Fracture haematoma proteomics

surgical invasiveness characterizes the early fracture healing cascade after multiple trauma

From MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands

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Correspondence should be sent to Rald Victor Maria Groven r.groven@maastrichtuniversity.nl

R. V. M. Groven,[1,2] C. Kuik,[3] J. Greven,[4] Ü. Mert,[5] F. G. Bouwman,[6] M. Poeze,[2] T. J. Blokhuis,[2] M. Huber-Lang,[7] F. Hildebrand,[5] B. Cillero-Pastor,[1,3] M. van Griensven[1]

[1]Department of Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands
[2]Division of Trauma Surgery, Department of Surgery, Maastricht University Medical Center+, Maastricht, Netherlands
[3]Maastricht Multimodal Molecular Imaging (M4i) Institute, Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, Netherlands
[4]Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedics, Trauma and Reconstructive Surgery, University Hospital RWTH Aachen, Aachen, Germany
[5]Department of Orthopaedics, Trauma and Reconstructive Surgery, University Hospital RWTH Aachen, Aachen, Germany
[6]NUTRIM, School for Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, Netherlands
[7]Institute of Clinical and Experimental Trauma Immunology, University Hospital Ulm, Ulm, Germany

Aims
The aim of this study was to determine the fracture haematoma (fxH) proteome after multiple trauma using label-free proteomics, comparing two different fracture treatment strategies.

Methods
A porcine multiple trauma model was used in which two fracture treatment strategies were compared: early total care (ETC) and damage control orthopaedics (DCO). fxH was harvested and analyzed using liquid chromatography-tandem mass spectrometry. Per group, discriminating proteins were identified and protein interaction analyses were performed to further elucidate key biomolecular pathways in the early fracture healing phase.

Results
The early fxH proteome was characterized by immunomodulatory and osteogenic proteins, and proteins involved in the coagulation cascade. Treatment-specific proteome alterations were observed. The fxH proteome of the ETC group showed increased expression of pro-inflammatory proteins related to, among others, activation of the complement system, neutrophil functioning, and macrophage activation, while showing decreased expression of proteins related to osteogenesis and tissue remodelling. Conversely, the fxH proteome of the DCO group contained various upregulated or exclusively detected proteins related to tissue regeneration and remodelling, and proteins related to anti-inflammatory and osteogenic processes.

Conclusion
The early fxH proteome of the ETC group was characterized by the expression of immunomodulatory, mainly pro-inflammatory, proteins, whereas the early fxH proteome of the DCO group was more regenerative and osteogenic in nature. These findings match clinical observations, in which enhanced surgical trauma after multiple trauma causes dysbalanced inflammation, potentially leading to reduced tissue regeneration, and gained insights into regulatory mechanisms of fracture healing after severe trauma.

Bone & Joint Research

Article focus

- Examining the applicability of label-free proteomics as an analytical tool for the analysis of the fracture haematoma proteome.
- Profiling the early fracture haematoma proteome after multiple trauma.
- Identifying potential differences in the fracture haematoma proteome based on the applied trauma treatment strategy: early total care versus damage control orthopaedics.

Key messages

- Label-free proteomics is a suitable analytical tool to identify proteome differences in the early fracture haematoma.
- Surgical invasiveness significantly influences local proteome changes, which is key in directing tissue regeneration at the fracture site.

Strengths and limitations

- The study comprises of a translation large animal model with great clinical relevance. This increases the translational applicability of the obtained results.
- Due to the nature of the study, the fracture haematoma could only be analyzed at one timepoint after trauma. Changes over time of fracture haematoma proteome could therefore not be determined.

Introduction

Bone fracture healing is a dynamic process, requiring sophisticated communication and collaboration of various cell types to enable adequate bone regeneration. Several risk factors have been identified for impaired fracture healing, such as fracture comminution, open fractures, and multiple trauma.[1] The fracture healing cascade is initiated directly after a fracture occurs, with the formation of a fracture haematoma (fxH).[2] Studies have shown that the fxH is of particular importance in the first phase of the healing cascade: the inflammatory phase.[3] It is key in orchestrating bone regeneration throughout the phases of the fracture healing cascade by attracting different cell types, such as immune cells, mesenchymal stem cells, and fibroblasts.[4] The fxH also influences these cells' functioning, acts as a degradable scaffold for callus formation, and aids in maintaining an immunological balance at the fracture site. Failure to maintain this immunological balance can again result in fracture healing impairments.[2]

Proper patient management after multiple trauma can also aid in minimizing the risks of fracture healing impairments. Apart from the multiple trauma itself, the applied surgical treatment enhances both local and systemic inflammation by exacerbating or prolonging inflammatory responses, a phenomenon referred to as the "second hit" or surgical trauma.[5] Two concepts in trauma surgery focus on this additional surgical trauma impact in relation to the overall condition of the patient: early total care (ETC) and damage control orthopaedics (DCO). ETC represents a more invasive type of surgery, whereby definitive care is applied for long bone fractures during primary fracture surgery. The invasive character of this surgical approach makes it unfit for patients whose overall condition does not allow for further tissue damage and the resulting additional systemic inflammation.[6] For such patients, DCO is considered a better choice which temporarily fixates all long bone fractures in a minimally invasive manner, e.g. by applying external fixators prior to definitive fracture fixation at a later timepoint when the patient's physiology is restored.[5] The initial local and systemic inflammatory burden is decreased in DCO when compared with ETC, but more follow-up surgeries are required, often prolonging inflammation at the fracture site. These two surgical approaches also have differential effects on fracture healing. In part, these are caused by the differences in additional local and systemic inflammation, but any disturbance of the physical integrity of the formed fxH, e.g. by postponed definitive fracture stabilization as in the DCO concept, may also play a role, compromising proper initiation of the fracture healing cascade.[3,4]

Cellular mechanisms that underlie the varying effects of these different surgical treatment approaches are not yet fully known and understood. Of particular interest is the influence of these surgical approaches on cellular communication and functioning in the fxH.[7] On a biomolecular level, these cellular processes are dependent on various proteins that, among others, regulate the post-traumatic immune response, initiate the attraction and osteogenic differentiation of mesenchymal stem cells (MSCs), promote angiogenesis, and recruit fibroblasts, which are important for callus formation. The proteome of the fxH after multiple trauma, as well as alterations in the fxH proteome that result from the different surgical treatments, may provide insights into the influence of multiple trauma and the applied surgical intervention on the initiation of the early fracture healing cascade by the fxH. To our knowledge, no study has reported on proteome analysis of the fxH after multiple trauma. Therefore, the aim of this study was to determine the fxH proteome after multiple trauma using label-free proteomics, comparing two different established surgical treatment strategies.

