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Review Article

Accelerated fracture healing accompanied with traumatic brain injury: A review of clinical studies, animal models and potential mechanisms

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ABSTRACT

The orthopaedic community frequently encounters polytrauma individuals with concomitant traumatic brain injury (TBI) and their fractures demonstrate accelerated fracture union, but the mechanisms remain far from clear. Animal and clinical studies demonstrate robust callus formation at the early healing process and expedited radiographical union. In humans, robust callus formation in TBI occurs independently of fracture fixation methods across multiple fracture sites. Animal studies of TBI replicate clinically relevant enlarged fracture callus as characterized by increased tissue volume and bone volume at the early stages. However, refinement and standardization of the TBI models requires further research. The quest for its underlying mechanisms began with the finding of increased osteogenesis *in vitro* using the serum and cerebral spinal fluid (CSF) from TBI individuals. This has led to the investigation of myriads of brain-derived factors including humoral factors, cytokines, exosomes, and mi-RNAs. Further, the emerging information of interplay between the skeletal system and central nervous system, the roles of peripheral nerves and their neuropeptides in regulating bone regeneration, offers valuable insights for future research. This review consolidates the findings from both experimental and clinical studies, elucidating the potential mechanisms underlying enhanced fracture healing in concurrent TBI scenarios that may lay down a foundation to develop innovative therapies for fracture healing enhancement and conquer fracture non-union.

The translational potential of this article.

This review comprehensively summarizes the observations of accelerated fracture healing in the presence of traumatic brain injury from both preclinical and clinical studies. In addition, it also delineates potential cellular and molecular mechanisms. Further detailed investigation into its underlying mechanisms may reveal innovative orthopaedic intervention strategies to improve fracture healing and thus offering promising avenues for future translational applications.

1. Introduction

Trauma is the leading cause of mortality and disability among the working population, resulting approximately 6 million global annual deaths [1,2]. Fracture is a common form of trauma-related injuries, with estimated number of 178 million new cases and 455 million prevalent cases in 2019 globally, which pave significant medical and socioeconomic burden to patients, public health systems and societies [3]. Despite the efforts in iterations in fracture fixation techniques, innovative implants, application of bionics, and progress in treatment modality

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concepts, the unsolved complications such as delayed union, nonunion and osteonecrosis [4–6], the increased incidence of osteoporosis and its fragility fractures in the global aging era [7,8], all pose great challenges to the unmet demands for enhanced recovery and healing after fracture.

Exhilaratingly, accelerated fracture healing with concomitant traumatic brain injury (TBI) has been continuously reported since the 1960s [9]. Various terms, including "hyperplastic callus," "ossifying hematoma, " and "calcifying hematoma," have been employed to describe this fracture healing pattern in TBI individuals, yet the underlying mechanisms remain elusive and warrant further exploration [10,11]. Understanding these mechanisms could facilitate early intervention in the fracture healing process, aiding bone regeneration and mitigating risks of delayed union, non-union, and reducing the need for secondary interventions in complex scenarios. While preclinical studies have suggested that the TBI-enhanced fracture healing may be attributed to the abnormal state of acid-base balance [12], growth factors [13–15], cytokines [16], miRNAs with or without exosomes [17,18], neuropeptides and neurotransmitters [12,19–22], a definitive connection between brain trauma and the stimulation of peripheral bone fracture healing has yet to be established (Fig. 1). Unraveling the key mechanisms and biological factors inducing this unique phenomenon is crucial for novel and translational therapy targeting early fracture healing stages and enhancing bone regeneration.

Therefore, this review aims to recapitulate and evaluate clinical reports and experimental *in vivo* studies, demonstrating the altered fracture healing patterns in TBI scenario. The current knowledge of potential mechanisms pertaining to the enhanced fracture healing is also summarized and discussed. This review upholds the expectation to provide essential information towards comprehensive understanding for further research on the mechanisms associated with the highly consistent observation on "TBI accelerated fracture healing".

2. Accelerated fracture healing in TBI individuals

Exuberant callus formation at the early stage and overall shorter fracture healing time are two distinct features seen in some polytrauma individuals sustaining concomitant fracture and TBI (Fig. 2, Table 1). This promotive effect on bone fracture healing is suggested to correlate with the severity of the brain injury, classified using Glasma Coma Scale (GCS) scores which describe the extent of impaired consciousness in all types of acute medical and trauma individuals, and also Marshall CT classification where the severity of head trauma is categorized based on CT findings of the injured brain [23,24]. The enhanced callus formation and their increased bone density are often observed in moderate and severe TBIs, and negative linear relationship between GCS and the callus ratio were also reported [13,23]. Tendency towards larger callus formation is also reported in specific types of brain injury, for example, the subdural hemorrhage over the other intra-axial lesions [13,25]. More interestingly, these unique findings are mainly reported to occur in the appendicular bones including fractures in the femur, tibia, humerus, clavicle, but incidence in axial bones such as vertebral fractures are yet to be reported. The unique fracture healing response in TBI individuals

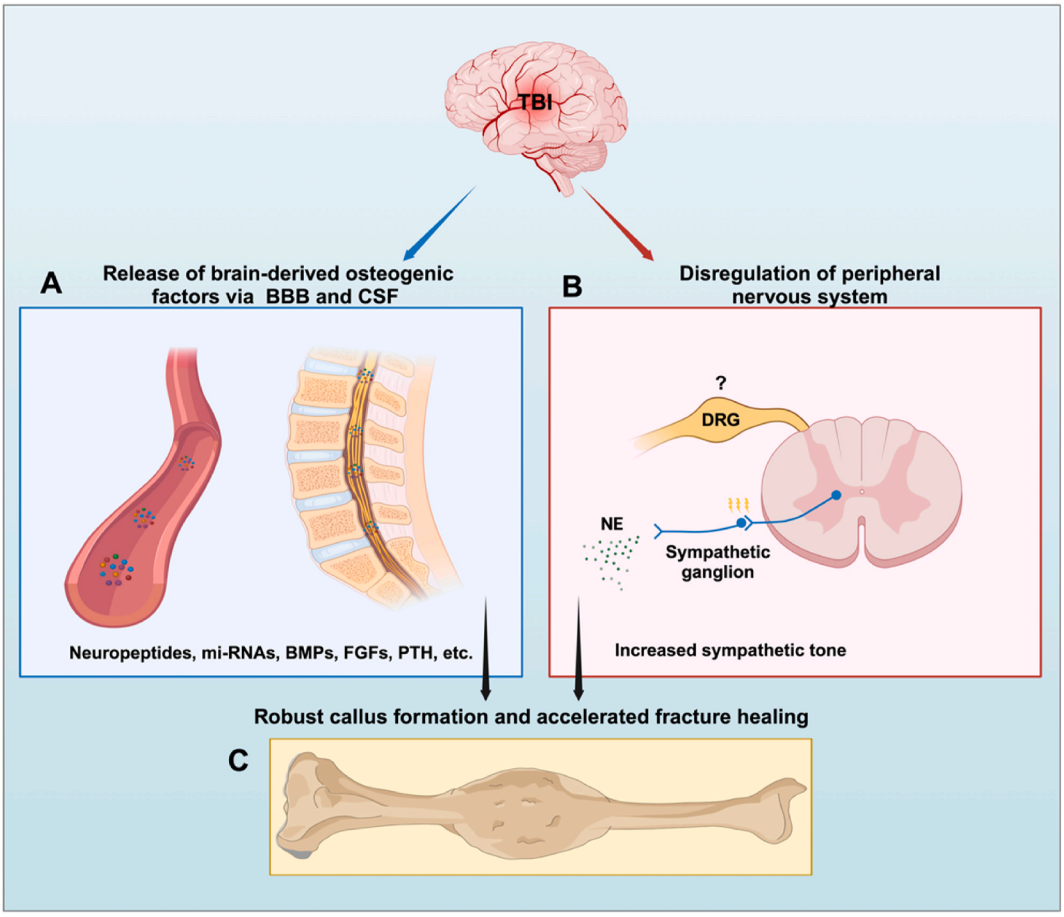

Fig. 1. Two mainstream mechanisms of accelerated fracture healing accompanied with traumatic brain injury (TBI). (A) Serum mediated accelerated fracture healing. The injured brain and subsequent blood brain barrier leakage leads to the release of brain-derived osteogenic factors to the peripheral via blood stream and cerebrospinal fluids, including neuropeptides, mi-RNAs and exosomes, BMPs, FGFs, PTH, etc. (B) Dysregulation of peripheral nervous system after TBI. Sympathetic nervous system hyperactivity and the mass release of peripheral norepinephrine (NE) leads to the accelerated fracture healing (C) TBI results in robust callus formation at the early stages and accelerates fracture healing. Created with BioRender.com.

