Stem Cell Reviews and Reports (2020) 16:1121–1138
https://doi.org/10.1007/s12015-020-10022-9

Reengineering Bone-Implant Interfaces for Improved Mechanotransduction and Clinical Outcomes

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Published online: 17 August 2020

Abstract

The number of patients undergoing joint replacement surgery has progressively increased worldwide due to world population ageing. In the Unites States, for example, the prevalence of hip and knee replacements has increased more than 6 and 10 times, respectively, since 1980. Despite advances in orthopaedic implant research, including the development of novel implantable biomaterials, failures are still observed due to inadequate biomechanical compliance at the bone-implant interface. This comprises static and dynamic mechanical mismatch between the bone and the implant surface. The importance and robustness of biomechanical cues for controlling osteogenic differentiation of mesenchymal stem cells (MSC) have been highlighted in recent studies. However, in the context of bone regenerative medicine, it remains elusive how mechanobiological signals controlling MSC osteogenic differentiation dynamics are modulated in their interaction with the bone and with implants. In this review, we highlight recent technological advances aiming to improve host bone-implant interactions based on the osteogenic and mechanoresponsive potential of MSC, in the context of joint replacement surgery. First, we discuss the extracellular and intracellular mechanical forces underlying proper receptivity and stimulation of physiological MSC differentiation and linked osteogenic activity. Second, we provide a critical overview on how this knowledge can be integrated towards the development of biomaterials for improved bone-implant interfaces. Third, we discuss cross-disciplinarily which contributes to the next generation design of novel pro-active orthopaedic implants and their implantation success.

Keywords Orthopaedic implants failures · Biomaterials surface design · Mechanobiology · Mesenchymal stem cells · Osteogenic differentiation

Conventional Therapeutic Approaches for Hip and Knee Replacements

Joint replacement surgery (hip and knee replacements) is considered the most effective intervention for treating severe osteoarthritis, reducing pain and disability and considerably recovery of patient's motor activity [1]. In 2014, and according to the estimates provided by the Organization for Economic Co-operation and Development (OECD) countries, an average of 189 hip and 130 knee joints were replaced by prostheses per 100.000 habitants [1]. In the United States, according to a projection from 2010 to 2030, the demand for primary Total Hip Arthroplasty (THA) and Total Knee Arthroplasty (TKA) is estimated to grow by 60% to 4 million and by 58% to 7.4 million procedures, respectively, by simply considering the population ageing [2].

Despite advances in bone implant research, prosthetic failure is often observed with time, leading to high-risk and high-cost revision surgeries and implant replacement [3–5]. Development of novel biomaterials and surgical techniques

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has improved the clinical outcomes, leading to enormous progress in patient care. Nevertheless, a number of unresolved problems still exist. For example, foreign body-associated infections are one of the most frequent reasons for implant failure [6]. It has been proposed that prompt and firm bacterial attachment combined with a poor host cell attachment can lead to implant-related infections (the "race for the space") [7]. However, "aseptic loosening" was also suggested as one of the most predominant causes for limited longevity of orthopaedic implants after 10–15 years [4, 5, 8, 9]. Over 25–75% of "aseptic loosening" cases were, in fact, due to non-diagnosed or negative tests of bacterial contamination [10–12]: there is often no sensitive testing of the implants for biofilms allowing quantitative and qualitative determination of the pathogens on the implant surface. It was reported that certain infections might never become clinically evident or may only result in a presumably aseptic loosening sooner or later [13, 14]. Therefore, data analysis from joint registries can lead to distorted results with limited reliability and the lack of information about true aseptic loosening. Nevertheless, even in this context, the share of really "aseptic" failures (where implant loses its contact with the surrounding bone) is still high.

In the orthopaedic clinics, two implant fixation techniques are currently used in both hip and knee implants: cemented and cementless. Despite the similar outcomes for both fixation methods in THA, most surgeons prefer cementless and, therefore, press-fit implants, in which a slightly oversized stem is placed into the femoral cavity [15, 16]. Cementless knee implants were not adopted as readily as hip implants but are now gaining acceptance. In fact, cementless TKA allow a better long-term biologic fixation than cemented implants [17–19]. In addition, the press-fitting strategy facilitates the implant insertion and reduces surgical time [17]. Hip and knee implants require remarkably different designs due to the distinctive spatial and biophysical constraints [20–22], as illustrated in Fig. 1. During locomotion, the femur's highest tensile strains were reported to be along the lateral and anterior side of the femur, whereas the highest compressive strains were along the medial and posterior surface [23–25]. As a consequence, tensile, compressive and shear stresses are present in different regions of an hip implant during locomotion, while a knee implant is mainly subjected to compressive stresses [20–22]. THA and TKA prostheses transfer different loads along the surrounding bone, leading to different bone formation/resorption percentages along the bone/implant length. In fact, significant bone resorption on hip implants is found in the lateral side of the proximal femur [26, 27], whereas on knee implants bone resorption is more significant underneath the tray on the tibial side [28]. As a result, the strain compliance becomes non-uniform along the bone-implant interface leading to weaker areas which mechanically would fail easier. Therefore, implants design should consider dynamics and temporal progress of the stress and strain distributions to avoid uncontrolled bone resorption. While changes in the geometry of the implant can address this non-uniform load distribution, previous studies

