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Matrisomal components involved in regenerative wound healing in axolotl and *Acomys*: implications for biomaterial development†

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Achieving regeneration in humans has been a long-standing goal of many researchers. Whereas amphibians like the axolotl (*Ambystoma mexicanum*) are capable of regenerating whole organs and even limbs, most mammals heal their wounds *via* fibrotic scarring. Recently, the African spiny mouse (*Acomys* sp.) has been shown to be injury resistant and capable of regenerating several tissue types. A major focal point of research with *Acomys* has been the identification of drivers of regeneration. In this search, the matrisome components related to the extracellular matrix (ECM) are often overlooked. In this review, we compare *Acomys* and axolotl skin wound healing and blastema-mediated regeneration by examining their wound healing responses and comparing the expression pattern of matrisome genes, including glycosaminoglycan (GAG) related genes. The goal of this review is to identify matrisome genes that are upregulated during regeneration and could be potential candidates for inclusion in pro-regenerative biomaterials. Research papers describing transcriptomic or proteomic coverage of either skin regeneration or blastema formation in *Acomys* and axolotl were selected. Matrisome and GAG related genes were extracted from each dataset and the resulting lists of genes were compared. In our analysis, we found several genes that were consistently upregulated, suggesting possible involvement in regenerative processes. Most of the components have been implicated in regulation of cell behavior, extracellular matrix remodeling and wound healing. Incorporation of such pro-regenerative factors into biomaterials may help to shift pro-fibrotic processes to regenerative responses in treated wounds.

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1. Introduction

Throughout the animal kingdom, adult skin wounding can be resolved either by fibrotic scarring or tissue regeneration. With some exceptions such as ear punch closure in rabbits,[1,2] antler regrowth in deer,[3,4] digit tip regeneration in children and mice,[5,6] most adult mammals repair their wounds by fibrotic scarring.[7,8] In humans, fibrosis in response to the destruction of both the epidermis and dermis (full-thickness skin wounds), such as third degree burns and traumas, results in scars that can have a severe impact on the patients' quality of life. Often, follow-up procedures, treatments, and medication are required to alleviate the functional impairment and discomfort caused by these scars.[9] In contrast, some species of vertebrates (fish, amphibians, salamanders) are capable of skin regeneration. Why are some organisms capable of regeneration while others are not? Why is scarless regeneration in humans, with some notable exceptions, lost after early development? Both cell intrinsic and cell extrinsic factors are believed to play a role in the fibrosis *vs.* regeneration outcome, but the cellular and molecular mechanisms underlying fibrosis compared to regeneration remain poorly understood. Understanding the drivers of fibrotic scarring *vs.* regeneration will aid in developing approaches and therapies to induce skin wound regeneration in humans. These novel therapies would meet an important medical need. In this context the discovery of *Acomys* as an adult mammal capable of skin regeneration becomes significant.

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Fibrotic wound repair is a (patho)physiological mechanism where an exacerbated healing response occurs and a fibrous tissue is formed. In contrast, regeneration involves complete structural and functional reconstruction of a tissue or organ after wounding and is traditionally understood as a specialized re-enactment of development.[10,11] Research into the underlying molecular and cellular mechanisms of both fibrosis and regeneration in the skin have often focused on cell-intrinsic behavior and underlying genetic pathways or molecular players. In contrast, the role and contribution of the extracellular matrix (ECM) and its components during both fibrosis and regeneration is still poorly understood. Initially regarded as a somewhat inert structural scaffold, it is now accepted that the ECM is a major determinant of cell behavior in all tissues, mediated by its structure, composition and modifications. For example, increasing the stiffness of 3D collagen hydrogels by introducing intra- and interfibrillar crosslinks activated the expression of pro-fibrotic genes by adipocytes.[12] The ECM can regulate the capture and activity of growth factors, which in turn influences local cellular processes, as is seen in the maintenance of stem cell niches.[13,14] It stands to reason that the ECM plays a role during skin regeneration and that the "regenerative matrix" is a highly specialized environment unique to regenerative species. The ECM and all its associated (glyco)proteins are collectively known as the matrisome. The Matrisome Project has assembled lists of all ECM-related genes, divided into two groups; core matrisome genes, (composed of collagens, glycoproteins and proteoglycans), and matrisome associated genes, including regulators, secreted factors and ECM-affiliated proteins.[15,16] In particular, proteoglycans are underrepresented in the Matrisome Project. Proteoglycans consist of a core protein with glycosaminoglycan (GAG) side chains, the latter of which convey important biological properties. Proteoglycans are an important component of the ECM, but the high variability of the GAG side chains makes it difficult to assess their involvement. Proteoglycans (and thus GAGs) may play a significant role during regeneration. The metabolism and regulation of proteoglycans, in particular the subset of genes pertaining to GAG metabolism, are a focus of this review.

