Eicosanoids in tissue repair

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Abstract

Trauma or infection can result in tissue damage, which needs to be repaired in a well-orchestrated manner to restore tissue function and homeostasis. Lipid mediators derived from arachidonic acid (termed eicosanoids) play central and versatile roles in the regulation of tissue repair. Here, I summarize the current state-of the-art regarding the functional activities of eicosanoids in tissue repair responses during homeostasis and disease. I also describe how eicosanoids are produced during tissue damage and repair in a time- cell- and tissue-dependent fashion. In particular, recent insights into the roles of eicosanoids in epithelial barrier repair are reviewed. Furthermore, the distinct roles of different eicosanoids in settings of pathological tissue repair such as chronic wounds, scarring or fibrosis are discussed. Finally, an outlook is provided on how eicosanoids may be targeted by future therapeutic strategies to achieve physiological tissue repair and prevent scarring and loss of tissue function in various disease contexts.

Introduction

Eicosanoids (*είκοσι* (greek) =20) are metabolites of polyunsaturated fatty acids (PUFAs) with 20 carbon atoms. Amongst the eicosanoids, metabolites of the ω-6 PUFA arachidonic acid (AA) are particularly potent signaling molecules with important roles in inflammation and immunity (for a recent review see[1]). Together with their immunological functions, eicosanoids are versatile mediators of tissue repair. Depending on the time after injury and the tissue microenvironment, eicosanoids can either promote or suppress healing responses (Table 1).
In order to fullfill these distinct functions, eicosanoid synthesis occurs in a time-dependent fashion during the different phases of tissue repair (Figure 1). The first days after injury are refered to as the «inflammatory phase», which is characterized by the infiltration of neutrophils and monocytes/ macrophages. Eicosanoids such as leukotrienes (LTs) and thromboxane A_2 (TXA_2) contribute to the recruitment and activation of these cells (Figure 1).[2,3] Activated wound macrophages produce chemokines, eicosanoids (e.g. PGE_2) and growth factors, thus promoting the migration and proliferation of fibroblasts. This phase of tissue repair is therfore called the «proliferative phase». In response to growth factors (e.g. TGFβI), fibroblasts differentiate into myofibroblasts and upregulate the production of extracellular matrix (ECM) components such as collagen. Newly deposited ECM then undergoes structural «remodeling» and the inflammatory response is terminated (e.g. by the action of lipoxin A_4 (LXA_4)), thus resulting in «resolution» and the formation of new functional tissue.

This review aims to provide an overview of the roles of eicosanoids in physiological as well as pathogenic situations of tissue repair. As eicosanoids produced via the cyclooxygenase (COX) pathway (prostanoids) play particularly important roles in tissue repair, these mediators will be discussed in detail and with a focus on recent insights into their involvement in the repair of epithelial barriers.[4,5]

Total and conditional knock-outs of eicosanoid pathway components in mice have provided detailed mechanistic insights into the contribution of individual metabolites and receptors to tissue repair. These studies showed that COX metabolites (e.g. prostaglandin E_2) can accelerate repair, whilst LTs produced via the 5-lipoxygenase (5-LOX) pathway can impair tissue repair and instead cause aberrant inflammation and tissue (Table 1).[4–7] In contrast to the detrimental role of pro-inflammatory 5-LOX metabolites, anti-inflammatory metabolites of 12/15-LOX (e.g. LXA_4) can reduce inflammation and promote tissue repair.[8] Similarly, anti-inflammatory derivatives of ω-3 PUFAs (e.g. resolvins), also termed «specialized pro-resolving mediators (SPMs)», have recently been implicated in tissue repair.[9–11] Indeed, the local application of lipoxins or resolvins to damaged tissues could restore tissue integrity and prevent inflammatory damage, particularly in settings of epithelial barrier repair. Similar beneficial effects on barrier repair have also been reported for PGE_2, which promoted healing of chemotherapy-induced mucosal lesions in human cancer patients and was recently identified as a key factor in intestinal tissue repair. [5,12]

Thus, although targeting eicosanoid pathways in tissue repair is not a new idea, we are only now beginning to understand the complexity of eicosanoid-mediated effects in tissue repair. Recent mechanistic insights into the eicosanoid-driven modulation of repair responses should foster the development of new and more precise therapeutic strategies for conditions with impaired or aberrant tissue repair in human patients.

Biosynthesis of eicosanoids during tissue damage and repair

To achieve rapid healing without scar formation or aberrant inflammation, the synthesis of eicosanoids needs to be controlled in a time- and tissue-dependent fashion. Upon tissue damage, AA is released from cellular membranes by the action of cytosolic phospholipase A2 (cPLA2) and is thus made available for the subsequent conversion into a wide array of bioactive metabolites.