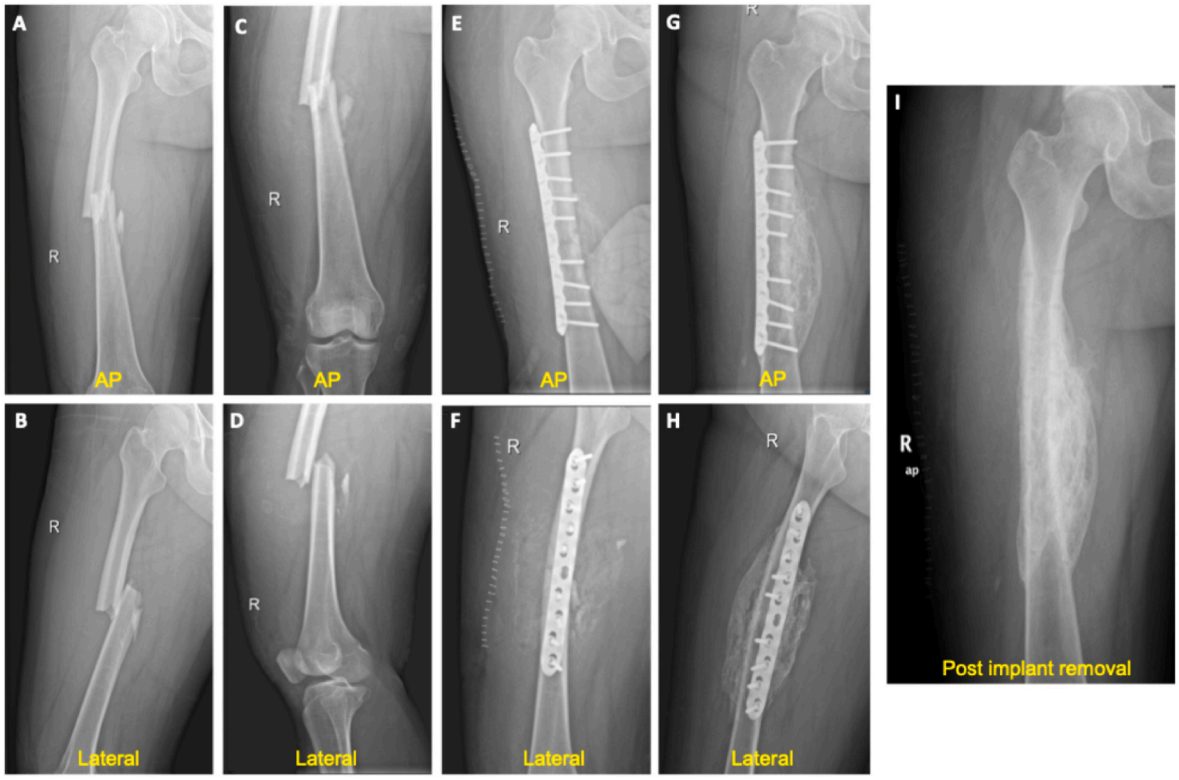

Fig. 2. Case demonstration of displaced right femur shaft fracture with concomitant TBI injury. (A, B) Anteroposterior and lateral view at admission. (C, D) Visible fracture callus already observed at 14 days post injury. (E, F) Exuberant callus formation at immediate post-op, 23 days post injury. (G, H, I) Large and dense fracture callus at 24 months post injury and after implant removal.

may be independent of fracture fixation methods (stable vs unstable fixation), and the robust callus formation was observed as early as 2–3 weeks after injury (Fig. 2) [24,26]. Other than radiographical evidence, the histopathological specimens from the reaming debris before intramedullary nail insertion also showed higher rate of osteoblasts presence (82.9 %) than those in simple fractures (27.5 %) [27]. Longer duration of coma and subsequent ventilation support has been reported as risk factors for neurogenic heterotopic ossification [28]. Likewise, ventilation is proposed to enhance fracture healing in combined fracture and TBI individuals [12,29], as respiratory alkalosis caused by hyperventilation for the treatment of brain trauma may lead to accelerated calcium deposition. Of note, others also reported the healing pattern was similar between ventilated and non-ventilated individuals in later studies [25,30]. In addition to TBI, the formation of robust callus was also reported in individuals sustaining severe spinal cord injury including those with concomitant quadriplegia or paraplegia and combined long bone fractures [31,32]. In complete neurological injury or Frankel Grade A individuals, in which both motor and sensory function were lost below the lesion, accelerated fracture union and larger callus was shown in 15 out of 22 individuals [33]. Furthermore, a more rapid bone union and excessive femoral fracture callus formation were noted in individuals with both traumatic brain injury (TBI) and complete spinal cord injury (SCI) (paraplegia or quadriplegia) in comparison to individuals with fractures alone (6.5 weeks vs. 22.4 weeks). Notably, serum samples obtained from SCI individuals notably boosted the proliferation of mesenchymal stem cells (MSCs) *in vitro*. The data indicating the potential of both TBI and SCI to enhance fracture healing imply a potential shared fundamental mechanism between these conditions.

3. Combined TBI and fracture animal models and fracture healing patterns

Three kinds of models are commonly used to produce TBIs, including controlled cortical impact (CCI) model, weight drop (WD) model and lateral fluid percussion injury model (LFPI) (Fig. 3). These three models can each produce different types of brain injuries (focal, diffuse, mixed) and show markedly difference in terms of strength and weaknesses (reviewed elsewhere) [41,42]. Various TBI methods and the criteria used in different laboratories for TBI induction is inconsistent, which may produce variable effects on fracture healing pattern, biomechanical properties, and other important features (Fig. 3D–Table 2).

The Controlled Cortical Impact (CCI) model involves delivering trauma to the intact dura through a unilateral craniotomy typically positioned between bregma and lambda, resulting in deformation of the underlying cortex. This method induces diffused brain damage, encompassing acute cortical, hippocampal, and thalamic degeneration. The CCI model offers a precise, reproducible, and controlled range of severity levels, mirroring the spectrum of injuries encountered in clinical cases (Fig. 3A). Deformation parameters in the CCI model can be finely tuned by controlling impaction duration, velocity, and impact depth [43]. Tsitsilonis et al. introduced a concurrent traumatic brain injury (TBI) and femoral osteotomy model in mice using the CCI system and external fixators [44]. By aligning with criteria for mild TBI, the TBI severity was calibrated, leading to escalated callus formation in the combined TBI and fracture group from the second week, with a relatively low overall mortality rate (21 %, 4/19). Additionally, micro-CT scans showed heightened bone mineral density (BMD) and an accelerated fracture gap union rate throughout the study. Notably, employing the same mild TBI parameters, the combined trauma group exhibited enhanced fracture callus strength by the fourth week compared to the fracture-alone group [45]. Accelerated fracture healing with higher bone tissue composition was also reported using the CCI method in severe TBI combined with fracture [46]. However, such phenomenon was only observed at the early stage (day 5).

In weight-drop (WD) models, the skull is exposed (with or without a craniotomy) to a free falling, guided weight rod or cone. Injury severity

Table 1
A glance at the clinical studies reported accelerated fracture healing with concomitant TBI.

