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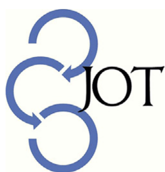

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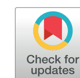
Review Article

How much do we know about the role of osteocytes in different phases of fracture healing? A systematic review



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ABSTRACT

Background: Although emerging studies have provided evidence that osteocytes are actively involved in fracture healing, there is a general lack of a detailed understanding of the mechanistic pathway, cellular events and expression of markers at different phases of healing.

Methods: This systematic review describes the role of osteocytes in fracture healing from early to late phase. Literature search was performed in PubMed and Embase. Original animal and clinical studies with available English full-text were included. Information was retrieved from the selected studies.

Results: A total of 23 articles were selected in this systematic review. Most of the studies investigated changes of various genes and proteins expression patterns related to osteocytes. Several studies have described a constant expression of osteocyte-specific marker genes throughout the fracture healing cascade followed by decline phase with the progress of healing, denoting the important physiological role of the osteocyte and the osteocyte lacuno-canalicular network in fracture healing. The reports of various markers suggested that osteocytes could trigger coordinated bone healing responses from cell death and expression of proinflammatory markers cyclooxygenase-2 and interleukin 6 at early phase of fracture healing. This is followed by the expression of growth factors bone morphogenetic protein-2 and cysteine-rich angiogenic inducer 61 that matched with the neo-angiogenesis, chondrogenesis and callus formation during the intermediate phase. Tightly controlled regulation of osteocyte-specific markers E11/Podoplanin (E11), dentin matrix protein 1 and sclerostin modulate and promote osteogenesis, mineralisation and remodelling across different phases of fracture healing. Stabilised fixation was associated with the finding of higher number of osteocytes with little detectable bone morphogenetic proteins expressions in osteocytes. Sclerostin-antibody treatment was found to result in improvement in bone mass, bone strength and mineralisation.

Conclusion: To further illustrate the function of osteocytes, additional longitudinal studies with appropriate clinically relevant model to study osteoporotic fractures are crucial. Future investigations on the morphological changes of osteocyte lacuno-canalicular network during healing, osteocyte-mediated signalling molecules in the transforming growth factor-beta-Smad3 pathway, perilacunar remodelling, type of fixation and putative biomarkers to monitor fracture healing are highly desirable to bridge the current gaps of knowledge.

The translational potential of this article: This systematic review provides an up-to-date chronological overview and highlights the osteocyte-regulated events at gene, protein, cellular and tissue levels throughout the fracture healing cascade, with the hope of informing and developing potential new therapeutic strategies that could improve the timing and quality of fracture healing in the future.

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Introduction

The epidemiology of traumatic and fragility fracture risk is rising with advancement of urbanisation and age globally. Fragility fractures exceed 2 million per annum incur an estimated total direct cost of nearly 25 billion dollars in the United States [1–3], 37.4 billion EUR in Europe [4] and 27.48 billion by 2020 USD on hip fracture care in China and rising to 581.97 billion 2050 [5].

Fracture healing is a complex physiological process that consists of different stages of inflammatory, reparative and remodelling [6]. Growing interest on the molecular biology and function of osteocytes suggests that osteocytes are involved in coordinating physiological processes in bone and other organs, including the kidney, heart and potentially muscle [7,8]. Osteocytes are the most abundant cell type in bone, where they reside in lacunae and communicate with one another via gap junctions through their extensive cell processes which extend through the bone matrix in canaliculi [9]. The osteocyte acts as a coordinator of bone metabolism and is in communication with bone lining at quiescent bone surfaces, cells of the marrow stroma and osteoblasts at the sites of new bone formation. Previous studies have also shown that osteocytes communicate with other cells through the expression of various signalling molecules, such as nitric oxide transporters, glutamate transporters and insulin-like growth factors in response to mechanical strain [10].

Fracture repair requires a multitude of cell types and their local expression of various growth factors and signalling molecules. Osteocytes are positioned within the bone matrix to sense both physical and biochemical signals that in turns regulate bone metabolism, regeneration and remodelling. Emerging evidence show that osteocyte and its secretory factors may play key regulatory roles in fracture repair. For example, osteocytes at fracture sites release osteopontin (OPN) for robust cellular recruitment of multiple cell types [11], periosteal callus cartilage formation and bone regeneration during fracture healing [12]. Although a considerable number of studies have started to discover the functional role of osteocytes, the underlying mechanism is still poorly understood.

As there is a lack of knowledge on the role of osteocytes in fracture healing, this review summarises the respective roles and functions of osteocytes at different phases of fracture healing. By acquiring a better and thorough understanding of the underlying mechanism of osteocytes in fracture healing, we can potentially target related upstream regulatory pathways and facilitate the development of novel therapeutic treatments that can be translated clinically in future studies.

Materials and methods

Search strategy

Literature search was performed on PubMed and Embase databases (last access was 25th October 2018), and the keywords included were osteocyte and fracture. The search strategy was "osteocyte* AND fracture*" and performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline [13].

Search criteria

Inclusion criteria were (1) preclinical or clinical studies that investigated the role of osteocyte in fracture healing, (2) studies that had reported healing as an outcome, (3) related to osteocyte morphology or function during fracture healing and (4) written in English with full-text available. Exclusion criteria were (1) non-English papers, (2) not osteocyte-related, (3) not fracture-related, (4) onset of disease and medical complication, (5) intervention applied with no control group, (6) drill holes models used, (7) microcracks, microdamage or diffused damage models, (8) review articles and (9) reports published as conference abstracts.

Selection of studies

Study selection was conducted by two reviewers independently and settled by discussion if there was discrepancy. Titles and abstracts were screened to exclude irrelevant papers. Full-text articles were then retrieved and assessed for eligibility based on inclusion and exclusion criteria [14].

Data extraction

The following information was extracted by reviewers [15]: methodology, fracture models used, species, interventions, histomorphometric results, gene and protein expression analysis, *in vivo* or *in vitro* studies, time points and all related assessments on osteocytes.

Data analysis

The included studies had a high discrepancy in terms of animal species, methodology, key findings and statistical methodology [15]. Therefore, it was not suitable to conduct a meta-analysis. A qualitative review was performed explicitly on the cellular changes, gene and protein expressions, signalling pathways and the effect of interventions in chronological order.

Results

Results of the search

A total of 704 and 1439 studies were identified from PubMed and Embase, respectively. Each title and abstract of the 2143 papers was reviewed; all duplicate entries and studies which failed to meet selection criteria were excluded. Seventy-one studies were identified for further assessment. Upon detailed review of each study in full text, an additional 48 were excluded because of inappropriate fracture models including drill holes defects, distraction, bone graft, onset of diseases and medical complications, e.g. diabetes, transplantation, cancer, osteogenesis imperfecta, osteonecrosis and conference reports. Our results included a total of 23 manuscripts for analysis [12,16–37]. The flow diagram in Fig. 1 summarises the selection process.

Characteristics of the papers

The 23 studies were published from 1996 to 2016 (Table 1). In total, there were 22 preclinical animal studies and one clinical study. Among the selected studies, 11 were in mice [12,16,18–22,24,26,27,31,35], 10 were in rats [17,20,23,25,29,30,32–34,36], 1 was in chicken [28] and 1 was in clinical patients [37].

Amongst all included studies, 22 performed a fracture, in which 14 studies adopted a closed fracture model [16,18,19,21,24–27,29,31–34,36], nine created an open fracture either with osteotomies, transverse fracture or defect [12,17,20,22,23,26,28,30,35].

Within the selected studies, the location of fracture site varied. Ten were conducted in the femur [16,20,23,25,27,30,32–34,36], seven in the tibia [18,19,21,24,26,31], two in the rib [12,35]and one in the mandible [17], radius [28] and ulna [22,29].

Fracture healing is an intricate process that comprises of cellular response, signalling pathways regulation and specific markers expression. Thus, we depicted the progressive changes of osteocytes and derived marker expression at the gene, protein and cellular levels during fracture healing. The potential effect of interventions on osteocytes, such as type of fracture fixation and sclerostin antibody are also illustrated in the following subsections.

Osteocyte survival after fracture

Three studies [17,19,35] evaluated the cell density by quantification of empty lacunae at different magnifications. Wichmann et al (closed

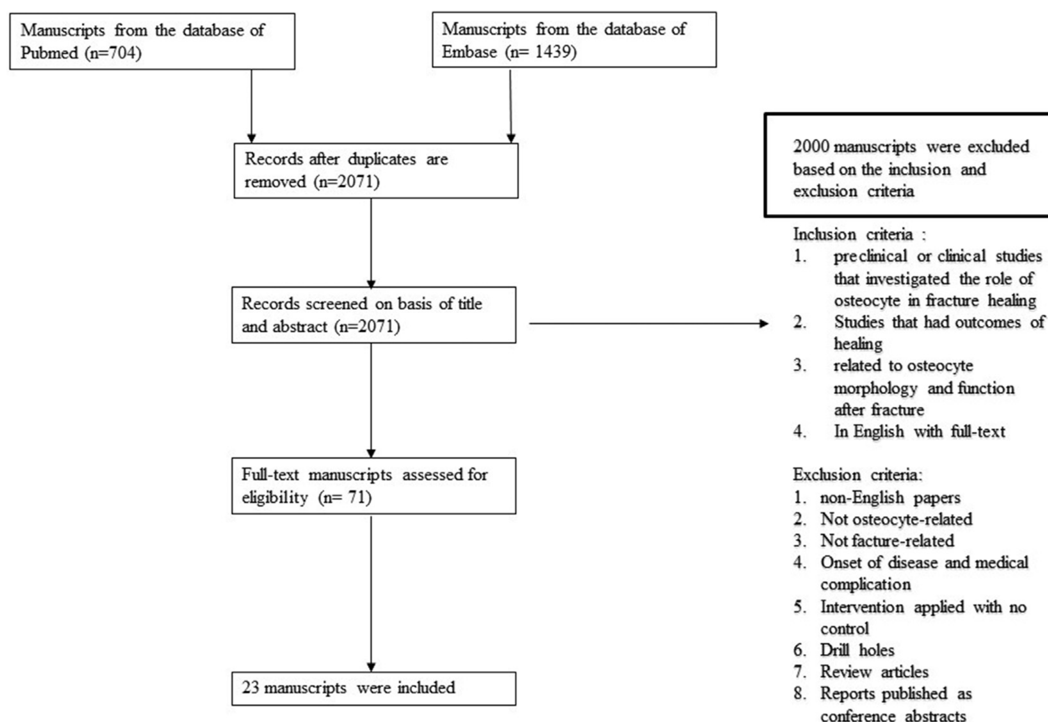

Figure 1. Flow diagram showing the process of literature search.