By the action of **COX enzymes**, which are the mechanistic targets of nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, AA is converted into prostaglandin (PG) H_2, the precursor of all bioactive prostanoids (Figure 2) (for a more detailed review of eicosanoid synthesis pathways see [1]). Prostanoids have highly diverse roles in pain, fewer and inflammation and they control hemostasis and vascular tone. The production of each individual prostanoid depends on terminal synthases, which are differentially expressed by different cell types and in different tissues. After trauma, activated platelets release TXA_2 by the action of thromboxane synthase, whilst prostacyclin (PGI_2) is released from endothelial cells, which express prostacyclin synthase (Figure 2). Tissue damage also results in the release of PGE_2 from stromal cells or cells of the myeloid lineage, which unpregulate microsomal prostaglandin E synthase-1 (mPGES-1).[13] Mast cells, which are also activated during trauma, express prostaglandin D synthase and thus release PGD_2 upon challenge.[14]

As an alternative to the COX pathway, AA can be metabolized via **LOX enzymes**, giving rise to hydroxyeicosatetraenoic acids (HETEs), LTs or lipoxins. Together with myeloid cells, which can release large amounts of LTs upon cellular damage, epithelial cells have been implicated more recently in the production of LTs in settings of inflammation and tissue remodelling, particularly in the lung.[15] In the intestine, LT biosynthetic enzymes are also expressed by

(epithelial) tuft cells (also called brush cells), which have recently been implicated in the induction of type 2 immune responses.[16] In addition to 5-LOX, the synthesis of bioactive LTs (LTB_4 and cysteinyl LTs (cysLTs, LTC_4, LTD_4, LTE_4)) requires the enzymes LTA_4 hydrolase (LTA_4H) and LTC_4 synthase (LTC_4S). During injury, LT-biosynthetic enzymes are induced, resulting in increased levels of both LTB_4 and cysLTs in damaged tissues such as the lung or skin.[17] In addition to the COX and 5-LOX pathways, AA can be metabolized by 12- or 15-LOX enzymes, which are expressed by multiple cell types including myeloid cells and stromal cells. 12-/15-LOX metabolites, which are produced during tissue damage and repair include 12- and 15-HETE as well as anti-inflammatory LXs.[8] Of note, mice express a 12-/15-LOX enzyme, which preferentially synthesizes 12-HETE, whilst the homologous enzyme in humans (15-LOX 1) mainly produces 15-HETE.[18] During tissue repair, LOX and COX enzymes do not only produce AA-derived eicosanoids, but also metabolize ω-3 PUFAs (e.g. DHA, and EPA) into anti-inflammatory SPMs.[19]

Another group of enzymes, involved in the metabolism of ω-3 and ω-6 PUFAs in settings of tissue repair is the **cytochrome P450 (CYP) pathway**, which is particularly abundant in endothelial cells.[20] CYPs, convert AA into epoxyeicosathetraenoic acids (EETs), which have long been recognized for their vascular activities, but have only more recently been implicated in tissue repair.[20]

In summary, tissue damage results in the release of multiple eicosanoids with diverse roles in tissue repair and inflammation, which will be discussed in detail in the following sections.

Roles of eicosanoids in tissue repair

Thromboxane - a critical mediator of haemostasis and early phases of tissue repair

After tissue injury, bleeding has to be stopped and the open wound needs to be closed quickly in order to avoid contamination with external microbes. An important process during this initial phase of tissue repair is the activation and aggregation of platelets. The COX metabolite thromboxane A_2 (TXA_2), which is synthesized by activated platelets acts in an autocrine fashion to trigger the irreversible aggregation of platelets.[21] Indeed, defects in TXA_2 receptor (TP) signalling have been linked to bleeding disorders in humans and TP deficient mice show prolonged bleeding and delayed platelet aggregation.[22] In addition to its autocrine roles in haemostasis, TXA_2 has been reported to promote the recruitment of macrophages and fibroblasts, which contribute to tissue repair by secreting growth factors and extracellular matrix (ECM) components.[21] Thus, in a model of acute toxic liver injury, TP receptor signaling was responsible for timely tissue regeneration.[3,23]

In summary, TXA_2 is produced immediately after tissue damage and provides a critical link between haemostasis and the subsequent inflammatory phase of tissue repair (Figure 1).

Initially considered as a mere by-product of TX synthesis, **12-hydroxy-heptadecatrienoic acid (12-HHT)** has more recently been shown to be critically involved in tissue repair responses. By binding to the low affinity receptor for LTB_4 (BLT2), 12-HHT can induce the migration of keratinocytes and thereby promote skin wound healing.[24] Moreover, in a rat model of diabetes, administration of a BLT2 agonist promoted the release of growth factors (TGFβ1 and basic fibroblast growth factor) from keratinocytes, thus accelerating fibroblast proliferation and wound closure.[25] In line with its roles in skin wound healing, 12-HHT has also implicated in tissue repair in the eye as mice treated with NSAIDs showed impaired corneal healing, which could be rescued by administration of 12-HHT.[26] Taken together, the thromboxane synthesis pathway provides critical

signals for tissue repair responses particularly during the first 24 hrs after tissue injury.

