MicroRNAs in fracture healing and its treatment

English summary

Globally, traumatic injuries, and in particular fractures, represent a significant health burden. Therefore, a necessity for further investigation into fracture healing and fracture treatment strategies persists. This is emphasized by a global rise in fracture incidences, a substantial increase in years lived with disability due to fractures, and the aging demographic worldwide. The research presented in this thesis focusses on exploring the expression and function of microRNAs (miRNAs) in relation to fracture healing and its treatment. MiRNAs are small, non-coding RNA molecules that regulate protein expression, and are thereby of great importance in, among others, tissue regeneration.

The work presented in this thesis is based on the hypothesis that miRNAs play a vital role in fracture healing and multiple trauma, that their expression is under the influence of the applied surgical treatment approach, and that miRNAs may serve potential therapeutic, diagnostic, and prognostic roles in the field of trauma surgery.

In **chapter 1**, a general introduction in trauma and fracture epidemiology is provided, as well as a short overview of fracture healing. Furthermore, the pathophysiology of multiple trauma as well as the treatment approaches of long bone fractures in multiple trauma are briefly elaborated on, followed by an introduction on miRNAs in general, and the outline of this thesis.

The work presented in this thesis is divided in three parts:

1. MiRNAs in fracture hematoma and autograft, chapters 2-4
2. The expression and role of miRNAs in multiple trauma fracture healing, chapters 5-6
3. Systemic involvement of miRNAs in multiple trauma, chapters 7-8

Part one of this thesis starts with **chapter 2**, consisting of a review which summarizes the main results of various in vitro and *in vivo* studies which investigated the role of specific miRNAs in relation to processes which are key in the fracture healing cascade. It is demonstrated that miRNAs play widespread roles in various aspects of fracture healing, but that a lack of translational, clinically relevant research exists.

In **chapter 3**, we investigated the expression of miRNAs in fracture hematoma samples of 61 patients, and correlated these expression patterns to patient characteristics. Also, an *in silico* mRNA target prediction unraveled interesting implications of these miRNAs in different aspects of the fracture healing cascade. This study showed the regulatory role of miRNAs in the human fxH, and displayed that their expression patterns depend on patient characteristics.

Chapter 4 focused on a novel surgical tool, designed to generate a bone grafting substitute during surgery, thereby potentially reducing the necessity for additional autograft harvesting from other donor sites. In samples from nonunion patients, we showed that this device yielded a bone grafting substitute with osteogenic miRNA and mRNA profiles. Compared to the clinically applied control, this study showed promising results for the novel surgical tool and warrants further research into its clinical use aimed at minimizing the requirement for invasive autograft harvesting.

Part two of this thesis focused on miRNA expression in multiple trauma fracture healing. **Chapter 5** describes a porcine multiple trauma model in which two different established multiple trauma treatment strategies are compared: Early Total Care vs. Damage Control Orthopedics. In this study, we examined the influence of these different treatment strategies on the expression of miRNAs at the fracture site, in fractured bone and the fracture hematoma, and in the systemic circulation. Distinct local and systemic miRNA expression responses were identified after multiple trauma, some of which were specific for the applied surgical treatment. Furthermore, several mRNA targets of the most deregulated miRNAs were validated *in vivo* to study the miRNA's mechanisms of action.

Chapter 6 displays the characterization of the fracture hematoma proteome after multiple trauma and describes how surgical treatment strategies influence this fracture hematoma proteome. Distinct, treatment specific proteome

changes were identified, related to osteogenic differentiation, the post-traumatic immune response, and cellular survival and proliferation.

The **third part** of this thesis started with the investigation of miRNA expression over time in extracellular vesicles after multiple trauma. These results are shown in **chapter 7**, where temporal miRNA expression patterns were identified which correlated with the surgical trauma, also called the ''second hit''. These data showed that surgical invasiveness, apart from influencing local gene expression at the surgical site, also greatly influenced systemic gene expression. As a proof of concept, the mRNA targets of the most deregulated miRNAs were *in vivo* validated in target organs of the multiple trauma model, which revealed mRNA expression patterns in the local tissue which corresponded with systemic miRNA expression.

Lastly, **chapter 8** describes the results of a novel immune modulating therapy, where a C5 inhibitor was combined with anti-CD14 to block part of the post-traumatic innate immune response. It was shown that this C5/CD14 therapy greatly affected osteogenic and inflammatory gene expression at both the fracture site, as well as in the systemic circulation over time. In particular, a strong local and systemic anti-inflammatory effect was observed after C5/CD14 therapy administration, which may contribute to the prevention of inflammatory complications after severe trauma. These results warrant further research into the long-term outcome of such C5/CD14 therapy and offer promising prospects for the clinical application of a partial blockade of the innate immune system in the treatment of severely injured patients.

Chapter 9 discusses the main results obtained by the studies presented in this thesis, and places them into the perspective based on current literature. Furthermore, future directions for research in this field are provided at the end of this chapter.

In **chapter 10**, the main objectives and findings of this thesis are described and their potential impact on both societal and scientific sectors is elaborated on.