Author	Year	Number of individuals	Fracture	Fixation approach	TBI injury severity (GCS)	Major findings in TBI/FX individuals
Newman [12]	1987	11 TBI/FX	Femur Tibia	Traction or cast	—	• Rapid fracture union in 11 individuals. • Time to callus formation: 2–4 weeks. • Time to clinical union: 4–7 weeks.
Skirving [34]	1987	22 TBI/FX vs 22 FX		AO nailing	—	• Greater callus and shorter bone union duration (12.4 weeks vs 15.7 weeks) in TBI/FX. • No correlation found between the size of callus and duration of coma.
Spencer [11]	1987	53 TBI/FX vs 30 FX	Femur Tibia Humerus	Conservative Internal fixation	3–10	• Abundant healing response in TBI individuals in tibia, femur, and humerus. • Accelerated union in TBI individuals in tibia (13.05 weeks vs 14.90 weeks), femur (12.40 weeks vs 15.25 weeks), and humerus (6.73 weeks vs 7.30 weeks). • Abundant healing response found in 73 % of individuals with severe TBI.
Giannoudis [30]	2006	17 TBI/FX vs 50 FX	Femur	Reamed and unreamed IMN	5.5 (3–10)	• Time to fracture union significantly shorter than either the reamed or unreamed group (10.5 weeks vs 20.5 and 26.9 weeks). • Higher mean callus to diaphyseal ratio in TBI/FX individuals.
Gautschi [23]	2009	17 TBI/FX vs 24 FX	Femur Tibia Humerus	IMN fixation in femur fractures	5.6 ± 2.2 (3–8)	• Two-fold shorter time to union and 37 %–50 % increased callus ratio. • Sera induced a higher proliferation rate in hFOB cells ($P < 0.05$). • GCS scores correlated with the callus ratio on radiographic projections ($P < 0.05$), time to union ($P = 0.04$), proliferation rate of hFOB cells at 6 h after injury ($P = 0.03$).
Wang [35]	2010	18 TBI/FX vs 18 FX	Femur	IMN fixation	8–12	• Larger callus size and faster healing (11.83 ± 1.58 vs14.0 ± 2•29 weeks). • 8 HO found in TBI/FX
Fu [36]	2011	25 TBI/FX vs 25 FX	Femur	Steel plate and screws	8–12	• Larger callus size at 6 weeks after surgery. • Higher callus BMD at 1, 3, 5 weeks after surgery
Huang [37]	2012	24 TBI/FX vs 21 FX	Mandible	Conservative	—	• Earlier callus formation within 3–4 weeks after injury in individuals with TBI (12/24) than without (6/21)
Yang [25]	2012	20 TBI/FX vs 54 FX	Femur	IMN	7–15	• Individuals with a brain injury had an earlier appearance of bridging callus formation (2.3 months) and a greater final mean callus thickness value (3.3 months) than those without. • No difference found in GCS <8 vs > 8 fracture time to callus formation and thickness.
Khallaf [31]	2015	20 TBI/FX vs 20 SCI vs 20 FX	Femur Tibia Fibula	Plates or IMN		• Larger callus formation and shorter time to union 6.5 (range 4–17) weeks vs 22.4 (range 13–41) weeks.
Khallaf [38]	2016	52 TBI vs 50 TBI/FX vs 60FX	Humerus Femur Tibia	Plates or IMN	3–8	• Thicker callus and shorter time to healing (6.9 ± 2.9 weeks vs 22.4 ± 8.7 weeks).
Li [39]	2017	25 TBI/FX vs 33 FX	Humerus Femur Tibia	Nail, plate, external fixator	5.3	• Larger callus formation and shorter time to union (16.1 weeks vs 12.6 weeks).
Zhang [26]	2018	22 TBI/FX vs 20 FX	Clavicle	Plate	mean 11.5	• Lower blood monocyte, larger fracture callus volume, and shorter healing time (82.2 days vs 127.0 days).
Zhang [40]	2020	60 TBI/FX vs 20 FX	Femur Tibia Humerus	IMN	3–15	• Higher rate of callus formation for moderate and severe TBI/FX but not in mild TBI/FX.
Khorramdelazad [16]	2021	30 FX vs 30 TBI vs 30 TBI/FX	Femur	IMN	5.97	• Shorter time to union (9.1 weeks and 17.2 weeks, respectively, $P = 0.03$).
Ravi [13]	2022	15TBI/FX vs 15 FX	Femur Tibia	IMN	2–10	• Larger callus formation at 6 weeks, 3 months, and 6 months post fixation.
Shim [24]	2022	12 TBI/FX vs 36 FX	Tibia	Not specified	2–14	• Earlier (17.5 days vs 93.4 days) and larger callus formation and accelerated fracture healing at tibia. • Negative correlation of callus ratio and GCS scores.

GCS: Glasgow coma score; TBI: traumatic brain injury; TBI/FX: combined TBI and fracture; FX: fracture; IMN: intramedullary nail; HO: heterotopic ossification.

in these models can be altered by adjusting the weight and the height (Fig. 3B). Lin et al. reported no obvious effect on fracture healing in mild TBI using WD method and high mortality using in severe TBI in mice [17]. Similar to CCI methods, larger callus size and higher BV, TV and Tb.N values were shown at the fracture callus in moderate TBI and combined fracture group at week 3 post injury. In a closed skull WD-induced TBI model, larger callus size and increased moment of inertia (an indicator of torsional strength) were documented, with the advantage of excluding craniotomy as a potential confounding variable seen in open-skull TBI models [47]. However, a decrease in callus size was observed when utilizing the WD method to induce diffuse axonal injury in rats compared to controls at the 21-day post-injury mark [48]. The researchers suggested that by this time point, the fracture callus had likely advanced to the remodeling phase, as evidenced by the brain-injured group displaying greater stiffness compared to the fracture-only group.

Lateral fluid percussion injury (LFPI) model replicates the clinical brain injury by impacting the brain using a pendulum striking the piston of a reservoir of fluid to generate a fluid pressure pulse to the intact dura through a craniotomy and at the lateral side of the parietal bone between bregma and lambda (Fig. 3C). LFPI produces a combination of focal cortical contusion and diffuse subcortical neuronal injury (including injury in the hippocampus and thalamus). In rats, markedly increase of callus volume and bone volume/tissue volumes (BV/TV%) were observed in moderate severity combined TBI and proximal tibia defect (PTD) group at day 7 and day 14.

In summary, variations in TBI methodologies, injury parameters, injury sites, and severity criteria within animal models may yield differing fracture healing outcomes, affecting factors such as healing speed, callus size, and biomechanical indices during early and late stages