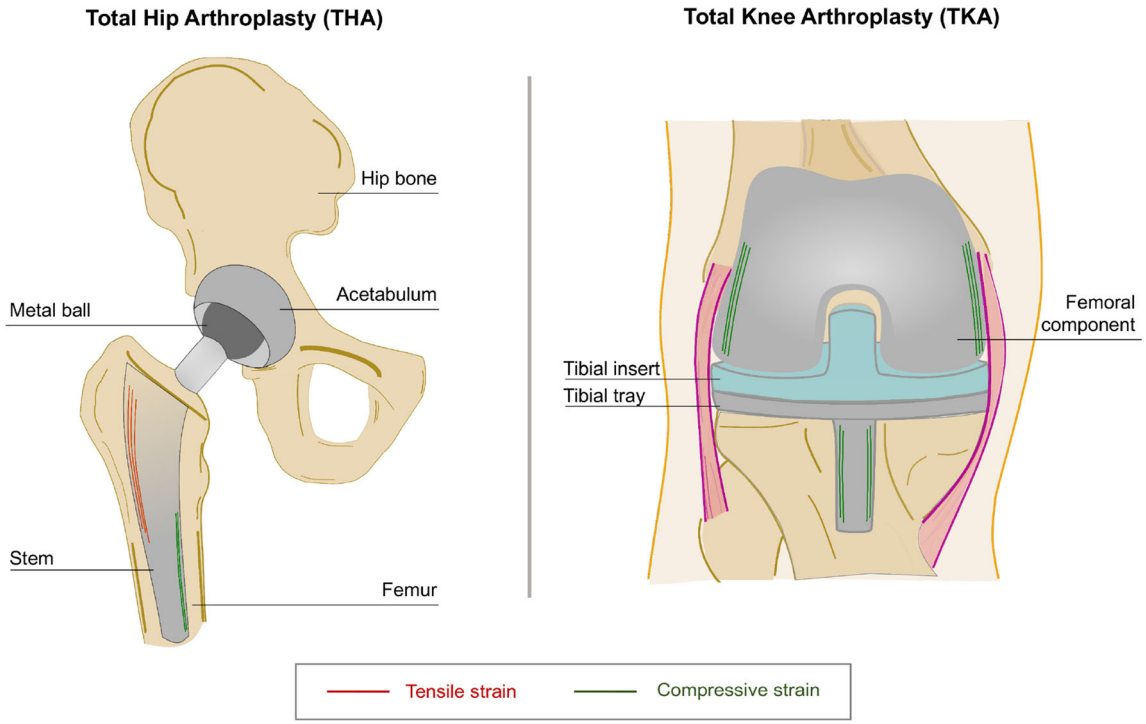

Fig. 1 Illustration of a total hip and knee arthroplasties on the left and right, respectively. Hip implants are composed by an acetabular, femoral head and femur components, whereas knee implants are comprised by femoral, tibial and patellar components (the latter not represented). During locomotion, the femur is subjected to tensile strains (represented in red) on the lateral and anterior sides and to compressive strains (represented in green) on the medial and posterior sides

regarding THA have shown that mismatched stiffness of these orthopaedic implants is one of the key parameters that influences the stability of strain compliance [29].

Here, we focus on combination of key mechanotransduction processes required for bone remodelling, proper biomechanical conditions, and, therefore, on critical aspects that should be considered to delay or even avoid the aseptic loosening events-related to revision surgeries.

Fundamental Aspects Underlying Bone Formation and Maintenance

Bone is a highly vascularized tissue with an intrinsic property of self-repair [30] and its regeneration in vivo is stimulated by mechanical loading related to gravity [31]. As a result of the mechanical usage, i.e., physical loads and motions imposed on the skeleton by normal physical activities, bone biological mechanisms are regulated by modulating the bone basic multicellular units [32, 33]. These units comprise osteoblasts (bone-forming cells), osteoclasts (bone-resorbing cells), bone lining cells (couple resorption to bone formation) and osteocytes (responsible for bone tissue maintenance) [32, 34, 35]. Various stem cell populations are present in the bone marrow, including very-small embryonic-like stem cells (proposed as the most primitive population), hematopoietic stem cells, endothelial progenitor cells and mesenchymal stem cells [36–38]. Pluripotent stem cells are able to differentiate not only into germline layers but also into three germ layers (meso-, ecto- and endoderm), whereas multipotent cells may only differentiate into two germ layers [36–38]. Mesenchymal stem cells (MSC) are present, for example, in bone marrow, periosteum and adipose tissue. Besides the ability of self-renewal, MSC are pluripotent cells with the capacity to differentiate into three tissue types: adipose, bone and cartilage and, consequently, currently used in bone regenerative therapies [39–41]. To avoid any confusions, according to the International Society of Cell and Gene Therapy, the term MSC is not equivalent to mesenchymal stromal cells, the latter corresponding to a heterogeneous population that supports hematopoietic development and presents notable secretory, immunomodulatory and homing properties. Bone marrow derived MSC play an important role in bone tissue regeneration, as represented in Fig. 2.

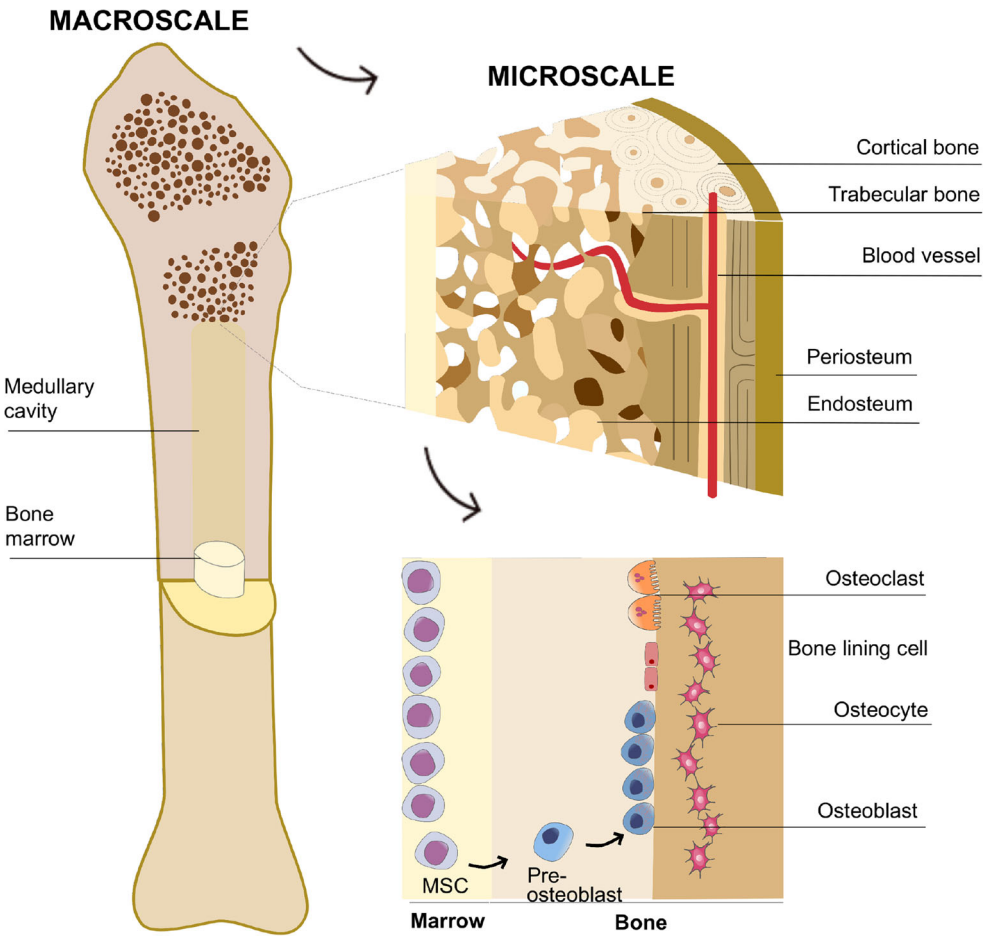

Fig. 2 Illustration of the bone structure at a macroscale, and the osteogenic commitment of mesenchymal stem cells (MSC) at a microscale. MSC reside in the bone marrow niches and differentiate into bone cells in the bone site under regeneration

Gravitational forces and muscle contractions result in small bone deformations which generate matrix strain and interstitial fluid flow within bone porosity [42]. While strain is applied directly through the bone cell attachments, fluid flow is sensed through the cell membrane and both cause cell deformation [42]. The balance between bone formation and resorption is tightly controlled, among others, by osteocytes, which sense mechanical strain and load generated factors (e.g. fluid flow and pressure) through a canalicular network [43, 44]. As a consequence, signal factors are released, regulating osteoclast and osteoblast activity [42]. Bone remodelling processes are modulated by mechanics, as the bone-marrow derived mesenchymal stem cells undergo osteoblastic differentiation in response to mechanical loading. Furthermore, the bone adaptation occurs only when dynamically loaded and thus, in response to cyclic, not static, loading [45, 46]. One extreme example that lack of proper mechanical loads results in bone loss is that astronauts, who live in a microgravity environment, experience a hip bone density loss up to 2% each month [47].

