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REVIEW

Clinical management and innovation in fracture non-union

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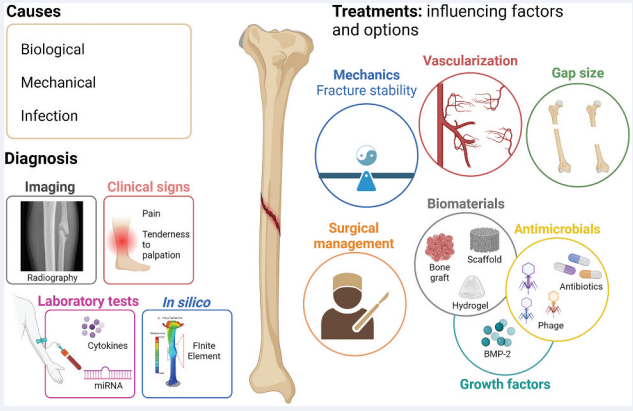
ABSTRACT

Introduction: With the introduction and continuous improvement in operative fracture fixation, even the most severe bone fractures can be treated with a high rate of successful healing. However, healing complications can occur and when healing fails over prolonged time, the outcome is termed a fracture non-union. Non-union is generally believed to develop due to inadequate fixation, underlying host-related factors, or infection. Despite the advancements in fracture fixation and infection management, there is still a clear need for earlier diagnosis, improved prediction of healing outcomes and innovation in the treatment of non-union.

Areas covered: This review provides a detailed description of non-union from a clinical perspective, including the state of the art in diagnosis, treatment, and currently available biomaterials and orthobiologics.

Subsequently, recent translational development from the biological, mechanical, and infection research fields are presented, including the latest in smart implants, osteoinductive materials, and *in silico* modeling.

Expert opinion: The first challenge for future innovations is to refine and to identify new clinical factors for the proper definition, diagnosis, and treatment of non-union. However, integration of *in vitro*, *in vivo*, and *in silico* research will enable a comprehensive understanding of non-union causes and correlations, leading to the development of more effective treatments.



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Bone; non-union; fracture; fracture-related infection (FRI); bone healing

1. Introduction

Despite remarkable advancements in the operative management of bone fractures in the last decades, fracture non-union still represents a major clinical challenge [1]. In most patients, fractures heal, and the bone integrity is restored, providing shape, structure, and function to the previously injured limb. However, some patients suffer irreversible interruption of the fracture healing process that it is described as *non-union* [2,3]. Whether a fracture progresses to heal successfully or proceeds to non-union, may be influenced by injury severity, mechanical stability of the fixation, comorbidities of the patient or the presence of a fracture-related infection (FRI). However, for most cases, the cause is often multifactorial and most often not explained by any single factor [4].

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

Article highlights

- Healing complications can occur, and failed bone healing is defined as fracture non-union.
- Many non-union cases have poorly understood etiologies, and treatment approaches can vary.
- Despite the advancements in fracture fixation, non-unions still occur and there is still a clear need for improved patient outcomes through refined management of non-union, as well as innovation in the prediction, diagnosis, and treatment of non-union.
- This review covers several aspects of non-union giving first a detailed description of the problem from a clinical perspective. It then describes the available clinical diagnosis and treatment options and finally it outlines all the recent translational development from the biological, mechanical, and infection research fields.

One of the fundamental challenges in dealing with non-unions, that has impacted the field enormously, is the difficulty in accurately and reliably diagnosing non-union based on specific objective diagnostic criteria. In fact, as early as 1847, the French surgeon Joseph-Francois Malgaigne noted the difficulty in differentiating between prolonged healing and non-union in long-bone fractures [5]. Despite the numerous advances in diagnosis and treatment that have been made since that time, a widely accepted definition of non-union has still not been established. In fact, numerous definitions have been proposed over the years that have differed in even the most basic aspects of non-union including the time required to define it, as well as the means, and measurements to evaluate healing. This situation leads to inconsistency in daily clinical practice, to the inability to design adequate clinical trials, to the inability to compare clinical trial data or to benchmark clinical practice outcomes and to a lack of high-quality data on management principles.

The overall worldwide non-union rate remains poorly described. In high-income countries (HICs), the incidence of non-union has been reported at between 2% and 10% [4,6], although the prevalence may be substantially higher in low- and middle-income countries and regions of conflict like is the case for FRI [7]. In terms of economic cost, the available data again comes primarily from HICs, with reported costs for non-union cases of approximately £16,000 in the UK [8], $25,556 in the US [9] and $4,788 AUD in Australia [6], with the difference reflecting the variety of health systems, and treatment strategies.

Since the rate of non-union has remained stubbornly high, despite advancements in fracture management, there is a clear need for innovation in the prediction, diagnosis, and treatment of non-union. This review aims to provide an overview of the various causes, current diagnostic tools, and clinical treatment strategies for non-union. Subsequently, the state of the art in research and development is provided to review the approaches that may in future optimize the diagnosis and management of non-union.

2. Definition and classification of non-union

A recent systematic review showed that only half of the currently published studies focusing on adult long-bone fracture non-union contain a definition of non-union [10]. Multiple definitions for non-union have been proposed in recent decades, all with their own advantages and disadvantages. The Food and Drug Administration (FDA) defines non-union as a fracture that is at least 9 months old and has not shown any signs of healing for the last 3 months [11,12]. This definition is primarily based on a time component and does not define radiographic healing and does not incorporate other important characteristics, such as clinical signs (e.g., pain). The lack of a clear, universally accepted, definition based on specific diagnostic criteria emphasizes the need for a consensus-based approach to the diagnosis of non-union centered on clinical, radiographical, and time-related criteria.

To classify non-union, the analyses of callus formation on conventional radiography are currently the most used indicators. Non-union can be broadly described to have three distinct phenotypes: hypertrophic, oligotrophic, and atrophic (Figure 1) [13]. Hypertrophic non-union is associated with a fracture site that has adequate vascularization and is filled with vital tissue, has abundant callus, and is generally associated with inadequate stability of the callus/fracture site. In contrast, atrophic non-union occurs when the blood supply is severely impaired leading to no bone healing response and a fracture site that is filled with fibrous tissue and no callus formation [14–16]. Atrophic non-unions are more commonly associated with patients with comorbidities, genetic predisposition, and biological risk factors, but are also associated with overly stiff constructs [17]. An oligotrophic non-union is characterized by adequate or reduced vascularization with minimal callus formation.

3. Causes of non-union

3.1. Risk factors for non-union

The risk of developing non-union may be associated with the underling injury, with damage to the periosteum and surrounding soft tissue [18,19]. Vascular injury, leading to insufficient blood supply, is another major risk factor for delayed healing, characterized by a hypoxic microenvironment and limited immune and progenitor cell recruitment [20]. However, the relevance of vascular damage in non-unions is still unclear as often no correlation to healing outcomes can be detected [21,22], which was recently highlighted as the vascularization paradox of non-union [23].

