Summary
Tissue engineering, or regenerative medicine, aims at regenerating or replacing tissues that malfunction or that are lost due to disease or injury. Here, scaffolds and/or cells are implanted in the patient to guide tissue repair and to stimulate autologous tissue formation and regeneration. Albeit the knowledge on tissue engineering is expanding, the poor vascularization of engineered tissues remains a principle constraint. Generally, the formation of new blood vessels (angiogenesis) is not fast enough to provide adequate oxygen and nutrient supply to and waste product removal from the scaffold. In the absence of a vascular system, the maximum diffusion rate of oxygen (~100 to 200 \( \mu \)m) determines the size of the scaffold. Therefore, there is a need for factors and matrices that facilitate neovascularization of the scaffold. Fibrin is a natural temporary extracellular matrix that not only seals a wound, but also provides a temporary healing matrix wherein angiogenesis is supported.

The precursor of fibrin is the soluble protein fibrinogen, which is present in our circulation. Fibrinogen in human plasma shows a high degree of heterogeneity caused by alternative mRNA processing, postranslational modifications and proteolytic degradation. Three major fibrinogen variants that occur in the circulation are high molecular weight (HMW, native form) fibrinogen, low molecular weight (LMW) fibrinogen and low molecular weight' (LMW') fibrinogen. The LMW and LMW' fibrinogen variants are generated by partial proteolytic degradation of the fibrinogen alpha chain(s). The different fibrinogen variants can be purified from plasma and HMW-, LMW- and LMW'-fibrin matrices can be prepared. Previously it has been shown that HMW-fibrin matrices have an open structure with thick fibers, whereas LMW-fibrin matrices have a more dense structure with thinner fibers. Strikingly, in vitro and in vivo angiogenesis assays showed a more extended tube-like structure formation and blood vessel formation in HMW-fibrin matrices, than in the natural mixture of fibrinogen variants. Moreover, LMW-fibrin matrices severely reduced the tube-like structure and blood vessel formation. These data provide evidence that the rate of vascularization can be influenced by using different fibrinogen variants.

The aim of this thesis is to evaluate the effects of naturally occurring fibrin(ogen) variants as scaffolds in tissue engineering, with specific emphasis on their effects on angiogenesis and stem cell behavior, in order to gain more insight in refinement of scaffold vascularization. Objectives of this study are:

(I) Unravel the mechanism of the endothelial cell response on the fibrin molecular weight variants.

(II) Investigate the adipose tissue-derived stem cell response on the fibrin molecular weight variants.

(III) Explore the effects of other naturally occurring fibrinogen variants on angiogenesis and wound healing.

(IV) Investigate whether recombinant and plasma-derived fibrinogen variants have similar effects on angiogenesis.

This thesis starts with an introduction of tissue engineering, angiogenesis and fibrinogen (Chapter 1), that is followed by the aim and objective of the study. Next to specific characteristics of fibrin matrices, various applications of fibrin in tissue engineering are described in Chapter 2. The current literature on wound sealing, skin tissue engineering, vascular tissue engineering,
heart valve replacement and biomolecule/drug delivery is discussed. In **Chapter 3-5** the focus is on endothelial- and stem cell characteristics of cells cultured on matrices that are composed of the molecular weight variants of fibrinogen; HMW- and LMW-fibrin respectively. **Chapter 6** describes the exploration of other fibrinogen variants for tissue engineering, namely the gamma(γ)-variants of fibrinogen. Finally in **Chapter 7**, native (HMW) fibrinogen derived from human plasma is compared with recombinantly produced native (HMW) fibrinogen.

The rate of angiogenesis can be influenced by using various fibrinogen molecular weight variants. In addition to the structural differences of these fibrin matrices, the mechanism(s) that underlie the altered rate of angiogenesis remain unclear. The study described in **Chapter 3**, investigates the functional characteristics and molecular mechanisms of human microvascular endothelial cells cultured on HMW- and LMW-fibrin matrices. We showed that the various fibrin matrices induce different gene expression patterns and alter the functional characteristics of microvascular endothelial cells. Moreover, a higher fibrinolytic sensitivity was found for HMW-fibrin matrices. HMW-fibrin matrices result in a pro-angiogenic phenotype of endothelial cells, whereas LMW-fibrin results in an anti-angiogenic phenotype of endothelial cells.

