highly dependent on biostability. The intracellular or extracellular release of ions can trigger the activation of ion channels which control processes such as protein transport and signaling receptors. For example, release of calcium and other divalent ions outside the cell can trigger calcium-sensing receptors (CaR) which play a role in processes including apoptosis, cell proliferation, differentiation, activation of ion-channels, and chemotaxis.[26] Ion release in the cytosol can regulate protein binding and function, as well as biochemical pathway activation through manipulation of cofactors. Specifically, zinc finger binding domains interact with zinc ions to mediate protein binding to other proteins and nucleic acids.[27] Release of ions from biomaterials can also regulate gene expression. For example, ions released from calcium−silicon composites can activate osteogenic-related genes and can stimulate the formation of mineralized matrix.[28,29] In another example, cuprous oxide (Cu_2O) nanoparticles are utilized to suppress the expression of angiogenic pathways in human umbilical vein endothelial cells (HUVECs) by downregulating the vascular endothelial growth factor (VEGFR2) signaling. This was accomplished via cell cycle arrest by the Cu_2O nanoparticles in the synthesis (S) phase.[30] Ultimately these examples suggest that the biostability of a mineral-based nanoparticle can be harnessed to cause the release of biologically active, mineral dissolution ions directly within the cell. These mineral nanoparticles when embedded within polymeric network can slowly dissociate and release ions, which can be used to direct cellular functions.[31] In addition, mineral-based biomaterials can be used to sequester and release therapeutics to control cell fate. For example, protein therapeutics can be loaded on the surface of inorganic

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

nanoparticles, which can be released to affect cell function.[13,32,33]

Both biophysical and biochemical cues of biomaterials can be utilized simultaneously to first direct cell responses, but ultimately stimulate healing for various tissues, such as neural, vascular, bone, and cartilage (Figure 2C). Biophysical cues can direct internalization of essential ions, nanomaterials, or proteins into the cytosol. Consequently, biochemical cues can then guide genomic changes within the cell to direct cellular fate. From a holistic perspective, ions are used to control various steps of the regeneration process. There are three steps in this process: inflammation, proliferation and maturation. During inflammation, ions control the tissue response by promoting anti-inflammatory effects.[34−36] In the proliferation phase, ions act to stimulate release of growth factors.[37,38] Finally, in maturation, ions act to direct cell fate to different lineages by inhibiting or promoting various signaling pathways.[39,40] In this way, ions act to direct tissue regeneration at each stage, and can be utilized for tissue engineering applications.

Overall, inorganic biomaterials can influence cellular functions though direct or indirect approaches. Given the importance of macrominerals in tissue homeostasis and growth it is imperative to leverage these necessary nutrients for regenerative medicine. Here, we will discuss various essential ions and mineral-based biomaterials that have an ability to facilitate tissue healing and regeneration based on their classification in the periodic table (Table 1).

3. ALKALI AND ALKALINE EARTH METALS

Alkali metal elements are housed on the left side of the periodic table, typically exhibiting a monovalent, (+1) charge. Alkali metals explicitly consist of the following elements: lithium (Li), sodium (Na), potassium (K), rubidium (Rb), cesium (Cs), and francium (Fr). These elements are highly reactive, positively charged, monovalent metals capable of forming strong alkaline hydroxides in the presence of water, hence, the name alkali metals. Alkaline metal ions (housed to the right of alkali metals on the periodic table) also play a significant role in bodily functions. For instance, both magnesium (Mg^{2+}) and calcium (Ca^{2+}) are essential for numerous cellular processes. Specifically, Mg^{2+} ions act as a cofactor for a large set of enzymes such as glucose 6-phosphatase, hexokinase, and deoxyribonucleic acid (DNA) polymerase.[41] Mg^{2+} ions also chelate adenosine triphosphate (ATP) and are involved in the activation of the adenosine triphosphatase (ATPase) enzyme. Similarly, calcium ions serve as one of the basic signaling molecules of our body. Therefore, it is incredibly valuable to discuss and understand the role of calcium and calcium-based biomaterials. Other alkaline metals include beryllium (Be) magnesium (Mg), calcium (Ca), strontium (Sr), barium (Ba), and radium (Ra). These alkaline metals react with water to create basic hydroxides. Beryllium is a known biological hazard as it replaces magnesium with in cells, resulting in the inhibition of vital functions such as DNA synthesis and thus has not been explored for biomaterial applications.[42] Strontium acts in manner similar to calcium; however, its cellular affect or function has not been well characterized.[43] Here, we will discuss some of these cell regulating ions and mineral-based biomaterials for specific tissue engineering applications.

3.1. Calcium and Calcium-Based Biomaterials. Calcium is the third most abundant metal in the earth's crust after aluminum and iron. In the human body it is an essential metal as it is a constituent of skeletal tissue, with a recommended intake of 1 g/day for an adult human. Current clinical uses of calcium-based biomaterials are found in bone cements or ceramic scaffolds used for bone regeneration. The total serum concentration of calcium in the human body typically ranges between 8.5 and 10.5 mg/dL.[44] Calcium is an essential part of cellular signal transduction. Calcium in its Ca^{2+} state acts as a secondary messenger in a number of cellular signaling cascades that allow the cell to interpret and respond to different external stimuli.[11] Within the human body, parathyroid hormone secreted by the parathyroid gland is responsible for the amount of calcium present in the bloodstream by modulating the rate of reabsorption of Ca^{2+} ion from the bone and kidneys. In bone, Ca^{2+} is a signaling molecule between osteoclasts and osteoblasts to regulate natural bone formation and metabolism (Figure 3). It does this via gap junction communication, as well

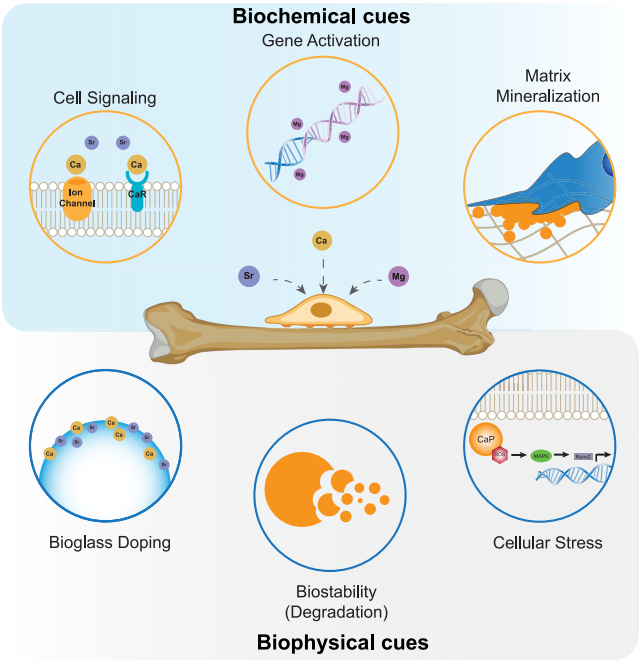


Figure 3. Alkali and alkaline earth metals and their role in bone formation. Individual ions from this group provide biochemical cues relevant to osteogenesis.

as activating a purinergic G protein-coupled receptor known as P2Y, which are necessary to propagate the Ca^{2+} ion wave.[45] Ca^{2+} also plays a key role in cell apoptosis in addition to the physiological processes of muscle contraction and generation of cardiac pulse creation.[46] It is also necessary for the activation of many kinase enzymes such as trypsin.[47−49] Given the myriad functions involving the Ca^{2+} ion, there is no surprise that calcium-based biomaterials are used to modulate cellular function for musculoskeletal tissue engineering.

3.1.1. Biochemical Properties of Calcium-Based Biomaterials. Use of nanosized hydroxyapatite (HAp) has been shown to promote osteogenesis, but this is mostly attributed to activation of stress related signaling.[50,51] The release of Ca^{2+} ion, a biochemical property, has been shown to activate the extracellular signal-regulated kinase 1/2/Fos-proto-oncogene, AP-1 subunit/Jun-proto-oncogene, AP-1 subunit (ERK1/2-FOS/JUN) pathway, while nano-HAp's biophysical properties induce osteogenesis[50] via mitogen activated protein kinase/

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

extracellular signal-regulated kinase/mitogen activated protein kinase 14 (MAPK-ERK1/2/p38) signaling.[51] The MAPK-ERK1/2/p38 signaling pathway mediates osteoblast function, which can be attributed to biophysical properties rather than biochemical mechanisms.[52] This pathway is considered the noncanonical pathway of transformation growth factor-β/bone morphogenic protein-2 (TGF-β/BMP-2), in which MAPK cascades activate ERK1/2 or mitogen activated protein kinase 14 (p38).[53] p38 is considered a stress activated kinase,[54] which is understandable given that nanoparticles tend to activate stress-related pathways.[55−57] Activation of p38 can be attributed to a cellular response to biophysical stimuli rather than biochemical cues because these nanoparticles are more biostable (a common biophysical property) and activate physical stress pathways (another common biophysical attribute). Studies have clearly demonstrated that nanosized hydroxyapatite significantly enhances osteogenic differentiation of human mesenchymal stem cells.[50] Therefore, it is important to note that osteogenesis can be induced through genetic pathway activation from both biochemically active ions and the biophysical properties of biomaterials.

Calcium-based nanoparticles and their dissolution products have been heavily investigated. These nanomaterials have been utilized for bone tissue engineering not only due to their similarity to bone, but also as a platform technology for drug delivery and hydrogel scaffold fabrication. In drug delivery applications, calcium-based nanoparticles have been investigated for cancer therapy due to their ability to disintegrate in the low pH environment of tumors.[58] This property can also be applied for imaging purposes to deliver contrast agents to these tumor regions. On the other hand, calcium-based nanoparticles, such as nano-HAp, have also been utilized as an additive to increase the mechanical integrity and strength of polymeric hydrogels for bone tissue regeneration.[16]

It is important to note that calcium-deposition from calcium based-biomaterials is beneficial only for bone tissue engineering. Calcification in other areas, such as heart valves and other cardiac tissues, can impair tissue functions and result in serious pathologic conditions.[59] Utilization of ion release from minerals and mineral-based nanoparticles certainly have benefits, but only when their therapeutic effect is localized.

3.1.2. Biophysical Properties of Calcium-Based Biomaterials. A range of calcium-based biomaterials, including calcium silicates, carbonates, fluorinates, and phosphates, have been investigated for their biophysical properties in tissue engineering applications. Calcium phosphates are perhaps the most relevant to therapeutic delivery and bone regenerative medicine applications, which include phases, such as amorphous calcium phosphate ($Ca_x(PO_4)_y{\cdot}nH_2O$), hydroxyapatite ($Ca_{10}(PO_4)_6OH_2$), tricalcium phosphate ($Ca_3(PO_4)_2$), and dicalcium phosphate ($CaHPO_4$).[60] These calcium phosphates have the same constituent elements but different physical lattice structure, which provide diverse biophysical characteristics. In addition, calcium-phosphate-based biomaterials have various biostabilities, and some formulations are more susceptible to degradation or dissolution compared to others. This variation in dissolution time is a good example of the biophysical properties attributed to biomaterials, as these properties can be leveraged when developing tissue engineering strategies. Both the biophysical and biochemical characteristics of these biomaterials can potentially alter cellular response and thus it is important to understand their interactions within cells and tissue systems.

Hydroxyapatite (HAp) is a major component of bone and subsequently the skeletal system (Figure 3).[61] HAp nanoparticles have been extensively used in bone-tissue engineering applications,[62] but have a slow dissolution rate under physiological conditions. Another common calcium phosphate, β-tricalcium phosphate (β-TCP), has a much faster dissolution rate compared to HAp, and has been utilized specifically for this biophysical property.[63] The rate of degradation or dissolution is important because the spatiotemporal release of calcium from such biomaterials alters cellular response. For example, cells will uptake dissolution ions from biomaterials with faster degradation rates, which understandably means the cells are responding to primarily biochemical cues presented by fast-dissolving biomaterials. However, the cellular effect of slow degrading biomaterials can be predominantly attributed to physical characteristics such as surface topography and size, as cells inevitably interact more with these properties in slow-dissolving biomaterials. On the basis of these unique calcium-phosphate properties, a combination of β-TCP and HAp has been used to modulate the degradation rate of biomaterial scaffolds. To investigate the effect of biomaterial dissolution, different formulations of β-TCP/HAp were investigated on osteoinductive properties.[60] Increasing the concentration of β-TCP in β-TCP/HAp nanocomposites showed enhanced differentiation of mesenchymal stem cells, as evident by upregulation of osteospecific genes like runt-related transcription factor 2 (RUNX2) and BMP2.[64] This is attributed to the increased Ca^{2+} ion concentration due to degradation of the β-TCP. Previous research has shown that Ca^{2+} ions increase the expression of BMP-2 through activation of first ERK1/2, then FOS/JUN, and finally the transcription factor activator protein-1 (AP-1) which turns on BMP-2 gene expression.[65]

3.2. Strontium-Based Biomaterials. Strontium (Sr) is classified as part of the alkaline earth metals, has an atomic number of 38, and has a charge of +2 (Sr^{2+}), which is characteristic of this group of elements. As Sr^{2+} is similar to Ca^{2+}, it can interfere with some of the pathways associated with Ca^{2+}.[66] Calcium is a major constituent in bone, but strontium can also be found stored in this area, just at a concentration of approximately a thousand-fold less than calcium.[67] Given its known presence in bone, strontium has already been used as medication for osteoporosis in the form of strontium ranelate ($C_{12}H_6N_2O_8SSr_2$). Osteoporotic structural damage in postmenopausal women is due to an increase in osteoclastic activity. Daily ingestion of strontium ranelate has been shown to decrease the risk of vertebral fractures by 41% over a three-year period by reducing osteoclastic activity.[68] However, this supplement has associated side effects, such as toxic epidermal necrolysis, though it is unclear whether this condition is due to ranelic acid or strontium.[69] This therapeutic still needs better characterization, and alternative delivery strategies can help prevent such side effects.

3.2.1. Biochemical Effect of Strontium-Based Biomaterials. Incorporation of strontium in biomaterials has been utilized primarily as an eluted ion that acts as a biochemical cue. For example, strontium is already being incorporated into biomaterial scaffolds due to its osteogenic ability.[70,71] Specifically, strontium/calcium polyphosphate loaded in silk fibrin scaffolds showed enhanced osteogenic differentiation compared to silk fibronin scaffolds.[70] In the absence of calcium, strontium doped silicate glasses were shown to increase osteoblast activity and differentiation.[66,72] The mode of action by which strontium exhibits osteoconductive

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

behavior can be attributed to the ability of this ion to cause the apoptosis of osteoclast cells, which actively suppress bone formation.[66] Strontium interacts with the calcium-sensing receptor (CaSR) and the osteoprotegerin (OPG)/ receptor activator of nuclear kappa-b (RANK)/ receptor activator of nuclear kappa-b ligand (RANKL) pathway which governs the cycle of bone formation/resorption (Figure 3).[69,73] The modulation of OPG/RANK/RANKL pathways is due to ability of strontium to promote production of osteoprotegerin (OPG), which subsequently binds to the receptor activator NF-kB ligand (RANKL) instead of RANK. The competitive inhibition of RANKL by activation of OPG prevents osteoclast proliferation and facilitates bone formation.[74] Additional studies have shown that strontium has the ability to interact with calcium-signaling pathways as a biochemical cue. Specifically, the CaSR receptor interaction with strontium mediates the MAPK/ERK1/2 pathway.[75] Therefore, it is possible that strontium utilization as a biochemical cue may be a useful tool in not only the bone resorption cycle by inhibiting osteoclast survival but also another inducer of bone formation through a well understood calcium pathway.

3.2.2. Biophysical Effect of Strontium-Based Biomaterials. Addition of strontium within biomaterials has been shown to alter material physical properties. Specifically, addition of strontium within the hydroxyapatite lattice structure has been shown to improve solubility and altered the crystallinity at a 10% strontium substitution for calcium.[76,77] Strontium enhances solubility by expanding the crystal lattice by replacing calcium ions.[77] The biological effect of these altered physical properties increases osteoblast proliferation and the deposition of mineralization granules by cells.[76] Along with apatite formation, enhanced biomaterial solubility improves biomaterial integration with bone and allows for the ingrowth of native tissue, and improves the mechanical integrity of the bone-biomaterial interface.[78,79] Thus, altering the biophysical characteristics of hydroxyapatite by doping with strontium is an alternative strategy to improve deposition of mineralized extracellular matrix (ECM) and integration of biomaterials with native bone tissue.

The addition of strontium into borosilicates was also shown to suppress its degradation.[76] Borosilicates have been utilized for their fast dissolution rate and quick conversion into hydroxyapatite, but as can be expected from highly dissolvable materials, borosilicates have reduced mechanical integrity.[80] The balance between dissolution and mechanical integrity is difficult to master, but with the addition of strontium, borosilicates do not degrade rapidly.[76] In addition, the biological effect of strontium doping of borosilicates showed increased cell proliferation.

Strontium in nanomaterial form is usually supplemented by the presence of other minerals, such as in the case of strontium hydroxyapatite nanorods, which are utilized uniquely for their fluorescent properties and ability to deliver therapeutics.[81] Ultimately, strontium nanoparticles have not been heavily studied for regenerative medicine and, typically, have been used for their luminescent properties for imaging purposes.[82] In some regenerative medicine applications, strontium ions have been eluted from nanomaterials, such as graphene nanoparticles.[71] However, strontium has been used to replace calcium in hydroxyapatite due to similar size and charge of the ion.[83] Interestingly, incorporation of strontium into hydroxyapatite increased osteo-specific markers after 21 days and inhibited osteoclast activity, which are desirable outcomes for the treatment of osteoporosis.[84] This might be attributed to increased degradation of the lattice because of strontium substitution.

3.3. Magnesium and Magnesium-Based Biomaterials.

Magnesium, another example of an alkaline earth metal, typically has a charge of +2 (Mg^{2+}) and an atomic number of 12. The total concentration of magnesium in a typical human adult is around 25 g, with the 60% of the magnesium ion (Mg^{2+}) residing in bones.[85] Magnesium ions have various well-known roles in the human body, acting as cofactors for various enzymes, modulating signaling and ion transport throughout the cell, and mediating energy metabolism and cell proliferation (Figure 3).[86] Lack of magnesium has been shown to play a role in the onset of diseases such as pre-eclampsia, stroke, heart disease, diabetes, atherosclerosis, asthma, osteoporosis, and many other pathological conditions.[86]

3.3.1. Biochemical Effects of Magnesium. Magnesium deficiency has been shown to play a role in osteoporosis, specifically in postmenopausal women with the disease.[87] However, the specific contribution of magnesium is not well understood, other than that magnesium deficiency correlates with the presence of osteoporosis in women. Magnesium alloys have been utilized for their physical attributes in bone-related medical devices because of their improved mechanical strength and bioresorption characteristics.[88,89] The addition of 5−10 mM of magnesium sulfate ($MgSO_4$) has been shown to upregulate osteo-related gene expression and enhance matrix mineralization.[88] Interestingly, these processes were promoted by the activation of hypoxia inducible factor-1 (HIF-1), which is known to play a role in osteogenic differentiation.[90] In another study, cobalt/magnesium doped hydroxyapatite was shown to enhance osteoblast proliferation and differentiation, which coincided with magnesium ions generating reactive oxygen species (ROS), which subsequently upregulated HIF-1.[91] However, because of the presence of cobalt ions, it is difficult to discern whether the osteogenic induction was because of magnesium or if magnesium and cobalt work synergistically to enhance osteogenesis.

3.3.2. Biophysical Effects of Magnesium-Based Biomaterials. Magnesium ions are a significant constituent of bone, and because of this, have been incorporated into biomaterials for bone regeneration. As seen with strontium, magnesium has been included alongside calcium in tissue regeneration approaches. Specifically, one study utilized magnesium to change the physical properties of bone cement formulations in order to bolster the degradation rate and mechanical integrity.[92] Bone cement with incorporated magnesium was shown to exhibit good biocompatibility and decent degradation that provided proper tissue in-growth along with improved compressive strength compared to standard calcium phosphate cement.[92] This study primarily focused on the added benefits of magnesium as a means to increase degradation of the cement as well as adding mechanical integrity, by providing unique biophysical cues to improve bone tissue response to this biomaterial. However, the role of magnesium as a biochemical cue for bone formation has been investigated sparingly, and was not investigated in this context.

