Biomaterials Science

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Biomater. Sci.*, 2023, **11**, 2277

Cellular metabolism: a link connecting cellular behaviour with the physiochemical properties of biomaterials for bone tissue engineering

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Biomaterial properties, such as surface roughness, morphology, stiffness, conductivity, and chemistry, significantly influence a cell's ability to sense and adhere to its surface and regulate cell functioning. Understanding how biomaterial properties govern changes in cellular function is one of the fundamental goals of tissue engineering. Still, no generalized rule is established to predict cellular processes (adhesion, spreading, growth and differentiation) on biomaterial surfaces. A few studies have highlighted that cells sense biomaterial properties at multiple length scales and regulate various intracellular biochemical processes like cytoskeleton organization, gene regulation, and receptor expression to influence cell function. However, recent studies have found cellular metabolism as another critical aspect of cellular processes that regulate cell behavior, co-relating metabolism to cellular functions like adhesion, proliferation, and differentiation. Now researchers have started to uncover previously overlooked factors on how biomaterial properties govern changes in cellular functions mediated through metabolism. This review highlights how different physiochemical properties of scaffolds designed from different biomaterials influence cell metabolism. The review also discusses the role of metabolism change in cellular functions and cell behavior in the context of bone tissue engineering. It also emphasizes the importance of cell metabolism as a missing link between the cellular behavior and physicochemical properties of scaffolds and serves as a guiding principle for designing scaffolds for tissue engineering.

Received 31st August 2022,
Accepted 17th January 2023

DOI: 10.1039/d2bm01410f

rsc.li/biomaterials-science

1. Introduction

Over the years, the increasing global demand for designing bioactive materials for bone tissue engineering (BTE) has steered substantial interest in tuning biomaterial properties for accelerated bone regeneration.[1] Designed biomaterials with unique physicochemical properties have been shown to regulate cell attachment, spreading, proliferation, and differentiation.[2] Thus, understanding how biomaterial properties govern changes in cellular functioning is one of the fundamental goals of BTE. With the increasing demand for different biomaterials in BTE, researchers have started investigating the complex dynamics of material–cell interactions. Several studies have highlighted that cells sense these material properties at multiple length scales and regulate various intracellular biochemical processes like cytoskeleton organization, gene regulation, and receptor expression to influence cell function.[3] However, recent studies have found cellular metabolism as another critical aspect of cellular processes that regulate cell behavior.[4,5] Metabolic requirement depends on the tissue microenvironment, which varies for different tissue due to their complex extracellular microenvironment.[6] To regenerate tissues, cells shift from a low metabolic basal function state to a high metabolic state with the activation of anabolic pathways for biomass and extracellular matrix (ECM) production.[7] It has been observed that bone cells near the fracture site alter their metabolic pathways to trigger bone tissue regeneration by remodeling the extracellular microenvironment.[8] Bone and stem cells become metabolically active and produce a high amount of cellular energy to support the anabolic synthesis of biomolecules to deposit a collagen-rich mineral matrix for bone regeneration.[9]

The discovered link between metabolism and cell function affected by the extracellular matrix in cancer biology[10] has prompted researchers to investigate how different physicochemical features of biomaterials can influence cell function *via* cellular metabolism. It is well established that stem cells sense and respond to the mechanical properties of biomaterials due to their mechanosensitive nature. Still, the correlation between stem cell mechanosensitivity and their metabolism is an overlooked area. The mechanical stimulus exerted by the

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extracellular material on cells may act as integrated information from the microenvironment, which may coordinate and regulate cellular bioenergetics to control cell functions. On that note, Romani *et al.*, in an exciting review, highlighted how cells cross-talk to regulate their metabolism by sensing extracellular mechanical forces to control cell function.[11]

Similar to mechanical properties, it may be possible that other properties of biomaterials as extracellular stimuli (like the released exogenous product, surface chemistry, surface roughness, surface topography, and conductivity) can regulate cellular metabolism to control gene expression and hence the cell fate processes. Thus, in this review, we collectively highlight how extracellular signals provided by different physicochemical properties of biomaterials regulate bone cell metabolism to control transcription and gene expression to augment bone regeneration, as schematically illustrated in Fig. 1. This review mainly concentrates on bone tissue regeneration due to the rapid global development of various biomaterials with different physicochemical properties to augment bone regeneration. Understanding how different biomaterials with different physicochemical properties influence cell metabolism to regenerate tissue will provide new insight into designing bioactive materials. In the present review, we started by linking how a change in cell metabolism and its readout can cross-talk with different transcription factors that regulate osteogenic genes to control bone cell function. Next, we focus on understanding how different physicochemical properties of biomaterials can influence cell metabolism to regulate transcription factors that control osteogenic gene expression allowing context-specific cell behavior. This review might encourage researchers around the globe to work on this interdisciplinary growing subfield and provide experimental evidence with a direct connection between the physicochemical properties of biomaterials linking cell function mediated through metabolism.

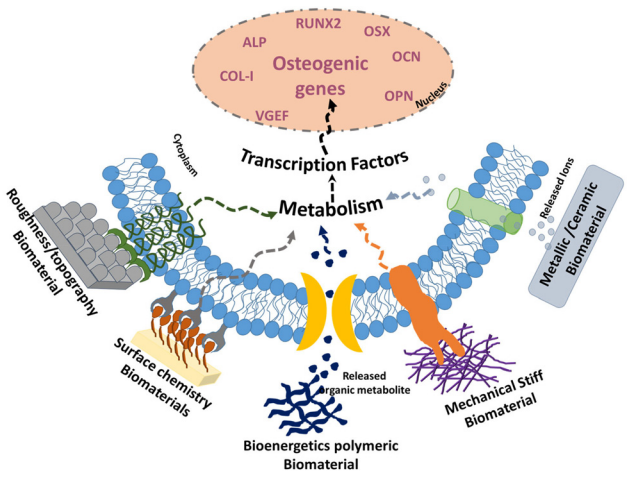

Fig. 1 Biomaterial properties can regulate cell metabolism to control cell fate. Schematic model illustrating the effects of different physicochemical properties of biomaterials regulating bone cell metabolism to control gene expression to augment bone regeneration.

2. Cell metabolism readout

Cell metabolism is a complex biochemical process that occurs in all living organisms to synthesize macromolecules using energy (anabolism); later, those synthesized macromolecules are broken down into simple molecules to release energy (catabolism). Cell meets its energy requirements for survival, proliferation, differentiation, and cell-specific functions through the dynamic balance between anabolism and catabolism. Examination of the cellular metabolism (as illustrated in Fig. 2(i)) has sparked a series of observations suggesting how extracellular and intracellular stimuli tightly regulate the production and consumption of energy (in the form of Adenosine triphosphate (ATP)) by different pathways (glycolysis, tricarboxylic acid (TCA), beta-oxidation, *etc.*).

Glucose and its metabolite are the classical readouts to understand the metabolism-mediated change in cell behavior. It is widely reported that high glucose uptake is needed for the rapid proliferation and differentiation of osteoprogenitor cells to osteoblasts.[12] For rapid cell proliferation, high glucose levels promote glycolysis and pentose phosphate pathway (PPP) to facilitate ATP and nucleotide synthesis for cell division, whereas for bone differentiation, stem cell consumes high glucose to stimulate ATP synthesis mediated through oxidative phosphorylation (Oxphos) in mitochondria for the biosynthesis of the collagen-rich mineralized matrix. Thus by assessing the uptake of glucose by stem cells, its metabolic state and fate can be evaluated. As a result, researchers developed several methods to measure glucose uptake into cells. Different types of glucose derivatives like radiolabeled 3-*O*-methylglucose, radiolabeled 2-deoxyglucose (2DG), and 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) were used to measure glucose uptake by cells using fluorometric and imaging techniques.[13] In addition to glucose, enzyme activity also provides information related to specific metabolic pathways that they belong to. The high enzyme activities of hexokinase, phosphorylase, and phosphofructokinase have been measured in cell extracts and connected with high rates of glycolysis.[14] Similarly, high acetyl-CoA and pyruvate dehydrogenase activity in cell extracts indicated Krebs cycle mediated energy production by cells.[15] Measurement of such enzymatic activity combined with expression levels of enzymes using high throughput transcriptomic and proteomic methods may provide a valuable way to investigate specific metabolic pathways in metabolically active cells like bone.

