The Role of the Lysyl Oxidases in Tissue Repair and Remodeling: A Concise Review

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Abstract Tissue injury provokes a series of events containing inflammation, new tissue formation and tissue remodeling which are regulated by the spatially and temporally coordinated organization. It is an evolutionarily conserved, multi-cellular, multi-molecular process via complex signalling network. Tissue injury disorders present grievous clinical problems and are likely to increase since they are generally associated with the prevailing diseases such as diabetes, hypertension and obesity. Although these dynamic responses vary not only for the different types of trauma but also for the different organs, a balancing act between the tissue degradation and tissue synthesis is the same. In this process, the degradation of old extracellular matrix (ECM) elements and new ones' synthesis and deposition play an essential role, especially collagens. Lysyl oxidase (LOX) and four lysyl oxidase-like proteins are a group of enzymes capable of catalyzing cross-linking reaction of collagen and elastin, thus initiating the formation of covalent cross-links that insolubilize ECM proteins. In this way, LOX facilitates ECM stabilization through ECM formation, development, maturation and remodeling. This ability determines its potential role in tissue repair and regeneration. In this review, based on the current *in vitro*, animal and human *in vivo* studies which have shown the significant role of the LOXs in tissue repair, e.g., tendon regeneration, ligament healing, cutaneous wound healing, and cartilage remodeling, we focused on the role of the LOXs in inflammation phase, proliferation phase, and tissue remodeling phase of the repair process. By summarizing its healing role, we hope to shed light on the understanding of its potential in tissue repair and provide up to date therapeutic strategies towards related injuries.

Keywords Lysyl oxidase · Cross-link · Repair · Remodeling · Tissue-engineering

1 Summary

Tissue injury provokes a series of events containing inflammation, new tissue formation and tissue remodeling which are regulated by the spatially and temporally coordinated organization. It is an evolutionarily conserved,

multi-cellular, multi-molecular process via complex signalling network [1]. Tissue injury disorders present grievous clinical problems and are likely to increase since they are generally associated with the prevailing diseases such as diabetes, hypertension and obesity [2]. Although these dynamic responses vary not only for the different types of trauma but also for the different organs, a balancing act between the tissue degradation and tissue synthesis is the same [3]. In this process, the degradation of old extracellular matrix (ECM) elements and new ones' synthesis and deposition play an essential role, especially collagens [4].

Lysyl oxidase (LOX) and four lysyl oxidase-like proteins are a group of enzymes capable of catalyzing cross-linking reaction of collagen and elastin, thus initiating the

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formation of covalent cross-links that insolubilize ECM proteins. In this way, LOX facilitates ECM stabilization through ECM formation, development, maturation and remodeling [5, 6]. This ability determines its potential role in tissue repair and regeneration.

In this review, based on the current *in vitro*, animal and human *in vivo* studies which have shown the significant role of the LOXs in tissue repair, e.g., tendon regeneration, ligament healing, cutaneous wound healing, and cartilage remodeling, we focused on the role of the LOXs in inflammation phase, proliferation phase, and tissue remodeling phase of the repair process. By summarizing its healing role, we hope to shed light on the understanding of its potential in tissue repair and provide up to date therapeutic strategies towards related injuries.

2 Introduction

Lysyl oxidase (LOX), a copper-dependent amine oxidase, initiates covalent cross-linking of elastin and collagen by catalyzing oxidative deamination of the ϵ -amino group of lysine and/or hydroxylysine [7] and ultimately insolubilizes these extracellular proteins resisting to proteolysis. The LOX family (LOXs) contains another four additional members, lysyl oxidase-like protein 1 (LOXL-1) [8], lysyl oxidase-like protein 2 (LOXL-2) [9], lysyl oxidase-like protein 3 (LOXL-3) [10], and lysyl oxidase-like protein 4 (LOXL-4) [11, 12], respectively. Each shows the highly similarity in the carboxyl (C)-terminus, including the copper binding domain, the lysyl-tyrosyl quinone (LTQ) residues, and the cytokine receptor-like (CRL) domain [13]. By comparison, the amino (N)-terminal regions of the LOXs are variable, showing little homology except for LOXL-2, LOXL-3, and LOXL-4, which are characterized by a conservation of four copies of scavenger receptor cysteine-rich (SRCR) domains [14]. Another N-terminal consensus related to cleavage by procollagen C-proteinases also appears among the LOX and LOXL-1 [15]. The distinct sequence of each member may contribute to their individual biological functions. The human LOX [16] is synthesized as a 48-kDa prepro-protein in the ribosome. Following translation, the N-terminal signal peptide sequence is removed in the endoplasmic reticulum and the propeptide domain is N-glycosylated in the golgi [16]. After these intracellular processing, a 50-kDa pro-protein (proLOX) is secreted to the extracellular compartment in which the proLOX is cleaved between residues Gly168 and Asp169 by procollagen C-proteinase, mainly bone morphogenic protein-1 (BMP-1) [5]. Later on, the 32-kDa active LOX is released to the ECM to participate in cross-linking of the extracellular proteins (Fig. 1).

It has been confirmed that LOXs are not only present in the ECM but also in the cytoplasm and nucleus [17]. In human being, LOX gene has been detected in adult human heart, placenta, testis, lung, kidney, and uterus, but only marginally in brain and liver; LOXL-1 is much higher in placenta, kidney, muscle, heart, lung, and pancreas, but much lower in liver or brain [8]; LOX and LOXL-1 are expressed in most tissues, while the expressions of the LOXL-2, LOXL-3, and LOXL-4 genes are more restricted [18]. LOXL-2 is detected in several human tissues and especially high in spleen, testis, uterus, and placenta, but is expressed at much lower levels in brain and liver [19]; LOXL-3 is high in testis, spleen, and prostate, moderately in placenta, but not in liver [20]; LOXL-4 is expressed at the highest levels in skeletal muscle, testis, and pancreas. LOXs are responsible for diverse biological functions, including developmental regulation, tumor suppression, cell motility, gene transcription regulation, signal transduction, and cell adhesion [21, 22].