fracture, tibia, external fixation and mice] found osteocyte necrosis at 0.72 ± 0.04 mm adjacent to fracture site at 72 h postfracture at 100× magnification [19] while Liu et al (open fracture, rib, no fixation and mice) showed that the ratio of empty lacunae/lacunae occupied with osteocytes adjacent to the fracture site did not vary significantly from Day 2 to Day 4 and was followed by a decrease of the ratio at Day 7 [35]. Donneys et al (open, mandibular osteotomy, fixator and rat) observed a mean number of empty lacunae to be 2.35 ± 1.27 at 40 days postfracture at 16× magnification [17].

Three of the studies [17,23,31] evaluated the osteocyte number after fracture by histomorphometry. Dishowitz et al (closed fracture, tibia, intramedullary pin and mice) found that the osteocyte density normalised by bone area (Ocy/BA) was 1300/mm^2 and 1000/mm^2 at 10 and 20 days postfracture, respectively, at 200× magnification [31]. Donneys et al (open, mandibular osteotomy, fixator and rat) observed a mean number of 70.35 ± 10.78 osteocytes/high power field at 16× magnification in the fractured group compared with 29.76 ± 10.83 in the radiated fracture group. The cell number was increased to 78.92 ± 13.40 in radiated fracture with the addition of amifostine at 40 days postfracture [17]. Neagu et al (open fracture, femur, plates and screws/K-wire and rat) revealed a mean of 65 osteocytes/microscopic field (mf) after plate and screws fixation and a mean of 33 osteocytes/mf after K-wire fixation at 56 days postfracture [23].

Two of the included studies [28,29] showed osteocyte apoptosis at the acute phase, which were in close vicinity to the fracture. Clark et al (open fracture, radii, no fixation and chicken) demonstrated that

Table 1
Summary of the study characteristics.

Author	Animal species	Fracture type	Site of fracture	Fixation
Dishowitz et al., 2013	Mice	Closed	Tibia	Intramedullary pin
Kitaura et al., 2014	Mice	Open (osteotomy)	Tibia	Intramedullary pin
Lau et al., 2016	Mice	Closed	Tibia	Intramedullary pin
Li et al., 2004	Mice	Open (transverse)	Rib	No fixation
Liu et al., 2016	Mice	Open (transverse)	Rib	No fixation
Loiselle et al., 2013	Mice	Closed	Femur	Intramedullary pin
Lu et al., 2004	Mice	Closed	Tibia	Nonstabilised
Takamiya et al., 2008	Mice	Closed	Femur	Intramedullary pin
Toyosawa et al., 2004	Mice	Closed	Tibia	No fixation
Wichmann et al., 1996	Mice	Closed	Tibia	External splint
Yu et al., 2010	Mice	Closed	Tibia	External fixator
Alaee et al., 2013	Rat	Open	Femur	Plates and screws
Donneys et al., 2014	Rat	Open (osteotomy)	Mandible	Fixator
Hadjiargyrou et al., 2000	Rat	Closed	Femur	Kirschner wires
Hadjiargyrou et al., 2001	Rat	Closed	Femur	Intramedullary pin
Liu et al., 2016	Rat	Closed	Femur	Kirschner wires
Meyer et al., 2006	Rat	Closed	Femur	Intramedullary pin
Neagu et al., 2016	Rat	Open (osteotomy)	Femur	Plates and screws/Kirschner wires
Suen et al., 2015	Rat	Open (osteotomy)	Femur	Kirschner wires
Wu et al., 2014	Rat	Closed	Ulna	No fixation
Yao et al., 2013	Rat	Closed	Femur	Kirschner wires
Clark et al., 2005	Chicken	Open (osteotomy)	Radius	No fixation
Caetano-Lopes et al., 2011	Patient	Open	Hip	Hip replacement surgery

osteocyte apoptosis was significantly higher at 12, 24, 48 and 72 h in comparison to 0 h after osteotomy, with an increase of intracortical osteoclast counts (n/mm2) after 48 h compared with the control [28]. Furthermore, highest apoptotic osteocytes were found at site closest to the osteotomy (1 mm) as compared with distant regions at 2-mm and 4-mm. Caspase-3 is a member of the cysteine-aspartic acid protease (caspase) family that facilitates cell apoptosis. Wu et al (closed fracture, ulna, no fixation and rat) showed a dramatic increase in caspase-3 expression at gene and protein levels as compared with control without fracture. Caspase-3 gene expression was increased by 2.5-fold at 4 h after fracture and peaked at Day 14 postfracture, while the expression of its protein was localised in the osteocytes adjacent to the stressed fracture region from Day 1 to Day 7 [29].

In summary, the above seven out of the 23 studies have shown an immediate decrease in osteocyte after fracture as shown by cell count and expression of apoptosis marker followed by a gradual increase of osteocytes as healing progressed.

Regulatory role of osteocytes in chondrogenesis

The relationship between osteocytes and chondrogenesis were described in 10 out of 23 studies [12,18,21,23,24,26,27,32,33,35]. Eight of the studies showed that osteocyte-related protein markers were present in chondrocytes, osteoblasts and osteocytes, regulating the process of chondrogenesis indirectly to different extents. Lau et al (closed fracture, tibia, intramedullary pin and mice) showed that the deletion of osteocyte Igf-1 in knockout mice did not show detectable reduction in insulin-like growth factor (IGF-I) expression in chondrocytes within the fracture callus. However, there was a significant increase in bone morphogenetic protein-2 (BMP-2) immunostaining in osteoblasts and chondrocytes within the conditional knockout mice (cKO) fracture callus when compared with wildtype mice [18]. Toyosawa et al (closed fracture, tibia, no fixation and mice) found that although some dentin matrix protein (DMP-1)–positive cells were identified among the hypertrophic chondrocytes, these chondrocytes did not express DMP-1 mRNA or its protein [21]. Hadjiargyrou et al (closed fracture, femur, intramedullary pin and rat) showed that cysteine-rich angiogenic inducer 61 (CYR61) was present in proliferating chondrocytes in cartilage, active osteoblasts and osteocytes within new trabecular bone, as well as in the interface between bone and cartilage undergoing endochondral ossification and newly made osteoid matrix [32]. Hadjiargyrou et al also found that strong E11 expressions were seen in osteoblast and preosteocyte along the interface between bone and cartilage undergoing endochondral ossification at Day 14 postfracture [33]. Yu et al (closed fracture, tibia, external fixator and mice) observed stronger immunostaining for BMPs and BMP signalling components in chondrocytes relative to osteoblasts and osteocytes. During the soft and hard callus phases of repair, BMP3 and noggin were found in osteoblast and osteocytes, which were first confined to new bone adjacent to hypertrophic cartilage, while BMPs were found throughout the callus [24]. Furthermore, Liu et al (opened fracture, rib, no fixation and mice) revealed that FGF23-immunoreactivity was present in fibroblastic cells from the bone marrow and in the granulation tissues at 2 and 3 weeks postfracture, and the population of nonosteocytic FGF23-positive cells reduced gradually as endochondral ossification proceeded [35]. Lu et al (closed fracture, diaphyseal tibia, no fixation and mice) found that matrix extracellular phosphoglycoprotein (MEPE) expression was first detected in fibroblast-like cells within the callus by 6 days postfracture and expressed within late hypertrophic chondrocytes and osteocytes in the regenerating tissues at 10 and 14 days postfracture [26]. Loiselle et al (closed fracture, femur, intramedullary pin and mice) reported deletion of connexin 43 (Cx43) in mature osteoblasts and osteocytes in this model did not affect the progression of chondrogenesis, as a larger Alcian blue stained callus was observed in Cx43cKO fractures. Instead, healing was impaired by the delayed transition to a mineralised callus, shown by the persistence of Alcian blue staining in the cKO callus at 28 days [27].

Moreover, three studies showed that the viability of osteocytes correlated with the progression of osteogenesis or chondrogenesis [12,23,35]. Li et al (open fracture, rib, no fixation and mice] found a majority of dead osteocytes were seen beneath the newly formed cartilage, while intact osteocytes were present in the cortical bone adjacent to the regenerated woven bone [12]. Neagu et al (opened fracture, femur, plate or screws/K-wires and rat) demonstrated bone healing progressed by endochondral ossification in the group fixed by K-wires showed the presence of hyaline cartilage with a lower mean number of osteocytes when compared with the group fixed by plates and screws, where direct bone repair progressed with the absence of cartilage [23]. Liu et al (opened fracture, rib, no fixation and mice) showed that chondrogenesis was taking place near the cortical bones featured several empty lacunae, while empty lacunae were hardly seen in the ones undergoing osteogenesis with newly formed woven bone [35].