Aside from macroscale biomaterials, such as scaffolds, magnesium has also been used in nanoparticle form for various reasons, such as antibacterial additives,[93,94] gene delivery devices,[95] bone tissue engineering,[96] and even heavy metal removal modalities in industrial applications.[97] Magne-

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

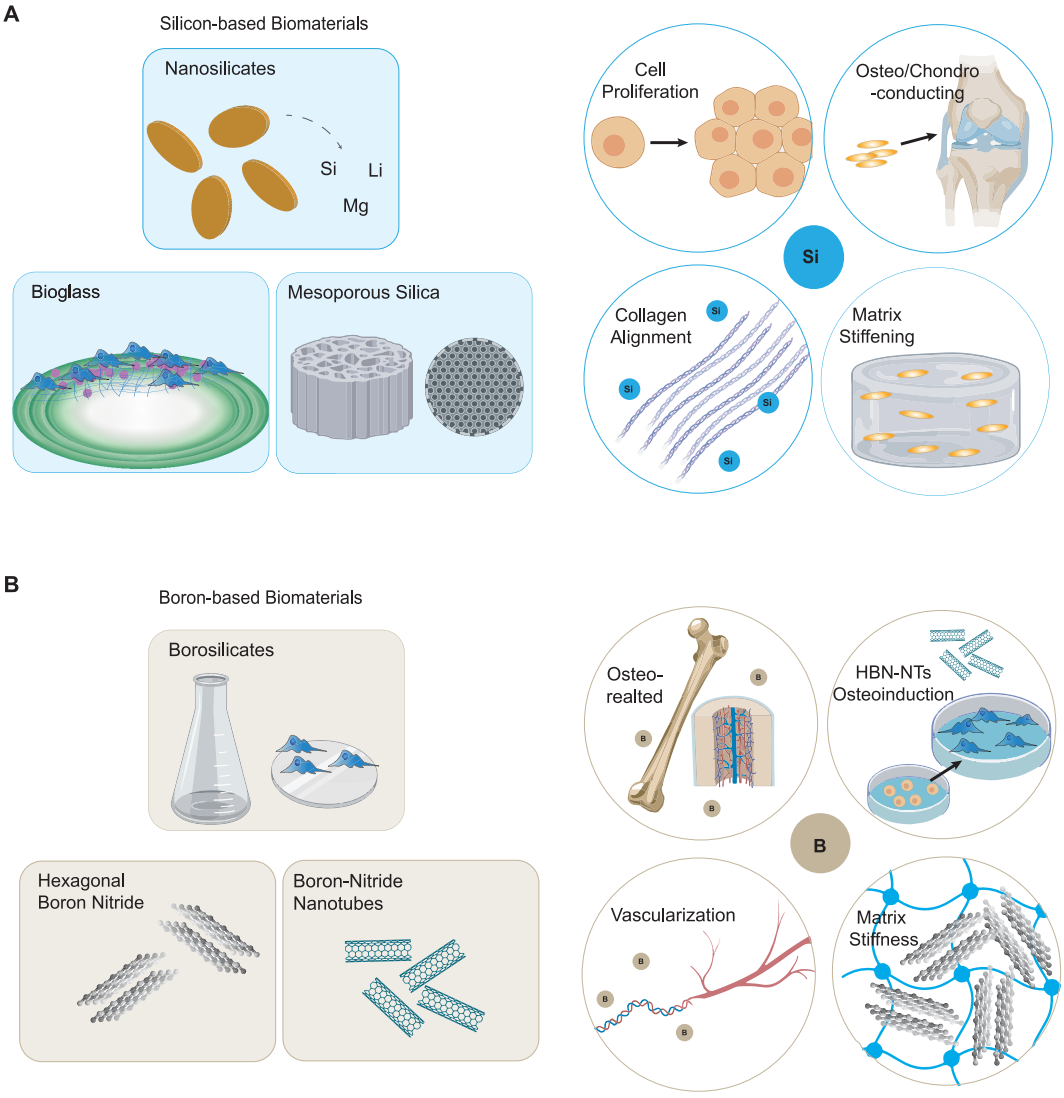


Figure 4. Metalloids have shown to direct formation of bone, cartilage, and vascular structures. (A) Silicon provides unique biochemical cues to stimulate regeneration of musculoskeletal tissues, such as bone cartilage and muscles. Silicon-containing biomaterials such as nanosilicates, bioactive glasses and mesoporous silica can induce osteogenic differentiation of stem cells in absence of growth factors. (B) Boron-based biomaterials such as boron-doped bioglass and hexagonal boron nitride are shown to stimulate angiogenesis and osteogenesis.

sium oxide (MgO) is most commonly utilized as an antibacterial additive. This nanomaterial is used primarily for its ability to increase pH and generate ROS that acts as the preventative molecule that targets bacterial cells.[93] In addition, MgO have been shown to cause membrane damage in bacteria such as *Escherichia coli* (*E. coli*).[94] MgO nanomaterials are typically formed by hydrothermal reaction,[98,99] thermal decomposition,[100] or sol−gel synthesis.[101,102]

Inorganic nanomaterials, such as MgO, have improved antimicrobial properties.[103] Increased surface area of metal oxide nanomaterials increases the ability of ROS formation, making it effective against microbes. The mechanism of MgO's antimicrobial activity has not been fully investigated, but it is clear that the presence of MgO significantly alters the membrane integrity of *E. coli*, which may lead to cell death.[104−108] Interestingly, compared to zinc-oxide (ZnO) nanoparticles, MgO showed enhanced antimicrobial properties but the exact mechanism is not known.[104]

4. METALLOIDS

Metalloids appear on the right of the periodic table and include elements such as boron (B), silicon (Si), germanium (Ge), arsenic (As), antimony (Sb), tellurium (Te), and polonium (Po). Some of these metalloids are biologically relevant, such as (Si), but some are toxic (such as As). For example, silicon-based biomaterials are extensively used as implants, contact lenses, and therapeutic delivery systems. Interestingly, biological systems and cells have adapted to accommodate and processes these elements. For example, a family of membrane proteins called major intrinsic proteins (MIPs) are utilized by bacteria, fungi, mammals, and plants to regulate arsenic and boron homeostasis, as well as silicon intake and transport.[109] Cells certainly utilize or at least tolerate the presence of some metalloids, but the exact mechanism by which they act is not well understood. We will discuss some of these metalloids and their biomedical applications.

4.1. Cellular Effects of Silicon and Silicon-Based Nanomaterials. Silicon is a metalloid with an atomic number of 14. The human body typically houses around 1 to 2 g of

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

silicon, and this element has been implicated as a relevant constituent of bone and connective tissue.[110] Silicon deficient diets directly result in reduction of collagen and glycosaminoglycans (which are important components of connective tissue).[111] This observation has been documented by additional research indicating that orthosilicic acid ($Si(OH)_4$) stimulates collagen synthesis, along with causing increased expression of alkaline phosphatase (ALP) and osteocalcin (OCN), however the molecular mechanism behind these findings has not been well characterized.[112] Collagen comprises over 90% of bone matrix and helps to provide mechanical support and structure to connective tissues.[113] Interestingly, in osteoporotic bone, collagen fibril formation is random rather than aligned, suggesting that this structural arrangement of collagen I plays a large role in the pathology of this disease.[114] Importantly, dietary supplementation with silicon was shown to increase bone mineral density in women before menopause and also in men, suggesting that silicon can play a preventative role against osteoporosis.[115] However, the mechanism by which silicon aids in collagen fibril alignment and mineral density improvement is not well understood.

4.1.1. Biophysical Effect of Silicon-Based Biomaterials. Silicon has already been incorporated into a large variety of biomaterials, including bioglasses,[116] bioglass ceramics,[117] and silicon-based nanoparticles[118] (Figure 4A). Bioglass (45S5, a specific formulation of bioglass) contains SiO_2(45 wt %), CaO (24.5 wt %), Na_2O(24.5 wt %), and P_2O_5 (6.0 wt %) has been extensively investigated for bone tissue engineering.[116,119] Interestingly, bioactive glass has been shown to promote cell proliferation by forcing the transition of cells from gap 1 (G1) to synthesis (S) phase.[120] However, silicon is not the only element in bioglass, so it is difficult to identify the primary cause of increased cell proliferation. In other bioactive ceramics, silicon has been incorporated into hydroxyapatite to increase bioactivity. The incorporation of silicon makes HAp ceramic more susceptible to degradation.[121] As previously mentioned, increasing the degradation rate improves native tissue integration into biomaterials, so many studies have incorporated new elements into hydroxyapatite to alter the degradation rate of this well-known biomaterial.[76,79,80] The addition of silicon to hydroxyapatite is no exception and is a testament to the utility of modifying biophysical properties of biomaterials to improve bioactivity.

Nanosilicates ($Na^{+0.7}(Mg_{5.5}Li_{0.3}Si_8)O_{20}(OH)_4)^{0.7}$), a type of layered silicates containing sodium, magnesium, and lithium, have been shown to induce osteogenic differentiation of stem cells.[122−124] Nanosilicates have been shown to dissolve within the lysosome at low pH, and subsequently release the ions that comprise it, providing biochemical cues to cells for osteogenesis and chondrogenesis.[125] In addition, nanosilicates have been used to design injectable hydrogels, bioinks for three-dimensional (3D) printing, drug delivery methods, and bone scaffolds.[126−130] These unique physical properties stem from the nanosilicates dual-charged nature which facilitate electrostatic interactions with a range of polymers. These electrostatic interactions between nanosilicates govern the formation of a house of cards structure that enhances the mechanical integrity of nanosilicate−polymer networks. The high surface area and charged characteristics of nanosilicates can be used to deliver a range of therapeutics agents.[13,32,33] Apart from nanosilicates, a range of nanoclays are used for biomedical applications.[122]

4.2. Cellular Effects of Boron. Boron is considered part of the metalloid group, with an atomic number of 11. Though not fully characterized, boron does play an essential role in animals and humans. Developmental biology highlights boron as a necessity in embryonic development, and when in deficit results in affected embryos or necrosis.[131] Additionally, a boron deficient diet in male pigs decreased the bone lipid in femurs and resulted in a higher bending moment, which suggests that boron plays a crucial role in bone metabolism.[131] Low concentrations of boron also activate the MAPK pathway, suggesting that it is important to cell metabolism in humans.[131] Therefore, boron could modulate stem cell differentiation because it has been proven essential in cell and bone metabolism (Figure 4B), and has already been incorporated into biomaterials for regenerative medicine applications.

4.2.1. Biochemical Effect of Boron. The biological effect of bioglass doped with boron has an effect on vascularization, as well as bone formation. Boron-based bioglass has been shown to cause proliferation of human umbilical vein endothelial cells (HUVECs) by the phosphorylation of extracellular signal-regulated kinase (ERK1/2), p38 protein, and focal adhesion kinase (FAK).[132] Though all of these proteins are associated with vascularization, ERK1/2 and p38 are pivotal growth factors associated with proliferation, differentiation, and cellular modulation,[133] whereas FAK is necessary for vascular and embryonic development.[134] Boron-doped bioglass microparticles showed increased vascular density levels in embryonic quail chorioallantoic membranes (CAMs) compared to bioglass particles, and had comparable vascularization to growth factor treatment.[132] Additionally, boron has been shown to upregulate vascular endothelial growth factor (VEGF) and transformation growth factor-beta (TGF-β) which are part of vascularization.[72] It is interesting that boron-bioglass is involved in modulating a variety of biological processes, and further solidifies the significance of this biomaterial in regenerative medicine applications.

Boron ion (3+) has also been implicated in the activation of the ERK1/2/p38 pathway, which regulates osteo-specific genes, such as BMP, RUNX2,[135] OCN, and bone-sialoprotein (BSP).[136] Additionally, intake of dietary boron has been said to enhance bone formation[72] and prevents calcium loss and bone resorption in women past menopause.[137] In biomaterials, boron-doped bioglass scaffolds showed a large increase in proliferation of osteoblasts compared to controls, with an increase in both RUNX2 and collagen 1- alpha 1 (COL1A1) expression.[138] COL1A1 is known to be activated during the process of cellular differentiation into osteoblasts.[139] Several studies have shown boron effects osteogenic differentiation in osteoblasts, however the role of boron in osteogenesis is not well understood. Nanoparticles eluting boric acid showed upregulation of osteoblast specific genes (COL1A1, osteopontin (OPN), and OCN) in preosteoblasts.[140] These results indicate that release of boron from biomaterials may be a useful therapeutic approach to improve bone tissue engineering, in addition to its already established incorporation for unique physical properties.

4.2.2. Biophysical Effect of Boron-Based Biomaterials. Borosilicate glasses are also used for bone tissue engineering due to their bioactive characteristics.[80] Boron-trioxide (B_2O_3) replaces silicon dioxide (SiO_2) in the standard bioglass structure (45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_5), and because of this, the normal three-dimensional network cannot be formed. This makes borosilicate susceptible to dissolution due to limited chemical durability.[141] Increased dissolution of bioglass improves the nucleation of hydrox-

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

yapatite. Borosilicate dissolution is relevant to the field of bone-bonding, which is a hallmark of bone-tissue engineering, and can be directly attributed to hydroxyapatite formation.[142]

Hexagonal boron nitride (hBN), an emerging two-dimensional (2D) nanomaterial, has been used for biomedical applications.[143] This material has semiconductive properties due to an increased band gap that allows for its use in biosensor and contrast agent.[144] Boron-nitride nanotubes are also investigated for drug delivery[145] and regenerative medicine.[146] Boron nitride nanotubes have shown to promote osteogenesis by release of boron ions.[146] Addition of boron nitride nanotubes provides biophysical cues to polymeric networks by increasing the matrix stiffness.[146] Increased matrix stiffness provides mechanical cues that enhance the osteogenic capabilities of stem cells.[147] This particular nanomaterial holds great potential in the field of tissue engineering due to its bioactive characteristics.

5. TRANSITION AND POST-TRANSITION METALS

Transition and post-transition metals make up the bulk of the periodic table, ranging from transition metals, such as iron, chromium, copper, and zinc, all the way to the noble metals gold, silver, and copper and post-transitional metal bismuth. Because of the wide number of elements in this category, their effects vary depending on charged state and atomic weight. Many transition metals have multiple charged states (i.e., Fe^{2+} vs Fe^{3+}), for which cells must account by having homeostatic pathways to mitigate any potential cytotoxicity.[148] However, many transition metals have important biological roles, such as those found in protein complexes. The transition metal class has several extremely significant biologically interactive ions, specifically iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), cobalt (Co), and nickel (Ni).[148] Other transition metals are not well characterized, but may have significant biological roles with potential impact on regenerative medicine if better studied.

5.1. Cellular Effects of Iron. Iron (Fe) (atomic number 26) is a major component of hemoglobin's quaternary structure in human body.[149] Heme-iron and inorganic iron both play roles in biological processes, and there are specific biochemical mechanisms for iron homeostasis adapted by the human body that process iron in its various forms.[20] Iron deficiency anemia is a known condition that has significant adverse effects, resulting in preterm labor or infant mortality in some pregnancy cases,[150] and decreased motor activity and attentiveness in children.[151] Iron-based biomaterials, such as iron oxide nanoparticles, have been extensively investigated for biomedical applications because of their unique superparamagnetic effect.

5.1.1. Biochemical Effect of Iron. Iron ions typically oscillate between Fe^{2+} and Fe^{3+} states and are highly reactive. In cells, iron is sequestered in the protein ferritin for storage and transport, which hold this ion in the Fe^{3+} state.[152] This cellular process is designed to prevent the presence of "free" iron ions which are highly reactive and can create ROS, thus making it necessary to sequester these ions in proteins for storage.[153] Iron is utilized in multiple biological processes such as electron transport,[154] oxygen transport,[155] and DNA metabolism.[156] Interestingly, in the brain, inorganic iron is utilized to bind serotonin and form catecholamine and when chelated out causes significant medical effects such as loss of consciousness.[157] Iron homeostasis has been shown to regulate stem cell pluripotency, by controlling glycerophospholipid metabolism though the mechanism by which iron accomplishes this is unknown.[158] Clearly iron ions have important biological significance, which makes this inorganic ion a potential candidate for modulating biological processes for regenerative medicine strategies.

5.1.2. Biophysical Effect of Iron-Based Biomaterials. To date, iron has been used in biomedical applications, such as diagnostic imaging, drug delivery, and stents.[159−161] In stents, the use of iron as a base material is beneficial for structure.[161] With the addition of a polylactic acid (PLA) coating the degradation of iron stents can be accelerated, creating byproducts with good biocompatibility.[161] Iron oxide nanomaterials, such as Fe_3O_4, have been extensively studied for their superparamagnetic properties.[159,160,162] These magnetic nanoparticles have been utilized as contrast agents for magnetic resonance imaging (MRIs),[163] drug carriers for localized delivery,[160] and increased hydrogel matrix stiffness to alter cell morphology.[164] Magnetic nanoparticles combined along with thermoresponsive polymers can be used to remotely delivery therapeutic agents upon exposure to an external magnetic field.[165,166] The high surface area of magnetic nanoparticles can be used to conjugate multiple polymeric chains on the surface of these nanoparticles.[164] Such nanoparticles can act as a cross-link epicenter and can help in improving the mechanical properties of polymeric hydrogels. The change in biophysical characteristics, such as stiffness of the hydrogel network, can be used to modulate stem cell behavior.