While many publications have focused on comparing regeneration competent to regeneration incompetent animals, two exceptional models of regeneration provide unique opportunities to explore the phenomenon of scarless regeneration: the axolotl (*Ambystoma mexicanum*) and the African spiny mouse (*Acomys* sp.). We will refer to *Ambystoma mexicanum* simply as 'axolotl'. This is in line with other scientific reports and the *Ambystoma* genus also encompasses other salamanders that are not investigated here. On the other hand, we will address the African spiny mouse by its genus name '*Acomys*'. Various species of *Acomys* are investigated in this review and the genus name is most commonly used in relevant scientific literature. *Acomys* is unique since it is a remarkable exception to the rule that adult mammals cannot regenerate their tissues, with individuals displaying remarkable wound healing responses throughout all life stages. Some mouse strains have acquired regenerative characteristics through selective breeding or genetic manipulation (MRL mouse, p21−/− mouse), but the natural regenerative potential of *Acomys* is far superior.[17] Every species of the *Acomys* genus examined so far has shown the ability to regenerate various tissues. The regenerative capacities of the axolotl are unparalleled, with individuals being able to regrow entire limbs. In contrast, frogs (*Xenopus* sp.) complete regeneration of amputated limbs with the formation of a cartilaginous spike.[18] Zebrafish, another well characterized model for regeneration, shows limitations to fin regeneration based on the amputation plane and adult zebrafish are unable to regenerate skin.[19,20] These limitations prompted us to focus our comparison on axolotl and *Acomys.* In this review, we investigate the role of matrisome components during wound healing in two regeneration competent animals by analyzing existing datasets from previous publications. We aim to compare the healing responses in terms of the ECM-related gene and protein profiles, with a focus on skin wound healing. We postulate that identification of matrisome-related targets involved in the regenerative process may lead to novel approaches in human wound healing, especially with regards to biomaterial development for skin wound healing. It is not our intention to compare or relate *Acomys* to humans. Instead, by comparing a regenerative competent amphibian (axolotl) and a regenerative competent mammal (*Acomys*) we may identify drivers of regeneration that transcend species. We will first briefly touch upon the process of regular wound healing ending in fibrosis and the process of regeneration *via* blastema as seen in some vertebrates.

2. Wound healing responses

The mammalian skin wound healing response, culminating in scar formation, can be divided into three distinct phases: inflammation, proliferation and remodeling[21] (Fig. 1A). The inflammatory phase occurs directly after wounding and is characterized by blood clot formation (hemostasis) and the invasion of neutrophils and monocytes.[22] Macrophages play an important role by phagocytizing cell debris and bacteria, as well as secreting various growth factors and chemo-attractants. The proliferation phase, also known as the granulation tissue formation phase, encompasses both wound re-epithelialization and the formation of a dense network consisting of fibroblasts and neovasculature in a collagen and fibronectin rich matrix.[23] This is a temporary matrix that is often highly disorganized compared to the original tissue and prone to rupturing. During the remodeling stage, the granulation tissue is remodeled into a smooth textured scar: rich in type I collagen fibers and lacking secondary skin appendages, such as hair follicles and sebaceous glands.[24]

Some species resolve complete limb amputation through blastema-mediated regeneration. A blastema, also known as a regeneration bud, is an autonomous structure composed of a heterogeneous mass of dedifferentiated stem/progenitor cells that goes through morphogenesis, thereby creating a multi-

