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Mechanotransducive Surfaces for Enhanced Cell Osteogenesis, a review.

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Abstract

Novel strategies employing mechano-transducing materials eliciting biological outcomes have recently emerged for controlling cellular behaviour. Targeted cellular responses are achieved by manipulating physical, chemical, or biochemical modification of material properties. Advances in techniques such as nanopatterning, chemical modification, biochemical molecule embedding, force-tuneable materials, and artificial extracellular matrices are helping understand cellular mechanotransduction. Collectively, these strategies manipulate cellular sensing and regulate signalling cascades including focal adhesions, YAP-TAZ transcription factors, and multiple osteogenic pathways. In this minireview, we are providing a summary of the influence that these materials, particularly titanium-based orthopaedic materials, have on cells. We also highlight recent complementary methodological developments including, but not limited to, the use of metabolomics for identification of active biomolecules that drive cellular differentiation.

Introduction

Repair of musculoskeletal defects, whether congenital, age-related, or caused by traumatic injury is still an unmet clinical challenge, with a globally aging and urbanising population.[1] Research is ongoing in the combined fields of biomaterials, biomechanics, cellular osteogenesis, and bone-implant osseointegration to address

the challenge presented in delivering restorative bone formation. Strategies employing bone marrow derived mesenchymal stromal cells (MSCs) combined with functional biomaterials promise to make significant and exciting advancements toward available clinical therapies.

Osseointegration is defined as the "direct structural and functional connection between living bone and the surface of the artificial implant".[2] Therefore, all bone implant materials are optimised to increase the bone-to-implant contact area for successful healing.[3] For example, metals such as titanium (Ti) and magnesium (Mg) are used for knee and hip replacement implants have been historically used.[4] Due to its superior durability and biocompatibility, Ti will be a particular focus of this review. Another category of materials used to replace or mimic bone and joints altogether following fracture, are made to resemble the natural bone or joint, and can be derived from metals, polymers, ceramics, and natural materials.[4] Cell adhesion and proliferation at the contact point is highly dependent on the properties of the implant including physical characteristics, stiffness, chemistry, functional groups, wettability, surface roughness and nanotopography.[2]

To create suitable materials for bone osseointegration and regeneration, conventional bulk materials can be enhanced by modifying their outermost layer, physically or chemically; or new materials and geometries can be constructed with more intricate characteristics from the ground up.[5] There is evidence that cells are sensitive to their environment and can respond to the shape of their surroundings.[6] The biocompatibility of the materials is of vital importance to temper immune response and guide away from foreign body reaction, and the properties of the outermost layer of an implant play a major role as the primary interfacing area for cellular interaction.[7] An overview of the most utilised materials and techniques to investigate bone regeneration is shown in Figure 1.

Here, we provide a summary of approaches to support and enhance cell osteogenesis through mechanotransduction.

Bone Microenvironment

Bone is a living tissue, and as such, requires a delicately balanced environment. Additionally, osteocytes and bone lining cells are present on the bone surface. Osteoblasts, osteoclasts inhabit the bone microenvironment and maintain skeletal

homeostasis by respectively forming and resorbing bone.[2] During skeletal remodelling and following fracture, osteoclasts resorb excess bone and eliminate microcracks, allowing osteoblasts to lay down the new bone matrix and potentially become entombed as bone-sensing osteocytes.[8] As cells embedded in the interconnected lacunocanalicular network, osteocytes detect biomechanical force in bone and translate it into biochemical response signal, coordinating local and global bone turnover.[9] Osteoblasts and osteoclasts originate from MSCs and haematopoietic stem cells respectively, which are recruited from reservoirs when necessary. The diversity and the components of the cellular bone microenvironment are shown in Figure 2.

Microenvironmental cues determine the multipotent MSCs to renew or differentiate towards osteoblast, chondrocyte, adipocyte, myoblast, fibroblast and marrow niche or other stromal cell lineages, and are therefore a focus of regenerative cell therapies of connective tissues.[10] Unique gene and protein expression profiles help characterise ontology and phenotype at each stage of MSC differentiation.[11] MSC precursor adhesion to the bone and implant surface promotes proliferation and subsequently these cells can differentiate to create new bone, in conjunction with the process of mechanotransduction (i.e. converting an external stimulus into biochemical signals).[12] Cells rearrange their cytoskeleton and attach to the surface using adherence proteins such as integrin dimers, an example being $\alpha_V\beta3$ integrin, the vitronectin receptor, which supports mechanotransduction and strengthens cytoskeleton-integrin interaction.[13] Matrix properties can activate cellular differentiation mechanisms such as the Wnt (Wingless integrated) pathway which determine cell feedforward and feedback response.[14] Transmitting cues of the cell-extrinsic matrix into cell-intrinsic signalling is crucial to eliciting the desired cellular response.