5.2. Cellular Effects of Copper. Copper (Cu) is a transition metal (atomic number 29) with normal stability as Cu^{2+} and an essential trace element for standard metabolic function.[167] It is also a common cofactor to many enzymes that are important to human biological processes such as catalase, cytochrome oxidase, and peroxidases.[168] When deficient in copper (Menkes syndrome), individuals exhibit neurodegeneration, seizures, and hypothermia, which can result in death depending on the severity and are in part due to inhibition of important functions, such as catecholamine production, peptide amidation, and mitochondrial respiration.[169] When copper is present in excess (Wilson's disease), individuals experience toxic levels of copper resulting in hepatic abnormalities and neurological defects.[170]

In biomedical applications, copper ion elution has been widely utilized as a birth control mechanism in intrauterine devices. The elution of copper ions prevents fertilization as a spermicide, with some evidence suggesting that implantation is also impaired.[171,172] This spermicidal property is caused by the activation of inflammatory processes, such as leukocyte and prostaglandins present in the uterus because of presence of copper ions.[173]

Other studies investigated the role copper ions in bone regeneration by doping mesoporous silica nanospheres with copper and showed that osteogenic gene expression was enhanced along with the creation of a hypoxic environment.[174] This hypoxic environment is indirectly beneficial for bone formation, specifically because hypoxia generally stimulates angiogenesis and vascularization. Vascularization is important for bone homeostasis because the vascular system provides nutrient exchange, oxygen transport, hormone cues, and growth factors to bone tissue.[175] Other studies have shown that copper also upregulates vascular endothelial growth factor (VEGF) gene expression through hypoxia and promote vascularization (Figure 5A).[176−178] Copper ions induce

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

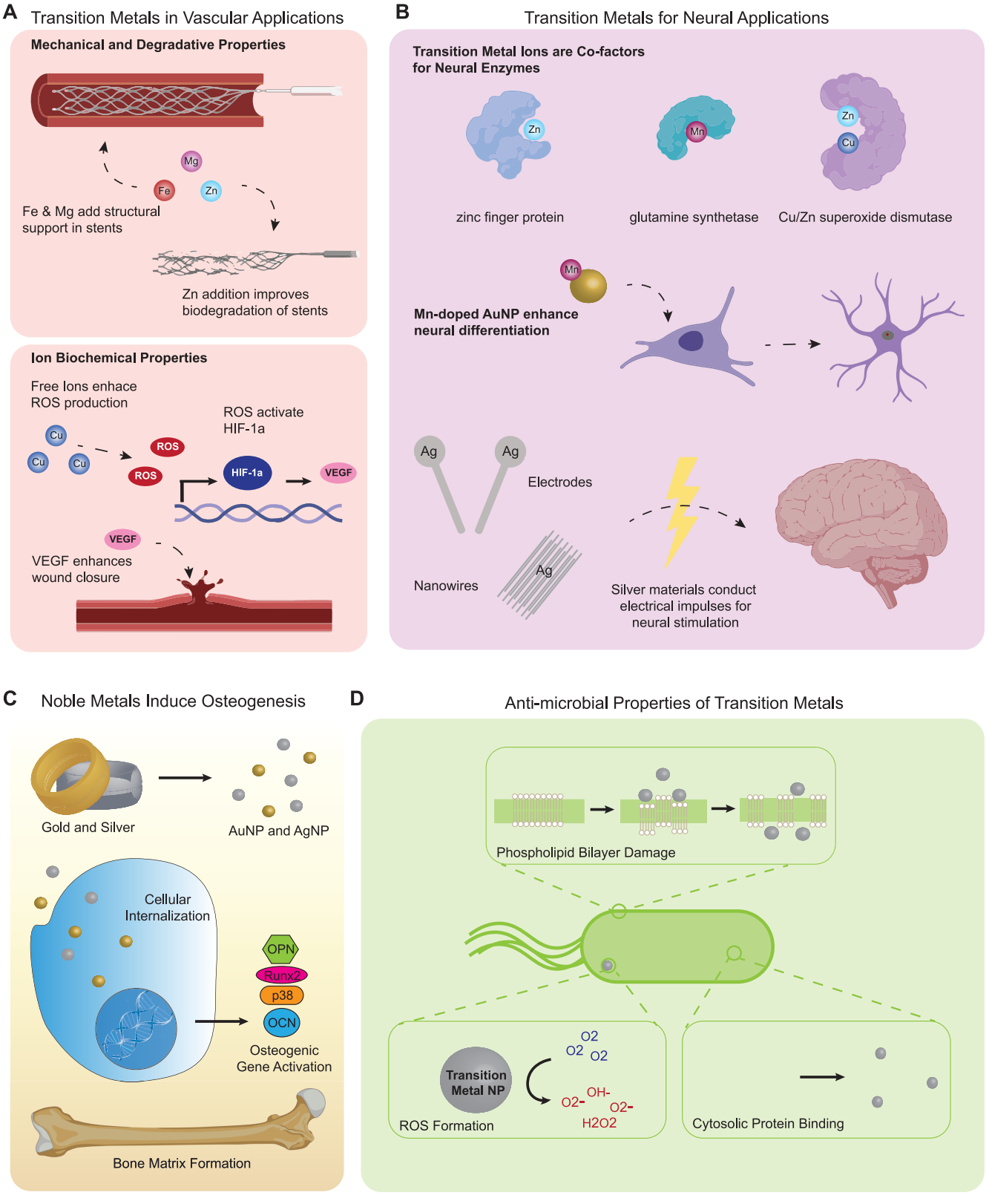


Figure 5. Effect of transition and post-transition metals. (A) Vascular applications of transition metals (Fe, Mg, Cu, and Zn). Doping metals with transition metal such as Mg and Zn facilitate dissolution of implants. While release of copper from biomaterials can stimulate angiogenesis. (B) Some of the transcription metals, such as Zn and Cu, are used in neural tissue engineering, and noble metals, such as Ag and Au, are used for neural stimulation. (C) Noble metals (Au and Ag) are shown to induce osteogenesis. (D) Antimicrobial properties of transition metals are also demonstrated.

hypoxia-inducible factor (HIF-1) dependent pathways and upregulate the protein production of HIF-1a which positively regulates VEGF downstream.[178] This property has been shown to be an effective addition in wound healing biomaterial

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

applications, as copper ions were shown to improve wound closure with a higher cell density in the granulation layer of tissue.[179,180] Copper is also an important ion implicated in neural development and function.[181] For example, copper ions promote neural differentiation by activating copper-regulating genes, such as Cu transporters and metallothioneins (MTs).[182] During the neural differentiation of progenitor cells, accumulation of copper ions in the cytoplasm was observed.[183,184] These studies highlight that copper ions can be used for a range of biomedical applications such as nerve tissue regeneration, wound healing, and bone-tissue vascularization.

5.3. Cellular Effects of Zinc. Zinc (Zn) is another transition metal with an atomic number of 30, and is a known essential mineral nutrient involved in multiple biological processes, specifically as a cofactor for enzymes and a relevant component of cellular metabolism (Figure 5B).[185] Severe zinc deficiency defines the disease known as acrodermatitis enteropathica with symptoms, including severe skin and gastrointestinal lesions, growth retardation, and an impaired immune system.[186] In this disease, dietary zinc is not absorbed correctly within the intestines because of a genetic defect. The wide breadth of symptoms associated with zinc deficiency are a testament to how biologically relevant zinc atoms are to standard human bodily function. Notably, transcription proteins often bind to DNA by utilizing "zinc finger binding domains" which require a zinc atom within the protein structure in order to properly function. These transcription factors regulate hormone receptors, such as estrogen, testosterone, and vitamin D, among other genetic pathways.[185]

5.3.1. Biochemical Effects of Zinc Ion. Zinc has a known role in enzymes associated with cellular filamentous structures, such as collagenase, proteoglycans, and keratins.[187] Additionally, zinc has a role in intracellular signaling by influencing the secretion of insulin and regulating apoptosis.[188] Incorporation of zinc coatings in titanium implants enhanced bone growth and osseointegration.[189] The presence of zinc has been shown to increase alkaline phosphatase activity and stimulates mineralization in rats; however, the mechanism by which zinc does this is unknown.[190] However, zinc was also shown to prevent both osteogenic and adipogenic differentiation of mesenchymal stem cells (MSCs), suggesting that the effect of zinc on bone formation is more complex than simply promoting stem cell lineage commitment.[191] Ions, such as zinc, play a role in so many biological processes that it is no surprise that addition of these elements into tissue systems has complex effects.

Zinc is incredibly important for neurodevelopment and neurogenesis. Zinc deficiency inhibits the neuronal stem cell marker nestin in mice (both before and after birth).[192] Additionally, zinc deficiency has been shown to decrease proliferation of neural stem cells and also enhance apoptosis of such cells partially due to increased ROS production.[193] These effects are likely because zinc (in addition to being a relevant cofactor for enzymes and transcription factors) as a free ion modulates synapse receptors, such as *N*-methyl-D-aspartate (NMDA), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainite glutamate receptors along with regulating ion transport channels.[193] Zinc ions are heavily involved in human bodily functions, including neural growth and neurogenesis and may be useful for regenerative applications in other tissues as previously mentioned.

5.3.2. Biophysical Effects of Zinc-Based Biomaterials. Zinc has been incorporated into biomaterials related to orthopedics, drug delivery, imaging, and cancer therapy.[194] Cardiovascular stents have been doped with zinc to tune the degradation profile of the stent.[195−197] Zinc oxides (ZnO) have defects at the nanoscale that result in the emission of blue fluorescence making this nanoparticle a good candidate for sensing and optical applications.[198,199] In drug delivery applications, nanostructures containing zinc oxide have been engineered for gene delivery.[200] Zinc nanoparticles itself can selectively cause apoptosis of cancer cells due to activation of tumor suppressor genes (tumor protein 53 (p53)) and apoptotic genes (BCL2-associated X (BAX) and caspase-3).[201] This is attributed to ROS production because of the presence of ZnO nanoparticles.[202]

5.4. Cellular Effects of Chromium. Chromium (Cr) is a transition metal (atomic number 24), and the most stable forms are Cr^{3+} and Cr^{6+}. Chromium works in conjunction with insulin hormone and is necessary for sugar metabolism.[203] Specifically, Cr^{3+} has been shown to increase the activity of insulin signaling and facilitates glucose uptake, thus making it a potential therapy for managing type-2 diabetes.[204] The charge of chromium is important because Cr^{3+} is less toxic and nonmutagenic, while Cr^{6+} is considered a class-1 carcinogen because of its ability to create ROS and leads to activation of apoptosis, which accounts for its known cytotoxicity.[205,206] Cr^{6+} is more reactive than Cr^{3+}, and thus produces more ROS making it more toxic.[207]

5.4.1. Biochemical Effect of Chromium Ions. The ROS generated by chromium have an effect on angiogenesis and potentially activate inflammatory processes. ROS stimulate hypoxia-inducible factor 1 (HIF-1), which activates vascular endothelial growth factor (VEGF) transcription,[205] and regulates genes associated with neovascularization, cellular metabolism, cell migration, and cell survival.[208] However, if not properly controlled, the intense activation of HIF-1 results in tumorigenesis.[209] It is important to note that ROS play a vital role in normal cell function by activating HIF-1 which regulates important cell processes.[210] A limited concentration of chromium could have a positive effect on tissue healing through activation of HIF-1 by promoting vascularization. The effects of chromium on bone formation have also been investigated. Increased doping of chromium on alumina tubes showed increased cellular activity.[211] This might be caused by chromium induced insulin stimulation, which promotes collagen synthesis and prevents bone resorption.[212,213]

5.4.2. Biophysical Effect of Chromium-Based Biomaterials. Chromium-oxide nanoparticles have been synthesized and have been shown to be internalized by phagocytosis.[214,215] Although these nanoparticles are internalized, they do not show any effect on osteogenic activity.[216] This finding suggests that the physical arrangement of ions has a great impact on the functionality of biomaterials. The use of chromium in biomaterials is mostly limited to cobalt−chromium alloys, which are used in stents,[217] dental prosthetics,[218] and bone implants.[219] These alloys are used primarily as structural components for their mechanical integrity and corrosion-resistant attributes. Increasing the presence of chromium actually reduces the corrosion of steel by creating a "healing" oxide film in the presence of oxygen, and at a concentration of 12% chromium, the well-known material "stainless steel" is formed.[219,220] However, degradation products from the implants are also known to cause inflammation, cytotoxicity, and hypersensitivity, and have the ability to travel in the bloodstream throughout the human body.[221] Therefore,

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

because of this well-known toxicity, it is understandable that the use of chromium in nanoparticle form has been incredibly limited in the field of biomaterials.

5.5. Cellular Effects of Cobalt. Cobalt (Co) (atomic number 27) is an important part of the essential vitamin B12, which is necessary supplementation for humans.[222] It is a transition metal just like chromium, with common oxidation states of Co^{2+} and Co^{3+}. Most notably, ionic cobalt has the ability to upregulate the transcription factor HIF-1a, which promotes vascularization and bone formation.

5.5.1. Biochemical Effect of Cobalt Ions. It has been established that Co^{2+} ions enhance osteoblast proliferation, and differentiation.[91] Specifically, $CoCl_2$-treated bone-mesenchymal stem cells (BMSCs) showed significantly increased mineralization after subcutaneous implantation.[223] Co^{2+} presence seemed to stimulate the expression of VEGF, which helps in osteogenesis of BMSC-derived osteoblasts.[224] Increased vascularization helps with tissue ingrowth and matrix deposition.[225] However, little is known about how Co^{2+} affects other cell processes. Cobalt is considered a possible carcinogen because of its ability to prevent DNA repair.[206] This ion induces ROS that can cause oxidative damage and angiogenesis.[206,224] In addition, cobalt is also known to induce a hypoxic environment, which activates angiogenic factors, such as VEGF and fibroblast growth factor (FGF).[72] Despite positive attributes of these cytokines, VEGF and FGF can push cells toward uncontrollable growth and cause tumorigenesis.

5.5.2. Biophysical Effects of Cobalt Nanoparticles. As mentioned previously with chromium, nanoparticles generated from CoCr implants present a cytotoxic risk to patients because they are easily phagocytosed by cells.[221] This can lead to adverse immune response and possible device failure. CoCr nanoparticles increase free radical formation and have been associated with DNA damage at certain concentrations, which can lead to foreign body reaction.[226] However, when cobalt is added to other types of mineral-nanoparticles, they can be used for biomedical applications without adverse effects. For example, iron-oxide nanoparticles containing cobalt have been utilized as a drug delivery vehicle for cancer therapeutics.[227,228] Despite some promising applications of cobalt containing biomaterials, it is important to investigate their immune response and effect on cells.

5.6. Cellular Effects of Manganese. The transition metal manganese (Mn) has an atomic number of 25, and can be found with several different oxidative states, specifically Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{6+}, and Mn^{7+}. Interestingly, manganese is an important cofactor in several enzymes key to brain function such as manganese-superoxide dismutase and glutamine synthetase.[229] Importantly, glutamine synthesis is necessary for the production of glutamine, an important precursor to the neural transmitters glutamate and gamma-aminobutyric acid (GABA).[229] Manganese-superoxide dismutase is necessary to mitigate damage reactive oxygen species in the mitochondria, which would otherwise lead to apoptosis of neural cells.[230] Some studies suggested that deficiency of Mn affect the presence of proteoglycans and glycosylation of other glycoproteins,[231,232] which are necessary for cell-adhesion abilities, growth, and differentiation.[233] Incorporation of manganese within nanoparticles or other biomaterials could improve cell adhesion and proliferation, and may be relevant for affecting neural cell fate.

5.6.1. Biochemical Effect of Manganese Ion. Supplementation of manganese increased the serum osteocalcin and bone mass density in ovariectomized mice, suggesting manganese plays a role in bone hemostasis.[234] Interestingly, manganese ion has been shown to neutralize the formation of ROS, and subsequently increases osteoblast proliferation.[224] Additionally, in the presence of manganese doped alumina enhances bone marrow compared to controls. The porous nature of the scaffold allows for tissue ingrowth, suggesting that this mineral is a valuable addition to bone-ceramic interfaces.[211] However, manganese has a copious number of oxidative states, so it would be interesting to understand the effect of charges on tissue regeneration. Moreover the exact mechanism of how manganese improves bone mineralization is not well understood. Thus, there is a need for additional studies on this element.

As previously mentioned, manganese has been noted as an important element for proper neural function. However, at high concentrations manganese intake can be neurotoxic, causing symptoms such as hallucinations, irritability, handwriting deterioration, and dementia.[235] Additionally, accumulation of Mn^{3+} ions inhibits key mitochondrial enzymes more effectively compared to Mn^{2+} ions, because of increased oxidative potential.[235] The use of nanomaterials to deliver appropriate doses of manganese has been explored. Interestingly, manganese (Mn)-doped gold (Au) nanoparticles have been shown to have an increased effect on neuronal differentiation, which can specifically be attributed to the presence of manganese (Figure 5B).[236] Manganese ions do this by competing with calcium ions for calmodulin binding which subsequently enhances neural differentiation because of the change in intracellular calcium ion concentration.[237] In this case, the Mn−Au composite prevented burst release of manganese, and controlled ion release in a dose-dependent manner. Mn is released in the endosome due to low pH, whereas Au-nanoparticles do not degrade because of their stability. In this way, stable nanoparticles can be used to provide safe dosages of mineral ions.

5.6.2. Biophysical Effect of Manganese-Based Biomaterials. The presence of manganese in biomaterials to date is limited. An iron−manganese scaffold has been developed for bone-tissue engineering, primarily for structural component.[238] Interestingly, addition of manganese enhances the degradation rate of the scaffold. Manganese content was found to be higher at grain boundaries and caused the decreased mechanical integrity and enhanced degradation properties of this biomaterial. Manganese nanoparticles have also been utilized as contrast agents[239] and drug delivery vehicles.[240,241] Additionally, bioactive glass nanoparticles doped with manganese have been shown to provide an antibacterial effect in addition to the well-known osteogenic applications of this biomaterial.[242]

5.7. Cellular Effects and Uses of Gold. Gold (Au) is an inert rare earth metal (atomic number 79), with high ductility and malleability. Gold nanoparticles (AuNPs) differ greatly from bulk gold in terms of physical properties. For example, gold nanoparticles have been utilized as fluorophores for imaging purposes,[243] as well as delivery for therapeutic oligonucleotides.[244] In addition, AuNPs have also been shown to promote osteogenic differentiation in human mesenchymal stem cells (hMSCs)[245] via activation of the p38/MAPK pathway, ultimately activating transcription of osteo-specific genes through RUNX2 (Figure 5C).[246] As previously mentioned, nanoparticles have been shown to activate this stress-related pathway because of their surface

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

energy, suggesting that biophysical cues from nanomaterial properties can affect osteogenesis. It is established that uptake of nanoparticles generates stress within cells, specifically through ROS production. ROS, which nanoparticles produce, are known to activate MAPK pathways, such as p38, which have the potential to activate osteogenic genes.[247] Therefore, this stress pathway could be used as a means to promote osteogenesis in response to ROS producing nanomaterials.

In addition to induction of stress pathway activation, an important aspect to consider is the biophysical effects induced by different sizes and shapes of AuNPs. For example, decrease in particle diameter has been shown to increase the toxic effects of AuNPs.[248] This property can be associated with increased surface-to-volume ratio resulting in higher production of ROS. Additionally, AuNPs (nanorods) have been shown to exhibit higher cytotoxicity than AuNPs (nanospheres) when treating a human cancer cell line.[249] This toxicity was associated with desorption of a capping agent cetrimonium bromide (CTAB) caused by larger surface area of nanorods compared to the gold nanospheres with the same capping agent. However, more in-depth studies need to be done to rule out the effect of capping agents and to evaluate the effect of nanomaterial geometry on cellular functions.

To prevent these cytotoxic effects when using AuNPs, surface modification via polymers are utilized. One study investigated the use of various polymer coatings on AuNPs to modulate the protein corona and observe the cellular uptake in hepatocytes and associated toxicity.[250] They showed that coating AuNPs with polymer mitigates the production of ROS. Additionally, at cytotoxic concentrations, pro-apoptotic genes were activated when exposed to branched polyethylenimine (BPEI) coated AuNPs. BPEI coating prevented the generation of a protein corona, which actually caused cytotoxic effects. Therefore, not only is the coating important to prevention of cytotoxic effects, but the subsequent protein corona created around nanoparticles has a significant effect on cellular uptake and effect, retention time, and excretion.

Given the stability of AuNPs against degradative agents,[244] it is difficult to discern the relevance or the effect of gold ion on cell differentiation or modulation. So far most of the literature has been focused on the biophysical cues that AuNPs generate and have overlooked any potential biochemical mechanisms that gold ions could have on cell modulation. It may be prudent for researchers to investigate the utility of gold ions, given the modulatory capabilities of the cations previously mentioned.

5.8. Cellular Effects and Uses of Silver and Silver Biomaterials. Silver (Ag) (atomic number 47) mirrors the properties of gold both at the macro and nanoscale, but is utilized in biomaterial applications for different reasons. In its bulk form it is a highly conductive and stable metal, whereas silver nanoparticles (AgNPs) exhibit a surface plasmon resonance, and the potential to generate ROS. The shape and size of AgNPs has an effect on its properties, one example being the inherent surface plasmon resonance of these particles.[251] Specifically, 30 nm AgNPs exhibit dipole plasmon resonance at a wavelength of 367 nm, while 60 nm AgNPs have quadrupole plasmon resonance at a wavelength of 357 nm.[252] However, the major biomedical application of silver nanoparticles lies in the field of antifouling agents. The incorporation of AgNPs into polymer membranes (such as chitosan) dramatically reduces bacterial adhesion without changing the bulk properties of the membrane. This change in bacterial adhesion is generally attributed to nanoparticle induced membrane lysis, cytosolic protein binding, and the presence of ROS, created at the surface of the membrane (Figure 5D).[253] As previously discussed, ROS have the capability to be cytotoxic at high concentrations. This property can be utilized as an antimicrobial additive by using these oxidative agents to attack potential bacterial colonizers on biomaterials that could lead to device failure.

AgNPs have been shown to cause permeated membranes in bacteria, indicating that these materials not only cause cytotoxic effects but also bind to membranes of fouling agents such as *E. coli*, a Gram-negative microorganism.[254] This mechanism is caused by free radicals, but it is interesting that *Staphylococcus aureus* (*S. aureus*), a Gram-positive organism, is shown to be more resistant to this type of antibacterial method.[255] However, other reports show that positive charged silver ions can cause the disruption of negatively charged cellular membranes because of electrostatic interactions.[256] One group supposed that this property of silver ions was due to the increased peptidoglycan layer of Gram-positive bacteria, which acts as a larger barrier to toxic effects.[257] The permeation aspects of the bacterial cell membrane seem to be dictated by thickness, which is shown in that Gram-positive bacteria are less susceptible to silver toxicity than Gram-negative. Ultimately, silver antimicrobial effects may be more beneficial in cases pertaining to Gram-negative bacteria with thinner cellular membranes, rather than thick, Gram-positive bacteria which can be targeted via other mechanisms. Whether cell lysis is caused by ROS generation or difference in charge, clearly silver nanoparticle have various ways to prevent biofilm production.