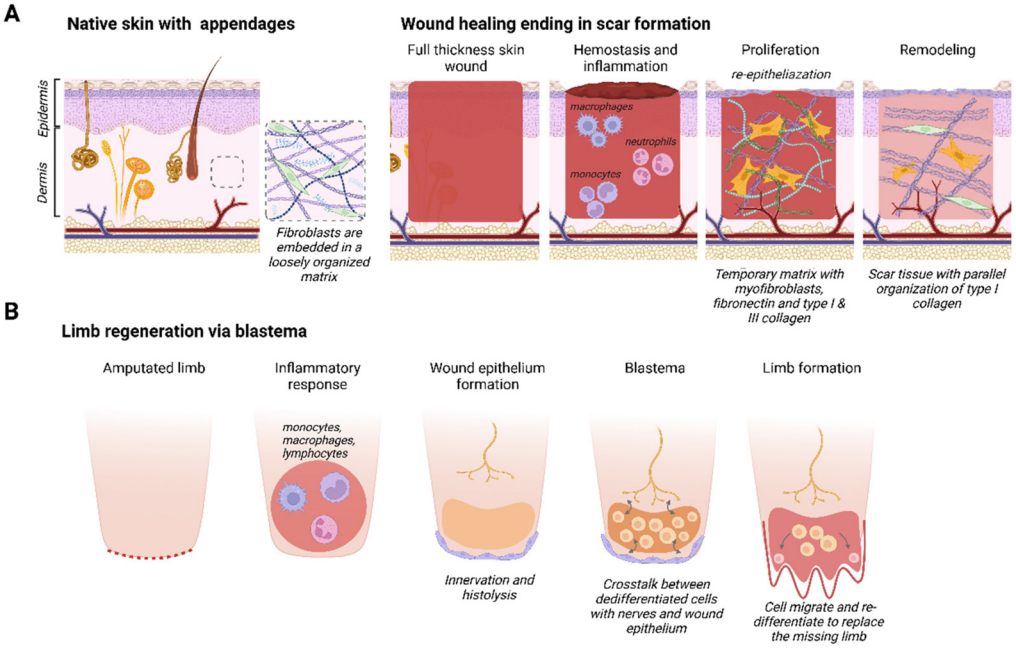

Fig. 1 Wound healing responses. Schematic representations of (A) native mammalian skin consisting of the epidermis and dermis with skin appendages and different phases of full-thickness skin wound healing ending in scar formation, and (B) phases in limb regeneration *via* a blastema in amphibians. Created with BioRender.com.

tude of cell types to replace the missing organ or limb[25,26] (Fig. 1B). The blastema is located under a layer of immature wound epithelium and it is created after the initial inflammatory response is resolved. Histolysis, re-innervation and ECM production will induce the migration and accumulation of cells under the wound epithelium that will differentiate into mesenchymal and ectodermal cell types.[27] A requirement for blastema formation is the molecular and cellular interaction between the wound epithelium, nerves, dermal cells and the ECM at the wound site.[28] In the final stage, cells inside the blastema differentiate and eventually form the missing organ, tissue or appendage.[27] Blastema formation has been identified in different species, such as zebrafish,[29] flatworms,[30] urodeles[26] and even some mammals (*Acomys* full-thickness ear wounds[31] and mouse digit tip[5]). Intriguingly, under the right circumstances the fingertips of children up to 10 years of age heal similarly to the mouse digit tip.[6]

While the fibrotic response is the norm in most mammals, some exceptions exist as seen in the regeneration capacity of fetal wounds and digit tip regeneration in mice and children. Human skin wounds regenerate without scarring up to 24 weeks of gestation, thereafter the healing response will gravitate towards scar tissue formation.[32] Research in fetal sheep indicated that, aside from gestational age, wound size also affects the regenerative capacity; with wounds exceeding 4 mm in diameter demonstrating an increased tendency to scar.[33] A unique feature in fetal wounds is the 'cable' of actin filaments that develops around the wound edge. Wound closure through contraction of this actin ring has been compared to a drawstring closure.[34–36] Recently, it has been postulated that myofibroblast-mediated wound contraction in adult mammals inhibits regeneration and drives scar formation.[37] In addition, a distinct immune response has been recorded in fetal wound regeneration that contributes to a non-inflammatory ECM environment.[32,38] The composition of fetal ECM, with an abundance of type III collagen and hyaluronan, is important in this aspect.[32] Children up to ~10 years of age are even able to fully regenerate a digit tip, as long as the wound is distal to the upper interphalangeal joint with sufficient nail bed[39,40] and little to no intervention.[41,42] Storer *et al.* showed that the ECM determines the blastema state and plasticity of mesenchymal cells in non-regenerative fingertip amputations,[43] suggesting the importance of focusing further on the role of the matrix in regeneration.

3. *Acomys*, African spiny mouse, a mammal capable of regeneration

Recently, the African spiny mouse (*Acomys*) has emerged as a remarkable exception to the rule that adult mammals are incapable of regeneration.[44] This rodent genus is found throughout Africa and the Middle East. Belonging to the subfamily Deomyinae, they are part of the Muroidea family and share a common ancestor with the Muridae, diverging about 23 million years ago.[45] The genus comprises over 20 different species, but most studies have focused on *A. cahirinus*.