MSC-based therapeutic intervention represents an ideal source for skeletal regenerative medicine as cellular phenotype and differentiation into terminal lineages, such as functional osteoblasts, can be manipulated using mechanotransducive cues dictated by the cell microenvironment. Cell mechanotransduction can originate from the material surface and vary according to their physical, and chemical characteristics and composition.[12]

Surface roughness

Physical modification of materials to enhance cell response can be achieved by chemical agents to create surface roughness to maximise the contact area with bone tissue. Nanotopography, which is relatively easy to fabricate on titanium implant surfaces, can change overall surface properties of wettability, surface charge and nanotribology[13] plays a key role in bone to implant osseointegration as it can modulate cell response by altering the cell traction forces upon contact to promote cell elongation, differentiation, or maintaining stemness.[15] We refer to nanotopography as added features at the nanoscale size (from 1 to 100 nm) on the material surface. The change in the material surface topography and therefore the roughness, can be achieved using sand blasting followed by acid-etching (SLA),[16] or hydrothermal alkaline etching.[17] These techniques can create topographies by either forming projected textures or pit-like patterns.

The intricate surface designs and patterns at the nanoscale can modulate cellular interactions,[18] impacting cell adhesion,[19] differentiation,[20] proliferation,[21] and bactericidal.[22] For instance, Li et al. has demonstrated that nanotopographic features of titanium implants can stimulate osteogenic differentiation by triggering β-catenin nuclear translocation and autophagy, processes crucial to osteogenesis.[23] Hence, these nanotopographic modifications hold immense potential for enhancing osseointegration, thereby ensuring the long-term success of the implants. Additionally, a recent study also showed that identical nanotopography can be achieved on 2D and 3D printed titanium substrate using alkaline etching technique, maintaining their antibacterial performance and hMSC integration to the surface.[24]

While physical modification can be improved to increase osseointegration, active molecules as modifications on the material's surface chemistry with added layers to deliver growth factors or other osteoconductive materials (including extracellular matrix proteins[25] and thin polymeric coatings[26]) can be performed. These are used to increase the overall surface wettability and hydrophilicity, which in turn enhances cell adhesion and osteogenic differentiation.[27] Hydrophilicity favours cell adhesion and proliferation.[28]

Implant surface functionalisation

The implant surface can be functionalised by adding thin layers of osteoconductive materials. For instance, plasma spray has historically been used to deposit thin layers of hydroxyapatite and other calcium phosphates onto Ti metal surfaces,[29 30] which improves surface roughness in addition to supporting osteoconduction by stimulating extracellular signal-regulated kinase (ERK)1/2 and insulin-like growth factor 1 (IGF-1) pathways.[31] Furthermore, single-protein adsorption on materials to promote cellular adhesion has been achieved using fibronectin, osteonectin, laminin 322,[32] osteopontin,[33] or modified silk fibroin proteins,[34] alongside multi-layered systems to act in synergy with Ti topographies[27] to promote osteoblast differentiation using low dose delivery of growth factors, which is important since side effects of high dosage can lead to swelling and ectopic bone formation due to the initial inflammatory response from the body.[35] For instance, it has been demonstrated that plasma polymerised polyethylene acrylate (pPEA) can be deposited on the surface of polyimide implants[36] or titanium[27] followed by fibronectin and bone morphogenetic protein 2 (BMP-2). The pPEA coating can unfold the binding sites of fibronectin, increasing the availability of the growth factor binding site, and the integrin binding domain, $FNIII_{12–14}$ and $FNIII_{9–10}$, respectively, as opposed to its globular conformation[37]. The availability of these sites promoted enhanced activity of BMP-2 in an *in vivo* model[36]. Additionally, materials containing phosphate groups such as biopolymers have gained attention as they promote protein adsorption, cell adhesion, calcium binding, encourage osteogenesis and *in vivo* bone regeneration.[38]

Engineered biomaterials

In addition to using hard metals with roughness texture modification or coatings as a source to repair bone defects, built 3D structures made of metals and their incorporation as scaffolds are also being studied.[39,40] New technologies allow us to create more intricate structures that can better mimic bone architecture and complement existing technologies. For example, the creation of porous structures can be achieved by 3D printing, consisting of the creation of a three-dimensional

structure layer by layer using extrusion for soft materials, biopolymers, or, relevant for this review, selective laser melting and electron beam melting starting with a powder metal.[41]

Trabecular metal, often constituted of tantalum, has become a remarkable milestone in the realm of medical implants. The sponge-like structure of this metal mimics the structure of trabecular bone, hence offering an optimal environment for bone in-growth and contributing to the stability of implants.[42] Tantalum's excellent biocompatibility, high-volume porosity, and high frictional characteristics enhance the mechanical interlocking between the implant and surrounding bone, thus promoting osteointegration.[42]

Meanwhile, the advent of 3D printing has catalysed a transformative shift in the medical sector, more so in the manufacturing of patient-specific implants. The ability to utilise computerised tomography (CT) scans to create bespoke implants allows for an unmatched degree of personalisation and precision in implantology. With this technology, it is possible to create complex geometries that are custom fitted to each patient's unique anatomy, enhancing the comfort, fit, and functionality of the implants.[43,44]

Further development of 3D printing technology has unlocked possibilities for customising trabecular metal and titanium implants. For instance, Electron Beam Melting (EBM) can be employed to create 3D printed trabecular titanium, exhibiting enhanced osteoinductive and osteoconductive performances.[45] The interplay between 3D printing and controlled titanium nanotopographies allows for more tailored implants, by adjusting surface designs at nano-, micro-, and macro-scales, to best suit specific patient needs and to optimize clinical outcomes. These advancements showcase the promising future of implantology, wherein every implant is tailored not only to the anatomical needs of a patient but also to the biological responses at the cellular level.