The effect of size has also been observed for antimicrobial properties. AgNPs have been shown to be more effective at inhibiting bacteria than silver ions alone, and this effect decreased as size of the nanoparticle increased.[258] Smaller sized nanomaterials were more easily transported into the cell compared to silver ions, and once internalized could produce a large quantity of damaging ROS, ultimately making a more effective antimicrobial treatment. Additionally, small silver nanoparticles have been shown to prevent biofilms through other mechanisms aside from ROS generation. In some cases, silver nanoparticles destroy the cellular membrane and cause cell lysis,[255] while others prevent enzyme function and respiratory chain operation.[103] Silver nanoparticles prove to be a useful antibacterial agent that target unwanted biofilms in many different ways because of their size variance and inherent antibacterial elemental composition.

Additionally, some evidence has shown that silver nanoparticles may play a role in osteogenesis in addition to their antimicrobial properties. In vitro and in vivo work has shown that silver nanoparticles may enhance osteogenesis; although there has not been much additional investigation into these findings.[259] Additionally, other studies have incorporated silver alongside titanium and magnesium for osseointegration applications, and successfully promoted matrix mineralization. Dually doping with magnesium and silver produced the greatest osteogenic effect, though doping with silver alone showed some improvement over titanium alone.[260] Therefore, AgNPs and incorporation of silver ion into nanomaterials may have additional benefits to bone-tissue regeneration applications apart from their well-known antimicrobial properties.

Silver biomaterials have also been utilized for neural stimulation. Silver nanowires have been used in micro-

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

electrodes and showed good biocompatibility along with electrical conductivity within a hydrogel system.[261] These nanowires have proven useful in the production of flexible and transparent electrodes with high conductivity, such as in one study showing a range of 5285−8130 S cm^{-1} in a polydimethylsiloxane (PDMS) layer.[262] The well-known conductivity of silver has made it a valuable material for bioelectronics both at the macro and nanoscale, providing yet another useful application for silver-based biomaterials in the field of biomedical engineering.

5.9. Effects of Molybdenum. Molybdenum is a transition metal with atomic number 42 and oxidation states of Mo^{4+}, Mo^{5+}, and Mo^{6+}.[263] Mo plays an important role as an enzyme cofactor[264] and is a component of nitrogenases in prokaryotes.[265] In the human body, deficiency in molybdenum results in fatalities, with patients exhibiting cerebral deficiency and loss of neurons due to a lack of sulfite oxidase enzymes.[266] Mammalian enzymes that utilize molybdenum are limited to sulfite oxidase and xanthine oxidase,[263] which in the case of sulfite oxidase (found in the liver) is essential for processing sulfur compounds.[267]

In biomaterials, molybdenum has been incorporated into stainless steels to increase strength[219] and improve resistance to pitting corrosion.[268] Molybdenum dioxide (MoO_2) nanoparticles have been used as a photothermal agent for cancer therapeutic as they exhibit a localized surface plasmon resonance (SPR) effect.[269] These nanoparticles degrade in phosphate buffer solution (PBS) and release molybdenum ions, which can cause some cytotoxicity. However, the study did not investigate the effect of molybdenum ions on cells.[269] Additionally, molybdenum disulfide (MoS_2) 2D nanomaterials show superhydrophobic properties by modulating their defect ratio.[270,271] Another study has used MoS_2 nanomaterials as a cross-linking agent using thiolated polymers to engineer cytocompatible hydrogels.[272] This is a relatively new biomaterials and there is a need to investigate the biophysical and biochemical effects of these nanomaterials on cellular functions.

5.10. Cellular Effects of Vanadium. Vanadium (V) is a transition metal (atomic number 23) with a charge of V^{+5} (vanadate) or V^{+6} (oxovanadium). Vanadium-based compounds enter the cell passively or through the anionic channels and have been shown to interact with cellular machinery in different ways.[273] For example, vanadate can bind to (Na,K)-adenosine triphosphatase (ATPase), a solute pump that regulates ion traffic into the cell, and creates competitive inhibition for the enzyme's activity, which is normally dependent on sodium to phosphorylate.[274] Toxicity of vanadate was evaluated in fetal mice, and though at normal levels, no embryotoxicity occurred, sodium orthovanadate (Na_3VO_4) showed fetal-growth retardation at known maternal toxicity.[275] This highlights the importance of dosage of these mineral ions because ionic compounds have the potential to cause toxic effects.

5.10.1. Biochemical Effects of Vanadium Ion. The effect of vanadium on bone health has been investigated in diabetic rats. Type 1 diabetes can cause bone resorption and decreased bone mineral density, making this animal model useful for bone-focused research.[276] Vanadium may accumulate in bone by replacing some of the phosphate molecules present in hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$),[277] which showed enhanced bone formation in diabetic rats.[276] Additionally, vanadate has also been shown to mimic the effects of insulin.[273,278] Interestingly, diabetic rats supplemented with vanadate showed similar blood-glucose levels to those of nondiabetic rats after 4 weeks.[279] Vanadium may act by either inhibiting insulin receptor protein tyrosine phosphatases (PTPases) or activating of cytosolic protein kinases,[278] suggesting that this ion could be useful in bone-related biomaterial applications.[280]

Other therapeutic avenues of vanadium have been explored, including stimulation of new blood vessel development. Administration of one vanadium compound improved angiogenesis in rats.[281] Within the cellular membrane, introducing V^{+5} or V^{+6} in this hydrophobic environment can induce a redox reaction resulting in ROS, which has the potential to induce angiogenesis.[273] Vanadate was shown to induce HIF-1α and VEGF protein expression through the production of ROS, which may occur through the process of the mitochondrial electron transport chain reducing vanadate and forming free radicals.[282]

Other studies have used vanadium in antitumor capacities.[283] It is important to note that angiogenesis and tumor angiogenesis share similar pathways (such as the need for hypoxia) but differ in other respects, such as the activation of oncogenes or mutation of tumor-suppressors in respect to tumor angiogenesis.[284] According to several studies done in rats, supplementation with vanadium provides protection against hepatocarcinogenesis by reducing the amount of carcinogen-derived reactive intermediates and potentially causes apoptosis through production of ROS.[283] In mice, the supplementation of vanadocene dichloride showed antitumor effects against carcinomas of the colon and lung by accumulation of vanadium in nucleic acids which inhibited DNA and ribonucleic acid (RNA) synthesis.[283,285] However, the mechanism as to how vanadium specifically prevents tumors is not a simple one, and there does not seem to be a solution as to how vanadium should be used to inhibit tumors. The utility of this ion has only been suggested, and at this point, more research would need to occur before vanadium could be definitively used for therapeutic applications.

5.10.2. Biophysical Effect of Vanadium-Containing Biomaterials. Similar to other transition metal nanoparticles, vanadium-based nanoparticles have been shown to have antimicrobial properties and have previously been used in paints for this reason. Specifically, vanadium pentoxide nanowires prevent biofilm formation through peroxide formation.[286] This nanomaterial property is not dissimilar from that of the elemental form, so it is difficult to discern whether the physical formulation of the nanowire or vanadium itself is the cause of the antifouling property. Vanadium doped scaffolds have also been utilized, primarily due to vanadium's known inhibitory role of PTPases as previously mentioned. One group utilized vanadyl acetylacetonate to dope scaffolds containing hydroxyapatite nanoparticles to induce endochondral ossification, by blocking PTPase and thus increasing osteo-specific protein production.[287] However, vanadium nanoparticles have yet to be used alone in order to promote osteogenesis, which could be a potential avenue of research.

Currently, vanadium is found within common titanium implants (called Ti-6Al-4 V) for orthopedic applications. However, it has been found that at certain concentrations the presence of vanadium within degradation products of these implants can increase cytotoxic effects.[288] Additionally, the titanium implants containing vanadium showed lower corrosion resistance, and lower cell growth ratios, suggesting

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

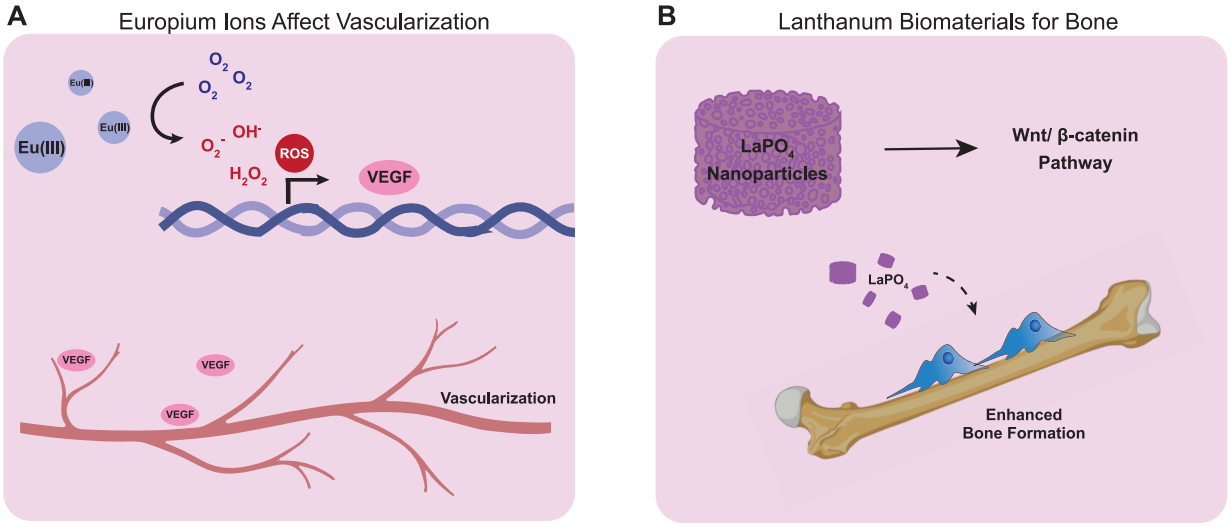


Figure 6. Role of lanthanide ions in tissue regeneration. (A) Europium ions aid in the process of vascularization. This occurs through the generation of reactive oxygen species (ROS). (B) Lanthanum-based biomaterials promote bone formation by stimulating Wnt/β-catenin pathway.

that the presence of vanadium in titanium implants may be detrimental.[289] It is unclear how the incorporation of vanadium actually affects implants structural composition and material properties aside from the knowledge that these alloys have low wear resistance.[290] Further characterization of the biophysical effects of vanadium-biomaterials is needed to conclude whether incorporating this element is useful. There is certainly room for further characterization of all of vanadium's properties to determine how the biochemical and biophysical attributes of this element could affect therapeutic systems outside of orthopedic implants.

6. CELLULAR EFFECTS AND USES OF LANTHANIDE METALS

Lanthanide elements (atomic numbers 57−71) have been explored as substitutes for other ions to control and direct cell functions.[291] For example, utilization of Ln^{3+} in place of Ca^{2+} has shown numerous different effects on enzymatic function. Ln^{3+} has been shown to bind to Ca^{2+} sites on cellular membranes and macromolecules.[291] However, a large difficulty in assessing their biochemical role in cells comes from the inability of lanthanide elements to cross the cell membrane.[291] Another study explored the effect of europium III hydroxide on angiogenesis in HUVECs.[292] They did not observe a change in cell proliferation with other lanthanides (Nd, Sm, Gd, and Tb) but observed increased proliferation with Eu(III) hydroxide ($EuIII(OH)_3$).[292] $EuIII(OH)_3$ produced similar vascularization results in chick chorioallantoic membrane (CAM) assays (Figure 6A). These angiogenic properties were due to the production of ROS as validated by imaging with green fluorescence.

Lanthanide nanomaterials have been previously used for luminescence used in diagnostics and imaging, including that of Eu(III) complexes.[293,294] In some cases, europium has been used within bioactive scaffolds for imaging alongside the activation of osteogenesis, and did show an enhancement in the expression of osteogenic-related genes.[295] However, the mode of action for Eu(III) is not well understood.[295] It would be interesting to see if Eu(III) nanoparticles had similar modes of ROS generation as the ion constituents or if they possess unique biophysical attributes that can modulate cellular phenotype and function in addition to their luminescent qualities.

Lanthanum-phosphate nanoparticles have been utilized within scaffolds to activate osteogenesis (Figure 6B).[296] Components of the Wnt/β-catenin pathway were shown to be activated in the presence of these nanomaterials,[296] whereas other work has indicated that lanthanum acts through the Smad-dependent BMP signaling pathway by simulating the phosphorylation of surface serine/threonine kinase receptors 1/5/8 (SMAD 1/5/8).[297] It would be interesting to fully characterize the effect of lanthanum compared to lanthanum-nanomaterials to understand whether activation of these osteogenic pathways is related to biochemical cues from lanthanum ions or biophysical properties of lanthanum-nanomaterials. The presence and effect of lanthanide elements in orthopedic implants has also been explored. Magnesium-lanthanide alloys (containing either lanthanum, neodymium, or cerium) were shown to corrode slowly without apparent systemic effects and exhibited good biocompatibility.[298] However, no additional bone growth was observed, suggesting that the presence of lanthanide elements provided only desirable physical material characteristics rather than significant activation of biological processes.

While utilizing lanthanide-based materials is an interesting method to promote vascularization mechanisms or provide structural support, the use of lanthanides is relatively difficult and quite expensive. There are much more readily available elements that are capable of forming ROS that could be utilized as a vascularization technique, and are potentially much less costly. The study of lanthanum in osteogenesis activation is in its infancy and certainly needs more characterization. Additionally, limited research has been performed to elucidate the effect of degradative products from lanthanide-based implants. However, the premise of using lanthanides does bring up an interesting idea of exploring the boundaries of how we currently use these elements. Still, it is important to consider the cost and potential ease of production of therapeutics, because these are intended to treat a wide number of patients and must have the potential for manufacturing.

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

7. FUTURE PERSPECTIVE

Ions released from inorganic biomaterials can be utilized to modulate cellular functions such as inflammation, wound healing, and angiogenesis. Although functions of some of the common ions are well-known, there are a multitude of ions which have not been investigated in detail. For example, the effect of rare earth elements as well as the lanthanide series have not yet been fully characterized in terms of their biological response. In addition, one of the major limitations of evaluating the biological response of ionic dissolution products is the use of tradition biological assays such as polymerase chain reaction (PCR) and Western blot to evaluate a preselected set of genes or proteins. These traditional approaches have inherent biases and lack the ability to provide a deep understanding of the effect of ions at a global scale. The recent emergence of "omics" techniques which provide readouts of different biological processes have allowed us to understand complex biological interactions of biomaterials in an unbiased approach. Specifically, transcriptomics, proteomics, and metabolomics have laid down the necessary foundation to provide an unbiased global view of the cellular activity with pivotal insights about the affected cellular pathways. Thus, emerging "mineralomics" approaches to evaluate the biological response of minerals (or ions) using omics-based techniques have the potential to transform our understanding of the role of minerals in tissue regeneration, leading to a new class of regenerative therapies. For example, recent study have shown that mineral nanoparticles can stimulate both chondrogenesis as well as osteogenesis though multiple signaling pathways.[125]

While individual minerals may display mild to moderate cellular responses, the combination of ions could augment/subdue these biological responses. Screening of different ions in combinations using high-throughput analysis can boost our ability to identify uniquely useful ion combinations. Combining minerals with synergistic characteristics will enhance regenerative capacities through simultaneous stimulation of different cellular pathways. Use of omics-based approaches will yield a comprehensive genetic profile that will enable us to distinguish "hit" mineral combinations with the ability to trigger complementary intracellular pathways and subsequently produce a more stable cellular differentiation. This knowledge will contribute in designing inorganic biomaterials with predetermined stoichiometry to direct cellular functions.

As ions play an important role in a multitude of biological processes, it is expected that they must have an ability to stimulate or suppress immune responses. Some earlier work has shown that patients with metallic implants develop sensitivity to specific antigens.[299−301] However, the effect of ions on immune response has not been thoroughly investigated, so it would be interesting to see if ions can be used for immunomodulation. Immune response can play an important role in defining the outcome of wound healing and regeneration processes, specifically in the balance between pro-inflammatory and anti-inflammatory processes are important. Specifically, the response of immune cells including macrophages, dendritic cells, and lymphocytes (T-cell and B-cells), to ionic dissolution products needs to be investigated. Development of inorganic biomaterials for immune modulation will provide new approaches for immunomodulation.

Another emerging application of inorganic micro- and nanomaterials are their use as reinforcing agents for designing biomaterials inks or bioinks for 3D printing.[302] Traditionally, polymeric hydrogels are used as bioinks for 3D printing because of their high water content and ability to maintain high cell viability. However, one of the primary limitations of polymeric hydrogels is their weak mechanical properties. The addition of inorganic biomaterials, such as microparticles and nanoparticles, can significant improve the mechanical strength and physiological stability of hydrogels.[303] In addition, some nanoparticles, such as nanoclay (nanosilicates), can incorporate shear-thinning properties to hydrogel networks, which is highly desirable for 3D printing.[304] Such shear-thinning biomaterials can shield encapsulated cells from shear forces and improve cellular viability postprinting. Also, inorganic nanomaterials can also sequester a range of therapeutics that to can be used to direct cellular functions.

8. CONCLUSION

The properties of mineral-based biomaterials are dependent on the constituent atoms. The composition of biomaterials provides unique biophysical and biochemical attributes that can direct cellular function. Ions released from mineral-based biomaterials can direct cellular function by activating specific genes or biochemical pathways. Incorporation of various ions within biomaterials can change physical properties, such as dissolution rate, charge, topography, and mechanical integrity. While some ions have been extensively studied, such as calcium (Ca^{2+}) and magnesium (Mg^{2+}), but other less common elemental ions have not yet been studied for this purpose. These lesser-known and poorly characterized mineral ions may have significant biophysical or biochemical effects when incorporated into biomaterials that could enhance and modify current tissue engineering strategies. Ions from minerals such as molybdenum (Mo) or lanthanum (La), though not thoroughly investigated to date, show incredible promise in directing cell fate when incorporated within biomaterials. Additionally, a deeper understanding of physical and chemical properties with respect to cellular function is key to developing fine-tuned regenerative medicine therapeutics. This Review highlighted several lesser known and characterized elements and mineral-based biomaterials along with their potential biophysical and biochemical effects within the body to explore their possible therapeutic avenues.

AUTHOR INFORMATION

Corresponding Author

Akhilesh K. Gaharwar − *Biomedical Engineering, Dwight Look College of Engineering, Material Science and Engineering, Dwight Look College of Engineering, and Center for Remote Health Technologies and Systems, Texas A&M University, College Station, Texas 77843, United States;* orcid.org/0000-0002-0284-0201; Email: gaharwar@tamu.edu

Author

Anna M. Brokesh − *Biomedical Engineering, Dwight Look College of Engineering, Texas A&M University, College Station, Texas 77843, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsami.9b17801

Funding

A.K.G. would like to acknowledge financial support from the National Institute of Biomedical Imaging and Bioengineering

(NIBIB) of the National Institutes of Health (NIH) Director's New Innovator Award (DP2 EB026265).

Notes

The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agency.

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to acknowledge Biorender.com for preparing some of the images in the manuscript.

■ REFERENCES

(1) Place, E. S.; Evans, N. D.; Stevens, M. M. Complexity in biomaterials for tissue engineering. *Nat. Mater.* **2009**, *8* (6), 457−470.

(2) Zakrzewski, J. L.; van den Brink, M. R. M.; Hubbell, J. A. Overcoming immunological barriers in regenerative medicine. *Nat. Biotechnol.* **2014**, *32* (8), 786−794.

(3) Hussey, G. S.; Dziki, J. L.; Badylak, S. F. Extracellular matrix-based materials for regenerative medicine. *Nature Reviews Materials* **2018**, *3* (7), 159−173.

(4) Griffith, L. G.; Naughton, G. Tissue Engineering–Current Challenges and Expanding Opportunities. *Science* **2002**, *295* (5557), 1009.

(5) Neufeld, G.; Cohen, T.; Gengrinovitch, S.; Poltorak, Z. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J.* **1999**, *13* (1), 9−22.