Cellular metabolism plays an essential role in producing energy in the form of ATP to maintain homeostasis, proliferation, and differentiation. During differentiation, the production of ATP shifts from a low metabolic state (glycolysis) to a high metabolic state (oxidative phosphorylation) with the activation of anabolic pathways for biomass and extracellular matrix (ECM) production. Thus partitioning of ATP generation between glycolysis and oxidative phosphorylation may help in understanding specific cellular bioenergetics. In an exciting work, Mookerjee *et al.* showed an interesting way to calculate

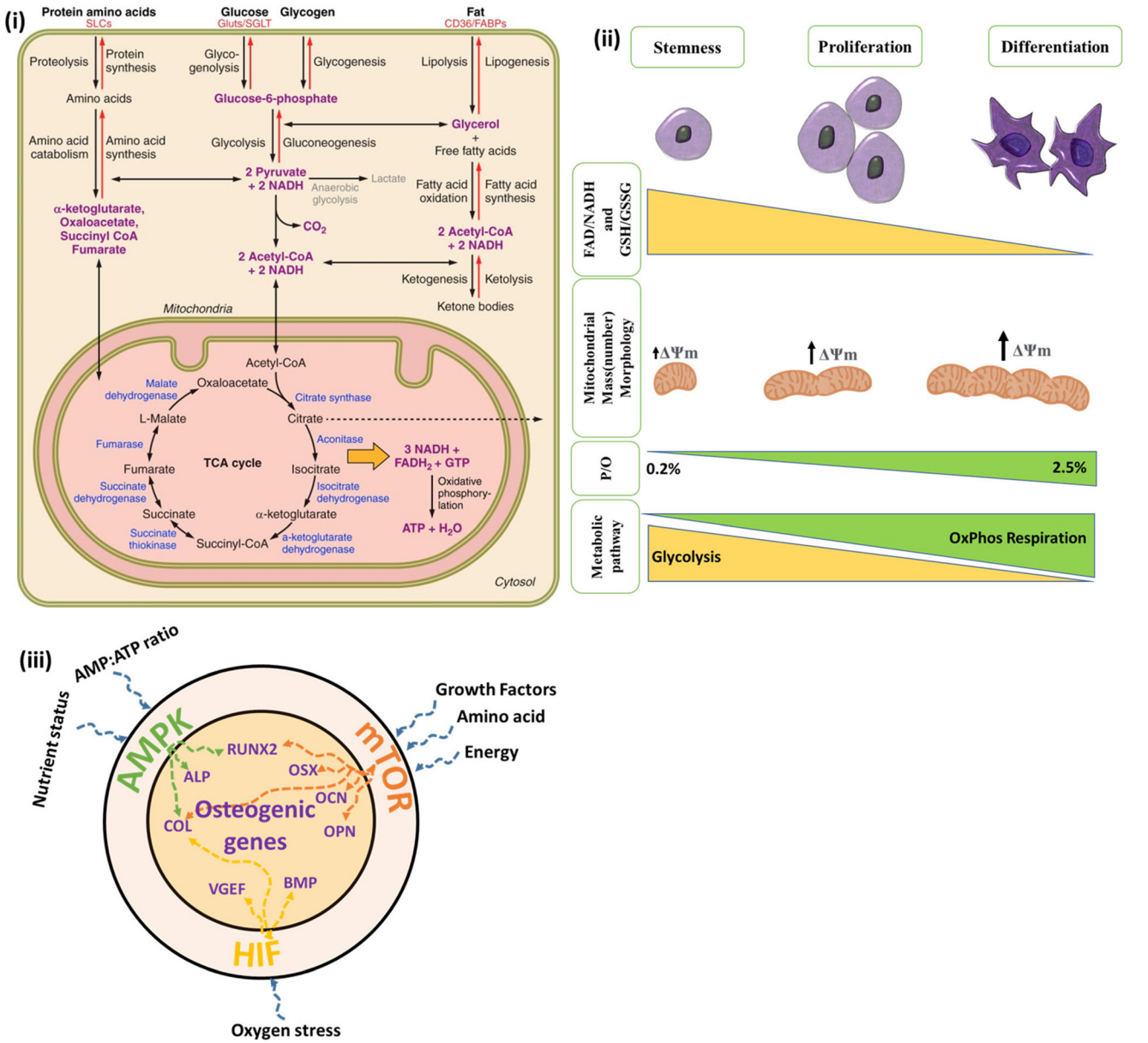

Fig. 2 Metabolic stimuli regulate cell bioenergetics and transcription factors. (i) Different metabolites and their intermediates regulate different cellular metabolic pathways to produce energy (ATP) as dictated by the bioenergetics need of cells (reprinted with permission from ref. 30, copyright (2017) American Physiological Society). (ii) Different readouts of the cellular metabolic profile, oxidative state, and energy status linked with stem cell behavior. (iii) The schematics model shows that different metabolic stimuli regulate different transcription factors to control different osteogenic genes.

rates of ATP generation by each pathway using the P/O ratio (mol of ATP generated per mol of oxygen consumed). Low metabolic state cells showed a P/O ratio of 0.16 for glycolysis, whereas a high metabolic state cell showed a P/O ratio of 2.5 for oxidative phosphorylation.[16] Several studies have also measured overall all ATP production in cells using a calorimetric approach to quantify the effect of ATP on differentiation and mineralization.[17,18] Low ATP levels in stem cells help to keep the uncommitted state of stem cells. In contrast, high ATP production stimulates osteogenic differentiation and mineral deposition.[19]

In stem cells, change in metabolism during differentiation can also be studied using metabolic co-factors like nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD).[20] In stem cells, the shift from cellular metabolism from glycolysis to oxidative phosphorylation during differentiation has been studied using the autofluorescence intensity ratio of FAD/NADH or FAD/(NADH + FAD), commonly referred to as the optical redox ratio. Differentiated cells show a decrease in the redox ratio, indicating the production of large amounts of ATP through oxidative phosphorylation to sustain cellular homeostasis and support differentiated specialized

cell functions.[21] Another cell-based bioenergetics assay uses Seahorse XF to analyze the metabolic state of cells using their extracellular fluxes. The Seahorse XF assay provides a readout for the oxygen consumption rate (OCR) in cells, an indirect measure of oxidative phosphorylation, and extracellular acidification rate (ECAR), a measure of glycolytic lactate production.[22] Using the reduced-to-oxidized ratio of glutathione (GSH/GSSG) obtained from protein (glutathione) metabolism, one can also measure the oxidative cellular metabolism of cells. A decrease in GSH/GSSG ratio is an important indicator of the higher oxidative state of cells.[23] Differentiated stem cells tend to use oxidative phosphorylation as a preferred energy production pathway, leading to increased reactive oxygen species (ROS) production and directing cells to a higher oxidative state. Produced intracellular ROS during oxidative metabolism converts reduced glutathione GSH to oxidized glutathione (GSSG).[24] Thus, by measuring the ratio of GSH/GSSG, one may find the metabolic-mediated oxidative state of cells.

The change in cellular metabolism from glycolysis to oxidative phosphorylation during bone regeneration can also be read using the mitochondrial membrane potential. The bioenergetic metabolites (succinate, citrate, malate, and α-ketoglutarate) take an active part in the TCA cycle to elevate the mitochondrial membrane potential for high ATP generation through oxidative metabolism in mitochondria, which subsequently accelerates bone regeneration.[25] Mitochondrial mass/number and shape also vary in metabolic active cells and differentiated cells through mitochondrial biogenesis and fusion pathway.[26] Fused elongated and high mitochondrial numbers enhance energy production to meet the high ATP demand during osteoblast differentiation.[27] For mitochondrial mass and morphology readout, various MitoTrackers are available that specifically label mitochondria in cells.

Nowadays, advancements in gas chromatography and mass spectrometry have markedly increased the number of metabolites that can be measured in extracellular fluxes. Using such metabolite data from gas chromatography and mass spectrometry in combination with advanced computational tools, several metabolic network dynamics analysis software has been developed to provide metabolic profile, oxidative state, and energy charge status of the cells.[28]

Overall, using these intracellular and extracellular readouts, one can evaluate the cell's metabolic state, providing useful information on how different metabolism and oxidative states of cells may direct cell fate, as illustrated in Fig. 2(ii).