Comorbidities (e.g., diabetes, obesity, frailty, changes in gut microbiome) may also increase the risk of non-union as well as other fracture healing complications such as FRI [12,17,24–29]. These comorbidities have an element of unresolved inflammation and/or proinflammatory environment that can lead to an altered cellular composition of the early fracture callus with a changed cytokine profile that can ultimately delay healing [30–32]. For example, diabetes and obesity have an underlying proinflammatory response and have been associated with delayed bone healing, especially in lower limb fractures [33]. Osteoporosis is a condition that is also associated with proinflammatory mediators, in which the bones become weak, fragile, and more susceptible to fractures. Animal studies have shown a negative effect of osteoporosis on fracture healing, however, the existing clinical evidence has not been

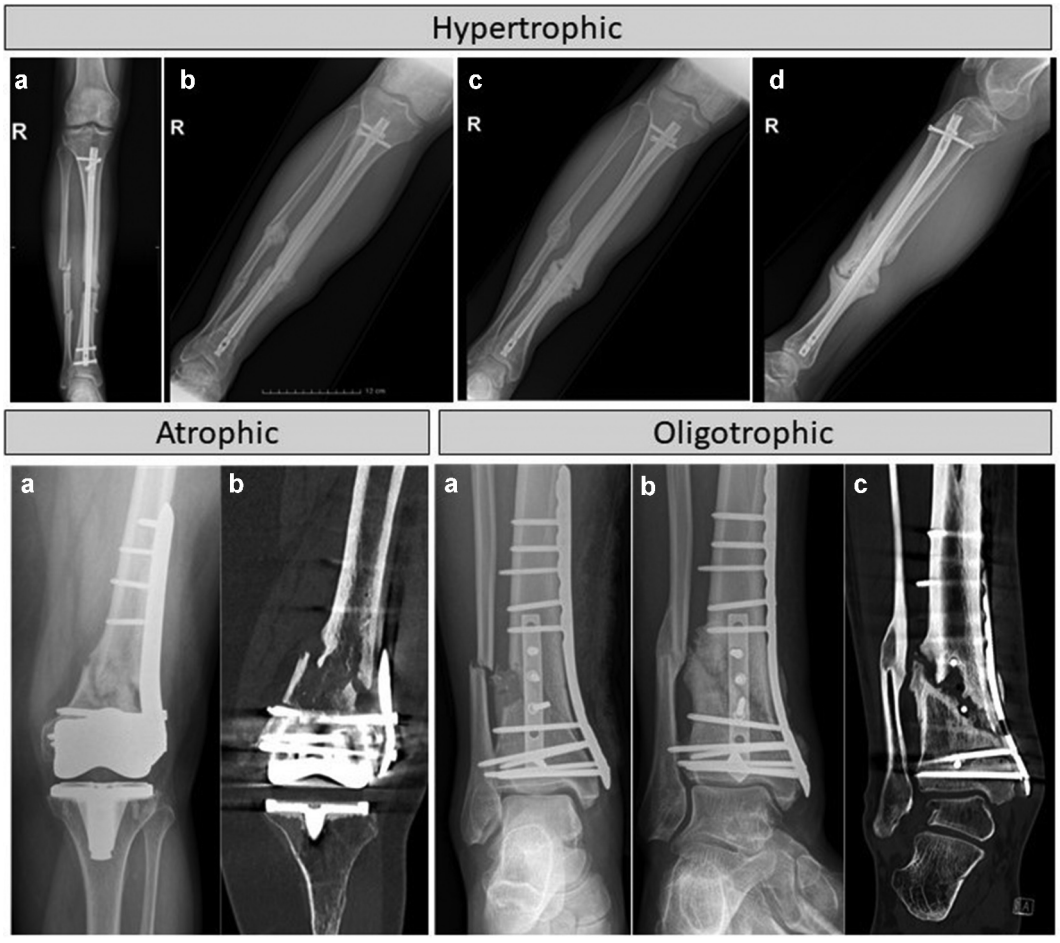

Figure 1. Hypertrophic, atrophic and oligotrophic non-union. **(top)** a fracture non-union of the tibia in a 43-year-old male patient after the operative treatment of an open tibia and fibula shaft fracture. (a) Anteroposterior radiograph of the tibia immediately after intramedullary nailing. (b) Anteroposterior radiograph of the tibia 8 months after the initial fixation. At that time, the patient reported pain during weightbearing. Therefore, the surgeon suspected a fracture non-union and decided to remove the two distal locking screws (dynamization) (c,d) anteroposterior and lateral radiographs of the tibia 18 months after the initial fixation. A typical hypertrophic non-union with a typical elephant foot shaped callus developed due to instability. **(bottom, left)** atrophic non-union of a distal periprosthetic fracture of the femur in a 70-year-old woman with total knee arthroplasty and subsequent osteosynthesis of the femur one year previously. (a) The fracture gap at the distal femoral metaphysis is clearly visible on radiographs and there is only minimal callus. (b) Coronal CT image one month later confirms the atrophic non-union, with no substantial callus formation and no osseous bridging. **(bottom, right)** oligotrophic non-union of a displaced multi-part fracture of the distal tibia in a 42-year-old man. (a) Anteroposterior radiograph of the ankle one day after osteosynthesis of the distal tibia. (b) Follow-up radiograph after one year shows non-union of the tibia with osseous callus on the medial side of the fracture line. The edges of the tibial fracture are sclerotic, and the fracture gap is larger than in the prior image. The laterally displaced distal fibular shaft fracture is fully healed. (c) Coronal CT image on the same day as the follow-up radiograph clearly shows the lack of bridging callus along the sclerotic fracture gap. Note that there is a screw within the non-union fracture gap.

convincing [26]. Aging is also associated with increased pro-inflammatory cytokines, likely due to a failure in the resolution of inflammation or aging-related senescence of the immune system resulting in impaired healing [34].

Further risk factors include medications such as nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids, which might affect bone healing [35]. However, while COX-2 inhibitors, but not nonselective NSAIDs, were associated with a higher risk of non-union [36]. The controversy on the data for nonselective NSAIDs requires further studies to reveal their true impact. Metabolic and endocrine abnormalities, such as vitamin D deficiency, calcium imbalances, central hypogonadism, thyroid disorders, and parathyroid hormone disorders are also related with non-union [37]. Vitamin D deficiency has a strong correlation with impaired bone healing, with up to 68% of non-union patients having a deficiency in vitamin D [37]. Smoking has been shown to decrease bone healing capacity by impairing the formation of new blood vessels [38], with nicotine regulating the expression of cytokines involved in vascularization and osteoblast differentiation [39]. Malnutrition, specifically a lack of protein, vitamins, and minerals, has been shown to impair bone healing capacity by decreasing the production of new bone tissue and delaying the formation of new blood vessels [40].

Finally, infection has long been recognized as a potential reason for non-union [41] although not all FRI cases progress to non-union. The clinical literature states that the rate of both non-union and FRI individually is approximately 5%, although the incidence increases significantly for certain complex open injuries [42,43]. The exact number of non-union cases that are infected is still relatively poorly described [44] although one study identified a 40% infection rate [45]. Furthermore, when more sensitive diagnostic modalities are applied, relatively even higher infection rates are revealed [46,47]. An overview of risk factors of non-union is shown in Table 1, which was readapted from Andrzejowski *et al*. 2019 [15] and Wildemann *et al*. 2021 [12].

Table 1. Risk factors of non-union.

Patient dependent factors		Patient independent factors	
Modifiable	*Non-modifiable*	*Non-modifiable*	
Smoking	Age	Severity of trauma and fracture pattern	Open reduction
Alcohol	Sex		Open fracture
Weight	Genetic predisposition		Wedge and multi-fragmentary fracture pattern
Nutritional deficiency	Comorbidities		Initial displacement
	Peripheral vascular disease	Compartment syndrome	
	Chronic inflammatory disease	Affected bone: highest in tibia	
	Chronic kidney disease	Fracture site in relation to vascularization zone	
	Diabetes	Inadequate reduction*	
	Medication	Poor mechanical stability by initial implant*	
	Opioids	Fracture-related infection	
	NSAID (COX-2 inhibitors)		

*Modifiable with proper surgical technique/revision.
Modified from [15] ©2019 Andrzejowski et al., licensed with CC BY 4.0 and from [12], © 2021, Springer Nature Limited.

