The direct effect of macrophages on wound healing and fibrosis

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Abstract

Wound healing is a multiple stage wound healing process in which damaged tissue is being repaired, but an impaired pathological repair can result in fibrosis. In fibrosis a damaged tissue is incompletely repaired following an elevated or persistent inflammation resulting in a hardened and impaired functioning tissue or a scar, primarily consisting of collagen type I. One of the main regulators in this multistage process are macrophages which act as first responders, in the early stages, by clearing and phagocytising wound debris. In the following stages they act as regulators by secreting several cytokines of which TGF-β is the most important by which they activate myofibroblasts to produce collagen. Besides these indirect regulatory effect of macrophages a more direct effect of macrophages on the collagen deposition is proposed. Macrophages producing collagen themselves are one of the possibilities but although research demonstrated that macrophages are capable of producing collagen and other ECM products no evidence for collagen I producing macrophages has been found. There is also a possibility for a fibrolytic phenotype macrophage altering and determining final ECM composition by for example MMP's and TIMPS. But also no evidence for such a phenotypic macrophage has been found, although macrophages are capable and do secrete MMP's but mainly in the early stages of wound healing favouring cell migration. Another possibility of contribution to the collagen deposition would be the transdifferentiation of macrophages into collagen producing myofibroblasts. Although there is evidence for a large portion of myofibroblasts originating from macrophages ,ranging from %50 to %80 of the total myofibroblast population, the functionality of these myofibroblasts has not been researched and therefore it remains unknown what the actual contribution of these macrophage derived myofibroblasts is to the total collagen deposition.

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Introduction

Already in the first century the Roman medical writer Aulus Cornelius Celsus described the process of inflammation with the words "*rubor, dolor, calor and tumour*", since then physicians and scientists have tried to unravel this mysterious and complex system of the body in battle against chemical, microbial, physical or mechanical damage. Not only does inflammation play a very important role in the body's reaction against pathogens in diseases but it is also the first phase of wound healing. In case of a bleeding, a rupture of a blood vessel, the inflammatory phase is preceded by haemostasis which stops the bleeding by closing the ruptured site. Haemostasis can be divided in three stages: first the surrounding blood vessels of the damaged site contract (vasoconstriction) after which in the second stage a plug of blood platelets forms, leading to a clot in the third stage, activated by several clotting factors and formation of fibrin binding the platelets. After the haemostasis phase follows the inflammatory phase which clears the damaged site of pathogens and other debris like dead or damaged cells. Inflammation and wound repair do not only follow after haemostasis but can also be induced by damage following pathogen associated tissue damage or for example liver damage due to chronic alcohol abuse. After the inflammatory phase follows the proliferative phase in which tissue, made up of collagen and extracellular matrix (ECM) molecules , is being rebuild, the wound is being contracted and a new network of blood vessels is constructed. After the proliferative phase follows the maturation phase in which the rebuild tissue is being remodelled and now unnecessary cells are removed by apoptosis or programmed cell death. The collagen produced in the proliferative phase is laid down unorganised and results in a thickened scar. In the final maturation phase this unorganised collagen is aligned along tension lines and cross-linked which results in a stronger tissue, but is still impaired in strength compared to normal tissue and reduces scar thickness. When confronted with a dysregulation of any of the phases, for example by an elevated or chronic inflammatory phase due to a persistent irritant, excess tissue is deposited in the proliferative phase or impaired remodelling in the maturation phase can lead to fibrosis resulting in a scar with a hardened and impaired functioning tissue.

Although fibrosis can lead to enormous health problems in humans this system of wound healing has been preserved in million years of evolution not only in humans but also in most mammals indicating the importance of this system in the battle against injuries. Though in modern society, fibrosis is a large burden and great problem in health care. Whereas 4000 years ago a quick wound healing process could save a live, in modern society modern healthcare outpaced the ancient system and is rather causing problems. Just fibrosis of the liver (cirrhosis) alone had cost the United States of America a staggering 10 billion dollars in 2004 and was the twelfth cause of death (Neff, Duncan, & Schiff, 2011). These numbers are even expected to rise due to a predicted doubling of the percentage of patients with cirrhosis from Hepatitis C virus (Davis, Alter, El–Serag, Poynard, & Jennings, 2010). Taking into account that fibrosis does not only affect the liver but can affect almost all organs indicates the enormous extent of fibrosis human health and health care costs.

Wound healing therefore has been a great subject of research and uncovered a large part of the underlying mechanisms and regulatory systems in wound healing. But still not everything is known about the complex processes like the fine tuning of all mechanisms in normal wound healing and fibrosis or what exactly determines the normal wound healing to progress towards the pathological fibrotic outcome. Also scientists and physicians remain unable the predict if an injury will be repaired as a normal tissue or a fibrotic tissue. Although it is known that scarring follows persistent and increased inflammation and by increased ECM production the exact mechanisms and causes of this dysregulation remain unknown (Wynn & Ramalingam, 2012). But certain is that outcome of the composition of the ECM defines if a tissue is a fibrotic one or not.