3. Cell metabolism cross-talk with transcription factors

In response to different metabolism pathways, cells adjust their biochemical activity and influence myriad downstream signaling pathways controlling transcription factors and gene expression, allowing context-specific cell behavior. Such changes in metabolic activity impact overall changes in cell functions but predicting how these specific internal or external stimuli regulate exact metabolic pathways is always challenging. Growing research activities in understanding metabolic pathways and the genomic landscape started interconnecting a few key transcription regulators linked to cell metabolism.[29] Riddle *et al.*, in their review, collectively highlighted different transcription regulators and their signaling mechanisms linked to bone cell metabolism governing their function.[30] Among the different transcription regulators, AMP-activated protein kinase (AMPK), the mammalian target of rapamycin (mTOR), and Hypoxia-Inducible Factor-1 (HIF-1) are three critical regulators in bone cells that respond to internal and external stimuli controlling the gene expression and cellular function mediated through metabolism.

AMP-activated protein kinase (AMPK) is a well-known metabolic regulator found in bone extract cells that control ATP production in response to cellular stress and variation in the energy status. An intracellular increase in the AMP-to-ATP ratio activates AMPK, which then activates catabolic pathways to generate ATP. Over the years, evidence suggested the involvement of AMPK in controlling cell differentiation.[31] Undifferentiated pre-osteoblast needs a high amount of glucose to generate ATP to activate runt-related transcription factor 2 (Runx2) for differentiation and gene expression of Alkaline Phosphatase (ALP) and Collagen I (COL-I) to synthesize a protein-rich ECM matrix and thus retains the high activity of AMPK, whereas differentiated osteoblasts decrease the activity of AMPK.[32,33]

The other similar metabolic regulator is the mammalian target of rapamycin (mTOR), an amino acid sensor, and has been described to integrate intracellular and extracellular signals with cell metabolism to control osteoblast functions.[34] Studies showed that osteoblast and stem cell differentiation are linked with metabolism through mTOR signaling to express osteopontin (OPN) and Osterix (OSX).[35] The activation of two distinct multi-protein mTOR complexes (mTORC1 and mTORC2) is associated with stage-specific roles in protein synthesis and ribosome biogenesis within the osteoblast lineage. mTORC1 activation mediated by amino acid, hormones, or energy deficiency regulates the cell's mitochondrial biogenesis leading to increased oxidative phosphorylation to meet the energy requirement for the osteogenic differentiation process.[36] Compared to mTORC1, the specific activation and roles of mTORC2 are less understood. However, increased levels of insulin-like growth factor (IGF1) and Wnt have been shown to activate mTORC2 for bone growth through the anabolic pathway.[37]

Like AMPK and mTOR complexes, hypoxia-inducible transcription factors (HIFs) act as transcriptional regulators for oxygen sensors and control the cellular response by activating genes directing bioenergetics.[38] HIF-1 serves as a molecular switch diverting energy production from oxidative to glycolytic metabolism under hypoxic conditions. Under hypoxic conditions, the HIF transcription factor is stabilized and accumulates inside cells to trigger gene expressions for a vascular endothelial growth factor (VEGF) and runt-related transcrip-

tion factor 2 (RUNX2).[39] HIF-1α stabilization maintains redox homeostasis during oxidative stress while enabling glycogen-dependent bioenergetics during glucose deprivation, which improves bone cell survival and substantially enhances bone regeneration.

Fig. 2(iii) schematically highlights the association of metabolite stimuli to regulate transcription factors that subsequently control several essential osteogenic genes. Biological factors like hormones, therapeutic drugs, growth factors, and chemicals controlling metabolism, transcription factors, and gene regulation have evolved into crucial parts of the cellular response system.[40] However, the response of external stimuli like the physicochemical properties of biomaterials to cell function mediated through cell metabolism is emerging and needs more attention. In the next section, we highlight and discuss how the external surface properties of biomaterials regulate cell function mediated through cellular metabolism.

4. Metal ion release regulating cell metabolism

The release of metal ions from a metallic or metal-doped biomaterial may provide an alternative approach to control cellular metabolism and cell functions. Several metallic ions (Ca^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+}) act as co-factors for enzymes, which play a vital role in regulating the metabolic activity of cells. Metal ions like Co^{2+} and Cu^{2+}, in micromolar (μM) concentrations, help cells to stabilize a transcription factor known as the hypoxia-inducible factor (HIF), which mediates the oxygen-sensing pathway to regulate the metabolic profile of stem cells controlling proliferation and differentiation.[41] As a result, biomaterials capable of regulating such cellular oxygen-sensing pathways, independent of environmental oxygen stress, have gained interest in bone tissue engineering.[42] Bioactive glass doped with Co^{2+} is one such material that alters stem cell metabolism by upregulation of HIF-1α for activation of the hypoxia pathway due to the release of Co^{2+} ions promoting angiogenesis, a crucial factor needed for vascularized bone tissue.[43]

Similarly, elution of Cu^{2+} ions from copper doped bioactive silicate and Cu-graphene coated calcium phosphate (CaP) scaffolds promoted the high expression of HIF-1α in bone marrow stem cells upregulating VEGF, ALP, and BMP-2 production, promoting angiogenic and osteogenic potentials (Fig. 3(i)(a–f)).[44] Based on these results, we and many others utilized copper incorporation in the biomaterial as an attractive strategy for vascularized bone regeneration.[45,46]

Currently, in the medical system, several other metallic implants (chromium, nickel, titanium, aluminum, and vanadium) are used in dental and orthopedic applications, but how these implants influence cell function mediated through cellular metabolism remains unclear. To understand bone–implant interactions of metallic implants, Sun *et al.* collectively tried to study the effect of different metal ions (Cr Al, Ni, Ti, and V) on osteoblast metabolism and function.[47] Among all the metal ions below their toxic level, Cr and Ti treated cells showed high retention of SDH activity in cells (Fig. 3(ii)(a–c)),[48] which later enhanced osteogenic gene expression (osteopontin and osteocalcin), boosting the mineralization ability of osteoblasts. SDH activity in cells provides critical information on the cell's metabolic state and oxidative potential. The high activity of SDH promotes the oxidation of succinate (metabolite found in the TCA cycle) that triggers intracellular calcium release promoting mineralization.[49] Thus, evaluating SDH activity in cells has renewed interest in BTE due to its ability to link to oxidative phosphorylation to meet the energy demand needed to upregulate the osteogenic process.

In the world of ceramic materials, calcium phosphate (CaP) has been frequently used for bone regeneration as they form the major constituent of native bone tissue. Numerous literature studies have shown the potential of released Ca^{2+} and $[PO_4]^{3-}$ ions on the osteogenic differentiation of bone progenitor cells.[50] The ability of CaP materials to control the fate of bone progenitor cells and the molecular mechanism through which these ions regulate the osteogenic commitment of bone progenitor cells remains largely unknown. Several new studies started to unravel previously unknown mechanisms; one such mechanism was found centered on phosphate metabolism. Shih *et al.* demonstrated the uptake of released inorganic phosphate ions from the CaP matrix by human mesenchymal stem cell (hMSC) regulated ATP synthesis in mitochondria as key substrates in cell energy metabolism to enhance osteogenic differentiation (osteocalcin and osteopontin expression) (Fig. 3(iii)(a–c)).[51] Exogenous supply of phosphate mediates the phosphorylation of adenosine organic molecules to yield energy in the form of ATP. Such high phosphorylation activity mediates the activation of AMPK, which subsequently controls the osteogenic differentiation potential of hMSCs by expressing the alkaline phosphatase (ALP) gene. These results are consistent with the emerging studies highlighting the pivotal role of phosphate, β-tricalcium phosphate, and adenosine as an ATP metabolite centered on phosphate metabolism controlling the osteogenic differentiation of stem cells.[52–55] Similar to phosphate, bisphosphonate releasing scaffolds are used to treat metabolic bone disorders like osteoporosis and Paget's disease.[56] Released bisphosphonate enters osteoblast cells and prevents the synthesis of cholesterol by inhibiting diphosphate synthase in the mevalonate pathway to attenuate cholesterol dependent osteoporosis.[57]

Although most of these studies concentrated on phosphate metabolism controlling the osteogenic ability of stem cells, but one should not neglect the beneficial effect of the released Ca^{2+} ions from the CaP matrix. The released Ca^{2+} ions from the CaP matrix alter mitochondrial signaling for cell metabolism regulation to meet the high energy demand for the proliferation and matrix mineralization of MSCs. Calcium-mediated mitochondrial metabolism promotes the expression and secretion of osteopontin (OPN), enhancing MSC migration under increased extracellular Ca^{2+} conditions for bone tissue regeneration.