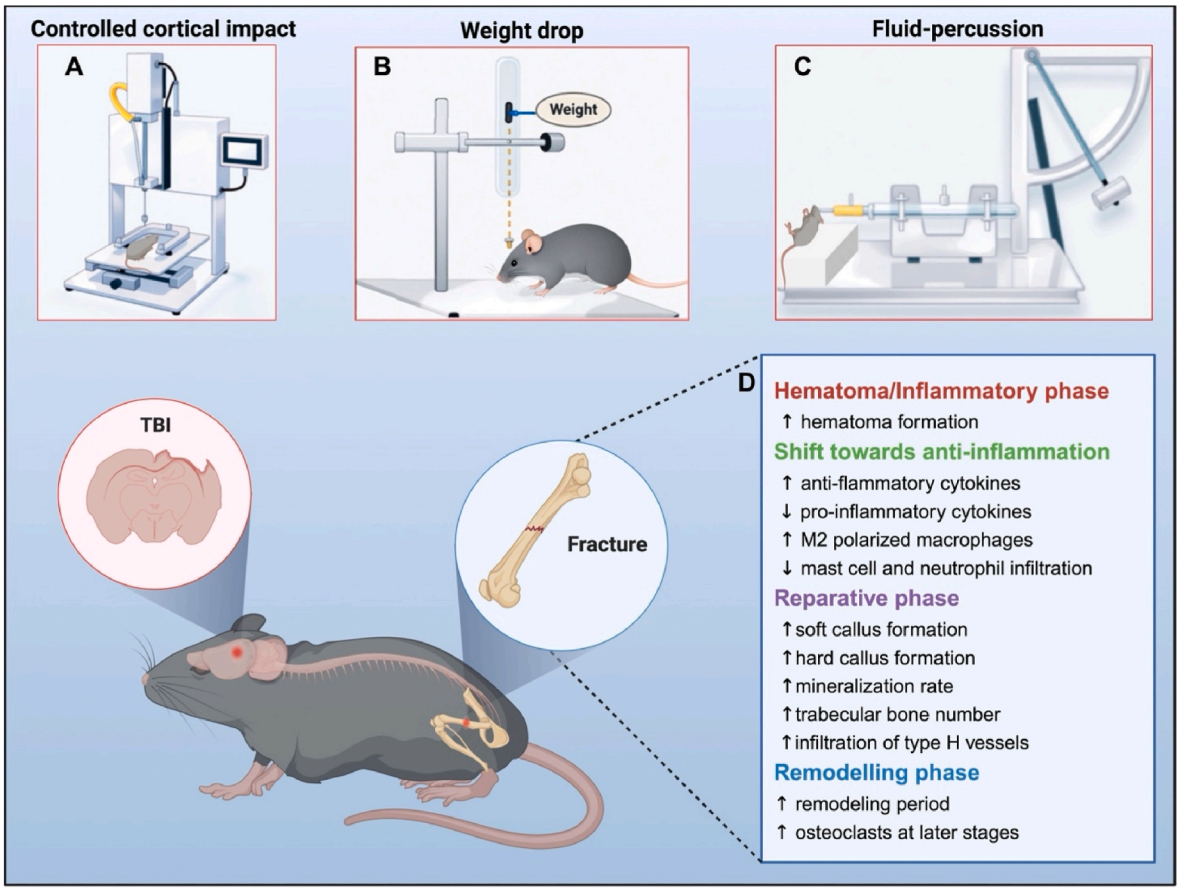

Fig. 3. Combined TBI and fracture animal models and altered fracture callus features. TBIs are produced using controlled cortical impact (A), weight drop (B), and lateral fluid percussion injury methods (C). *In vivo* studies show altered fracture callus features throughout the fracture healing phases in combined TBI and fracture animal model. (D). Created with BioRender.com.

Table 2
Animal models for concomitant TBI and fracture.

Refs	Animals	TBI model and severity	TBI establishment	Fracture	Fracture healing description
Tsitsilonis [44,45]	C57BL mice	CCI (Cortical controlled impact) Mild TBI	3 mm bolt with a flat tip to an impact velocity of 3.5 m/s inducing a penetration depth of 0.25 mm at a contact time of 0.15 s.	0.70 mm femur osteotomy with external fixators.	• Significant increase callus volume in the from the 2nd˙4th week higher bone density from week 1 and increased maximum torque at week 4.
McDonald [47]	C57BL mice	Close-head Weight-drop (WD) Not mentioned	WD from 2 cm height using rod (215 g) with an impact tip of 4 mm in diameter at the sagittal and coronal suture.	Closed mid-shaft transverse tibia fracture without fixation.	• Greater bone and total tissue volume, higher mean polar moment of inertia, and increased amount of trabecular bone in callus TBI/FX group at day 21.
Boes [48]	SD rats	Weight-drop Not mentioned	500 g weight from a height of exactly 1.5 m on nickel plated tap.	Closed femur fracture fixed with IMN.	• Reduced mean fracture callus diameter and stiffer fracture callus in TBI/FX group at day 21.
Lin et al. [17]	C57BL mice	Weight-drop with craniotomy Moderate TBI	Craniotomy between bregma and lambda 1 mm from midline. WD from 30 cm height using 3 mm (diameter) cone weighed 20g at right parietal cortex.	Closed transverse fracture of the tibia fixed with IMN.	• Larger callus and higher values of BV, TV and Tb.N seen in TBI/FX group at day 21. • Larger number of disorderly and dense trabeculae in the callus of the TBI/FX group. • Higher Runx2 and Osterix positive expression factors scattered in the trabecular bone in TBI/FX group at 3 weeks post-op.
Bai et al. [50]	SD rats	Lateral fluid percussion injury with craniotomy Moderate TBI	5 mm craniotomy between the bregma and lambda 2.5 mm lateral to midline. A pulse of increased intracranial pressure of 21–23 ms duration.	Proximal tibial defect (PTD)	• Marked increase in callus volume in TBI/PTD rats compared at day 7 and day 14. • Increased BV/TV and osteogenesis indicated by the number of osteoblasts per bone perimeter OCN staining.
Liu et al. [18,51]	SD rats	Weight-drop with craniotomy Mild TBI	Craniotomy with a diameter of 1.5 cm at 0.5 cm from the median suture. A force of 4 N applied through a drop from height of 5 cm.	Closed femur fracture fixed with IMN.	• Increased total and callus bone volume in TBI/FX rat at day 14. • Increase in BMD in TBI/FX relative to the isolated fracture group at day 21.

of callus formation and remodeling. Nevertheless, the ability of TBI models to mirror the clinical observation of "accelerated fracture healing in concurrent TBI" implies the existence of shared systemic and local mechanisms that potentially elucidate the heightened osteogenesis following TBI.

4. Fracture callus histology features in combined TBI and fracture animal model

Most preclinical studies have found evidence of TBI induced robust formation of fracture callus at early stage and shortened fracture healing duration using X-ray and micro-CT [17,45,49]. In addition, heterotopic ossification in close vicinity or remote from fracture site was not reported *in vivo* studies, and the general morphology of fracture callus showed no significant difference from normal callus except its size. Zhang et al. observed an "accelerated cartilaginous callus formation, prominent initiation of mineralization and increased angiogenesis" in combined TBI and open femur fracture mice [49]. Lin et al. reported a denser and disordered callus formation with more osteoblasts were observed at day 21 in the combined TBI and tibia fracture group than fracture only group. Higher expressions of Runx2 and Osterix, key osteoblast-differentiation transcription factors in callus were also observed at day 21, but not at day 35. McDonald et al. reported higher total bone callus volume and cartilage area at day 21 following close-head WD combined TBI and fracture mice, while the difference was diminished at day 35 [47]. Further, at day 21, increased bone remodeling activity in closed head TBI and fracture mice as evident by increased TRAP + staining at the fracture callus, indicating a higher degree of remodeling activity at the later healing stage [47].

5. Altered early-stage fracture healing response after TBI

The formation of a hematoma, characterized by blood supply disruption and clot development, establishes a specialized healing microenvironment that kickstarts the biological cascade essential for fracture healing. Essentially, the hematoma serves as an initial scaffold facilitating cell adhesion, fibronectin network formation guiding microvessel development, platelet activation, and the sustained release of cytokines and growth factors crucial for supporting bone regeneration [53]. Among them, HIF-1α acts as chemotaxis factor by recruiting osteoclasts, chondroblasts and immature osteoprogenitor cells to the fracture site and facilitate MSCs differentiation into osteoblasts. Further, HIF-1α elevates VEGF expression in osteoblasts to promote angiogenesis during fracture repair. Li et al. investigated the temporal and dynamic change of serum levels of HIF-1α, starting from admission to 24 weeks after surgery in individuals with combined TBI and fracture group and fracture alone [54]. Serum HIF-1α level was significantly higher than that in fracture group at day 3 and day 10 post-op and reached the peak earlier in combined injury group than isolated fracture group (day 10 vs day 14). HIF-1α level at fracture hematoma also showed similar trend. Xu et al. later proposed HIF-1α/SDF-1/CXCR4 signaling axis may be involved in the accelerated fracture healing after TBI [55]. Type H vessel is a bone specific vascular type that promote the coupling of angiogenesis and osteogenesis [56], also facilitate fracture healing [57]. Factors including HIF-1a, VEGF, PDGF-BB, SLIT3 and Notch signaling pathway are involved in the regulation of type H vessels within bone [56–59]. Zhang et al. reported TBI promoted cartilage callus formation, prominent initiation of mineralization and significant filtration of type H vessels at day 7 and day 14 [49].

In a clinical study, Cadosch et al. found that the serum from combined TBI and fracture injury induce a shift towards anti-inflammatory response evidenced by lower T lymphocyte proliferation rate and increased expression of immunosuppressive cytokines including IL-4, IL-10 and TGF-B [60]. Further, the study by Miclau et al. focused on acute phase of fracture healing and reported altered systemic and local immune response in combined TBI and fracture mice [61]. Dampened neutrophil and mast cell infiltration into the fracture hematoma were found at 6 h and 24 h post injury in combined TBI and fracture mice model. They proposed reduced mast cell numbers and mast-cell derived CXCL10 expression in combined TBI and fracture mice leads to decreased bone remodeling in the later phase [61].