The ECM consists of molecules which provide strength and is a scaffold to all cells in the human body. The main components of ECM are proteins and other carbohydrate containing molecules like proteins and proteoglycans and are secreted by cells. The most abundant molecule in the ECM is the protein collagen which forms strong fibers and gives structural support to surrounding cells and account for approximately 40% of all total human proteins (Campbell page 165-166). Collagen has a lot of different types which are present in different compositions in tissues, dictating the function of this tissue. Collagen is a triple helical protein of which 28 different types are known in vertebrates, are homotrimeric or heterotrimeric, are held together by hydrogen bonds and are surrounded by a highly organized hydration network. Interruptions and imperfections are known for causing conformational changes but the importance of these interactions are still under debate. Collagens can form into fibrils, for structure in a tissue, like collagen I which has not any imperfections in its hydration network and assembles into fibrils and is the main compound in a scar. But also collagens with imperfect hydration networks can form fibrillar collagen like collagen types II, III and V. Collagen II is present in cartilage whereas collagen III is mostly present in embryonic skin together with collagen I and V (Kadler, Baldock, Bella, & Boot-Handford, 2007).

One of the main producers of collagen in the ECM deposition of wound healing are myofibroblasts which carry out pro-fibrotic, contractile and pro inflammatory activities during the proliferative phase (Wynn & Barron, 2010). Due to its collagen producing capacity and as the origin of most collagen in a scar, myofibroblast have been the main subject of research in understanding and preventing the fibrotic process in wound healing. Although myofibroblasts seem to play a determining role in scar formation recent research predicates a maybe even more important role towards macrophages as the regulators of the whole wound healing process. Macrophages are part of the human innate body's response and are mononuclear phagocytes which respond to environmental cues like tissue damage and microbial products. In response to those cues they can express an array of phenotypes which are marked by two extremes: on one side the classical (M1) activated and on the other side alternative (M2) activated macrophages (Gordon & Taylor, 2005). M1 macrophages are induced by TLR ligands and IFN y and carry out an inflammatory like phagocytic phenotype whereas M2 macrophages are induced by IL-4 and IL-13 and express a more anti-inflammatory phenotype (Biswas & Mantovani, 2010). Macrophages originate from short time circulating monocytes expressing different receptors like FC y RIII (CD16) or the chemokine CCR2, CX3R and CCR8 receptors (Geissmann et al., 2010). But in most tissues also reside resident macrophages which are very heterogenous and adapt to the tissue environment in which they reside (Davies, Jenkins, Allen, & Taylor, 2014).

Although the role of macrophages in wound healing and fibrosis and their heterogeneity/plasticity is greatly researched their exact role on wound healing and fibrosis remains mostly unknown. Macrophages are known for their regulating role in wound healing and particularly their activation and stimulation of myofibroblast leading to collagen deposition and thereby contributing to fibrosis. Although macrophages have a huge indirect effect on collagen production and thus scar formation, a more prominent direct role of macrophages on collagen production and fibrosis have been described by recent research but which remain largely unclear. Therefore the aim of this thesis is to describe if besides the indirect effect of macrophages there is also a more direct effect of macrophages on collagen production and fibrosis.

Indirect effects

The indirect effects of macrophages in the development of fibrosis is largely due to its regulating role of the wound healing process. After blood clotting and the formation of a provisional ECM, macrophages and neutrophils are attracted into the wound site as first responders. During this early inflammatory phase M1 like macrophages perform pro-inflammatory functions like phagocytosis, anti-gen presenting and releasing cytokines and growth factors. After the inflammatory phase macrophages also play an important role in the successive phases of: proliferation, reepithelialisation and remodelling which are not precisely defined by time but are a dynamic overlapping processes (Eming, Krieg, & Davidson, 2017). In the inflammatory phase macrophages mostly differentiate into M1 like macrophages under the influence of pro-inflammatory cytokines released by for example neutrophils such as IL-1a, IL-1b, IL-6 and TNF-α (WERNER & GROSE, 2003). In this inflammatory phase M1 macrophages amplify the inflammation by attracting fibrocytes through CCL2 secretion and supporting myofibroblast proliferation by TNF-α and IL-10β secretion (Cao, Wang, & Harris, 2014). After a few days the main phenotype of the macrophage populations shifted from mainly M1 macrophages to M2 like macrophages, either through apoptosis of M1 macrophages after clearing wound debris and an influx of new M2 stimulated macrophages or through differentiation of M1 macrophages into M2 like phenotypes (L. Baum & J. Arpey, 2005; Lucas et al., 2010). In contrast to the M1 like macrophages the new M2 like macrophage population secrete anti-inflammatory factors to help diminish the previous inflammation and promote an anti-inflammatory environment for healing and regeneration of the wound. But when the injury persists M2 macrophages adapt a pro-fibrotic role and secrete factors such as TGF-β and galectin-3 (Vernon, Mylonas, & Hughes, 2017). Of these secreted factors TGF-β is probably the most important indirect contributor of macrophages to the fibrotic process as TGF-β promotes the ECM production, like collagen, by activating myofibroblast and stimulating fibroblast, pericytes, endothelial and epithelial cells into differentiating into myofibroblasts. This differentiation into myofibroblasts can also be stimulated by DAMPs, angiotensin II, PDGF, FGF2 and IGF-1 (Kurose & Mangmool, 2016; WERNER & GROSE, 2003).