Thus, the above studies have demonstrated a spatial association between osteocyte density and expressed markers in regulating chondrogenesis and osteogenesis during healing, as shown by a mixture of new cartilage mixed with periosteal bony callus during healing.

Gene expression of osteocytes

Osteocyte-related gene expressions were examined in seven out of 23 studies [16,21,29,32–34,37]. Amongst these studies, gene expression of several inflammatory and growth factors, including interleukin 6 (IL-6), interleukin 10, cyclooxygenase-2 (COX-2), bone morphogenetic protein-2 and CYR61 were quantified from osteocytes. IL-6 encodes a proinflammatory cytokine that functions in inflammation and the maturation of B cells. Wu et al (closed fracture, ulna, no fixation and rat) found upregulation of IL-6 and COX-2 mRNA expressions in osteocytes within the intracortical area adjacent to the fracture region, while no positive signal was observed in the control group without fracture [29]. Caetano-Lopes et al (hip fracture, hip replacement surgery and patients) also showed IL-6 gene expression reached its peak at Day 1 and fell from Day 4 postfracture in bone specimen of fragility fracture patients [37]. Takamiya et al (closed fracture, intramedullary pin and mice) detected a moderate anti-inflammatory interleukin 10 mRNA expression in osteocytes, and the expression remained constant from 1 h to 10 days postfracture [16].

Two of the studies [18,37] examined the expression of BMP-2 responsible for bone and cartilage development. Caetano-Lopes et al (hip fracture, hip replacement surgery and patients) found peak expression at 3-day postfracture, followed with decreased expression until 8 or more days postfracture [37]. Lau et al (closed fracture, tibia, intramedullary pin and mice) found no significant change of BMP-2 mRNA expression between the intact and fractured tibia in wild type mice at Day 14 postfracture [18]. CYR61 is involved in angiogenesis, inflammation, apoptosis and extracellular matrix formation. Hadjiargyrou et al (closed fracture, femur, intramedullary pin and rat) found CYR61 mRNA was temporally increased in the fracture calluses with peak expression at Day 7 and Day 10 postfracture [32]. Generally, proinflammatory IL-6 and COX-2 were upregulated at the acute phase, while growth factor markers including BMP-2 and CYR61 were found from early to intermediate phase of healing.

The other eight studies [21,32–34] evaluated the expressions of different osteocyte-specific genes. Meyer et al (closed fracture, femur, intramedullary pin and rat) showed osteocyte-specific markers of Connexin 43 and U19893 Actinin alpha4 mRNA expressions were significantly upregulated at Day 3 postfracture [34]. E11/gp38 is a transmembrane glycoprotein expressed during the earliest stage of osteoblast-to-osteocyte transition. Hadjiargyrou et al (closed fracture, femur, intramedullary pin and rat) showed E11 mRNA expression was consistently upregulated during the early stage of repair when compared with that of the contralateral intact femur [33]. Peak expression was seen at Day 3, followed by a decline at 14 and 21 days postfracture. Three studies [18,29,37] investigated sclerostin (SOST) expression, which is known to inhibit bone formation secreted by osteocytes. Wu et al (closed

fracture, ulna, no fixation and rat) identified SOST mRNA was localised predominantly at the intracortical region near the fracture site [29]. Caetano-Lopes et al (open fracture, hip replacement surgery and patient) found a diminished mRNA expression of SOST at Day 1 postfracture in fragility fracture patients [37]. Lau et al (closed fracture, tibia, intramedullary pin and mice) found a slight increased SOST expression in the fractured callus at Day 14 postfracture [18].

DMP-1 is an extracellular matrix protein that is essential for mineralisation of bone and dentin. Toyosawa et al (closed fracture, tibia, no fixation and mice) observed DMP-1 mRNA expression was elevated until Day 14 postfracture, then fell at Day 21 and 28 [21]. In general, these osteocyte-specific genes for cell–cell communication and osteocyte processes formation (Connexin 43, U19893 Actinin alpha 4, E11/gp38 and DMP-1) were upregulated while SOST was downregulated specifically at early stages postfracture. The expression of SOST and DMP-1 was increased at the intermediate stage.

Temporal and spatial expression of protein in osteocytes

Nine studies [12,21,24,26,27,29,32,33,35] examined the proteins expressed by osteocytes during the healing cascade.

Wu et al (closed fracture, ulna, no fixation and rat) showed COX-2 protein was present in the intracortical bone area adjacent to the stressed fracture line, with its expression peak at Day 1 and decreased by Day 7 significantly as compared with unloaded control [29]. IL-6 protein was upregulated along the stressed fracture line in comparison to the control and the expression remained constant from Day 1 to Day 7 [29].

Hadjiargyrou et al (closed fracture, femur, intramedullary pin and rat) revealed that immature osteocytes expressed CYR61 in trabecular bone of the callus at postfracture Day 10 and Day 21. The expression of CYR61 ceased once these immature osteocytes were fully differentiated into mature osteocytes [32].

One of the studies reported immunoreactivities for BMPs were seen in osteocytes during the soft and hard callus phases. Yu et al (closed fracture, tibia, external fixator and mice) demonstrated several BMP proteins including noggin, BMP-2, BMP-3, BMP receptors and effectors pSmad 1/5/8 were expressed within the woven bone [24]. Among all BMPs, noggin and BMP-2 to 8, BMP receptors and effectors pSmad 1/5/8 were detected in osteocytes at Day 7. By Day 14, the immunoreactivity for BMP receptors and effectors pSmad 1/5/8 declined, and only BMP-2 and BMP-6 were detected [24]. The strongest intensity was shown during the early soft callus phase, followed by a decrease during the hard callus phase.

Hadjiargyrou et al (closed fracture, femur, intramedullary pin and rat) observed a strong immunoreactivity for E11 in the soft and hard callus from Day 5–14 postfracture [33]. At Day 5 postfracture, the expression of E11 was found in new trabecular bone during intramembranous ossification, without the presence of proteoglycan in the cartilage. At Day 7 postfracture, E11 expression was seen in mature osteocytes along the cortical bone adjacent to the fracture. At Day 10 and 14 postfracture, E11 immunoreactivity was detected in preosteocytes within areas of new woven bone and the interface between bone and cartilage during endochondral ossification.

Two [18,29] of the studies illustrated an alteration of sclerostin expression near the fracture sites. Wu et al (closed fracture, ulna, no fixation and rat] discovered that sclerostin protein was significantly reduced by 40% from Day 1–7 in osteocytes adjacent to the stressed fracture region, as compared with the control [29]. Both Wu et al and Lau et al (closed fracture, tibia, intramedullary pin and mice] reported that sclerostin expression increased and mostly were localised in the cortical bone as endochondral ossification proceeded at later stage from Week 2 to 4 postfracture [18,29].

Three of the studies [12,21,35] described the expression of DMP-1 protein was predominantly present in the cortical bone from Day 2 to Day 14 postfracture. Both Liu et al (opened fracture, rib, no fixation and mice) and Li et al (open fracture, rib, no fixation and mice) demonstrated DMP-1 positive signals were present in the osteocyte lacuna-canalicular network in cortical bone [12,35]. Liu et al showed the expression showed a slight increase from Day 2 to Day 7 postfracture, and Toyosawa et al (closed fracture, tibia, no fixation and mice) reported that DMP-1 protein expressions were elevated until 14 days postfracture when bony callus was formed but declined during the remodelling stage [21].

Cx 43 is involved in bone homeostasis and formation and was examined by Loiselle et al (closed fracture, femur, intramedullary pin and mice) [27]. Cx 43 was expressed in osteocytes at Day 21 postfracture and ceased at Day 35 postfracture [38]. MEPE expression was studied by Lu et al (closed fracture, diaphyseal tibia, no fixation and mice) [26]. Osteocytes expressed MEPE proteins within the newly regenerated bone at Day 10 and 14 postfracture during the hard callus phase, and the protein was then externalised in the osteocyte lacunae. Its expression remained vigorous during the remodelling phase at 28 days postfracture [26].

Generally, osteocytes expressed a diversity of proteins to regulate inflammation, angiogenesis, callus formation, bone formation and mineralisation differentially throughout the healing cascade (Table 2).

Osteocyte-related signalling pathways

There were three studies [18,22,27] investigating the signalling pathways of IGF-1, zinc-finger transcription factor Gli-1 and Cx43, and all of them were performed in mice model. Lau et al (closed fracture, tibia, intramedullary pin and mice) showed that conditional deletion of osteocyte-derived IGF-1 promoted the Wnt and BMP-2 signalling pathway and upregulated the mRNA expressions of osteoblastic markers including core-binding factor alpha 1, alkaline phosphatase (ALP), OPN and osteocalcin at the fracture site [18]. There were a 50% reduction in SOST, a 3-fold increase in BMP-2, and more than a one-fold increase of core-binding factor alpha 1, ALP, OPN and osteocalcin mRNA expression at 14 days postfracture compared with the wild-type control. The deficiency of IGF-1 in osteocytes was shown to enhance bony union of the fracture gap in the conditional knockout mice.

Gli-1 is a transcriptional activator that acts downstream of the Hedgehog signalling to promote osteoblast proliferation and repress its

Table 2
Summary of the function of proteins expressed in osteocytes during fracture healing.