3.2. Biomechanics and fracture non-union

From a mechanical perspective, the course of secondary fracture healing (consisting of both intramembranous and endochondral ossification [48]) is directed by the local interfragmentary strain which is controlled by several factors, including fixation stability, loading, and callus formation [15,49–53]. It is generally believed that successful healing occurs when interfragmentary strain is kept between certain limits. When these mechanical stimuli are outside these ranges, it can lead to altered fracture healing. In case the stimulus is too small to trigger a healing response, the fracture gap is under-stimulated, leading to atrophic non-union with little to no fracture callus formation [12]. On the other hand, if mechanical stimulation is too high (instability), and the callus that formed is not stable enough to decrease the amount of local strain, a mineralized bridge cannot form and hypertrophic non-union develops [12].

The proposed magnitudes for appropriate mechanical stimulation (often considered to be between 2% and 10%) trace back to the work of Perren [53–55], who postulated that bone formation cannot occur when the strain is larger than the tensile failure limit of bone and that cartilage cannot form when strain exceeds the ultimate tensile limit of cartilage. However, while Perren's strain theory acknowledged the existence of these limits, it did not postulate their magnitude for uneventful healing. In fact, the rule of "2-10%" of strain has been contradicted by recent findings [49], and so this must be revisited [56] and addressed in dedicated studies in the future.

4. Diagnosis and treatment

4.1. Clinical signs

The clinical presentation of a patient with non-union may include pain, tenderness to palpation at the non-union site, limited use of the extremity, or even gross motion at the non-union site. Historical and clinical examination often suggest the presence of non-union but rarely confirm the diagnosis. The only clinical finding confirming the diagnosis is gross motion at the fracture site.

4.2. Radiological (and nuclear imaging) signs

Imaging plays an important role in monitoring fracture healing and establishing the diagnosis of non-union but also has the ability to predict those fractures at high risk for non-union [57]. The mainstay of clinical imaging of fracture healing are still conventional radiographs, which are readily available, depict the callus, and allow the progress of fracture union to be monitored over time [58]. Radiographs are also typically used in non-union classification systems [59], e.g., with radiographic union scoring (RUS) that can be adapted to different anatomic regions. RUS can be used to predict union on radiographs and identify patients who are likely to benefit from early surgical treatment.

While conventional radiographs are the most used imaging modality to monitor fracture healing, they are limited, as the assessment is based solely on two radiographic projections without three-dimensional information, and part of the fracture can be obscured by metal instrumentation. For imaging of non-union of the spine, as well as for a more detailed evaluation of non-union of other anatomical areas, computed tomography (CT) has many advantages, such as a detailed depiction of the bridging callus even in the first stages of fracture healing, confirmation of a mechanically stable bridging callus, as well as visualizing areas of bone resorption or sclerosis associated with delayed union or non-union [60]. Additionally, CT allows a multiplanar assessment of even complex anatomic regions and shows increased density of the surrounding soft tissues during the formation of fluid collections. One problem of using CT is the presence of metal artifacts after fracture fixation with metal screws and plates [61,62].

4.3. Laboratory signs

The purpose of preoperative laboratory testing is to identify causes for compromised fracture healing such as FRI, metabolic, and endocrine abnormalities as well as other underlying comorbidities and should cover serum-level evaluation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, calcium, phosphate, magnesium, thyroid function test, intact serum parathyroid hormone, blood cell count, albumin, creatinine, alkaline phosphatase, and liver transaminases [37,63].

4.4. Diagnosis of an underlying infection

Once the non-union diagnosis has been established, a key question is whether an associated infection is present at the fracture site. There are clinical findings confirming or

suggesting the presence of infection, which have been an important part of the consensus definition of FRI [64]. The confirmatory clinical criteria for FRI are [1]: purulent drainage from the wound or pus encountered during surgery [2], sinus track communication with the fracture or wound dehiscence with communication to the bone or implant [3] microbiological culture of organisms and [4] histological features of infection [65]. The suggestive clinical criteria for FRI are [1]: pain (without weight bearing, new-onset or increasing over time) [2], local warmth [3], local erythema [4], local swelling [5], persistent, increasing, or new-onset wound drainage, beyond the first few postoperative days [6], new-onset joint effusion in patients with intra-articular fractures or with implants penetrating a joint (e.g., retrograde femoral nailing) [7], fever (≥38.3°C; 101°F). The confirmatory criteria were validated in an international multicenter study of 637 patients who underwent revision surgery for suspicion of FRI [66].

Routine laboratory workup on serum inflammatory markers, such as white blood cell count (WBC), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) has a limited diagnostic value since they are not specific for FRI [67–71]. However, elevation of these parameters in combination with other suggestive signs of infection, like local redness, warmth, and swelling, should raise suspicion for an ongoing underlying infection [66,72]. Certain non-unions are associated with subclinical occult infections and are an unexpected finding diagnosed by intraoperative cultures [73] and have been reported in 7% to 42% of the patients with a presumed aseptic nonunion [47,74–77].

FRI presents as a separate group of patients with distinct imaging features. Infected non-unions can be best identified on imaging when there is rapid development of zones of bone resorption and erosions. This is often only seen with some considerable delay on radiographs but can be detected sooner on CT, especially in non-osteoporotic patients [78]. Standard nuclear medicine techniques, such as scintigraphy [60], SPECT or PET are not specific for detecting infection, but the diagnostic accuracy can be substantially improved by labeling human leukocytes with technetium-99 m and indium-111 (Figure 2). More recently, other promising radiometals have been introduced for imaging of infection such as gallium-67 and gallium-68 for SPECT and PET-imaging, respectively [79]. While imaging is very useful for diagnosing FRI, it plays a lesser role in the diagnosis of low-grade infections, as there are no specific imaging signs that are associated with low-grade infection.

4.5. Treatment of non-union

4.5.1. Promotion of fracture healing by non-surgical interventions

The non-surgical treatment options to increase bone healing are often called bone growth stimulators and primarily used in cases where infection is not suspected. These devices use low intensity pulsed ultrasound (LIPUS), electrical stimulation (ESTIM), pulsed electromagnetic fields (PEMF), combined magnetic fields (CMF) or extracorporeal shockwave therapy (ESWT) to stimulate the fracture site and surrounding tissue [80–82]. The outcome of these treatment modalities in cases of non-union is still controversial.