Methods

Experimental design

The data presented in this manuscript were gathered in the context of a multicentre study. The study has been performed according to the principles of arthroplasty, refinement, and reduction of animal research. All sections of this manuscript adhere to the ARRIVE guidelines for reporting animal research.[8] The multiple trauma model was approved by the German governmental office of animal care and use (Landesamt für Natur, Umwelt, und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen, Germany) under permit 81.02.04.2020.A215. The multiple trauma model has been described in more detail elsewhere.[9] This established translational, clinically relevant large animal model allows for the accurate and standardized assessment of (patho)physiological responses to trauma and the applied treatment strategies. Porcine models are of particular translational value due to the great physiological, anatomical, and genomic similarities compared with humans.[10-13]

In short, upon arrival at the hosting facility, all animals were examined by a veterinarian, and were subsequently housed for seven days prior to the experiment to acclimatize. For this study, a total of 16 male German Landrace pigs (*Sus scrofa*) of three months of age and 35 kg (± 5 kg) body weight were used. After induction of general anaesthesia, the animals were subjected to multiple trauma, consisting

of bilateral femur fractures, blunt unilateral chest trauma, liver laceration, and controlled haemorrhagic shock. Animals were subsequently allocated to either the ETC (n = 8) or DCO (n = 8) group. The ETC group received intramedullary nailing (T2 System; Stryker, Germany), while the DCO group received external fixation (Radiolucent Fixator; Orthofix, USA) of both femoral fractures. Furthermore, the animals were resuscitated according to standardized guidelines,[14,15] followed by a 72-hour period in which they were kept under general anaesthesia and were monitored and treated under intensive care unit conditions. After the 72-hour observational period, the animals were killed. Directly after sacrificing, an atraumatic, standardized surgical approach was used to expose the fracture and its surrounding fxH. The fxH was harvested directly from the fracture site with the intramedullary nail or external fixator still in situ to prevent the exposure of other tissues, such as bone marrow or whole blood, to the fxH. The fxH was transferred to 2 ml microcentrifuge tubes, snap frozen in liquid nitrogen, and stored at -80°C until further use (Figure 1).

Protein isolation

Samples were sectioned with a thickness of 15 μm at -20°C using a Leica CM1860 UV cryostat (Leica Microsystems, Germany). Per sample, ten sections were collected in microcentrifuge tubes (Eppendorf SE, Germany) and washed three times with 100 μl 50 mM ammonium formate (Sigma-Aldrich, Switzerland) to remove excess haemoglobin. Samples were then dissolved in 100 μl of urea lysis buffer (5 M Urea (GE Healthcare, Netherlands) in 50 mM ammonium bicarbonate buffer (ABC) (Sigma-Aldrich). This was followed by three freeze-thaw cycles for protein isolation including one minute of sonication between the cycles. The protein containing supernatant was collected after 30 minutes of centrifugation at 11,000 relative centrifugal force (RCF) at 4°C. A Bradford assay (Bio-Rad, Switzerland) was performed for protein quantification, and the samples were stored at -80°C until further analysis (Figure 1).

Gel electrophoresis and in-gel digestion

Per sample, 20 μg of proteins were loaded on a 12% sodium dodecyl sulfate-polyacrylamide gel (Bio-Rad), the electrophoresis subsequently ran for ten minutes at 50 V followed by four minutes at 180 V. Protein bands were stained with Coomassie blue (Sigma-Aldrich) and collected from the gel, followed by an in-gel digestion with a MassPREP robot (Waters, UK). First, the destaining of Coomassie blue was performed using 50% acetonitrile (Biosolve B.V., Netherlands) in 50 mM ABC buffer. Second, cysteines were reduced using 10 mM dithiothreitol (Sigma-Aldrich) in 50 mM ABC buffer, followed by alkylation for 20 minutes using 55 mM iodoacetamide (Sigma-Aldrich) in 100 mM ABC buffer. This step was carried out in the dark to avoid UV degradation of the iodoacetamide. The samples were subsequently washed with 50 mM ABC buffer and dehydrated using acetonitrile. The dehydrated proteins were digested using 6 ng/μl trypsin (Promega, Netherlands) in 50 mM ammonium bicarbonate (ABC) buffer. The solution was incubated at 37°C for five hours. Lastly, the peptides were extracted from the gel using 3 × 50 μl of 2% acetonitrile and 1% formic acid (Biosolve B.V.). The samples were concentrated to 50 μl using a speedvac vacuum concentrator (Eppendorf SE) (Figure 1).

Data acquisition

A measure of 5 μl of the digested samples was injected and separated on an Acclaim PepMap C18 analytical column (2 μm, 75 μm × 500 mm, 100 Å) (Thermo Fisher Scientific, USA) coupled to a Thermo Fisher Scientific Dionex Ultimate 3000 Rapid Separation ultrahigh-performance liquid-chromatography (HPLC) system (Thermo Fisher Scientific). The samples were on-line desalted by trapping them in a C18 column. On the analytical column, the peptides were separated by a four-hour linear gradient of 4% to 45% buffer B in buffer A (Buffer B 80% acetonitrile and 0.08% formic acid, Buffer A 100% water and 0.1% formic acid) with a 300 nl/min flowrate. The HPLC system was coupled to an Orbitrap MS Q-Exactive instrument (Thermo Fisher Scientific) equipped with a Proxeon nanoelectrospray Flex ion source (Thermo Fisher Scientific). The ions were analyzed in data-dependent acquisition (DDA) mode in positive polarity. For the full mass spectrometry (MS) scans, a mass range of 250 to 1,250 mass-to-charge (*m/z*) was used at a resolution of 17,500 at 200 *m/z* with a maximum injection time. Tandem mass spectrometry (MS/MS) scans were performed with an isolation window of 4 Da with a maximum injection time of 200 ms for the ten most intense ions at a resolution of 70,000. The system was externally calibrated using a Pierce LTQ Velos ESI positive ion calibration solution in positive ion mode (Thermo Fisher Scientific) (Figure 1).

Data processing

The raw data files were processed with proteome discoverer software (version 2.2, Thermo Fisher Scientific) for protein identification. The Swiss-Prot and TrEMBL *Sus scrofa* databases, version 13-01-2023 (TaxID 9823), were used. The database search was carried out using the following parameters: trypsin for enzyme, maximum of two missed cleavages, fixed modification for carbamidomethylation of cysteine for fixed modifications and variable modification for oxidation of M and acetylation of protein N-term, the mass tolerance of the precursor was set to 10 ppm and to 0.02 Da for fragment tolerance. The tolerated peptide length was 6–144 amino acids. The false discovery rate (FDR) was set to a maximum of 1%. Label-free quantitation was conducted using the Minora Feature Detector node in the processing step, and the Feature Mapper node combined with the Precursor Ions Quantifier node in the consensus step with default settings within Proteome Discoverer 2.2 (Thermo Fisher Scientific).

Protein interaction analyses for the significantly modulated proteins were performed using Search Tool for the Retrieval of Interacting Genes (STRING) (version 11.5) analysis. The protein interactions were identified through comparison of the dataset with the *sus scrofa* genome. The minimum required interaction score was set to a confidence of 0.4. Furthermore, the Reactome pathway tool was used to analyze differentially expressed proteins.