The first direct evidence of the correlation between the LOX activity and granulation tissue development in the wound healing was observed in 1974 [23]. Later, Fushida-Takemura firstly examined the time course and the localization of LOX mRNA during wound healing, revealing its gene expression pattern in injured tissues [24]. Recently, roles of LOX and other family members are studied extensively in tissue repair and tissue engineering, aiming at providing a promising therapy for abnormality and disability in tissue injuries.

3 Lysyl oxidases and tissue repair process

3.1 Conspectus of tissue injury and repair

With participation of numerous mediators, blood factors, stromal cells and other ECM elements, cellular and tissue responses are instantly activated following injury, leading to the goal of restoring organized matrix accomplished [25]. Necrotic tissues and fibrin clots are degraded by inflammatory components. Subsequently, new tissues are synthesized along with the deposition of collagen and elastin [26]. It is the balance between degradation and synthesis of the ECM components that results in a favorable healing outcome. In other words, the two processes appear to be two sides of the same coin, their opposing activities facilitate the shift of the balance, then repair process is promoted [27]. Pathophysiologic factors, i.e., the age, hormone level, and other diseases, e.g., pulmonary arterial hypertension [28], varicose vein [29] and congenital heart disease [30], have all demonstrated distinct discrepancies in the process, resulting in abnormal healing, which is still a challenging clinical problem.

Fig. 1 The synthesis of activated lysyl oxidases. The output of the process is the removal of the N-terminal signal peptide sequence of human LOX in the endoplasmic reticulum. Then the propeptide domain is N-glycosylated in Golgi and the 50-kDa pro-protein (proLOX) is generated. The proLOX is cleaved by procollagen

C-proteinase, mainly bone morphogenic protein-1 (BMP-1), leading to the formation of activated LOX and LOX-propeptide (LOX-PP). In the carboxyl (C)-terminus, the copper binding domain (Cu), the lysyl-tyrosyl quinone (LTQ) residues, and the cytokine receptor-like (CRL) domain are highly conserved in each member of the LOX family

To provide a promising avenue to refine these problems, several researchers have focused on the variation of the primary structural component of the ECM, collagen. Collagen is a triple helix of polypeptide chains. In general, collagen type I is the fundamental component of the ECM, while other kinds of collagens just comprise a small proportion, for instance, in skin the collagen type III only accounts for 10%. However, this proportional relation converts after wounding. Collagen I decreases and collagen III has a significant increase [31]. And the early increased presence of collagen type III mRNA becomes a typical wounded phenotype [32]. The increase in the expression of collagen type III coordinates closely with the increase of the LOX following skin injury, in which the increased mRNA peak of the LOX precedes that of collagen type III by several days. The result suggests that LOX is produced before collagen synthesis in preparation for cross-linking in the early phase of wound healing [24]. Similarly, Giamuzzi reported that the promoter activity of the collagen type III gene was increased remarkably in COS-7 cells due to ectopic over-expression of the mature form of the LOX and that this effect was abolished with β -aminopropionitrile (BAPN), the most potent inhibitor of the LOXs. Evidence was also shown in another report that the over-expression of the LOX promoted the activity of the collagen III promoter through LOX-induced binding of Ku antigen, leading to the increased gene expression of collagen type III [33]. An increase in collagen type IV and other subtypes were also observed [34]. Since collagen

type III fibers provide less strength for the wound than collagen I fibers, they will be degraded and replaced with collagen I as repair process goes on. The switch from collagen type III to type I provides greater mechanical strength. Most importantly, the ratio of collagen I to collagen III fibers and their degree of matured architecture significantly influence the rapidity of tissue remodeling and the resulting strength of the new-formed wound [35].

Another remarkable change of the ECM constituents in tissue repair is the activation of fibroblasts, which play a crucial role in healing. Fibroblasts participate in a range of reparative activities under phenotypic modulation [36], including the initial secretion of matrix metalloproteinases (MMPs), which can lyse fibrin clots produced in the inflammation phase. Besides, cytokines and growth factors generated from fibroblasts also act as an indirect signalling way. Above all, collagens synthesized by fibroblasts deposit after the cross-linking reaction catalyzed by the LOX, which promotes the healing cascade of reparative process in tissues such as cardiovascular, skin and bone [37, 38].

It has been confirmed that four LOX isoforms have the same traditional function of catalyzing cross-linking reaction of collagen and elastin [5]. And each of them plays a significant role in the healing process. The LOXL-1 localizes specifically to the sites of elastogenesis and serves as an element of the scaffold to ensure spatially defined deposition and homeostasis of elastins. Liu [39] reported that mice lacking the LOXL-1 did not deposit normal elastic fibers in the uterine tract post partum and developed

pelvic organ prolapse, enlarged airspaces of the lung, loose skin, and vascular abnormalities with concomitant tropoelastin accumulation. The LOXL-2 functions for the formation of lysine-derived cross-links and plays a functional role in maintenance of the structural integrity of connective tissues [40]. Yu [41] revealed that LOXL-3-mediated collagen cross-linking was associated with cardiac ECM remodeling. And the LOXL-4, the latest member of the LOX family, also functions as an amine oxidase like other family members to promote the formation of lysine-derived cross-links [42]. As the healing process goes on, the arrangement of the cross-link varies. In other words, the LOX-related arrangement of cross-link impenetrates the remodeling phase from the disordered collagen network formation to a more ordered collagen fiber arrangement. This transition with unclear mechanism is consistent with the increase in the mechanical strength at the injury site. During bone fracture healing, an examination of the transcriptome demonstrated that all five LOXs were highly expressed [43]. In particular, LOXL-2 expression was regulated in a temporal manner and remarkably increased in the chondrogenic phase of fracture healing *in vivo* [44]. On the other hand, injury stimulates the release of ligands, such as transforming growth factor- β_1 (TGF- β_1), tumor necrosis factor alpha (TNF- α), interleukin-1 (IL-1), interleukin-16 (IL-16), bone morphogenetic protein (BMP-12), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF-2) etc [45], and some of them exert effects together to regulate the activity or expression of the LOXs within the healing phase (Table 1).