Following up anecdotal reports of 'skin jumping' in *Acomys*, Seifert and colleagues reported *A. kempi* and *A. percivali*[46] exhibited remarkable wound healing properties after extensive

skin damage caused by mild handling of wild-caught animals. Following this initial report several groups have found that depending on type of organ and injury, *Acomys* shows remarkable responses to injury: extensive regeneration has been observed in skin, ear, muscle, digit tip and spinal cord[47] (Fig. 2). Acute ischemia wound models of heart and kidney do not seem to regenerate but show resistance to wounding and diminished fibrosis.[48] In contrast, *Mus musculus* shows extensive fibrotic scarring to similar injuries. Evolutionarily separated by only 23 million years, *Acomys* and *Mus* provide a powerful comparative framework to identify the cellular and molecular differences between regeneration and fibrotic scarring.

In all regenerative systems, the immune system is thought to play an important role through its ability to modulate inflammation, as severe inflammation can be detrimental to the healing process. Several groups have extensively described the immune response observed in *Acomys.*[48–52] In general, *Acomys* wounds demonstrated a distinct role for macrophage activity when compared to *Mus.*[50] Mature macrophages (F4/80+) were absent in *Acomys* full-thickness skin wound beds, along with a distinct lack of pro-inflammatory cytokines.[51] In *Acomys* ear wounds, CD86+ macrophages (classically activated M1) were absent from the blastema, being confined to the wound edges in the connective tissue distal to cartilage. CD206+ macrophages (M2, pro-regenerative) were restricted to the region beneath the wound epidermis and practically absent from the rest of the blastema.[48] On the other hand, in *Mus*, both M1 and M2 macrophages are present throughout all connective tissues of the injured area.[53] Interestingly, the depletion of macrophages through clodronate liposome injections severely delayed ear hole closure in *Acomys.*[50] The neutrophil response in *Acomys* is delayed compared to *Mus* and differences were found in the number of neutrophils in various compartments, such as blood and bone marrow, when comparing several species of *Acomys* to *Mus.*[52]

A second factor of particular significance for skin regeneration is the skin biology of *Acomys. Acomys* dorsal skin is weak and tears easily when subjected to a mean tensile strength of 0.11 MPa, a force approximately 20 times weaker than that required to tear skin of *Mus.*[46] Using atomic force microscopy to measure spatial tissue stiffness, intact *Acomys* skin did not exceed 15 kPa and wound centers measured no more than 5 kPa. Putting these findings into context, skin of C57Bl/6 mice measures 28 kPa, with wound beds of 10.5 kPa, further demonstrating the unique biomechanical properties of *Acomys* skin.[54] Following fast re-epithelialization after skin injury, *Acomys* granulation matrix was loosely organized with less collagenous deposition and increased gene expression of the matrix metalloproteinases *Mmp2* and *Mmp9* compared to *Mus.*[51,55] Overall, *Acomys* showed a distinct profile for protein remodeling and protein synthesis.[56,57] Upon completion of regeneration, *Acomys* skin developed hair follicles, sebaceous glands and an intact panniculus carnosus.[46,51] After wounding, enriched proteins belonged to pathways related to tight junction formation, endocytosis, ribosomes, proteasomes, Wnt signaling, MAPK signaling and vasopressin-regulated water reabsorption. Components of the ubiquitin-proteasome degradation pathway were strongly upregulated in *Acomys* and proteins in the ribosome related pathways were also implicated.[56,57] Overall, enhanced protein turnover seems an important characteristic of skin wound healing in Acomys. The unique characteristics of *Acomys*' skin may benefit wound healing, but the fact that *Acomys* is capable of regenerating other organs indicates that its skin biology may not be the only factor contributing to its regenerative potential.

Full-thickness skin wounds in *Acomys* regenerate without the presence of a blastema whereas ear punch wound closure in *Acomys* has been reported to occur through the formation of a blastema.[27,31,46] Research using an ear hole punch model in *A. cahirinus* was reported by Matias Santos and colleagues,[58] in response to the initial ear punch study.[46] In these studies, reports on muscle regeneration were inconsistent, with the original study (Seifert 2012)[46] mentioning the absence of muscle fibers in contrast to the study of Matias Santos (2016),[58] who reported presence of muscle fibers within the regenerating region. Additionally, all regular skin appendages, such as hair follicles and sebaceous glands, had regenerated and nerve fibers were present.[58] Gawriluk and colleagues demonstrated that the wound matrix of *A. cahirinus* exhibits several hallmarks of blastema-mediated regeneration.[31] The *Acomys* ear wound beds showed several signs of the blastema-like wound epidermis, such as restriction of proliferating cells to the wound borders, lack of basal-apical polarity in basal keratinocytes and presence of keratin 17 in the wound matrix.