(6) DeBerardinis, R. J.; Thompson, C. B. Cellular metabolism and disease: what do metabolic outliers teach us? *Cell* **2012**, *148* (6), 1132−1144.

(7) Gharibzahedi, S. M. T.; Jafari, S. M. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends Food Sci. Technol.* **2017**, *62*, 119−132.

(8) Williams, M. H. Dietary Supplements and Sports Performance: Minerals. *J. Int. Soc. Sports Nutr.* **2005**, *2* (1), 43.

(9) Gaharwar, A. K.; Singh, I.; Khademhosseini, A. Engineered Biomaterials for In situ Tissue Regeneration. *Nature Reviews Materials* **2020**, in press.

(10) de Boer, J.; Siddappa, R.; Gaspar, C.; van Apeldoorn, A.; Fodde, R.; van Blitterswijk, C. Wnt signaling inhibits osteogenic differentiation of human mesenchymal stem cells. *Bone* **2004**, *34* (5), 818−826.

(11) Pinton, P.; Giorgi, C.; Siviero, R.; Zecchini, E.; Rizzuto, R. Calcium and apoptosis: ER-mitochondria Ca2+ transfer in the control of apoptosis. *Oncogene* **2008**, *27* (50), 6407−6418.

(12) Jahnen-Dechent, W.; Ketteler, M. Magnesium basics. *Clin. Kidney J.* **2012**, *5* (Suppl 1), i3−i14.

(13) Cross, L. M.; Carrow, J. K.; Ding, X.; Singh, K. A.; Gaharwar, A. K. Sustained and Prolonged Delivery of Protein Therapeutics from Two-Dimensional Nanosilicates. *ACS Appl. Mater. Interfaces* **2019**, *11* (7), 6741−6750.

(14) Kerativitayanan, P.; Carrow, J. K.; Gaharwar, A. K. Nanomaterials for Engineering Stem Cell Responses. *Adv. Healthcare Mater.* **2015**, *4* (11), 1600−1627.

(15) Engler, A. J.; Sen, S.; Sweeney, H. L.; Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **2006**, *126* (4), 677−689.

(16) Gaharwar, A. K.; Dammu, S. A.; Canter, J. M.; Wu, C.-J.; Schmidt, G. Highly Extensible, Tough, and Elastomeric Nanocomposite Hydrogels from Poly(ethylene glycol) and Hydroxyapatite Nanoparticles. *Biomacromolecules* **2011**, *12* (5), 1641−1650.

(17) Jones, J. R.; Ehrenfried, L. M.; Hench, L. L. Optimising bioactive glass scaffolds for bone tissue engineering. *Biomaterials* **2006**, *27* (7), 964−973.

(18) Berdanier, C. D.; Dwyer, J. T.; Feldman, E. B. *Handbook of Nutrition and Food*; CRC Press, 2007.

(19) *Vitamin and Mineral Supplement Fact Sheets*; Office of Dietary Supplements, National Institutes of Health: Bethesda, MD, 2008.

(20) Zimmermann, M. B.; Hurrell, R. F. Nutritional iron deficiency. *Lancet* **2007**, *370* (9586), 511−520.

(21) Looker, A. C.; Dallman, P. R.; Carroll, M. D.; Gunter, E. W.; Johnson, C. L. Prevalence of Iron Deficiency in the United States. *JAMA* **1997**, *277* (12), 973−976.

(22) Monopoli, M. P.; Åberg, C.; Salvati, A.; Dawson, K. A. Biomolecular coronas provide the biological identity of nanosized materials. *Nat. Nanotechnol.* **2012**, *7*, 779.

(23) Jiang, W.; Kim, B. Y. S.; Rutka, J. T.; Chan, W. C. W. Nanoparticle-mediated cellular response is size-dependent. *Nat. Nanotechnol.* **2008**, *3*, 145.

(24) Saikia, J.; Yazdimamaghani, M.; Hadipour Moghaddam, S. P.; Ghandehari, H. Differential Protein Adsorption and Cellular Uptake of Silica Nanoparticles Based on Size and Porosity. *ACS Appl. Mater. Interfaces* **2016**, *8* (50), 34820−34832.

(25) Reilly, G. C.; Engler, A. J. Intrinsic extracellular matrix properties regulate stem cell differentiation. *J. Biomech.* **2010**, *43* (1), 55−62.

(26) Hofer, A. M.; Brown, E. M. Extracellular calcium sensing and signalling. *Nat. Rev. Mol. Cell Biol.* **2003**, *4* (7), 530−538.

(27) Cassandri, M.; Smirnov, A.; Novelli, F.; Pitolli, C.; Agostini, M.; Malewicz, M.; Melino, G.; Raschellà, G. Zinc-finger proteins in health and disease. *Cell Death Discovery* **2017**, *3*, 17071.

(28) Wang, C.; Lin, K.; Chang, J.; Sun, J. Osteogenesis and angiogenesis induced by porous β-CaSiO3/PDLGA composite scaffold via activation of AMPK/ERK1/2 and PI3K/Akt pathways. *Biomaterials* **2013**, *34* (1), 64−77.

(29) Wang, Y.; Yu, X.; Baker, C.; Murphy, W. L.; McDevitt, T. C. Mineral particles modulate osteo-chondrogenic differentiation of embryonic stem cell aggregates. *Acta Biomater.* **2016**, *29*, 42−51.

(30) Song, H.; Wang, W.; Zhao, P.; Qi, Z.; Zhao, S. Cuprous oxide nanoparticles inhibit angiogenesis via down regulation of VEGFR2 expression. *Nanoscale* **2014**, *6* (6), 3206.

(31) Egli, R. J.; Luginbuehl, R. Tissue engineering-nanomaterials in the musculoskeletal system. *Swiss Med. Wkly.* **2012**, *142*, w13647.

(32) Lokhande, G.; Carrow, J. K.; Thakur, T.; Xavier, J. R.; Parani, M.; Bayless, K. J.; Gaharwar, A. K. Nanoengineered injectable hydrogels for wound healing application. *Acta Biomater.* **2018**, *70*, 35−47.

(33) Peak, C. W.; Singh, K. A.; Adlouni, M. a.; Chen, J.; Gaharwar, A. K. Printing Therapeutic Proteins in 3D using Nanoengineered Bioink to Control and Direct Cell Migration. *Adv. Healthcare Mater.* **2019**, *8* (11), 1801553.

(34) Prasad, A. S. Zinc: An antioxidant and anti-inflammatory agent: Role of zinc in degenerative disorders of aging. *J. Trace Elem. Med. Biol.* **2014**, *28* (4), 364−371.

(35) DeSousa, J.; Tong, M.; Wei, J.; Chamley, L.; Stone, P.; Chen, Q. The anti-inflammatory effect of calcium for preventing endothelial cell activation in preeclampsia. *J. Hum. Hypertens.* **2016**, *30* (5), 303−308.

(36) Zeng, C.; Li, H.; Wei, J.; Yang, T.; Deng, Z.-h.; Yang, Y.; Zhang, Y.; Yang, T.-b.; Lei, G.-h. Association between Dietary Magnesium Intake and Radiographic Knee Osteoarthritis. *PLoS One* **2015**, *10* (5), e0127666−e0127666.

(37) Whitfield, J.; MacManus, J.; Rixon, R.; Boynton, A.; Youdale, T.; Swierenga, S. The positive control of cell proliferation by the interplay of calcium ions and cyclic nucleotides. A review. *In Vitro* **1976**, *12* (1), 1−18.

(38) Boynton, A. L.; McKeehan, W. L.; Whitfield, J. F. *Ions, Cell Proliferation, and Cancer*; Academic Press, 2013.

(39) Bikle, D. D.; Xie, Z.; Tu, C.-L. Calcium regulation of keratinocyte differentiation. *Expert Rev. Endocrinol. Metab.* **2012**, *7* (4), 461−472.

(40) Gu, X.; Spitzer, N. C. Breaking the code: regulation of neuronal differentiation by spontaneous calcium transients. *Dev. Neurosci.* **1997**, *19* (1), 33−41.

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

(41) Romani, A. Magnesium homeostasis in mammalian cells. *Front. Biosci., Landmark Ed.* **2007**, *12*, 308−331.
(42) Venugopal, B. *Physiologic and Chemical Basis for Metal Toxicity*; Springer Science & Business Media, 2013.
(43) Yin, J.; Hu, Y.; Yoon, J. Fluorescent probes and bioimaging: alkali metals, alkaline earth metals and pH. *Chem. Soc. Rev.* **2015**, *44* (14), 4619−4644.
(44) *Dietary Reference Intakes for Calcium and Vitamin D*; National Academics Press (US), 2011.
(45) Jørgensen, N. R.; Henriksen, Z.; Sørensen, O. H.; Eriksen, E. F.; Civitelli, R.; Steinberg, T. H. Intercellular calcium signaling occurs between human osteoblasts and osteoclasts and requires activation of osteoclast P2X7 receptors. *J. Biol. Chem.* **2002**, *277* (9), 7574−7580.
(46) Mattson, M. P.; Chan, S. L. Calcium orchestrates apoptosis. *Nat. Cell Biol.* **2003**, *5* (12), 1041.
(47) Takai, Y.; Kishimoto, A.; Iwasa, Y.; Kawahara, Y.; Mori, T.; Nishizuka, Y. Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *J. Biol. Chem.* **1979**, *254* (10), 3692−3695.
(48) Castagna, M.; Takai, Y.; Kaibuchi, K.; Sano, K.; Kikkawa, U.; Nishizuka, Y. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J. Biol. Chem.* **1982**, *257* (13), 7847−7851.
(49) Takai, Y.; Kishimoto, A.; Kikkawa, U.; Mori, T.; Nishizuka, Y. Unsaturated diacylglycerol as a possible messenger for the activation of calcium-activated, phospholipid-dependent protein kinase system. *Biochem. Biophys. Res. Commun.* **1979**, *91* (4), 1218−1224.
(50) Xia, L.; Lin, K.; Jiang, X.; Fang, B.; Xu, Y.; Liu, J.; Zeng, D.; Zhang, M.; Zhang, X.; Chang, J.; et al. Effect of nano-structured bioceramic surface on osteogenic differentiation of adipose derived stem cells. *Biomaterials* **2014**, *35* (30), 8514−8527.
(51) Xia, L. G.; Lin, K. L.; Jiang, X. Q.; Xu, Y. J.; Zhang, M. L.; Chang, J.; Zhang, Z. Y. Enhanced osteogenesis through nano-structured surface design of macroporous hydroxyapatite bioceramic scaffolds via activation of ERK and p38 MAPK signaling pathways. *J. Mater. Chem. B* **2013**, *1* (40), 5403−5416.
(52) Lai, C.-F.; Cheng, S.-L. Signal transductions induced by bone morphogenetic protein-2 and transforming growth factor-β in normal human osteoblastic cells. *J. Biol. Chem.* **2002**, *277* (18), 15514−15522.
(53) Wu, M. R.; Chen, G. Q.; Li, Y. P. TGF-beta and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. *Bone Res.* **2016**, *4*, 21.
(54) Raingeaud, J.; Gupta, S.; Rogers, J. S.; Dickens, M.; Han, J.; Ulevitch, R. J.; Davis, R. J. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J. Biol. Chem.* **1995**, *270* (13), 7420−7426.
(55) Ahamed, M.; Posgai, R.; Gorey, T. J.; Nielsen, M.; Hussain, S. M.; Rowe, J. J. Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in Drosophila melanogaster. *Toxicol. Appl. Pharmacol.* **2010**, *242* (3), 263−269.
(56) Wang, Z. Y.; Li, N.; Zhao, J.; White, J. C.; Qu, P.; Xing, B. S. CuO Nanoparticle Interaction with Human Epithelial Cells: Cellular Uptake, Location, Export, and Genotoxicity. *Chem. Res. Toxicol.* **2012**, *25* (7), 1512−1521.
(57) Marano, F.; Hussain, S.; Rodrigues-Lima, F.; Baeza-Squiban, A.; Boland, S. Nanoparticles: molecular targets and cell signalling. *Arch. Toxicol.* **2011**, *85* (7), 733−741.
(58) Morgan, T. T.; Muddana, H. S.; Altinoğlu, E. İ.; Rouse, S. M.; Tabaković, A.; Tabouillot, T.; Russin, T. J.; Shanmugavelandy, S. S.; Butler, P. J.; Eklund, P. C.; Yun, J. K.; Kester, M.; Adair, J. H. Encapsulation of Organic Molecules in Calcium Phosphate Nanocomposite Particles for Intracellular Imaging and Drug Delivery. *Nano Lett.* **2008**, *8* (12), 4108−4115.
(59) Schoen, F. J.; Levy, R. J. Calcification of tissue heart valve substitutes: progress toward understanding and prevention. *Annals of thoracic surgery* **2005**, *79* (3), 1072−1080.
(60) Dorozhkin, S. V. Bioceramics of calcium orthophosphates. *Biomaterials* **2010**, *31* (7), 1465−1485.
(61) Wei, G.; Ma, P. X. Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. *Biomaterials* **2004**, *25* (19), 4749−4757.
(62) Thakur, T.; Xavier, J. R.; Cross, L.; Jaiswal, M. K.; Mondragon, E.; Kaunas, R.; Gaharwar, A. K. Photocrosslinkable and elastomeric hydrogels for bone regeneration. *J. Biomed. Mater. Res., Part A* **2016**, *104* (4), 879−888.
(63) Bose, S.; Tarafder, S. Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: A review. *Acta Biomater.* **2012**, *8* (4), 1401−1421.
(64) Reddy, S.; Wasnik, S.; Guha, A.; Kumar, J. M.; Sinha, A.; Singh, S. Evaluation of nano-biphasic calcium phosphate ceramics for bone tissue engineering applications: In vitro and preliminary in vivo studies. *J. Biomater. Appl.* **2013**, *27* (5), 565−575.
(65) Barradas, A. M. C.; Fernandes, H. A. M.; Groen, N.; Chai, Y. C.; Schrooten, J.; van de Peppel, J.; van Leeuwen, J. P. T. M.; van Blitterswijk, C. A.; de Boer, J. A calcium-induced signaling cascade leading to osteogenic differentiation of human bone marrow-derived mesenchymal stromal cells. *Biomaterials* **2012**, *33* (11), 3205−3215.
(66) Weng, L.; Boda, S. K.; Teusink, M. J.; Shuler, F. D.; Li, X.; Xie, J. Binary Doping of Strontium and Copper Enhancing Osteogenesis and Angiogenesis of Bioactive Glass Nanofibers while Suppressing Osteoclast Activity. *ACS Appl. Mater. Interfaces* **2017**, *9* (29), 24484−24496.
(67) Cabrera, W. E.; Schrooten, I.; De Broe, M. E.; D'Haese, P. C. Strontium and Bone. *J. Bone Miner. Res.* **1999**, *14* (5), 661−668.
(68) Meunier, P. J.; Roux, C.; Seeman, E.; Ortolani, S.; Badurski, J. E.; Spector, T. D.; Cannata, J.; Balogh, A.; Lemmel, E.-M.; Pors-Nielsen, S.; et al. The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. *N. Engl. J. Med.* **2004**, *350* (5), 459−468.
(69) Ma, B.; Zhang, Q.; Wu, D.; Wang, Y.-l.; Hu, Y.-y.; Cheng, Y.-p.; Yang, Z.-d.; Zheng, Y.-y.; Ying, H.-J. Strontium fructose 1, 6-diphosphate prevents bone loss in a rat model of postmenopausal osteoporosis via the OPG/RANKL/RANK pathway. *Acta Pharmacol. Sin.* **2012**, *33* (4), 479−489.
(70) Wang, X.; Gu, Z.; Jiang, B.; Li, L.; Yu, X. Surface modification of strontium-doped porous bioactive ceramic scaffolds via poly-(DOPA) coating and immobilizing silk fibroin for excellent angiogenic and osteogenic properties. *Biomater. Sci.* **2016**, *4* (4), 678−688.
(71) Kumar, S.; Chatterjee, K. Strontium eluting graphene hybrid nanoparticles augment osteogenesis in a 3D tissue scaffold. *Nanoscale* **2015**, *7* (5), 2023−2033.
(72) Hoppe, A.; Güldal, N. S.; Boccaccini, A. R. A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. *Biomaterials* **2011**, *32* (11), 2757−2774.
(73) Hofbauer, L. C.; Schoppet, M. Clinical implications of the osteoprotegerin/rankl/rank system for bone and vascular diseases. *JAMA* **2004**, *292* (4), 490−495.
(74) Ma, B.; Zhang, Q.; Wu, D.; Wang, Y.-l.; Hu, Y.-y.; Cheng, Y.-p.; Yang, Z.-d.; Zheng, Y.-y.; Ying, H.-J. Strontium fructose 1,6-diphosphate prevents bone loss in a rat model of postmenopausal osteoporosis via the OPG/RANKL/RANK pathway. *Acta Pharmacol. Sin.* **2012**, *33*, 479.
(75) Saidak, Z.; Marie, P. J. Strontium signaling: Molecular mechanisms and therapeutic implications in osteoporosis. *Pharmacol. Ther.* **2012**, *136* (2), 216−226.
(76) Zhang, W.; Shen, Y.; Pan, H.; Lin, K.; Liu, X.; Darvell, B. W.; Lu, W. W.; Chang, J.; Deng, L.; Wang, D.; Huang, W. Effects of strontium in modified biomaterials. *Acta Biomater.* **2011**, *7* (2), 800−808.
(77) Christoffersen, J.; Christoffersen, M. R.; Kolthoff, N.; Bärenholdt, O. Effects of strontium ions on growth and dissolution of hydroxyapatite and on bone mineral detection. *Bone* **1997**, *20* (1), 47−54.