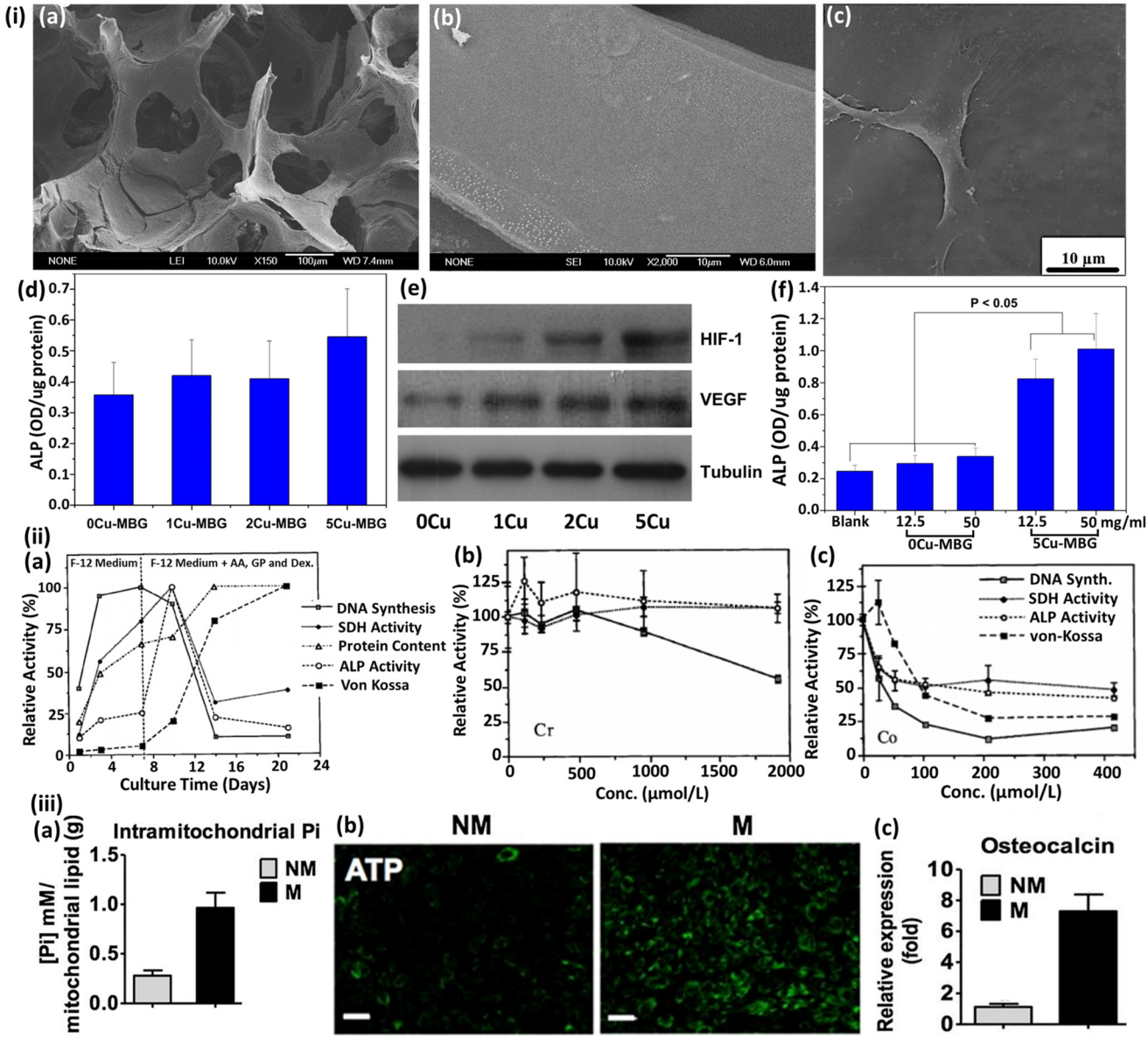

Fig. 3 The effect of released metallic ions on cell metabolism correlating with cell fate. (i) Mesoporous 5% copper substituted bioactive glass (5Cu-MBG) (SEM morphology shown in (i)(a) and (b)) supported hBMSC attachment and spreading with pseudopodia extension (i)(c). hBMSCs on the Cu-MBU scaffold showed an increase in ALP, HIF-1, and VEGF contents with an increase in the copper content (i(d) and (e)). Release extract collected from Cu-MBG scaffolds as conditioned media supported the high expression of ALP activity (i)(f) suggesting the presence of copper ions (around 150 ppm) from the 50 mg ml^{-1} 5Cu-MBG extract influenced the stem cells to upregulate HIF-1α for activation of the hypoxia pathway promoting osteogenic differentiation (reprinted with permission from ref. 44,57 copyright (2013) Elsevier). (ii)(a) Metabolic profile of osteoblast cells in complete culture media at different culture times. (ii)(b) and (c) Comparison of the effect of Cr and Co concentrations in culture media on osteoblast metabolism at day 3. Cr ions at a low concentration below its toxic effect retain a high SDH metabolic effect for ALP secretion in comparison with Co (reprinted with permission from ref. 48, copyright (1997) Wiley). (iii)(a) hMSCs cultured on the CaP mineralized matrix (M) accumulated a high amount of released phosphate ions into mitochondria in comparison with hMSCs on a non-mineralized matrix (NM) (after 1-day culture). A high amount of phosphate ions in mitochondria promoted the generation of a high amount of ATP in hMSCs. (iii)(b) (quinacrine staining for ATP after 4 d of culture, scale bar = 200 μm). (iii)(c) High amount of energy produced inside the cell promoted downstream signaling for the expression of osteocalcin (21 days) for the osteogenic differentiation of hMSCs (reprinted with permission from ref. 51, copyright (2013) National Academy of Sciences).

Overall, understanding how metal and ceramic ions are released from implants to regulate gene expression and cell function mediated through cellular metabolism is complex, but these studies started to clarify such aspects and need more detailed studies. In addition, it is important to remember that under *in vivo* conditions, some implants like Ti, Co, and Al may have passivated surfaces, which may have an additional effect on cell–implant interactions. Thus it needs further comprehensive studies under *in vitro* and *in vivo* conditions concerning bone tissue regeneration.

5. Degradable polymeric metabolites regulating cell metabolism

Growing evidence on how released metal ions from metallic and ceramic implants can deliver signals to cells to regulate cell metabolism and functioning has encouraged researchers to look into other degradable biomaterials on cell function mediated through metabolism. From the pool of different organic-based biomaterials (natural and synthetic), several undergo degradation, releasing products that may be metabolic regulators or key substrates for biosynthesis or energy production that can influence intracellular metabolic events for bone tissue regeneration. Understanding such temporal dependence of exogenous products in cellular metabolism and coordinated tissue formation may provide a better direction for designing materials for metabonegenic tissue regeneration.

On that note, using citrate-based biomaterials, Ma *et al.* highlighted a connection linking how released citrate from the materials influenced cellular metabolism and osteogenic differentiation (Fig. 4(i)).[58] Citrate, a key metabolite in the TCA cycle has proven to be an active osteopromotive component.[59] Hence culturing human mesenchymal stem cells (hMSCs) on citrate-based biomaterials mimics the bioactive interface layer typically found in the native bone matrix. The released citrate from the materials showed the inherent ability to regulate cellular metabolism shifting from glycolysis to oxidative respiration resulting in high ATP production and oxygen consumption. Such a shift in metabolism promoted mTOR-dependent protein synthesis and favored RUNX2 accumulation for osteogenic differentiation.[60]

In addition to bone regeneration, citrate as a metabolite also gained special attention in vascular tissue engineering.[1] The participation of citrate in multiple biological functions encouraged researchers to investigate its dual effect on osteogenesis and angiogenesis. Wu *et al.* prepared citrate incorporated magnesium calcium phosphate cement as a vascularized bone regeneration construct.[61] A matrix with a low citrate concentration (0.1–0.5 mM) was beneficial for promoting the osteogenic function of osteoblasts, whereas at a higher concentration (1–5 mM), it promoted the angiogenic process of vascular endothelial cells (Fig. 4(ii)). Leveraging the synergetic nature of citrate in forming bone tissue and constructing new blood vessels for nutrient supply. A new class of highly versatile and functional citrate-based biomaterials (CBBs) is emerging with tunable degradation from a few days to over a year to explore the impact on bone formation.[62]