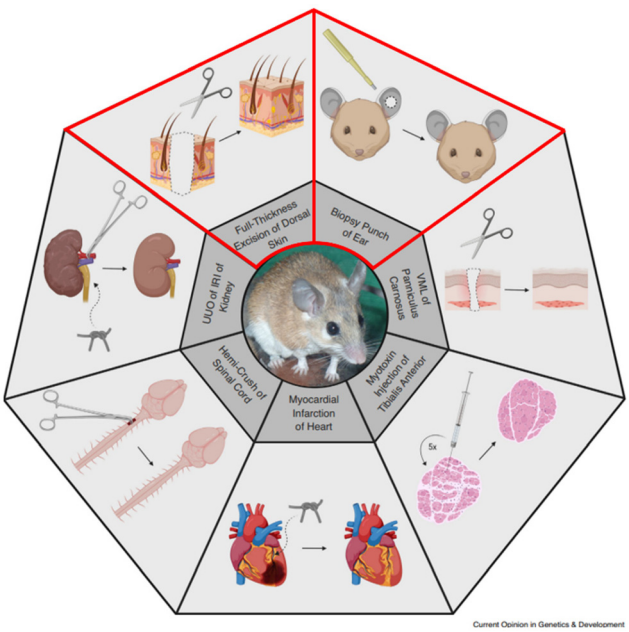

Fig. 2 Overview of tissues/organs with a remarkable healing response in *Acomys*. This review aims to elucidate the involvement of the matrisome during regeneration of the skin and ear (lined in red). VML = volumetric tissue loss, UUO = unilateral obstruction, IRI = ischemia reperfusion injury. Reproduced from Sandoval & Maden (2020)[48] with permission from Elsevier, 2020, under the STM permissions guidelines.

Evidence of reinnervation, an essential process in epimorphic regeneration, was obtained as enrichment of genes involved in axon guidance, neuroactivity and growth was observed. Re-entry of mesenchymal cells into the cell cycle, another defining characteristic of the blastema, was also observed. Taken together these observations provide strong evidence for the formation of blastema in *Acomys* ear punch wounds and cements the importance of *Acomys* as a model system capable of tissue regeneration. In this review, we compare its wound healing responses to those of the axolotl, an animal well known for its regenerative abilities.

4. Axolotl, salamanders capable of full organ regeneration

The axolotl (*Ambystoma mexicanum*) is a paedomorphic (or 'neotenic') salamander belonging to the Urodele order of the *Amphibia.* The axolotl grows through a process of juvenilization, meaning it delays somatic development and retains physiologically juvenile features during its development to sexual maturity.[59] Therefore, the axolotl is an animal that reaches sexual maturity without going through metamorphosis. Metamorphosis occurs only when they are in contact with an external and artificial stimulus, which is uncommon in the wild.[60] The axolotl represents an important model organism in the field of regenerative medicine due to its highly efficient regeneration capacity. Regeneration has been observed in limbs and several organs such as the skin, heart, brain, lungs, and eyes[61,62] (Fig. 3).

Limb regeneration has been extensively investigated in axolotl. Following limb amputation, wound closure continues to blastema formation and limb development.[26] Wound closure takes several hours, and full regeneration of the limb typically occurs between 40 and 50 days.[63] The formation of a blastema is essential to the regeneration of a limb. Research using the Accessory Limb Model has uncovered three requirements for successful blastema-mediated limb regeneration: the presence of a wound epithelium, the occurrence of nerve signaling and the availability of positional cues provided by pattern forming cells.[64] Absence of any of these factors abrogates limb regeneration.[65] Formation of a closed wound epithelium involves migrating keratinocytes that cover the wound surface.[26] Under the influence of signals emanating from local nerve fibers, proliferation of this wound epithelium into a multilayered epithelium result in the apical epithelial cap.[66] This epithelial cap is characterized by specific molecular markers and by an immature basement membrane beneath the epidermis.[67] Nerve signaling causes basal epithelial cells to exit the cell cycle. This step is essential for the development of the blastema as cells of the apical epithelial cap will start secreting growth factors and enzymes that degrade ECM and facilitate migration.[28] Next, fibroblasts originating from the limb circumference gather underneath the apical epithelial cap. The interaction between these cells with different positional identities is crucial for maintaining the blastema and ensuring regeneration as the 'positional memory' dictates the pattern of the missing limb.[26]