Protein marker	Stage of healing	Function in fracture healing
IL-6	Early	Inflammation and the maturation of B cells
Cox-2	Early	Produce proinflammatory prostaglandins and promotes endochondral ossification
CYR61	Early-mid	Stimulate chondrogenesis and angiogenesis
E11	Early-mid	Promote osteogenesis, intramembranous and endochondral ossification
BMP-2	Early-mid	Promote revascularisation, neoangiogenesis, bone and cartilage development
Cx 43	Early-late	Regulate bone formation, homeostasis and gap junction intercellular communication
DMP-1	Early-mid	Promote mineralisation of matrix
Sclerostin	Early-mid	Decreased bone formation at site of resorption and promote endochondral ossification
MEPE	Late	Bone mineralisation and regeneration

BMP-2 = bone morphogenetic protein-2; Cox-2 = cyclooxygenase-2; CYR61 = cysteine-rich angiogenic inducer 61; DMP-1 = dentin matrix protein; IL-6 = interleukin 6; MEPE = matrix extracellular phosphoglycoprotein.

maturation toward osteocytes. Kitaura et al (open fracture, hind limb, intramedullary pin and mice) showed Gli-1 haploinsufficiency mice caused a decrease in bone mass with reduced bone formation and accelerated bone resorption. Moreover, Gli-1 ± precursors showed premature differentiation of osteocytes and increased osteoclastogenesis in cell cultures [22]. Impaired fracture healing with inadequate soft callus formation was also observed in Gli-1 ± mice as compared with wild-type mice.

Cx 43, the most common gap junction protein in bone, is crucial for osteoblastic differentiation and bone formation by intercellular gap junctional communication. Loiselle et al (closed fracture, femur, intramedullary pin and mice) found that osteoblast and osteocyte-specific deletion of Cx 43 resulted in impaired bone formation, bone remodelling and mechanical properties during fracture healing [27]. Cx 43 knock-out mice displayed a lowered tartrate resistant acid phosphatase-positive osteoclasts in the callus, decreased bone volume, total volume, mineralisation and a significant decrease in torsional rigidity between 21 and 35 days postfracture.

Therefore, modulating these osteocyte-related signalling pathways including Wnt, BMP-2, Hedgehog and intracellular Cx 43 could alter the coordination of osteoblast, enhance callus formation and eventually led to better healing outcomes.

Effect of fracture fixation on osteocyte

From the selected studies, seven studies adopted the classic Bonnarens & Einhorn closed fracture model fixed with intramedullary pin [16,18,22,27,31,33,34]. There were two studies fixed with external fixator [19,24], five studies with Kirschner wires (K-wire) [23,30,32,36], two studies with plates and screws [20,23] and seven without fixation [12,21,26,28,29,35,37].

The effect of fixation was investigated by two studies [23,24]. Neagu et al (opened fracture, femur, plate or screws/K-wires and rat) demonstrated a higher number of osteocytes in fractures fixed with plates and screws, as compared with those fixed with K-wires. There was a mean difference of 32 cells/microscopic field (mf) measured at Week 8 [23]. Yu et al (closed fracture, tibia, external fixator and mice] found the expression of BMP, responsible for skeletogenesis, varied with different fixations [24]. In fractures without fixations, all BMPs signals were detected in chondrocytes and osteoblasts/osteocytes, whereas no signal was detected in fractures with stabilised fixation. In stabilised fractures, BMP proteins, receptors and effectors were not detectable in osteoblasts or osteocytes within the new bone that was primarily healed via intramembranous ossification. Hence, stabilised fixation would result in a higher number of osteocytes with no detectable BMPs expressions in osteocytes during fracture healing.

Effect of sclerostin antibody intervention on osteocyte

Four of the studies [20,25,30,36] assessed the anabolic Wnt/beta-catenin pathway, and all of them had utilised sclerostin antibody as a postfracture therapeutic treatment. Monoclonal antibodies of sclerostin (Scl-Ab) could promote bone formation by antagonising the inhibitory action of sclerostin [39]. Three of the studies [20,25,30] adopted the same dosage (25 mg/kg/dose s.c. twice weekly), and the treatment was injected subcutaneously to the rats till Week 8, 9 and 12.

All of the studies demonstrated a significant increase in bone volume fraction (BV/TV) and mineralisation in the Scl-Ab treatment group. Alaee et al (open defect, femur, plate and screws and rat) found complete bony unions were achieved in five out of 30 defects at 12 weeks postfracture, demonstrating that Scl-Ab could enhance BV/TV in a defect model [20]. Consistently, Suen et al [open fracture, femur, K- wire and rat], Liu et al (closed fracture, femur, K-wires and rat) and Yao et al (closed fracture, femur, K-wire and rat) found the BV/TV were also significantly higher in the treatment group [25,30,36]. All of the four studies showed the treatment increased bone mineral density, with Suen et al and Yao et al exhibited an enhanced mineral apposition rate, mineralising surface and bone formation rate.

Both Suen et al and Liu et al reported that Scl-Ab improved bone strength postfracture as evaluated by mechanical testing. Suen et al showed the rats had an improved trabecular and cortical bone mass at Week 9, with a significantly higher stiffness, energy to failure and ultimate load [30], while Liu et al also demonstrated the maximum loading, energy to maximum load and stiffness were significantly higher in Scl-Ab-treated ovariectomised (OVX) rats than those in saline controls at Week 8 [25]. Of the included studies, two of them had evaluated the effect on Scl-Ab in an osteoporotic rat model. Both Yao et al and Liu et al found a significant enhancement on ALP expression, in serum [36] and bone marrow stem cells isolated from fractured bone in OVX rats by ALP positive colony forming unit assay [25], respectively, after treatment.

In summary, all the included studies had exhibited an enhancement in bone mass, mineralisation and strength after the administration of Scl-Ab and the importance of sclerostin in coordinating callus formation and mineralisation during the late phase in a rat model. Furthermore, the inhibitory action of sclerostin could enhance ALP expression in osteoporotic fracture model.

Discussion

In this systematic review, we have evaluated the changes of cell density, osteocyte-related gene expression, protein expression, signalling pathways and the effect of intervention in preclinical and clinical studies.

Osteocyte survival after fracture was illustrated by six [17,19,28,29,31,35] of the included studies, and only 1 study [23] evaluated the cell count at the late stage of healing. Although there was a trend of a gradual increase in cell density with healing, some data were not strictly comparable because of the different indexes and methodologies adopted for analysis. Moreover, cell density was mainly evaluated by different cell counting methodologies and the expression of apoptotic markers. However, apoptotic markers' expression reflects the cell density indirectly and might be hard to differentiate from those expressed in normal cell cycle arrest. Thus, a unified quantitative measurement, such as total number of empty osteocyte lacunae per bone area, and the percentage of empty lacunae per total lacunae might be a better approach to estimate the osteocyte density objectively in the future.

During the acute phase of fracture, osteocytes undergo apoptosis and promote the recruitment of osteoclast, which has been supported by various *in vitro* studies. Osteocyte-like cells MLO-Y4 released apoptotic bodies under mechanical stretch [40], secreting soluble tartrate resistant acid phosphatase and receptor activator of NF-KappaB ligand (RANKL) during unfavourable conditions [41,42]. As fracture disrupted the cell–cell communication and fluid shear stresses, these immediate local changes triggered the programmed cell death of osteocytes by expressing apoptotic marker such as caspase-3 at the acute phase [29], acting substantially for the recruitment of osteoclasts to the vicinity and initiating bone resorption and remodelling.

Fracture disrupts blood supply of bone and its surrounding tissues, leading to the acute inflammatory response. Haematoma is formed, and a variety of signalling molecules are released, including cytokines and growth factors for revascularisation and neo-angiogenesis at tissue level. Osteocytes provide the required genes, proteins [29,37] and growth factors [18,24,32,37] to facilitate the inflammatory phase at molecular level. Because new vasculature is required for both bone formation and repair [43], the angiogenic properties of these osteocyte factors not only mediate the inflammatory stage of healing but also help to preserve the viability of osteocytes in presence of the vascular canals [44]. Furthermore, both the gene and protein expressions of sclerostin were found to be downregulated immediately after fracture [18,29,37] but restored with healing [18,29]. This regulatory mechanism prevents local bone deposition in the vicinity of bone resorption at the early stage, facilitates endochondral ossification by preserving cartilage [45] and signifies the maturation of osteocytes and remodelling phase at the later stage.

Another inhibitor dickkopf 1 (Dkk 1) which acts synergistically with sclerostin competes on the binding with various Wnt ligands to the LDL Receptor-Related Protein 5 (LRP5)/6-Frizzled co-receptors and promotes bone formation [46]. Therefore, it is of great interest to investigate the regulation of Dkk 1, as well as the synergistic interaction between sclerostin and Dkk 1 during fracture healing.

At the intermediate phase of healing, callus develops during the repair stage. Osteocytes regulate the process by cell death and expression of BMPs and other osteocytic markers such as CYR61 that promotes soft callus formation and regulates chondrogenesis. Previous studies have demonstrated that the coordinated downregulation of Wnt and BMP2 signalling would permit for an accurate and balanced regulation of the recruitment and differentiation of mesenchymal stem cells (MSCs) [18,47,48]. Wnt signalling favours osteogenesis and tends to inhibit chondrogenesis [49,50], while BMP signalling appears to initiate chondrogenesis. Strikingly, both pathways are regulated by osteocyte-secreted marker sclerostin. During early healing phases, the decrease in blood supply because of the disturbance of vascular network is correlated with hypoxia at the fracture site that leads to osteocyte cell death. Two of the studies have consistently observed empty lacunae at close vicinity of fracture site triggered initial chondrogenesis while the distant region promotes osteogenesis [12,23,35]. Such regulation would determine the differentiation of mesenchymal cells into chondrocytes for chondrocytic differentiation that led to an optimal endochondral bone formation with a functional cartilaginous callus [51]. The periosteum underlaid by the cortical bone with dead osteocytes and its associated proteins that might trigger the differentiation into a chondrogenic lineage because of the lack of binding competition of BMP signalling [12]. On the contrary, as healing progressed, the reduced osteocyte-derived IGF-I [52] would drastically downregulate the local expression of SOST [34], therefore upregulating the canonical Wnt signalling for osteogenesis. At the later phases of the healing, upregulated sclerostin might be vital to coordinate periosteal cells to inhibit chondrogenic differentiation by disrupting BMP signalling through competitive binding with the type I and type II BMP receptors [53].