Additionally, 3D printing has shown to aid in extracellular matrix (ECM) secretion by adhered MSCs and expression of the osteogenic ectoenzyme alkaline phosphatase.[2] Further, engineered honeycomb-like TiO_2 topography can tune

systemic immune macrophage polarisation towards the regenerative M2 phenotype, helping promote osteogenic differentiation and bone-implant osseointegration.[46]

Cell mechanotransduction

When considering materials, such as Ti and modified Ti, it is necessary to consider cell mechanotransduction. Typically, surface engineering approaches centre around modulating cell adhesion. This is particularly crucial considering osteoblasts need high degrees of cell adhesion to properly express their phenotype.[47] There are various mechanisms by which MSCs respond to their biophysical environment to transduce mechanical stimuli post-adhesion such as via actomyosin cytoskeleton, microtubule cytoskeleton and ion channels.[48]

The cellular adhesive response to surface topography, roughness, or functional layers can be evaluated by the length or contractility of actin filaments resultant from changes in cell adhesion, which guide cell adhesion using traction forces.[49] These focal adhesions (FA) are able to transduce mechanical stimuli to activate proteins and activate downstream signalling pathways.[50] FAs can be identified using immunofluorescence assays against focal adhesion kinase (FAK) and phosphorylated FAK, or through staining of adhesion-related proteins such as vinculin. The phosphorylation of FAK is regulated by force generation to activate the Rho family of GTPases.[51] Expression and activation (phosphorylation) of proteins such as myosin, that contract actin filaments, give an indication of cytoskeletal arrangement.[52] Alongside the cytoskeleton, the cell nucleus plays a key role in transducing mechanical cues to the whole cell through the actin cap.[53] High cellular and nuclear tension regulated by surface-cell adhesive properties can promote osteogenic differentiation.[54] For instance, lamin A/C are intermediate filament proteins in charge of determining nuclear size, shape, and cytosolic rigidity.[55] Found within the nuclear envelope, these proteins can be studied as sensors for nucleus-specific stability and mechanics, which have direct epigenetic consequences.[53] Osteoblasts continue to alter their own matrix, as increasing intrafibrillar mineralisation of collagen produces megapascal-level contractile stress within collagen fibres, biomechanically analogous to re-enforced concrete. [56] Thus, the multifaced intracellular response to environmental cues allows for bioengineering strategies for osteogenesis. Table 1 summarises several specific examples of surface design that influence mechanotransduction for osteogenesis.

Table 1. Nanotopographies that influence osteodifferentiation.

Strategy	Size	Cell type	Effect
Nanopits	24 ± 5 nm	Calvarial bone from newborn (2–4 days) Wistar rats	Increased osteopontin secretion, and ALP mineralisation.[57] They also could better sustain challenging loading conditions during initial bone healing.[58]
Nanopillars	15 nm height, 21 nm diameter, and positioned at 30 nm intervals	Human Bone Marrow Stromal Cells (BMSCs) and Human Bone Marrow Hematopoietic Cells (BMHCs)	Increased the presence of ALP, osteopontin, and mineralisation studies.[59]
Nanowires, nanoflakes, Nanonests	Nanowires (20–40 nm in diameter). Nanonest pores (~500 nm) nanoflakes 100–200 nm in length and ~13 nm in thickness, growing radially outward.	Mouse bone marrow mesenchymal stem cells	Significant increase of Runx2, osteocalcin, osterix. ROCK can modulate immunomodulatory response from BMSCs.[60]
Surface roughness	266.54 nm roughness	Murine preosteoblast cells (MC3T3-E1 cells)	Nanotopography activated the β-catenin pathway and promoted osteogenic differentiation in MC3T3-E1 cells.[61]
Nanowires	Spikes on the surface were 3 μm, and 20 nm in diameter	Human skeletal cells Stro+1	Significantly enhanced osteocalcin gene expression.[62]

Signalling in osteogenesis

Osteogenic signal transduction incorporates various intracellular signalling pathways (Figure 2). Cascade regulation and crosstalk amplify the complexity of osteogenic signals, incorporating much of the cellular machinery. Growth and stimulatory factors such as BMPs, fibroblast growth factors (FGFs), parathyroid hormone (PTH), toll-like receptors (TLRs) and Wnts present in the bone matrix microenvironment bind their cognate receptors and activate their respective effector proteins, small mothers against decapentaplegic (SMADs), mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), nuclear factor kappa B (NF-κB) and β-catenin, which induces kinase and transcription factor nuclear localisation. Osteogenic gene expression occurs in conjunction with the leading osteogenesis transcription factors Runt-related transcription factor 2 (RUNX2) and osterix (OSX). Differentiated osteoblasts engage in bone turnover by regulating, and being regulated by, osteoclasts (Figure 3).

The Wnt signalling pathway is central for controlling spatiotemporal skeletal patterning, development, and osteogenesis.[63] The combinatorial Wnt ligands binding to frizzled receptors[64] and further downstream specialisation allows three distinct Wnt pathways: the canonical β-catenin mediated and the non-canonical planar cell polarity (PCP) and Wnt/Ca^{2+} pathways.