Statistical analysis

Differences in protein abundancy between the ETC and DCO groups were analyzed using a one-way analysis of variance (ANOVA) in Proteome Discoverer software (version

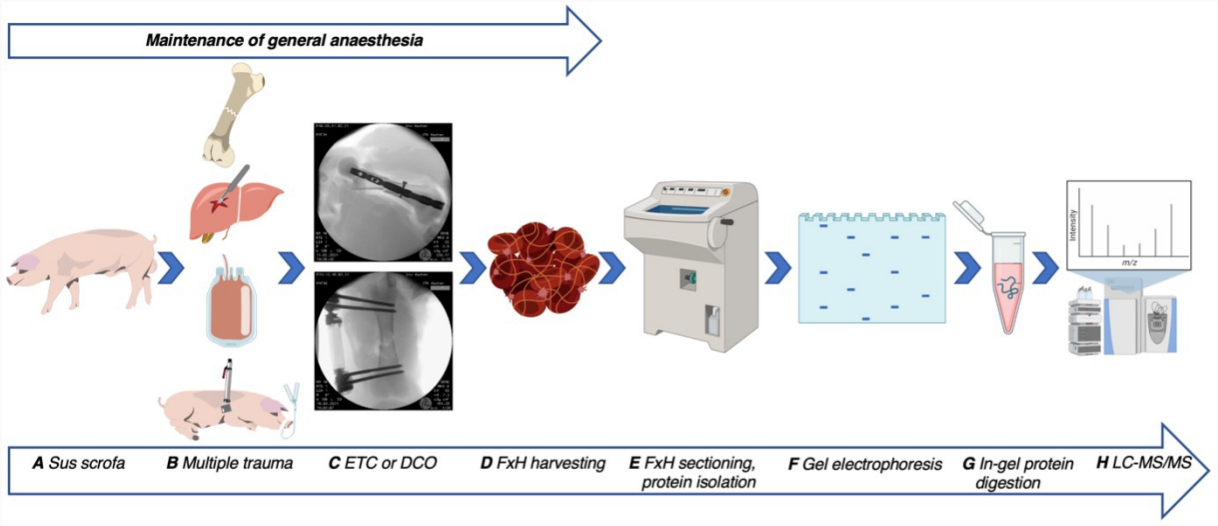

Fig. 1
Schematic experimental workflow. a) Male German Landrace pigs (*Sus scrofa*) were b) exposed to multiple trauma consisting of bilateral femur fractures, blunt chest trauma, liver laceration, and controlled haemorrhagic shock. Animals were operatively and medically stabilized, c) randomly allocated to either the early total care (ETC) or damage control orthopaedics (DCO) group, and subsequently monitored under intensive care unit standards for 72 hours until sacrifice. d) Fracture haematoma (fxH) was harvested from the fracture site after sacrificing, snap-frozen in liquid nitrogen, and stored at -80°C for further analysis. e) Per fxH sample, ten sections with a thickness of 15 μm were collected for protein isolation after which f) 20 μg of protein were loaded on a gel for gel electrophoresis. g) Protein bands were collected from the gel and digested. h) Lastly, samples were analyzed by means of liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.2). Proteins were considered significantly modulated if they had an abundance ratio of 1.5, an adjusted p-value < 0.05, and were detected in at least five samples.

Results

One animal in the ETC group did not survive until the 72-hour mark and was not included for analysis. Therefore, the final distribution over the treatment groups was ETC (n = 7), and DCO (n = 8).

The full proteome

Label-free liquid chromatography-tandem mass spectrometry (LC-MS) proteomics was performed to assess the full proteome of the fxH samples. In total, 1,692 unique proteins were identified in the ETC and DCO groups combined. These proteins were used to investigate the main pathways regulating fxH proteome. Proteins often interact with each other or function synergistically, performing multiple functions within one or more biomolecular pathways. The interaction analysis was carried out using STRING and visualized with Cytoscape (Figure 2). The network nodes in Figure 2 reflect proteins, and lines connecting the nodes represent the protein-protein interactions. In total, 221 protein-protein interactions were found that were involved in biomolecular pathways related to the expressional regulation of key transcription factors for osteogenic differentiation, the expression of pro- as well as anti-inflammatory proteins, the metabolism of hypoxia-induced factors, and important proteins for the activation of the complement system (Figure 2, Table I).

Differentially regulated proteins between treatment groups

The ETC group yielded 1,535 proteins and the DCO group 1,574 proteins. Of these proteins, 1,417 were detected in both datasets, and 118 and 157 were only detected in either the ETC or the DCO group, respectively (Figure 3a). Proteins were analyzed on their differential expression in both groups; an abundance ratio > 1.5 and a p-value < 0.05 were applied for statistical significance. A total of 30 proteins were significantly differently regulated between the two treatment groups (Figure 3b). Compared with the DCO group, 15 proteins were significantly upregulated in the ETC group. On the other hand, seven proteins were significantly upregulated in the DCO group compared with the ETC group. These significant upregulations among the treatment groups are listed in Table II, including the protein description, accession code, gene name, and abundance ratio adjusted p-value. Furthermore, eight proteins were exclusively detected in either one of the two treatment groups (Table III). In the ETC group, the exclusively present proteins included histone H1.1, allograft inflammatory factor 1, MHC class II antigen, and apolipoprotein F. The exclusively present proteins in the DCO group were Golgi membrane protein 1, vacuolar protein sorting-associated protein 28 homolog, adenosine triphosphate (ATP) binding cassette subfamily E member 1, and PX domain-containing protein. Upregulated pathways, analyzed with Reactome, including pathway name, p-values, and entities ratios, are listed in Table III.

A comprehensive visualization of the main implicated pathways and biomolecular processes is provided in Figure 4. Furthermore, a summary of all identified and quantified proteins, including protein accession codes, protein descriptions, abundances, and ratios, is provided in Supplementary Table i.

Discussion

This study is the first to explore the proteome of the fxH, particularly in a multiple trauma setting in which two different

Table I. Biomolecular pathways from the full fracture haematoma proteome, analyzed with reactome.

Pathway identifier	Pathway name	Entities ratio	p-value
R-HSA-909733	Interferon α/β signalling	8.47E-03	2.08E-03
R-HSA-977606	Regulation of complement cascade	9.13E-03	2.40E-01
R-HSA-6798695	Neutrophil degranulation	3.14E-02	2.42E-01
R-HSA-9711097	Cellular response to starvation	1.16E-02	2.94E-01
R-HSA-168249	Innate immune system	8.80E-02	4.99E-01
R-HSA-1280218	Adaptive immune system	6.13E-02	5.56E-01
R-HSA-2262752	Cellular responses to stress	6.62E-02	8.72E-01
R-HSA-8964539	Glutamate and glutamine metabolism	2.76E-03	3.14E-03
R-HSA-913531	Interferon signalling	2.11E-02	3.53E-03
R-HSA-877300	Interferon γ signalling	1.16E-02	5.04E-03
R-HSA-173736	Alternative complement activation	3.94E-04	1.17E-02
R-HSA-174577	Activation of C3 and C5	4.60E-04	1.37E-02
R-HSA-1280215	Cytokine signalling in immune system	6.82E-02	1.45E-01
R-HSA-166663	Initial triggering of complement	7.88E-03	2.11E-01
R-HSA-2132295	MHC class II antigen presentation	8.99E-03	2.37E-01

MHC, major histocompatibility complex.

surgical treatment strategies were compared. LC-MS/MS was used as an analytical tool for the determination of the fxH proteome in a translational porcine multiple trauma model, comparing different fracture treatment strategies. This study is the first to determine the fxH proteome, and offers novel insights into the effect of severe trauma and surgical invasiveness on the fracture healing cascade.

Overall fxH proteome

Protein interaction analyses revealed 211 protein-protein interactions involved in biomolecular processes that are key to the early fracture healing cascade (Figure 4). Various proteins were detected in the fxH proteome of both the ETC and DCO groups.