Synthetic polyesters of lactic acid and glycolic acid or combined are popular materials for tissue regeneration, especially in orthopedic applications. The interest in these biomaterials is due to their ability to degrade under physiological conditions to release glycolic acid and lactic acid as naturally occurring metabolites. The uptake of exogenous lactic acid in the form of lactate serves as an energy source; the cell converts lactate to glucose by the Cori cycle or converts it to pyruvate and acetyl-CoA to fuel the TCA cycle in mitochondria for energy metabolism.[63] Lactic acid is oxidized to pyruvate and acetyl-CoA to fuel the TCA cycle and has been linked with generating a high amount of ATP through oxidative phosphorylation. Stem cell or osteogenic progenitor cells deriving a high amount of ATP from oxidative phosphorylation than normal glycolysis undergo fast osteogenic differential to deposit collagen-rich mineral nodules.[64,65]

In contrast, few studies have shown that a high amount of lactic acid release (>15 mM) from a polyester-based material causes a decrease in surrounding pH, resulting in increased accumulation of lactate in cells, causing a decrease in the metabolism of MSCs, resulting in decreased proliferation and osteogenic differentiation capacity.[66,67] Therefore, it is essential to design a polylactic acid (PLA) based scaffold with controlled degradation limiting the poor culture effect with a high concentration of lactate release. As a result, researchers have started to find additives and fillers to tune the biodegradation rate of polylactic acid (PLA).[68,69]

Inspired by the concept of degradable products from biomaterials accelerating tissue regeneration modulating through cell metabolism. Many scientists have started designing and creating a new biomaterial class called bioenergetic materials. Liu *et al.* prepared one such bioenergetics active material (BAM) from poly(glycerol succinate) for bone repair applications.[70] The released succinate fragments from BAM were internalized into cells and hydrolyzed to produce metabolic intermediates, which subsequently enter mitochondria and produce other key metabolites (malate, citrate, and α-ketoglutarate) for the TCA cycle. The bioenergetics of the TCA cycle resulted in the production of oxaloacetic acid as a precursor for collagen and high ATP to confer the higher energetic capacities required by mesenchymal stem cells to activate the osteogenic differentiation process (Fig. 4(iii)).

6. Organic metabolic drug release regulating cell metabolism

Understanding the role of released degraded products from polyesters, citrate-based polymers, and bioenergetics polymer matrices in regulating cellular bioenergetics for bone regeneration encouraged researchers to explore the further possibility of reinforcing metabolic drugs or bioenergetics growth factors into the scaffold to regulate bone cell energetics for BTE. One of the most commonly used metabolic drug supplements for bone is Vitamin D. Vitamin D helps to build strong bones and prevent bone loss (osteoporosis) by regulating the calcium and phosphate metabolism in bone cells. The binding of vitamin D to the vitamin D receptor (VDR) on bone cells increases the calcium influx into cytosol and mitochondria, which further augments energy generation through oxidative phosphorylation. By regulating cellular bioenergetics, vitamin D promotes metabolically less active preosteoblasts into metabolically active osteoblasts by high production of mitochondria with high-transmembrane potentials to deposit a Ca–P rich bone matrix. Considering the metabolic role of vitamin D in regulat-

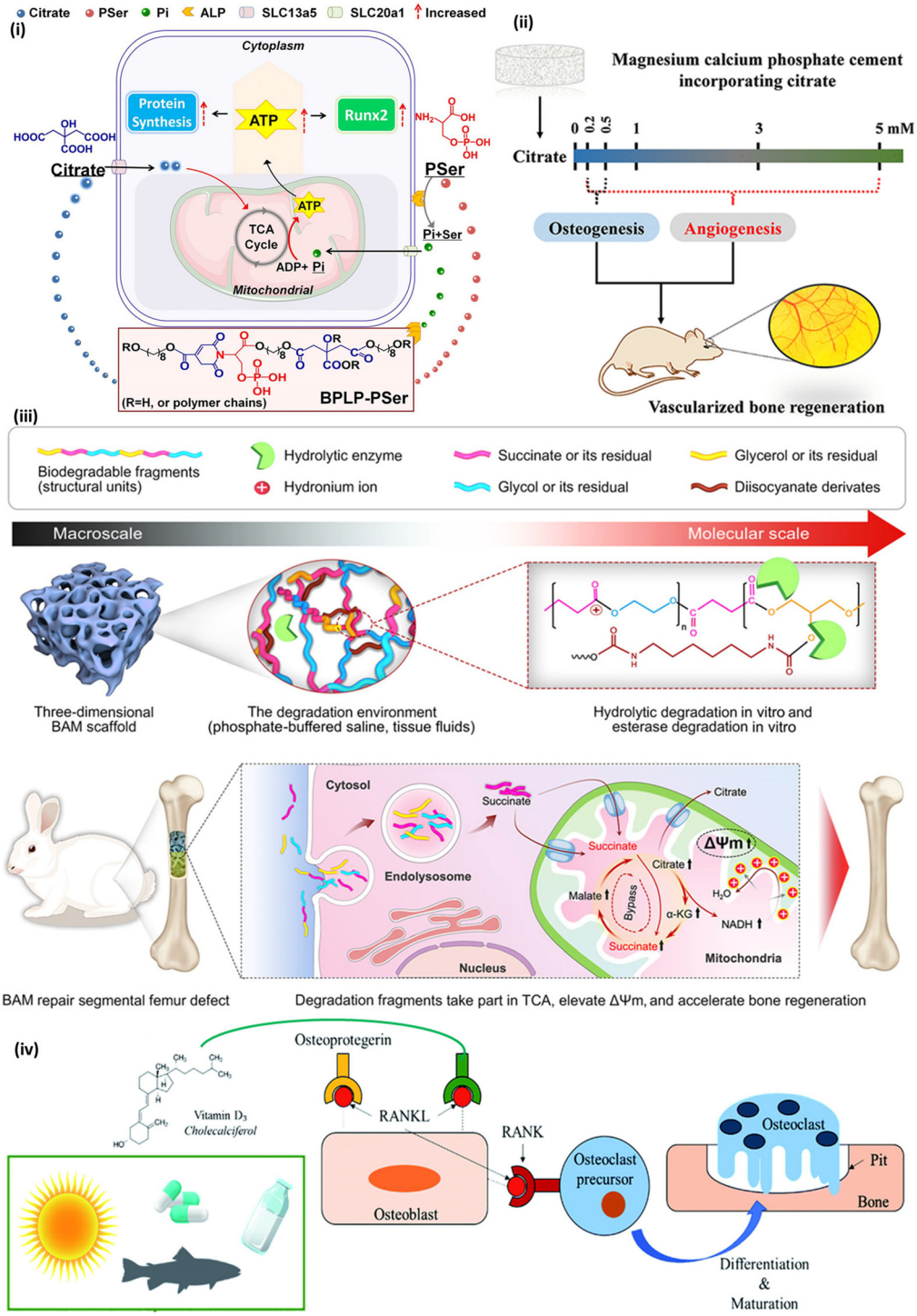

Fig. 4 Effect of organic metabolites released from biomaterials to regulate cell metabolism and control cell fate. (i) Schematic model highlighting the metabonegenic nature of citrate-based materials (citrate/phosphoserine-based photoluminescent biodegradable polymer (BPLP-PSer)), which induces citrate-mediated regulation of cell energy metabolism toward osteophenotype progression (reprinted with permission from ref. 58, copyright (2018) National Academy of Sciences). (ii) Citrate as a metabolite shows a concentration-dependent multi-beneficial effect for promoting osteogenic and angiogenic functions as vascularized bone regeneration (reprinted with permission from ref. 61, copyright (2020) American Chemical Society). (iii) Poly(glycerol succinate) scaffolds prepared from metabolic units of succinate and glycerol acted as bioenergetics active materials (BAMs) for bone tissue regeneration. Enzymatic and hydrolytic released succinate fragments from poly(glycerol succinate) scaffolds were internalized into cells and hydrolyzed to produce metabolic intermediates, which subsequently enter mitochondria and produce other key metabolites (malate, citrate, and α-ketoglutarate) for the TCA cycle. High bioenergetics of the TCA cycle further supported the energy demand of mesenchymal stem cells to activate the osteogenic differentiation process for bone tissue regeneration (reprinted with permission from ref. 70, copyright (2020) American Association for the Advancement of Science). (iv) Vitamin D released from the scaffolds helps in binding the receptor activator of nuclear factor (RANK) with RANKL expressed in osteoblast cells (reprinted with permission from ref. 71 copyright (2019) Royal Society of Chemistry).