In conclusion, numerous constituents of the ECM, such as collagens, fibroblasts, and varieties of chemical mediators play significant roles in the homeostasis of tissues. Their reciprocities promote the processes of tissue repair. The multi-cellular and multi-molecular response can be divided into three characteristic phases which overlap in time and space. First step is inflammation, then new tissue formation, the last is tissue remodeling [6]. To the best of our knowledge, the three stages are interconnected and interpenetrative. Here we provide an overview of the cross-linking role of the LOXs in terms of these three phases, which would help to understand the insight of the LOXs in the tissue healing and pathological process.

3.2 Up-regulation of LOXs in inflammation

As an important part of the innate immune response, acute inflammation is the initial response towards injury. To some extent, acute inflammation itself serves as a tool for tissues to maintain ECM mechanical integrity and return the impaired tissue to a normal state [60]. Although synthetic activities also participate, degradative activities mediated by inflammatory markers, e.g., cytokines, elastases, and interleukins, are typical of inflammation, especially in the early inflammatory phase in which mature elastin and collagen fibers are degraded into soluble peptides [61]. The acute inflammatory response usually persists for 24 to 48h in some tissues, but the duration could even last for up to two weeks in certain cases of healing [62]. Various inflammatory cytokines, growth factors, adhesion molecules, and cells are in close cooperation with

Table 1 The variation of the LOX mRNA/protein under the modulation of influencing factors

Influencing factors	Effects/responses	Research objects	Culture type	Study
PGE ₂	Enzyme↓	Neonatal rat lung fibroblast	<i>In vitro</i>	[46]
PDGF	mRNA↑Enzyme↑	Adult rat vascular smooth muscle cell	<i>In vitro</i>	[47]
BFGF	mRNA↓Enzyme↓	Osteoblastic MC3T3-E1 cell	<i>In vitro</i>	[48]
TGF- β_1	mRNA↑Enzyme↑	Osteoblastic MC3T3-E1 cell	<i>In vitro</i>	[49]
Angiotensin II	Enzyme↑	Rat neonatal cardiac fibroblast	<i>In vitro</i>	[50]
IGF-1	mRNA↑	High-growth C57BL/6 J mouse	<i>In vivo</i>	[51]
Estrogen	mRNA↑	Vaginal wall of guinea pig	<i>In vitro</i>	[52]
HIF-1	mRNA↑Enzyme↑	Human MDA231 breast and SiHa cervical cancer cell	<i>In vitro</i>	[53]
IFN- γ	mRNA↓	Rat aortic smooth muscle cell	<i>In vitro</i>	[54]
TNF- α	Enzyme↓	ACL and MCL fibroblast	<i>In vitro</i>	[55]
CTGF	mRNA↑Enzyme↑	Human gingival fibroblast	<i>In vitro</i>	[50]
LDL	mRNA↑	Vascular wall of hypercholesterolemic pig	<i>In vivo</i>	[56]
RA	mRNA↓	Rabbit retinal pigment epithelium cell	<i>In vitro</i>	[57]
IL-6	mRNA↓	Pre-osteoblastic MC3T3-E1 cell	<i>In vitro</i>	[58]
IL-4	mRNA↑	Human ovarian surface epithelium cell	<i>In vitro</i>	[59]

each other, in this way modulating the responses of the LOXs and other constituents of the ECM and consequently prompting ECM regeneration. Thus, the LOXs are regulated by these components and play an essential role in the early period of the healing response, i.e., inflammation phase.

The increase of collagen cross-linking is congruent with the up-regulation of the LOXs after tissue injury [63]. A few investigators have reported some approaches to accelerating healing of injured tissues through up-regulating the LOX in the acute phase after injury. For example, direct injection of mesenchymal stem cells (MSCs) into the damaged anal sphincter tissues isolated from rats increased the expression of the LOX and matrix deposition. 21 days later, twitch tension and maximal field-stimulated force of the MSC-injected sphincters were virtually identical to sphincters from the unoperated controls, while the phosphate-buffered saline (PBS)-injected sphincters didn't recover. The accelerated recovery of the direct MSC-injected sphincters might be due to the stimulated the LOX expression [64]. Elvira [65] reported that the wounded skin of discoidin domain receptor 2-deficient mice which expressed less LOX had defective dermal wounding responses. Therefore, the LOX could act as an appealing target for a favorable healing outcome. Not only the LOX, the LOXL-2 also shows the same variation. In the study of healing process after glaucoma filtration surgery, Tine [66] observed the up-regulation of the LOX and LOXL-2 in Tenon's capsule and the conjunctiva in the acute phase. In addition, targeting LOXL-2 with an inhibitory monoclonal antibody inhibited angiogenesis, inflammation and profibrotic components. Similarly, Barry [67] put forward that the inhibition of LOXL-2 resulted in a marked reduction of inflammatory cells and molecules, e.g., activated fibroblasts, endothelial cells, growth factors, and cytokines. Meanwhile, TGF- β_1 pathway signalling and collagen deposition both decreased. Further studies demonstrated that all five LOXs were highly expressed in fibroblasts isolated from injured anterior cruciate ligament (ACL) and medial collateral ligament (MCL) [68]. Some investigators referred that the intrinsic difference in the reaction of the LOXs to injuries within different tissues could be partly blamed for their different healing abilities. The activities of the LOXs are stronger in MCL fibroblasts than that in ACL fibroblasts, which could be an explanation for the different healing abilities between the well functionally self-healing MCL and the poorly self-healing ACL [69]. It should be noted that the LOXL-1 doesn't have significantly higher expressions like other family members in the injured state. And the distinction in the activity of the LOXL-1 is also obsolete between impaired ACL and MCL fibroblasts. Further *in vitro* studies showed that the expressions of the LOXs were mostly down-regulated in injured posterior

cruciate ligament (PCL) fibroblasts after co-culture with synovial cells. The decline was probably due to the interaction of injured PCL fibroblasts and synovial cells [69].