Given the context of fracture healing microenvironment, the macrophages polarize into two phenotypic (M1 and M2) and functionally diverse subsets. The timely progression and the right balance of M1/M2 macrophages are crucial for successful fracture healing. The M1 macrophages contribute to the establishment of initial proinflammatory phase by secreting pro-inflammatory cytokines, recruitment of immune cells, removal of debris and production of growth factors. In contrary, the M2 macrophages promotes the resolution of inflammation and fracture repair and secrete anti-inflammatory cytokines such as arginase-1, IL-4, IL-10, and transforming growth factor beta (TGF-β). Zhang et al. investigated the M1 and M2-type macrophage proportion at clavicle fracture callus upon surgery at 1 and 2 weeks after initial TBI injury [26]. A markedly higher proportion of anti-inflammatory M2 macrophages (CD206+) and significantly decreased of proinflammatory M1 macrophages (COX2+) were observed in the fracture callus which correlated with the larger callus and shorter time to fracture union.

6. Low bone mass in non-injured bone after TBI

Some previous clinical studies however revealed that isolated TBIs demonstrated suboptimal BMD in uninjured bone and increased potential fractures risks [62,63]. Rats experiencing TBIs displayed with bone mass loss and impaired fracture healing, as indicated by the reduction in cortical thickness, content, and trabecular volume [64,65]. Of note, no difference was found in locomotor function, indicating factors other than immobilization attributes to altered bone metabolism in TBI rats [65]. Pituitary dysfunction and growth hormone deficiency are common complications in TBI survivors, which may blunt bone healing process and cause delayed union [66]. Similarly, bone loss caused by CCI in mice were rescued by growth hormone therapy and increased IGF-1 content, further substantiating the connection between decrease of bone content and TBI injury [67]. Recent study by Keller et al., proposed that TBI exerts its negative effects on intact bone by the hyperactivation of sympathetic nervous system via Adrb2 signaling, which latter inhibits osteoblast proliferation and bone formation, and promote osteoclastogenesis and bone resorption [68].

Genet et al. highlighted the significant involvement of local inflammation and resident macrophages in the development of neurological heterotopic ossification (NHO) following SCI [69]. In their study, the absence of local injury failed to induce ossification in the SCI model. Depletion of resident macrophages using clodronate-loaded liposomes resulted in a 90 % reduction in NHO volume and prevented its formation. In NHO animal models, local injuries or inflammation were found to be crucial in TBI and SCI, as isolated TBI or SCI alone did not induce NHO. However, the combination of TBI or SCI with multiple traumas (fracture, muscle crush, or both) increased the prevalence of NHO [10,69,70]. This aligns with clinical observations where trauma can exacerbate NHO development resulting from TBI and SCI, with NHO often occurs near the fracture sites [71,72]. Garland et al. observed that around 90 % of TBI individuals with an elbow injury developed NHO [73]. This underscores the paradoxical impact of TBI on both healthy and fractured bones. The presence of peripheral injury (such as a fracture) and local inflammation may be necessary to enhance fracture healing after TBI. Future studies comparing TBI's effects on cellular and molecular changes in injured versus healthy bone could provide clarity in this complex interplay.

7. Potential mechanisms

7.1. Serum mediated fracture healing acceleration after TBI

Blood brain barrier (BBB) dysfunction is a common pathological sequala initiated at the early phase of TBI [81]. Following primary injury after brain trauma, secondary injuries trigger processes such as neuronal death, reactive gliosis, pericyte dysfunction, and activation of proteases, leading to mitochondrial damage, lipid peroxidation, extracellular matrix (ECM) cleavage, antibody migration into the brain, and heightened inflammation. These changes then induce cleavage of endothelial tight junctions, increased permeability, and finally cause the leakage of the blood–brain barrier [82,83]. It is postulated that TBI induced blood–-brain barrier dysfunction may lead to subsequent release and migration of brain-derived osteogenic or growth promoting factors including neuropeptides, growth factors and endocrine factors into the blood circulation and exert enhanced healing effect at the fracture microenvironment (Table 3). Indeed, the serum and cerebrospinal fluid from combined TBI and fracture injury individuals and animals directly promoted the proliferation and osteogenic differentiation of various cell types, including rat calvaria osteoblasts [76], hFOB cells [60,77], fibroblasts, MG-63 and of hBM cells [74], multipotential mesenchymal stem cells [48](Table 2). Further reinforcing this hypothesis, the proliferation of bone marrow stem cells was boosted when exposed to brain cortex tissue supernatant post-TBI, underscoring the osteogenic potential of brain-derived factors [84]. Subsequent efforts to replicate accelerated fracture healing through plasma transfer or serum injection demonstrated that systemic or synchronized intramuscular administration of TBI serum at the fracture site induced faster remodeling and accelerated healing, as evidenced by a rapid decrease in callus size and earlier formation of lamellar bone[[79,80]]. Notably, *in vivo* injection of TBI-derived serum into surrounding muscle reproduced the osteo-inductive effects and accelerated fracture healing phenotype, emphasizing the systemic release of factors promoting fracture repair [79,80]. On this basis, evaluation of potential growth factors and inflammatory mediators related to bone metabolism, alongside monitoring serum fluctuations, revealed dynamic patterns associated with enhanced fracture healing. Herein, potential humoral factors that have been focused on prior studies are selected for review and discussed.

7.2. Parathyroid hormone (PTH)

Trentz et al. first reported an imbalance of bone formation and reportion serum markers after brain injury [85]. Instant elevation of PICP, a serum marker for enhanced osteoblastic activity and bone formation, were found after brain injury indicating enhanced bone regeneration immediately after brain injury. While individuals with combined TBI and fracture showed lower ICTP compared to fracture-only individuals during initial 7-day study period after injury. Elevated concentration of parathyroid hormone in the serum has been reported in individuals with combined TBI and fracture injury at the early phase of the injury [[13,31,40,85]]. PTH is crucial regulator in maintenance of calcium and phosphorus equilibrium in the serum. PTH stimulates bone turnover by both the promotion of bone formation and bone resorption by producing both osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL). Although continuous high level of PTH, hyperparathyroidism, is associated with high bone turnover, osteoporosis, and high risk of fractures, but intermittent administration of PTH has been proven to enhanced osteogenesis rather than increased bone catabolic effect. Shorter healing time were reported in multiple fracture sites with intermittent injection of teriparatide, a recombinant human PTH analogue [86–90]. Post-hoc analysis by Aspenberg's group reported an earlier callus formation in high-dose PTH-treated when compared to low-dose PTH and control group in distal radius fractures [89].

Table 3
Osteogenic and mitogenic effect of TBI/FX derived serum.

Authors	Year	Study design and serum source	Serum extraction post-injury	Major findings
Boes [48].	2006	43 rats 23TBI/FX vs 20 FX	2 days	•Increased proliferation of C3H10T1/2 (multipotential mesenchymal stem cell) •No effect on mature osteoblast (MC3T3-14) nor fibroblasts (NIH3T3)
Eid [74]	2006	22 individuals 10 TBI/FX and 12 control individuals	1,3,5,7,10,14,21 days	•Stimulation of proliferation of fibroblasts, MG-63 and of hBM cells •Significant decrease of apoptosis of hBM cells compared with the control serum. •Similar matrix production and osteogenic gene expression of hBM cells •Decreased activity of alkaline phosphatase in MG-63 and hBM cells
Klein [75]	1999	16 rats 8 TBI and 8 control rats	1, 2, 7 and 14 days	•Osteogenic effect from serum expected to close 48 h post-CNS injury
Bidner [76]	1990	32 individuals 8 individuals each for TBI, TBI/FX, FX, healthy	1–65 days	•Increased mitogenic activity of rat calvaria osteoblasts cultured with serum of TBI and TBI/FX individuals.
Gautschi [77]	2007	84 individuals 11 TBI, 26 non-traumatic brain pathology (NTBP), 47 control.	—	•Highest proliferation rate of hFOB cells in TBI group
Gautschi [60]	2009	61 individuals 12 TBI/FX, 21TBI, 19 FX, 9 control	6, 24, 72 and 168 h post-injury	•Increased proliferation rate of hFOB1.19 cells at 6, 24 and 72 h post-injury ($P < 0.05$). •Increased expression of ALP, cathepsin K and RUNX2 mRNA. •Exclusive expression of serine protease 7 in TBI/FX serum.
Cadosch [78]	2010	13 TBI, 14 FX, 10 healthy	—	•Serum from individuals with traumatic brain injury induced a significant increase in the rate of proliferation of primary skeletal muscle cells. •The serum from individuals with a traumatic brain injury did not