Leukotriene B_4 - an early mediator of the inflammatory phase after injury

During the initial phase of tissue repair, which is mainly aimed at sealing off the wound, inflammatory cells (particularly neutrophils) are recruited to the site of injury in order to prevent infection and initiate further cell recruitment. As a major chemoattractant for neutrophils, the 5-LOX metabolite LTB_4 plays a key role in early inflammatory cell recruitment both during sterile injury and infection.[26] However, whilst the early and transient production of LTB_4 is instrumental for rapid wound closure, dysregulated LTB_4 formation can contribute to increased inflammatory injury e.g. in settings of lung fibrosis.[27] This study also showed that in addition to its pro-inflammatory effects on neutrophils, LTB_4 (via its high affinity receptor BLT1) promoted the production of growth factors (e.g. TGFβ1) by macrophages, and epithelial cells, thus stimulating collagen production by fibroblasts. Furthermore, in a model ot type 2 diabetes, exaggerated production of LTB_4 was observed in wounds of diabetic mice and involved in the increased susceptibility to infection with antibiotic resistant *S. aureus*.[28] Indeed, multiple studies have implicated the 5-LOX-LTB_4-BLT1 axis in aberrant neutrophil recruitment and impaired tissue repair in experimental models of skin wound healing. In particular, 5-LOX deficiency or pharmacological targeting of the 5-LOX pathway resulted in accelerated cutaneous healing, likely by reducing the production of reactive oxygen species (ROS) as well as of pro-inflammatory cytokines and chemokines in wounded skin.[6,29] Similarly, 5-LOX deficient mice and mice treated with LT-modifying drugs were protected against acute inflammatory lung injury in a model of bacterial sepsis.[17] However, these studies also suggested that in addition to the LTB_4-BLT1 axis, cysteinyl LTs contributed to 5-LOX-driven inflammatory tissue damage and aberrant tissue repair.

Cysteinyl leukotrienes - culprits in pathogenic repair and remodelling

Upon tissue damage, activated platelets can metabolize neutrophil-derived LTA_4 into cysLTs, a phenomenon termed “trans-cellular metabolism”.[30] The activation of resident mast cells may further contribute to the early wave of cysLT generation during tissue injury (Figure 1, Figure 3).[14] The first wave of cysLTs can then promote the recruitment of eosinophils, which produce numerous molecules involved in tissue repair and tissue remodelling (for a recent review see [31]). In addition to eosinophils, monocytes, macrophages and dendritic cells can produce high levels of cysLTs, particularly when exposed to TGFβ1.[32] Thus, the recruitment of eosinophils and cells of the monocyte/ macrophage lineage during the inflammatory and proliferative phase of tissue repair results in a second wave of cysLTs, which has been linked to pathological remodelling, e.g. in asthma or fibrosis. During these later phases of tissue repair, stromal cells produce various mediators of tissue remodelling (including TGFβ1, wnt5A and transglutaminase 2 (TG2)), which can promote the generation of cysLTs by myeloid cells.[15,32] On the other hand, cysLTs produced by myeloid cells or damaged epithelium can stimulate collagen production by (myo-)fibroblasts by activating their high-affinity receptor (CysLT1R).[33] Thus, cysLTs are crucially involved in the bidirectional crosstalk between stromal cells and myeloid cells, which can drive pathological remodelling in inflammed airways.

The central role of cysLTs in pathological tissue remodelling suggests that in order for tissue repair to proceed normally, cysLT production has to be turned off during the repair process. Such negative regulation of cysLT biosynthesis may be provided by the mTOR/ p70S6 kinase pathway, which is a key driver of tissue repair and has been implicated in the suppression of cysLT production in human macrophages.[34,35]

In addition to promoting tissue remodelling in airway inflammation, cysLTs have been reported to act as negative regulators of bone repair as mice treated with the CysLT1R antagonist montelukast showed improved fracture healing.[36] In particular, inhibition of CysLT1R signaling promoted the proliferation and differentiation of chondrocytes in an experimental mouse model of fracture repair. In keeping with these pharmacological studies, mice genetically deficient in 5-LOX showed faster fracture healing, whilst healing was impaired and correlated with increased LT levels in COX-2 deficient mice.[37] Taken together, the exaggerated or prolonged generation of LTs after tissue injury can result in aberrant inflammation and host defense as well as pathological tissue repair in various tissues, including skin, lung and bone.

Prostaglandin E_2 - a key eicosanoid in physiological tissue repair

Given that AA can be shunted from the COX- into the 5-LOX pathway, increased generation of LTs may contribute to impaired tissue repair, which has been observed in the absence of COX enzymes.[37–39] However, recent studies using pharmacological inhibition or genetic ablation of prostanoid receptors suggest that COX metabolites (particularly PGE_2) actively participate in tissue repair responses.[5,40]

During the early inflammatory phase of tissue repair, immigrating macrophages and stromal cells produce PGE_2, which has been shown to activate myofibroblasts and thus promote wound contraction[41,42] (Figure 1, Figure 3). In addition, PGE_2 promoted wound closure in the skin by inducing the expression of the IL-6 family cytokine oncostatin M in wound macrophages.[43] Upon mucosal injury in the intestine, PGE_2 accelerated tissue repair by promoting the removal of fibrin clots and by driving the differentiation of wound-associated epithelial cells in small intestinal lesions.[5]