The LOXs were activated in a time-dependent manner and were relatively highly expressed in the late phase of inflammation, which was consistent with their anti-inflammatory effects of promoting the transition from inflammation to proliferation phase and the up-regulation of transforming growth factor- β_1 (TGF- β_1) in this period. Among all the cytokines, TGF- β_1 which appears in the damaged area accompanying the inflammation phase is highly related to the up-regulation expressions of the LOXs [70]. TGF- β_1 plays a pivotal role in the healing process of many tissues such as muscle, tendon, ligament, skin, and bone [71]. It has been reported that TGF- β_1 induces the expression of collagen type I mRNA in cardiac fibroblasts [72]. The *in vitro* investigations into injured ACL and MCL fibroblasts showed that TGF- β_1 remarkably up-regulated the expressions of the LOXs [73]. TNF- α is another key cytokine in the acute inflammation phase. Several studies have reported the decrease in the expressions of the LOX/LOXs caused by TNF- α in endothelial cells, osteoblasts, and fibroblasts [55, 74, 75]. Showing the similar effect like TNF- α , pro-inflammatory cytokine IL-1 contributed to lower expressions of the LOXs in injured ACL fibroblasts compared with those in injured MCL fibroblasts [76]. Prostaglandin E₂ (PGE₂), a crucial chemical mediator of the early phase of healing process, is assumed to be a negative regulator of the LOX [46]. A research into the effects of pulsed ultrasound on bone fracture repair showed that high-dose PGE₂ induced by pulsed ultrasound may be detrimental to the formation of cross-links which were required for the initiation of mineralization. This healing retardation of pulsed ultrasound is attributed to the decreased activity of the LOX caused by PGE₂ [77]. In the inflammation phase of healing, the levels of IL-1, VEGF, BMP-1, IGF-1, and MMP-2 are also elevated and these chemical mediators may further modulate the levels of the LOXs released from the damaged tissues [78].

However, when inflammatory factors exist continuously, inflammation persists and will transform into chronic disease. Chronic inflammation is characterized by hyperplasia and lasts for a longer time, in which fibroblasts produce excessive ECM proteins, leading to disorders of structure and function. Pathological fibrosis and liver cirrhosis are typical examples. An early study has been described the increase of LOX expression in fibroblasts in different types of fibrosis [79]. Similarly, Zeneca referred to the remarkable increase of LOX in organ fibrosis and pointed out inhibitors of the enzyme could limit fibrogenesis, although side effects of manipulation of collagen metabolism were still unneglectable [80]. Airway fibrosis caused by TGF- β_1 was attenuated after the treatment of BAPN [81]. Even in

rheumatoid arthritis, an autoimmune inflammatory condition, an increased LOX-mediated cross-linking activity was observed, conformed with the increased urinary cross-link excretion [82]. It's worth mentioning that LOXs expression doesn't always show the same pattern, and different kinds of chronic inflammations have the own features. Reduction of LOX expression was noted in chronic periodontitis, which was probably blamed for the tissue lesions and impaired healing abilities [83]. An animal study into the treatment of abdominal aortic aneurysm provided a therapy in which LOX expression was improved in order to stimulate ECM synthesis [84].

3.3 Participation of the LOXs in various biological actions in the proliferation phase

Biomechanical strength of the injured tissues gradually recovers through the formation of granulation tissue in the proliferation phase [85]. Granulation tissue is a resultant structure successfully performed through fibroplasia and angiogenesis. The reciprocities of fibroblasts, keratinocytes, endothelial cells (ECs), and other stromal cells are also involved. More importantly, the significance of the temporary fibrin-fibronectin structure is to act as a scaffold to guide cellular infiltration and differentiation [86].

In response to injury, activated fibroblasts start to proliferate, migrate to the damaged site, secrete cytokines, and lay down ECM components [87]. In the migration process, the fibrin clots and necrotic tissues are lysed by MMPs released from fibroblasts while the fibronectin and hyaluronic acid (HA) take their place and then participate in the development of granulation tissue and angiogenesis [88]. At the same time, ECs and keratinocytes start to migrate from the edge of the wound, over a provisional irregular matrix comprised mostly of fibronectin and fibrin, to achieve epithelialization and form granulation tissue together with fibroblasts [89]. Once entering the center of the wound, fibroblasts begin to produce collagen as well as other ECM components to develop a fibrous tissue. Stiffness, integrity, and strength of the granulation tissue all increase accompanying this process. Eventually, the previous granulation tissue is replaced and the transition is successfully achieved along with cross-linking of collagens. The LOX is involved in the maturation of granulation tissue. In human post-burn hypertrophic scars, the activity of the LOX is rather low in the newly formed granulation tissue and it will be promoted after scarring and reach a significantly higher value compared with normal skin [90].

Angiogenesis, a necessary step in the formation of granulation tissue, provides the fibroblasts with sufficient nutrient supply [91]. The LOXs also play a role in angiogenesis, especially the LOXL-2. A previous study showed angiogenesis regulators, such as VEGF, could induce the

expression of the LOXL-2 in ischemia-induced angiogenesis of mouse limbs, suggesting its potential in the EC-mediated angiogenic response [92]. Moreover, anti-LOX and anti-LOXL-2 antibody treatment increased the expressions of β 2-microglobulin and intercellular cell adhesion molecule (ICAM)-1, factors known to induce blood vessel formation [93–95]. The LOXL-2 is also expressed as a hypoxia-target in angiogenic ECs and accumulated in the ECM to regulate angiogenesis [96].

When granulation tissue contains sufficient ECM, fibroblasts will differentiate to myofibroblasts which are replete with actin bundles along the cytoplasmic face of the plasma membrane and have great migratory and contractile abilities [97]. Contraction is a normal physiologic phenomenon characterized by a decreased area of a full-thickness wound, leading to tissue distortion as well as cosmetic disfigurement. Contraction even limits joint mobility on certain occasions [98], so researchers are dedicated in finding therapies to reduce contraction. Wound contraction mediated by collagen and elastin cross-links plays an important role in constrictive remodeling [99]. Consequently, contraction is dependent on the LOX and specific inhibition of the LOX with BAPN reduces the collagen cross-link-dependent contraction [90]. Moreover, the number of LOX-dependent cross-links correlates with the degree of contraction [100], so different LOX capacity between the fetal and adult wound healing could be an explanation for the different contraction. As a result, the LOX appears to be a promising potential target for preventing contraction.