(*continued on next page*)

Table 3 (*continued*)

Authors	Year	Study design and serum source	Serum extraction post-injury	Major findings
				increase the proliferation rates of human T-lymphocytes or monocytic cells when compared with the serum from individuals with an isolated fracture or that from controls
Fang [79]	2020	Serum taken 20 rats each from TBI and blank.	2, 5, 8, 12, 15, 19, 22, 26 days	•Intramuscular injection of serum from TBI rats reproduced enhanced fracture healing by showing larger callus and higher BMD at 2nd week
Sarı [80]	2021	72 rats	24 h or 7 days	•Osteo-inductive effect after head trauma transmitted between individuals by means of serum transfer

TBI: traumatic brain injury; TBI/FX: combined traumatic brain injury and fracture; FX: fracture; I.M.: intramuscular injection.

7.3. Leptin

Extensive efforts have been made to explain the accelerated fracture healing in TBI scenario considering leptin's crucial role in crosstalk between body metabolism and regulation of bone mass accrual. Briefly, leptin demonstrates dual role as a central inhibitor and peripheral promotor in bone formation [91–94]. Leptin's effect on bone is mediated by sympathetic tone, which is supported by the persistent high bone mass phenotype observed in *Adrb2 null* mice and cannot be corrected by the central administration of leptin [95,96]. Ob/ob (leptin deficient) mice is characterized as hyperglycemia, hyperinsulinemia, a metabolic syndrome, obesity, and high bone mass [91]. Infusion of leptin in the third ventricle in the brain decreased the bone volume and bone mass to the level of wild type. Whereas peripheral administration of leptin in ob/ob mice restored bone growth, bone formation rate, osteoblastic number and activity, and upregulated VEGF activity [93,94,97]. Moreover, exogenous leptin treated wild type mice also showed accelerated callus formation and remodeling of callus, significantly higher BMD, BV/TV, BV, cortical thickness, and cortical number [94]. In addition, site specific bone regulating effect were observed in ob/ob mice, as evidenced by decreased BMC, BMD, and biomechanical properties in the femur, while in the spine, the results were opposite [93,98]. *In vivo* studies have reported a higher concentration of leptin in CSF and serum detected after concomitant TBI and fracture injury [99–101]. Increased fracture callus formation after TBI were eradicated in ob/ob mice, as evidenced by high non-union rates, and loss of superior callus formation, biomechanical properties and overall lack of bone remodeling, and authors summarized as "leptin deficient mice showed bone formation almost everywhere but in the fracture gap" [102]. In combined trauma group, hyperinsulinaemic stress reaction were more pronounced and led to a greater reduction in osteocalcin (OCN) synthesis and release, and this anomaly were lost in ob/ob mice [103]. Tretz et al. reported also lower levels of osteocalcin in TBI individuals with or without concomitant fracture in the first week of injury [85]. The authors proposed that in TBI scenario, sympathetic nervous system activation after brain injury via increased central and peripheral leptin causes the disruption of insulin-OCN-Feed-Forward-Loop [104]. This may lead to disinhibition of OPG synthesis, followed by decreased osteoclast activation [105–107].

7.4. Exosomes and micro-RNAs (miRNA)

Exosomes have gained increasing attention as cell-free therapy with merits including little immune hypersensitivity reaction for bone regeneration. Briefly, they are generated from endosomal compartment by the budding of multivesicular body (MVB) membrane, subsequent formation of intraluminal vesicles (ILVs), and then the fuse of ILVs with the cell membrane and final release to the extracellular environment and thus formation of exosomes. Exosomes exert diversified physiological functions and cellular functions including signal transduction, immune response and many others by their complex compositions including proteins, lipids, mRNAs and miRNAs [108]. Among them, exosomes can regulate bone regeneration and remodeling by transmission of miRNAs into target cells and modulate protein expression. Within the skeleton, stem cells derived exosomes containing miRNAs were found to promote bone regeneration by regulating the osteogenic differentiation [109–111], bone matrix mineralization [112,113], and promotion of angiogenesis [114–116]. In addition, exosome incorporated biomaterials were found to alter local bone immune response by promoting polarization of anti-inflammatory M2 macrophages for prevention of excessive inflammation which may hinder bone healing progress [117–119].

Liu et al. reported that serum and callus miRNA-26a-5p and miRNA-92a-3p levels were elevated in TBI individuals [51,52]. Using their agonists, increased osteoblast differentiation of MC3T3-E1 cells were shown by influencing IBSP/PI3K/AKT and PTEN/PI3K/AKT pathways. Direct administration of both agomiR-26a-5p and agomiRNA-92a-3p at fracture site in mice showed accelerated fracture healing. Further, consecutive local injections of serum exosome (TBI-Exos) from individuals sustaining combined fracture and TBI into murine fracture mode showed larger callus volume and improved bone index values. MiR-21-5p, an osteogenic-related mi-RNA derived from pituitary gland, can induce bone formation, and increase trabecular numbers [18]. TBI-Exos contained markedly enriched miR-21- 5p in patient serum, and the TBI-Exos demonstrated to accelerate bone remodeling *in vivo*, and *in vitro* by promoting hMSCs proliferation and osteogenic differentiation. Downregulation of SMAD7 signaling by miR-21-5p was proposed as the underlying mechanism. Lin et al. reported co-culture of MC3T3-E1 cells with exosomes extracted from combined TBI and fracture mice showed increased proliferation and osteogenic potential [17]. Three potential mi-RNA were screened after mi-RNA sequencing, namely miR-22–3p, miR-34a-5p, miR-378a-3p. Transfection into MC3T3-E1 cells demonstrated markedly increased cell matrix mineralization. However, the source of these exosomes and miRNAs were not traced in Lin's study. Interestingly, Xia et al. traced the source of small extracellular vesicles (sEVs) to the brain CA1 and DG regions of the rat hippocampus and proposed the fibronectin-1 enriched on the exosome membrane surface for targeted delivery to the bone and osteoprogenitor cells at the fracture site [50]. Further, miR-328a-3p and miR-150-5p were found to be enriched in the sEVs from rats and individuals after TBI and promoted osteogenesis by directly targeting the 3′UTR of FOXO4 or CBL, respectively.

7.5. The role of peripheral nerves and neuropeptides in normal fracture healing and TBI

Sensory, sympathetic and parasympathetic nerves are all innervated in the bone and orchestrate bone homeostasis and healing, including the transmission of bone derived pain, regulation of angiogenesis and osteogenesis, monitor of bone remodeling and healing during fracture, and mobilization of hematic poietic stem cells from bone marrow niches [120]. Further, aberrant changes in spatial distribution pattern, shapes, and density of the nerve fibers in the bone reflect underlying abnormal

state of bone healing [121]. Growing evidence suggest the direct contribution of altered central and peripheral nerves and neuropeptides contribute to accelerated fracture healing after TBI (Fig. 4).

7.6. Sympathetic nerves and norepinephrine (NE)

Sympathetic nerves express tyrosine kinase and promote norepinephrine expression in bone [122,123]. Cylindrical or corkscrew morphology around the vessels in bone marrow and periosteum are its innervation features [124]. In a nutshell, the increase of sympathetic tone inhibits bone mass accrual. Whereas the parasympathetic nerves show axons expressing vesicular acetylcholine transporter (VAChT) and choline acetyltransferase (ChAT) in long bones and antagonize the sympathetic tone to increase bone mass accrual [125].