Among these were the regulation of runt-related transcription factors (RUNX) 1, 2, and 3. While RUNX2 is a well-known osteogenic marker gene, enhancing the osteogenic differentiation of MSCs into mature osteoblasts, RUNX1 and RUNX3 also play key roles in bone formation. Both hold important roles in endochondral ossification by enhancing chondrocyte maturation, as well as promoting chondrocyte commitment to the osteoblastic lineage.[16,17] These osteogenic marker genes are upregulated from early to late phases of osteogenic differentiation, matching the uniform upregulation of proteins involved in their signalling cascades.

Furthermore, proteins involved in the post-traumatic immune response are of great clinical interest. It is known that, compared with monotrauma, multiple trauma elicits a much stronger immunological response, and that dysbalanced post-traumatic immune responses, such as systemic inflammatory response syndrome or compensatory anti-inflammatory response syndrome, can contribute to fracture healing impairments.[2,18] A key factor in this post-traumatic, innate immune response is the complement system.[18] Several proteins involved in the activation of the complement system, the coagulation cascade, as well as wound healing and tissue remodelling processes, such as CLEC3B, APOH, and F13B, were identified in both treatment groups.[19–21] The finding is that these proteins, involved in basic post-traumatic immune responses, are identified in similar quantities among the two treatment groups. A focus is put on fxH samples from the early fracture healing cascade, a phase that is mainly characterized by an acute, post-traumatic inflammatory response that gradually shifts to bone formation and remodelling, the latter encompassing processes that are expected in later stages of the fracture healing cascade.[2] Furthermore, treatment-based proteome changes related to the complement system, coagulation cascade, and tissue regenerative processes were also observed, which will be elaborated on in the following section.

Neutrophils are among the main cells that are involved in the acute post-traumatic immune response. Neutrophils are the most abundant circulatory cells and are considered the 'first cellular line of defence' after injury. They appear very early in the fxH, and their activation and subsequent degranulation have been shown to contribute to fracture healing impairments.[22,23] Nevertheless, neutrophils play an important regulatory role in the post-traumatic inflammatory cascade, and recent studies have shown that neutrophils may play key roles in tissue regeneration, rather than just causing collateral tissue damage after trauma.[24] Neutrophils contribute to the activity of the complement cascade by secreting properdin when stimulated, while also being attracted to an injury site by complement activation products.[25] Upon recruitment, their main functions are phagocytosis, degranulation, and the formation of neutrophil extracellular traps, all of which require the neutrophils to be activated. For both treatment groups, a great number of proteins were detected that showed involvement in the degranulation of neutrophils, such as ERP44, RAP2A, and TSPAN14.[26,27] However, for complement system activation, as well as for neutrophil degranulation, profound differences were found based on the applied surgical treatment.

Another key biomolecular signalling cascade in osteogenesis, as well as inflammation, is the NF-κβ pathway. A large amount of the identified proteins in the fxH were involved in NF-κβ signalling, either by promoting the NF-κβ signalling cascade (e.g. by PSMD9) or enabling its degradation (e.g. by RPS27a).[28,29] Furthermore, distinct differences in key proteins involved in the NF-κβ signalling pathways were observed between the treatment groups, indicating a correlation between surgical trauma and the activity of

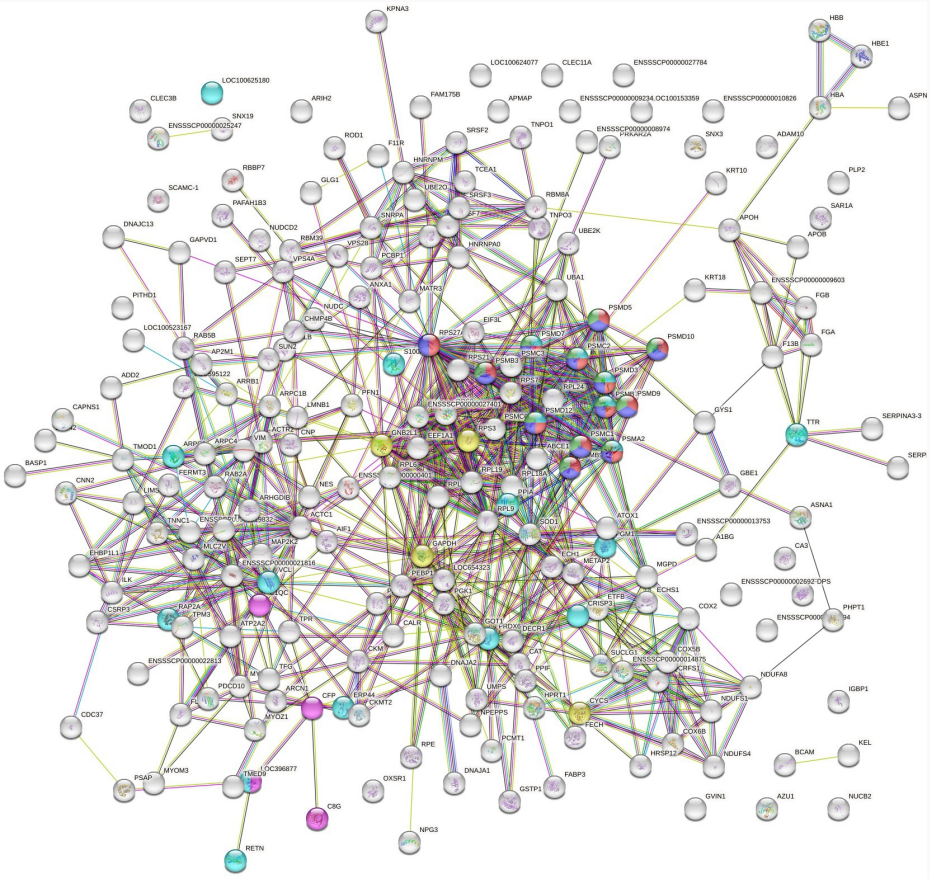

Fig. 2
Protein interaction network made with Search Tool for the Retrieval of Interacting Genes (STRING), visualized with Cytoscape. The depicted protein-interaction network contains the full fracture haematoma proteome from both treatment groups. Proteins that correspond with pathways involved in fracture healing in relation to the multiple trauma animal model have been highlighted. Purple = complement system activation and functioning; turquoise = neutrophil functioning; Red = osteogenic marker gene expression; Blue = cellular proliferation; Green = inflammatory regulation and osteoclastogenesis; Yellow = apoptosis. Coloured lines between the nodes correspond to a specific interaction. Turquoise = known interactions from curated databases; Purple = known interactions experimentally determined; Green = predicted interactions by gene neighborhood; Red = predicted interactions by gene fusions; Blue = predicted interactions by gene co-occurrence; Yellow = acquired by text mining; Black = assigned by co-expression.