Canonical signalling activates upon the binding of one of 19 Wnt ligands to one of 10 frizzled receptors and low-density lipoprotein receptor related protein (LRP5/6) co-receptors. This prevents downstream β-catenin destruction and allowing it to activate gene transcription alongside T cell factor/lymphoid enhancer factor family (TCF/LEF).

The non-canonical Wnt/PCP pathway activates with frizzled co-receptors receptor tyrosine kinase-orphan receptor (ROR), related to receptor tyrosine kinase (RYK) and Van Gogh-like protein (Vangl) to directly activate downstream directional/asymmetric actin polymerisation via Rho family of GTPases and Rho-associated protein kinase (ROCK), and simultaneously activates the central kinases, mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinases (JNK) and serine/threonine kinase Akt (AKT/PKB) that regulate gene transcription.

The Wnt/Ca^{2+} pathway recruits G proteins, activating phospholipase C (PLC) and protein kinase C (PKC) cascades that integrate with Ca^{2+}-mediated machinery, including downstream transcription factors NF-κB, cAMP response element-binding

protein (CREB) and nuclear factor activated T cells (NFAT), and antagonises canonical Wnt by inhibiting β-catenin and TCF/LEF.[65] Thus, in osteogenesis, the Wnt pathway pivotally incorporates anabolic growth factor and calcium-mediated signalling, and response to mechanical stimulation.

Cells are responsive to mechanical cues from the extracellular matrix, predominantly through bound integrin adhesion complexes. These complexes connect mechanical stimuli to intracellular pathways and affect downstream proteins implicated in signalling and structural roles,[66] such as, Yes-associated protein (YAP) and WW-domain-containing transcription regulator 1 (TAZ). While the translocation of YAP is associated with the Hippo pathway, it is demonstrated to also be activated by the non-canonical Wnt/PCP pathway, be directly translocated following low density lipoprotein receptor-related protein 6 (LRP6) stimulation, and simultaneously inhibit canonical Wnt by inducing secretion of Wnt ligand inhibitors.[65,67]

Examples of Wnt involvement in mechanotransduction include: the prototypically mechanosensitive ion channel Piezo1 activating the transcription factor complex of NFAT, YAP and β-catenin for bone formation;[68,69] integrin binding creating a positive feedback loop between FAK and β-catenin, enhancing Wnt pathway expression;[70] indeed, numerous studies have reported synergistic activation of a Wnt-integrin signalling axis upon biomechanical stimulation of skeletal cells, where integrin subunits α5, αv, β1 and β3 and Wnt ligands 3α, 5β and 10β predominate.[71, 70, 72]

Osteoblasts terminally differentiated into osteocytes become specialised mechanosensory cells of bone, utilising Wnt/β-catenin similar to osteoblasts for anabolism,[73] and primarily regulate bone mass through the expression of the Wnt antagonists sclerostin (SOST) and Dickkopf-related protein 1 (DKK1), in response to multiple intracellular mechanotransduction mechanisms.[74,75] Thus, the abundant interplay of Wnt crosstalk with other pathways of biomechanical force-sensing machinery underlies its importance.

The predisposition of osteocytes as bona fide mechanosensory cells, recently characterised by a pan-skeletal gene expression signature,[76] extends their role beyond being buried in the bone matrix to the entire organism. For example, osteocytes can independently trigger inflammatory osteolysis via activation of myeloid differentiation primary response 88 (MYD88), upstream of innate immune and Wnt signalling,[77] bypassing osteoblasts and directly delivering RANKL to osteoclasts, in response to bacterial infection.[78]

Recent studies show how osteocytes employ their mitochondria to regulate immunity against metastatic cancer cells,[79] as well as the development of transcortical blood vessels linking to circulation outside of bone.[80] Co-morbid conditions such as type 2 diabetes can affect osteocyte mechanosensitivity, which can be rescued by re-activating Ca^{2+} cycling via the Wnt-preferential ion pump sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2).[81,82] Conversely, excess production of SOST, the Wnt-antagonist, by osteocytes has recently been linked to impaired cognitive function in aging and in Alzheimer's disease.[83] Thus, the mechanisms that involve osteocyte mechanosensation are consequential to multiple body systems and overall health.

YAP, uniquely, plays a key role in mechanotransduction by triggering the cell plasticity response. The activation of YAP/TAZ coactivators has been shown to significantly increase osteogenesis. This was explored by transfecting adipose MSCs with BMP-2/VEGF, and measuring their osteogenic ability using alizarin red staining, RNA-seq, and rats as an animal model to measure transplanted cells into induced bone defects. YAP/TAZ activated cells yielded a significant increase in bone healing.[84] YAP nuclear translocation levels oscillated alongside Ca^{2+} fluctuations, after receiving a mechanical stimulus.[85] The YAP/TAZ duo can also bind to RUNX2 in the cell nucleus increasing osteogenesis.[85]