4.5.2. Surgical aspects in non-union treatment

Numerous treatment options are available for non-union, and they mainly depend on the viability of the fracture ends, the presence of a bone defect and the presence of an infection. Most of the evidence related to the various treatment strategies is available from case series and case reports, which are considered low-quality evidence. Current treatment of non-unions is often based on the 'diamond concept' for bone regeneration [15,83,84] introduced by Giannoudis *et al.* in 2007 [51,85], which refers to the availability of osteoinductive mediators, osteogenic cells, an osteoconductive matrix (scaffold), optimum mechanical environment, adequate vascularity,

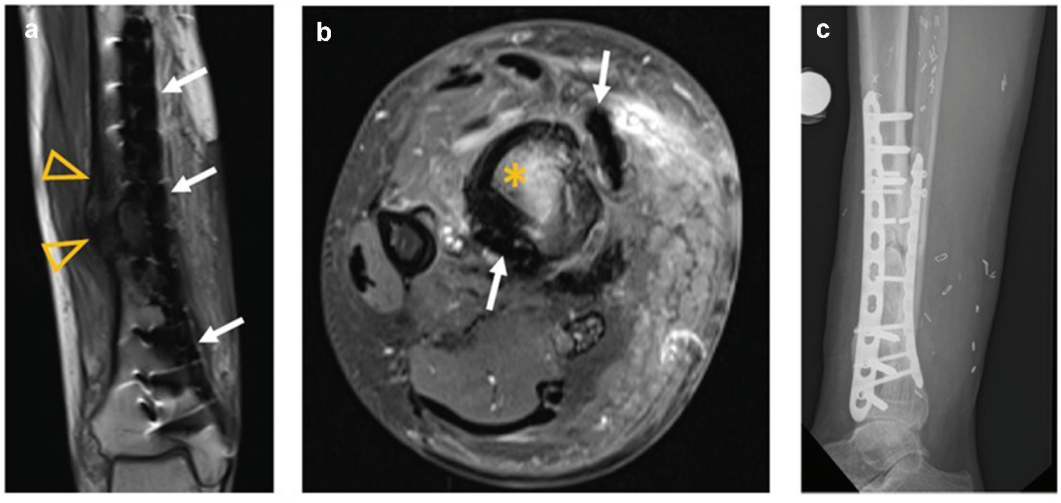

Figure 2. Clinical case of 38-year-old male patient with non-union of a tibial fracture with chronic osteomyelitis with hypointense signal of (a) the bone marrow (arrowheads) in the T1-weighted coronal MR image and (b) contrast enhancement of the bone marrow (asterisk) on the transverse fat-saturated T1-weighted MR image after intravenous gadolinium administration. Both mr-sequences have been acquired with increased readout bandwidth to reduce the metal artifacts of the osteosynthesis (arrows). Note that on (c) the lateral radiograph several screws are fractured proximal to the non-union.

and addressing any existing comorbidities of the host. However, a lot of open questions remain.

Key factors for the surgical treatment of non-union are fracture (construct) stability, control of any associated infection, vascularity of the soft tissue envelope, viable bone, and reconstruction of any bone and soft tissue defects [8]. Optimizing the mechanical environment by limiting the strain across the fracture gap is a critical component of the surgical management [12]. Stabilization can be achieved by internal or external fixation, depending on the fracture type and anatomical location, soft tissue status and the presence of a bone defect. Infection diagnosis and treatment are pivotal. Proper sampling for microbiological and histopathological analysis are crucial to detect an underlying infection and to establish targeted antimicrobial treatment. Debridement with excision of necrotic bone and soft tissues is also of utmost importance and this may result in large bone and soft-tissue defects. The size of the bone defect needs to be considered when selecting the management option. In smaller defects, it can be sufficient to use standard autologous bone grafting, harvested from the iliac crests or the medullary canal of the femur using the Reamer Irrigator Aspirator (RIA) system as treatment modality [86,87]. The induced membrane technique, introduced by Masquelet [88], distraction osteogenesis, or free vascularized bone transfer are reconstructive procedures needed for larger bone defects (Figure 3). Certain techniques may be preferred depending on the size of the defect and surgeon preference.

Besides defect management and ensuring stability, soft-tissue management is pivotal. Debridement with excision of necrotic bone and soft tissues may result itself in large bone and soft-tissue defects [89]. Since a vital soft-tissue envelope is required for a successful outcome in non-union (and infection) management, advanced reconstruction techniques (i.e., flaps) might consequently be required to achieve soft tissue coverage. Finally, in certain cases bone deformity correction is necessary to restore the mechanical axis of the limb.

4.5.3. Bone morphogenetic proteins and non-union

BMPs are recognized as key factors of chondrogenic and skeletogenic functions during normal embryonic development [90]. Although, recombinant human BMP-2 (rhBMP-2) and rhBMP-7 (BMP-7 has been withdrawn off the market) have shown promising results, their use is still controversial [91–94]. The use of rhBMP-2 was reported to cause several side effects, such as inflammatory complication, swelling, and seroma formation, radiculopathy, ectopic bone formation, osteoclasts activation, osteolysis, and wound complications [95]. These adverse effects are connected to the supraphysiological doses required to induce bone formation. rhBMP-2-induced bone healing has species-specific concentration requirements, and the dose-dependent efficacy was observed in a clinical study of fracture healing, where 1.50 mg/mL (dose approved by FDA) and 0.75 mg/mL rhBMP-2 applied to an absorbable collagen sponge were compared [96]. This study showed that the median time to fracture healing was reduced with 1.50 mg/mL but not 0.75 mg/mL rhBMP-2 [96]. A recent study compared rhBMP-2 application to the standard of care (SOC, defined as non-union resection and autograft of cancellous bone from the iliac crest) without rhBMP-2 [97]. Overall, rhBMP-2 application induced faster bone healing compared to SOC with significantly higher union rates at

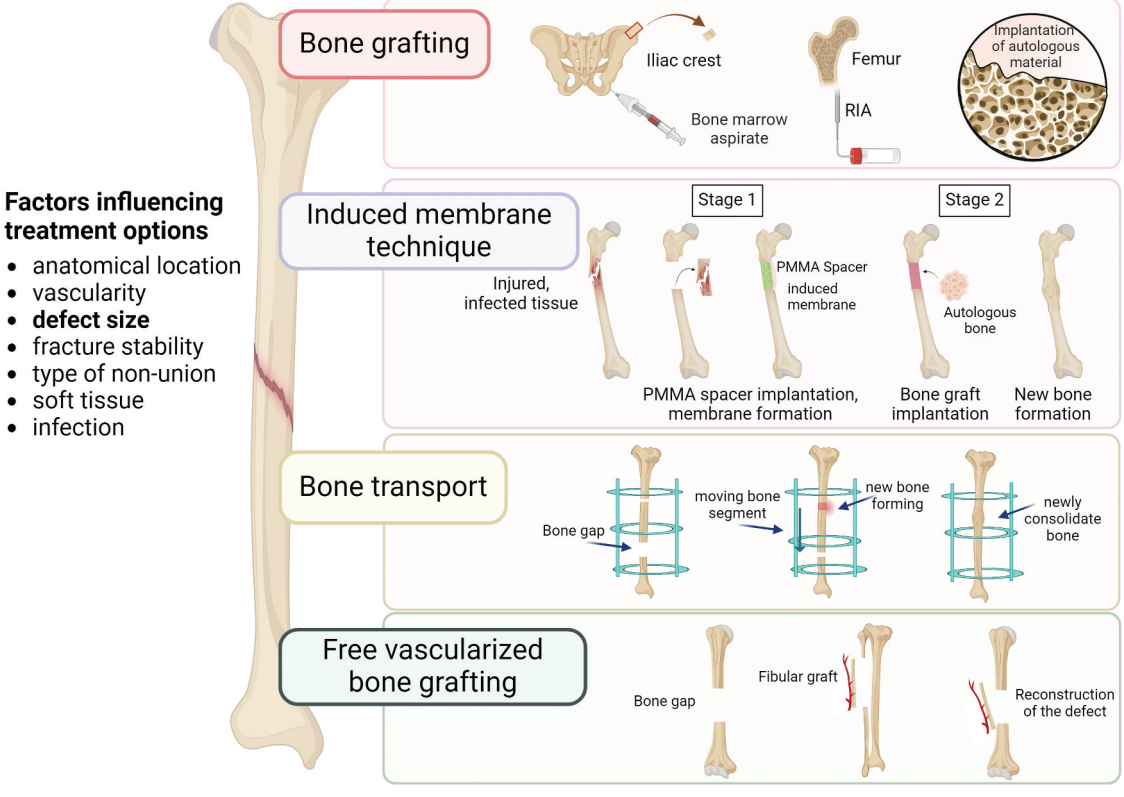

Figure 3. Strategies for bone defect reconstruction. Created with Biorender.com.