Biomechanical stimuli play a very important role in organisms' development, homeodynamics and homeostasis, and on the level of a cell, mechanical signalling is one of the three fundamental pathways (electrical, (bio)chemical and mechanical) for cell to communicate with its environment [48]. Here we may describe *mechanobiology*, *biomechanics* and *biomechanology* as three areas comprising studying of these phenomena. The first one describes combined synergetic effects of acting mechanical forces on the subcellular and the cell levels as they modulate morphological and structural features of the tissues especially like bone, cartilage, ligament and tendon [49, 50] but it also can have a critical influence on cell behaviour, even in tissues and organs that do not serve an apparent biomechanical role in the body [48, 51]. *Biomechanics* addresses mainly macroscopic aspects of performance of tissues and biomaterials under proper structural, functional and locomotory actions, and it usually involves simplified models of both materials and tissues [49, 52, 53]. *Biomechanology* is a recently introduced term [48] postulated as a discipline combining practically feasible, controllable and measurable biomechanical stimuli and parameters on the level of tissues and organs, which has closer clinical and physiological relevancy. In other words, biomechanology aims on design, characterization, analysis, quantification of biomaterials and tissues in a way to cover the most of physiological relevance, address unmet practical clinical needs and to be fitted with regulatory approval procedures: what are correct properties of a biomaterial or tissue, how they needed to be measured and how these data has to be used in a clinical practice?

Extracellular and Intracellular Mechanical Forces that Regulate MSC Differentiation and Linked Osteogenic Activity

Bone regeneration in situ comprises the MSC lineage commitment towards osteogenesis and recent studies have highlighted the role of external mechanical cues in controlling MSC differentiation [54–57]. Although it remains elusive the biochemical and mechanical principles regulating MSC differentiation in the bone vicinity during locomotion, MSC may be subject to indirect regulation through paracrine signalling from mature cells [58] or may sense the applied mechanical stimulus directly and only then start the differentiation process [59]. MSC, which reside in bone marrow niches near the bone surface, are exposed to multiple biophysical signals, including fluid flow induced shear stress, hydrostatic pressure, substrate strain, stiffness and topography [60]. The mechanical stimuli are not limited to externally-imposed forces but also include the intrinsic tensions generated by active cell contraction that occur in the absence of external forces [61]. In response to their microenvironment, MSC modulate their niche by generating intrinsic tensions by active cell contraction, or physical forces on the surrounding extracellular matrix (ECM) or neighbouring cells [61, 62].

MSC are highly mechanosensitive and able to adjust to the continuously changing dynamic mechanical environment. The process by which cells sense the mechanical properties of the ECM, including stress/strain, substrate rigidity, topology and adhesiveness is denominated cell mechanosensing [63]. The surrounding mechanical cues are sensed by cells via surface cell receptors (mechanoreceptor). Afterwards, the mechanical signal wave propagates inside the cytoplasm and when it arrives to the nucleus it is transduced into changes in the intracellular biochemistry and gene expression [42]. The adaptation of cells to different mechanical stimuli is governed by a four events chain [64]:

1 Mechanocoupling which corresponds to the conversion of the applied forces into a signal which acts directly on cells;
2 Mechanotransduction, that is the conversion of the mechanical cues into electrical, chemical or biochemical responses;
3 Transduction of the intracellular signals into final signals;
4 Cellular response.

The mechanoresponse involves the rapid and dynamic remodelling of the cytoskeleton in response to the local mechanical cues, such as variations of ECM stiffness or changes in cell shape [65, 66]. Cellular contractility and cytoskeletal tension modulate the fate of MSC [66–69] and an optimal contractility threshold triggers their osteogenic differentiation. Besides the important role of the cytoskeleton in mechanotransduction, given that forces act directly on the cell

membrane and that the applied force is transmitted to the cytoskeleton [64], mechanical-based signal propagation through the cytoskeleton is much faster than chemical diffusion- or translocation-based signal propagation [70, 71]. The force propagation from the ECM toward the cell interior and finally to the nucleus occurs in a form of a stress wave and depends on the stiffness differences along the cytoskeleton [71–73]. The rate of mechanotransduction (via stresses and strains) is yet overlooked method affecting cells and tissues. It was shown that the chemical transport able to trigger cellular response is substantially slower than a mechanical stress wave, giving about a million times faster response due to the cytoplasmic viscoelasticity [71].

Transmembrane integrins not only conduct information from the ECM to the cytoskeleton [74, 75] but are also responsible for transmitting cell-intrinsic forces to matrix proteins [61]. Integrins are organized into small adhesive structures in membrane protrusions, named nascent adhesions (NA), which either disassemble after a short lifespan or mature into larger and longer-lived structures, termed focal adhesions (FA). The formation of FA is dependent on myosin-II mediated cell contractility [75]. By clustering into FA, integrins recognize multiple ligands. For example, integrin receptors bind to ECM ligands such as RDG, which activates focal adhesion kinase (FAK), an important regulator of cell adhesion [61]. Integrins are able to communicate either with the ECM and with the cytoskeleton, contributing to outside-in and inside-out signalling, respectively [61, 74], as illustrated in Fig. 3. Stem cells are able to maintain their physiological functions when experiencing intracellular and extracellular stresses by inducing autophagy [41]. As a result, a number of organelles are recycled, such as mitochondria, important controllers of cellular quality. Moreover, mechanical homeostasis in stem cells is maintained by modifying FA ligand affinity, by regulating FA assembly/disassembly and by regulating the underlying cytoskeleton and actomyosin contractility [76]. In fact, integrins play an important role in the osteogenic differentiation of MSC, given that the activation of particular integrins enhanced the expression of osteogenic markers [77, 78]. As integrins are closely related to the cytoskeleton, although it is widely accepted that the cytoskeleton is one of the main structural components responsible for inducing a particular cellular behaviour [75, 79], its structural organization during MSC osteogenic differentiation in response to physical cues remains poorly characterized.