Osteocyte-specific E11 and Cx 43 are upregulated for the maintenance and reconstruction lacuno-canalicular network (LCN) disrupted by fracture. Three studies [33,34,38] showed all of these genes were expressed in the osteocytes at the early stage, and the expressions were maintained till the intermediate to late phase postfracture. Osteocytes can detect changes in loading stresses or strains, and the ion channels and integrin receptors are essential for the transduction of the mechanical signals into biochemical signals [54]. Shear stress induced by fracture could trigger the translocation of Cx 43 to the membrane surface and the release of signalling molecules such as prostaglandin (PGE2), one of the key osteocytic mediators for load-induced bone formation secreted in response to mechanical strain. PGE2 was subsequently expressed after the secretion of mechanically activated COX-2 [55], thereby stimulating Cx 43 expression and the functional gap junctions between osteocytes [56]. Moreover, recent studies have shown that Cx 43 deletion in cultures would increase apoptosis and affect gene expressions of osteocytes [57]. Hence, osteocyte-specific marker genes E11 and Cx 43 expressions were up-regulated for the intercellular communication between osteocytes, modulation of osteoblastic cell signalling [58] and osteocyte survival during the healing cascade and would decline once the LCN between osteocytes were re-established.

To regenerate bone during healing, resorption of soft cartilaginous callus and mineralisation of hard bony callus is crucial. During the resorption phase, a surge of extracellular Pi levels are resulted from the dissolving hydroxyapatite into mineral ions Ca2+ and Pi. The increase of phosphate in the bone microenvironment was sensed by the osteocytes and triggered the initiation of mineralisation by osteoblasts and phosphate homeostasis by regulating the expression of the mineralisation-related genes, such as DMP-1 and MEPE to form new hydroxyapatite [59]. DMP-1 is a highly phosphorylated protein localised in the gap region between collagen type 1 fibrils for the initiation of mineralisation [60]. DMP-1 reached peak expression at Day 14 postfracture and fell thereafter [12,21,35]. MEPE protein was expressed mainly from the soft and hard callus phases to the remodelling phase [26], and the expression persisted at the later stage, as compared with ALP. Both DMP-1 and MEPE belong to the SIBLING (small integrin-binding ligand, N-linked glycoproteins) family that are expressed in osteocytes and extracellular matrix of bone and dentin. Both of them participate in mineralisation with DMP-1 primarily expressed from the early to intermediate phase in cortical bone, while MEPE was predominantly present at the later stage within the newly regenerated bone. Finally, remodelling stage occurs, which the callus is modified and replaced by lamellar bone. Sclerostin expression was upregulated at the later phase of healing [18,29] to inhibit osteoblastic bone formation by antagonising beta-catenin-Wnt signalling pathway, as well as to facilitate the remodelling stage. It regulated differential RANKL and osteoprotegerin (OPG) production, resulting in either bone formation or resorption depending on the dynamic RANKL/OPG ratio [59]. Furthermore, the well-established LCN and increase in osteocyte integrity at the late phase of healing might also plays an important role in remodelling. The osteocytic dendritic processes present the membrane-bound RANKL that interacts directly with osteoclast precursors and hence initiates the osteocyte-dependent osteoclastogenesis. Previous investigations have suggested that osteocytes density might be a key determinant in the regulation of bone remodelling [61,62]. Moreover, the termination of resorption phase and the transition to osteoblastic activity required an osteoclastogenesis inhibitory factor, OPG, primarily produced by osteocytes [63], could also in turn regulate osteocyte-intracellular functions upon stimulation with RANK by limiting the release of RANKL to the surface of dendrites in osteocytes [64]. Osteocytes not only facilitate remodelling through osteoclast and osteoblast recruitment but also mediate local perilacunar remodelling by resorbing and replacing their surrounding matrix around their lacuna [65,66]. Osteocyte-mediated perilacunar remodelling is vital for the maintenance of bone quality and fracture resistance [67]. A recent study reported that there was a self-healing mechanism of sub-lamellar level cracking in bone which did not involve traditional bone remodelling. Moreover, the microstructure interface, such as the lacunae, could dissipate energy to attenuate microcrack and fracture propagations [68]. Hence, to what extent do perilacunar remodelling contribute to fracture healing is yet to be investigated in future studies.

Furthermore, fracture healing requires the spatial coordination of markers that are expressed in osteocytes near the fracture site. For instance, CYR61 and E11 were found within trabecular bone; COX-2, E11, DMP-1 and sclerostin in the newly formed outer or native inner cortical bone and BMPs and its receptors and effectors, E11 and MEPE, within the newly regenerated bone. According to the spatial and temporal expression pattern, it is postulated that osteocytes in the trabecular and woven bone might have the capacity to act as a mediator of the fractured surface within the injured matrix and the secreted bone matrix, finally reconnecting the bone canaliculi of the lamellar bone in the cortex (Fig. 2).

Three studies [18,22,27] investigated the signalling pathway of IGF-1, Gli-1 and Cx 43 in mice and assessed several critical cytokines' signalling pathways such as Wnt, Cx43, Hedgehog and Notch. Surprisingly, all of these signalling pathways are in crosstalk with the transforming growth factor-beta (TGF-beta)/BMP signalling [69], which is vital for osteogenesis, chondrocyte differentiation, skeletal development and bone homeostasis [70]. TGF-beta is essential for the stability of osteocytes [71], and osteocytes might exert their actions on the matrix through the TGF-beta–mediated regulation of proteases such as MMP-13. TGF-beta–Smad signalling positively regulates proliferation and early differentiation of osteoprogenitor. Meanwhile, it inhibits osteoblast maturation into osteocyte, as well as osteoclast differentiation by decreasing RANKL/OPG secretion ratio [72]. Smad3 is an intracellular signalling transducer in the TGF-beta pathway that regulates osteogenesis and chondrogenesis. In previous report, Smad3-null mice demonstrated a decreased osteoblast life span, promoted osteocyte cell