TGF-β activates fibroblast and other cells by binding the TGF-β II receptor which activates the TGF-β I receptor forming the TGF-β receptor complex. This TGF-β receptor complex phosphorylates Smad 2 and Smad 3, together forming R-Smad, forming a transcriptional complex with Smad 4 and translocates to the nucleus where it regulates the TGF-β gene (Medina et al., 2011). The transcription of the TGF-β gene results in proliferation, contraction, ECM production, autocrine TGF-β secretion upregulation and differentiation in myofibroblasts (figure 1)(Walraven, Gouverneur, Middelkoop, Beelen, & Ulrich, 2014). Knockout of the Smad 3 protein results in a faster and less fibrotic healing of a wound (Ashcroft et al., 1999) due to a reduced macrophage infiltration in the wound and a reduced matrix deposition compared to wildtype mice. In the same research they administered exogenous TGF-β resulting in an increase of the matrix deposition in the wild type mice but not in Smad 3 homozygote knockout mice without evidence for increased fibroblast numbers. Not only did depletion of the regulatory Smad 3 protein reduce the scar formation but also an overexpression of TGF-β results in hypertrophic scar formation and counteracting TGF-β is associated with a reduction of scar formation (Choi et al., 1996; Ishida, Gao, & Murphy, 2007). Suggesting an important role of macrophages on the scar formation by activating myofibroblasts through the secretion of TGF-β.

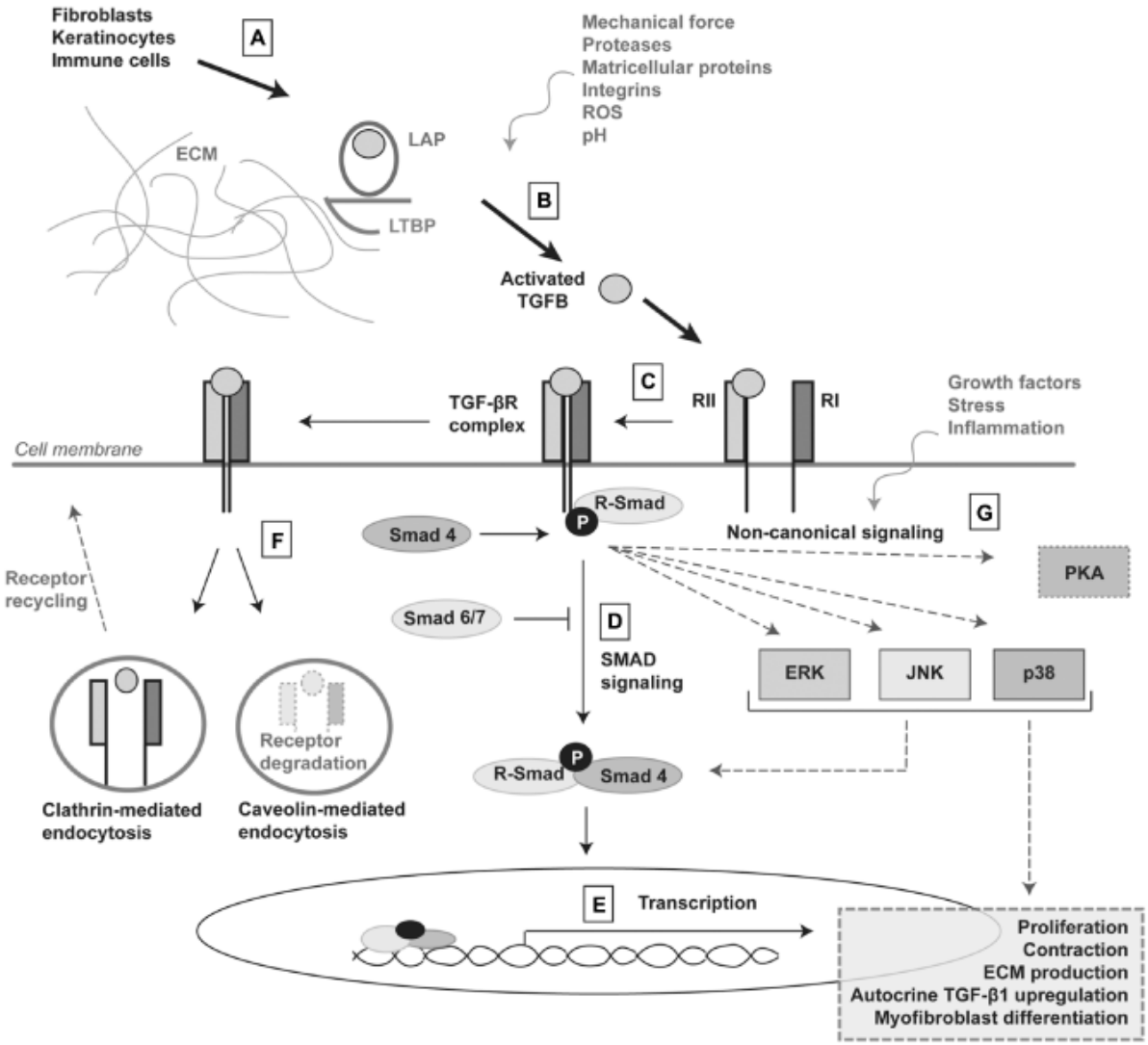

Figure 1 shows the signaling pathway of TGF-β in fibroblasts. R-Smad is the combination of Smad 2 and Smad 3 (Medina et al., 2011).