During chemically-induced MSC osteogenic differentiation, a decrease in cellular stiffness, size, and circularity were observed [80], given the changing of F-actin staining parameters (mean intensity, total intensity, and the number of F-actin branches). One group characterized quantitatively the actin cytoskeleton remodelling during osteogenic commitment and concluded that its structure and dynamics appear to regulate several mechanical parameters of MSC [54]. As shown in Fig. 4, undifferentiated MSC present a fibroblast-like shape whereas fully differentiated bone cells present a near spherical shape. Within 21 days of osteogenic induction, thick actin fibers in MSC were progressively replaced with a thinner actin meshwork. Furthermore, and in agreement to the literature

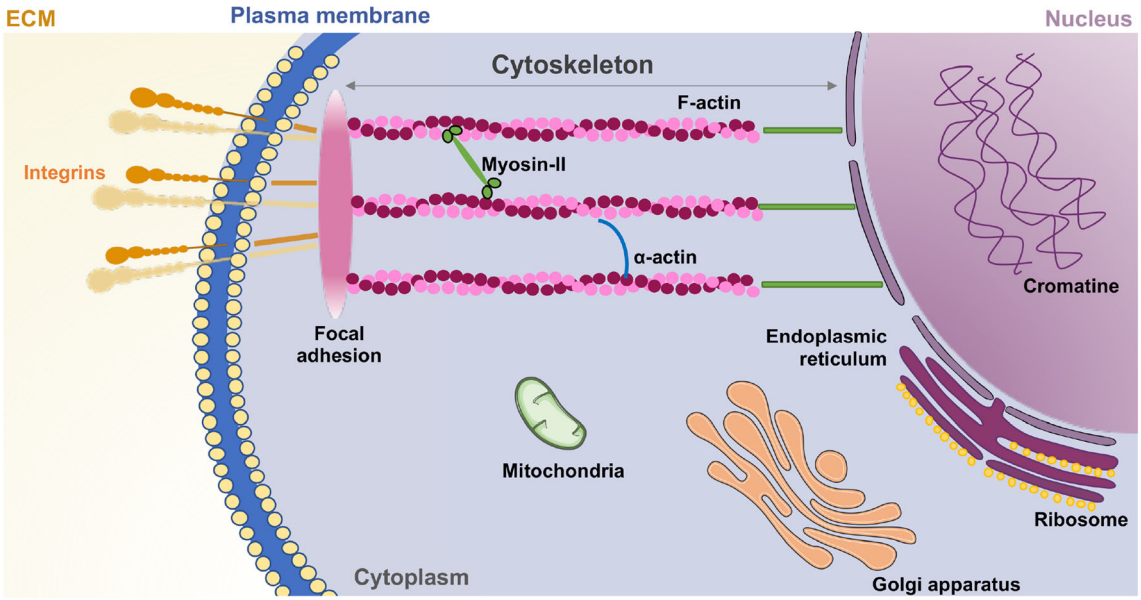

Fig. 3 Schematic representation of the main features governing the mechanotransduction system organization. Integrins are organized into larger structures named focal adhesions (FA), which formation is dependent on myosin-II mediated cell contractility. By clustering into FA, integrins recognized and bind to multiple extracellular matrix (ECM) ligands, therefore transmitting information from the ECM, to the cytoskeleton and to the nucleus, and vice versa. The mechanoresponse involves not only the rapid remodelling of the cytoskeleton but also the activation of specific genetic programs which involves different cell compartments, such as the ribosomes, Golgi apparatus and mitochondria

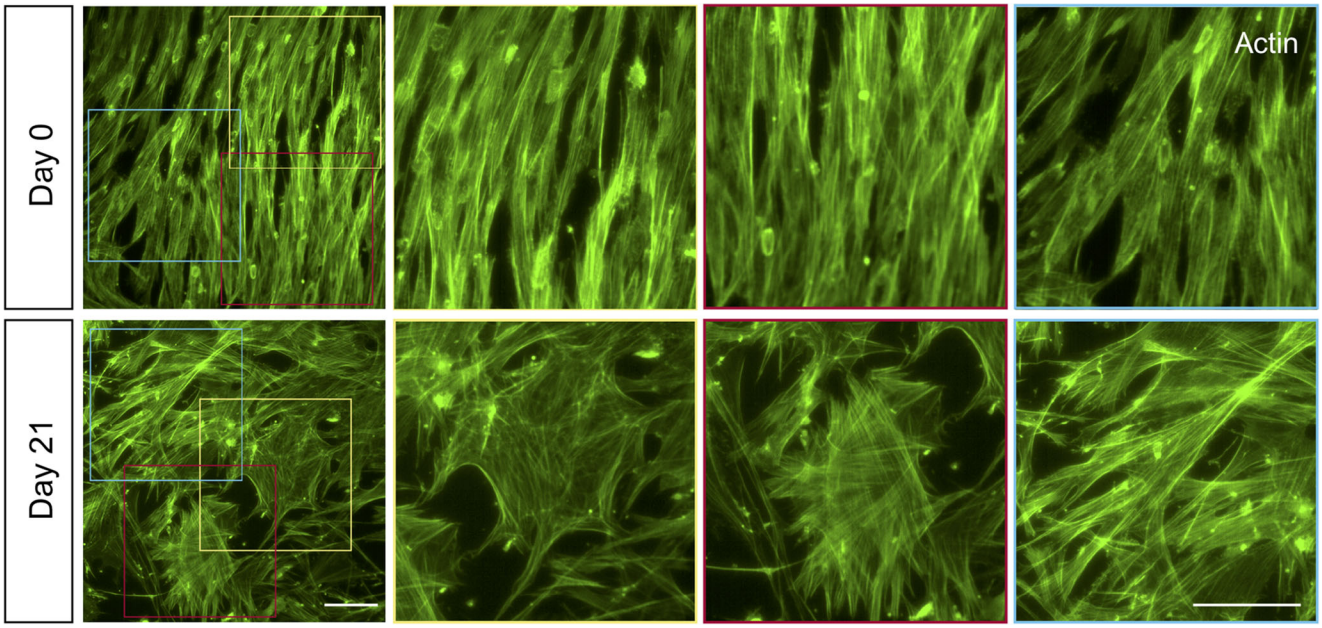

Fig. 4 Actin cytoskeleton rearrangement during MSC osteogenic differentiation. Bone marrow-derived MSC were seeded on glass coverslips coated with human fibronectin and cultured in a xeno-free osteogenic differentiation medium for 21 days, as described in [81, 82]. Human MSC (day 0) and fully differentiated bone cells (day 21) were fixed and stained with Alexa Fluor 488 Phalloidin. Scale bar: 100 μm

[54, 83, 84], MSC display long and parallel stress fibers while, during osteogenesis, these fibers acquire a crisscross pattern. The actin organization is a key mechanism for decreasing cell stiffness during osteogenic differentiation [54]. Moreover, the membrane-cytoskeleton interaction in MSC is mostly through focal complexes, often associated with the formation of thick stress fibers, whereas in mature osteoblasts the membrane–cytoskeleton crosslinking is also mediated by actin-binding proteins (e.g. ezrin, radixin and moesin) [54]. Altogether, these structural and mechanical changes lead to significant differences in cellular mechanics between MSC and osteoblasts. As suggested by Titushkin et al., 2007 [54], MSC are extremely sensitive to the environment and highly responsive to multiple mechanical cues due to a stiff cytoskeleton owing to the rapid propagation of the stress waves in prestressed inhomogeneous materials [71, 72]. On the other hand, osteoblasts are morphologically stable despite being constantly subjected to different stresses and strains.