the femora (85% *vs*. 44%), and tibia (93% *vs* 33%) but not in the humerus. Additionally, rhBMPs provide advantages such as shorter operative time and reduced intraoperative blood loss [98]. Iliac crest autograft was associated with longer operative procedures compared to rhBMP-2 on collagen sponge mixed with cancellous allograft (257.9 ± 93.0 *vs* 168.9 ± 86.5 min) and greater intraoperative blood loss (554.6 ± 447.8 *vs* 331.6 ± 357.2 mL) [98]. However, some controversy remains among the clinicians on their use as SOC in the clinical practice.

Meanwhile, research has focused on different techniques to decrease the amount of protein required to induce bone formation by various immobilization techniques [99–101] or by improving the osteogenic potency of the protein [102,103]. However, the clinical application of those approaches is still far beyond.

Even if rhBMPs provide advantages such as shorter operative time and reduced intraoperative blood loss [98], they also report higher costs compared to autologous bone grafting [104]. The controversy on the use of BMPs as standard of care in the clinical practice remains among the clinicians.

5. Basic and translational research

In the current clinical situations, relatively few patients with bone fractures develop non-union, and in those cases different surgery-based interventions are often offered as a solution. However, a better understanding of the biological and mechanical genesis of non-union may identify new interventional strategies to reduce the incidence or improve recovery from non-union. The following sections will cover the prognosis, diagnosis, and latest understanding of the biology of non-union.

5.1. Biomarkers for non-union

The identification of biomarkers that predict healing progression early after a fracture would be of enormous value for patient stratification and early intervention. Ideally, blood-based prognostic biomarkers would be the best option as they would require a minimally invasive procedure and their analysis can be easily combined in a prognostic panel [105].

Terminally differentiated CD8(+) cells in peripheral blood have been correlated with delayed fracture healing [106]. In the same study, the same cell population was detected in the fracture hematoma. Transforming growth factor (TGF)-β proteins have been studied as prognostic markers by many groups, though the overall results from the different studies do not support a role for these proteins as reliable biomarkers [107–110]. Placenta growth factor (PlGF) showed potential to be used as a marker of non-union in humans [110], where as early as 24 h after the injury, PlGF was found to be significantly elevated in serum of fracture patients which developed non-union compared to those with uneventful healing. Blood samples can be used not only for testing the levels of circulating protein but also for gene and non-coding RNA expression. For example, *ANXA3* (encoding for Annexin A3, a protein with anti-inflammatory and anti-coagulant properties) was identified in blood samples as a potential marker for non-union in patients with and without type 2 diabetes [111]. miRNA represents a class of promising biomarkers for several conditions and has also been studied in the context of fracture healing. A recent systematic review identified miRNAs that were more frequently associated with non-unions [112]. Of note, miR-31-5p, miR-221, and miR-451-5p have been associated with cases of compromised fracture healing and are therefore miRNAs with biomarker potential.

An interesting approach is to identify biomarkers for patient-specific response to intervention. For example, the induced-membrane (Masquelet) technique can be used in an attempt to induce bone healing after non-union [113], but not all patients respond as well to this approach. The early identification of the non-responders would benefit the post-operative management and give the opportunity of additional care to the patients at risk. Two studies from the same group focused on this topic, identifying a different profile of serum MMP and chemokine expression between responders and non-responders to the Masquelet approach [114,115]. Patients who did not respond to the treatment had higher MMP2, lower MMP8, and MMP9, and lower MMP9/MMP2 ratios after the first step of the induced-membrane technique [114]. Similarly, they also showed higher CCL-3 and IFN-γ serum levels. These promising results support further clinical implementation, although larger studies with higher patient numbers have not yet been published.

5.2. Diagnostic biomarkers for FRI

Another valuable class of biomarkers would be those that can lead to the identification of FRI, especially in culture-negative cases as this would enable earlier commencement of appropriate therapy. The most common serum inflammatory markers for FRI are WBC, CRP, and ESR [71], but their value especially in the diagnosis of chronic/late-onset FRI is limited [67–70]. A study by Zhao *et al*. investigated the inflammatory cytokine interleukin-6 (IL-6) as a potential diagnostic FRI marker [116]. In this retrospective controlled analysis, IL-6 displayed a lower sensitivity (57.5%) compared to ESR and CRP (72.7% and 65.6%, respectively) but a higher specificity (ESR = 70.3%, CRP = 75.4%, IL-6 = 83.6%).

Evaluation of anti-*Staphylococcus aureus* humoral immune response has revealed interesting trends in specific antigen analyses in the serum of infected patients. Either a single anti-immunoglobulin G (IgG) test [117] or a multiplex immunoassay against 14 known *S. aureus* antigens [118–120] have been explored as a diagnostic approach for various bone infections.

A few miRNA studies focused on FRI [121–123]. Bone tissue miRNAs from infected tibial non-union group compared to the control group showed that 20 miRNAs were significantly differentially expressed in the two groups and six miRNAs (miR-649, miR-29b-3p, miR-498, miR-365a-5p, miR-328-5p, and miR-345-3p) were significantly down-regulated in tissue from patients with infected tibial non-union compared to controls (closed tibial fractures) [124]. Among those, a combination of miR-649, miR-328-5p, and miR-345-3p together yielded an overall diagnostic accuracy of 85% in sensitivity and 90% in specificity, with an AUC of 0.953. However, some of the miRNAs were also identified in cases of aseptic fracture non-union. Although more research is still

required, miRNA expression profiling of different long bones, soft tissues, and sera may contribute to providing comprehensive microRNA network information and play an important role in the diagnosis of infected non-unions.

5.3. Orthobiologics and non-union

Biology-based treatments for impaired healing and non-unions include the application of cells, growth factors, and other anabolic agents (summarized as orthobiologics [125]).

Recent efforts have included those focusing on the optimization of grafting procedures and the standardization of stem cell parameters (e.g., concentration, proliferative, and differentiative potential) [126,127]. Bone marrow aspirate concentrate (BMAC) and the application of BMSCs were shown to improve healing in preclinical and clinical studies [128,129] but the regeneration of large bone defects was found to remain challenging mainly due to the absence of adequate vascularization [129]. Alternative cell sources and preparation protocols (e.g., adipose-derived stem cells (ADSCs), dental pulp stem cells (DPSCs), induced pluripotent stem cells (iPSCs)), the *in vitro* expansion, pre-differentiation, and gene-modifications of cell-subpopulation, as well as procedures, such as *in situ* fabrication of vascularized bone grafts, are currently under investigation [126,129–133]. In addition to the endogenous bone-forming potential, particularly pericytes were shown to strongly mediate healing via paracrine mechanisms with indirect activation of growth factors, such as FGF2 and VEGF [134,135].