Transcriptomic analysis has revealed the importance of the immune system in axolotl wound healing and regeneration. There is a gradual increase of macrophages and fibroblast-like cells post amputation, whereas neutrophil populations decreased after wounding.[68] Neutrophils evoke anti-inflammatory macrophages, which leads to a reduction in pro-inflammatory cytokines.[69] Macrophages with anti-inflammatory and pro-resolving phenotypes were dominant in the first seven days of axolotl limb regeneration, thereby contributing to repressing the inflammatory response.[70] When macrophages were systemically depleted, limb regeneration failed. A fibrotic ECM was observed with increased type I collagen deposition and the presence of αSMA-positive cells[71] that could also inhibit apical epithelial cap formation and blastema-induction.[72] ECM remodeling is of key importance to rapidly reconstruct the matrix[70] and the ECM contains positional information required for limb pattern formation through location-specific differences in heparan sulfate sulfation. This was shown by grafting decellularized axolotl skin or decellularized mouse skin onto axolotl limb wounds.[73] Depending on the developmental stage, positional origin and heparan sulfate sulfation pattern of the graft, limb patterning was either supported or inhibited. These results demonstrated that the ECM contains information, conveyed *via* heparan sulfate sulfation patterns, that can influence limb regeneration and this information is also present in the ECM of mice.[73]

Axolotl skin, which regenerates without blastema formation, provides a platform to uncover drivers of regeneration in the absence of blastema. The skin of juvenile axolotls consists of a pseudostratified epithelium which contains epithelial and Leydig cells, and the dermis presents a loosely organized

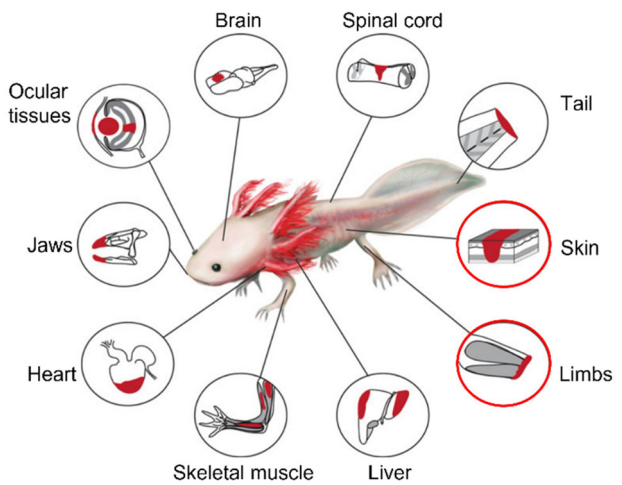

Fig. 3 Summary of tissues/organs that can be regenerated by axolotls (*Ambystoma mexicanum*). The regeneration of skin and limbs, outlined in red, and the involvement of matrisome components is further explored in this review. Reproduced from Debuque & Godwin[62] with permission from Springer International Publishing Switzerland, 2016, under the STM permissions guidelines.

network of thin collagen fibrils in which fibroblasts and mucus glands are embedded.[74] A layer of compressed collagen fibers separates the hypodermis from the underlying muscle layer. Scarless skin wound healing has been reported in adult axolotls after making excisional wounds on the flanks. Wounds did not form scabs, instead epithelization was completed within 24 h and full skin healing including all skin appendages was completed within 80 days.[67] Overall, the regenerating tissue in axolotl was characterized by high levels of matrix remodeling enzymes, relatively low abundance of fibronectin and persistently high levels of tenascin C throughout the wound bed. Type III collagen deposition was followed by type I collagen deposition and maturation from day 14 onwards. Following a full-thickness wound, the collagen architecture in the dermis did not return to the normal lattice-like pattern, but was disorganized, even though the healed skin appeared identical to unwounded skin. However, after blastema-mediated skin regeneration following limb amputation, the dermis, including collagen architecture, does fully regenerate.[75]

The healing capacity of the axolotl is unique and while directly translating regeneration in salamanders to mammals is difficult, some clues may be found in the regenerative response of axolotls. Axolotls display a minor inflammatory response with macrophages of a mostly anti-inflammatory phenotype. The fibrotic response is notably absent, ECM deposition is regulated, and matrix remodeling enzymes are prevalent (Fig. 4A). These hallmarks are also observed in *Acomys* regeneration, suggesting regeneration shares similar mechanisms across both models. However, there are differences in intensity and duration of the different events in regenerative and non-regenerative mammals, indicating the importance of timing of the various phases (Fig. 4B). The various wound healing responses described for the animals are summarized in Table 1 (full thickness skin wound healing) and Table 2 (blastema-mediated regeneration).