NF-κβ signalling at the fracture site. In part, this might be due to the multifaceted role of NF-κβ in relation to the early fracture healing cascade. It is a necessary transcription factor for osteoclastogenesis and bone resorptive activity, while also blocking osteogenic differentiation. Furthermore, looking at inflammation, it is activated by key inflammatory mediators such as tissue necrosis factor-α and interleukin-1β, and subsequently acts as a transcription factor for various pro-inflammatory cytokines.[30]

Treatment-specific fxH proteome changes: ETC versus DCO

Among the significantly upregulated proteins in the ETC group were complement factor B (CFB) and scinderin (SCIN). As discussed above, the complement cascade is a major player in the post-traumatic immune response. Multiple trauma can deplete complement factors, on the one hand resulting in an enhanced activation of the complement system, while on the other hand also causing this physiological cascade to dysfunction, a phenomenon referred to as complementopathy.[31] In fact, a key factor that drives the potential malfunctioning of this system is early trauma management.[32] The complement system can be activated via three pathways, all leading to the formation of a C3 convertase. The alternative pathway is the main activated pathway in the acute phase after trauma, and its C3 convertase consists of the identified CFB, C3b, and the previously mentioned properdin.[25] This matches the enhanced surgical trauma in the ETC group, since it aligns with an increased activation of the complement system, in particular the alternative pathway, underlying the increased expression of CFB in the fxH during the early fracture healing cascade.[18] Furthermore, the findings are in line with previous reports on the impact of the complement system in bone homeostasis and fracture healing.[33] However, translating these findings to therapeutic approaches could be challenging.

Another protein that was significantly upregulated in the fxH of the ETC group was SCIN, a protein with several ties to fracture healing. First, SCIN is known to be an important protein for tissue invasion of immune cells in general.[34] When looking more specifically at neutrophils, SCIN has been shown to be key for the migration as well as polarization of neutrophils.[35] The increased expression of SCIN in fxH samples of the ETC group aligns with the increased local inflammatory load after ETC. Regarding bone formation, SCIN is important for both chondrocyte maturation and the maintenance of chondrocyte phenotype, indicating potential implications

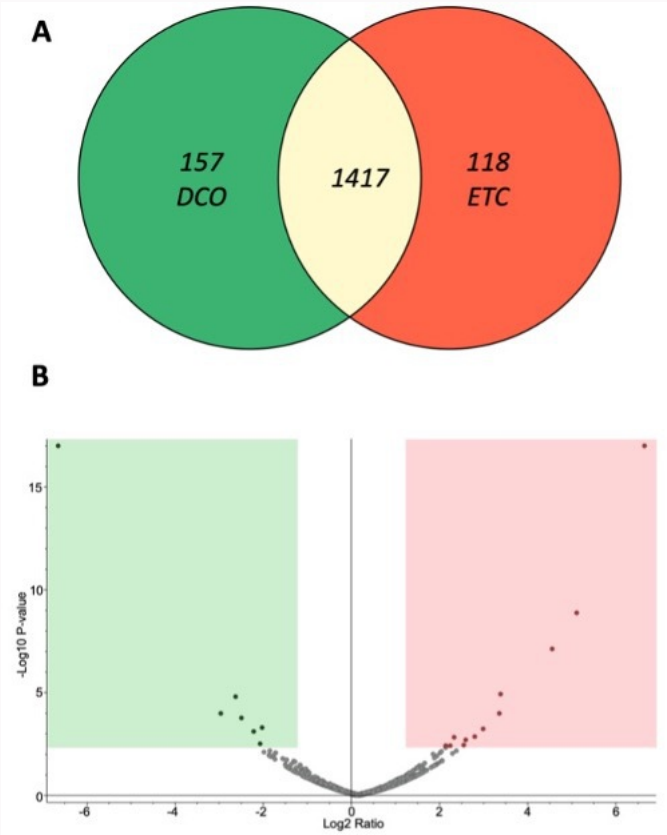

Fig. 3
Venn diagram and volcano plot. a) The Venn diagram displays 1,417 proteins that were identified in the fracture haematoma samples of both the damage control orthopaedics (DCO; green) and early total care (ETC; red) groups. A total of 157 and 118 proteins were exclusively detected in the DCO and ETC groups, respectively. b) The volcano plot illustrates the significantly differentially abundant proteins in the DCO and ETC groups, and plots the $-\log_{10}$ (corrected p-value) against the $\log_2$ ratio (abundance ratio).

for endochondral ossification.[36,37] Furthermore, research has shown that SCIN is also involved in osteoclast differentiation and activity, as its expression is upregulated during receptor activator of NF-κβ induced osteoclast differentiation, while its knockdown decreased the number of differentiated osteoclasts as well as osteoclastic secretion of bone-degrading enzymes.[38]

Among the exclusively detected proteins in the ETC group was allograft inflammatory factor 1 (AIF1). This protein enhances the production of pro-inflammatory cytokines, activates macrophages, and is important for their survival, and furthermore acts as a chemotactic agent for fibroblasts.[39] All these functions are vital in important processes taking place in the fxH throughout the early fracture healing cascade. Furthermore, silencing AIF1 has proven to reduce the differentiation of various cell types, among which are bone marrow MSCs (BMSCs) and monocytes, which coincides with the normal fracture healing cascade and the fact that the acute phase after trauma is mainly dominated by inflammation rather than cellular differentiation.[40]

The enhanced expression of the above-described proteins in the ETC group matches clinical observations in which severely traumatized patients who undergo early invasive surgery tend to suffer from a dysbalanced inflammatory response.[5] Furthermore, it shows that the post-traumatic immune response and fracture healing exhibit a delicate interplay rather than being two individual entities. The fxH proteome in the ETC group shows that early, definitive surgical treatment after multiple trauma influences the microenvironment at the individual injury site.

Among the significantly upregulated proteins in the DCO group was tripartite motif-containing protein 72 (gene MG53). Released from muscle tissue upon trauma, MG53 is a myokine that has gained great interest in the field of regenerative medicine. This is due to its anti-inflammatory characteristics, its role in maintaining stem cell lineage and function, and its role in repairing acute cellular membrane damage.[41] As a circulating agent after tissue damage, MG53 is of particular interest in relation to multiple trauma, a condition in which the interplay between individual injuries throughout the body greatly influences the patient's overall condition.[42] In fact, preclinical studies have shown promising results of the intravenous administration of recombinant MG53 in the treatment of acute lung injury, in part by reducing inflammatory cytokine production.[43] The upregulated expression of MG53 in the DCO group compared with the ETC group thus matches a reduced surgical trauma and subsequent decreased local inflammatory load, bearing in mind its anti-inflammatory and regenerative character.

Golgi membrane protein 1 (GOLM1) was among the exclusively detected proteins in the DCO group. GOLM1 stimulates glutamine metabolism, a metabolic pathway that has several ties to bone regeneration and homeostasis.[44] Glutamine is a direct stimulator of collagen type 1A1

Table II. Significantly modulated proteins in the fracture haematoma proteome of the early total care and damage control orthopaedics groups. The abundance ratio depicts the quantified abundances of proteins found in the groups. The adjusted p-value reflects the significance of the obtained abundance ratio.