These positive effects of PGE_2 on wound closure and early repair responses were mostly dependent on two of the four PGE_2 receptors (EP1-4), namely EP2 and EP4. Whilst both EP2 and EP4 signaling can contribute to bone repair[44], the PGE_2-EP4 axis appeared to be particularly important in early barrier repair in the skin and intestine.[5,43]

During the subsequent proliferative phase of tissue repair, activated myofibroblasts, can themselves produce PGs (PGE_2 and PGD_2), which induce a regulatory macrophage phenotype expressing high levels of IL-10 and Arg1, but low levels of iNOS. Indeed, IL-10 and Arg1 have both been implicated in the prevention of tissue damage as well as in the regulation of pathological tissue remodelling in settings of type 2 immunity.[45–47] Thus, the PG-driven induction of these regulatory factors may provide a negative feedback loop, which limits further activation and migration of myofibroblasts during physiological tissue repair.[48] This implicates PGE_2 in the transition from the inflammatory to the proliferative phase of tissue repair and in the prevention of aberrant (myo)fibroblast activation. Due to its regulatory effects on fibroblasts, PGE_2 also functions as an important negative regulator of pulmonary fibrosis and airway remodelling in asthma.[49,50] Mechanistically, the anti-fibrotic effects of PGE_2 were mainly mediated via EP2 and EP4, which both increase cellular cAMP levels and thus regulate matrix metalloproteinases (MMPs) and collagen deposition.[40]

The prominent effects of PGE_2 on tissue repair responses have prompted the idea to administer PGE_2 agonists in settings of impaired tissue repair or pathological remodelling. When applied topically to the oral or vaginal mucosa before or after chemotherapy, PGE_2 could prevent mucosal damage and accelerate re-epithelialization in cancer patients.[12] Furthermore, local treatment with PGE_2 could restore cutaneous tissue repair in mice that showed impaired repair after treatment with NSAIDs.[51] However, the therapeutic potential of PGE_2 agonists in pulmonary fibrosis or airway remodelling during severe asthma may

be limited as PGE_2 agonists could only prevent, rather than reverse structural changes in the lung.[52] Thus, the development of novel modulators of EP receptor signaling may be useful for the prevention or treatment of pathological tissue repair in various disease contexts (for review see [53]).

Prostaglandin D_2 - a negative regulator of tissue repair

In contrast to the versatile roles of PGE_2 in tissue repair, much less is known about the function of PGD_2 in the context of tissue repair. Upon injury, PGD_2 is formed subsequent to PGE_2 with peak concentrations observed around 2 weeks after wounding.[13] In an experimental model of skin wound healing, PGD_2 and its degradation products were proposed to reduce inflammation via the activation of PPARγ, thus contributing to resolution after the inflammatory phase of tissue repair. In addition to the potential pro-resolving effects of PGD_2 metabolites, PGD_2 itself was reported to suppress collagen production and the recruitment of pulmonary fibroblasts by engaging its high affinity receptor DP1.[54] This suggests that despite its pro-inflammatory roles in type 2 immune responses, PGD_2 can act as a negative regulator of airway remodelling. In line with the suppressive effects of PGD_2 on tissue repair responses in the lung, PGD_2 was found to inhibit the formation of new hair follicles during cutaneous repair.[55] An important role for PGD_2 in the repair of barrier tissues was further supported by a recent study, which implicated the PGD_2-DP1 axis in mucosal healing during ulcerative colitis.[4] In summary, PGD_2 formation during the later phases of tissue repair appears to contribute to the timely termination of proliferative responses as well as of ECM production.

Prostacyclin - a modulator of clotting and ECM deposition

In oder for tissue repair to proceed normally, clots formed during the early phases of tissue repair have to be gradually removed. This process is called fibrinolysis and critically depends on the activation of plasmin by urokinase-type plasminogen activator (uPA). Whilst platelet derived TXA_2 is critical for clot formation, prostacyclin (PGI_2), another COX metabolite formed by endothelial cells, has fibrinolytic potential. Indeed stable analogues of PGI_2 could promote the expression of uPA in human fibroblasts and thereby contribute to physiological tissue repair responses.[56] In addition to its role in fibrin clot degradation, PGI_2 has been implicated in the regulation of ECM deposition as a PGI_2 analogue decreased fibronectin production in airway smooth muscle cells (ASM) and fibroblasts.[57] Thus, similar to PGE_2 and PGD_2, PGI_2 may act as a negative regulator of ECM production by structural cells and thereby contribute to the COX-driven regulation of tissue remodeling. These parallel effects of the three prostanoids (PGE_2, PGD_2, PGI_2) may not be surprising given that all of them activate GPCRs that couple to Gα proteins and thus increase cAMP.

In summary, the time- and tissue-dependent production of the different prostanoids contributes to rapid wound closure and tissue regeneration, whilst preventing scarring and fibrosis. Although the central roles of prostanoids in haemostasis and tissue repair have long been appreciated, novel roles and mechanisms of these versatile mediators of tissue repair remain to be uncovered. As the COX pathway represents a major drug target in chronic inflammation and cancer, new insights into prostanoid-driven tissue repair responses may hopefully be translated into clinical practice and improve the management of these common diseases.