MSCs can be directed toward osteogenesis under tension via Rho A kinase (ROCK), a contraction-driving kinase that modulates actin filament elongation and actomyosin contraction (under isotonic and isometric tension respectively) until an equilibrium is reached between MSCs and the ECM.[86,87] ROCK and myosin shuttle YAP.[52] Conversely, simple active physical nanovibrational cues are sufficient in inducing metabolite-driven osteogenic differentiation pathways in precursor cells. Mechanical vibration has shown to upregulate ROCK signalling pathway.[88,89] Additionally, mechano-stimulation maintains a population of osteolectin-expressing MSCs in an osteogenic state and prevents their adipogenesis.[90]

Metabolomics as an identification tool

Cell interaction with biomaterials can be evaluated using several techniques. While standard techniques including protein quantification via immunostaining and western blotting have proven robust for targeted studies, the emergence of omics techniques

offers a molecular profiling approach to characterising cellular activity. Metabolomics typically utilises mass spectrometry or nuclear magnetic resonance (NMR) to analyse metabolites in cells, tissues and biofluids, that affect and reflect cellular function.[91] The metabolome of MSCs changes quickly in response to environmental cues, such as differentiation, thus allowing for derivation of real-time phenotypic information.[86] Various mechanotransduction pathways that are integrin-specific, bind and signal within milliseconds,[92] therefore, a rapid cell extraction methodology incorporating metabolomics may better facilitate their elucidation.[93]

Untargeted experimental approaches to understanding osteoblast metabolism at baselines or during the complete osteogenic differentiation process have simultaneously become valuable repositories and revealed novel metabolic phenomena, such as the identification of time-dependent osteoblast biomarker metabolites.[94]

Targeted metabolomics has been used to compare the differentiation of MSCs of various sources and varying osteogenic stimuli to primary osteoblasts, providing granular insights into every differentiation variable.[95] Technical capabilities continue to evolve, as untargeted metabolomics using NMR was coupled to mass spectrometry, with the aim to identify metabolites, including isomers, that might be missed with any single analytical technique.[96,97] Furthermore, analysis of the metabolic activity of amino acids can reveal molecules in protein synthesis; while metabolic energy can be linked to the carbohydrates, nucleotides and lipids, framing and allowing for multi-omics integration.[86, 98, 99.]

Recent work demonstrated how surface nanotopography regulates MSC ontology fate switching from self-renewal to differentiation via metabolic cues, identifying bioactive metabolites that regulate MSC growth through oxidative glycolysis, cellular cytoskeletal tension, and paracrine immunomodulation of T cells.[100] The work showed that changes in intracellular tension derived from alterations in cell adhesion led to a switch from oxidative glycolysis in the MSC state to increased use of oxidative phosphorylation in the osteoblast-differentiating state. Indeed, learning from materials, it was shown that metabolites such as adenine, niacinamide, glutamic acid and citrate regulate the MSC phenotype, while metabolites such as cholesterol sulphate regulate the osteoblast phenotype; by altering the tension and adhesion of the cells, thus helping us to understand mechanisms of mechanotransduction.[10089] A complementary study corelating metabolomics and

transcriptomics during MSC differentiation in varying ECM stiffnesses found that cells in stiffer environments exhibited standout upregulation of citric acid, mannose, and gluconic acid metabolism produced by the aerobic oxidation of glucose during osteogenesis alongside transcriptional enrichment of TNF and NF-κB pathways and specifically downregulation of the *WNT5B* gene.[101] This very signature, now associated with osteogenic differentiation,[102] re-emerged as fundamental to cells that were mechanotransduced via nanovibration with an increased activation of NF-κB and WNT post-translational palmitoylation imputed from metabolomics data, as well changes in lipids, and carbohydrate pathways.[89] Increasingly, generated metabolomics data can be analysed and classified by neural networks to aid understanding of the inherent complexity in the metabolome, and a data base could be created.[103–105] Thus, metabolomics has emerged with novel insights for osteogenesis and promises to be indispensable for future discoveries.

Conclusion

Osteogenesis of MSCs is a complex process involving a plethora of cues and stimuli. The environment outside cells, in the extracellular matrix, fundamentally determines cell fate and activity, and in turn, cell-intrinsic mechanisms then shape the very matrix itself. Enabling new bone matrix formation is essential and biomaterial surfaces play a critical role by regulating cell adhesion, proliferation and subsequently cell osteogenic differentiation.

While significant progress has been made in recent years, some key limitations require immediate attention. Many studies are carried out in 2D culture environments that do not fully consider the complex 3D microenvironments cells *in vivo*. There are also challenges in scaling up some nanotopography and surface modification techniques for clinical applications. Additionally, the long-term stability and impacts of some biochemical modifications need to be studied to assess durability, stability, and potential complications.

Future research in high-throughput materials screening, 3D-culture models, and modelling of dynamic cell-material interactions will be crucial in developing and optimizing the design of mechanotransductive surfaces. Emerging areas like 4D bioprinting utilizing shape-morphing scaffolds and incorporation of dynamic, force responsive elements could be explored. Importantly, a complete understanding of how physicochemical, biochemical, and microenvironmental cues are integrated by cells will guide the next generation of smart mechanotransducing biomaterials to unlock their full therapeutic potential for enhancing osteogenesis.