In the intact bone, the release of norepinephrine by the sympathetic nervous system through Adrb1(mice and human) Adrb2 (mice) receptors signal the inhibition of bone formation and stimulation of bone resorption [96,126]. The activation of Adrb2 signals leads to inhibition of osteoblasts proliferation and higher expression of RANKL for osteoclast differentiation [95,127]. This was further evidenced by the high bone mass type in null *Adrb2* mice, and the use of beta-blockers in preventing bone loss in the ovariectomy (OVX) mice [128,129]. Conversely, the rise of parasympathetic tone antagonizes with the sympathetic tone to increase bone mass accrual [125].

Retrospective studies have identified the paroxysmal sympathetic hyperactivity (PSH), or autonomic dysreflexia, as a significant risk factor for heterotopic ossification in TBI individuals [71,72]. Central regulation of autonomic function disperse throughout the neuroaxis, and injury to any of the nerve structures in four hierarchical levels including spinal, bulbopontine, pontomesencephalic, and forebrain can lead to dysregulation of autonomous nervous system [130]. The loss of supraspinal control of sympathetic outflow after TBI leads to uncontrolled release of norepinephrine (NE) and catecholamines, and affect peripheral organs and systems [131].

Interestingly, within the intact bone, the suppression of NE content were found for 24 h after TBI and TBI stimulation of bone formation rate was initiated shortly 2 days after TBI and returned to normal level 8 days later as indicated by mineral apposition rate [132]. The stimulation of bone formation was preceded by the rise 2-arachidonoylglycerol (2-AG) and decrease of NE from sympathetic terminals in bone by the regulation of cannabinoid CB1 receptor at the trabecular metaphysis.

Previous studies reported macrophages exert tissue specific response to distant injury stimuli in which local proliferated macrophages dominated over recruitment in expansion of macrophage numbers [133]. For instance, alveolar macrophages showed protective role in reducing pneumonia post myocardial infarction. In contrast to the concept of decreased peripheral bone mass after increased sysmpathetic nervous system activity, recent studies have reported the increased sympathetic tone in TBI scenario may be the potential mechanism behind TBI-accelerated fracture healing. Zhang et al. proposed that TBI exerted the activation of hypothalamus-sympathetic nerve descending pathway and subsequent acute myelopoiesis and anti-inflammation microenvironment in the fracture callus [49]. TBI induced elevation of sympathetic tone was evidenced by the elevation of NE concentration in the serum and bone marrow, and noradrenergic fibers Tyrosine Hydroxylase + in the bone marrow at day 7 and day 14. The TBI-induced hyperadrenergic response in the bone marrow induced the expansion of hematopoietic progenitors and robust hematopoiesis of Ly6C^{low} monocytes in bone marrow through β3-adrenergic signaling, which was consistent with previous findings in the intracerebral hemorrhage individuals and experimental models [134]. Subsequently, anti-inflammatory microenvironment was shaped through β2-adrenergic signaling as evident by polarization of bone marrow macrophages towards M2 phenotype (F4/80+CD206+) at day 7 and day 14 in the bone marrow. Keller et al. also proposed hyperadrenergic activity after TBI induce systemic bone loss in the non-injured bone while facilitates fracture healing, while NE directly elevated VEGFA and CGRP expression and subsequentially stimulated callus neovascularization through the formation of CD31+Endomucin + type H vessels during fracture healing [68].

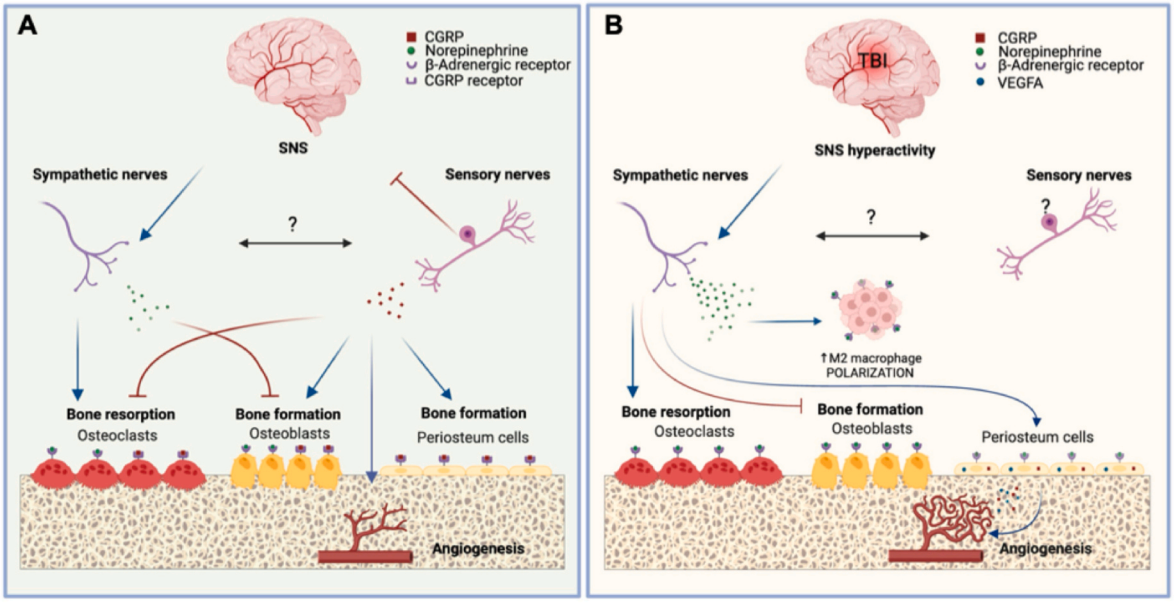

Fig. 4. Sympathetic and sensory nerve regulation of fracture healing in normal and TBI scenario. (A). Sympathetic and sensory nerve regulation of bone in non-injured brain. The release of norepinephrine decreases bone mass via B-adrenergic receptors to inhibit osteogenesis and promote osteoclastogenesis. Sensory nerves release CGRP to increase bone mass accrual by promoting osteogenesis (periosteum stem cells and osteoblasts), angiogenesis (endothelial cells) and inhibition of osteoclastogenesis. The sensory nerves in turn down regulates sympathetic nervous system (SNS) activity through skeletal interceptive pathway (B). SNS hyperactivation after TBI leads to mass release of NE. In the fractured bone, NE facilitates fracture healing by promoting M2 macrophage polarization and neovascularization via periosteum cells through the release of VEGFa and CGRP. In the intact bone, NE also induce systemic bone loss in TBI scenario. The interaction between NE and CGRP during both common fracture healing and those combined TBI and fracture scenario remains to be explored. Created with BioRender.com.

7.7. Sensory nerves and calcitonin gene-related peptide (CGRP)

Spinal afferent nerves disperse throughout the bone marrow and the periosteum with distinct pattern of spatial distribution. Both CGRP+ and unmyelinated, nonpeptidergic (CGRP-), are also seen in the bone marrow cavity and periosteum [135]. CGRP + positive fibers display a longitudinal morphology parallelling with the bone marrow axis with varices along the fibers, with single bouton termination, in close encounter with blood vessels or at distance from them.

The important role of CGRP, a major neurotransmitter of sensory nerves, in bone homeostasis and regeneration has been reviewed previously [136]. In brief, CGRP is significantly expressed in the early and intermediate stages of fracture healing, promotes osteogenic differentiation of periosteum-derived stem cells, proliferation of BMSCs, and attenuates apoptosis of osteoblasts [137–141]. *In vitro*, the activation of PI3K/AKT signaling pathway by CGRP, promoted BMSCs differential towards endothelial progenitor cells (EPCs), subsequent tube formation, and its co-coculture with BMSCs lead to increased osteogenic differentiation [139].

In fracture healing, neuropeptide CGRP is indispensable as α-CGRP-deficient mice demonstrate significant reduction of osteoblasts, high rate of incomplete bridging and non-union [142]. Increase of CGRP levels in the blood serum is observed in both human samples and also from animal study following fractures and the major origin is suggest to be from fracture callus only, as *Calca* mRNA levels and the majority of CGRP immunofluorescence were only found to elevated in the regenerating bone tissue, but not in other organs including hippocampus, liver, spleen, lung, muscle, white and brown tissues [142]. CGRP is reported to be another potential factor effecting fracture healing response in TBI scenario. Song et al. showed elevated CGRP concentration in the brain tissue (72 h), whereas CGRP level in the serum and muscles surrounding the fracture site was not changed during early phases of fracture (at 72 h and 168 h after TBI) [143]. Zhu et al. also reported persistent rise of CGRP concentration in the hippocampus in TBI rat at 168 h post injury, and proposed TBI induced CGRP expression leaked into the blood circulation after BBB dysfunction [19]. Indeed, CGRP is considered a responsive protective agent for its vasodilatory effect and overexpressed following cerebral ischemia [144]. Their micro-CT data demonstrated an accelerated fracture healing and mineralization as significantly higher bone mineral density and bone mineral content of fracture callus formed in TBI and fracture group compared with control group at 4 weeks after fracture.