Epoxyeicosatrienoic acids (EETs) - endothelial mediators of regeneration

In addition to producing the COX metabolite PGI_2, endothelial cells can metabolize AA into epoxyeicosatrienoic acids (EETs) via cytochrome P450 epoxygenases (Figure 1, Figure 2). Although EETs have mainly been studied for

their vascular activities, these mediators have also been implicated in the regeneration of various tissues and organs, including the liver, kidneys, lung and eye.[20] In particular, the administration of 11,12-EET and 14,15-EET improved liver regeneration after experimental hepatectomy, a phenotype that was reproduced in transgenic mice expressing increased levels of the CYP enzyme CYP2C8. Furthermore, whilst EETs promoted angiogenesis and wound closure, impaired generation of EETs in diabetic mice correlated with delayed tissue repair.[58] As impaired tissue repair is a major complication in type 2 diabetes the generation of EETs via CYPs or their degradation by soluble epoxide hydrogenase (sEH) represents an attractive drug target that may help to prevent or treat diabetes-associated tissue repair conditions such as diabetic ulcers.

Lipoxins and resolvins - anti-inflammatory mediators of barrier repair

In addition to generating pro-inflammatory LTs via the 5-LOX pathway, damaged tissues show increased production of 12/15-LOX metabolites, including 12- and 15-HETE and LXA_4. An important functional role for 12/15-LOX-derived eicosanoids in tissue repair was suggested by studies showing impaired re-epithelialization after corneal damage in mice lacking the 12/15-LOX enzyme ($Alox15^{-/-}$).[8] Whilst the role of 12- and 15-HETE in tissue repair is largely unclear, LXA_4 has been implicated in tissue regeneration as it could reduce inflammation and promote re-epithelialization in the eye.[8] Moreover, the administration of LXA_4-containing microparticles in a rat model of skin wound healing promoted wound closure, angiogenesis and collagen deposition, potentially by inducing IL-4, TGFβ1 and tissue repair macrophages.[59] Intriguingly, 12-/15-LOX is generally highly expressed during type 2 immune responses, e.g. after exposure to house dust mite or during intestinal nematode infection.[15,60] This may suggest that 12/15-LOX also plays important roles in epithelial barrier repair in mucosal tissues such as the airways and intestine.

As an alternative to the ω-6 PUFA AA, COX, LOX and CYP enzymes can metabolize ω-3 PUFAs (particularly DHA or EPA) into further pro-resolving mediators, which have been implicated in tissue repair. Resolvin D_2 (RvD_2), which is formed by the combined action of 5-LOX and 12-/15-LOX was reported to promote revascularization in diabetic mice that otherwise showed defective repair in a model of hind limb ischemia.[10] The abundant formation of resolvins during mucosal healing in an IBD model further suggested the involvement of these mediators in barrier repair after inflammatory injury.[19] Indeed, high levels of RvD_2 were also produced after skin injury in mice and pigs and topically administered RvD_2 could promote re-epithelialization.[9] Mechanistically, the RvD_2-driven migration of epithelial keratinocytes depended on the lipoxin receptor Alx/Fpr2 and the downstream activation of PI3K-AKT-mTOR-S6 kinase signaling.

Taken together, pro-resolving PUFA metabolites appear to be crucially involved in repair and resolution in various tissues with particular importance for epithelial tissue repair.

Conclusion and future perspectives

Recent studies have uncovered an enormous versatility and complexity of eicosanoid-driven responses during tissue repair. In particular, eicosanoids have been implicated in the repair of epithelial barriers, which is central for host defense and the prevention of chronic inflammation. Despite this progress, the physiological roles of AA-metabolic pathways in tissue repair are incompletely understood as research has traditionally been focused on their involvement in pathological tissue remodelling (e.g. during fibrosis). Thus, future studies should further define the mechanisms, by which eicosanoids regulate physiological repair responses in various tissues. In particular, it would be important to compare the eicosanoid expression patterns during tissue repair in different

organs and determine their impact on different regenerative capacities (e.g. scar formation in the skin vs. liver regeneration).
In addition, the immunological and metabolic regulation of eicosanoid synthesis during tissue damage and repair remains to be characterized. Such studies may provide important insights into the intricate cellular and metabolic crosstalk that enables proper tissue function and prevents aberrant healing, which can lead to fibrosis, diabetic ulcers or cancer.
Indeed, the involvement of eicosanoids in virtually all stages of the tissue repair process provides ample opportunity for pharmacological intervention in conditions, where tissue repair has become impaired or exaggerated. This may include the use of already approved drugs (e.g. LT antagonists or NSAIDs) as well as newly-developed drugs, which can e.g. mimic the beneficial effects of pro-resolving eicosanoids. To date, several clinical trials have been conducted to explore the effects of ω-3 PUFA supplementation on chronic inflammation and tissue repair in human patients, however with mixed results. Despite the beneficial effects of PGE_2 application in patients suffering from mucosal lesions and despite a plethora of promising findings from experimental models, no clinical trials are currently ongoing to translate repair functions of eicosanoids into the clinic. Thus, by better understanding and harnessing the roles of eicosanoids in tissue repair we may develop new strategies for the prevention and treatment of major diseases of today's societies.