Successful osseointegration following injury, joint replacement, or implantation, typically using Ti based materials, can be achieved by combinatorial harnessing the advances in engineered biomaterial surfaces, stimulation of master regulators of cell signalling in osteogenic mechanotransduction and fine-tuning approaches such as metabolomic manipulation.

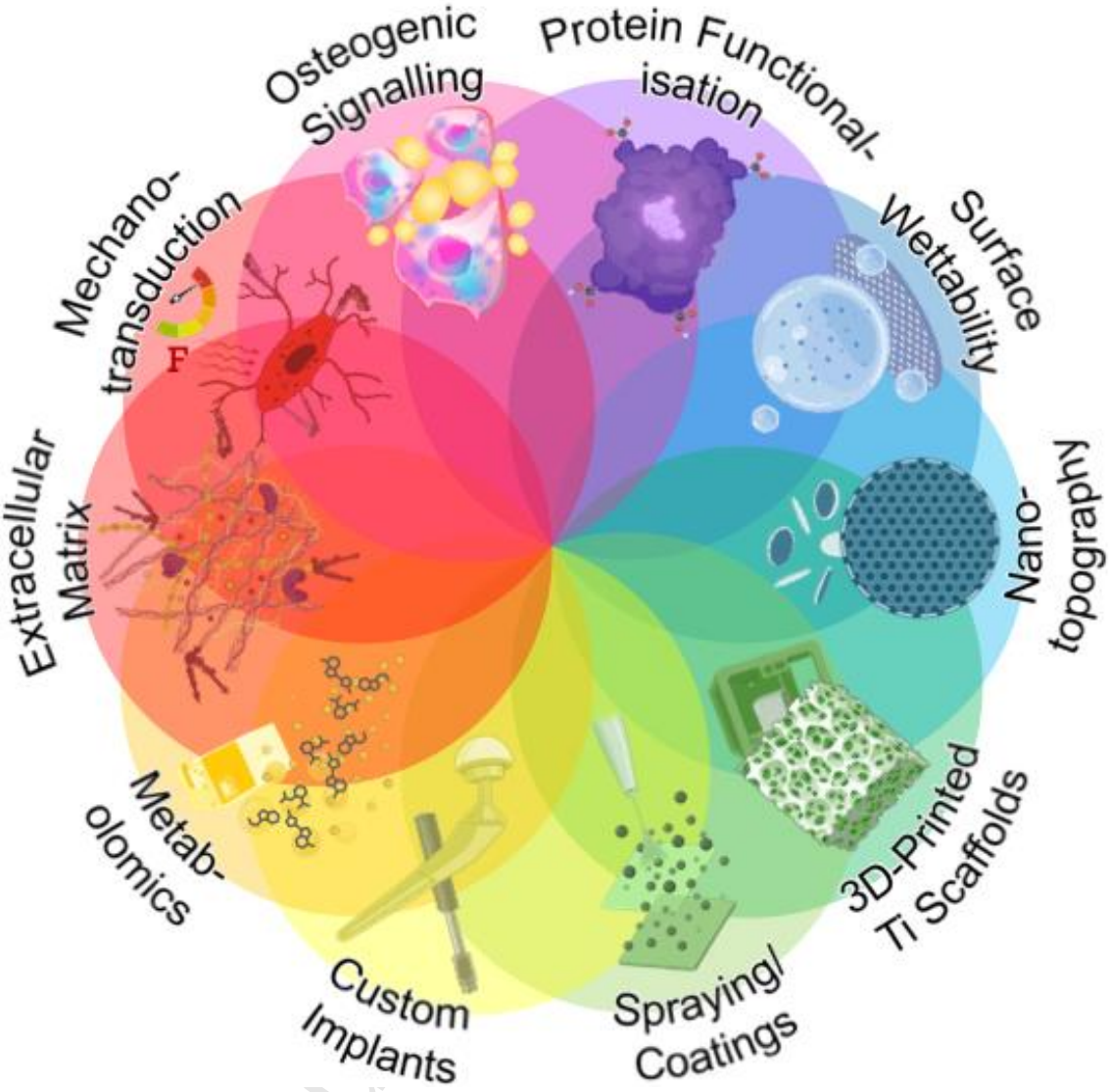

Figure 1. Technologies to investigate bone regeneration. Successful osteogenesis and osteointegration can employ any combination of various technologies and approaches, from a single molecule intervention to a bone-material implant. The major technological frontiers in the exponentially increasing combinatorial possibilities for therapy are depicted in the schematic.