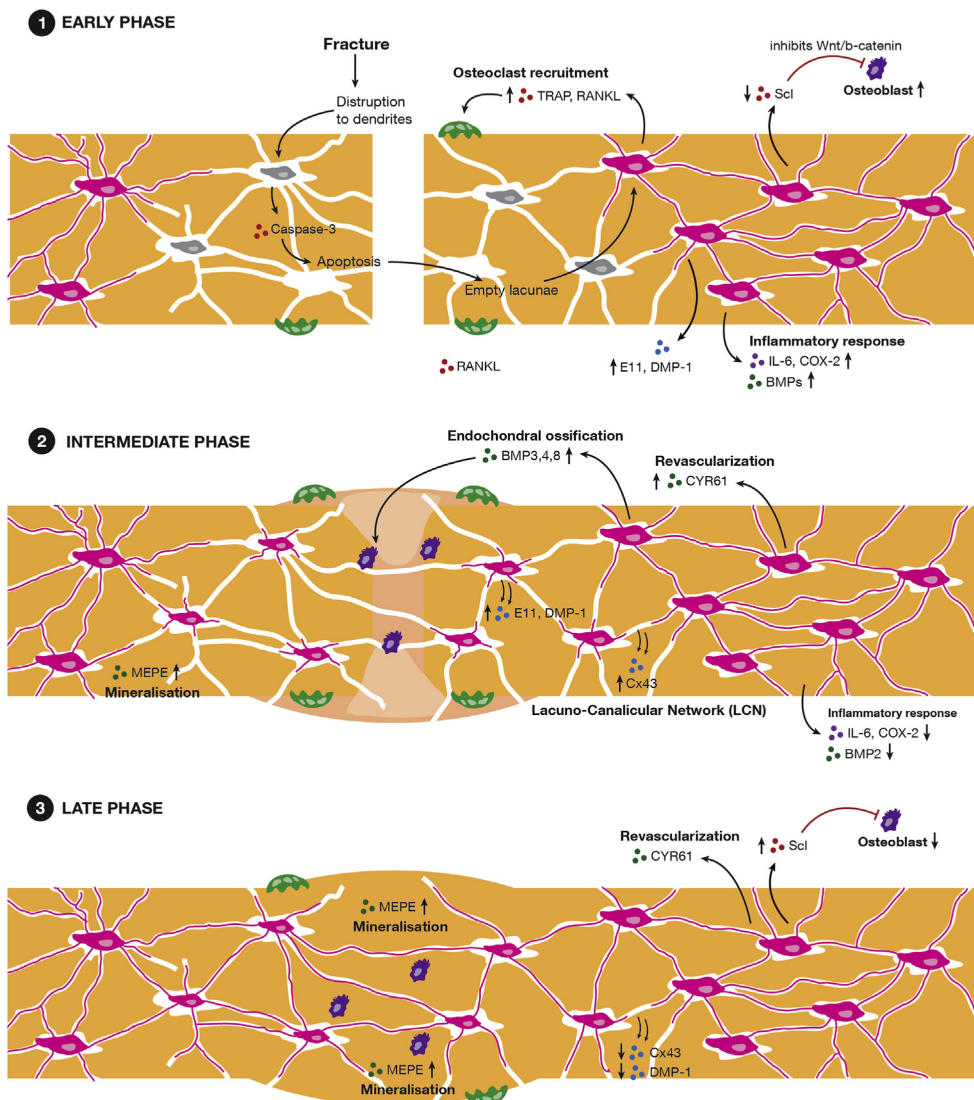

Figure 2. Overview of the role of osteocytes in fracture healing at different stages concluded from various studies. At the early phase of fracture healing, osteocytes undergo apoptosis and express cell apoptotic markers capase-3 in the vicinity of the fracture site. Upregulation of proinflammatory markers such as interleukin 6 (IL-6) and cyclooxygenase-2 (COX-2), triggers the coordinated bone healing response at the inflammatory stage. Growth factors bone morphogenetic proteins (BMPs) are expressed for the revascularisation and neoangiogenesis of callus in response to fracture. Upregulation of osteocyte-specific markers E11 and dentin matrix protein 1 (DMP-1) coupled with the downregulation of sclerostin have been suggested to promote osteogenesis. At the intermediate phase of healing, osteocytes continue to express growth factors BMPs and cysteine-rich angiogenic inducer 61 (CYR61) that may promote soft callus formation and chondrogenesis. BMPs expression would decline as healing progresses. Osteocyte-specific E11 and Cx 43 are upregulated for the maintenance of the lacuno-canalicular network (LCN). E11/gp38 is the earliest marker to be expressed as osteoblasts differentiate into osteocytes that is responsible for the formation and elongation of dendritic processes. The expression of Cx 43 enhances intercellular communication between osteocytes, modulation of osteoblastic cell signalling and aids the survival of osteocytes. DMP-1 expression increases as osteoblasts differentiate towards osteocytes which signifies osteocyte maturation and mineralisation. Sclerostin expression restores to its normal level to suppress osteoblastic action and possibly preserves the cartilaginous callus. At the late phase, healing progresses with the remodelling and mineralisation of the hard bony callus. Once the LCN between osteocytes are re-established, markers such as DMP-1, E11 and Cx 43 expressions would decline, while the expression of sclerostin remains constant representing the maturation of osteocytes. Matrix extracellular phosphoglycoprotein (MEPE) is expressed by osteocytes embedded in the matrix of mineralised bone and the persisted expression potentially demonstrates a significance in undergoing rapid mineralisation of callus at the late phase of healing.

fate [73] and an accelerated fracture healing [74]. The above evidence suggested that TGF-beta and Smad3 might be an important signalling pathway activated in osteocytes during fracture healing, by repressing osteoblast differentiation, maintaining the viability of osteocytes and dynamic remodelling of perilacunar bone matrix. Yet, the detailed molecular mechanisms remains to be elucidated in further investigations.

Various fixation methods were used among the studies, and the effect of fixation were investigated in two studies [23,24]. Plate and screws were shown to be a better fixation method, as demonstrated by a higher number of osteocytes present when compared with K-wires [23]. Rigid plate fixation restricts macromovements and provides a stable environment to preserve osteocytes and favours the growth and reconnection of canaliculi amongst the osteocytes. However, none of the studies investigated the effect of external fixator. The type of fixation was shown to alter the protein expression pattern in osteocytes, which BMP expressions were only present in the osteocytes with unstabilised fixation [24]. Another study also substantiated that the decreased fixation stability was associated with a downregulation of CYR61, leading to delayed healing [75]. To sum up, the varied stability by different fixations is likely to affect osteocytes' viability and protein expressions.

Therapeutic treatments have been targeting sclerostin since the past decade. Four of the studies [20,25,30,36] have successfully demonstrated a better healing outcome with increased bone mass and mineral density in both osteoporotic and nonosteoporotic animals. However, its effectiveness in treating fracture healing varies because of discrepancy of type and site of fractures, molecular mass and immunogenicity of Scl-Ab. Moreover, sclerostin antibody was shown to be most effective in the presence of cortical integrity during fracture repair [20]. Hence, it is

more effective to treat fractures healing through intramembranous ossification. Future studies focusing on the healing effect of Scl-Ab via endochondral ossification, as well as on the proliferation of fibrous tissues, angiogenesis, chondrogenesis, osteoinductivity and influence on the cardiovascular system would further supplement the effect of sclerostin antibody and develop a more potent therapeutic treatment.

There are a few limitations in this review. Among the 23 selected studies, 22 were animal studies which cannot fully simulate the real clinical scenario. Also, meta-analysis was hardly performed because of the heterogeneity of the included studies. Besides, most of the studies adopted traditional histological analysis in 2D plane, which might not truly represent the 3D LCN of osteocytes. Furthermore, osteocytes share the same origin of osteoblasts, leading to a high level of complexity to distinguish some functional genes and proteins expressed in osteocytes.

In summary, emerging studies support that osteocyte play a crucial role in different phases of fracture healing (Table 3), but there exists knowledge gaps. Osteoporotic fracture is known to show impaired healing properties [76,77], but only two of the 23 selected studies have demonstrated the role of osteocytes in osteoporotic fracture healing through Scl-Ab treatment [25,36] and one in fragility patients [37].

Table 3
A summary of the general changes of mRNA and protein expression during healing at different stages.

Tissue level	Progress of fracture healing			Other remarks
	Early	Intermediate	Late	
	Callus formation	Mineralisation	Callus remodelling	
Cell count	1300/mm² ocy: bone area [31]	1000/mm² ocy:bone area [31]	70.35/HPF [17]	Overall osteogenic cell density/bone area increases at 20 days postfracture [31]
Osteocyte:osteoblast ratio	0.35	0.2 [31]	65/mf (OPS) [23] 33/mf (OIKW) [23]	
Empty lacunae	0.72 ± 0.04 mm [19]		2.35/HPF [17]	
Gene expression (mRNA)	IL-6 ↑ [16,21,29,32–34,37] IL 10 mRNA [16] Cox-2 [29] SOST ↓ [18,37] CYR61 ↑ [32] E11 ↑ [33] DMP-1 ↑ [21] Type II collagen ↑ [21] Capase-3 ↑ [21] Sox-9 [31] Osteocalcin(Ocn) [31] Osteopotin (Opn) ↑ [33] Osterix(Osx) [37] TRAP [37] RANKL↑ [37] IGF-1 ↑ [37] BMP-2↑ [18,37] ALP [37] OPG↑ [37] CBFA 1 ↑ [37]	IL-6 ↓↑ [16,21,29,32–34,37] Cox-2 [29] SOST ↑ [18,29,37] CYR61 ↓ [32] E11 [33] DMP1 ↑ [21] Type I collagen 1↑ [31] Type II collagen ↓ [21] Type X collagen ↑ [31] Sox 9 ↑ [31] Osteocalcin(Ocn)↑ [21,31,33] Osteopotin (Opn) ↑ [21] Osterix(Osx) [31]↑ TRAP↑ [37] IGF-1 ↓ [37] BMP-2↓ [18,37] ALP↑ [37] CBFA 1 ↑ [37] OPG↓ [37]	Cx 43 [34] U19893 Actinin alpha4 [34] DMP1 ↓ [21] OC↑ [21] Type II collagen↓ [21] Type X collagen↓ [21]	
Signalling pathways involved	Wnt/beta-catenin BMP [18]	BMP [18] Wnt/beta-catenin [18] Hedgehog [22]	Cx 43 [27]	
Protein markers involved	IL 6 ↑ [29] COX-2 ↑ [29] Scl ↓ [18,29] E11↑ [33] OPN [21] ALP ↑ [12,25] TRAP ↑ [27] PCNA [31] BMP 2 ↑ [24] BMP 3–4 [24] BMP 8 ↑ [24] Noggin Smad 1/5/8 BMP 5–7 ↑ [24] BMPRIA ↑ [24] BMPRIB ↑ [24] BMPRII ↑ [24] Dmp1 ↑ [12,21,35] CYR61 [32] FGF 23 [35] Capase-3↑ [29] Type II collagen [12]	IL 6 [29] COX-2↓ [29] Scl ↑ [18,29] CYR61 ↑ [32] E11↑ [33] MEPE↑ [26] OPN [21] ALP ↑ [12,25] PCNA↑ [31] Cyclin D1↑ [31] Smad 1/5/8 ↑ [24] BMPRIA ↓ [24] BMPRIB ↓ [24] BMPRII ↑ [24] BMP2 ↓ [24] BMP 3–4 ↑ [24] BMP 5–7 ↓ [24] BMP 8 ↑ [24] Dmp1 ↑ [12,21,35] CYR61 [32] FGF 23[35] CX 43 ↑ [27] Type II collagen↓ [12]	CYR61 [32] MEPE↑ [26] OPN [21] Dmp1 ↓ [12,21,35] CX 43 ↓[27]	
Cytokine	IL 10 mRNA [16] IL 6 ↑ [29] COX-2 ↑ [29] CD 31 [12]	IL 10 mRNA [16] IL 6 ↓ [29] COX-2↓ [29] Bmp 2 mRNA↑ [37]		

ALP = alkaline phosphatase; BMP-2 = bone morphogenetic protein-2; Cox-2 = cyclooxygenase-2; Cx 43 = connexin 43; CYR61 = cysteine-rich angiogenic inducer 61; DMP-1 = dentin matrix protein; IGF = insulin-like growth factor; IL-6 = interleukin 6; MEPE = matrix extracellular phosphoglycoprotein; OPN = osteopontin; RANKL = receptor activator of NF-KappaB ligand; TRAP = tartrate resistant acid phosphatase; FGF = fibroblast growth factor; PCNA = proliferating cell nuclear antigen; CBFA 1 = core-binding factor α1; HPF = high power field; OPS = osteosynthesis using plates and screws; OIKW = osteosynthesis using Kirschner wire.