ing bone remodeling, several researchers started reinforcing vitamin D or its derivatives in biomaterials to promote bone tissue regeneration (Fig. 4(iv)).[71] The release of vitamin D from different scaffolds (calcium phosphate, gelatin, and gelatin-hydroxyapatite-vitamin D loaded graphene oxide) provided a local drug delivery system enabling a high metabolic rate proliferative profile of osteoblasts and high mineralization activity (high osteocalcin, and RunX2 expression) to stem cells for BTE.[72–74] For bone tissue, hypoxic microenvironments often create oxygen-tensed conditions for cellular survival, development, and differentiation. Under hypoxia conditions, stem cells or bone progenitor cells respond by expressing hypoxia-inducible factor-1α (HIF-1α), which serves an essential role in promoting angiogenesis of new blood vessels sprouting from existing blood vessels to cope with the local increased demand for oxygen and metabolites for bone regeneration. Deferoxamine, as a hypoxia mimic drug, regulates bone cells' ability to respond to oxygen tension by activating the HIFα pathway as a coupling factor for angiogenesis and osteogenesis during skeletal development.[75] By developing a deferoxamine releasing 3D-printed polycaprolactone (PCL) scaffold, Yan *et al.* showed deferoxamine released from the PCL scaffold upregulated HIF1-α expression in HUVECs, and osteoto marker RUNX-2 in rat BMSCs promoting rapid angiogenesis and osteogenesis in the animal model.[76]

Stimulating stem cells with osteo-anabolic growth factors like bone morphogenetic protein 2 (BMP-2) can also coordinate the changes in the mitochondrial number and membrane potentials to enhance energy production in order to meet the high ATP demand during osteoblast differentiation.[77] Thus, reinforcing BMP-2 in the polymeric scaffold with control release kinetics provides a platform to maintain the metabolic microenvironment for increased MSC osteodifferentiation for bone repair. Lin *et al.* developed a BMP-2/Mg releasing GelMA hydrogel system which demonstrated rapid metabolic activation of rat bone marrow stem cells (BMSCs) triggered due to the uptake of released BMP-2/Mg from hydrogels. Released BMP-2 and Mg synergetically acted as energy propellants to increase cellular energetic levels of BMSCs to support osteogenesis *via* the Akt-glycolysis-Mrs2-mitochondrial axis.[78] Similar to BMP-2, other growth factors like insulin regulate fuel consumption and energy expenditure in metabolically active bone tissues. Insulin regulates osteoblasts' glucose metabolism to control osteocalcin production, promoting bone deposition.[79] Incorporating insulin in collagen scaffolds supported osteogenesis and bone turnover ability under *in vitro* and *in vivo* conditions.[80] Metformin, another anti-hyperglycemic agent, has been shown to down-regulate cholesterol metabolism and upregulate glucose metabolism *via* AMPK, which results in bone formation ability.[81] The metformin reinforced gelatin/nHA scaffold showed the osteoinductive potential by upregulating the activity of ALP, COL-I, and RUNX2 *via* AMPK signaling.

Overall, these studies as a proof-of-concept demonstrated degradation fragments from natural and synthetic biomaterials and released organic metabolic drugs from the biomaterial matrix can stimulate bone cell bioenergetics to enhance bone regeneration *via* different metabolism-pathways.

7. Substrate stiffness regulates cell metabolism

Over the years, biomechanics has evolved to set principles and guidelines to design materials for bone tissue regeneration. One such principle suggested that designing substrate stiffness mimicking native tissue mechanical properties promotes lineage-specific differentiation of stem cells. As a result, stem cells are now well known to respond differently to the mechanical properties of biomaterials.[82] Based on substrate stiffness, it is likely that stem cells may regulate their cellular metabolism by utilizing specific metabolites that may be linked with a particular cell lineage. Linking the mechanosensing ability of cells with metabolism may explain how cells uniquely sense substrate stiffness. On that note, Alakpa *et al.* studied the effect of hydrogel stiffness on stem cell differentiation combined with metabolomics analysis to identify the role of specific biomolecules in differentiation.[83] Supramolecular peptide gels of different stiffness, namely soft (1 kPa), stiff (13 kPa), and rigid (32 kPa) (Fig. 5(i)(a)) stimulated perivascular stem cells to differentiate into neuronal, chondrogenic, and osteogenic, respectively, supporting the principle of the stiffness-directed stem cell fate (Fig. 5(iii)(b)). Metabolites collected from the stem cells highlighted a significant difference in the metabolite profile on stiff and rigid gels suggesting the distinct metabolism during the differentiation (Fig. 5(ii)(c)). High expression of cholesterol sulfate on rigid hydrogels as a steroid biosynthesis pathway promoted transforming growth factor β (TGF-β) signaling, a key component of regulating bone morphogenic protein (BMP), and activated RUNX2 for osteogenesis.[84] Taken together, these studies connected the data suggesting that mechanosensing and metabolism are linked. Still, more work is needed to support and clarify this picture.

8. Substrate conductivity regulates cell metabolism

Conductive biomaterials are gaining much interest in bone tissue engineering due to the intrinsic piezoelectric nature of bone tissue. Conductive materials provide a stimulating surface for transferring electrical, electrochemical, and electromechanical signals directly to the bone cells at the polymer–tissue interface to improve cellular activity.[85–87] Several interesting studies have highlighted the conductive surface ability to influence electroactive tissues (nerve, cardiac, and bone) to regulate cellular metabolism through calcium-mediated electrical activity.[88] An increase in cytosolic calcium through voltage-gated calcium channels regulates cellular bioenergetics by activating key metabolic enzymes, metabolite shuttles, and signaling cascades.[89] Da Silva *et al.* cultured hMSC-BM on con-