But what is the exact role of myofibroblast as the TGF-β induced effector of macrophages? Myofibroblasts are the main producers of ECM components in wound healing and are characterized by smooth muscle actin (α-SMA) and can originate from many precursor cells like circulating fibrocytes, local fibrocytes and pericytes or through the endothelium/epithelium to mesenchymal transition (EndoMT and EMT) (Conway & Hughes, 2012). The most secreted ECM products by myofibroblast are collagen types I, III, IV and V but the final ECM composition is not only determined by the secretion of myofibroblast but also other factors like MMPs, TIMPS and crosslinking determine the final outcome of the ECM composition. Other than secreting ECM components myofibroblasts are also responsible for creating tension and contracting a wound by its smooth muscle like phenotype (Hinz, 2017).

Direct effects

That the effect of macrophages on fibrosis is existent was already shown by Pierce and his colleagues in 1989 (Pierce et al., 1989). Their research showed that the complete depletion of monocytes resulted in a reduced fibrotic wound. So clearly macrophages do have an important role on fibrosis partially by its effect on myofibroblasts, as described earlier, and thereby the ECM production and composition. But there are also indications for a more direct role of macrophages on the ECM production and scar formation for example possibly by macrophages producing collagen themselves, macrophages differentiating into myofibroblasts or a direct effect on the final ECM composition by secreting MMPs (figure 2). All of them are less well known possible effects of macrophages but might also be major contributions to the scar formation, making macrophages even more determining in the wound healing process than their indirect regulatory role which they are held accounted for now (Nikolic-Paterson, Wang, & Lan, 2014).

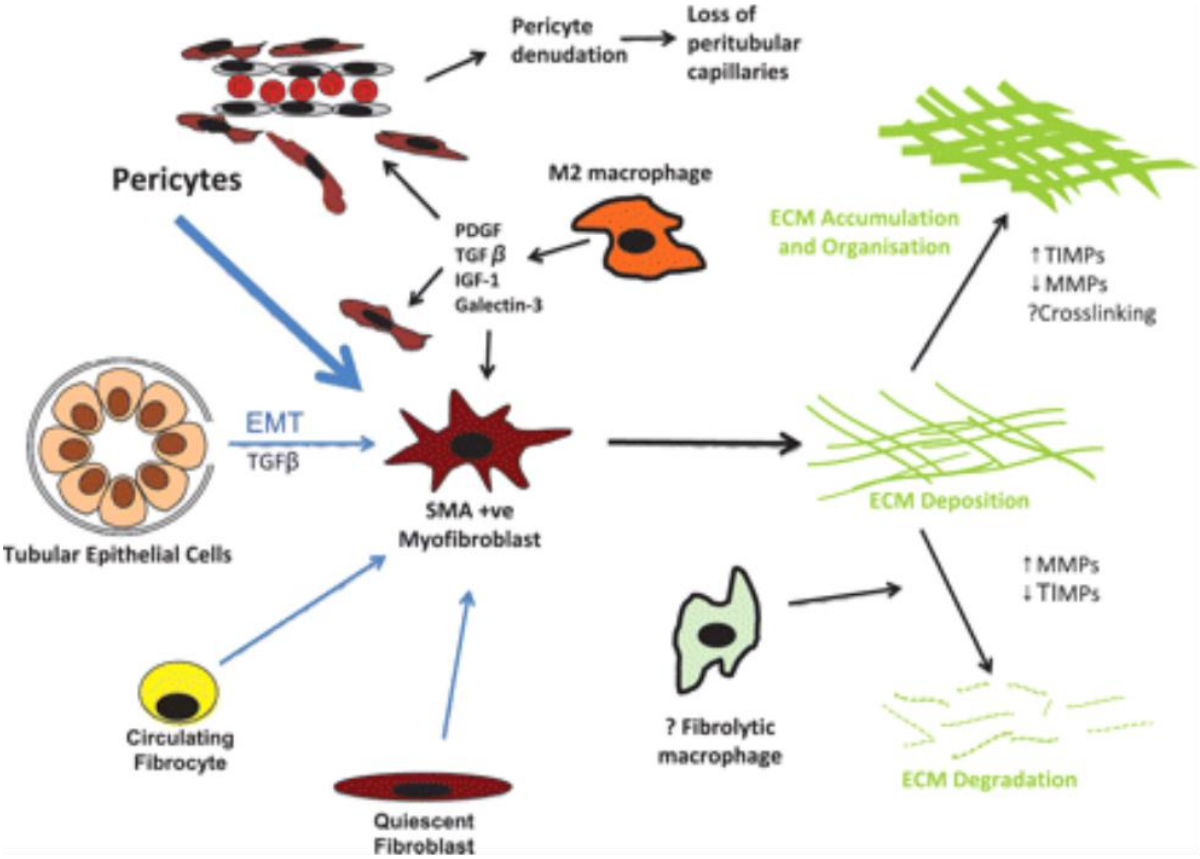

Figure 2 shows the possible origins of myofibroblasts and the influence of macrophages in the wound healing process (Conway and Hughes, 2012*)*

Macrophages producing ECM

That macrophages are actually capable of synthesizing collagen and have the machinery to do so was already demonstrated in 1999 (WEITKAMP, CULLEN, PLENZ, ROBENEK, & RAUTERBERG, 1999). They demonstrated that macrophages expressed and secreted collagen type VIII which is a short chain non-fibrillar collagen type that is a constituent of the extracellular matrix in for example in the sclera, skin and glomerulus but it is also important in the vasculature and promoting smooth muscle cell migration (Shuttleworth, 1997). Collagen VIII also has a possible role in fibrosis by adhering to ECM components, especially collagen I, and thereby creating an overlay over collagen I favouring a substrate for migration (Adiguzel et al., 2005). Not only did Weitkamp and his colleagues demonstrated that macrophages are capable of producing collagen they also showed that LPS and

INF-γ stimulation decreased the collagen VIII secretion and elevated the secretion of matrix metalloproteinase 1 (MMP-1), which can degrade ECM, indicating a switch of a collagen producing macrophage towards a collagen degradative macrophage. More recent research (Skrbic et al., 2015) also confirmed the importance of collagen VIII in fibrosis. Skrbic and his colleagues showed that in a heart with left ventricular overload, leading to hypertrophy and fibrosis, reduced collagen VIII is correlated to the degree of dilatation and reduced fibrosis.