Platelet-rich plasma (PRP) is a cost-effective method to obtain high concentrations of specific growth factors (e.g., PDGF, VEGF, TGF beta 1/2, and IGF-1) and preclinical and clinical studies reported PRP-mediated accelerated fracture healing [136–138]. However, to facilitate clinical translation and acceptance of PRP as a therapy, Andersen *et al*. [136] indicated the need to investigate higher volumes of PRP in randomized clinical trials with longer duration. Further factors currently being investigated include the application of miRNAs for theranostic approaches as well as exosomes as therapeutic tools for the treatment of non-unions [112,139].

Recently, immunomodulation strategies have targeted cells of the adaptive, and innate immune system or have used cytokines to promote healing via downregulation of excessive and prolonged inflammation. Several preclinical studies showed a modulating impact of regulatory T cells (Tregs) in bone regeneration [140,141], which promoted the development of biomaterials and implants with incorporation of immunomodulatory strategies, such as macrophage polarization and Treg induction [142–145]. A further research focus has been to apply an Interleukin-1 receptor antagonist (IL-1Ra) to facilitate BMP-2-driven bone regeneration that would otherwise suffer excessive and prolonged inflammation [146,147]. Preclinical studies by Lackington *et al*. and Panos *et al*. showed, that immunomodulation may permit effective use of growth factors at lower doses to prevent and treat non-unions [148,149].

Mechanical stimulation either applied locally to the fracture site or globally has been shown to induce intracellular osteoanabolic signaling pathways promoting healing [150,151]. This has inspired developments toward novel mechanotherapeutics (e.g., mechanically activated bone cell derived extracellular vesicles) aiming to prevent and treat non-unions [152]. Further understanding of the spatio-temporal regulation of fracture healing could allow for individualized healing phase-specific treatments and application of mechanotransductive agents. The integration of evolving omics-based technologies [153] will enable novel multimodal approaches aiming at individualized biomarker-based treatment strategies and monitoring to early counteract impaired healing and non-union formation.

5.4. Biomaterials

Synthetic biomaterials are not currently used clinically on their own for non-union management, but they might be useful when combined with autologous bone graft for larger bone defect [154]. Autologous bone transplantation remains one option, particularly for atrophic non-unions and/or large bone defects. However, research focuses on achieving the same biological efficacy of autograft and avoiding donor site morbidity by combining bone-marrow derived mesenchymal stromal cells with biominerals [155].

Additional options, mostly used in case of FRI when larger defects are debrided, include off the shelf synthetic compositions, such as bioglasses and bioceramics, including hydroxyapatite, beta tricalcium phosphate [156] and their combinations into biphasic calcium phosphates that may also be loaded with antibiotics. Calcium sulfates have also been employed, although their efficacy in the treatment of non-unions is controversial [157]. Calcium biominerals are preferably used as granules with controlled porosity, which facilitates fluid exchange, cell invasion, vascular, and bone ingrowth. The use of calcium phosphates as bone graft substitutes is well established, as these minerals have a composition that closely resembles the mineral phase of bone, and provide the basic building blocks for deposition of bone mineral upon biological or chemical degradation [158].

Bone graft substitutes can be made from biologically derived biomaterials and include demineralized bone matrix, allografts, and xenografts, although their use widely varies geographically depending on availability. While no cellular components are present, these naturally derived materials contain a varying amount of biological cues for bone formation. Due to their bulk nature and lack of porosity, calcium phosphate- or sulfate-based cements are not considered as suitable for the treatment of non-unions.

5.5. Interventions targeting infection

Efficacy of conventional antibiotic treatment is often limited in case of infected non-union, or FRI, due to antibiotic resistance or tolerance development, biofilm formation, and limited antibiotic penetration to the site of infection [159]. Biomaterials-based strategies for FRI are already available, typically antibiotics loaded with biomaterials which allow a sustained release at the infection site, thereby reducing the possibility of systemic adverse effects [160].

Numerous innovative therapies targeting infection have been evaluated in recent years, with some closer to clinical application. Prominent examples include active and passive vaccines, silver coated implants and phage therapy. Phages are viruses that specifically infect and kill targeted bacteria and are not impacted by antibiotic resistance or tolerance mechanisms. More and more studies have been conducted and published on enlightening the positive effect of bacteriophages as antimicrobials against difficult-to-treat infections, including FRI, with success rates reported between 79% and 87% [161]. Incorporating phages into biomaterials, such as bone grafts of hydrogels, represents another approach to locally treat the infection, facilitating a controlled and prolonged release of phage [162], although only one clinical case report has been published to date [163].

There have also been efforts to apply nanotechnology to the treatment of FRI. Nanoparticles have been extensively tested for cancer treatment, where the anti-cancer drugs are delivered directly to cancer cells, avoiding damaging the healthy tissues, as often happens during chemotherapy and/or radiation therapy [164]. Further, applications of nanotechnology involve using chemicals (photosensitizers) in combination with light to produce reactive oxygen species (ROS), which has been studied as a treatment for various types of localized infections [165]. Nanotechnology can also be developed and adopted to specifically target intracellular bacteria, which were previously challenging to address with conventional therapies [166].

For the prevention of infection, a five-antigen *S. aureus* vaccine has been tested for safety and immunogenicity in patients undergoing elective surgery for closed fractures [167]. The randomized, double-blind, placebo-controlled, multicenter phase 2 clinical trial proved that the tested S. aureus vaccine was safe and well tolerated in patients undergoing elective surgery for closed fractures and it elicited rapid and robust specific humoral immune responses.

5.6. Animal models of non-union

Animal studies can play an important role in understanding the genesis of non-union and evaluate targeted therapies. The models used should ideally mimic the (clinical) situation and the pathogenesis as closely as possible. Since the reasons for non-unions in humans are multifaceted and still a matter of research, there is no single animal model that researchers can adopt to recapitulate non-union overall [168,169]. Many animal models concentrate on creating a situation where the bone does not heal, often by creating a bone defect that is so large that the bone will not be able to bridge it within a reasonable observation period. This concept is referred to as 'critical size defect' [170] and has been applied to many species [171–174] and in many different anatomical situations. However, the specifics of the model (e.g., size of defect, anatomical location, species of animal) vary substantially among researchers, hindering cross-referencing of results among research labs [175]. Since other factors will also impact the results (i.e., age, gender, strain/breed, and fixation method), it is of uppermost importance to include appropriate negative and positive controls in these studies.

Other approaches to creating non-unions have involved periosteal damage [176], creating an unfavorable mechanical environment [177] or physical disruption (i.e. thermal injury [178]. In rodents, transgenic models have been applied targeting different genes of interest [168,179], (e.g., matrix metalloproteinase 9 $(MMP9)^{(-/-)}$ mice [180], mice with limb-specific knockout of BMP-2 [181] or 3.6Col1A1-tk mice [182]). As previously described, co-morbidities are also implicated in the onset of non-unions in humans. In contrast, young and healthy animals are used in the vast majority of preclinical studies involving animals. This lack of co-morbidities is a shortcoming of most current animal models for non-unions, especially in large animal models [183]. As rodent models of diabetes and obesity exist and have been used for bone research, it is expected that these may also be applied to non-union studies in the future [184,185].

Due to plethora of published animal models, it is of uppermost importance to choose a model tailored to the research question and clinical problem addressed [186]. The targeted type of non-union and the primary outcome major are considered as good starting point.