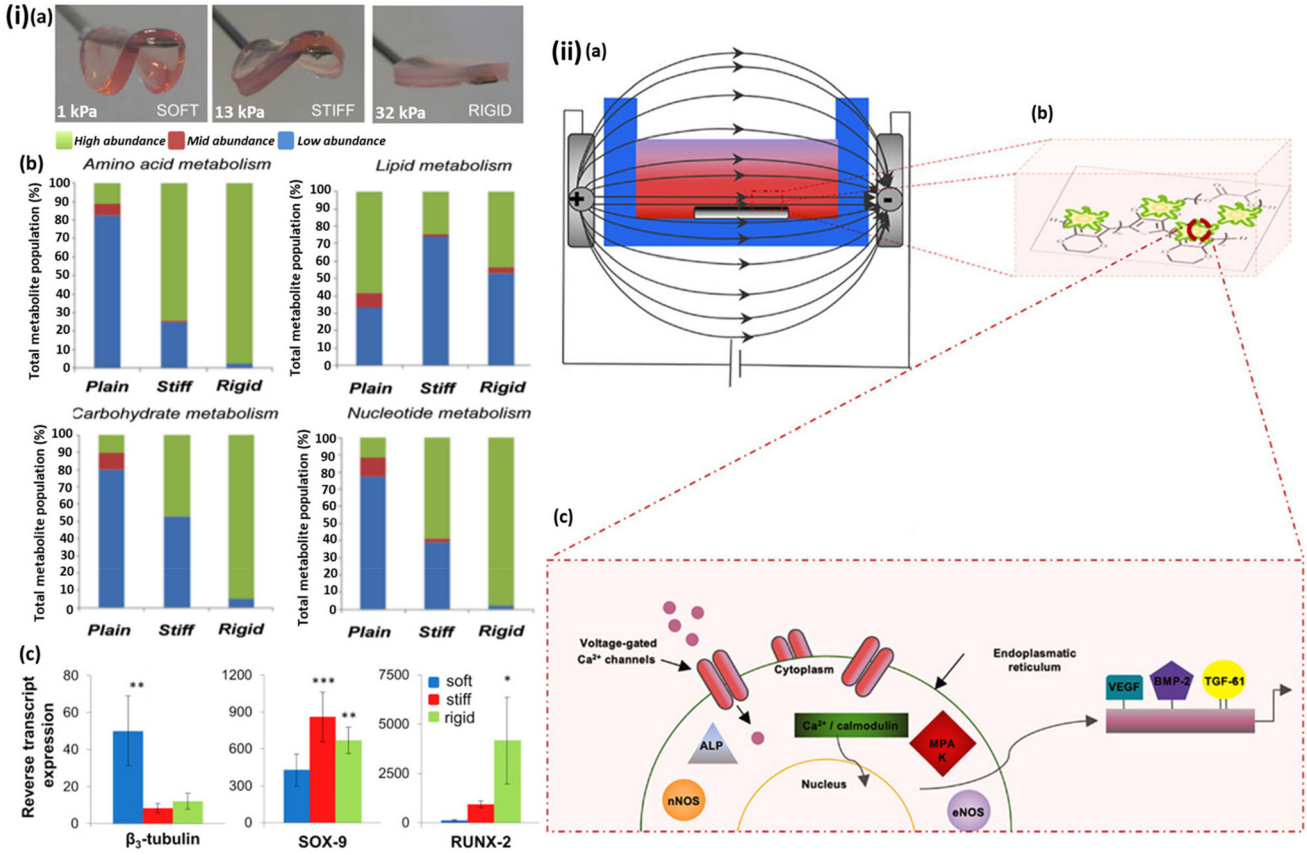

Fig. 5 Response of the mechanical and electrical properties of biomaterials on cellular metabolism to regulate cell behavior. (i)(a) Macroscopic images of prepared soft, stiff, and rigid gels in culture media. (b) and (c) Metabolic state of perivascular stem cells on the respective hydrogels showing distinct metabolic profiles controlling different gene expression depending on the mechanical properties of the hydrogel substrate (reprinted with permission from ref. 83, copyright (2016) Wiley). (ii)(a) Schematic of the electric field applied on the conducting surface seeded with hMSC-BM, (b) enlargement of the selected area with attached hMSC-BM over PEDOT-*co*-PDLLA, and (c) intracellular metabolic activity behavior under capacitive electric stimulation (reprinted with permission from ref. 90 copyright (2021) AIP).

ducting the PEDOT:PDLLA surface and applied a short-term electric field to understand proliferation and osteogenic differentiation by analyzing cell metabolic behavior (Fig. 5(ii)(a–c)).[90] The hMSC-BM on the conducting surface showed significantly high mitochondrial oxidative metabolism with elevated intracellular calcium activating MAPK signaling cascade for deposition of a calcium rich mineral matrix compared to the non-conducting PEDOT:PDLLA surface. These results suggested that the intrinsic conductive nature of the polymer can regulate hMSC-BM metabolism in promoting osteogenic differentiation. On a similar line, researchers have also found that coating a conductive layer of the PPy/PDA film can stimulate rapid proliferation and metabolism activity of pre-osteoblasts to differentiate into osteoblasts with high ALP activity. These emerging results co-relating conductive properties of biomaterials with cellular metabolism to regulate cellular functions have encouraged researchers to conduct more rigorous experiments to link cellular metabolism as another critical aspect of cell–material interactions to control bone cell behavior.

9. Surface properties regulate cell metabolism

Cell–material interactions are essential to understand the bioactivity of designed biomaterials in a preclinical study. This involves studying the dynamic interaction of cells with the biomaterial surface and their response to regulate the biological activity of cells like adhesion, spreading, growth and differentiation. The surface properties of biomaterials, such as surface roughness, morphology, and chemistry, markedly determine the cells' ability to sense and adhere to the material surface to regulate cell function. However, no generalized rule was established to predict cellular processes (adhesion, spreading, growth and differentiation) on the material surface.[91] Recent studies supported the hypothesis that adhered cells on the material surface sense the surface properties and modifies their metabolism through the metabolite and energy balance to control specific pathways for synthesizing biomolecules needed to control cell function.[92] Given that synthesizing bio-

molecules consumes additional energy, it provides a possible explanation for why cells may regulate metabolism depending on the material's surface properties.

9.1. Surface roughness regulates cell metabolism

The surface roughness of bone-implant materials influences cell behavior by affecting cell adhesion and migration through focal adhesion and cytoskeleton organization. For developing focal adhesion complexes through integrin, the cells need to synthesize cytoskeletal proteins and organize them, which regulates cell metabolism.[93] However, understanding how biomaterials' surface roughness cross-talk between cell metabolic pathways for bone regeneration has not gained much attention. Only a few studies demonstrated the effect of biomaterial roughness on cell function regulated through metabolism. Ball *et al.* evaluated the osteoblast biological response on a series of titanium alloy (Ti6Al4V) samples having order and random micro-roughness created by machining techniques (Fig. 6(i)(a)).[94] Human osteoblast culture on the order microstructure showed an elongated morphology with higher metabolic activity than random microstructure samples (Fig. 6(i)(b and c)). Osteoblast oxygen-dependent metabolic activity on the order microstructure increased steadily, reaching a peak after seven days, followed by a steady decrease – a classical characteristic of osteogenic differentiation following a similar pattern for ALP expression.[95] Analyzed ALP activity at a very early time point after 48 h of culture of osteoblasts on order, and the random microstructure titanium alloy showed more expression of ALP on the order microstructure due to high initial metabolic activity supporting an early high metabolic state regulating cell function (Fig. 6(i)(d)).

On the other hand, creating nano or micro-scale irregularity on polymer surfaces by adding nanoparticles can also affect surface roughness, which can have a pronounced effect on cell metabolism and function. Reinforcing nanodiamond particles in a copolymer matrix of poly(L-lactide-*co*-ε-caprolactone) affected surface roughness, stimulating bone regeneration in a rat calvarial defect model by promoting the metabolic activity of BMSCs.[96] The osteogenic metabolic activity of BMSCs was evaluated using [18F] sodium fluoride ($^{[18F]}$NaF), a classic marker to study bone metabolism. The enhanced mineralization capacity of BMSCs promoted the rapid exchange of ^{18}F ions with hydroxyl (OH) on the surface of the hydroxyapatite matrix-forming fluorapatite. Such active bone formation sites are visualized and quantified using PET/CT. Although these two studies mentioned above did not provide much detail on specific metabolite or energy-dependent pathways involved, they demonstrate how oxygen-dependent activity and ^{18}F tracer