5. Identification of matrisome-related genes and proteins during regeneration using existing datasets

Comparing the matrisome and GAG-related components present during regeneration in *Acomys* and axolotl will aid in uncovering similarities between these two animals. Through this approach, we aim to identify shared pro-regenerative factors between species, which may be translated to other species (*i.e.* humans).

5.1 Selection of datasets

To select the databases that will be analyzed, papers that document regenerative wound healing in *Acomys* or axolotl were retrieved, with a focus on skin and blastema. Publications with either a proteomics or transcriptomics approach were extracted. For *Acomys* publications on both skin regeneration and ear hole closure were included in the search. A total of four papers were identified, of which three focused on the regeneration of full-thickness skin wounds[55–57,76] and one publication described ear hole closure.[31] The datasets of Brant *et al.* (2015)[55] and Brant *et al.* (2019)[76] were compared for overlap, as these publications investigate similar samples with different techniques. All genes identified by the 2015 publication were present in the 2019 publication. As the 2019 publication offered more data only this set was included for the

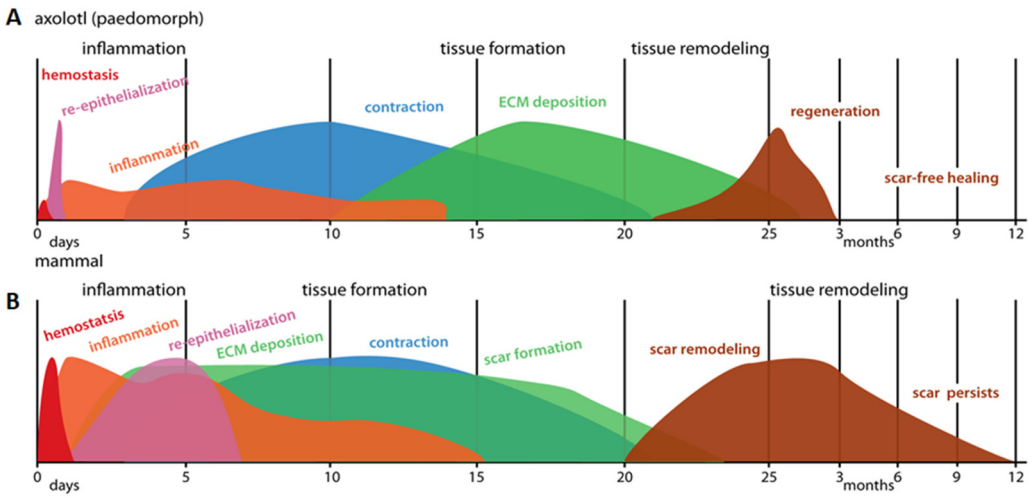

Fig. 4 Schematic representation of the wound healing response in (A) paedomorphic axolotls and (B) mammals. Axolotl wound healing shows minimal hemostasis and very rapid re-epithelization followed by low levels of inflammation. ECM deposition occurs after wound contraction and tissue remodeling does not lead to a scar, but results in normal skin appearance. Contrary to that, in mammals, a strong inflammatory response is maintained even after re-epithelization is complete. Deposition of new ECM and wound contraction occur at the same time and scar tissue persists after completion of the months-long remodeling phase. Reproduced from Seifert *et al.* (2012)[67] with permission from © 2012 Seifert *et al.*, under the Creative Commons Attribution License.

Table 1 Summary of processes observed during full-thickness skin wound healing

Full-thickness skinwound healing	Mus musculus (mammal)	*Acomys* sp. (mammal)	Axolotl (amphibian)
Re-epithelialization	Slow (5–7 days) with a scab	Fast (3 days) with a scab	Fast (1 day) without a scab
Inflammation	High	Low	Low
	Fast neutrophil response	Delayed neutrophil response	Low neutrophil response
	High expression of M1 macrophages	High expression of M2 macrophages	High expression of pro-resolving macrophages
	Pro-inflammatory cytokines ++	Pro-inflammatory cytokines −−	Pro-inflammatory cytokines −−
Proliferation	Early matrix deposition	Delayed matrix deposition	Delayed matrix deposition
	Dense granulation matrix	Loose granulation matrix	Loose granulation tissue
	Collagen deposition+	Collagen depostion −	Matrix degradation+
Remodeling	Scar remodeling	Scar-free remodeling	Scar-free remodeling
	Type I collagen with parallel orientation	Type I collagen with basket weave orientation	Type I collagen did not return to normal lattice organization
	No regeneration of skin appendages	Regeneration of hair follicles and sebaceous glands	Regeneration of mucous glands

