Summary
RBC transfusion practice has been widely applied in clinical settings to compensate for excessive blood loss as well as to improve the oxygen carrying capacity. Although the infusion of refrigerated stored RBCs has been considered a life-saving practice for years, concerns about the efficacy and safety of prolonged stored RBCs are currently emerging. This is because RBCs biochemical processes are not completely suppressed during refrigerated storage. Consequently, the RBCs undergo various cellular and biochemical changes that could hamper the RBC to adequately function after infusion. Yet, evidence of a significant detrimental clinical effect associated with the infusion of prolonged stored RBCs is still inconclusive. The rheologic properties are important determinants of the quality of RBCs. Particularly because impaired RBC rheologic properties, which are enhanced aggregability, reduced deformability and elevated adherence to endothelial cells, proposes a circulatory risk by hindering adequate tissue perfusion and contributing to ischemia or even infarction in the micro-vascular environment.

**Chapter 1** reviews ex vivo RBC preservation and emphasizes the importance of the RBC rheologic properties and in particular those that can be measured by the LORCA. Alternatively, the utilization of cryopreservation for long-term storage of RBCs is discussed. The general objectives of this thesis were to gain a better understanding of the RBC quality from a rheologic perspective in transfusion medicine, as well as to explore the utilization of cryopreserved RBCs for routine clinical practice.

The first part of this thesis mainly focuses on the RBC aggregation process and the rheologic properties of refrigerated stored RBCs. RBC aggregation has been studied for decades in healthy and diseased subjects. Despite these studies, the underlying mechanism and the physiological role are still elusive. Replacement of plasma proteins by standard polymers to induce RBC aggregation helps to unravel the fundamentals of the aggregation process. In **chapter 2** the usefulness of 200-kDa HES polymers to induce RBC aggregation was investigated. The EPM of RBCs in 200-kDa HES solution was measured to get more insight into the RBC aggregation mechanism. The measured EPM values of RBCs in HES solutions were less negative than could be predicted by the suspension viscosity, which supports the bridging theory as the mechanism underlying 200-kDa HES induced RBC aggregation. These findings are in line with previous observations in which stronger and larger RBC aggregates are formed with HES solutions of increasing molecular weight. Furthermore, the LORCA was used to demonstrate aggregation differences between RBCs from healthy individuals and patients with a disease. We demonstrate that like the standard
500-kDa dextran polymers, the 200-kDa HES polymers are able to discriminate cellular induced aggregation differences between RBCs from healthy and T1DM subjects. Altogether, our results demonstrate that 200-kDa HES polymers are useful as a pro-aggregant in RBC rheologic studies.

Enhanced RBC aggregation has been observed in a variety of chronic diseases, among which T1DM disease. Yet, RBC aggregation could also play a pivotal role in certain acute situations, such as during damage control resuscitation of severely bleeding trauma patients. Chapter 3 discusses the potential importance of RBC aggregation in supporting hemostasis after blood component infusion in severely bleeding trauma patients. In general, RBCs aggregates exclude leukocytes and possibly platelets from the axial core and direct them towards the vascular wall. This process is essential, since leukocytes and platelets need to get into close contact with the damaged endothelium, in order to exert their function. Adoption of a liberal policy with regard to FFP to RBC infusion could promote RBC aggregation. Although physiological levels of RBC aggregation would be beneficial for supporting hemostasis, promotion of aggregation could be detrimental to patients in which the RBC rheology is already compromised, as was observed in certain trauma states. Future studies with the LORCA will be helpful to elucidate the role of RBC aggregation in damage control resuscitation of severely injured trauma patients.

A better insight of the RBC rheologic properties during ex vivo preservation may improve transfusion outcome. Earlier studies have shown that the RBC rheologic properties become impaired as soon as the second week of refrigerated storage. Yet, most of these studies were not representative for modern RBC storage due to the absence of leukofiltration prior to storage. In chapter 4 the RBC rheologic features and other hematologic variables of leukoreduced RBCs were studied during seven weeks of refrigerated blood bank storage. Our data shows, that although the aggregability of leukoreduced refrigerated stored RBCs was slightly altered over time, an enhanced aggregability was clearly not observed. In addition, we found that the reduction in RBC deformability, which was only observed at high shear stress, was still within physiological ranges. We did observe that the ATP content became progressively depleted during refrigerated storage, whereas the MCV, pH and MCHC were affected to a lesser degree. We postulate, that the observed changes in RBC variables during refrigerated storage minimally affected the RBC ability to aggregate and deform, even after prolonged refrigerated storage. Based on these and recent findings,
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we conclude that the rheologic properties of leukoreduced RBC units were well preserved during routine blood bank storage.

In order to circumvent storage induced lesion and thus to extend the shelf-life of RBCs, cryopreservation of RBCs has become a feasible alternative. In Chapter 5 cryopreservation of RBC and in particular its utilization in modern transfusion practice is discussed. In the past, the clinical applicability of cryopreserved RBCs was hampered by the expensive, less efficient and more time consuming nature of this preservation method. Yet, the subsequent unfamiliarity with regard to the quality of cryopreserved RBCs has further limited clinical usage. In this regard, the rheologic features and various hematologic variables of HGM cryopreserved RBCs were investigated in Chapter 6. We showed that the aggregability of cryopreserved RBCs was reduced, compared to fresh and refrigerated stored RBCs. The deformability of stored RBCs was enhanced compared to fresh RBCs, but no significant differences in deformability between cryopreserved and 21- or 35-day refrigerated stored RBCs was observed. We also show that the osmotic fragility, hemolysis, MCV and MCHC of cryopreserved RBCs were altered, compared to fresh and refrigerated stored RBCs, but that the ATP content of cryopreserved RBCs remained close to those of fresh RBCs. We demonstrated that although cryopreserved RBCs were more fragile than refrigerated stored and fresh RBCs, the HGM procedure did not adversely affect the ATP content or the aggregability and deformability of cryopreserved RBCs. Therefore, cryopreserved RBCs could become a more utilized blood resource in clinical settings.

To date however, utilization of cryopreserved RBCs is primarily restricted to controlling an inventory in situations where the RBC availability is limited or unpredictable. Such is the case for storage of RBC with rare blood groups or for usage in military conflicts. In this regard, the feasibility of a new Bio-freezer was investigated in Chapter 7, as a way to improve the clinical applicability of cryopreserved RBCs. Human and rat RBCs were preserved with different glycerol concentrations in the Bio-freezer at -25°C or in combination with storage at -80°C in a mechanical freezer. Cryopreserved human RBCs were tested in vitro for deformability and various hematologic variables. The posttransfusion survival was determined in rats with chromium-51 labeled preserved rat RBCs. Our data demonstrated that the liquid Bio-freezer alone was not sufficient to preserve the RBC deformability or to maintain high in vivo RBC survival. However, usage of the liquid Bio-freezer in combination with the -80°C mechanical freezer, enabled preservation of RBCs with 20% glycerol, while maintaining the RBC integrity,
deformability and high 48-hour posttransfusion survival values. The use of only 20% glycerol could be beneficial in reducing the osmotic stress that is associated with the glycerolization and deglycerolization procedure and which causes cellular losses. We therefore consider that the liquid Bio-freezer could become a valuable tool for the cryopreservation of RBCs.

Ultimately important findings in this thesis were discussed in a broader perspective in chapter 8.