Exploring Biomaterials with the Ability to Provide Optimal Niches for MSC to Robust Bone Repair

Among the diverse biophysical stimuli present in MSC niche, in vitro mechanobiological studies have contributed to ascertain which are the preferable signals for promoting physiological osteogenic differentiation and, therefore, regulating bone turnover [54–57]. Synthetic matrices are being used to create engineered systems that can generate different stress and strain conditions and thus manipulate the extracellular microenvironment of MSC [61, 85], as summarized in Table 1.

Due to the variety of factors selected in mechanobiological experiments, including the dimensionality of the model system (in vitro vs in vivo), mechanical stimuli (tensile, compression and shear), loading conditions (strain magnitude, frequency and duration), substrate materials and culture medium composition, the optimum conditions for controlling osteogenic lineage of MSC differentiation remain unspecified. Nevertheless, it can be concluded that tensile strain and fluid flow induced stress promote osteogenesis, but compressive loading can also be beneficial for bone formation. For low cellular contractility levels, stem cells retain their multipotency, whereas upon exposure to physiological fluid flow forces, for example, the intracellular tension in MSC was found to increase, inducing osteogenic differentiation [95]. One, however, must be careful drawing conclusions on biomechanical effects of fluid flow alone, often reported in literature [96]. First, fluid flow causing shear stress does not always lead to cell mechanostimulation, as pure shear stress does not lead to volumetric changes of the object, so fluid flow pressure is not at all equivalent to direct mechanical stimulation [48, 97]. Second, the shear stress cannot be measured so many assumptions are needed, which are not always reasonably justified. Third, changing the fluid flow not only changes the shear stress (many biological fluids are not Newtonian), but also changes amount of nutrients brought to the cells per time and unit area, and amount of their metabolic products to be removed [98, 99]. Forth, as there are many ways of expression of strain and stresses tensors, failure to

Table 1 Osteogenic results from mechanical loading on MSC

Mechanical loading	Strain	Conditions	Material	Osteogenic results	Ref
Cyclic stretching	2%, 8%	2 h, three times per day, for 3 days	Silicone either with or without DEX	Only the 2% elongation increased ALP and OC levels and upregulated Col I, III	[86]
	0%, 10%, 12%	1 Hz for 4 h/day for 7 or 14 days	Linear 3D Col I matrices	Osteogenic differentiation was significantly promoted at 12% strain in 14 days	[87]
	3%	0.25 Hz (continuously), for 16 days	Silicon rubber, pre-coated with Col I	Proliferation was inhibited whereas the matrix mineralization increased	[88]
Cyclic compression	0.22% (physiological), 0.88%, 1.1% (supra-physiological)	1 Hz, for 4 weeks	PCL-TCP	Higher levels of ALP activity, mineralization, and expression of ON, Col I and OC were obtained for 0.22% strain.	[89]
	5%	2 h every 5 days, for 10 days	3D PU scaffolds either with or without DEX	Expression of Col I mRNA and calcium deposition doubled comparing to the non-loaded constructs	[90]
Fluid shear stress	Unknown	1.2 Pa for 30 and 90 mins	Sandwich of a silicone gasket between the glass slide and an acrylic plate	Significant increase in ALP gene expression and a marked decrease in Col I	[91]
	Unknown	Maximum peak shear force at 0.051 Pa, starting from day 4 of culture, 1 h per day, 5 days per week, for 21 days	Gelatine-coated plastic	Upregulation of ALP, Col and calcium production	[92]
	Unknown	90 Pa for 24 h, 3 days after being in culture	PC and glass slide	ALP activity in osteogenic medium was significantly increased and at days 4 and 8 of culture the mRNA expression of BMP-2 and OP was significantly higher	[93]
	Unknown	Maximum mean surface stress of 0.013 Pa for 4 and 8 days	Porous foams and nonwoven f ibrous meshes of PLA	Dynamic culturing using flow perfusions enhanced growth and differentiation within the first 8 days	[94]

Polymers: polycarbonate (PC), polycaprolactone-β (PCL), poly-L-lactic acid (PLA), polyurethane (PU). Bioactive material: tricalcium phosphate (TCP). Chemotherapeutic agent: dexamethasone (DEX). Osteogenesis markers: alkaline phosphatase (ALP), bone morphogenetic protein-2 (BMP-2), collagen (Col), osteocalcin (OC), osteonectin (ON), osteopontin (OP)

describe rationale why that particular form has been selected and how it relates to physiological relevance does not allow further translation of these studies into practice.

The majority of the in vitro studies found in literature focused on linear elastic 2D polymeric substrates, which strain range is much different from those achieved by metallic orthopaedic implants (physiological strains ranging from 0.02–0.35%) and don't directly correlate with the microscale loading promoted on bone cells niche [61]. Although cyclic stress at lower/equal physiological frequencies resulted in osteogenic differentiation [87], the physiological frequencies during locomotion are around 1–3 Hz [89]. Despite many loading systems aim to only study the effect of a particular mechanical stimulus, depending on the scaffold material and architecture, secondary effects are expected in 3D cell-seeded tissue engineered constructs in vitro [100, 101].

Mechanically rigid matrices that mimic collagenous bone [66], 3D-dimensional microcarrier cell cultures [102], or specific texture patterns [103] were found to increase cytoskeletal tension and actomyosin contraction which induced the osteogenic differentiation of MSC without the need for exogenous stimuli. Both cell-cell and cell-substrate adhesions are important in stem cell maintenance, proliferation and differentiation [104]. Substrate topography (with micro- or nano-features) has direct effects on cell orientation and morphology and on cytoskeleton arrangements [105]. Some topographical surface features including the pattern shape and dimensional parameters as diameter, spacing, height and depth and spatial arrangement, schematized in Fig. 5, influence the cell response and behaviour [103]. The main cellular responses of human MSC cultured on different patterns are summarized in Table 2.