That macrophages possibly are not only capable of synthesizing and secreting Collagen VIII was shown by (Schnoor et al., 2008). They demonstrated that almost all collagen types mRNAs are expressed by monocytes and macrophages with the exception of types XIII and XXII. The most abundant expressed collagen was type VI of which variations in expression and distribution are associated with certain pathological conditions like fibrosis . Although highly expressed Schnoor and his colleagues found no actual secretion of collagen VI into filaments, indicating that although macrophages express collagen mRNA's they actually do not secrete all those collagen types. Therefore further research was done by Schnoor and his colleagues and they concluded that macrophages secrete collagen VI during differentiation and maturation. They also demonstrated that macrophages secrete collagen via the Golgi apparatus independent of intracellular microtubular transport and that under the influence of TGF-β the amount of secreted collagen VI was increased. Although the secreted collagen VI is not assembled into filaments but is bound to the cell surface, further investigation showed that the necessary processing proteins to do so are present in macrophages comparable to the levels of those processing proteins in fibroblast, indicating that macrophages actually are equipped to process collagen correctly. Also other non-collagen ECM compounds like fibronectin and BIG-H3 were expressed at levels comparable to fibroblasts. Also suggesting a possible direct effect of macrophages on the ECM composition although no evidence have been found for the secretion of the fibrillar types collagen I and III.

Macrophage-myofibroblast transdifferentiation (MMT)

Myofibroblasts do not only originate from fibroblast but can also be derived from other cells like epithelial or endothelial cells (Moore-Morris, 2016). Also leaving open the possibility for myofibroblast originating from macrophages through which way macrophages would directly contribute to the collagen production as transdifferentiated myofibroblasts. Other researchers demonstrated that in the foreign body reaction myeloid cells like monocytes and macrophages do contribute to the myofibroblast population (Mooney et al., 2010). This was demonstrated in a foreign body reaction model in rats, in which Mooney and colleagues implanted a foreign body object in the form of a sterile cube of protein in the peritoneal cavity. In the foreign body reaction an inflammatory response against a foreign body object is followed by the encapsulation of this object by fibrotic tissue like in a scar. Mooney and colleagues showed that within the capsule a large quantity of the α-SM actin cells also were positive for the myeloid (macrophage/monocyte) EGFP marker. At day 14 this accounted for 12.7% +/- 0.5% for all α-SM actin cells this even increased to a percentage of 63% +/- 14.5% at day 28.

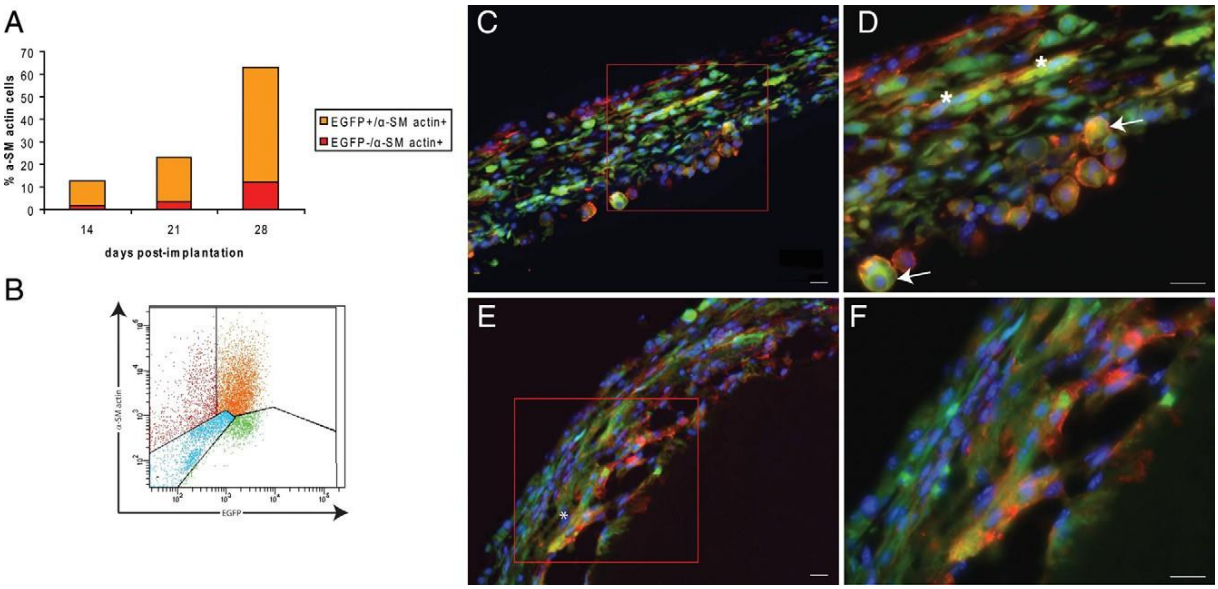

Figure 3 **A** shows the percentage of total cells expressing *α-SM* actin at days 14, 21 and 28 **B** shows the FACS *α-SM* actin profile at day 28 **C** shows the coexpression of EGFP (green) and *α-SM* actin (red) at day 14 **D** shows a magnification of C with some elongated cells (*) and round cells at day 14 **E** shows coexpression of (EGFP) green and *α-SM* actin at day 28 **F** is a magnification of E showing predominantly elongated cells (Mooney et al. 2010).