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Tables

Eicosanoid	Cellular source	Function	Experimental model
prostaglandin E_2 (PGE_2)	macrophage (myo-)fibroblast	• myofibroblast migration ↑ • re-epithelialization ↑ • keratinocyte proliferation ↑ • epithelial differentiation ↑ • ECM production/ fibrosis ↓	skin punch biopsy[40] full thickness excisional skin wounding[51] intestinal epithelial stem cells/ wound-associated epithelial cells[5] bleomycin-induced lung fibrosis[52]
prostaglandin D_2 (PGD_2)	mast cell macrophage	• ECM production ↓ • fibroblast migration ↓ • hair folicle neogenesis ↓	human lung fibroblasts[54] full thickness excisional skin wounding[55]
thromboxane A_2 (TXA_2)	platelet macrophage	• platelet aggregation ↑ • fibroblast migration ↑ • macrophage recruitment ↑	tail incision[22] acute toxic liver injury[3,23]
12-hydroxy-heptadecatrienoic acid (12-HHT)	platelet macrophage neutrophil	• fibroblast migration ↑ • fibroblast proliferation ↑ • keratinocyte recruitment ↑	skin punch biopsy[24] corneal wound injury[26] *in vitro* scratch assay[26]
prostacyclin (PGI_2)	endothelial cell	• fibrinolysis ↑ • ECM deposition ↓	*in vitro* scratch assay, human fibroblasts [56,57]
leukotriene B_4 (LTB_4)	neutrophil macrophage	• inflammation ↑ • wound closure ↓ • TGFβI production ↑ • ECM deposition / fibrosis ↑	skin punch biopsy[6] bleomycin-induced lung fibrosis[27] acute lung injury (sepsis)[17]
cysteinyl leukotrienes (cysLTs: LTC_4, LTD_4, LTE_4)	eosinophil macrophage platelet/ neutrophil aggregates epithelial cell	• inflammation ↑ • wound closure ↓ • ECM deposition / fibrosis ↑	human (myo)fibroblasts[33] skin punch biopsy[6]
epoxyeicosatrienoic acids (EETs)	endothelial cell	• regeneration of lung, liver & kidney • neovascularization ↑ • collagen production ↑ • inflammation ↓	unilateral/ partial pneumonectomy, nephrectomy/ hepatectomy, skin punch biopsy[20] full thickness excisional skin wounding/ obesity[58]
lipoxin A_4		• TGFβI production ↑ • inflammation ↓ • re-epithelialization ↑ • wound closure ↑	skin injury[59] corneal wound injury[8]

Table 1: Overview of eicosanoids and their cellular sources and functions in different experimental models of tissue repair

Figure and Table legends

Table 1: Overview of eicosanoids and their cellular sources and functions in different experimental models of tissue repair; The table summarizes the functions of eicosanoids identified in different models of tissue injury and repair with a focus on barrier organs (skin, lung, intestine). Further examples of tissue repair functions of eicosanoids in other organs (eye, liver, kidney) are also provided.