Engineered topographies should promote an optimal arrangement, number and size of FA, so that the osteogenic signalling mechanotransduction processes enhance osteoinduction, osteoconduction and finally, osteointegration [103]. As presented in Table 2, architectural signals, including cell shape and geometry, defined by the surface pattern, have a profound impact on cell behaviour [120, 121]. The osteogenic differentiation of human MSC is highly sensitive to the topographical feature diameter/ridge width when cultured on nanopits on ridges, respectively. When cultured on nanopillars and nanotubes, MSC osteogenic commitment is regulated by the feature height, shape and spatial arrangement. Surface nanoporosity was shown to have a direct impact on cell adhesion [113, 122]. Consequently, a strong interaction between the cytoskeleton and substrate contributes preferentially to osteogenic differentiation. In addition, nano-displaced topography significantly increases osteospecific differentiation, given that a degree of disorder facilitates FA formation, intracellular tension and subsequent cellular spreading [116, 123]. Despite the promising results found in literature, it is difficult to systematically compare the effects of different patterns and corresponding dimensional parameter. Moreover, in vivo results may be different from those reported in the in vitro studies. Therefore, till date, no optimal surface patterns have been specified for inducing osteogenic differentiation of MSC. MSC self-renewal, proliferation and differentiation depend not only on the substrate topography, but also on its stiffness. Experimental studies found that MSC differentiate towards different cell lineages in direct response to tissue mechanics [66, 124] due to variations in extracellular mechanical force exerted on the cultured cells [62]. Osteogenesis is favoured upon a more rigid [125], and highly adhesive substrates [126], as one study concluded that MSC cultured on elastically-tuneable polyacrylamide gels with bone-like (25–40 kPa), muscle-like (8–17 kPa) and brain-like (0.1–1 kPa) stiffness, resulted in osteogenic, myogenic and neuronal differentiation, respectively [66]. However, using the 'stiffness' value alone, without proper detail, is a great oversimplification for estimation of tissue formation. All tissue models have a great number of assumptions – even for tissues known to be highly non-linear and viscoelastic, often 'Young modulus' is addressed in the calculations to simplify interactions of implant surface geometry with the environment or tissue-implant interface development [127]. For example, the guidelines of National Physical Laboratory (UK) list nine methods of measurement and calculation of elastic modulus [128], all giving different outcomes, and it is not at all straightforward for an implant designer which method would be the most suitable. Actual mechanical stimuli for bone formation are implant-, surface- and geometry specific, so getting the primary stability does not yet guarantee subsequent failure due to differences in the pattern of bone formation. This has important implications to the design of bone fracture repair devices and engineered skeletal tissues [129].

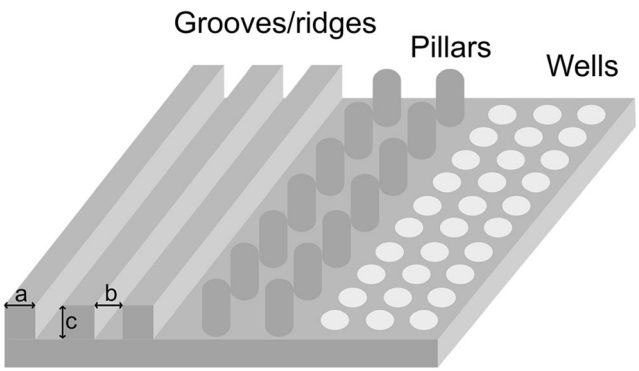

Fig. 5 Some typical topographical patterns include grooves/ridges, pillars and wells and the patterning parameters are: pillar diameter/ridge width (**a**), pillar interspacing/groove width (**b**), and pillar height/groove depth (**c**)

Table 2 Substrate topography effect on human MSC

Material	Pattern shape	Pattering dimensions				Parameter on study	Osteogenic results	Ref
		Aspect ratio	a (μm)	b (μm)	c (μm)			
PDMS stamps with pattern adhesive islands of octadecanethiolate	Rectangle	1:1; 3:2; 4:1				Aspect ratio	Yield of osteogenesis increased with aspect ratio	[68]
	Flower Star Pentagonal					Types of curvature (convex or concave edges)	The star shape was preferred for an osteogenic fate	
	Circular shape "Holly leaf"	2:1				Local curvature and aspect ratio	The "holly leaf" was the only that promoted osteogenic fate	
PI	Grooves and ridges		2–15	2–15		Ridges dimensions	Osteogenesis was enhanced on thinner ridges (2 μm) comparing to wider ridges. Gradual reduction of osteogenic differentiation with increasing ridge width, whereas groove width was less relevant	[106]
			5	2/15			Osteogenic differentiation was enhanced on 2 μm ridges	
SiO_2	Nanopillars		0.01/0.03	0.05–0.12	0.02–0.035	Pillar dimensions	Osteogenic differentiation was clearly favoured on high nanopillars (0.05 μm) of moderate distance (0.01 μm)	[107]
TiO_2	Nanopillars		0.02 0.03 0.04	0.04 0.07 0.105	0.015 0.055 0.09	Pillar heights	0.015 μm nanopillars demonstrated enrichment of RUNX2 and OC	[108]
TiO_2	Nanopillars		0.03 0.04 0.055	0.04 0.075 0.115	0.015 0.055 0.1	Pillar heights	OP and OC nodules decreased as height increased.	[109]
TiO_2	Nanopillars				0.008 0.015	Pillar heights	Both heights showed more OC relative to planar control, but enhanced size of OC deposits was reported on the highest pillars	[110]
TiO_2	Nanotubes		0.03 0.05 0.07 0.1	20		Tubes diameter	0.1 μm diameter tubes displayed the highest up regulation of ALP, OC and OP	[111]
Ti	Hemisphere-like topographic nanostructures		0..88 0.130 0.238	462	0.05 0.1 0.2	Nanoparticle size	Higher RUNX2 expression was found on flat and 0.2 μm compared with 0.05 μm surfaces; ALP activity significantly increased as the size increased, and calcium phosphate deposition was superior on 0.1 μm and 0.2 μm surfaces.	[112]
Ti	Nanopits		0.03 0.15 0.3			Nanopits diameter	OC was upregulated on nanopits with 30 and 0.15 μm diameter by greatest mineralization	[113]

Table 2 (continued)