After analysis at day 14 a large portion of the co-expressing cells still retained their macrophage like round morphology and only a small portion exhibited an elongated myofibroblast like morphology. At day 28 the majority of the co-expressing cells exhibited a spindle like myofibroblast morphology, as confirmed by (Jabs, Moncada, Nichols, Waller, & Wilcox, 2005; Mesure, de Visscher, Vranken, Lebacq, & Flameng, 2010).

Just like fibroblasts differentiate into myofibroblasts under the influence of TGF-β, macrophages also seem to transdifferentiate under the influence of the same cytokine secreted by themselves (NINOMIYA, TAKAHASHI, FUJIOKA, ISHIKAWA, & YOKOYAMA, 2006). Ninomiya and colleagues examined the effects of TGF-β on macrophages by using macrophages, identified by the CD11b antigen, after which they stimulated one cell culture with TGF-β1 (1 ng/ml) while the other cell culture was kept at 10% FBS as a control. After 10 days they saw an increase in the myofibroblast SMemb, α-SM actin and calponin markers (figure 4) in the TGF-β treated group, in contrast the macrophage CD11B antigen decreased in this group. Also a test with a TFG-β receptor antagonist named SB-431542 was held to examine if the effect is accomplished by the same receptor pathway

as in fibroblasts, which was confirmed (figure 6). Also the effect of TGF-β on the SMC marker expressions seems to be dose dependent (figure 5).

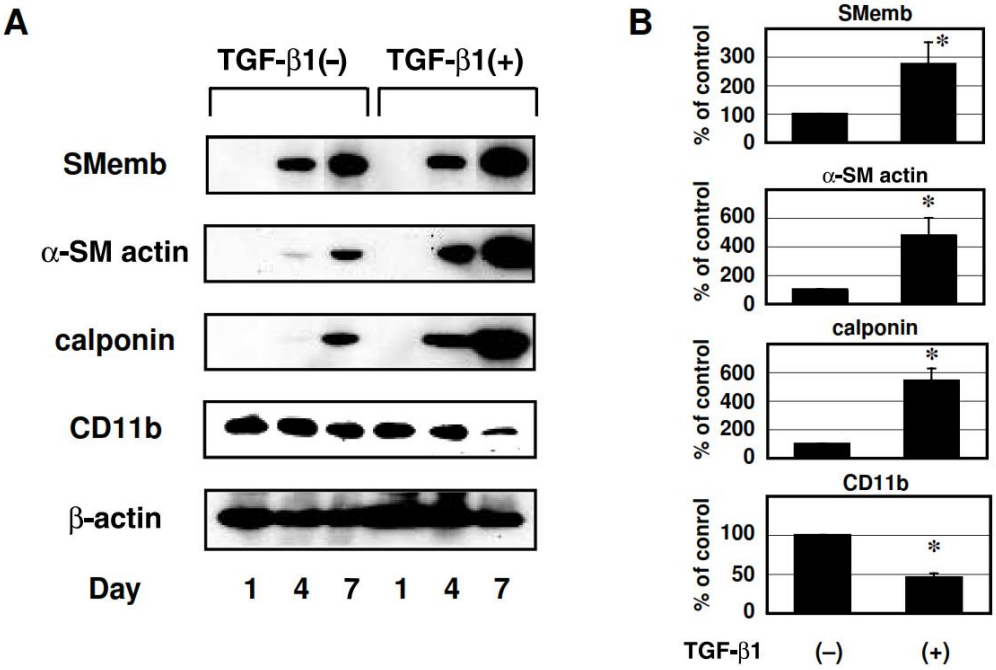

Figure 4 shows that SMC markers are enhanced by TGF-β **A** confirmed by Western Blotting on the right panel **B** the results are quantified and are expressed as relative percentages to the control (Ninomiya et al., 2010).