Low LOX activity in granulation tissue reaches a notably higher value and results in scarring. Scarring, the culmination of abundant deposition of the ECM components mostly from fibroblasts and myofibroblasts, is not a necessary stage to repair damaged tissues. It is well-known that scar forms not only in dermal wounds, but also in many other injured conditions, e.g., spinal cord ruptures, scarification of hand tendons after injury, corneal abrasions, human vascular restenosis lesions, myocardial infarction, diffuse fasciitis etc [101]. Despite the fact that scarring is unsightly and detrimental to tissue functions, it is able to restore mechanical integrity of the injured region [102].

Collagens are involved in the scarring process. The ratio of collagen type III to type I, the architecture of collagen network, and other aspects of collagen co-modulate scar formation. The role of the LOX in scar formation has been previously identified by Fushida [24], who referred the increased expression of the LOX during scar formation in wounded rat skin. In rat central nerve system (CNS) injury, Gilad [103] detected the high expressions of the LOX and pointed out that the LOX was synthesised and secreted in a spatio-temporal manner by cells attracted to the CNS injury

site. These cells participated in scar formation in this way. Colwell [104] demonstrated that compared with early-gestation scarless wounds, fetal scarring wounds had higher expressions of the LOX in the early response of excisional wound healing in mice, which promoted scar formation through LOX-mediated cross-linking. This difference was just correlated with another finding which showed less TGF- β_1 was expressed in scarless wounds and contributed to the low expression and activity of the LOX [105]. Excessive scarring is one of the characteristics of surgical failure [106]. It was reported by Tine [66] that the LOXL-2 could be an efficient target for treatment of hypertrophic scar formation after glaucoma filtration surgery. Other fibrotic-related pathologies are also related to the low expression and activity of the LOX at different stages of scar formation [90].

3.4 Facilitation role of the LOXs in tissue remodeling

Tissue remodeling can be defined as an orchestrated change of structural components of the ECM, especially collagens and various proteoglycans. Old or damaged proteins are broken down and new proteins take place in this process, which is dependent on the metabolic balance between the proteoglycan molecules and the components of the collagen network [107]. Since the collagen network provides tissues with tensile strength and shear stiffness, when the deposition of collagens outstrips that of proteoglycans, an increase in mechanical integrity will manifest [108]. On the contrary, the maintenance of proteoglycans and the decrease in the concentrations of collagens result in tissue swelling, which is characterized by an increase in resistance to compressive loading and a decrease in tensile strength [109]. The two situations mentioned above are exactly observed *in vivo* and *in vitro*, respectively. They represent two different types of remodeling, constrictive remodeling and excessive enlargement remodeling [110]. Cross-linking reaction of collagen and elastin participates in the healing process via promoting constrictive remodeling. However, excessive accumulation of cross-linked collagens probably result in a bad outcome after injury, such as restenosis after balloon angioplasty [111].

To a large extent, the collagen network is stabilized through cross-linking reaction dependent on the LOXs. Hence the LOXs are critical intermediaries responsible for remodeling during the process of healing [112]. Tensile strength of the wounds can be enhanced with an increased level of the LOX activity stimulated by dexamethasone (DEX), a half analog of curcumin [61]. The change of the LOX activity caused by DEX indicates that the up-regulation of the LOX might act as a way to make up for the insufficiency of mature collagen fibers in case of

suppression of healing process. A study of wound breaking strength (WBS) demonstrated that ethanol exposure decreased the LOX activity and collagen production, which could be partially blamed for reduced WBS and increased incidence of wound failure [88]. So it's conceivable that BAPN treatment or the inhibition of the LOX would pose an obstacle for tissue remodeling. Maki observed increased fragmentation of elastin fibers and excessive enlargement remodeling in the LOX-knockout mice which had high morbidity rate of aortic aneurysms [37]. In an atherosclerotic rabbit model after balloon injury, restenosis decreased in the BAPN-treated animals compared with controls. This discrepancy could be explained that the LOX activity was statistically reduced by the addition of BAPN, then collagen fibers became disorganized and the collagen content was decreased. In the end, constrictive remodeling was significantly inhibited [113].

The LOX is regulated by TGF- β_1 and many other factors in the repair process, including the remodeling phase. Effects of TGF- β_1 on human ocular fibroblasts increase the LOX expression, expediting the pace of ECM remodeling [114]. Likewise, the expression of the LOX in injured Achilles tendons treated with TGF- β_1 -transfected bone marrow-derived mesenchymal stem cells (BMSCs) was up-regulated, which accelerated collagen synthesis, cross-link formation, and matrix remodeling [115]. Estrogen is another mediator that promotes remodeling probably via the activation of TGF- β_1 [116]. The LOX was up-regulated along with induced collagen gene expression after the addition of exogenous estrogen to the injured vaginal wall of guinea pigs, contributing to a remarkable increase in the density of elastin and collagen fibers as well as tensile strength of the tissue [52].

The growth factors are usually applied in the inflammatory or early proliferative phase of healing, while the mechanical loading generally plays a great role in the entire healing process [117]. It has been indicated that physiologic levels of mechanical strain on osteoblasts may regulate the transformation of a calcifiable collagenous matrix into a mature one with a specific molecular packing arrangement through collagen cross-linking as mineralization begins [118]. By contrast, Martinez reported that simulating the effects of weightlessness with the implementation of the rat hindlimb unloading (HLU) model decreased the LOX expression and retarded the repair of connective tissues [119]. A recent research into periodontal ligament (PDL) cells showed that low-level mechanical stretching could promote the formation and stabilization of the ECM via up-regulating the gene transcriptions of collagen and the LOX. Moreover, the LOX activity and total collagen production were also enhanced [120]. Therefore, mechanical stimulation can be appropriately applied to facilitate tissue remodeling.