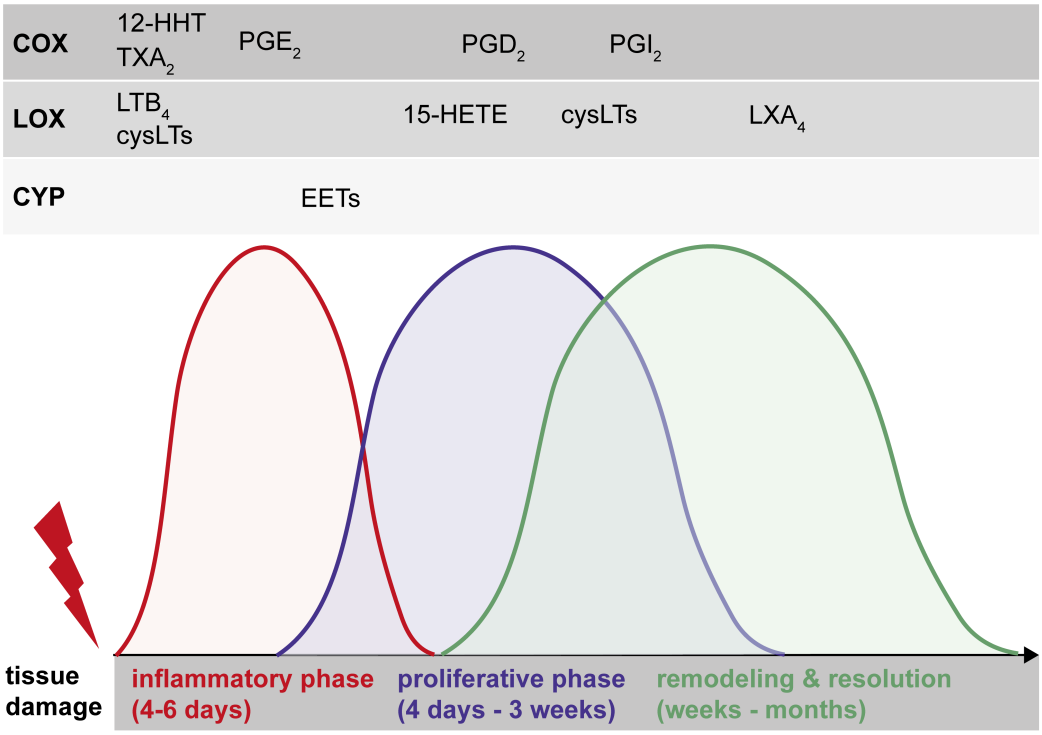

Figure 1. Time course of eicosanoid production during the different phases of tissue repair; Eicosanoids are produced via different enzymatic pathways, which are expressed and activated in a time-dependent fashion during the tissue repair process. Abbreviations: COX=cyclooxygenase, LOX=lipoxygenase, CYP=cytochrome P450, PG=prostaglandin, TX=thromboxane, HHT=hydroxy-heptadecatrienoic acid, EET=epoxy-eicosatetraenoic acid, HETE=hydroxy-eicosatetraenoic acid, LT=leukotriene, cysLT=cysteinyl leukotrienes, LX=lipoxin.