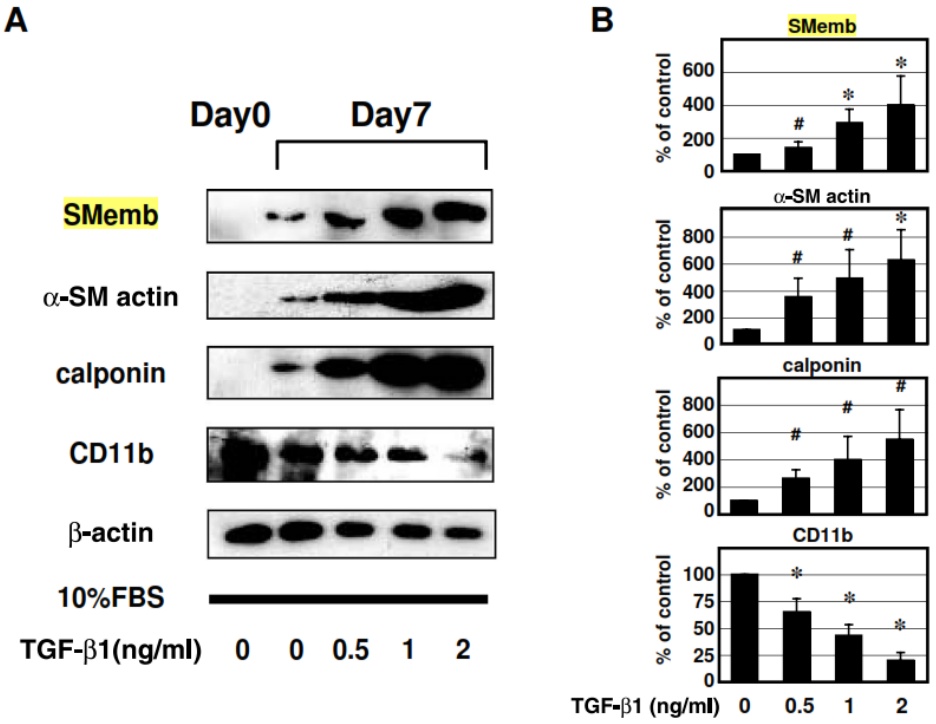

Figure 5 shows that the effects of TGF-β are dose dependant. **A** shows the effect of TGF-β on SMC markers by Western blotting after 7 days by dose in **B** the results are quantified and displayed as relative percentages compared to the control (Ninomiya et al., 2010).

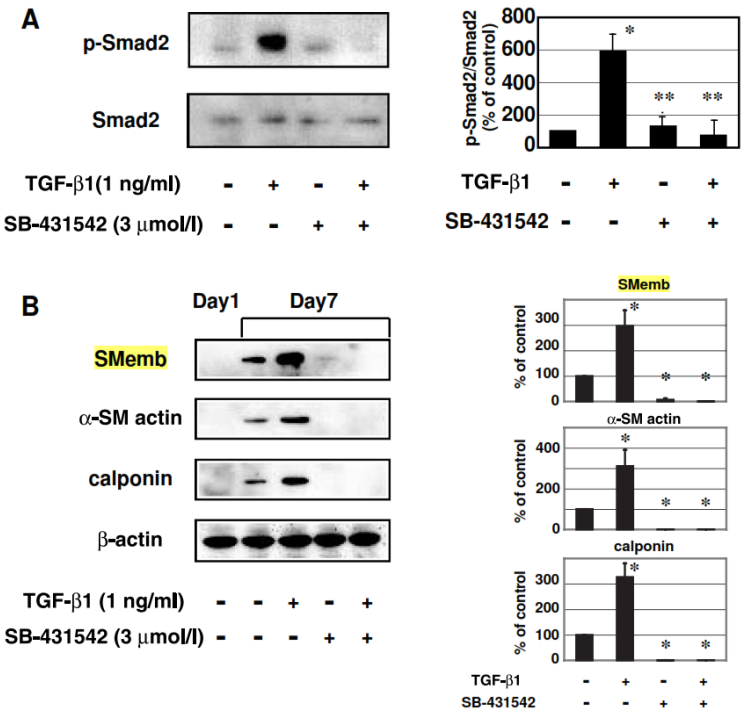

Figure 6 shows SB-431542 blocking the TGF-β effect on SMC markers. **A** marcophages were pretreated on day 2 for a hour with SB-431542 after which they were stimulated with TGF-β for a half hour. Then a Western Blot was performed with the results in the left panel in the right panel the results are quantified to the total Smad2 level of the control. In **B** macrophages are cultured with or without SB-431542 for 7 days and in the presence or absence of TGF-β. The left panel shows the results on the SMC markers by Western Blot. In the right panel results are presented representative as compared to the control (Ninomiya et al., 2010).

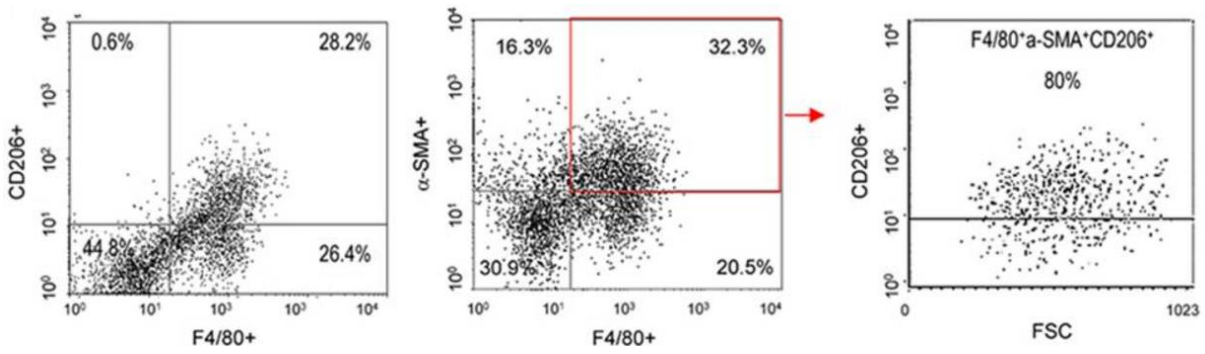

Figure 7 shows a flow cytometric analysis of cells isolated at day 7. Showing that 80% of the population co-expressing F4/80 and *α-SMA* also express CD206 indicating a M2 like macrophage enriched population (Meng et al., 2016).