However, the normal continuum of wound healing is disrupted in some pathological conditions, e.g., diabetes, in which repair process might enter into a non-healing state characterized by persistent inflammation, enhanced proteolytic activity and impaired ECM remodeling [121]. The vitreous level of the LOX activity is markedly decreased in proliferative diabetic retinopathy, leading to inadequate cross-links and ECM changes notorious in this disease. Aiming at these circumstances, wound healing models have been established both *in vitro* and *in vivo* to understand the insight of the LOXs in tissue remodeling. A report of the rat dorsal skin showed that the mechanical strength of the fibroblast-inserted gene activated matrix (GAM) with LOX transgene was much higher than that of the green fluorescence protein (GFP)-transgene controls. The development of mechanical strength was achieved through the increase of collagen cross-links after the treatment with LOX-producing GAM [122]. On the other hand, the increased expression and activity of the LOX could be a mechanism for compromised ECM barrier function. A recent research into the retina of diabetic mice showed that the integrity of the ECM, which was characterized by the decreased collagen fibril diameter and the irregular arrangement of collagens resulting from high glucose levels, could be compromised caused by the overexpression of the LOX [123]. These discoveries mentioned above may provide novel insight in the treatment of poorly healing wounds from the perspective of the LOXs.

4 Lysyl oxidases and tissue engineering

4.1 Conspectus of tissue engineering

Tissue engineering, a new and exciting technique, provides an effective reparative treatment for acute or chronic tissue injury. Unlike the autograft tissue and allograft tissue which are restricted by the inability to successfully integrate with the host tissue, the strategically engineered neotissue mimics the complex structure of native tissue and has promising prospects of application in tissue repair [124], especially for tissues which lack an intrinsic ability to self-repair following disease- or injury-induced degradation, e.g., bone, cartilage, vascular, knee menisci, intervertebral discs, tendons, and ligaments [125, 126]. Although tissue engineering technique holds great potential, the engineered tissues are prone to have a inferior function in integration with the host tissue *in vivo* and are unable to withstand the complex distribution of mechanical loading. These insufficiencies hinder the widespread application of tissue engineering technique [127]. Various methods such as using biological tissue adhesives at the

interface encourage integration to some extent, whereas low levels of collagens synthesized between native-to-engineered tissues don't change, so the poor integrative ability and low mechanical strength of neotissues are still the same [128]. A desirable collagen network depends on collagen concentration, relative proportion of different types of collagen, the density and diameter of collagen fibers, the spatial alignment, and more importantly, cross-linking [129, 130]. Hence, enhancing the functional properties and integrative ability of the neotissue through increasing collagen cross-linking could be a viable solution to the graft problems mentioned above [131].

The formation of mature cross-links is catalyzed by the LOXs. Lysine residues within elastin and collagen are converted into hydroxy-lysine residues by lysyl hydroxylase, then peptidyl lysine and/or hydroxy-lysine residues both serve as the precursors of cross-links and undergo the oxidative deamination catalyzed by the LOXs, and unstable aldehydes are generated. The resulting aldehydes then react with neighboring lysine residues or with other peptidyl aldehyde residues spontaneously through Schiff's base formation or aldol condensation to generate intra- and intermolecular covalent cross-links, leading to the formation of immature covalent cross-links which are converted into stable multivalent cross-links. Mature cross-links, the most abundant cross-links in varieties of tissues, result in the formation of large heterodispersed insoluble extracellular proteins that are resistant to proteolysis [132] (Fig. 2).

The pivotal role of the mature cross-link has been confirmed in the stabilization and mechanical support of the collagen network [133]. A recent study demonstrated that increasing LOX-mediated collagen cross-links through supplementing the medium with copper sulfate and hydroxylysine would synergistically increase both the tensile and compressive properties of the engineered articular cartilage. Some investigators even pointed out that the cross-link could be useful as a functional marker to access mechanical properties of the engineered tendon and could be used in tissue-engineered scaffold design to regulate regeneration [134]. Saito [135] referred the direct effect of collagen cross-links on the strength of bones, tendons, and ligaments. Regardless of engineered tissues or native tissues, a collagen network with highly favorable biological properties requires sufficient cross-links to stabilize the matrix and to provide it with mechanical support [136], so the activity and expression of the LOX are essential to the maturation of the ECM and properties of the engineered tissue.

4.2 Effects of the LOX on tissue-engineered constructs in *in vitro* culture

Current tissue engineering approaches to generate highly cellularized and metabolically active neotissues *in vitro*

Fig. 2 Formation of stable multivalent cross-links in the ECM. Lysine residues within elastin and collagen are firstly converted into hydroxy-lysine residues by lysyl hydroxylase, then peptidyl lysine and/or hydroxy-lysine residues both serve as the precursors of cross-links and undergo the oxidative deamination catalyzed by the LOX family (LOXs), and unstable aldehydes are generated. The resulting aldehydes then react with neighboring lysine residues or with other

peptidyl aldehyde residues spontaneously through Schiff's base formation or aldol condensation to generate intra- and intermolecular covalent cross-links, leading to the formation of immature dehydroxylysino-leucine (DHLNL) cross-links which are converted into stable pyridinoline (PYR) cross-links, the main type of cross-links in the extracellular matrix (ECM) of cartilage

typically involve a combination of cells, signals, and scaffolds. With structure and function being intimately linked, a direct tie between mechanical integrity and both the composition and structure of the ECM has been observed in a wide variety of engineered tissues [137]. And the LOX is an essential intermediary for tissue-engineered constructs to achieve a better mechanical integrity.