Material	Pattern shape	Pattering dimensions				Parameter on study	Osteogenic results	Ref
		Aspect ratio	a (μm)	b (μm)	c (μm)			
							was found on 0.3μnm diameter nanostructures	
PDMS	Silicon hexagonanally micropost arrays		1.83	4	0.97 6.1 12.9	Rigidity	Osteogenic differentiation was promoted on rigid micropost arrays ($K = 1556\ nN\mu m^{-1}$), which corresponds to the lowest height	[114]
PMMA	Islands		2.2 1.7 0.144	4.3 2.9 0.184	0.045 0.033 0.01	Height	All nanotopographies stimulated the osteoprogenitor cell differentiation towards an osteoblastic phenotype. However, it was more evident for the 0.033 μm height islands which presented bone nodules	[115]
PMMA	Nanopits		0.120	0.3	0.1	Square array (SQ), hexagonal array (HEX), disordered square arrays with 20 nm (DSQ20) and 50 nm (DSQ50) displacement from their square position, and randomly positioned (RAND)	On the DSQ50 surfaces, MSC displayed areas of positive OC and OP staining and nodule formation	[116]
PC	Nanopits		0.120	0.3	0.1	Square (SQ) vs near square random displacement of 50 nm (NSQ50)	Runx2 was expressed significantly higher on NSQ50 compared to planar controls and SQ surfaces	[117]
PCL	Nanopits		0.120	0.3	0.1	Near square random displacements of up to ±50 nm (NSQ50)	NSQ50 induced higher levels of BMP2 mRNA expression and increased OPN and mineralization, compared to flat surface	[118]
PCL	Nanopits		0.120	0.3	0.1	Near square random displacements of up to ±50 nm (NSQ50)	RUNX2, OSX, ALP, OC and OP were up regulated in NSQ50 compared to planar control.	[119]
PDMS	Lines		2 1 0.25 0.25 0.46	1 2 0.25 0.25 0.07	0.08 0.12 0.25 0.11 0.040	Distinct topographies and dimensions	The first 'gratings' dimensions and wells exhibited the maximum difference in the cell spreading area. Between these, cells spread widely and generated higher contractility in the 'well' topography.	[95]
	Pillars		2 0.5	12 10	2 0.5			
	Wells		1	6.5	1			

Polymers: polydimethylsiloxane (PDMS), polyimide (PI), polymethylmethacrylate (PMMA). Osteogenesis markers: alkaline phosphatase (ALP), osteocalcin (OC), osteopontin (OP), osterix (OSX)

The Concept Idea for New Generation Design of Novel pro-Active Orthopaedic Implants

Despite advances in bone implant research, including the development of novel implantable biomaterials, the available orthopaedic implants do not elicit a proper mechanical stimulation at the bone-implant interface. In fact, the innovation rate in the orthopaedic industry has been declining, showing the importance of a clinical need-based strategy [130]. In this sense, the clear evidence that orthopaedic implants still fail, often due to aseptic loosening, creates new challenges and the development of new solutions. Here we have analysed the most relevant conditions for the orthopaedic implants which are in a way optimal for neo-bone formation, stability and endurance limits (as presented in literature [131–135], validated by in silico and in vivo studies), as represented in Table 3. These values might be considered as a guideline for implants, biomaterials and conditions of their design for proper orthopaedic applications. Note that these values are also depending on the number of loading cycles and history, as well as must be supported by other necessary conditions. For example, proper fluid supply, generated by the relative micromotions of the implant and bone - as related to mechano-regulative index [136, 137] is needed for cells proliferation and growth.

As mentioned, hip and knee implants failures are often related to aseptic loosening events. During locomotion, surrounding bone experiences different loads along the bone/implant length, leading to different bone resorption percentages. The awareness about non-uniform strain miscompliance along an implant is pushing forward the (re)design of new implants. Recently, auxetic cellular structures have gained substantial interest due to their unique properties given the negative Poisson's ratio, which corresponds to the ability of expand laterally when stretched [138]. Auxetic metabiomaterials offer a feasible route to design different parts of an implant with different strains as response to a given loading [139], privileging the most suited strains for a given area of hip and knee implants. Focusing, for example, on the hip implant, its femoral part is repeatedly loaded, predominantly, under axial compression [23, 140], creating tensile loading on the lateral side and compression on the medial side [141]. The side that experiences tension will retract from bone, causing a diminished mechanical stimulation at the bone-implant interface and an increased chance of wear particles entering the interface space, becoming more susceptible to failure. Although no animal models or clinical trials were conducted up to now, in theory, if the femoral part is able to create compression on both sides, the bone-implant contact is enhanced [139]. In this regard, if under physiological loading the implant is able to expand on the areas more prone to suffer bone resorption, the surrounding bone will be mechanically stimulated. This pro-active characteristic will promote an adequate stress/strain conditions at the bone-implant interface to assure a healthy bone formation. Furthermore, bone-implant interfaces may be improved by controlling some properties of the implant surface, including surface energy and topography. As some groups concluded, micro- and nano-topography may promote osteogenic differentiation of human MSC. Different surface patterns, including ridges, pillars, pits and wells, forced stem cells to change their shape, strongly influencing the stem cell fate. Combined effects of topography and mechanical stimulation were found to augment the osteogenic commitment of MSC. One group studied the effect of fluid shear stress on human MSC seeded on 1 μm wells and on 2 μm gratings [95]. Cells generated higher contractility onto the well topography, on contrary to the grating topography where MSC poorly spread and presented lower contractility. After an exposure of 48 h of continuous 1 Pa fluid flow induced shear stress, MSC exhibited increased contractility only on the well topography and increased number of FA, leading to their osteogenic differentiation. In this sense, in addition to the externally applied mechanical loading, physical cues may be employed to increase the cytoskeleton tension of MSC, promoting osteogenesis. Based on this, Fig. 6 illustrates a novel concept for discussion of a pro-active hip implant. The proposed design represents a one-for-all solution which should succeed in the majority of joint replacement cases, given the following features:

Table 3 Combined data on physiological conditions for bone performance

Conditions	Frequency (Hz)	Strain* (millistrain)	Strain rate (1/s)	Comments
Free walking	0.5–1.0	0.3–0.5	~0.001	
Brisk walking, jogging	1.2–1.8	0.6–1.0	~0.01	
Slow running	2–3	1.0 ~ 1.5	~0.03	
Bone yield limit	–	3.0 ~ 6.7	–	Depends on bone and loading conditions
Fracture	–	25 ~ 30 (static), ~10 (dynamic)	~1	
Trauma	–	>30	>1	

*here strain (1 millistrain = 0.001 x strain) means octahedral resolved strain, i.e. deviatoric value of principal true (logarithmic) strain values [136]