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

(78) Ni, G. X.; Lu, W. W.; Xu, B.; Chiu, K. Y.; Yang, C.; Li, Z. Y.; Lam, W. M.; Luk, K. D. K. Interfacial behaviour of strontium-containing hydroxyapatite cement with cancellous and cortical bone. *Biomaterials* **2006**, *27* (29), 5127−5133.
(79) Barrère, F.; van Blitterswijk, C. A.; de Groot, K. Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics. *Int. J. Nanomedicine* **2006**, *1* (3), 317−332.
(80) Fu, Q.; Rahaman, M. N.; Fu, H.; Liu, X. Silicate, borosilicate, and borate bioactive glass scaffolds with controllable degradation rate for bone tissue engineering applications. I. Preparation and in vitro degradation. *J. Biomed. Mater. Res., Part A* **2010**, *95A* (1), 164−171.
(81) Zhang, C.; Li, C.; Huang, S.; Hou, Z.; Cheng, Z.; Yang, P.; Peng, C.; Lin, J. Self-activated luminescent and mesoporous strontium hydroxyapatite nanorods for drug delivery. *Biomaterials* **2010**, *31* (12), 3374−3383.
(82) Sun, L. N.; Guo, Q. R.; Wu, X. L.; Luo, S. J.; Pan, W. L.; Huang, K. L.; Lu, J. F.; Ren, L.; Cao, M. H.; Hu, C. W. Synthesis and photoluminescent properties of strontium tungstate nanostructures. *J. Phys. Chem. C* **2007**, *111* (2), 532−537.
(83) Terra, J.; Dourado, E. R.; Eon, J.-G.; Ellis, D. E.; Gonzalez, G.; Rossi, A. M. The structure of strontium-doped hydroxyapatite: an experimental and theoretical study. *Phys. Chem. Chem. Phys.* **2009**, *11* (3), 568−577.
(84) Capuccini, C.; Torricelli, P.; Sima, F.; Boanini, E.; Ristoscu, C.; Bracci, B.; Socol, G.; Fini, M.; Mihailescu, I. N.; Bigi, A. Strontium-substituted hydroxyapatite coatings synthesized by pulsed-laser deposition: In vitro osteoblast and osteoclast response. *Acta Biomater.* **2008**, *4* (6), 1885−1893.
(85) Volpe, S. L. Magnesium. In *Present Knowledge in Nutrition*, 10th ed.; Erdman, J. W., Jr., MacDonald, I., Zeisel, S. H., Eds.; John Wiley & Sons: Ames, IA, 2012.
(86) Saris, N.-E. L.; Mervaala, E.; Karppanen, H.; Khawaja, J. A.; Lewenstam, A. Magnesium: An update on physiological, clinical and analytical aspects. *Clin. Chim. Acta* **2000**, *294* (1), 1−26.
(87) Rude, R. K.; Gruber, H. E. Magnesium deficiency and osteoporosis: animal and human observations. *J. Nutr. Biochem.* **2004**, *15* (12), 710−716.
(88) Yoshizawa, S.; Brown, A.; Barchowsky, A.; Sfeir, C. Magnesium ion stimulation of bone marrow stromal cells enhances osteogenic activity, simulating the effect of magnesium alloy degradation. *Acta Biomater.* **2014**, *10* (6), 2834−2842.
(89) Witte, F.; Kaese, V.; Haferkamp, H.; Switzer, E.; Meyer-Lindenberg, A.; Wirth, C. J.; Windhagen, H. In vivo corrosion of four magnesium alloys and the associated bone response. *Biomaterials* **2005**, *26* (17), 3557−3563.
(90) Wan, C.; Gilbert, S. R.; Wang, Y.; Cao, X.; Shen, X.; Ramaswamy, G.; Jacobsen, K. A.; Alaql, Z. S.; Eberhardt, A. W.; Gerstenfeld, L. C.; Einhorn, T. A.; Deng, L.; Clemens, T. L. Activation of the hypoxia-inducible factor-1 alpha pathway accelerates bone regeneration. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (2), 686−691.
(91) Kulanthaivel, S.; Mishra, U.; Agarwal, T.; Giri, S.; Pal, K.; Pramanik, K.; Banerjee, I. Improving the osteogenic and angiogenic properties of synthetic hydroxyapatite by dual doping of bivalent cobalt and magnesium ion. *Ceram. Int.* **2015**, *41* (9), 11323−11333.
(92) Wu, F.; Wei, J.; Guo, H.; Chen, F.; Hong, H.; Liu, C. Self-setting bioactive calcium−magnesium phosphate cement with high strength and degradability for bone regeneration. *Acta Biomater.* **2008**, *4* (6), 1873−1884.
(93) Makhluf, S.; Dror, R.; Nitzan, Y.; Abramovich, Y.; Jelinek, R.; Gedanken, A. Microwave-Assisted Synthesis of Nanocrystalline MgO and Its Use as a Bacteriocide. *Adv. Funct. Mater.* **2005**, *15* (10), 1708−1715.
(94) Stoimenov, P. K.; Klinger, R. L.; Marchin, G. L.; Klabunde, K. J. Metal Oxide Nanoparticles as Bactericidal Agents. *Langmuir* **2002**, *18* (17), 6679−6686.
(95) Bhakta, G.; Mitra, S.; Maitra, A. DNA encapsulated magnesium and manganous phosphate nanoparticles: potential non-viral vectors for gene delivery. *Biomaterials* **2005**, *26* (14), 2157−2163.
(96) Hickey, D. J.; Ercan, B.; Sun, L.; Webster, T. J. Adding MgO nanoparticles to hydroxyapatite−PLLA nanocomposites for improved bone tissue engineering applications. *Acta Biomater.* **2015**, *14*, 175−184.
(97) Hua, M.; Zhang, S.; Pan, B.; Zhang, W.; Lv, L.; Zhang, Q. Heavy metal removal from water/wastewater by nanosized metal oxides: A review. *J. Hazard. Mater.* **2012**, *211−212*, 317−331.
(98) Ding, Y.; Zhang, G.; Wu, H.; Hai, B.; Wang, L.; Qian, Y. Nanoscale Magnesium Hydroxide and Magnesium Oxide Powders: Control over Size, Shape, and Structure via Hydrothermal Synthesis. *Chem. Mater.* **2001**, *13* (2), 435−440.
(99) Yu, J. C.; Xu, A.; Zhang, L.; Song, R.; Wu, L. Synthesis and Characterization of Porous Magnesium Hydroxide and Oxide Nanoplates. *J. Phys. Chem. B* **2004**, *108* (1), 64−70.
(100) Beruto, D.; Botter, R.; Searcy, A. W. H2O-Catalyzed Sintering of ∼ 2-nm-Cross- Section Particles of MgO. *J. Am. Ceram. Soc.* **1987**, *70* (3), 155−159.
(101) Wang, J. A.; Novaro, O.; Bokhimi, X.; López, T.; Gómez, R.; Navarrete, J.; Llanos, M. E.; López-Salinas, E. Structural Defects and Acidic and Basic Sites in Sol−Gel MgO. *J. Phys. Chem. B* **1997**, *101* (38), 7448−7451.
(102) Wang, J. A.; Novaro, O.; Bokhimi, X.; López, T.; Gómez, R.; Navarrete, J.; Llanos, M. E.; López-Salinas, E. Characterizations of the thermal decomposition of brucite prepared by sol−gel technique for synthesis of nanocrystalline MgO. *Mater. Lett.* **1998**, *35* (5), 317−323.
(103) Morones, J. R.; Elechiguerra, J. L.; Camacho, A.; Holt, K.; Kouri, J. B.; Ramírez, J. T.; Yacaman, M. J. The bactericidal effect of silver nanoparticles. *Nanotechnology* **2005**, *16* (10), 2346−2353.
(104) Jin, T.; He, Y. Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. *J. Nanopart. Res.* **2011**, *13* (12), 6877−6885.
(105) Azam, A.; Ahmed, A. S.; Oves, M.; Khan, M.; Memic, A. Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and-negative bacterial strains. *Int. J. Nanomed.* **2012**, *7*, 3527.
(106) Adams, C. P.; Walker, K. A.; Obare, S. O.; Docherty, K. M. Size-dependent antimicrobial effects of novel palladium nanoparticles. *PLoS One* **2014**, *9* (1), e85981.
(107) Pan, Y.; Neuss, S.; Leifert, A.; Fischler, M.; Wen, F.; Simon, U.; Schmid, G.; Brandau, W.; Jahnen-Dechent, W. Size-dependent cytotoxicity of gold nanoparticles. *Small* **2007**, *3* (11), 1941−1949.
(108) Ivask, A.; Kurvet, I.; Kasemets, K.; Blinova, I.; Aruoja, V.; Suppi, S.; Vija, H.; Käkinen, A.; Titma, T.; Heinlaan, M.; et al. Size-dependent toxicity of silver nanoparticles to bacteria, yeast, algae, crustaceans and mammalian cells in vitro. *PLoS One* **2014**, *9* (7), e102108.
(109) Bienert, G. P.; Schüssler, M. D.; Jahn, T. P. Metalloids: essential, beneficial or toxic? Major intrinsic proteins sort it out. *Trends Biochem. Sci.* **2008**, *33* (1), 20−26.
(110) Jugdaohsingh, R. Silicon and bone health. *J. Nutr Health Aging* **2007**, *11* (2), 99−110.
(111) Sripanyakorn, S.; Jugdaohsingh, R.; Thompson, R. P. H.; Powell, J. J. Dietary silicon and bone health. *Nutrition Bulletin* **2005**, *30* (3), 222−230.
(112) Reffitt, D. M.; Ogston, N.; Jugdaohsingh, R.; Cheung, H. F. J.; Evans, B. A. J.; Thompson, R. P. H.; Powell, J. J.; Hampson, G. N. Orthosilicic acid stimulates collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro. *Bone* **2003**, *32* (2), 127−135.
(113) Tzaphlidou, M. Bone architecture: collagen structure and calcium/phosphorus maps. *J. Biol. Phys.* **2008**, *34* (1−2), 39−49.
(114) Kounadi, E.; Fountos, G.; Tzaphlidou, M. The Influence of Inflammation-Mediated Osteopenia (IMO) on the Structure of Rabbit Bone and Skin Collagen Fibrils. *Connect. Tissue Res.* **1998**, *37* (1−2), 69−76.
(115) Jugdaohsingh, R.; Tucker, K. L.; Qiao, N.; Cupples, L. A.; Kiel, D. P.; Powell, J. J. Dietary silicon intake is positively associated with bone mineral density in men and premenopausal women of the

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

Framingham Offspring cohort. *J. Bone Miner. Res.* **2004**, *19* (2), 297−307.
(116) Hench, L. L. The story of Bioglass. *J. Mater. Sci.: Mater. Med.* **2006**, *17* (11), 967−978.
(117) Lin, K. L.; Xia, L. G.; Li, H. Y.; Jiang, X. Q.; Pan, H. B.; Xu, Y. J.; Lu, W. W.; Zhang, Z. Y.; Chang, J. Enhanced osteoporotic bone regeneration by strontium-substituted calcium silicate bioactive ceramics. *Biomaterials* **2013**, *34* (38), 10028−10042.
(118) Zeng, Q. H.; Yu, A. B.; Lu, G. Q.; Paul, D. R. Clay-based polymer nanocomposites: Research and commercial development. *J. Nanosci. Nanotechnol.* **2005**, *5* (10), 1574−1592.
(119) Xynos, I. D.; Edgar, A. J.; Buttery, L. D. K.; Hench, L. L.; Polak, J. M. Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass® 45S5 dissolution. *J. Biomed. Mater. Res.* **2001**, *55* (2), 151−157.
(120) Xynos, I. D.; Hukkanen, M. V. J.; Batten, J. J.; Buttery, L. D.; Hench, L. L.; Polak, J. M. Bioglass 45S5 Stimulates Osteoblast Turnover and Enhances Bone Formation In Vitro: Implications and Applications for Bone Tissue Engineering. *Calcif. Tissue Int.* **2000**, *67* (4), 321−329.
(121) El-Ghannam, A. Bone reconstruction: from bioceramics to tissue engineering. *Expert Rev. Med. Devices* **2005**, *2* (1), 87−101.
(122) Gaharwar, A. K.; Cross, L. M.; Peak, C. W.; Gold, K.; Carrow, J. K.; Brokesh, A.; Singh, K. A. 2D Nanoclay for Biomedical Applications: Regenerative Medicine, Therapeutic Delivery, and Additive Manufacturing. *Adv. Mater.* **2019**, *31* (23), 1900332.
(123) Gaharwar, A. K.; Mihaila, S. M.; Swami, A.; Patel, A.; Sant, S.; Reis, R. L.; Marques, A. P.; Gomes, M. E.; Khademhosseini, A. Bioactive silicate nanoplatelets for osteogenic differentiation of human mesenchymal stem cells. *Adv. Mater.* **2013**, *25* (24), 3329−36.
(124) Xavier, J. R.; Thakur, T.; Desai, P.; Jaiswal, M. K.; Sears, N.; Cosgriff-Hernandez, E.; Kaunas, R.; Gaharwar, A. K. Bioactive Nanoengineered Hydrogels for Bone Tissue Engineering: A Growth-Factor-Free Approach. *ACS Nano* **2015**, *9* (3), 3109−3118.
(125) Carrow, J. K.; Cross, L. M.; Reese, R. W.; Jaiswal, M. K.; Gregory, C. A.; Kaunas, R.; Singh, I.; Gaharwar, A. K. Widespread changes in transcriptome profile of human mesenchymal stem cells induced by two-dimensional nanosilicates. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (17), E3905.
(126) Gaharwar, A. K.; Avery, R. K.; Assmann, A.; Paul, A.; McKinley, G. H.; Khademhosseini, A.; Olsen, B. D. Shear-thinning nanocomposite hydrogels for the treatment of hemorrhage. *ACS Nano* **2014**, *8* (10), 9833−42.
(127) Peak, C. W.; Stein, J.; Gold, K. A.; Gaharwar, A. K. Nanoengineered Colloidal Inks for 3D Bioprinting. *Langmuir* **2018**, *34*, 917−925.
(128) Sheikhi, A.; Afewerki, S.; Oklu, R.; Gaharwar, A. K.; Khademhosseini, A. Effect of ionic strength on shear-thinning nanoclay-polymer composite hydrogels. *Biomater. Sci.* **2018**, *6*, 2073−2083.
(129) Thakur, A.; Jaiswal, M. K.; Peak, C. W.; Carrow, J. K.; Gentry, J.; Dolatshahi-Pirouz, A.; Gaharwar, A. K. Injectable shear-thinning nanoengineered hydrogels for stem cell delivery. *Nanoscale* **2016**, *8*, 12362−12372.
(130) Wilson, S. A.; Cross, L. M.; Peak, C. W.; Gaharwar, A. K. Shear-Thinning and Thermo-Reversible Nanoengineered Inks for 3D Bioprinting. *ACS Appl. Mater. Interfaces* **2017**, *9* (50), 43449−43458.
(131) Goldbach, H. E.; Wimmer, M. A. Boron in plants and animals: Is there a role beyond cell-wall structure? *J. Plant Nutr. Soil Sci.* **2007**, *170* (1), 39−48.
(132) Haro Durand, L.; Vargas, G.; Romero, N.; Vera Mesones, R.; Porto López, J.; Boccaccini, A.; Zago, M.; Baldi, A.; Gorustovich, A. Angiogenic effects of ionic dissolution products released from a boron-doped 45S5 bioactive glass. *J. Mater. Chem. B* **2015**, *3* (6), 1142−1148.
(133) Shefer, G.; Oron, U.; Irintchev, A.; Wernig, A.; Halevy, O. Skeletal muscle cell activation by low-energy laser irradiation: A role for the MAPK/ERK pathway. *J. Cell. Physiol.* **2001**, *187* (1), 73−80.
(134) Zhao, X.; Peng, X.; Sun, S.; Park, A. Y. J.; Guan, J.-L. Role of kinase-independent and -dependent functions of FAK in endothelial cell survival and barrier function during embryonic development. *J. Cell Biol.* **2010**, *189* (6), 955.
(135) Qi, H.; Aguiar, D. J.; Williams, S. M.; La Pean, A.; Pan, W.; Verfaillie, C. M. Identification of genes responsible for osteoblast differentiation from human mesodermal progenitor cells. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (6), 3305−3310.
(136) Hakki, S. S.; Bozkurt, B. S.; Hakki, E. E. Boron regulates mineralized tissue-associated proteins in osteoblasts (MC3T3-E1). *J. Trace Elem. Med. Biol.* **2010**, *24* (4), 243−250.
(137) Nielsen, F. H.; Hunt, C. D.; Mullen, L. M.; Hunt, J. R. Effect of Dietary Boron on Mineral, Estrogen, and Testosterone-Metabolism in Postmenopausal Women. *FASEB J.* **1987**, *1* (5), 394−397.
(138) Wu, C.; Miron, R.; Sculean, A.; Kaskel, S.; Doert, T.; Schulze, R.; Zhang, Y. Proliferation, differentiation and gene expression of osteoblasts in boron-containing associated with dexamethasone deliver from mesoporous bioactive glass scaffolds. *Biomaterials* **2011**, *32* (29), 7068−7078.
(139) Hu, H.; Hilton, M. J.; Tu, X.; Yu, K.; Ornitz, D. M.; Long, F. Sequential roles of Hedgehog and Wnt signaling in osteoblast development. *Development* **2005**, *132* (1), 49−60.
(140) Gümüşderelioğlu, M.; Tunçay, E. Ö.; Kaynak, G.; Demirtaş, T. T.; Aydın, S. T.; Hakkı, S. S. Encapsulated boron as an osteoinductive agent for bone scaffolds. *J. Trace Elem. Med. Biol.* **2015**, *31*, 120−128.
(141) Huang, W.; Day, D. E.; Kittiratanapiboon, K.; Rahaman, M. N. Kinetics and mechanisms of the conversion of silicate (45S5), borate, and borosilicate glasses to hydroxyapatite in dilute phosphate solutions. *J. Mater. Sci.: Mater. Med.* **2006**, *17* (7), 583−596.
(142) Liu, X.; Huang, W.; Fu, H.; Yao, A.; Wang, D.; Pan, H.; Lu, W. W.; Jiang, X.; Zhang, X. Bioactive borosilicate glass scaffolds: in vitro degradation and bioactivity behaviors. *J. Mater. Sci.: Mater. Med.* **2009**, *20* (6), 1237−1243.
(143) Chimene, D.; Alge, D. L.; Gaharwar, A. K. Two-Dimensional Nanomaterials for Biomedical Applications: Emerging Trends and Future Prospects. *Adv. Mater.* **2015**, *27* (45), 7261−7284.
(144) Ciofani, G.; Raffa, V.; Menciassi, A.; Cuschieri, A. Cytocompatibility, interactions, and uptake of polyethyleneimine-coated boron nitride nanotubes by living cells: Confirmation of their potential for biomedical applications. *Biotechnol. Bioeng.* **2008**, *101* (4), 850−858.
(145) Ciofani, G.; Raffa, V.; Yu, J.; Chen, Y.; Obata, Y.; Takeoka, S.; Menciassi, A.; Cuschieri, A. Boron nitride nanotubes: a novel vector for targeted magnetic drug delivery. *Curr. Nanosci.* **2009**, *5* (1), 33−38.
(146) Li, X.; Wang, X.; Jiang, X.; Yamaguchi, M.; Ito, A.; Bando, Y.; Golberg, D. Boron nitride nanotube-enhanced osteogenic differentiation of mesenchymal stem cells. *J. Biomed. Mater. Res., Part B* **2016**, *104* (2), 323−329.
(147) Shih, Y. R. V.; Tseng, K. F.; Lai, H. Y.; Lin, C. H.; Lee, O. K. Matrix stiffness regulation of integrin-mediated mechanotransduction during osteogenic differentiation of human mesenchymal stem cells. *J. Bone Miner. Res.* **2011**, *26* (4), 730−738.
(148) Bleackley, M. R.; MacGillivray, R. T. A. Transition metal homeostasis: from yeast to human disease. *BioMetals* **2011**, *24* (5), 785−809.
(149) Perutz, M. Regulation of oxygen affinity of hemoglobin: influence of structure of the globin on the heme iron. *Annu. Rev. Biochem.* **1979**, *48* (1), 327−386.
(150) Brabin, B. J.; Hakimi, M.; Pelletier, D. An analysis of anemia and pregnancy-related maternal mortality. *J. Nutr.* **2001**, *131* (2), 604S−615S.
(151) Sachdev, H.; Gera, T.; Nestel, P. Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. *Public health nutrition* **2005**, *8* (2), 117−132.
(152) McCord, J. M. Effects of Positive Iron Status at a Cellular Level. *Nutr. Rev.* **1996**, *54* (3), 85−88.