Description	Upregulated treatment group	Accession code	Gene name	Abundance ratio: ETC/DCO	Abundance ratio adj. p-value: ETC/DCO
Atypical kinase COQ8A, mitochondrial	DCO	A0A5G2R212	COQ8A	0.01	1.49×10^{-16}
Myozenin-1	DCO	Q4PS85	MYOZ1	0.13	1.49×10^{-3}
Tripartite motif-containing protein 72	DCO	A0A1W6R2B4	MG53	0.16	2.40×10^{-4}
Antileukoproteinase	DCO	A0A8D1CCN7	SLPI	0.18	2.41×10^{-3}
Ig-like domain-containing protein	DCO	A0A4 × 1SL55	HLA-A	0.22	1.07×10^{-2}
Cytochrome c oxidase subunit 7A1, mitochondrial	DCO	A0A8D0JIX1	COX7A1	0.24	3.99×10^{-2}
Ankyrin repeat domain-containing protein 2	DCO	A0A4 × 1UBC7	ANKRD2	0.25	6.65×10^{-3}
Scinderin	ETC	A0A4 × 1TLV9	SCIN	99.73	1.49×10^{-16}
Glucosamine-6-phosphate isomerase	ETC	A0A4 × 1SSI5	GNPDA1	99.73	1.49×10^{-16}
Solute carrier family 25 member 24	ETC	F6Q4L6	SLC25A24	99.73	1.49×10^{-16}
TGc domain-containing protein	ETC	A0A8D0Z7Y2		34.54	2.06×10^{-8}
Complement factor B	ETC	A0A8D1B0Z8	CFB	23.59	1.12×10^{-6}
C-type lectin domain-containing protein	ETC	A0A287ANQ8	LOC100625180	10.41	1.80×10^{-4}
C-type lectin domain-containing protein	ETC	I3LEF9	LOC110255185	10.20	1.42×10^{-3}
C-type lectin domain-containing protein	ETC	A0A4 × 1VP71		7.89	7.68×10^{-3}
Delta-1-pyrroline-5-carboxylate synthase	ETC	A0A8D1GXF7	P5CS	6.92	1.79×10^{-2}
PI4 C2 alpha-protease inhibitor (Fragment)	ETC	Q9TR67		6.02	2.60×10^{-2}
Mast cell protease 3	ETC	A0A4 × 1SUV1	LOC100739080	5.86	4.51×10^{-2}
DNA replication licensing factor MCM2	ETC	A0A287AD55	MCM2	5.03	1.91×10^{-2}
Aminopeptidase	ETC	A0A1P8VJR2	APN	4.76	4.86×10^{-2}
60S ribosomal protein L35	ETC	A0A8D1BRU0	RPL35	4.38	4.91×10^{-2}

DCO, damage control orthopaedics; ETC, early total care.

transcription in fibroblasts, the most abundant collagen in bone tissue, enhances osteogenic marker gene expression, and promotes matrix mineralization.[44] Furthermore, glutamine supplementation has been shown in the clinical setting to be of value in multiple trauma treatment, reducing infectious complications by improving humoral and cell-mediated immunity, hospitalization duration, and mortality.[45,46] The expression of GOLM1 in the fxH in the acute phase after trauma therefore fits in the physiological fracture healing cascade in which the production of a soft callus needs to be initiated around that time, and BMSCs need to be attracted rather than differentiated to the fracture site.

In comparison with the ETC group, the proteome of fxH from the DCO group thus comprises considerably more anti-inflammatory and regenerative proteins that have links with key biomolecular processes in the fracture healing cascade. These findings match the reduced invasiveness of the primary surgical fracture fixation in the DCO group, thereby allowing for earlier anti-inflammatory and regenerative processes. This is in line with the clinical observation that reduced surgical invasiveness in severely traumatized patients during the acute phase after multiple trauma allows for more rapid primary restoration of systemic homeostasis, followed by enhanced tissue regeneration.

A limitation of this study is that it only enables the determination of the fxH proteome at one timepoint after trauma. Therefore, future research should focus on gaining more insights into the development of the fxH proteome over time, throughout different fracture healing phases. Furthermore, more research ought to investigate the impact of secondary nailing of long bone fractures on the fxH proteome, compared with early primary nailing. Lastly, a translational validation study in human samples is required to examine potential correlations between the fxH proteome, patient characteristics, and clinically relevant outcomes, such as impaired fracture healing. This study was a first step in the application of proteomics as an analytical tool for examining the fxH.

Table III. Significantly modulated proteins that were only detected in the fracture haematoma proteome of either the early total care or the damage control orthopaedics group. The abundance ratio depicts the quantified abundances of proteins found exclusively in each group. The adjusted p-value reflects the significance of the obtained abundance ratio.

Description	Treatment group	Accession code	Gene name	Abundance ratio: ETC/DCO	Abundance ratio adj. p-value: ETC/DCO
PX domain-containing protein	DCO	A0A8D1MEU7	PXDC	0.010	1.49E-16
ATP binding cassette subfamily E member 1	DCO	A0A5G2QNL9	ABCE1	0.010	1.49E-16
Vacuolar protein sorting-associated protein 28 homolog	DCO	K7GM88	VPS28	0.010	1.49E-16
Golgi membrane protein 1	DCO	A0A8D1QI47	GOLM1	0.010	1.49E-16
Histone H1.1	ETC	A0A480TW51	H1.1	99.73	1.49E-16
Allograft inflammatory factor 1	ETC	A5D9N3	AIF1	99.73	1.49E-16
MHC class II antigen	ETC	A0A7R8NC20	SLA-DQB1	99.73	1.49E-16
Apolipoprotein F	ETC	A0A4 × 1W2 H9	APOF	99.73	1.49E-16

ATP, adenosine triphosphate; DCO, damage control orthopaedics; ETC, early total care; MHC, major histocompatibility complex.

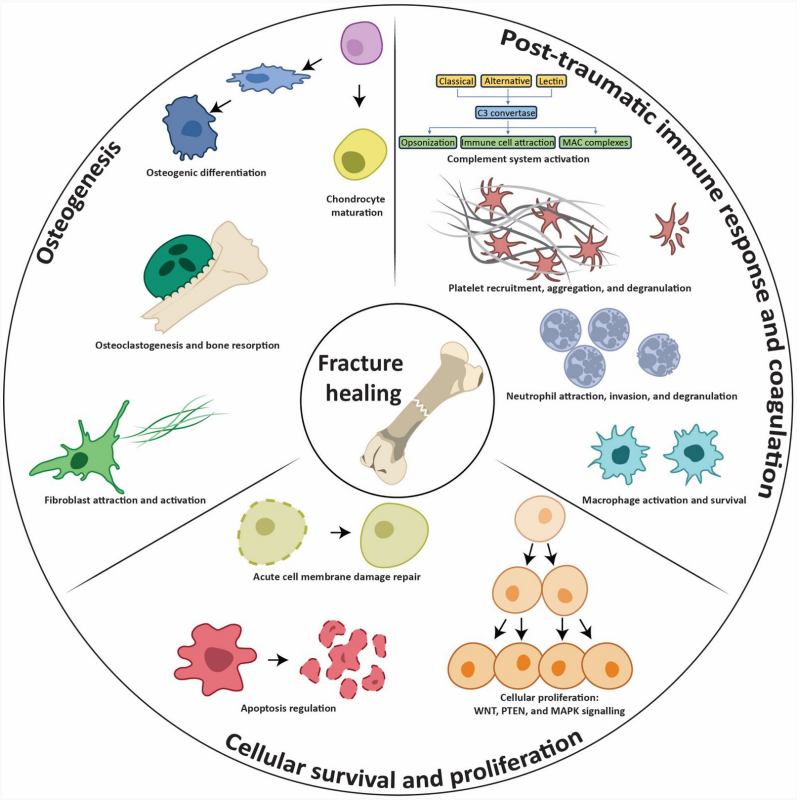

Fig. 4
Main pathways and biomolecular processes from the fxH proteome, related to important aspects of fracture healing. MAC, membrane attack complex; MAPK, mitogen-activated protein kinase; PTEN, phosphatase and tensin homolog; WNT, wingless integrated.