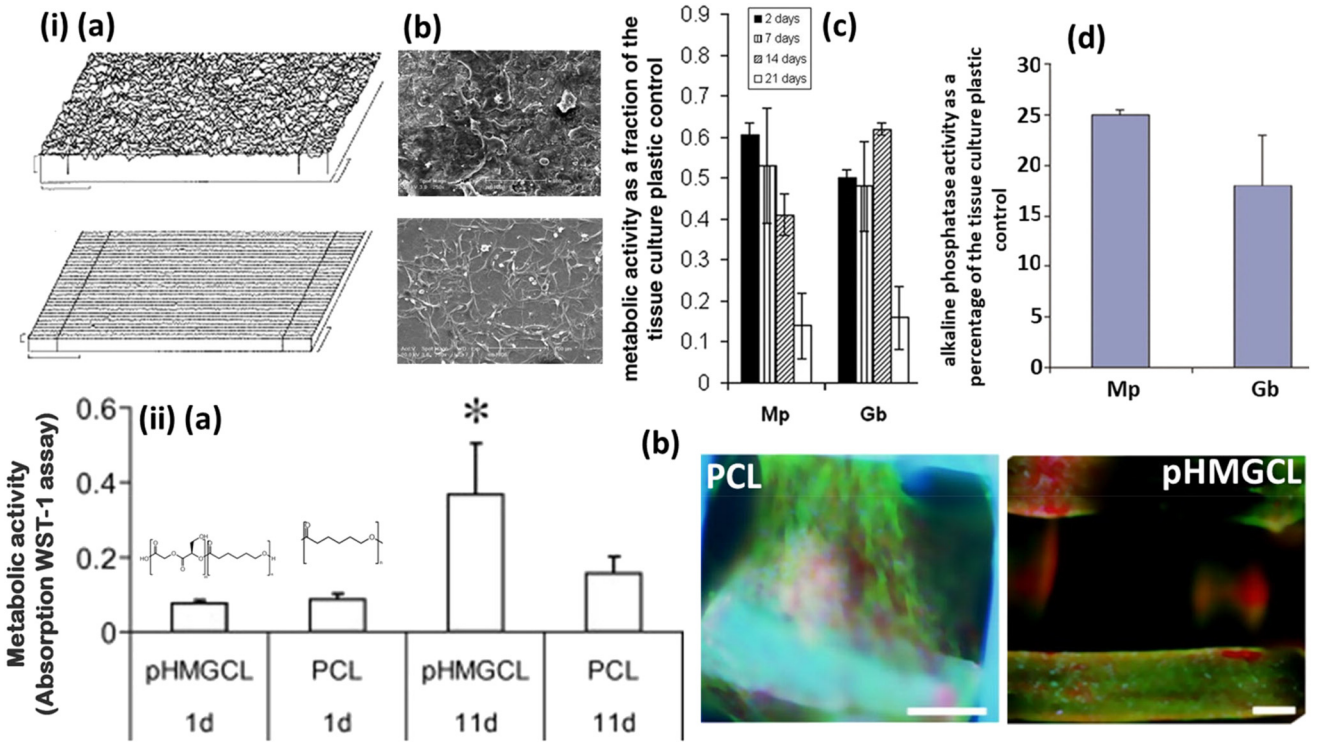

Fig. 6 Effect of the surface properties of biomaterials regulating cell metabolism to control cell function. Influence of the surface pattern: (i)(a) 3D representations of the random (grit blasted, Gb) and ordered (micro polished, Mp) microstructure titanium alloy surfaces generated by profilometry. (i)(b) and (c) Human osteoblasts cultured on the ordered microstructure showed an elongated morphology, high early time point metabolic activity, and high ALP expression in comparison with a random microstructure (reprinted with permission from ref. 94 copyright (2007) Wiley). The effect of surface chemistry; (ii)(a) and (b) PCL having a hydroxyl functional group on the surface showed more metabolically active stem cells with high ALP expression for osteogenic differentiation in comparison with the pristine PCL surface (scale bar = 200 μm, actin filaments = green, nucleus = blue) (reprinted with permission from ref. 107, copyright (2011) Elsevier).

uptake can be directly related to the metabolic activity of osteogenic cells on micro-roughness materials regulating bone regeneration.

9.2. Surface morphology regulates cell metabolism

In addition to surface roughness, surface morphology (pattern or architecture) has played a vital role in influencing the cell fate. Biomaterials designed to mimic the extracellular matrix architecture of bone has favored the osteogenic behavior of stem cells.[97,98] In that regard, the electrospun nanofibrous scaffold has attracted considerable attention in BTE due to its ability to mimic the hierarchical structure of the extracellular bone matrix. Raic *et al.* demonstrated that electrospun bovine serum albumin (BSA) fibers resembling the bone marrow extracellular matrix supported enhanced focal adhesion formation and osteogenic differentiation of MSCs.[99] In addition, literature is also available that discusses the effects of the fiber orientation (random and aligned) on the cellular response.[100] Aligned nanofibers of poly(L-lactide) (PLLA) were found to enhance the adhesion, cell migration, and extracellular collagen assembly similar to the lamellar bone arrangement.[101,102] As a result, electrospun fibers specially aligned nanofiber architecture is a desirable design for bone tissue engineering.

Nevertheless, the mechanism by which cells respond to the fiber orientation is still unclear. Few studies have now started to look into the role of metabolite-dependent mechanisms in the cellular response to the fiber architecture. Osteoblasts cultured on aligned fibers induced increased cellular metabolic activity compared to the random fiber architecture; as a result, osteoblasts on the aligned matrix showed metabolic-dependent mineralization.[103] Although these studies used Resazurin dye to detect cell metabolism based on the ability of NADPH or NADH dehydrogenase to reduce it to resorufin. High Resazurin metabolic readout on aligned fibers suggested that osteoblasts had energized mitochondria with high NADH to participate in energy production through oxidative phosphorylation, a marker for energy-dependent tissue regeneration.

9.3. Surface chemistry regulates metabolism

Numerous studies highlighted the effect of materials chemistry on the development of cytoskeletal and focal adhesion complexes for osteogenesis.[104–106] However, it is surprising to see no dedicated literature demonstrating the bone cells' ability to sense the surface chemistry of biomaterials to modulate their intracellular metabolism for osteogenesis. However, an indirect study by Seyednejad *et al.* compared the surface chemistry of hydroxyl functionalized PCL (pHMGCL) with pristine PCL for bone tissue engineering applications.[107] Hydroxyl functional groups on 3D printed PCL surfaces provided different surface chemistry than pristine PCL, which significantly enhanced the surface wettability of the hydroxyl PCL polymer and influenced the metabolic state to regulate the differentiation of hMSCs. Compared to neat PCL, the hydroxyl functional PCL surface showed more metabolic active cells with high ALP activity for osteogenic differentiation (Fig. 6ii(a and b)).[107] In a similar study conducted on a different theme, Hambleton *et al.* studied the effect of chondrocyte metabolism on titanium dioxide, aluminum oxide, zirconium oxide, and calcium phosphate materials often used in orthopedic applications. The chemical nature of these materials affected the chondrocyte metabolism differently, regulating the cell phenotype, cell maturation, and collagen production.[108] Although these studies did not provide detailed underlying reasons for influencing specific cell metabolism, and may require further advanced investigation to directly connect surface chemistry with cell metabolism.

Overall, directly or indirectly, studies highlighted that bone or stem cells could sense the surface properties (surface roughness, morphology, and chemistry) of biomaterials to regulate their metabolic activity to meet the bioenergetics demand for cellular adhesion, spreading, growth and differentiation. Nevertheless establishing such complex cross-talks between materials' surface properties and cell metabolism needs more detailed studies.

10. Concluding remarks and future outlook

Recent studies on material–cell interactions started to link the cells' ability to sense biomaterial properties at the molecular level to regulate various intracellular biochemical processes. Tuning cell metabolism with modulating the biomaterial properties to regenerate tissue is gaining much interest in BTE. Emerging results correlating metabolism with cell functions like adhesion, proliferation, and differentiation have encouraged researchers to uncover previously neglected factors on how biomaterial properties govern the changes in cell function mediated through metabolism. In this review, it has been highlighted how biomaterials' physiochemical properties act as extracellular signals to influence cell metabolism to generate energy that regulates different transcription factors controlling gene expression, thus allowing BTE context-specific cell behavior. Many studies considered in this review involve early stages of linking cross-talks between the biomaterial properties and cell metabolism to regulate cell functioning and need more detailed studies to establish such a concept.

To take this further, advanced studies are needed to explain the fundamental mechanisms of how different properties of biomaterials provide varying signals to control different metabolic pathways to regulate cell functions. Advanced analytical instruments like Raman spectroscopy coupled with microscopy can provide a functional link of metabolism with cell function on biomaterial surfaces using deuterated metabolite molecules. Using this technique, one can image and detect deuterated molecules in cells, providing information on the bio-processing of deuterated molecules by different metabolic pathways to tune cell function. In addition, more research is needed to evaluate the metabolic response of cells on biomaterial surfaces using metabolomics analysis to identify specific molecules coupled to cell metabolism controlling

cell function. Studies also need to provide a complete picture of how biomaterials' physicochemical properties can influence specific cell metabolism to regulate transcription factors, which control specific gene expression allowing context-specific cell behavior. Finally, just studying metabolic activity using a water-soluble tetrazolium salt (WST or MMT) assay may not be sufficient to link cross-talks between biomaterial properties and cell metabolism to regulate cell function. More detailed scientific experimental design is needed to evaluate specific metabolites, metabolic pathways, and bioenergetics of cells controlling cell function. Overall, it is a new growing field, and there is much to do to establish the concept of cross-talks between the biomaterial properties and cell metabolism to regulate cell fate. Establishing such a scientific concept may help design new bioactive materials for specific needs depending on the bioenergetics of cells.

Author contributions

S. C., D. G. and P. T. compiled the literature survey and wrote the manuscript; S. C. discussed figures and literature examples with S. K.; S. C. and S. K. wrote and edited the manuscript.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

S. K. acknowledges the support from the Science & Engineering Research Board, the Startup Research Grant (SERB-SRG/2021/001886), ICMR (Adhoc-ID.2021-9106), and the IIT Delhi seed grant.

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