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

(153) Abbaspour, N.; Hurrell, R.; Kelishadi, R. Review on iron and its importance for human health. *J. Res. Med. Sci.* **2014**, *19* (2), 164−174.
(154) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. Electron-transport chains and their proton pumps. *Molecular Biology of the Cell*, 4th ed.; Garland Science: New York, 2002.
(155) Underwood, E. J. *The Mineral Nutrition of Livestock*; Cabi, 1999.
(156) Puig, S.; Ramos-Alonso, L.; Romero, A. M.; Martínez-Pastor, M. T. The elemental role of iron in DNA synthesis and repair. *Metallomics* **2017**, *9* (11), 1483−1500.
(157) Gutteridge, J. M. Iron and oxygen radicals in brain. *Ann. Neurol.* **1992**, *32* (S1), S16−S21.
(158) Han, Z.; Yu, Y.; Xu, J.; Bao, Z.; Xu, Z.; Hu, J.; Yu, M.; Bamba, D.; Ma, W.; Ding, F.; Zhang, L.; Jin, M.; Yan, G.; Huang, Q.; Wang, X.; Hua, B.; Yang, F.; Li, Y.; Lei, L.; Cao, N.; Pan, Z.; Cai, B. Iron Homeostasis Determines Fate of Human Pluripotent Stem Cells Via Glycerophospholipids-Epigenetic Circuit. *Stem Cells* **2019**, *37* (4), 489−503.
(159) Tartaj, P.; Morales, M. P.; Gonzalez-Carreño, T.; Veintemillas-Verdaguer, S.; Serna, C. J. The Iron Oxides Strike Back: From Biomedical Applications to Energy Storage Devices and Photoelectrochemical Water Splitting. *Adv. Mater.* **2011**, *23* (44), 5243−5249.
(160) Gupta, A. K.; Gupta, M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials* **2005**, *26* (18), 3995−4021.
(161) Qi, Y.; Qi, H.; He, Y.; Lin, W.; Li, P.; Qin, L.; Hu, Y.; Chen, L.; Liu, Q.; Sun, H.; Liu, Q.; Zhang, G.; Cui, S.; Hu, J.; Yu, L.; Zhang, D.; Ding, J. Strategy of Metal−Polymer Composite Stent To Accelerate Biodegradation of Iron-Based Biomaterials. *ACS Appl. Mater. Interfaces* **2018**, *10* (1), 182−192.
(162) Zhang, L.; Dong, W.-F.; Sun, H.-B. Multifunctional superparamagnetic iron oxide nanoparticles: design, synthesis and biomedical photonic applications. *Nanoscale* **2013**, *5* (17), 7664−7684.
(163) Wu, W.; Xiao, X.; Zhang, S.; Zhou, J.; Fan, L.; Ren, F.; Jiang, C. Large-Scale and Controlled Synthesis of Iron Oxide Magnetic Short Nanotubes: Shape Evolution, Growth Mechanism, and Magnetic Properties. *J. Phys. Chem. C* **2010**, *114* (39), 16092−16103.
(164) Jaiswal, M. K.; Xavier, J. R.; Carrow, J. K.; Desai, P.; Alge, D.; Gaharwar, A. K. Mechanically Stiff Nanocomposite Hydrogels at Ultralow Nanoparticle Content. *ACS Nano* **2016**, *10* (1), 246−256.
(165) Gaharwar, A.; Wong, J.; Müller-Schulte, D.; Bahadur, D.; Richtering, W. Magnetic nanoparticles encapsulated within a thermoresponsive polymer. *J. Nanosci. Nanotechnol.* **2009**, *9* (9), 5355−5361.
(166) Wong, J. E.; Gaharwar, A. K.; Müller-Schulte, D.; Bahadur, D.; Richtering, W. Magnetic nanoparticle−polyelectrolyte interaction: a layered approach for biomedical applications. *J. Nanosci. Nanotechnol.* **2008**, *8* (8), 4033−4040.
(167) Williams, D. Copper Deficiency in Humans. *Sem. Hematology* **1983**, 118−128.
(168) Barceloux, D. G.; Barceloux, D. Copper. *J. Toxicol., Clin. Toxicol.* **1999**, *37* (2), 217−230.
(169) de Bie, P.; Muller, P.; Wijmenga, C.; Klomp, L. W. J. Molecular pathogenesis of Wilson and Menkes disease: correlation of mutations with molecular defects and disease phenotypes. *J. Med. Genet* **2007**, *44* (11), 673−688.
(170) Ala, A.; Walker, A. P.; Ashkan, K.; Dooley, J. S.; Schilsky, M. L. Wilson's disease. *Lancet* **2007**, *369* (9559), 397−408.
(171) Kaneshiro, B.; Aeby, T. Long-term safety, efficacy, and patient acceptability of the intrauterine Copper T-380A contraceptive device. *Int. J. Women's Health* **2010**, *2*, 211−220.
(172) Oster, G.; Salgo, M. P. The copper intrauterine device and its mode of action. *N. Engl. J. Med.* **1975**, *293* (9), 432−438.
(173) Cao, B.; Zheng, Y.; Xi, T.; Zhang, C.; Song, W.; Burugapalli, K.; Yang, H.; Ma, Y. Concentration-dependent cytotoxicity of copper ions on mouse fibroblasts in vitro: effects of copper ion release from TCu380A vs TCu220C intra-uterine devices. *Biomed. Microdevices* **2012**, *14* (4), 709−720.
(174) Shi, M.; Chen, Z.; Farnaghi, S.; Friis, T.; Mao, X.; Xiao, Y.; Wu, C. Copper-doped mesoporous silica nanospheres, a promising immunomodulatory agent for inducing osteogenesis. *Acta Biomater.* **2016**, *30*, 334−344.
(175) Filipowska, J.; Tomaszewski, K. A.; Niedźwiedzki, Ł.; Walocha, J. A.; Niedźwiedzki, T. The role of vasculature in bone development, regeneration and proper systemic functioning. *Angiogenesis* **2017**, *20* (3), 291−302.
(176) Wang, H.; Zhao, S.; Zhou, J.; Shen, Y.; Huang, W.; Zhang, C.; Rahaman, M. N.; Wang, D. Evaluation of borate bioactive glass scaffolds as a controlled delivery system for copper ions in stimulating osteogenesis and angiogenesis in bone healing. *J. Mater. Chem. B* **2014**, *2* (48), 8547−8557.
(177) Kong, N.; Lin, K.; Li, H.; Chang, J. Synergy effects of copper and silicon ions on stimulation of vascularization by copper-doped calcium silicate. *J. Mater. Chem. B* **2014**, *2* (8), 1100−1110.
(178) Martin, F.; Linden, T.; Katschinski, D. r. M.; Oehme, F.; Flamme, I.; Mukhopadhyay, C. K.; Eckhardt, K.; Tröger, J.; Barth, S.; Camenisch, G.; Wenger, R. H. Copper-dependent activation of hypoxia-inducible factor (HIF)-1: implications for ceruloplasmin regulation. *Blood* **2005**, *105* (12), 4613−4619.
(179) Sen, C. K.; Khanna, S.; Venojarvi, M.; Trikha, P.; Ellison, E. C.; Hunt, T. K.; Roy, S. Copper-induced vascular endothelial growth factor expression and wound healing. *American Journal of Physiology-Heart and Circulatory Physiology* **2002**, *282* (5), H1821−H1827.
(180) Pickart, L. R., Method of using copper (II) containing compounds to accelerate wound healing. U.S. Patent US5164367A, 1992.
(181) Cordano, A.; Placko, R. P.; Graham, G. G. Hypocupremia and neutropenia in copper de-ficiency. *Blood* **1966**, *28*, 280−283.
(182) Hatori, Y.; Yan, Y.; Schmidt, K.; Furukawa, E.; Hasan, N. M.; Yang, N.; Liu, C.-N.; Sockanathan, S.; Lutsenko, S. Neuronal differentiation is associated with a redox-regulated increase of copper flow to the secretory pathway. *Nat. Commun.* **2016**, *7* (1), 10640.
(183) Ogra, Y.; Tejima, A.; Hatakeyama, N.; Shiraiwa, M.; Wu, S.; Ishikawa, T.; Yawata, A.; Anan, Y.; Suzuki, N. Changes in intracellular copper concentration and copper-regulating gene expression after PC12 differentiation into neurons. *Sci. Rep.* **2016**, *6* (1), 33007.
(184) Birkaya, B.; Aletta, J. M. NGF promotes copper accumulation required for optimum neurite outgrowth and protein methylation. *J. Neurobiol.* **2005**, *63* (1), 49−61.
(185) Hambidge, M. Human Zinc Deficiency. *J. Nutr.* **2000**, *130* (5), 1344S−1349S.
(186) Nistor, N.; Ciontu, L.; Frasinariu, O.-E.; Lupu, V. V.; Ignat, A.; Streanga, V. Acrodermatitis Enteropathica: A Case Report. *Medicine (Philadelphia, PA, U. S.)* **2016**, *95* (20), e3553−e3553.
(187) Williams, R. J. P. An Introduction to the Biochemistry of Zinc. In *Zinc in Human Biology*; Mills, C. F., Ed. Springer: London, 1989; pp 15−31.
(188) Solomons, N. W. Update on Zinc Biology. *Ann. Nutr. Metab.* **2013**, *62* (S1), 8−17.
(189) Qiao, Y.; Zhang, W.; Tian, P.; Meng, F.; Zhu, H.; Jiang, X.; Liu, X.; Chu, P. K. Stimulation of bone growth following zinc incorporation into biomaterials. *Biomaterials* **2014**, *35* (25), 6882−6897.
(190) Yamaguchi, M.; Oishi, H.; Suketa, Y. Stimulatory effect of zinc on bone formation in tissue culture. *Biochem. Pharmacol.* **1987**, *36* (22), 4007−4012.
(191) Wang, T.; Zhang, J.-C.; Chen, Y.; Xiao, P.-G.; Yang, M.-S. Effect of zinc ion on the osteogenic and adipogenic differentiation of mouse primary bone marrow stromal cells and the adipocytic trans-differentiation of mouse primary osteoblasts. *J. Trace Elem. Med. Biol.* **2007**, *21* (2), 84−91.
(192) Wang, F. D.; Bian, W.; Kong, L. W.; Zhao, F. J.; Guo, J. S.; Jing, N. H. Maternal zinc deficiency impairs brain nestin expression in prenatal and postnatal mice. *Cell Res.* **2001**, *11* (2), 135.

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

(193) Gower-Winter, S. D.; Levenson, C. W. Zinc in the central nervous system: From molecules to behavior. *BioFactors* **2012**, *38* (3), 186−193.
(194) Su, Y.; Cockerill, I.; Wang, Y.; Qin, Y.-X.; Chang, L.; Zheng, Y.; Zhu, D. Zinc-Based Biomaterials for Regeneration and Therapy. *Trends Biotechnol.* **2019**, *37* (4), 428−441.
(195) Prasad, A. S. Discovery of human zinc deficiency: its impact on human health and disease. *Adv. Nutr.* **2013**, *4* (2), 176−190.
(196) Mostaed, E.; Sikora-Jasinska, M.; Drelich, J. W.; Vedani, M. Zinc-based alloys for degradable vascular stent applications. *Acta Biomater.* **2018**, *71*, 1−23.
(197) Bowen, P. K.; Drelich, J.; Goldman, J. Zinc Exhibits Ideal Physiological Corrosion Behavior for Bioabsorbable Stents. *Adv. Mater.* **2013**, *25* (18), 2577−2582.
(198) Zhang, Y.; Nayak, T.; Hong, H.; Cai, W. Biomedical applications of zinc oxide nanomaterials. *Curr. Mol. Med.* **2013**, *13* (10), 1633−1645.
(199) Zeng, H.; Duan, G.; Li, Y.; Yang, S.; Xu, X.; Cai, W. Blue Luminescence of ZnO Nanoparticles Based on Non-Equilibrium Processes: Defect Origins and Emission Controls. *Adv. Funct. Mater.* **2010**, *20* (4), 561−572.
(200) Mirzaei, H.; Darroudi, M. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. *Ceram. Int.* **2017**, *43* (1), 907−914.
(201) Akhtar, M. J.; Ahmad, M.; Kumar, S.; Khan, M. A. M.; Ahmad, J.; Alrokayan, S. A. Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. *Int. J. Nanomed.* **2012**, *7*, 845.
(202) Ahamed, M.; Akhtar, M. J.; Raja, M.; Ahmad, I.; Siddiqui, M. K. J.; AlSalhi, M. S.; Alrokayan, S. A. ZnO nanorod-induced apoptosis in human alveolar adenocarcinoma cells via p53, survivin and bax/bcl-2 pathways: role of oxidative stress. *Nanomedicine* **2011**, *7* (6), 904−913.
(203) Mertz, W. The essential trace elements. *Science* **1981**, *213* (4514), 1332−1338.
(204) Hua, Y.; Clark, S.; Ren, J.; Sreejayan, N. Molecular mechanisms of chromium in alleviating insulin resistance. *J. Nutr. Biochem.* **2012**, *23* (4), 313−319.
(205) Saghiri, M. A.; Asatourian, A.; Orangi, J.; Sorenson, C. M.; Sheibani, N. Functional role of inorganic trace elements in angiogenesis—Part II: Cr, Si, Zn, Cu, and S. *Critical Reviews in Oncology/Hematology* **2015**, *96* (1), 143−155.
(206) Sargeant, A.; Goswami, T. Hip implants − Paper VI − Ion concentrations. *Mater. Eng.* **2007**, *28* (1), 155−171.
(207) Ye, J.; Wang, S.; Leonard, S. S.; Sun, Y.; Butterworth, L.; Antonini, J.; Ding, M.; Rojanasakul, Y.; Vallyathan, V.; Castranova, V.; et al. Role of reactive oxygen species and p53 in chromium (VI)-induced apoptosis. *J. Biol. Chem.* **1999**, *274* (49), 34974−34980.
(208) Pouysségur, J.; Dayan, F.; Mazure, N. M. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* **2006**, *441*, 437.
(209) Semenza, G. L. Expression of hypoxia-inducible factor 1: mechanisms and consequences. *Biochem. Pharmacol.* **2000**, *59* (1), 47−53.
(210) Sena, L. A.; Chandel, N. S. Physiological roles of mitochondrial reactive oxygen species. *Mol. Cell* **2012**, *48* (2), 158−167.
(211) Pabbruwe, M. B.; Standard, O. C.; Sorrell, C. C.; Howlett, C. R. Bone formation within alumina tubes: effect of calcium, manganese, and chromium dopants. *Biomaterials* **2004**, *25* (20), 4901−4910.
(212) McCarty, M. F. Anabolic effects of insulin on bone suggest a role for chromium picolinate in preservation of bone density. *Med. Hypotheses* **1995**, *45* (3), 241−246.
(213) Evans, G.; Swenson, G.; Walters, K. Chromium Picolinate Decreases Calcium Excretion and Increases Dehydroepiandrosterone (DHEA) in Post Menopausal Women. *FASEB J.* **1995**, A449−A449.
(214) Horie, M.; Nishio, K.; Endoh, S.; Kato, H.; Fujita, K.; Miyauchi, A.; Nakamura, A.; Kinugasa, S.; Yamamoto, K.; Niki, E.; Yoshida, Y.; Iwahashi, H. Chromium(III) oxide nanoparticles induced remarkable oxidative stress and apoptosis on culture cells. *Environ. Toxicol.* **2013**, *28* (2), 61−75.
(215) Maldiney, T.; Bessière, A.; Seguin, J.; Teston, E.; Sharma, S. K.; Viana, B.; Bos, A. J. J.; Dorenbos, P.; Bessodes, M.; Gourier, D.; Scherman, D.; Richard, C. The in vivo activation of persistent nanophosphors for optical imaging of vascularization, tumours and grafted cells. *Nat. Mater.* **2014**, *13*, 418.
(216) Kaivosoja, E.; Myllymaa, S.; Takakubo, Y.; Korhonen, H.; Myllymaa, K.; Konttinen, Y. T; Lappalainen, R.; Takagi, M. Osteogenesis of human mesenchymal stem cells on micro-patterned surfaces. *J. Biomater. Appl.* **2013**, *27* (7), 862−871.
(217) Marrey, R. V.; Burgermeister, R.; Grishaber, R. B.; Ritchie, R. Fatigue and life prediction for cobalt-chromium stents: A fracture mechanics analysis. *Biomaterials* **2006**, *27* (9), 1988−2000.
(218) Hensten-Pettersen, A.; Jacobsen, N. Perceived side effects of biomaterials in prosthetic dentistry. *J. Prosthet. Dent.* **1991**, *65* (1), 138−144.
(219) Sumita, M.; Hanawa, T.; Teoh, S. H. Development of nitrogen-containing nickel-free austenitic stainless steels for metallic biomaterials—review. *Mater. Sci. Eng., C* **2004**, *24* (6), 753−760.
(220) Hallab, N. J.; Mikecz, K.; Vermes, C.; Skipor, A.; Jacobs, J. J. Orthopaedic implant related metal toxicity in terms of human lymphocyte reactivity to metal-protein complexes produced from cobalt-base and titanium-base implant alloy degradation. In *Molecular Mechanisms of Metal Toxicity and Carcinogenesis*; Springer, 2001; pp 127−136.
(221) Keegan, G. M.; Learmonth, I. D.; Case, C. P. Orthopaedic metals and their potential toxicity in the arthroplasty patient. *J. Bone Jt. Surg., Br. Vol.* **2007**, *89-B* (5), 567−573.
(222) Wu, C.; Zhou, Y.; Fan, W.; Han, P.; Chang, J.; Yuen, J.; Zhang, M.; Xiao, Y. Hypoxia-mimicking mesoporous bioactive glass scaffolds with controllable cobalt ion release for bone tissue engineering. *Biomaterials* **2012**, *33* (7), 2076−2085.
(223) Fan, W.; Crawford, R.; Xiao, Y. Enhancing in vivo vascularized bone formation by cobalt chloride-treated bone marrow stromal cells in a tissue engineered periosteum model. *Biomaterials* **2010**, *31* (13), 3580−3589.
(224) Bose, S.; Fielding, G.; Tarafder, S.; Bandyopadhyay, A. Understanding of dopant-induced osteogenesis and angiogenesis in calcium phosphate ceramics. *Trends Biotechnol.* **2013**, *31* (10), 594−605.
(225) Kempen, D. H. R.; Lu, L.; Heijink, A.; Hefferan, T. E.; Creemers, L. B.; Maran, A.; Yaszemski, M. J.; Dhert, W. J. A. Effect of local sequential VEGF and BMP-2 delivery on ectopic and orthotopic bone regeneration. *Biomaterials* **2009**, *30* (14), 2816−2825.
(226) Papageorgiou, I.; Brown, C.; Schins, R.; Singh, S.; Newson, R.; Davis, S.; Fisher, J.; Ingham, E.; Case, C. P. The effect of nano- and micron-sized particles of cobalt−chromium alloy on human fibroblasts in vitro. *Biomaterials* **2007**, *28* (19), 2946−2958.
(227) Pouponneau, P.; Leroux, J.-C.; Soulez, G.; Gaboury, L.; Martel, S. Co-encapsulation of magnetic nanoparticles and doxorubicin into biodegradable microcarriers for deep tissue targeting by vascular MRI navigation. *Biomaterials* **2011**, *32* (13), 3481−3486.
(228) Wu, H. X.; Liu, G.; Wang, X.; Zhang, J. M.; Chen, Y.; Shi, J. L.; Yang, H.; Hu, H.; Yang, S. P. Solvothermal synthesis of cobalt ferrite nanoparticles loaded on multiwalled carbon nanotubes for magnetic resonance imaging and drug delivery. *Acta Biomater.* **2011**, *7* (9), 3496−3504.
(229) Prohaska, J. R. Functions of trace elements in brain metabolism. *Physiol. Rev.* **1987**, *67* (3), 858−901.
(230) Keller, J. N.; Kindy, M. S.; Holtsberg, F. W.; St. Clair, D. K.; Yen, H.-C.; Germeyer, A.; Steiner, S. M.; Bruce-Keller, A. J.; Hutchins, J. B.; Mattson, M. P. Mitochondrial Manganese Superoxide Dismutase Prevents Neural Apoptosis and Reduces Ischemic Brain Injury: Suppression of Peroxynitrite Production, Lipid Peroxidation, and Mitochondrial Dysfunction. *J. Neurosci.* **1998**, *18* (2), 687.