When vascular smooth muscle cells (VSMCs) transfected with the LOX gene were seeded in collagen gels, the tensile strength and elastic modulus of the tissue-engineered constructs were greatly enhanced due to the increase of cross-links [138]. Moreover, the LOX-mediated cross-links are independent on the collagen content or cell proliferation rate. This result is consistent with another study focused on the development of embryonic tendon functional properties which showed that the LOX-mediated cross-links altered mechanical properties without affecting matrix content and organization [139]. Eleftherios [140] put forward a novel use of the LOX through a combination of the LOX, chondroitinase-ABC (C-ABC) and TGF- β_1 could promote the maturation of the *in vitro* neofibrocartilage in a manner akin to native morphogenesis, and eventually result in a more biomechanically robust matrix. Not only aiding tissue maturation, the exogenous LOX along with C-ABC and TGF- β_1 also facilitated integration in the interface between the engineered implant and native tissue. It's conceivable that BAPN treatment would inhibit the formation of collagen cross-links and mechanical integrity of the tissue [141]. Since non-cross-linked collagens are more sensitive to the degradation by MMPs and collagen cross-links inhibit the activity of MMP-2, BAPN treatment leads to a reduced collagen content [142].

Although the primary effect of BAPN appears to be on collagen assembly, Marvy [143] reported that the altered state of the ECM caused additional effects on gene expressions of cartilage-specific collagens, i.e., collagen type II and type XI. The expressions of these collagens were up-regulated while the collagen content of the BAPN-treated cultures was actually less than that of controls. Additionally, it has been suggested that the up-regulation of collagen synthesis is related to impaired cell-matrix interactions due to the increased extractability of collagens. From a mechanobiological point of view, the LOX activity decrease caused by BAPN reduced insoluble collagens and mechanical strength, and ultimately provided a negative feedback stimulus for further collagen synthesis [144]. On the other hand, fibrogenic cells synthesized and assembled significantly larger collagen fibrils in the presence of BAPN compared with control cultures. The increase of fibril diameter with BAPN treatment may be caused by the lack of proteins, such as decorin which is on the surface of the fibril that normally limits the fibril diameter [144]. So the rational utilization of BAPN could be a potential therapeutic strategy towards facilitating mechanical properties. And several studies *in vitro* have referred some therapies with the addition of BAPN to treat the defects in tissues. For example, Ahsan reported that pre-treatment with BAPN caused an accumulation of unmodified hydroxylysines, the precursors of cross-links in the explants. Meanwhile, the formation of collagen cross-links reduced [145]. Upon BAPN withdrawal, these precursors converted rapidly into cross-links catalyzed by the LOXs, leading to an acceleration of subsequent integrative proceedings and an increase in tensile properties of the explants [146].

Not only mechanical integrity, a stable and robust integration interface is another crucial point for tissue engineering technique. Proper integration is the most imperative aspect ensuring that the implant remains stabilized and is able to competently function *in vivo*. It is generally agreed that the development of adhesive strength between the native tissue and engineered explant tightly correlates with the collagen synthesis, cross-linking, and deposition. Aristos [138] referred that the LOX could promote integration between native-to-implant cartilage surfaces through targeting the collagen assembly and organization. Thus, we can induce the activity/expression of the LOX to promote integration and eventually optimize tissue properties.

Micro-array data showed that the LOX was significantly induced under hypoxic conditions. Definitely, oxygen concentration which is widely investigated for potential application to tissue engineering is critical to regulate the ECM production and cross-linking [147]. To the best of our knowledge, hypoxia has a strong impact on cell biology via the transcription factor hypoxia-inducible factor-1 (HIF-1) and other targets of the hypoxia-inducible factors which are responsible for the exponential increase of gene expressions in the decreasing oxygen concentration conditions [148, 149]. Human vascular-derived myofibroblasts which were cultured at the physiological oxygen concentration of 7% showed an increase in the total amount of collagens and collagen cross-links compared with the myofibroblasts cultured at 21% of oxygen [150]. In addition, hypoxia in which the oxygen level dropped below 4% enhanced the gene expressions and protein production of the ECM components, including the LOX and collagen [147]. Similarly, tenocytes *in vivo* live commonly in a low O₂ environment and are difficult to expand due to the phenotype drift and functional loss [151]. Whereas the *in vitro* low O₂ tension culture, i.e., 2% O₂, enhanced the proliferative capacity of the tenocytes derived from the newborn pigs and inhibited the expressions of MMP-1 and IL-6, and eventually lead to decreased collagen degradation and increased collagen deposition [152]. These discoveries might be beneficial to the maturation of engineered tissues. Although the effects of hypoxia *in vitro* vary due to different cell sources, oxygen concentration and the time of exposure, we can take advantage of the hypoxia-mediated increase of the LOX expression to stimulate matrix production and maturation of the explants and promote their mechanical integrity. For instance, 4% O₂-mediated up-regulation of the LOX expression during the 3rd and 4th weeks of the self-assembling process increased the pyridinoline (PYR) cross-links and tensile integrity of the tissue-engineered cartilage [153].

Elastin, another substrate of the LOXs, is a vital structural protein conferring elasticity and resiliency to the

ECM. Tissues rich in elastins include aorta and large blood vessels (28–32% of the dry mass), lung (3–7%), elastic ligaments (50%), tendons (4%), and skin (2%) [21]. The elaboration and homeostasis of elastins are essential for the regeneration of these highly elastic tissues. And the LOX plays a role in the process since the enhanced production/activity of the LOX contributes to the increased cross-linked elastins. Knocking out the LOX gene in mice is perinatally lethal and results in the dysfunction of elastic tissues and fragmented elastic fibers [37]. While in culture of adult rat aortic smooth muscle cells, supplementation with the exogenous bovine LOX can improve elastin synthesis, deposition, and cross-linking in a dose-dependent manner [154]. Targeting the LOX with a mixture of TGF- β_1 and HA oligomers is of tremendous utility to restore elastin matrix homeostasis in the tissue-engineered constructs. HA oligomers and TGF- β_1 together modestly increased the LOX production and activity, and synergistically improved tropoelastin synthesis and matrix assembly in *in vitro* adult rat aortic SMCs. Meanwhile, the tropoelastins were also up-regulated likely via the increased the LOX production [155]. Similarly, Chandrasekhar referred that copper nanoparticles (CuNPs), the effective delivery vehicles for Cu²⁺ ion, together with HA oligomers could facilitate the regeneration of cross-linked elastins via stimulation of the LOX in adult vascular SMCs [156].