The proteome of the early fxH was characterized by immunomodulatory and osteogenic proteins, as well as proteins involved in the coagulation cascade. Distinct, treatment-specific proteome alterations were observed. The fxH proteome of the ETC group showed increased expression of pro-inflammatory proteins related to, among others, activation of the complement system, neutrophil functioning, and macrophage activation, while showing decreased

expression of proteins related to osteogenesis and tissue remodelling. The fxH proteome of the DCO group, on the other hand, contained various upregulated or exclusively detected proteins related to tissue regeneration and remodelling, as well as proteins related to anti-inflammatory and osteogenic processes. In short, the early fxH proteome of the ETC group was characterized by the expression of immunomodulatory – mainly pro-inflammatory – proteins, whereas the early fxH proteome of the DCO group was more regenerative and osteogenic in nature. These findings match clinical observations, in which enhanced surgical trauma after severe multiple trauma causes dysbalanced inflammation, potentially leading to reduced tissue regenerative capabilities, and gained insights into regulatory mechanisms of fracture healing after severe trauma.

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Supplementary material

A complete overview of all identified and quantified proteins in fracture haematoma samples of the early total care (ETC) and damage control orthopaedics (DCO) groups, including protein accession codes, protein descriptions, abundances, ratios, and respective treatment groups. An ARRIVE checklist is also included.

References

1. **Nicholson JA**, **Makaram N**, **Simpson A**, **Keating JF**. Fracture nonunion in long bones: a literature review of risk factors and surgical management. *Injury*. 2021;52 Suppl 2:S3–S11.
2. **ElHawary H**, **Baradaran A**, **Abi-Rafeh J**, **Vorstenbosch J**, **Xu L**, **Efanov JI**. Bone healing and inflammation: principles of fracture and repair. *Semin Plast Surg*. 2021;35(3):198–203.
3. **Schell H**, **Duda GN**, **Peters A**, **Tsitsilonis S**, **Johnson KA**, **Schmidt-Bleek K**. The haematoma and its role in bone healing. *J Exp Orthop*. 2017;4(1):5.
4. **Ehnert S**, **Relja B**, **Schmidt-Bleek K**, **et al**. Effects of immune cells on mesenchymal stem cells during fracture healing. *World J Stem Cells*. 2021;13(11):1667–1695.
5. **Pape HC**, **Moore EE**, **McKinley T**, **Sauaia A**. Pathophysiology in patients with polytrauma. *Injury*. 2022;53(7):2400–2412.
6. **Pfeifer R**, **Klingebiel FK-L**, **Halvachizadeh S**, **Kalbas Y**, **Pape H-C**. How to clear polytrauma patients for fracture fixation: results of a systematic review of the literature. *Injury*. 2023;54(2):292–317.
7. **Groven RVM**, **Peniche Silva CJ**, **Balmayor ER**, **et al**. Specific microRNAs are associated with fracture healing phases, patient age and multitrauma. *J Orthop Translat*. 2022;37:1–11.
8. **Percie du Sert N**, **Hurst V**, **Ahluwalia A**, **et al**. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLOS Biol*. 2020;18(7):e3000410.
9. **Groven RVM**, **Greven J**, **Mert Ü**, **et al**. Circulating miRNA expression in extracellular vesicles is associated with specific injuries after multiple trauma and surgical invasiveness. *Front Immunol*. 2023;14:1273612.
10. **Tsukamoto T**, **Pape HC**. Animal models for trauma research: what are the options? *Shock*. 2009;31(1):3–10.
11. **Hauser CJ**. Preclinical models of traumatic, hemorrhagic shock. *Shock*. 2005;24 Suppl 1:24–32.
12. **Lunney JK**. Advances in swine biomedical model genomics. *Int J Biol Sci*. 2007;3(3):179–184.
13. **Hildebrand F**, **Andruszkow H**, **Huber-Lang M**, **Pape H-C**, **van Griensven M**. Combined hemorrhage/trauma models in pigs-current state and future perspectives. *Shock*. 2013;40(4):247–273.
14. **Stewart RM**. American College of Surgeons. 2018. https://cirugia.facmed.unam.mx/wp-content/uploads/2018/07/Advanced-Trauma-Life-Support.pdf (date last accessed 11 April 2024).
15. **Polytrauma Guideline Update Group**. Level 3 guideline on the treatment of patients with severe/multiple injuries: AWMF Register-Nr. 012/019. *Eur J Trauma Emerg Surg*. 2018;44(Suppl 1):3–271.
16. **Tang C-Y**, **Wu M**, **Zhao D**, **et al**. Runx1 is a central regulator of osteogenesis for bone homeostasis by orchestrating BMP and WNT signaling pathways. *PLoS Genet*. 2021;17(1):e1009233.
17. **Zhou Z**, **Yao B**, **Zhao D**. Runx3 regulates chondrocyte phenotype by controlling multiple genes involved in chondrocyte proliferation and differentiation. *Mol Biol Rep*. 2020;47(8):5773–5792.
18. **Huber-Lang M**, **Lambris JD**, **Ward PA**. Innate immune responses to trauma. *Nat Immunol*. 2018;19(4):327–341.
19. **Brown GD**, **Willment JA**, **Whitehead L**. C-type lectins in immunity and homeostasis. *Nat Rev Immunol*. 2018;18(6):374–389.
20. **Bekeschus S**, **Lackmann JW**, **Gümbel D**, **Napp M**, **Schmidt A**, **Wende K**. A neutrophil proteomic signature in surgical trauma wounds. *Int J Mol Sci*. 2018;19(3):761.
21. **Kleber C**, **Sablotzki A**, **Casu S**, **et al**. The impact of acquired coagulation factor XIII deficiency in traumatic bleeding and wound healing. *Crit Care*. 2022;26(1):69.
22. **Hoff P**, **Gaber T**, **Strehl C**, **et al**. Immunological characterization of the early human fracture hematoma. *Immunol Res*. 2016;64(5–6):1195–1206.
23. **Bastian OW**, **Croes M**, **Alblas J**, **Koenderman L**, **Leenen LPH**, **Blokhuis TJ**. Neutrophils Inhibit Synthesis of Mineralized Extracellular Matrix by Human Bone Marrow-Derived Stromal Cells *In Vitro*. *Front Immunol*. 2018;9:945.
24. **Peiseler M**, **Kubes P**. More friend than foe: the emerging role of neutrophils in tissue repair. *J Clin Invest*. 2019;129(7):2629–2639.
25. **Murphy KW**, **Weaver C**. *Janeway's Immunobiology*. Ninth ed. New York, New York: Garland Science, 2017.
26. **Aarts CEM**, **Downes K**, **Hoogendijk AJ**, **et al**. Neutrophil specific granule and NETosis defects in gray platelet syndrome. *Blood Adv*. 2021;5(2):549–564.
27. **Luerman GC**, **Powell DW**, **Uriarte SM**, **et al**. Identification of phosphoproteins associated with human neutrophil granules following chemotactic peptide stimulation. *Mol Cell Proteomics*. 2011;-10(3):M110.001552.
28. **Sahu I**, **Sangith N**, **Ramteke M**, **Gadre R**, **Venkatraman P**. A novel role for the proteasomal chaperone PSMD9 and hnRNPA1 in enhancing IκBα degradation and NF-κB activation - functional relevance of predicted PDZ domain-motif interaction. *FEBS J*. 2014;281(11):2688–2709.
29. **Collins PE**, **Mitxitorena I**, **Carmody RJ**. The ubiquitination of NF-κB subunits in the control of transcription. *Cells*. 2016;5(2):23.
30. **Jimi E**, **Katagiri T**. Critical roles of NF-κB signaling molecules in bone metabolism revealed by genetic mutations in osteopetrosis. *Int J Mol Sci*. 2022;23(14):14.
31. **Burk A-M**, **Martin M**, **Flierl MA**, **et al**. Early complementopathy after multiple injuries in humans. *Shock*. 2012;37(4):348–354.
32. **Huber-Lang MS**, **Ignatius A**, **Köhl J**, **Mannes M**, **Braun CK**. Complement in trauma-Traumatised complement? *Br J Pharmacol*. 2021;178(14):2863–2879.
33. **Mödinger Y**, **Löffler B**, **Huber-Lang M**, **Ignatius A**. Complement involvement in bone homeostasis and bone disorders. *Semin Immunol*. 2018;37:53–65.
34. **Wang Y**, **Li Z**, **Bai L**, **Zhang D**, **Zhang T**, **Ren F**. Scinderin is a novel oncogene for its correlates with poor prognosis, immune infiltrates and matrix metalloproteinase-2/9 (MMP2/9) in glioma. *Brain Sci*. -2022;12(10):1415.
35. **Glogauer J**, **Sun C**, **Wang Y**, **Glogauer M**. The actin-binding protein Adseverin mediates neutrophil polarization and migration. *Cytoskeleton (Hoboken)*. 2021;78(5):206–213.
36. **Chan B**, **Parreno J**, **Glogauer M**, **Wang Y**, **Kandel R**. Adseverin, an actin binding protein, regulates articular chondrocyte phenotype. *J Tissue Eng Regen Med*. 2019;13(8):1438–1452.
37. **Nurminsky D**, **Magee C**, **Faverman L**, **Nurminskaya M**. Regulation of chondrocyte differentiation by actin-severing protein adseverin. *Dev Biol*. 2007;302(2):427–437.
38. **Song MK**, **Lee ZH**, **Kim HH**. Adseverin mediates RANKL-induced osteoclastogenesis by regulating NFATc1. *Exp Mol Med*. 2015;47(12):e199.