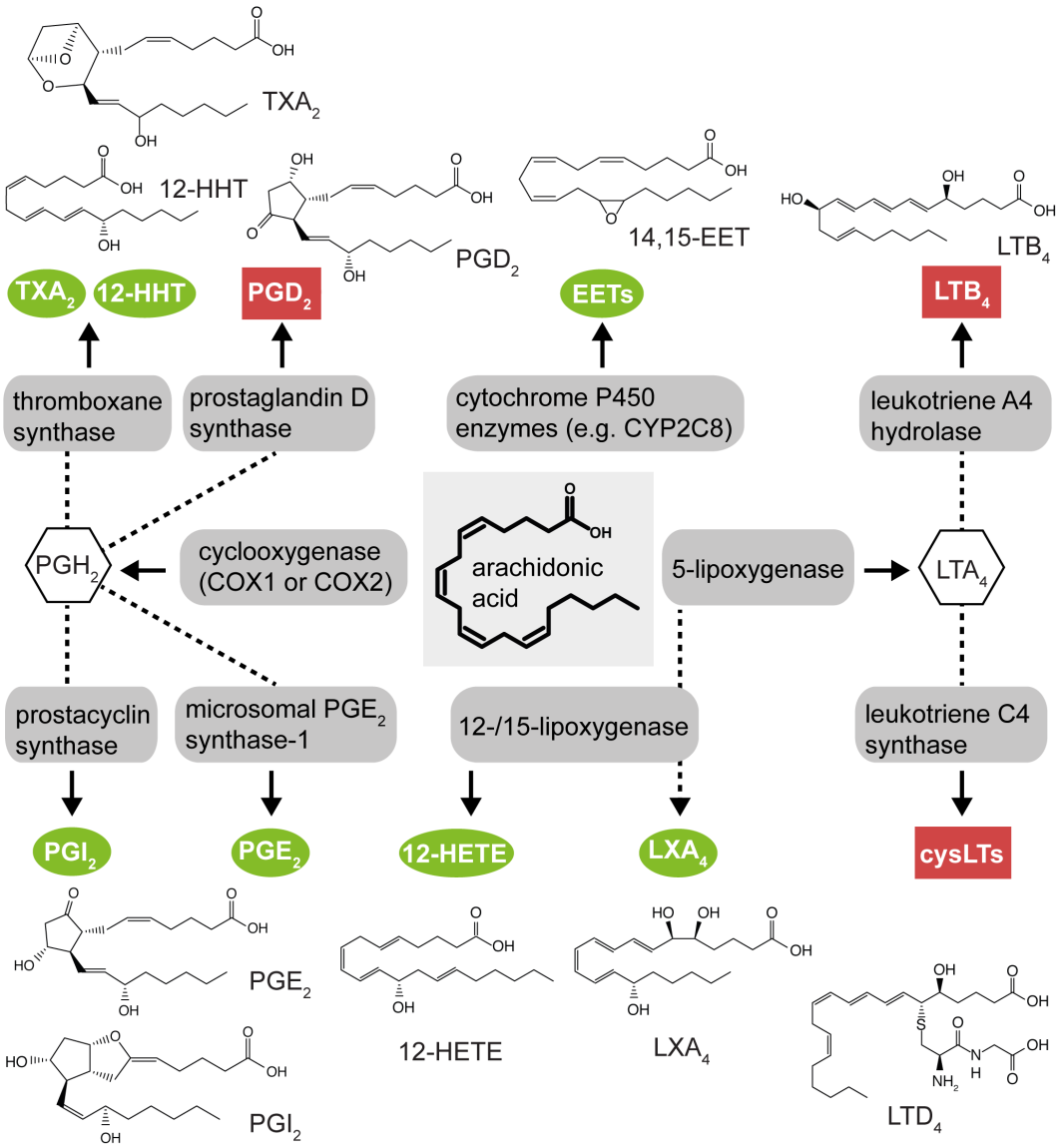

Figure 2. Biosynthesis of eicosanoid lipid mediators with major roles in tissue repair; Upon cellular stimulation, the ω-6 PUFA arachidonic acid (AA) is liberated from cellular membranes and metabolized into eicosanoids via four major enzymatic pathways: the prostanoids are synthesized via the cyclooxygenase (COX) pathway, leukotrienes and lipoxins via lipoxygenases (LOX) and epoxy-eicosatetraenoic acids (EETs) via cytochrome P450 (CYP) enzymes. Mediators that predominantly act as positive regulators of tissue repair are shown in green, whilst negative regulators of tissue repair are shown in red. Intermediates without biological activity are uncoloured; Abbreviations: PG=prostaglandin, TX=thromboxane, HHT=hydroxy-heptadecatrienoic acid, EET=epoxy-eicosatetraenoic acid, HETE=hydroxy-eicosatetraenoic acid, LT=leukotriene, cysLT=cysteinyl leukotrienes, LX=lipoxin.

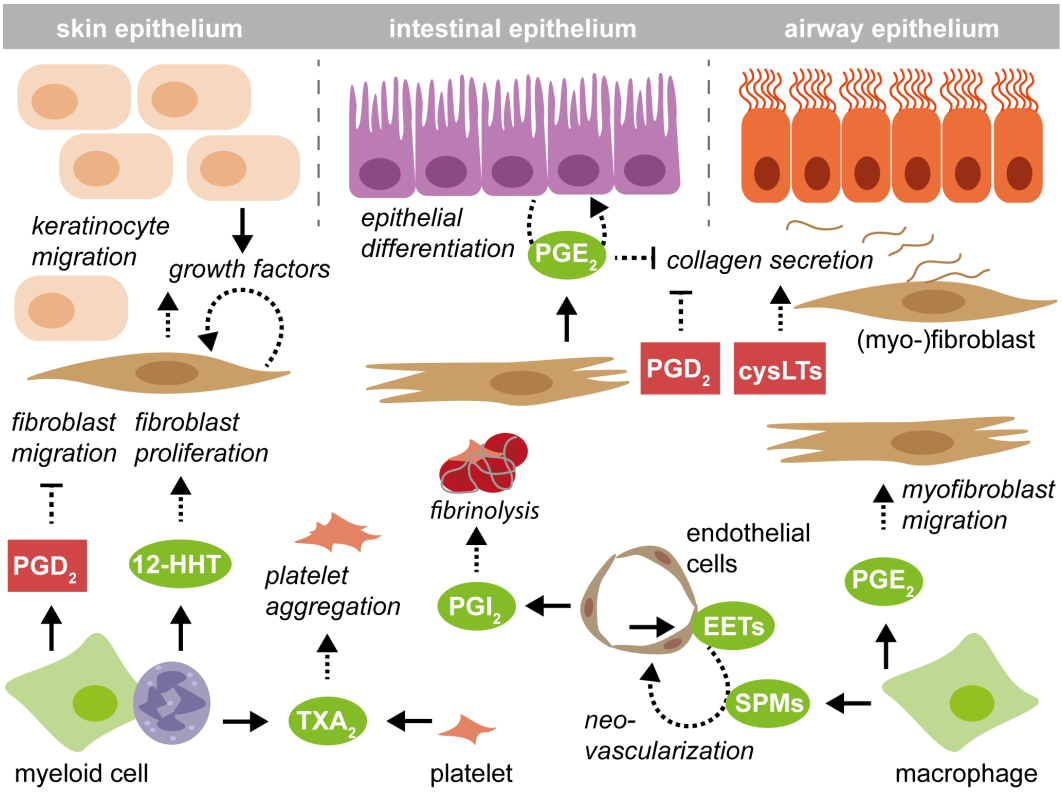

Figure 3. Source- and target cells as well as biological functions of eicosanoids in tissue repair; Eicosanoids play key roles in the repair of epithelial barriers in the skin, intestine and airways. Thromboxane (TXA_2) promotes platelet aggregation, whilst prostaglandin E_2 (PGE_2) drives myofibroblast migration and epithelial differentiation. PGs act as negative regulators of collagen secretion, while leukotrienes promote collagen production and inflammation. Mediators that predominantly act as positive regulators of tissue repair are shown in green, whilst negative regulators of tissue repair are shown in red. Abbreviations: PG=prostaglandin, TX=thromboxane, HHT=hydroxy-heptadecatrienoic acid, EET=epoxy-eicosatetraenoic acid, HETE=hydroxy-eicosatetraenoic acid, LT=leukotriene, cysLT=cysteinyl leukotrienes, LX=lipoxin, SPMs= specialized pro-resolving mediators.