Another application of naturally occurring fibrinogen variants in tissue engineering is their coating on synthetic vascular grafts. Previously it has been shown that fibrinogen coating of a scaffold results in a non-thrombogenic, endothelialized inner layer of a vascular graft. In **Chapter 4A** the barrier integrity and formation of a quiescent endothelial monolayer on HMW- and LMW-fibrinogen coatings are described, in order to identify better and more defined fibrinogen coatings for vascular tissue engineering. We showed that the endothelial monolayer on purified HMW-fibrinogen coatings has a better integrity with a more quiescent phenotype than on LMW-fibrinogen coatings. These findings provide a good starting-point for vascular tissue engineering with HMW-fibrinogen coatings. **Chapter 4B** gives a discussion of the observations of Sahni et al. on the addition of intact fibrinogen that induces an increase in endothelial permeability via fibrinogen domain β_{15-42} binding to VE-cadherin.

When the rate of scaffold vascularization is influenced by using naturally occurring fibrinogen variants, it is also important to know the effects on other scaffold components. Stem cells are important in tissue engineering since they have high proliferative capacity and differentiate into multiple cell types. **Chapter 5** describes the influence of naturally occurring fibrinogen variants (HMW- and LMW-fibrin) and the oxygen tension on adipose tissue-derived mesenchymal stem cell expansion and differentiation. In hypoxic culture conditions (1% oxygen) the adipose tissue-derived stem cells proliferated faster, showed reduced cell aging, and their stemness was preserved. The various fibrinogen coatings did not influence the expansion and differentiation of adipose tissue-derived mesenchymal stem cells. Differentiation of the stem cells towards adipogenic and osteogenic lineages was improved in 20% oxygen, whereas 1% oxygen improved chondrogenic differentiation. These data emphasize the importance of oxygen concentrations during stem cell growth and differentiation and increases knowledge on fibrinogen variants for tissue engineering applications.

In order to explore additional fibrinogen variants, the splice variant γ’-fibrinogen was subjected to further investigation. Previously γ’-fibrinogen was shown to possess unique structural
characteristics; and increased plasma levels of γ'-fibrinogen associated with venous and arterial thrombosis. In Chapter 6 the effects of γ-fibrinogen variants on angiogenesis and in vivo wound healing are described. We showed that matrices composed of γA- and γ'-fibrinogen govern different structural and functional characteristics. Small, non-significantly increases for in vitro tube formation and in vivo wound healing were observed in γA-fibrin, compared to γ'-fibrin matrices. However, more data are necessary before decisive conclusions can be drawn.

The last experimental chapter (Chapter 7) describes the set up of a recombinant HMW-fibrinogen production platform, and the comparison of recombinant HMW-fibrinogen with plasma-derived HMW-fibrinogen is made. The adhesion and proliferation of endothelial cells on recombinant and plasma-derived HMW-fibrinogen were similar. Also in vitro tube formation developed to the same extent on both fibrin matrices, although it was observed that in certain conditions the cell-mediated fibrinolysis was more extensive on recombinant HMW-fibrin. These findings provide new insight in the endothelial cell characteristics on recombinant fibrinogen, which can be directive for the development of tissue engineering applications.

Chapter 8 summarizes the results of this thesis and places the findings in perspective. Here, our findings are discussed in the context of the rapidly developing fields of tissue engineering and angiogenesis. Among other points the scaffold degradation, stability and oxygen levels within fibrin scaffolds are discussed. Also the limitations of our studies and aspects that should receive further attention are described.

This thesis shows that the use of fibrin matrices, composed of naturally occurring fibrinogen variants, is a proper approach to influence the vascularization of tissue engineered scaffolds. Changing the composition of the fibrin matrix has impact on endothelial cells, but not on stem cell characteristics. Nowadays, most fibrin scaffolds are composed of plasma-derived fibrinogen and contain the natural mixture of fibrinogen variants. In this thesis it is demonstrated that specific naturally occurring fibrinogen variants are suitable to define the environmental conditions within the scaffold. Choosing specific fibrinogen variants can alter the vascularization and oxygen concentration within the tissue engineered scaffold.