Other research by (Meng et al., 2016) also confirmed the transdifferentiation of macrophages into myofibroblasts under the influence of TGF-β. Showing that myofibroblasts originating from macrophages predominantly expressed a M2 phenotype. Figure 7 shows that of all cells expressing α-

SMA, 32.3% also express the murine macrophage receptor F4/80 of which 80% also express CD206 which is a other well-known receptor of macrophages.

Other research confirms that predominantly M2 macrophages undergo MMT and even shows 60% of α-SMA cells originating from macrophages (Wang et al., 2015). But how much these myofibroblast actually contribute to the total collagen production has not been researched and remains unknown.

MMPs

Another important effect on the final scar formation and ECM composition is those of the matrix metalloproteinases (MMPs) which are secreted by almost all cell types in a wound site and are counteracted by tissue inhibitory of metalloproteinases (TIMPs). MMPs are known for their role in ECM turnover but they also promote cell migration and angiogenesis (Giannandrea & Parks, 2014). Although MMPs are also secreted by macrophages the function of these specific MMPs is mostly in the early stages of wound healing to improve cell migration and a possible direct role in scar formation and indications for a "fibrolytic" macrophage remains unknown. The first MMP was discovered by Gross and Lapiere in 1962 and is known as collagenase (MMP-1), and is able to degrade fibrillar collagen by cleaving the peptide bond between Gly777-Ile776 or Gly775-Lys776 in collagen types I, II and III (Gross & Lapiere, 1962)(Welgus, Jeffrey, & Eisen, 1981). Other important MMPs in wound healing are for example are MMP-3 of which a deficit leads to impaired wound healing or MMP-7 which is known for its mediation of epithelial cell migration in wounds (Page-McCaw, Ewald, & Werb, 2007). But the most and clear effect seems to be that of MMP-1 of which reduced levels lead to hypertrophic scars and increased levels reduce fibrosis in mouse models (Eto et al., 2012; Iimuro et al., 2003; Kaar et al., 2008). Another MMP with a direct effect might be MMP-8 of which overexpression and depletion reduces lung and liver fibrosis and seems to soften scars in skin (Gutierrez-Fernandez et al., 2007).

It is known that macrophages are able to express MMP-1 in coronary plaques under the influence of C5a complement factor (Speidl et al., 2011). In this study they showed in vitro that after C5a complement activated macrophages expressed increased MMP-1 mRNA levels and could be blocked by C5a/CD88 antibodies. They also concluded that the activation was mediated by the transcription factor AP-1. Other research subscribes this MMP-1 secreting phenotype to the classical M1 activated macrophages also upregulating MMP types -3, -7, -10, -12, -14 and -25 and the alternative activation leads to a decreased MMP -2, -8 and 19 but has an increased -11, -12 and -25 expression (Huang, Sala-Newby, Susana, Johnson, & Newby, 2012). Indicating a possible anti fibrotic effect of M1 macrophages which is lost after differentiation towards the alternative M2 phenotype although the exact effect of the secreted MMPs on the scar formation has not been researched yet.

Discussion

Although there is no clear evidence for all possible stated direct effect of macrophages on the scar formation, the MMT of macrophages seem to be a possible contributor to the fibrillar collagen production showing myofibroblasts originating from macrophages from 60% up to 80%, although there is no confirmation that these myofibroblasts are actual functional collagen secreting myofibroblasts. As shown by Hosper and his colleagues, the collagen I deposition from transdifferentiated epithelial myofibroblasts was not present or hardly detectable (Hosper et al., 2013). There is also no direct evidence for macrophages contributing to and producing fibrillar collagen themselves although it is proven they are capable to do so and secrete other non-fibrillar ECM components. Also no evidence of macrophages influencing the ECM composition of the final scar by a fibrolytic macrophage phenotype through MMP or TIMP activity has been found. Although macrophages are capable of producing for example MMP-1 this is only present in M1 macrophages

in early wound healing phases and not in the late phases of remodelling M2 macrophages, also another beneficial MMP-8 seems to be decreased in the late stage M2 macrophages suggesting that a fibrolytic macrophage phenotype is lost in the late phases after M1 to M2 differentiation.

Further research on the functionality of MMT derived myofibroblasts is needed to determine if macrophages can directly contribute to the collagen production in scars through MMT. Also the precise level of collagen production from MMT derived myofibroblasts compared to other originated myofibroblasts has to be determined. A study in which the direct effect of macrophages could be tested, would be a complete depletion of macrophages in a wound but keeping its indirect effect intact by artificially mimicking the microenvironment as if the macrophages are still present. When the functionality and direct influence on collagen production of MMT derived myofibroblasts is confirmed succeeding research can be done to block the MMT process and be researched as a possible way to improve the wound healing process and less fibrotic scars.

Also further research on the possible fibrolytic capacity of macrophages can be done for example by examining the effect of different phenotypes of macrophages on existing and developing scar tissues. If the fibrolytic capacity of proposed M1 like macrophages is confirmed further research on exploiting this phenotype in the wound healing process can be done. For example by favouring M1 like macrophages in a wound eventually resulting in a less fibrotic scar.

Although no exclusive evidence have been found for a direct effect of macrophages on fibrosis by direct macrophage collagen production or remodelling by MMP activity, a direct effect of macrophages on the collagen production by MMT is possible. Although the level of the contribution to the total collagen production remains unknown as it also remains unknown if the MMT derived myofibroblasts are even functional in secreting collagen.

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