4.3 Effects of the LOX on tissue-engineered constructs in *in vivo* culture

The afore-mentioned views were studied *in vitro*, which only partially mimicked the actual micro-environment *in vivo*. In the *in vitro* studies, integration mostly occurs parallel to the surface. While in the typical clinical application, integration is often performed in a direction perpendicular to the tissue surface. Furthermore, when cells or tissues are suspended in collagen gels, there is little constraint counteracting swelling in the thickness direction and no appreciable distortion of the apposed geometry of the tissue samples [140]. In addition, oxygen level in the two-dimensional (2D) cell cultures *in vitro* is well-distributed. However, there is an oxygen gradient within tissue-engineered constructs at the three-dimensional (3D) level *in vivo* due to poor oxygen diffusion in the middle of the tissue [157]. So in order to understand the insight of the LOX in tissue engineering, it's necessary to perform studies *in vivo* to describe the actual properties and functions of the engineered tissues.

The LOX has been reported to promote integration and enhance the formation of cross-links between native-to-implant surfaces in an animal model. Neofibrocartilage pre-

Fig. 3 Signal transduction initiated by transforming growth factor- β_1 (TGF- β_1). The increase of the lysyl oxidase (LOX) mRNA and activity induced by TGF- β_1 is mediated through the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt), drosophila mothers against decapentaplegic protein 3 (Smad3), and mitogen-activated protein kinases (MAPKs) pathways. The reciprocities among these signalling pathways have been noticed. Akt phosphorylation and the increase in total Akt expression are PI3K-dependent. Moreover, the activation of p38-MAPK is necessary for the induction of TGF-mediated Smad3 signalling. Meanwhile, there is a cross communication between the PI3K and Smad3 signalling pathways in which the

inhibition of Smad3 reduces the activation of Akt by TGF- β_1 and that PI3K inhibition reduces the activation of Smad3. In MAPK signalling pathway, TGF- β_1 induces the phosphorylation of p38-MAPK, c-Jun N-terminal kinase (JNK) and extracellular regulated protein kinases 1/2 (ERK1/2). Moreover, TGF- β_1 -mediated increase in expression of bone morphogenetic protein-1 (BMP-1) may also activate TGF- β_1 and provide positive feedback to generate more LOX in the formation of the extracellular matrix (ECM). ① represents the MAPK pathway; ② represents the Smad3 pathway; ③ represents the PI3K pathway [149, 154, 159, 163]

treated with a combination of the LOX, C-ABC, and TGF- β_1 carries over an effect into an *in vivo* model following implantation, achieving the tensile properties of the integration interface on par with the values of the intact native fibrocartilage [134]. Another recent study provided applications of the exogenous LOX for improving tensile properties of a spectrum of native and engineered tissues both *in vitro* and *in vivo* [158]. *In vivo* subcutaneous implantation of the LOX-treated neocartilage into the nude mice promoted further maturation of the neotissue, enhancing tensile strength and PYR content approximately 3-fold and 14-fold, respectively, compared with *in vitro* controls [158].

5 Signal transduction of the LOXs in tissue repair

Enhanced TGF- β_1 expression is a typical response to injury accompanied by the increase of the LOXs expressions and synthesis of multiple ECM proteins [159]. Elvira reported the increase of the LOX mRNA and activity induced by TGF- β_1 in adult cardiac fibroblasts was mediated through the phosphatidylinositol-3-kinase (PI3K)/protein kinase B

(Akt), drosophila mothers against decapentaplegic protein 3 (Smad3), and mitogen-activated protein kinases (MAPKs) pathways [154]. The reciprocities of the three signalling pathways contribute to tissue remodeling through the regulation of the LOXs [160–162]. In addition, TGF- β_1 -mediated increase in the expression of BMP-1, a key activator of the LOX, may activate TGF- β_1 and provide a positive feedback to generate more LOX in the formation of the ECM [163]. In MAPK signalling pathway, TGF- β_1 induces the phosphorylation of p38-MAPK, c-Jun N-terminal kinase (JNK) and extracellular regulated protein kinases 1/2 (ERK1/2) [149] (Figure 3). The role of the p38-MAPK signalling pathway in anti-inflammation has also been explored by Georgia, who reported that IL-4 and IL-1 α induced the expression of the LOX in the post-ovulatory wound healing process via the p38-MAPK signalling pathway [59].

Unlike TGF- β_1 , PGE₂ significantly inhibits the LOX activity in the inflammation phase of healing. Recently, Utako put forward that this inhibition from PGE₂ acted as a means to regulate elastogenesis in the ductus arteriosus (DA) [164]. PGE₂ signal transduction promoted the lysosomal degradation of the LOX protein through the clathrin-

mediated endocytosis. And the negative effect of PGE₂ is achieved through the PGE₂-EP4-c-Src-PLC γ signal pathway which plays a primary role in the post-translational decrease of the LOX. It is clearly demonstrated that prostaglandin E receptor 4 (EP4), a kind of PGE₂ receptor, can directly stimulate the activity of c-Src, a tyrosine kinase acting as a cellular signal transducer that can be activated by EP4 [165]. So the direct association between EP4 and c-Src may activate its downstream signalling in the EP4-mediated degradation of the LOX.

6 Conclusion and future prospects

Tissue repair is a dynamic response to injury with lots of ambiguous mechanisms. Current researches indicate the essential role of the LOXs on expediting healing proceedings in various phases. The LOXs are differentially regulated by different ligands depending on the different phase of healing process. In this review, we have concluded the experimental results from the *in vitro*, animal and human *in vivo* studies and summarized the effects of the LOXs in tissue repair. In addition, the LOX can be a new target for improved mechanical strength and integration in tissue engineering. So the application of the LOX and its activators could act as a new therapeutic approach to desirable grafting and regeneration of tissue-engineered constructs. In general, the role of the LOXs in tissue repair and tissue engineering still deserves further attention and should be investigated in more detail.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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