5.7. The role of biomechanical stimulation in bone healing and non-union

Fracture healing disturbances occur when the magnitude of mechanical stimulation is either too low, thus suppressing callus formation, or too large and therefore leads to hypertrophic non-union. In clinical settings, achieving an adequate magnitude of mechanical stimulation is not trivial because the extent of stimulation depends on numerous (and often difficult-to-control) factors, i.e. fracture type, implant material, screw working length and the amount of physiological loading [187–190]. Recently, two technologies that facilitate the installation of appropriate mechanical conditions at the fracture gap have moved from "lab bench to bedside" - namely far-cortical-locking (FCL) [191] and biphasic plating [192].

With the great benefit of locking screws in providing higher stability in osteoporotic bone, the locking plate constructs can be overly stiff, thus suppressing the interfragmentary motion in the fracture gap and, as a result, limiting callus formation [193–195]. To decrease this effect, far-cortical-locking technology (FCL) was introduced. FCL uses a locking screw that anchors only in the far cortex, thus enabling larger motion in the fracture gap [196]. While FCL technology helps in avoiding under stimulation, the magnitude of interfragmentary motion in the fracture gap can still be affected by the configuration of screws and the extent of physiological loading. The novel biphasic plate addresses this issue by simultaneously providing controlled fracture motion and enhanced implant strength. This is achieved by a transverse slot feature in a region of increased plate thickness that reduces plate stiffness at low loads until approximately 20 kg [188,197,198]. When the plate is loaded beyond 20 kg, the slot closes, and the plate, due to the increased thickness, becomes very rigid, thereby preventing excessive motion and overstimulation. The substantial benefit of the biphasic plate is the standardized and controlled mechanical stimuli in the fracture gap which is unaffected by screw configuration

or functional loading. Following successful preclinical trials [191,197] both FCL and biphasic plating are now available for clinical use [5,6] but still need to prove their long-term clinical benefits.

While the magnitude of mechanical stimulation is unquestionably an important parameter impacting the course of healing, several recent studies have pointed out the importance of the temporal distribution of mechanical stimulation and its potential in decreasing healing disturbances. In clinical settings, patients are usually only allowed full functional loading when signs of callus formation are visible on x-ray. This strategy, however, limits the extent of stimulation in the early stage of bone healing. Recent results from animal studies question whether this is the optimal strategy. Using a sheep osteotomy model, Windolf *et al*. [199] observed that the number of interfragmentary stimuli applied in the first two-week post-op correlated positively with the strength of healing tissue nine-week post-op, thus demonstrating the importance of mechanical stimulation in the early post-op phase. Furthermore, using an active fixation on a sheep model, Barcik *et al*. demonstrated that delaying the initiation of mechanical stimulation retards callus development [200]. The recently introduced concept of reverse/inverse dynamization [201–203] even postulates that stimulation should be provided exclusively in the early post-op phase to encourage and accelerate early callus formation. Later, interfragmentary motion should be prevented as to avoid repeated disruption of the repair tissue and support/enable its mineralization. This technique showed acceleration of healing in osteotomy models in large animals [204,205] as well as improving healing of a segmental defect in rats [206]. It is relatively straightforward to realize inverse dynamization in preclinical settings by using an external fixator whose stiffness can be easily modified and thus promoting or suppressing interfragmentary motion [207]. However, most surgically managed bone fractures in humans are treated with internal fixators, for which up to now clinical translation of the concept of inverse/reverse dynamization is 'an open case.' In the research domain however, the use of shape-memory alloy implants is proposed to achieve alterable implant stiffness [208].

5.8. Computer simulations

Objective quantification of the biomechanical progress of fracture healing is crucial for accurate clinical care and diagnostics. Most currently available clinical methods rely on surrogate measures that are related to but are not accurate descriptors of, callus stability and require input from the patient or the surgeon, making them inherently subjective. One promising such avenue is patient-specific biomechanical computer simulations based on computed tomography (CT) scans.

Diagnostic computer simulation tools aim to evaluate healing status at given time points. CT image-based moment of inertia calculations estimate callus stability using planar composite beam theory [209]. However, this slice-by-slice analysis technique cannot capture the 3D callus structure. Finite element (FE) simulations can predict the mechanical behavior of complex 3D constructs and have been employed in biomechanics for over 40 years [210]. These simulations have aided in the investigations of fracture risk [211] from falls [212,213] and into orthopedic devices [214], such as artificial knees and hips [210] and plates and nails [215–218]. Additionally, they have provided meaningful insights into fracture repair after fixation [219,220]. An important advantage of FE modeling in biomechanical investigations is the ability to capture patient-specific geometries and material properties [221–223]. CT-based FE models can incorporate, beyond the implants, the 3D geometry and material properties of the bone fragments and the healing fracture callus, including its heterogenous distribution of bone mineral density, and predict strain in the fracture gap (Figure 4). FE-based stability measures better predicted the time to clinical union when compared to patient reported outcome measures (PROMs) or even radiographic union scoring (RUS) methods [224,225], indicating great promise in the diagnosis of fracture non-union in a noninvasive, objective manner.

Prospective simulations of bone healing aim to predict fracture outcomes based on the motion of the fracture region due to loading. Studies have been performed showing that construct-specific FE models of the fractured bone with different implant configurations can predict callus formation and healing outcomes [219]. However, these simulations are only analyzed at the direct postoperative time point. More complex simulation techniques are capable of capturing the time evolution of callus competence [226–230]. These analyses are built on strain theory [52] and describe the spatial and temporal changes of tissue types within the healing region throughout the course of healing via mechanobiological algorithms [226,231]. The ability of these simulations to analyze the entire healing process opens up opportunities to evaluate the effects of fixation on healing time [232] as well as weight bearing protocols [233] and dynamization [234].

5.9. Sensors

Lacking more suitable diagnostic methods, radiographic assessment is along with clinical examination still the gold standard in evaluating fracture healing. The need for a more reliable, quantitative measure without exposing patients to ionizing radiation has been recognized since decades and researchers have explored the diagnostic potential of a variety of technologies. Among the most studied approaches are ultrasound as both a diagnostic and therapeutic tool, vibrational analysis as well as strain measurements of the fixation hardware as an indirect indicator of fracture healing progression [235–238]. More recently, also electrical impedance spectroscopy (EIS) has been proposed to monitor maturation of the repair tissue after bone fractures [239,240].

In contrast, sensors integrated in the fixation hardware offering wireless readings could be used in a home care setting and enable quasi real-time remote monitoring. An active, implantable sensor system consists of an implantable load sensor that can be attached to conventional locking plates and allows for continuous measurement of the implant load based on an integrated strain sensor [241,242]. Fracture consolidation is hereby detected as progressive unloading of the osteosynthesis plate under physiological weight bearing. The