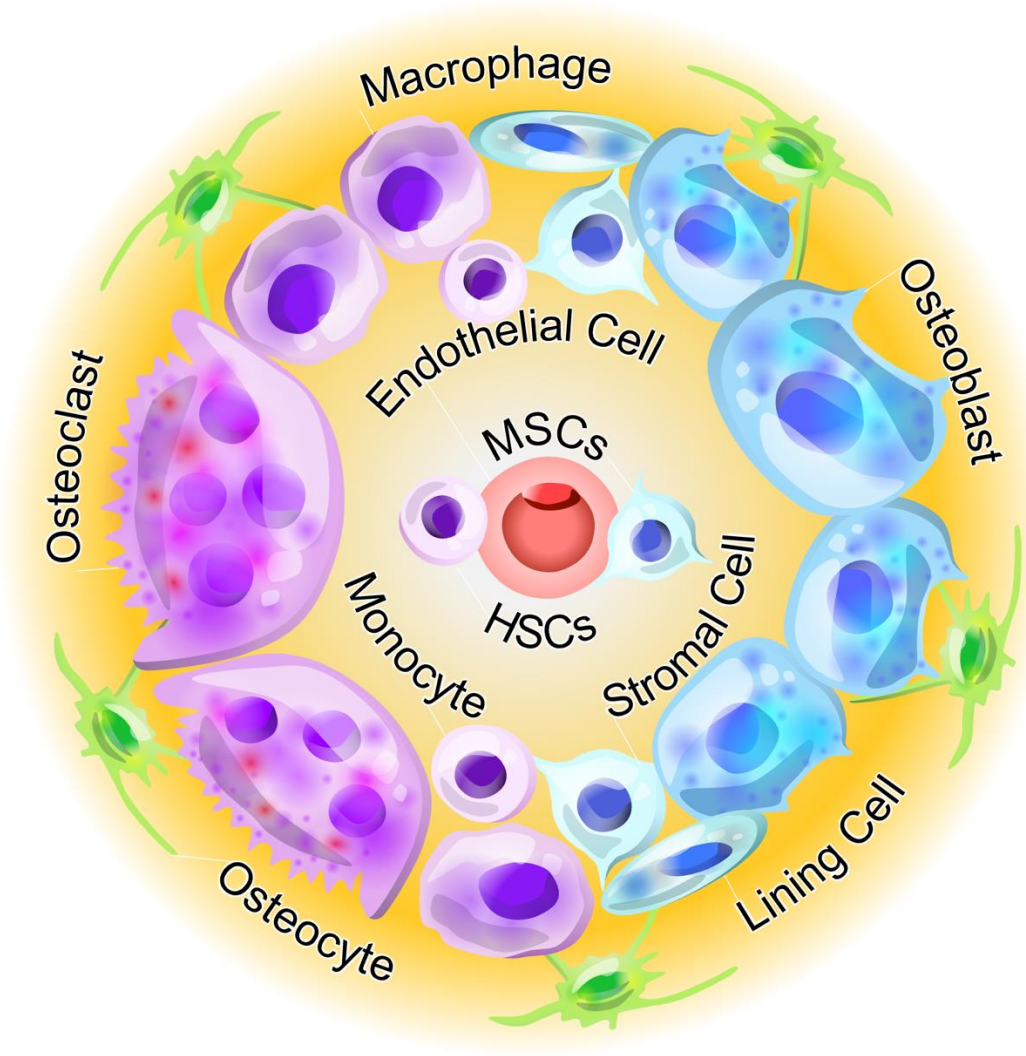

Figure 2 Cell Microenvironment. The bone cellular microenvironment is maintained by an equilibrium between the bone-forming **osteoblasts** (blue) and the bone-resorbing **osteoclasts** (green)**,** which maintain crosstalk through a plethora of molecular signals. Osteoblasts and other **stromal cells** derive from multipotent **mesenchymal stromal cells** (MSCs) and can further differentiate into bone **lining cells** (light blue) in areas of low activity or into embedded **osteocytes** (purple) that biomechanical force within bone matrix and signal distally via a network of canaliculi. **Osteoclasts** derive from **hematopoietic stem cells** (HSCs) that differentiate into circulating monocytes and further differentiate into **macrophage**-like cells, eventually fusing to form multinucleated functional osteoclasts. Porous tissue allows for vascularization by endothelial cells, allowing circulating cells in blood and immune cells, including HSCS, monocytes and MSCs to ingress into the microenvironment.

