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Summary
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Summary

Myocardial infarction (MI) results in a permanent loss of function in the heart and thus affects these patients for the rest of their lives. Even though fast interventions and medications to reduce recurrence have substantially increased the quality of life, there is no therapy available that addresses the heart of the problem: the loss of cardiomyocytes. The endogenous regenerative capacity of the heart is insufficient to deal with the damage after a MI. Even though there are new blood vessels being formed and there is limited proliferation of cardiac progenitor cells and cardiomyocytes, in the end the affected tissue is not sufficiently perfused and the lost cardiomyocytes are replaced by scar tissue. To fully regenerate the heart, an intervention is needed directly after the occurrence of an infarction, in order to reduce the damage to the myocardium. Furthermore, new cardiomyocytes are needed, either through stimulating proliferation of endogenous cells or cell transplantation. Lastly, other processes needed in regeneration such as angiogenesis also need to be stimulated. A central point in stimulating regeneration is cellular communication: how do (exogenous) cells induce survival and how can we alter the communication signals to increase regeneration?

Cell transplantation has been a focus of cardiac regenerative studies as it holds the potential to completely replace any lost cardiomyocytes. In chapter 2, we explored the different approaches taken in cell transplantation studies. Several different cell types have been used in both pre-clinical and clinical studies, such as bone marrow mononuclear cells (BM-MNCs), mesenchymal stromal cells (MSCs), and cardiac progenitor cells (CPCs). Of these, only the CPCs have the ability to differentiate to cardiomyocytes, but all three have shown to increase cardiac function in preclinical models. More interesting, the results from these transplantation studies show an effect on cardiac function and vessel formation with minimal retention and differentiation in the heart, suggesting that the paracrine effect of the cells is a major factor.

Besides replacing cardiomyocytes, restoring blood flow to the infarcted area is vital. Therefore, studying the mechanisms of angiogenesis in vitro can provide relevant insights for stimulating this process in vivo. It is important to use the proper assay to answer an angiogenesis related question. The methods and uses of a metatarsal assay, spheroid assay and embryoid body assay are described in chapter 3. Since angiogenesis is a process that is influenced by many signalling pathways, among which TGF-β, we sought out to identify the role of the TGF-β coreceptor endoglin in chapter 4. Using a variety of methods, among which embryonic stem cells from endoglin knockout mice and shRNA mediated endoglin knockdown, we showed that vasculogenesis is not hampered by the loss of endoglin. However, proper angiogenesis, among which network formation, was impaired when the endoglin expression was reduced, showing that endoglin and TGF-β signalling are indispensable for angiogenesis.

Since the effect of MSC and CPC transplantation after MI seems to be partly due to the paracrine factors they secrete, and extracellular vesicles (EVs) are a major part of the paracrine factors, we investigated if the EVs from MSCs and CPCs can affect angiogenesis in chapter 5. Both in vitro and in vivo angiogenesis was significantly improved in the presence of these EVs. In detail analysis showed the presence of several pro-angiogenic
factors, among which EMMPRIN. Knockdown of EMMPRIN resulted in a pronounced reduction in angiogenesis and cell migration.

The effect of CPC derived EVs after MI is studied in chapter 6. Here, we investigated the effect seen short term after MI, after 48 hours, to see if EVs can reduce the initial damage and convey survival signals to the tissue. Comparing CPC transplantation and EV injection on infarct size reduction showed similar results, concluding that EVs are able to mimic their donor cell effects. Furthermore, total proliferation was increase in the border zone and infarcted area as seen by an increase in Ki67 and Yap. Interestingly, in depth analysis showed that mainly endothelial cells and cardiomyocytes were increased in proliferation.

In summary, this thesis studies various means in which cellular communication, through cytokine signalling and EV secretion, plays a role in the regenerative processes after a MI. It shows the importance of proper angiogenesis and how the EVs from various cell types can stimulate this. Moreover, these EVs have similar effects as their donor cells after MI and can stimulate proliferation in the heart. Thus the use of EVs as a therapeutic to restore cardiac function is a viable alternative to using cells.