Whether osteocytes attribute to impaired healing capacity in osteoporotic bone is still unanswered. Ageing decreases the connectivity of canalicular network that is in close relation with microdamage accumulation and contributes to skeletal fragility [78]. Osteoporotic bone exhibited delayed healing by a reduced osteogenic capacity of MSCs, impaired callus vascularisation, cartilaginous and bony callus maturation and decreased expression of anabolic signalling molecules in bone formation [79]. Recent studies have suggested that, as a conjecture, the pathogenesis of the poor bone quality was associated with the osteocyte-regulated TGF-beta signalling [80,81], imbalanced osteoclast and osteoblast activity and different mechanoresponsiveness of several genes in aged rats, including TGF-beta, matrix metalloproteinase (MMP)-9 and MMP-13, which might impair osteocytic perilacunar remodelling [67,82]. As most osteoporotic fractures occur at the metaphyseal bone region with trabecular bone [83], studies using a clinically relevant osteoporotic metaphyseal fracture models should be considered [15]. As osteocyte viability is essential in regulating fracture repair, longitudinal studies are required to provide evidence unveiling the dynamic changes of osteocytes from early to later stages. In addition, because alteration of lacuna size may reflect the healing status of osteocytes, 3D quantitative assessment of the osteocyte LCN by using the latest imaging techniques is essential. Future studies to investigate how the osteocyte-mediated signalling molecules were transported dynamically within the lacunae and canaliculi, as well as to its effector cells should be performed. As different fixation methods influence osteocytes' viability and their protein expression, more related studies with good clinical implication should be conducted. Meanwhile, more investigations on osteocyte-specific biomarkers may help to understand or even monitor the status of fracture healing. Along with the new discoveries of osteocytes, it is of paramount importance to elucidate the underlying mechanisms that osteocytes involved in fracture healing. Through a better understanding of the respective roles of osteocytes, it is hopeful that more potential therapeutic strategies could be translated to improve the rate and quality of fracture healing as an ultimate goal.

Conflicts of interest Statement

The authors have no conflict of interest relevant to this article.

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References

[1] Ensrud KE. Epidemiology of fracture risk with advancing age. J Gerontol: Series A. 2013;68(10):1236–42.

[2] Siu A, Allore H, Brown D, Charles ST, Lohman M. National Institutes of Health Pathways to Prevention Workshop: Research Gaps for Long-Term Drug Therapies for Osteoporotic Fracture Prevention. Ann Intern Med 2019;171:51–7. https://doi.org/10.7326/M19-0961 [Epub ahead of print 23 April 2019].

[3] Wasfie T, Jackson A, Brock C, Galovska S, McCullough JR, Burgess JA. Does a fracture liaison service program minimize recurrent fragility fractures in the elderly with osteoporotic vertebral compression fractures? Am J Surg 2019;217(3):557–60.

[4] Kanis JA. Diagnosis and clinical aspects of osteoporosis. Pocket reference to osteoporosis. Springer; 2019. p. 11–20.

[5] Si L, Winzenberg TM, Jiang Q, Chen M, Palmer AJ. Projection of osteoporosis-related fractures and costs in China: 2010–2050. Osteoporos Int 2015;26(7):1929–37.

[6] Kalfas IH. Principles of bone healing. Neurosurg Focus 2001;10(4):1–4.

[7] Bonewald LF. The amazing osteocyte. J Bone Miner Res 2011;26(2):229–38.

[8] Bonewald LF. The role of the osteocyte in bone and nonbone disease. Endocrinol Metab Clin N Am 2017;46(1):1–18.

[9] Doty SB. Morphological evidence of gap junctions between bone cells. Calcif Tissue Int 1981;33(5):509–12.

[10] Pitsillides AA, Rawlinson SC, Suswillo RF, Bourrin S, Zaman G, Lanyon LE. Mechanical strain-induced NO production by bone cells: a possible role in adaptive bone (re)modeling? FASEB J : Off Publ Fed Am Soc Exp Biol 1995;9(15):1614–22.

[11] Hirakawa K, Hirota S, Ikeda T, Yamaguchi A, Takemura T, Nagoshi J, et al. Localization of the mRNA for bone matrix proteins during fracture healing as determined by in situ hybridization. J Bone Miner Res 1994;9(10):1551–7.

[12] Li M, Amizuka N, Oda K, Tokunaga K, Ito T, Takeuchi K, et al. Histochemical evidence of the initial chondrogenesis and osteogenesis in the periosteum of a rib fractured model: implications of osteocyte involvement in periosteal chondrogenesis. Microsc Res Tech 2004;64(4):330–42.

[13] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009;151(4):264–9.

[14] Wang J, Leung KS, Chow SK, Cheung WH. The effect of whole body vibration on fracture healing - a systematic review. Eur Cells Mater 2017;34:108–27.

[15] Wong RMY, Choy MHV, Li MCM, Leung KS, KHC S, Cheung WH, et al. A systematic review of current osteoporotic metaphyseal fracture animal models. Bone Joint Res 2018;7(1):6–11.

[16] Takamiya M, Fujita S, Saigusa K, Aoki Y. A study on mRNA expressions of interleukin 10 during fracture healing for wound age determination. Leg Med 2008;10(3):131–7.

[17] Donneys A, Tchanque-Fossuo CN, Blough JT, Nelson NS, Deshpande SS, Buchman SR. Amifostine preserves osteocyte number and osteoid formation in fracture healing following radiotherapy. J Oral Maxillofac Surg 2014;72(3):559–66.

[18] Lau KW, Rundle CH, Zhou XD, Baylink DJ, Sheng MH. Conditional deletion of IGF-I in osteocytes unexpectedly accelerates bony union of the fracture gap in mice. Bone 2016;92:18–28.

[19] Wichmann MW, Arnoczky SP, DeMaso CM, Ayala A, Chaudry IH. Depressed osteoblast activity and increased osteocyte necrosis after closed bone fracture and hemorrhagic shock. J Trauma 1996;41(4):628–33.

[20] Alaee F, Virk MS, Tang H, Sugiyama O, Adams DJ, Stolina M, et al. Evaluation of the effects of systemic treatment with a sclerostin neutralizing antibody on bone repair in a rat femoral defect model. J Orthop Res 2014;32(2):197–203.

[21] Toyosawa S, Kanatani N, Shintani S, Kobata M, Yuki M, Kishino M, et al. Expression of dentin matrix protein 1 (DMP1) during fracture healing. Bone 2004;35(2):553–61.

[22] Kitaura Y, Hojo H, Komiyama Y, Takato T, Chung UI, Ohba S. Gli 1 haploinsufficiency leads to decreased bone mass with an uncoupling of bone metabolism in adult mice. PLoS One 2014;9(10):e109597.

[23] Neagu TP, Tiglis M, Popp CG, Jecan CR. Histological assessment of fracture healing after reduction of the rat femur using two different osteosynthesis methods. Rom J Morphol Embryol 2016;57(3):1051–6.

[24] Yu YY, Lieu S, Lu C, Miclau T, Marcucio RS, Colnot C. Immunolocalization of BMPs, BMP antagonists, receptors, and effectors during fracture repair. Bone 2010;46(3):841–51.

[25] Liu Y, Rui Y, Cheng TY, Huang S, Xu L, Meng F, et al. Effects of sclerostin antibody on the healing of femoral fractures in ovariectomised rats. Calcif Tissue Int 2016;98(3):263–74.

[26] Lu C, Huang S, Miclau T, Helms JA, Colnot C. Mepe is expressed during skeletal development and regeneration. Histochem Cell Biol 2004;121(6):493–9.

[27] Loiselle AE, Paul EM, Lewis GS, Donahue HJ. Osteoblast and osteocyte-specific loss of Connexin 43 results in delayed bone formation and healing during murine fracture healing. J Orthop Res 2013;31(1):147–54.

[28] Clark WD, Smith EL, Linn KA, Paul-Murphy JR, Muir P, Cook ME. Osteocyte apoptosis and osteoclast presence in chicken radii 0-4 days following osteotomy. Calcif Tissue Int 2005;77(5):327–36.

[29] Wu AC, Kidd LJ, Cowling NR, Kelly WL, Forwood MR. Osteocyte expression of caspase-3, COX-2, IL-6 and sclerostin are spatially and temporally associated following stress fracture initiation. BoneKEy Rep 2014;3:571.

[30] Suen PK, Zhu TY, Chow DH, Huang L, Zheng LZ, Qin L. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength of intact bones in adult male rats. Sci Rep 2015;5:15632.

[31] Dishowitz MI, Mutyaba PL, Takacs JD, Barr AM, Engiles JB, Ahn J, et al. Systemic inhibition of canonical Notch signaling results in sustained callus inflammation and alters multiple phases of fracture healing. PLoS One 2013;8(7):e68726.

[32] Hadjiargyrou M, Ahrens W, Rubin CT. Temporal expression of the chondrogenic and angiogenic growth factor CYR61 during fracture repair. J Bone Miner Res 2000;15(6):1014–23.

[33] Hadjiargyrou M, Rightmire EP, Ando T, Lombardo FT. The E11 osteoblastic lineage marker is differentially expressed during fracture healing. Bone 2001;29(2):149–54.

[34] Meyer Jr RA, Desai BR, Heiner DE, Fiechtl J, Porter S, Meyer MH. Young, adult, and old rats have similar changes in mRNA expression of many skeletal genes after fracture despite delayed healing with age. J Orthop Res 2006;24(10):1933–44.

[35] Liu Z, Yamamoto T, Hasegawa T, Hongo H, Tsuboi K, Tsuchiya E, et al. Immunolocalization of osteocyte-derived molecules during bone fracture healing of mouse ribs. Biomed Res 2016;37(2):141–51.

[36] Yao Q, Ni J, Hou Y, Ding L, Zhang L, Jiang H. Expression of sclerostin scFv and the effect of sclerostin scFv on healing of osteoporotic femur fracture in rats. Cell Biochem Biophys 2014;69(2):229–35.

[37] Caetano-Lopes J, Lopes A, Rodrigues A, Fernandes D, Perpetuo IP, Monjardino T, et al. Upregulation of inflammatory genes and downregulation of sclerostin gene expression are key elements in the early phase of fragility fracture healing. PLoS One 2011;6(2):e16947.