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

(231) Strause, L.; Saltman, P.; Glowacki, J. The effect of deficiencies of manganese and copper on osteoinduction and on resorption of bone particles in rats. *Calcif. Tissue Int.* **1987**, *41* (3), 145−150.
(232) Kaufman, R. J.; Swaroop, M.; Murtha-Riel, P. Depletion of manganese within the secretory pathway inhibits O-linked glycosylation in mammalian cells. *Biochemistry* **1994**, *33* (33), 9813−9819.
(233) Bagambisa, F. B.; Joos, U. Preliminary studies on the phenomenological behaviour of osteoblasts cultured on hydroxyapatite ceramics. *Biomaterials* **1990**, *11* (1), 50−56.
(234) Bae, Y.-J.; Kim, M.-H. Manganese supplementation improves mineral density of the spine and femur and serum osteocalcin in rats. *Biol. Trace Elem. Res.* **2008**, *124* (1), 28−34.
(235) Hamai, D.; Bondy, S. C. Oxidative Basis of Manganese Neurotoxicity. *Ann. N. Y. Acad. Sci.* **2004**, *1012* (1), 129−141.
(236) Bhang, S. H.; Han, J.; Jang, H. K.; Noh, M. K.; La, W. G.; Yi, M.; Kim, W. S.; Kwon, Y. K.; Yu, T.; Kim, B. S. pH-triggered release of manganese from MnAu nanoparticles that enables cellular neuronal differentiation without cellular toxicity. *Biomaterials* **2015**, *55*, 33−43.
(237) Yu, B.; Ma, H.; Du, Z.; Hong, Y.; Sang, M.; Liu, Y.; Shi, Y. Involvement of calmodulin and actin in directed differentiation of rat cortical neural stem cells into neurons. *Int. J. Mol. Med.* **2011**, *28* (5), 739−744.
(238) Chou, D.-T.; Wells, D.; Hong, D.; Lee, B.; Kuhn, H.; Kumta, P. N. Novel processing of iron−manganese alloy-based biomaterials by inkjet 3-D printing. *Acta Biomater.* **2013**, *9* (10), 8593−8603.
(239) Lu, J.; Ma, S. L.; Sun, J. Y.; Xia, C. C.; Liu, C.; Wang, Z. Y.; Zhao, X. N.; Gao, F. B.; Gong, Q. Y.; Song, B.; Shuai, X. T.; Ai, H.; Gu, Z. W. Manganese ferrite nanoparticle micellar nanocomposites as MRI contrast agent for liver imaging. *Biomaterials* **2009**, *30* (15), 2919−2928.
(240) Ye, F.; Barrefelt, A.; Asem, H.; Abedi-Valugerdi, M.; El-Serafi, I.; Saghafian, M.; Abu-Salah, K.; Alrokayan, S.; Muhammed, M.; Hassan, M. Biodegradable polymeric vesicles containing magnetic nanoparticles, quantum dots and anticancer drugs for drug delivery and imaging. *Biomaterials* **2014**, *35* (12), 3885−3894.
(241) Bae, K. H.; Lee, K.; Kim, C.; Park, T. G. Surface functionalized hollow manganese oxide nanoparticles for cancer targeted siRNA delivery and magnetic resonance imaging. *Biomaterials* **2011**, *32* (1), 176−184.
(242) Nawaz, Q.; Rehman, M. A. U.; Burkovski, A.; Schmidt, J.; Beltran, A. M.; Shahid, A.; Alber, N. K.; Peukert, W.; Boccaccini, A. R. Synthesis and characterization of manganese containing mesoporous bioactive glass nanoparticles for biomedical applications. *J. Mater. Sci.: Mater. Med.* **2018**, *29* (5), 13.
(243) Sau, T. K.; Rogach, A. L.; Jäckel, F.; Klar, T. A.; Feldmann, J. Properties and applications of colloidal nonspherical noble metal nanoparticles. *Adv. Mater.* **2010**, *22* (16), 1805−1825.
(244) Han, G.; You, C. C.; Kim, B. j.; Turingan, R. S.; Forbes, N. S.; Martin, C. T.; Rotello, V. M. Light-regulated release of DNA and its delivery to nuclei by means of photolabile gold nanoparticles. *Angew. Chem.* **2006**, *118* (19), 3237−3241.
(245) Ha, S.-W.; Jang, H. L.; Nam, K. T.; Beck, G. R. Nano-hydroxyapatite modulates osteoblast lineage commitment by stimulation of DNA methylation and regulation of gene expression. *Biomaterials* **2015**, *65*, 32−42.
(246) Yi, C.; Liu, D.; Fong, C.-C.; Zhang, J.; Yang, M. Gold Nanoparticles Promote Osteogenic Differentiation of Mesenchymal Stem Cells through p38 MAPK Pathway. *ACS Nano* **2010**, *4* (11), 6439−6448.
(247) Son, Y.; Kim, S.; Chung, H.-T.; Pae, H.-O. Chapter Two: Reactive Oxygen Species in the Activation of MAP Kinases. In *Methods in Enzymology*; Cadenas, E., Packer, L., Eds.; Academic Press, 2013; Vol. *528*, pp 27−48.
(248) Pan, Y.; Leifert, A.; Ruau, D.; Neuss, S.; Bornemann, J.; Schmid, G.; Brandau, W.; Simon, U.; Jahnen-Dechent, W. Gold Nanoparticles of Diameter 1.4 nm Trigger Necrosis by Oxidative Stress and Mitochondrial Damage. *Small* **2009**, *5* (18), 2067−2076.
(249) Alkilany, A. M.; et al. Cellular Uptake and Cytotoxicity of Gold Nanorods: Molecular Origin of Cytotoxicity and Surface Effects. *Small* **2009**, *5* (6), 701−708.
(250) Choi, K.; Riviere, J. E.; Monteiro-Riviere, N. A. Protein corona modulation of hepatocyte uptake and molecular mechanisms of gold nanoparticle toxicity. *Nanotoxicology* **2017**, *11* (1), 64−75.
(251) Mock, J.; Barbic, M.; Smith, D.; Schultz, D.; Schultz, S. Shape effects in plasmon resonance of individual colloidal silver nanoparticles. *J. Chem. Phys.* **2002**, *116* (15), 6755−6759.
(252) Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C. The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment. *J. Phys. Chem. B* **2003**, *107* (3), 668−677.
(253) Tang, S.; Zheng, J. Antibacterial Activity of Silver Nanoparticles: Structural Effects. *Adv. Healthcare Mater.* **2018**, *7* (13), 1701503.
(254) Sondi, I.; Salopek-Sondi, B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* **2004**, *275* (1), 177−182.
(255) Kim, J. S.; Kuk, E.; Yu, K. N.; Kim, J.-H.; Park, S. J.; Lee, H. J.; Kim, S. H.; Park, Y. K.; Park, Y. H.; Hwang, C.-Y.; Kim, Y.-K.; Lee, Y.-S.; Jeong, D. H.; Cho, M.-H. Antimicrobial effects of silver nanoparticles. *Nanomedicine* **2007**, *3* (1), 95−101.
(256) Gold, K.; Slay, B.; Knackstedt, M.; Gaharwar, A. K. Antimicrobial Activity of Metal and Metal-Oxide Based Nanoparticles. *Advanced Therapeutics* **2018**, *1* (3), 1700033.
(257) Jung, W. K.; Koo, H. C.; Kim, K. W.; Shin, S.; Kim, S. H.; Park, Y. H. Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. *Appl. Environ. Microbiol.* **2008**, *74* (7), 2171−2178.
(258) Choi, O.; Hu, Z. Size Dependent and Reactive Oxygen Species Related Nanosilver Toxicity to Nitrifying Bacteria. *Environ. Sci. Technol.* **2008**, *42* (12), 4583−4588.
(259) Zhang, R.; Lee, P.; Lui, V. C. H.; Chen, Y.; Liu, X.; Lok, C. N.; To, M.; Yeung, K. W. K.; Wong, K. K. Y. Silver nanoparticles promote osteogenesis of mesenchymal stem cells and improve bone fracture healing in osteogenesis mechanism mouse model. *Nanomedicine* **2015**, *11* (8), 1949−1959.
(260) Zhao, Y.; Cao, H.; Qin, H.; Cheng, T.; Qian, S.; Cheng, M.; Peng, X.; Wang, J.; Zhang, Y.; Jin, G.; Zhang, X.; Liu, X.; Chu, P. K. Balancing the Osteogenic and Antibacterial Properties of Titanium by Codoping of Mg and Ag: An in Vitro and in Vivo Study. *ACS Appl. Mater. Interfaces* **2015**, *7* (32), 17826−17836.
(261) Ahn, Y.; Lee, H.; Lee, D.; Lee, Y. Highly Conductive and Flexible Silver Nanowire-Based Microelectrodes on Biocompatible Hydrogel. *ACS Appl. Mater. Interfaces* **2014**, *6* (21), 18401−18407.
(262) Xu, F.; Zhu, Y. Highly Conductive and Stretchable Silver Nanowire Conductors. *Adv. Mater.* **2012**, *24* (37), 5117−5122.
(263) Schwarz, G.; Mendel, R. R.; Ribbe, M. W. Molybdenum cofactors, enzymes and pathways. *Nature* **2009**, *460* (7257), 839−847.
(264) Schroeder, H. A.; Balassa, J. J.; Tipton, I. H. Essential trace metals in man: molybdenum. *J. Chronic Dis.* **1970**, *23* (7), 481−499.
(265) Howard, J. B.; Rees, D. C. Structural Basis of Biological Nitrogen Fixation. *Chem. Rev.* **1996**, *96* (7), 2965−2982.
(266) Rajagopalan, K. Molybdenum: an essential trace element in human nutrition. *Annu. Rev. Nutr.* **1988**, *8* (1), 401−427.
(267) Johnson, J. L.; Rajagopalan, K.; Cohen, H. J. Molecular basis of the biological function of molybdenum effect of tungsten on xanthine oxidase and sulfite oxidase in the rat. *J. Biol. Chem.* **1974**, *249* (3), 859−866.
(268) Park, J. B.; Bronzino, J. D. *Biomaterials: Principles and Applications*; CRC Press, 2002.
(269) Zhang, C.; Wang, X.; Zhang, E.; Yang, L.; Yuan, H.; Tu, W.; Zhang, H.; Yin, Z.; Shen, W.; Chen, X.; et al. An epigenetic bioactive composite scaffold with well-aligned nanofibers for functional tendon tissue engineering. *Acta Biomater.* **2018**, *66*, 141−156.
(270) Jaiswal, M. K.; Singh, K. A.; Lokhande, G.; Gaharwar, A. K. Superhydrophobic states of 2D nanomaterials controlled by atomic

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

defects can modulate cell adhesion. *Chem. Commun.* **2019**, *55* (60), 8772−8775.
(271) Kurapati, R.; Muzi, L.; de Garibay, A. P. R.; Russier, J.; Voiry, D.; Vacchi, I. A.; Chhowalla, M.; Bianco, A. Enzymatic Biodegradability of Pristine and Functionalized Transition Metal Dichalcogenide MoS2 Nanosheets. *Adv. Funct. Mater.* **2017**, *27* (7), 1605176.
(272) Jaiswal, M. K.; Carrow, J. K.; Gentry, J. L.; Gupta, J.; Altangerel, N.; Scully, M.; Gaharwar, A. K. Vacancy-Driven Gelation Using Defect-Rich Nanoassemblies of 2D Transition Metal Dichalcogenides and Polymeric Binder for Biomedical Applications. *Adv. Mater.* **2017**, *29* (36), 1702037.
(273) Crans, D. C.; Trujillo, A. M.; Pharazyn, P. S.; Cohen, M. D. How environment affects drug activity: Localization, compartmentalization and reactions of a vanadium insulin-enhancing compound, dipicolinatooxovanadium(V). *Coord. Chem. Rev.* **2011**, *255* (19), 2178−2192.
(274) Cantley, L.; Cantley, L. G.; Josephson, L. A characterization of vanadate interactions with the (Na, K)-ATPase. Mechanistic and regulatory implications. *J. Biol. Chem.* **1978**, *253* (20), 7361−7368.
(275) Sanchez, D.; Ortega, A.; Domingo, J.; Corbella, J. Developmental toxicity evaluation of orthovanadate in the mouse. *Biol. Trace Elem. Res.* **1991**, *30* (3), 219−226.
(276) Facchini, D. M.; Yuen, V. G.; Battell, M. L.; McNeill, J. H.; Grynpas, M. D. The effects of vanadium treatment on bone in diabetic and non-diabetic rats. *Bone* **2006**, *38* (3), 368−377.
(277) Fukui, K.; Fujisawa, Y.; Ohya-Nishiguchi, H.; Kamada, H.; Sakurai, H. In vivo coordination structural changes of a potent insulin-mimetic agent, bis(picolinato)oxovanadium(IV), studied by electron spin-echo envelope modulation spectroscopy. *J. Inorg. Biochem.* **1999**, *77* (3), 215−224.
(278) Thompson, K. H.; Orvig, C. Vanadium in diabetes: 100 years from Phase 0 to Phase I. *J. Inorg. Biochem.* **2006**, *100* (12), 1925−1935.
(279) Heyliger, C. E.; et al. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. *Science* **1985**, *227* (4693), 1474−1477.
(280) Srivastava, S.; Kumar, N.; Thakur, R.; Roy, P. Role of Vanadium (V) in the Differentiation of C3H10t1/2 Cells Towards Osteoblast Lineage: A Comparative Analysis with Other Trace Elements. *Biol. Trace Elem. Res.* **2013**, *152* (1), 135−142.
(281) Saghiri, M. A.; Orangi, J.; Asatourian, A.; Sorenson, C. M.; Sheibani, N. Functional role of inorganic trace elements in angiogenesis part III: (Ti, Li, Ce, As, Hg, Va, Nb and Pb). *Critical Reviews in Oncology/Hematology* **2016**, *98*, 290−301.
(282) Gao, X.; Cao, Y.; Song, X.; Zhang, Z.; Xiao, C.; He, C.; Chen, X. pH- and thermo-responsive poly(N-isopropylacrylamide-co-acrylic acid derivative) copolymers and hydrogels with LCST dependent on pH and alkyl side groups. *J. Mater. Chem. B* **2013**, *1* (41), 5578−5587.
(283) Evangelou, A. M. Vanadium in cancer treatment. *Critical Reviews in Oncology/Hematology* **2002**, *42* (3), 249−265.
(284) Bergers, G.; Benjamin, L. E. Tumorigenesis and the angiogenic switch. *Nat. Rev. Cancer* **2003**, *3*, 401.
(285) Harding, M.; Mokdsi, G. Antitumour Metallocenes: Structure-Activity Studies and Interactions with Biomolecules. *Curr. Med. Chem.* **2000**, *7* (12), 1289−1303.
(286) Natalio, F.; André, R.; Hartog, A. F.; Stoll, B.; Jochum, K. P.; Wever, R.; Tremel, W. Vanadium pentoxide nanoparticles mimic vanadium haloperoxidases and thwart biofilm formation. *Nat. Nanotechnol.* **2012**, *7*, 530.
(287) Schussler, S. D.; Uske, K.; Marwah, P.; Kemp, F. W.; Bogden, J. D.; Lin, S. S.; Livingston Arinzeh, T. Controlled Release of Vanadium from a Composite Scaffold Stimulates Mesenchymal Stem Cell Osteochondrogenesis. *AAPS J.* **2017**, *19* (4), 1017−1028.
(288) López, M. F.; Gutiérrez, A.; Jiménez, J. A. In vitro corrosion behaviour of titanium alloys without vanadium. *Electrochim. Acta* **2002**, *47* (9), 1359−1364.
(289) Okazaki, Y.; Rao, S.; Ito, Y.; Tateishi, T. Corrosion resistance, mechanical properties, corrosion fatigue strength and cytocompatibility of new Ti alloys without Al and V. *Biomaterials* **1998**, *19* (13), 1197−1215.
(290) Singh, R.; Dahotre, N. B. Corrosion degradation and prevention by surface modification of biometallic materials. *J. Mater. Sci.: Mater. Med.* **2007**, *18* (5), 725−751.
(291) Evans, C. H. Interesting and useful biochemical properties of lanthanides. *Trends Biochem. Sci.* **1983**, *8* (12), 445−449.
(292) Patra, C. R.; Bhattacharya, R.; Patra, S.; Vlahakis, N. E.; Gabashvili, A.; Koltypin, Y.; Gedanken, A.; Mukherjee, P.; Mukhopadhyay, D. Pro-angiogenic Properties of Europium(III) Hydroxide Nanorods. *Adv. Mater.* **2008**, *20* (4), 753−756.
(293) Armelao, L.; Quici, S.; Barigelletti, F.; Accorsi, G.; Bottaro, G.; Cavazzini, M.; Tondello, E. Design of luminescent lanthanide complexes: From molecules to highly efficient photo-emitting materials. *Coord. Chem. Rev.* **2010**, *254* (5), 487−505.
(294) Bünzli, J.-C. G. Lanthanide light for biology and medical diagnosis. *J. Lumin.* **2016**, *170*, 866−878.
(295) Wu, C.; Xia, L.; Han, P.; Mao, L.; Wang, J.; Zhai, D.; Fang, B.; Chang, J.; Xiao, Y. Europium-Containing Mesoporous Bioactive Glass Scaffolds for Stimulating in Vitro and in Vivo Osteogenesis. *ACS Appl. Mater. Interfaces* **2016**, *8* (18), 11342−11354.
(296) Hu, H.; Zhao, P.; Liu, J.; Ke, Q.; Zhang, C.; Guo, Y.; Ding, H. Lanthanum phosphate/chitosan scaffolds enhance cytocompatibility and osteogenic efficiency via the Wnt/β-catenin pathway. *J. Nanobiotechnol.* **2018**, *16* (1), 98.
(297) Liu, D.; Ge, K.; Sun, J.; Chen, S.; Jia, G.; Zhang, J. Lanthanum breaks the balance between osteogenesis and adipogenesis of mesenchymal stem cells through phosphorylation of Smad1/5/8. *RSC Adv.* **2015**, *5* (53), 42233−42241.
(298) Willbold, E.; Gu, X.; Albert, D.; Kalla, K.; Bobe, K.; Brauneis, M.; Janning, C.; Nellesen, J.; Czayka, W.; Tillmann, W.; Zheng, Y.; Witte, F. Effect of the addition of low rare earth elements (lanthanum, neodymium, cerium) on the biodegradation and biocompatibility of magnesium. *Acta Biomater.* **2015**, *11*, 554−562.
(299) Merritt, K.; Rodrigo, J. J. Immune response to synthetic materials: sensitization of patients receiving orthopaedic implants. *Clin. Orthop. Relat. Res.* **1996**, *326*, 71−79.
(300) Griem, P.; Gleichmann, E. Metal ion induced autoimmunity. *Curr. Opin. Immunol.* **1995**, *7* (6), 831−838.
(301) Eil, R.; Vodnala, S. K.; Clever, D.; Klebanoff, C. A.; Sukumar, M.; Pan, J. H.; Palmer, D. C.; Gros, A.; Yamamoto, T. N.; Patel, S. J.; et al. Ionic immune suppression within the tumour microenvironment limits T cell effector function. *Nature* **2016**, *537* (7621), 539.
(302) Chimene, D.; Kaunas, R.; Gaharwar, A. K. Hydrogel Bioink Reinforcement for Additive Manufacturing: A Focused Review of Emerging Strategies. *Adv. Mater.* **2020**, *32*, 1902026.
(303) Gaharwar, A. K.; Peppas, N. A.; Khademhosseini, A. Nanocomposite hydrogels for biomedical applications. *Biotechnol. Bioeng.* **2014**, *111* (3), 441−53.
(304) Chimene, D.; Lennox, K. K.; Kaunas, R. R.; Gaharwar, A. K. Advanced bioinks for 3D printing: a materials science perspective. *Ann. Biomed. Eng.* **2016**, *44* (6), 2090−2102.
(305) Fu, Q.; Zhou, N.; Huang, W.; Wang, D.; Zhang, L.; Li, H. Effects of nano HAP on biological and structural properties of glass bone cement. *J. Biomed. Mater. Res., Part A* **2005**, *74* (2), 156−163.
(306) Ciofani, G.; Danti, S.; D'Alessandro, D.; Ricotti, L.; Moscato, S.; Bertoni, G.; Falqui, A.; Berrettini, S.; Petrini, M.; Mattoli, V.; Menciassi, A. Enhancement of Neurite Outgrowth in Neuronal-Like Cells following Boron Nitride Nanotube-Mediated Stimulation. *ACS Nano* **2010**, *4* (10), 6267−6277.
(307) Turkevich, J.; Stevenson, P. C.; Hillier, J. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss. Faraday Soc.* **1951**, *11* (0), 55−75.
(308) Guzman, M.; Dille, J.; Godet, S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. *Nanomedicine* **2012**, *8* (1), 37−45.
(309) Vivek, R.; Thangam, R.; Muthuchelian, K.; Gunasekaran, P.; Kaveri, K.; Kannan, S. Green biosynthesis of silver nanoparticles from

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344

Annona squamosa leaf extract and its in vitro cytotoxic effect on MCF-7 cells. *Process Biochem.* **2012**, *47* (12), 2405−2410.
(310) Barrio, D. A.; Braziunas, M. D.; Etcheverry, S. B.; Cortizo, A. M. Maltol Complexes of Vanadium (IV) and (V) Regulate In Vitro Alkaline Phosphatase Activity and Osteoblast-like Cell Growth. *J. Trace Elem. Med. Biol.* **1997**, *11* (2), 110−115.
(311) Barrio, D. A.; Etcheverry, S. B. Vanadium and bone development: putative signaling pathways. *Can. J. Physiol. Pharmacol.* **2006**, *84* (7), 677−686.
(312) Nechay, B. R.; Nanninga, L. B.; Nechay, P. S. E. Vanadyl (IV) and vanadate (V) binding to selected endogenous phosphate, carboxyl, and amino ligands; calculations of cellular vanadium species distribution. *Arch. Biochem. Biophys.* **1986**, *251* (1), 128−138.

https://dx.doi.org/10.1021/acsami.9b17801
ACS Appl. Mater. Interfaces 2020, 12, 5319−5344