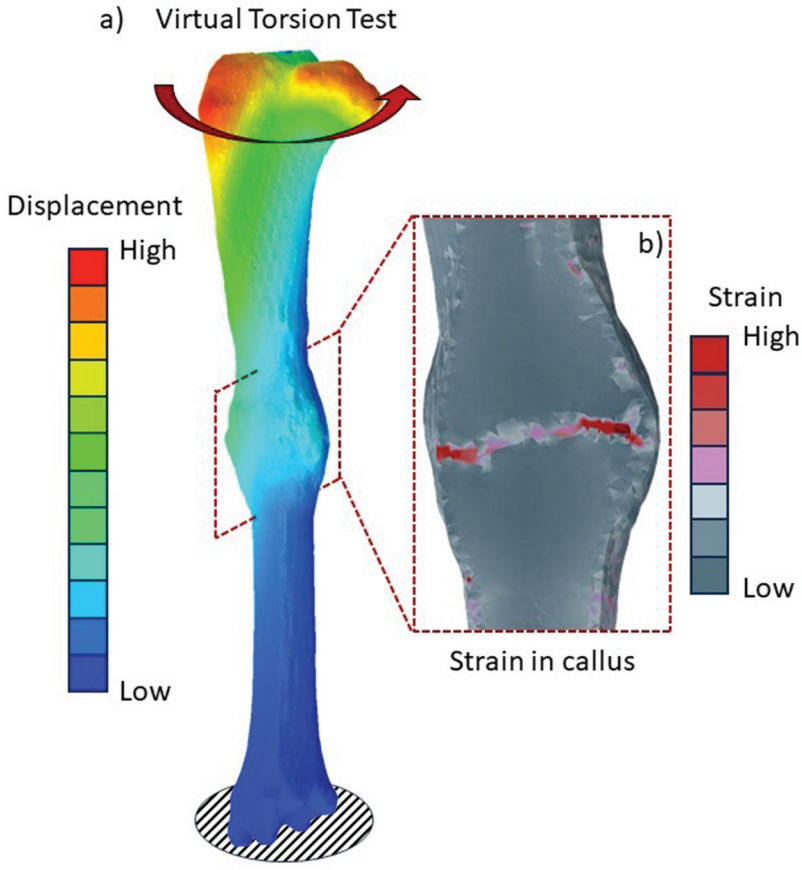

Figure 4. Finite element model. An example of a ct-based finite element model of an ovine (sheep) tibia with fracture callus undergoing a virtual torsion test. (a) a displacement contour plot. (b) a slice view of the callus region showing the internal strains that influence tissue formation and healing outcome.

strain signal is processed in the implant and the resulting statistical parameters are automatically transmitted via an encrypted Bluetooth connection to a smartphone once a day. From there, the data is forwarded to a cloud server and is available to the surgeon via a dedicated webapp to support therapeutic decision-making. Clinical experience of a similar, but passive measurement system has already been obtained by Kienast *et al*. [243]. They have measured 56 patients following fracture healing of the femur and were able to distinguish between four types of healing curves.

Less matured but also potentially offering integration with fixation hardware and frequent measurements are EIS measurements for fracture healing assessment. Unlike strain-gauge -based approaches that rely on physical loading of the implantation hardware, EIS offers a direct measurement of callus tissue characteristics but, on the other hand, requires the placement of electrodes in the fracture gap [240].

Common to all these assessment methods is that they are more sensitive in the early healing stages compared to radiologic methods [240,241,243,244]. Hence, establishing a reliable and quantitative alternative indicator for the course of fracture healing bears the potential to reconsider and adjust current definitions of union and non-union and likely will permit earlier detection of healing complications. While proposed technologies are expected to be sensitive to different types of healing complications, such as infection, atrophic or hypertrophic non-unions, they cannot diagnose the cause of the healing disturbance. Nevertheless, they support a timely diagnosis and – if needed – intervention, and thereby pave the way toward more patient-specific fracture aftercare.

6. Conclusion

Despite the enormous advances in basic, clinical, and translational research, there still are many barriers hindering improved patient care in case of non-union. The multifactorial nature of non-unions, including variations in patient characteristics, injury, anatomical location, fixation methods and comorbidities makes any definition challenging. Furthermore, this variation requires larger patient cohorts to be included in clinical studies in order to provide a comprehensive answer to the outstanding clinical questions. It may be that this is out of reach for most studies, and subdivision into discrete cohorts may be required, for example, infected non-union, or non-union of the lower extremities for example. With the continuing adoption of big data science and modern-omics technologies, we can expect a substantial increase in knowledge of the basic pathologies contributing to non-union, although how this may be translated to better diagnosis, prognosis, or therapy remains to be seen. Additionally, *in silico* models offer novel approaches for the identification of patients at risk for developing non-unions due to mechanical problems by predicting the individual healing process based on postoperative data or using overtime evaluation of bone fracture healing

using sensors. Implanted and embedded sensors may offer complementary data to such prediction tools that may allow also earlier identification of healing complications and more rapid intervention. New biomaterials offering a stable support for bone growth, delivering growth factors involved in osteogenesis and vasculogenesis, and possibly also delivering local antimicrobials in case of infection treatment, are also slowly emerging, and this trend should persist for the coming years.

Overall, the multifactorial nature of non-union requires a multidisciplinary team including clinicians and researchers to provide meaningful improvements in patient care. There is an active research community studying non-union and many new and important developments are on the horizon that may be translated from the lab to the clinic.

7. Expert opinion

Since the advent of osteosynthesis more than 60 years ago, the portfolio of implants and instruments available to surgeons has advanced greatly, and we have witnessed a parallel increase in the quality of care afforded to trauma patients. However, there has been stagnation in the prevention, diagnosis, and treatment of complications, particularly for non-union. In order to change the status quo, among the first challenges to be addressed are the basic clinical factors whereby the definition, diagnosis, and treatment approaches for non-union should be refined or resolved. Without this first step, any future innovation in the field may struggle to adequately determine clinical benefit and impact on patient care.

From the basic science perspective, the advancement in sequencing, proteomics, and other-omics technologies have not fully revealed the basic pathophysiology of non-union, or at least not to the point where innovative interventional strategies were identified for any particular subset of non-union patients. This has not been for lack of effort. The application of unbiased and untargeted data-driven methods allows the exploration of molecular pathways and molecules, and the application of multi-omics can provide an even more comprehensive characterization of non-union and capture molecular signatures and interactions spanning various biological levels. It may be that such an approach could decipher on-union mechanisms more deeply than are currently known, and this information may yet lead to translational approaches eventually leading to better interventional strategies. Once again, however, the basic clinical variables at play make this task more challenging, particularly if we expect to encompass the variability present in both patient and injury-related factors. In the absence of a single unifying pathology, the ultimate limit of our ambition in this complex clinical problem may be to pride better prognostic approaches, and targeted interventions suitable for a narrower range of patients. Although this will not eliminate non-union, it represents a realistic, achievable, and ambitious goal.

These biological approaches would look to extrapolate representative patient data to a population level, and it seems unfeasible to apply these technologies on a patient-by-patient basis, at least at the present time. To apply a more patient-specific approach, the future may more likely lie in computer simulations and implantable sensors. It is now established that validated healing simulations can provide preoperative planning tools that can lead to better patient care. Although such computational techniques are still labor/computationally intensive and not currently feasible for clinical use, this is likely a solvable issue as computing power continues to increase with time. Furthermore, through improvements in bioinformatics and AI, the requirement of large patient cohorts may also be reduced, making this in silico approach even more attractive. In parallel, implantable sensors are revolutionizing the diagnosis of fracture healing and the detection of non-unions. Continuous healing progress data provided by implanted sensors are beneficial for patients and physicians because this would allow them to continuously monitor their healing. Although these sensors will not prevent non-union, it may well be that it detects them earlier, allowing for a more simple, early intervention. This may be the greatest benefit of this approach in the near term, as we await improved prognostic technologies that can achieve this goal at the earliest setting post-injury.

In the foreseeable future, the combined use of computational simulations and implant sensors holds great promise in predicting reducing the impact of non-union. Integration of *in vitro*, *in vivo*, and in silico research will enable a comprehensive understanding of non-union causes and correlations, leading to the development of more effective treatments. Aside from these aspects, experience informs our opinion that the greatest impacts will inevitably come from earlier identification of complications and as such, any prognostic, be it based on in silico or biological approach, is the obvious prime target from the basic science perspective. Whether such a milestone can be achieved within a 5-year timeframe remains to be determined.

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