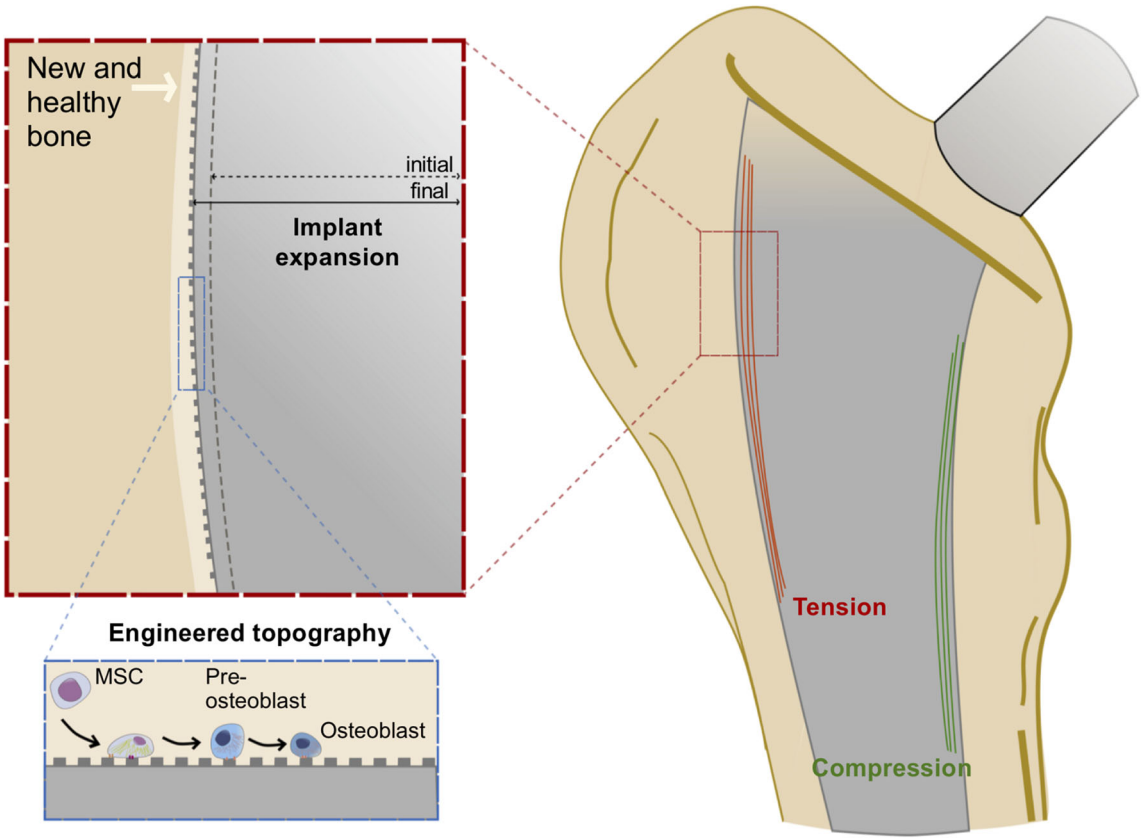

Fig. 6 Example of a novel pro-active hip implant combining an auxetic material and engineered topography. The optimized and improved design is for the purpose of osteointegration at the bone-implant interface, particularly at the lateral side of the proximal femur which, under physiological loading, experiences mostly tensile stress

- Adaptive surface to generate a proper mechano-regulative index;
- Use of an auxetic material to achieve a pro-active lateral expansion under physiological loading to stimulate the surrounding bone and promote healthy bone formation;
- Engineered topography to elicit proper cell adhesion, biomechanics and fluidics and therefore promote MSC osteogenic differentiation but also able to enhance the implant expansion effect;
- Feasible production and commercialization on a large scale

Altogether, it presents advantages, on average, superior to the existing ones and thus, could substitute the current orthopaedic solutions available in the market as it would extend the longevity of the orthopaedic implant and improve the patient's quality of life.

Given that the short- and long-term maintenance of the implant is assured if the host bone is mechanically stimulated to recruit MSC from the bone marrow and consequently induce their osteogenic differentiation, the study of MSC response to physical and mechanical cues will improve stem cell therapeutics in a biomaterial-based regenerative medicine [55]. Focusing on orthopaedic applications, future studies should be performed using metallic substrates and reproducing the conditions of a real implantation scenario. The know-how underlying the proposed design combined with the suggested studies will lead, in the future, to the development of a "patient-specific" solution. Customized orthopaedic prostheses would present an "adaptive" design, in which the proper implant expansion and surface pattern would be positioned and designed based on image acquisition techniques, e.g. computed tomography (CT) and magnetic resonance imaging (MRI) [142]. Customizing would consider, among other requirements, the patient-specific bone anatomy, mechanical properties and load pattern and, finally the biochemical complex [143]. Although in the current scenario it is only feasible to propose a one-for-all solution that could be large-scale produced, it is expected that in the future, the focus will be on the development of personalized medicine in orthopedy.

Conclusion

The study of external loading conditions and/or ECM physical cues has shown that MSC osteogenic commitment may be enhanced. Tensile strain and fluid flow are more prone to induce

osteogenesis however, the majority of the mechanobiology studies use polymeric substrates which strain range is much different from those achieved by metallic implants. In this sense, real stress/strain conditions in hip and knee implants should be reproduced in vitro in order to mimic the dynamic environment of MSC when in contact with these orthopaedic implants' interfaces. Moreover, micro- and nano-topographies have direct effects on cell morphology and cytoskeleton arrangements and thus, in regulating the stem cell fate.

Based on the osteogenic and mechanoresponsive potential of MSC, host bone-implant interactions may be improved in joint replacement surgery. Although this review focused on hip and knee implants, the conclusions presented here can be translated into any orthopaedic application, as this knowledge can be integrated towards the development of better biomaterials and bone-implant interfaces. Furthermore, the mechanical regulation of MSC by the identification of the most appropriate loading parameters combined with proper substrate topography will contribute to the next generation design of novel pro-active orthopaedic implants, to their implantation success and, in the future, to the development of personalized medicine in orthopedy.

Acknowledgements This work was supported by FCT (Fundação para a Ciência e Tecnologia) through the grant SFRH/BD/141056/2018 and the projects FCT/PTDC/EMSTEC/5422/20 and POCI-01-0145-FEDER-030498. Additionally, this work was supported by FEDER fund through the project reference NORTE-01-0247-FEDER-034074, by European Cooperation in Science and Technology through the project reference COST Action MP1301 and also Marie Sklodowska-Curie COFUND Programme 'NanoTRAINforGrowth', from the European Union's Seventh Framework Programme for research, technological development and demonstration.

Authors' Contributions Francisca Melo-Fonseca: Literature search; Data analysis; Writing – Original Draft. Georgina Miranda: Conceptualization; Writing - Review & Editing; Supervision. Helena S. Domingues: Writing - Review & Editing. Inês Mendes Pinto: Writing - Review & Editing. Michael Gasik: Writing - Review & Editing; Supervision. Filipe Samuel Silva: Conceptualization; Writing - Review & Editing; Supervision.

Compliance with Ethical Standards

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

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Highlights - Aseptic orthopaedic implants failures are still being observed, often due to improper static and dynamic biomechanical compliance at the bone-implant interface;- Osteointegration involves proper activity of MSC, which mechanosensitivity exploiting with the extracellular and intracellular biomechanical cues, responsible for modulating MSC osteogenic differentiation, remains poorly specified and implemented;- Biomaterials surface design improvements should be targeted on optimization to induce proper osteogenic lineage commitment of MSC;- This cross-disciplinary review aims to highlight impacts of the design for the next generation of pro-active orthopaedic implants.