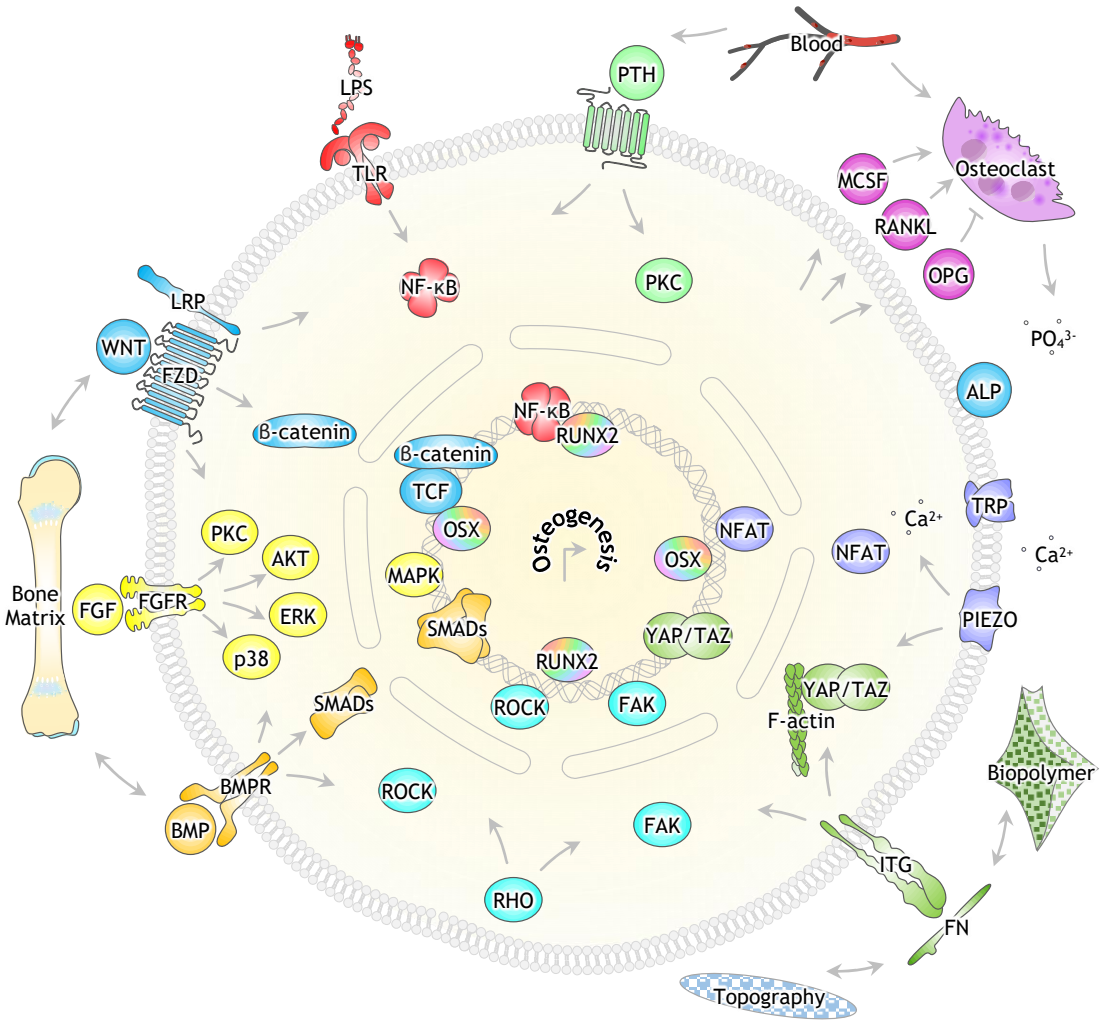

Figure 3. Pathways to osteogenesis. Prototypical extracellular signalling molecules include the transforming growth factor β (TGF-β) subfamily, bone morphogenetic protein (BMPs), which when bound to their specific receptors (BMPR), trigger phosphorylation of transducer SMADs.[106] Other growth factor families such as fibroblast growth factor (FGF) bind receptors (FGFR) to activate central cell kinases, including AKT, PKC (protein kinase C), p38, extracellular signal-regulated kinases, (ERK), and other mitogen-activated kinases (MAPKs).[107,108] WNT ligand binding to FZD (frizzled)/LRP (low-density lipoprotein receptor-related protein) receptor complexes leads to canonical β-catenin activation and transcription regulation alongside TCF/LEF (T cell factor/lymphoid enhancer factor family) transcription factors or non-canonical activation of nuclear factor kappa light-chain enhancer of activated B cells (NF-κB), PKC or RHO GTPases.[109]

Toll-like receptor (TLR) binding by ligands such as lipopolysaccharide (LPS) allow NF-κB dimer translocation to nucleus.[102,109] Circulating endocrine parathyroid hormone (PTH) binds its receptors to regulate osteogenesis through PKC and crosstalk with pathways such as NF-κB.[110] Ion channels such as transient receptor potential (TRPs) and PIEZO can regulate intracellular Ca^{2+} concentration in response to osmotic, thermal and mechanical perturbation, activating Ca^{2+}-dependent NFAT (nuclear factor of activated T-cells) and the Hippo pathway yes-associated protein/WW domain-containing transcription regulator protein 1 (YAP/TAZ) transcription factors.[111] Integrin dimer tethering to extracellular matrix components, including fibronectin (FN), or in combination with biopolymer or topographical interventions, induces YAP/TAZ and focal adhesion kinase (FAK) activation, linking the cytoskeleton to extrinsic forces.[112] The Rho family of GTPases including RHOA modulate cytoskeletal response to biomechanical force and typically activate ROCK and FAK.[113] Osteoclast differentiation and activity is coupled to osteoblast-secreted factors including, macrophage colony stimulating factor (MCSF), receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG),[114] while osteoblasts respond to osteoclast activity, for example by regulating the balance of phosphate metabolite availability with alkaline phosphatase (ALPL).[115] The master osteogenesis transcription factors RUNX2 (Runt-related transcription factor 2) and OSX (osterix) orchestrate osteoblast differentiation and activity in concert with the other transcription regulating signals.[116] Pathway complexity and crosstalk are simplified for visualisation.

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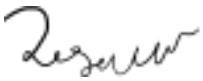
Sincerely,



Dr R Cuahtecontzi Delint

Highlights

Mesenchymal stromal cells response to mechanotransductive cues from materials.

The understanding of pathways activated by materials to drive osteodifferentiation.

Metabolomics as a tool to identify active molecules to induce osteodifferentiation