Direct electrical stimulus lead to increased CGRP release from dorsal root ganglion, and led to higher rate of ectopic bone formation and intertransverse process fusion independent of decortication and bone grafting [145], significantly ameliorated tibial bone loss and bone microstructure induced by unloading in rats [146]. Further, enhanced CGRP release at the bone defect site mediated type H vessel formation, which latter was reported to couple osteogenesis and angiogenesis in fracture repair, bone defect regeneration, spine fusion, and ossification of endplates [56,147–149]. *In vivo* study using in both fracture and OVX rats, fracture fixation using biodegradable magnesium intramedullary nail led to rapid release neuronal CGRP in both the peripheral cortex of the femur and DRG, which promoted fracture healing with improved biomechanical indexes [150]. Further, Jiang et al. reported an increasing trend of CGRP concentration at dorsal root ganglion (DRG), but not in the serum, after both TBI or SCI combined with tibia fractures at day 14 and day 28 than that of the peripheral nerve injury and simple fracture group in rats. Again, fracture combined with TBI or SCI demonstrated accelerated fracture healing as evidenced by higher content of hard callus, trabecular bone at 2 weeks after injury [151].

7.8. Nerve growth factor (NGF)

NGF is an axon guiding molecule that plays important role in growth, differentiation survival, plasticity of CNS and PNS of cholinergic nerves [152]. NGF, via its high affinity tyrosine kinase receptor A (TrkA), regulate both intramembranous and endochondral fracture healing process [123,153,154]. NGF is abruptly expressed in the early phases of post-fracture, including stress fracture, calvaria defects repair and long bone fracture animal models. In stress fracture model demonstrating intramembranous fracture healing, at the most robust bone matrix deposition timepoint, majority of NGF-reporter cells resided within the hard callus. And the majority of NGF reporter positive cells were found to be periosteal/stromal cells in the early stages and osteocalcin (OCN+) bone-lining osteoblasts at day 3 and day 14 after fracture respectively [153]. Interestingly, sensory innervation preceded vascularization and ossification as indicated by their respective peak activity. Different from stress fractures, in calvaria bone defect repair, macrophages were dominant cells secreting NGF at inflammatory phase which its expression is induced by proinflammatory cytokines including IL-1β and TNF-α [155]. Abrupt NGF expression promoted axonal ingrowth and sprouting at the bone defect, and further promoted angiogenesis and bone regeneration. In long bone fracture model, NGF-TrkA were shown to be expressed at the chondro–osseous transition zone [156]. Exogeneous b-NGF administration in cartilaginous phase of fracture repair showed acceleration endogenous endochondral fracture healing as evidenced by upregulation of endochondral ossification markers [157].

In addition, NGF binding with its low-affinity receptor p75 showed migration of MSCs to the defect site, and the disruption of this signaling pathway impaired cell migration, ossification, and injury associated macrophage activation in calvaria defect repair [158]. Further, disruption of TrkA signaling using 1NMPP1-treated $TrkA^{F592A}$ mice, a chemical-genetical inhibition of TrkA activity, led to significant decrease of nerve innervation (YFP^{+} nerve fibers), vascularization ($CD31^{+}$) and ossification (ALP activity) after fracture [153]. In individuals with combined clavicular fracture and TBI individuals, earlier rise and higher content of NGF were found in the serum and fracture callus tissue, which peaked at 14 days after fracture and increased expressions of CD31, NGF, and VEGF makers correlated with shorter fracture healing time [21]. Similar results were seen in rabbits and rats with combined TBI and fracture injury demonstrating elevated NGF concentration in both serum and CSF with other bone regeneration factors including IGF-1, BMP-7 and TGFβ1 [20,159].

7.9. Perspectives

Over the past decades, the unique clinical finding regarding the exuberant callus formation in the early stage, with overall accelerated bone union with combined brain injury, have undoubtedly drawn continuous attention and effort from the clinical community to explore its essential mechanisms. Nonetheless, high quality clinical studies with larger sample size are still needed to investigate its incidence, risk factors and injury patterns regarding both the CNS and bone fractures, for which may provide essential information for further exploration into its mechanisms.

Despite controversies using myriads of "combined TBI and fracture" animal models, the reproduction of phenotype, "accelerated fracture healing by TBI" seems successful by demonstrating attributes of fracture callus which are observed in clinical settings; specifically, the enlarged callus formation at the early stage of fracture healing and overall shorter time to bone union. The investigation of its mechanisms may provide new avenue for treatment strategies in the early stages to mitigate fracture complications. Nonetheless, a comprehensive understanding of its underlying mechanism, the impact of TBI on fracture healing, and the potential for enhancing fracture repair in the initial stages necessitates further detailed *in vivo* investigations, entailing refinement and standardization of animal models. For example, both preclinical and clinical studies suggest a correlation between the location and severity of TBI and the subsequent fracture healing response. Factors such as the affected brain regions due to injury location and the severity of TBI (e.g., impact speed, depth, contact duration) could potentially influence the

brain-derived factors or specific central-to-peripheral neural pathways that trigger the fracture healing response initiated by TBI. Moreover, additional validation is essential to ascertain whether the composition and fracture healing responses of these fracture calluses at different stages of healing in animal models align with clinical samples. Extended periods of follow-up involving histomorphometry analysis and radiological assessments are imperative for this validation process.

Previous studies searched into brain-released factors upon TBI, demonstrating osteogenesis effects *in vitro*, from the blood circulation and CSF, fracture healing micro-environment including dynamic changes of immune and inflammation markers in the fracture callus, and direct CNS control towards the hematopoiesis and differentiation and impact on fracture healing through the activation of sympathetic nerves. However, the origins and release patterns of brain-derived factors, along with their specific effects on the fracture microenvironment, require additional validation through well-designed preclinical and clinical studies. Moreover, investigating the temporal and spatial changes in the expression and release origins of neuropeptides and neurotransmitters, the distribution characteristics of peripheral nerves, and their potential orchestrating roles, as well as exploring the role of neuroimmune interactions within the fracture callus niche in combined TBI and fracture scenarios, represents a compelling area of interest for further research. To note, the main contributing factors, the primary and secondary factors, as well as the synergistic interactions of these potential factors during accelerated fracture healing process following TBI, warrant further investigation.

Noteworthy, the effect of TBI on intact bone is less investigated but of great significance as it will advance the discovery of new factors controlling bone homeostasis, therefore providing new avenues for tackling the more prevalent silent bone diseases (i.e. osteoporosis). In vivo studies reported a rapid loss of bone mass after TBI at the non-injured skeleton achieved in only 1–2 weeks after TBI [64,65,68], which the speed of bone loss is faster compared to that of the traditional unloading and ovariectomy animal models. Furthermore, an investigation into the long-term functional outcomes in individuals experiencing accelerated fracture healing is imperative, encompassing an assessment of factors such as postoperative ambulation and overall recovery.

The downstream cells within the bone that responds to TBI, and its stimulus needs further study, including skeletal lineage cells in the periosteum and other cells in the bone marrow niche. Single-cell RNA-seq (scRNA-seq) techniques can be used to identify the contributing cells and heterogeneity of cellular composition in the fracture callus and bone marrow niche that are involved during fracture repair, and their dynamic role in different fracture healing stages after TBI. Spatial transcriptomics will also aid in identifying potential interactions (cross-talks) amongst multiple types of cells.

We aspire that this study can offer valuable insights to catalyze more comprehensive research in this domain, facilitating the translation of discoveries from bench to bedside, thereby paving the way for innovative therapies targeting fracture healing and challenging bone conditions.

Conflicts of interest

None to be declared.

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