[38] Loiselle A, Billingsley A, Paul E, Zhang Y, Donahue H. Loss of connexin 43 in mature osteoblasts and osteocytes results in delayed mineralization and bone remodeling during fracture healing. J Bone Miner Res 2010;25:S103.

[39] Paszty C, Turner CH, Robinson MK. Sclerostin: a gem from the genome leads to bone-building antibodies. J Bone Miner Res 2010;25(9):1897–904.

[40] Kogianni G, Mann V, Noble BS. Apoptotic bodies convey activity capable of initiating osteoclastogenesis and localized bone destruction. J Bone Miner Res 2008;23(6):915–27.
[41] Al-Dujaili SA, Lau E, Al-Dujaili H, Tsang K, Guenther A, You L. Apoptotic osteocytes regulate osteoclast precursor recruitment and differentiation in vitro. J Cell Biochem 2011;112(9):2412–23.
[42] Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell ... and more. Endocr Rev 2013;34(5):658–90.
[43] Trueta J. The role of the vessels in osteogenesis. Bone & Joint Journal 1963;45(2): 402–18.
[44] Marotti G, Remaggi F, Zaffe D. Quantitative investigation on osteocyte canaliculi in human compact and spongy bone. Bone 1985;6(5):335–7.
[45] Kusu N, Laurikkala J, Imanishi M, Usui H, Konishi M, Miyake A, et al. Sclerostin is a novel secreted osteoclast-derived bone morphogenetic protein antagonist with unique ligand specificity. J Biol Chem 2003;278(26):24113–7.
[46] Okman-Kilic T, Sagiroglu C. Anabolic agents as new treatment strategy in osteoporosis. Topics in Osteoporosis. InTech; 2013.
[47] Lau KHW, Kothari V, Das A, Zhang X-B, Baylink DJ. Cellular and molecular mechanisms of accelerated fracture healing by COX2 gene therapy: studies in a mouse model of multiple fractures. Bone 2013;53(2):369–81.
[48] Lin GL, Hankenson KD. Integration of BMP, Wnt, and notch signaling pathways in osteoblast differentiation. J Cell Biochem 2011;112(12):3491–501.
[49] McGee-Lawrence ME, Ryan ZC, Carpio LR, Kakar S, Westendorf JJ, Kumar R. Sclerostin deficient mice rapidly heal bone defects by activating beta-catenin and increasing intramembranous ossification. Biochem Biophys Res Commun 2013; 441(4):886–90.
[50] Morse A, Yu NYC, Peacock L, Mikulec K, Kramer I, Kneissel M, et al. Endochondral fracture healing with external fixation in the Sost knockout mouse results in earlier fibrocartilage callus removal and increased bone volume fraction and strength. Bone 2015;71:155–63.
[51] Chen Y, Whetstone HC, Lin AC, Nadesan P, Wei Q, Poon R, et al. Beta-catenin signaling plays a disparate role in different phases of fracture repair: implications for therapy to improve bone healing. PLoS Med 2007;4(7):e249.
[52] Koh A, Niikura T, Lee SY, Oe K, Koga T, Dogaki Y, et al. Differential gene expression and immunolocalization of insulin-like growth factors and insulin-like growth factor binding proteins between experimental nonunions and standard healing fractures. J Orthop Res 2011;29(12):1820–6.
[53] Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J 2003;22(23):6267–76.
[54] Haffner-Luntzer M, Liedert A, Ignatius A. Mechanobiology of bone remodeling and fracture healing in the aged organism. Innovat Surg Sci 2016:57.
[55] Duncan R, Turner C. Mechanotransduction and the functional response of bone to mechanical strain. Calcif Tissue Int 1995;57(5):344–58.
[56] Cherian PP, Siller-Jackson AJ, Gu S, Wang X, Bonewald LF, Sprague E, et al. Mechanical strain opens connexin 43 hemichannels in osteocytes: a novel mechanism for the release of prostaglandin. Mol Biol Cell 2005;16(7):3100–6.
[57] Bivi N, Condon KW, Allen MR, Farlow N, Passeri G, Brun LR, et al. Cell autonomous requirement of connexin 43 for osteocyte survival: consequences for endocortical resorption and periosteal bone formation. J Bone Miner Res : Off J Am Soc Bone Miner Res 2012;27(2):374–89.
[58] Civitelli R. Cell-cell communication in the osteoblast/osteocyte lineage. Arch Biochem Biophys 2008;473(2):188–92.
[59] Sapir-Koren R, Livshits G. Osteocyte control of bone remodeling: is sclerostin a key molecular coordinator of the balanced bone resorption-formation cycles? Osteoporos Int 2014;25(12):2685–700.
[60] Schaffler MB, Kennedy OD. Osteocyte signaling in bone. Curr Osteoporos Rep 2012; 10(2):118–25.
[61] Noble B, Stevens H, Loveridge N, Reeve J. Identification of apoptotic changes in osteocytes in normal and pathological human bone. Bone 1997;20(3):273–82.
[62] Power J, Loveridge N, Rushton N, Parker M, Reeve J. Osteocyte density in aging subjects is enhanced in bone adjacent to remodeling haversian systems. Bone 2002; 30(6):859–65.
[63] Kramer I, Halleux C, Keller H, Pegurri M, Gooi JH, Weber PB, et al. Osteocyte Wnt/ beta-catenin signaling is required for normal bone homeostasis. Mol Cell Biol 2010; 30(12):3071–85.
[64] Honma M, Ikebuchi Y, Kariya Y, Hayashi M, Hayashi N, Aoki S, et al. RANKL subcellular trafficking and regulatory mechanisms in osteocytes. J Bone Miner Res 2013;28(9):1936–49.
[65] Qing H, Bonewald LF. Osteocyte remodeling of the perilacunar and pericanalicular matrix. Int J Oral Sci 2009;1(2):59.
[66] Bélanger LF. Osteocytic osteolysis. Calcif Tissue Res 1969;4(1):1–12.
[67] Tang SY, Herber RP, Ho SP, Alliston T. Matrix metalloproteinase-13 is required for osteocytic perilacunar remodeling and maintains bone fracture resistance. J Bone Miner Res 2012;27(9):1936–50.
[68] Seref-Ferlengez Z, Kennedy OD, Schaffler MB. Bone microdamage, remodeling and bone fragility: how much damage is too much damage? BoneKEy Rep 2015;4:644.
[69] Heino TJ, Hentunen TA. Differentiation of osteoblasts and osteocytes from mesenchymal stem cells. Curr Stem Cell Res Ther 2008;3(2):131–45.
[70] Rahman MS, Akhtar N, Jamil HM, Banik RS, Asaduzzaman SM. TGF-beta/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. Bone Res 2015;3:15005.
[71] Tang SY, Alliston T. Regulation of postnatal bone homeostasis by TGFbeta. BoneKEy Rep 2013;2:255.
[72] Wu M, Chen G, Li Y-P. TGF-beta and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. Bone Res 2016;4: 16009.
[73] Borton AJ, Frederick JP, Datto MB, Wang XF, Weinstein RS. The loss of Smad 3 results in a lower rate of bone formation and osteopenia through dysregulation of osteoblast differentiation and apoptosis. J Bone Miner Res 2001;16(10): 1754–64.
[74] Kawakatsu M, Kanno S, Gui T, Gai Z, Itoh S, Tanishima H, et al. Loss of Smad 3 gives rise to poor soft callus formation and accelerates early fracture healing. Exp Mol Pathol 2011;90(1):107–15.
[75] Lienau J, Schell H, Epari DR, Schütze N, Jakob F, Duda GN, et al. CYR61 (CCN1) protein expression during fracture healing in an ovine tibial model and its relation to the mechanical fixation stability. J Orthop Res 2006;24(2):254–62.
[76] Shi HF, Cheung WH, Qin L, Leung AH, Leung KS. Low-magnitude high-frequency vibration treatment augments fracture healing in ovariectomy-induced osteoporotic bone. Bone 2010;46(5):1299–305.
[77] Cheung WH, Sun MH, Zheng YP, Chu WC, Leung AH, Qin L, et al. Stimulated angiogenesis for fracture healing augmented by low-magnitude, high-frequency vibration in a rat model-evaluation of pulsed-wave Doppler, 3-D power Doppler ultrasonography and micro-CT microangiography. Ultrasound Med Biol 2012; 38(12):2120–9.
[78] Vashishth D, Verborgt O, Divine G, Schaffler MB, Fyhrie DP. Decline in osteocyte lacunar density in human cortical bone is associated with accumulation of microcracks with age. Bone 2000;26(4):375–80.
[79] Haffner-Luntzer M, Liedert A, Ignatius A. Mechanobiology of bone remodeling and fracture healing in the aged organism. Innovat Surg Sci 2016;1(2):57–63.
[80] Dole NS, Mazur CM, Acevedo C, Fowler TW, Lopez J, Monteiro DA, et al. Novel role of TGFbeta in osteocytes: regulation of perilacunar remodeling and bone quality. J Orthop Res Conf 2017;35 [no pagination)].
[81] Mazur CM, Dole NS, Acevedo C, Gludovatz B, Ritchie RO, Alliston T. TGFbeta regulates fracture toughness via osteocytes. J Orthop Res Conf 2017;35 [no pagination)].
[82] Ode A, Duda GN, Geissler S, Pauly S, Ode J-E, Perka C, et al. Interaction of age and mechanical stability on bone defect healing: an early transcriptional analysis of fracture hematoma in rat. PLoS One 2014;9(9):e106462.
[83] Larsson S. Treatment of osteoporotic fractures. Scand J Surg 2002;91(2):140–6.