39. **Piotrowska K**, **Słuczanowska-Głabowska S**, **Kurzawski M**, **et al**. Overexpression of allograft inflammatory factor-1 (AIF-1) in patients with rheumatoid arthritis. *Biomolecules*. 2020;10(7):1064.
40. **Liu J**, **Chang X**, **Dong D**. MicroRNA-181a-5p curbs osteogenic differentiation and bone formation partially through impairing Runx1-dependent inhibition of AIF-1 transcription. *Endocrinol Metab (Seoul)*. 2023;38(1):156–173.
41. **Whitson BA**, **Tan T**, **Gong N**, **Zhu H**, **Ma J**. Muscle multiorgan crosstalk with MG53 as a myokine for tissue repair and regeneration. *Curr Opin Pharmacol*. 2021;59:26–32.
42. **Li Z**, **Wang L**, **Yue H**, **et al**. MG53, a tissue repair protein with broad applications in regenerative medicine. *Cells*. 2021;10(1):122.
43. **Whitson BA**, **Mulier K**, **Li H**, **et al**. MG53 as a novel therapeutic protein to treat acute lung injury. *Mil Med*. 2021;186(Suppl 1):339–345.
44. **Zhou T**, **Yang Y**, **Chen Q**, **Xie L**. Glutamine metabolism is essential for stemness of bone marrow mesenchymal stem cells and bone homeostasis. *Stem Cells Int*. 2019;2019:8928934.
45. **Al Balushi RM**, **Paratz JD**, **Cohen J**, **Banks M**. Glutamine Supplementation in Multiple Trauma of Critical Care. In: Rajendram R, Preedy VR, Patel VB, eds. *Diet and Nutrition in Critical Care*. New York, New York: Springer New York, 2015: 203–218.
46. **Cotoia A**, **Cantatore LP**, **Beck R**, **et al**. Immunological effects of glutamine supplementation in polytrauma patients in intensive care unit. *J Anesth Analg Crit Care*. 2022;2(1):41.

Author information

R. V. M. Groven, MD, PhD Candidate,
Department of Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands;
Division of Trauma Surgery, Department of Surgery, Maastricht University Medical Center+, Maastricht, Netherlands.

C. Kuik, MSc, PhD Candidate, Maastricht Multimodal Molecular Imaging (M4i) Institute, Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, Netherlands.

J. Greven, PhD, Post-Doctoral Researcher, Experimental Orthopaedics and Trauma Surgery, Department of Orthopaedics, Trauma and Reconstructive Surgery, University Hospital RWTH Aachen, Aachen, Germany.

Ü. Mert, MD, Surgical Resident
F. Hildebrand, Prof. Dr., Head of the Department of Orthopedic, Trauma, and Reconstructive Surgery
Department of Orthopaedics, Trauma and Reconstructive Surgery, University Hospital RWTH Aachen, Aachen, Germany.

F. G. Bouwman, PhD, Technician, NUTRIM, School for Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, Netherlands.

M. Poeze, Prof. Dr., Head of the Division of Trauma Surgery
T. J. Blokhuis, PhD, Trauma Surgeon
Division of Trauma Surgery, Department of Surgery, Maastricht University Medical Center+, Maastricht, Netherlands.

M. Huber-Lang, Prof. Dr., Institute of Clinical and Experimental Trauma Immunology, University Hospital Ulm, Ulm, Germany.

B. Cillero-Pastor, PhD, Principal Investigator, Department of Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands; Maastricht Multimodal Molecular Imaging (M4i) Institute, Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, Netherlands.

M. van Griensven, Prof. Dr., Head of Department, Department of Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands.

Author contributions

R. V. M. Groven: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing.
C. Kuik: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing.
J. Greven: Investigation, Writing – review & editing.
Ü. Mert: Investigation, Writing – review & editing.
F. G. Bouwman: Data curation, Formal analysis, Investigation, Writing – review & editing.
M. Poeze: Conceptualization, Writing – review & editing.
T. J. Blokhuis: Conceptualization, Writing – review & editing.
M. Huber-Lang: Conceptualization, Funding acquisition, Writing – review & editing.
F. Hildebrand: Conceptualization, Funding acquisition, Writing – review & editing.
B. Cillero-Pastor: Conceptualization, Methodology, Supervision, Writing – review & editing.
M. van Griensven: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

R. V. M. Groven and C. Kuik contributed equally to this work.

R. V. M. Groven and C. Kuik are joint first authors.

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Data sharing

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Ethical review statement

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