Table 2 Summary of processes observed during blastema-mediated regeneration

	Acomys sp. (mammal)	Axolotl (amphibian)
Blastema-mediated regeneration	Regeneration after ear punch *via* blastema-like process	Limb regeneration *via* blastema
Wound epithelium	Loss of basal-apical polarity in keratinocytes	Migrating keratinocytes close the wound
	Presence of keratin 17 (blastema marker)	Multi-layered epithelium forms (apical epithelial cap)
	Recruitment of mesenchymal stem cells	Immature basement membrane
Innervation	Upregulation of genes associated with axon guidance, neuron growth and neuroactivity	Apical epithelial cap (AEC) is innervated
	Cell proliferation is restricted to wound borders	Cells exit cell cycle
Blastema formation	Cell cycle re-entry, cell proliferation and cell division	Pro-migratory environment is formed (ECM degradation, growth factor secretion)
	Increase of matrix degradation enzymes	Fibroblasts with various positional identities migrate to the AEC
	Cartilage pattern re-established the ear	Limb pattern is established by ECM and cells
Differentiation	Regeneration of missing tissues	Cells redifferentiate to form missing limb

comparison. Details of the selected papers are presented in Table 3.

Several papers focusing on axolotl were reviewed such as Monaghan *et al.* (2009)[77] and Wu *et al.* (2013).[78] The limited availability of public transcriptomic datasets of *Acomys* led us to select publications on axolotl that best matched the time-points and tissues analyzed in the selected *Acomys* publications. Proteomic and transcriptomic studies of the axolotl were selected based on their description of the regenerating limb blastema and/or non-blastema mediated skin regeneration, which resulted in the selection of three papers (Table 3). The paper by Monaghan *et al.* (2012)[79] describes both limb regeneration (*via* blastema) and regeneration of a full-thickness skin wound. Both Rao *et al.* (2009)[63] and Stewart *et al.* (2013)[80] exclusively describe hind-limb regeneration *via* blastema at the proteomic and transcriptomic level, respectively.

5.2 Identification of the matrisome and GAG-related components

We next addressed the differences in gene annotations of the selected datasets. The publications focusing on *Acomys* used mouse gene symbols to annotate data, whereas the axolotl datasets were annotated using human gene symbols. Detailed lists of matrisome related genes of various species are available through the Matrisome Project.[15] The mouse and human matrisome master lists contain 1110 and 1027 genes, respectively. As human-mouse orthologs are comparable the more extensive mouse matrisome was used for the selection of matrisome genes from all datasets.

The genes of the matrisome are divided into core matrisome genes and matrisome-associated genes.[15] The former is further subdivided into ECM glycoproteins, collagens and proteoglycans while the latter is subdivided into ECM-affiliated proteins, ECM regulators and secreted factors (Fig. 5, full gene list is available in Table S1†). Closely associated with the matrisome, but not fully included in the matrisome lists, are glycosaminoglycans (GAGs). These polysaccharides are generally present in the ECM and mostly attached to a core protein, together known as proteoglycans.[81] Although the specialized role of GAGs in a multitude of biological processes is widely acknowledged, they are technically difficult to assess and are consequently often disregarded. After biosynthesis of the repeated disaccharide units, GAGs are extensively modified by enzymes that epimerize certain saccharides, remove acetyl groups and add sulfate groups at specific locations on the building blocks, resulting in highly heterogenous molecules.

Table 3 Selected publications focusing on regeneration in *Acomys* and axolotl

Publication	Title	Tissue	Timepoints	Technique	Dataset
Brant *et al.* 2019 (*Acomys*)[76]	Comparative transcriptome analysis of dermal wound healing reveals *de novo* skeletal muscle regeneration in *Acomys cahirinus*	Dorsal skin, 8 mm skin punch	Day 0, 7, 14	*De novo* transcriptome assembly and comparative transcriptomics	Supplementary files of ref. 76: S7 data and S8 data
Gawriluk *et al.* 2016 (*Acomys*)[31]	Comparative analysis of ear-hole closure identifies epimorphic regeneration as a discrete trait in mammals	Ear punch, 4 mm	Day 0, 5, 10, 15, 20	*De novo* transcriptome assembly and comparative transcriptomics	Supplementary file of ref. 31: dataset 1
Yoon *et al.* 2020 (*Acomys*)[56,57]	Comparative proteomic analysis in scar-free skin regeneration in *Acomys cahirinus* and scarring *Mus musculus*	Dorsal skin, 8 mm skin punch	Day 0, 7, 14	Shotgun proteomics using LC-MS/MS	Table 1 of ref. 56 and 57
Monaghan *et al.*, 2012 (axolotl)[79]	Gene expression patterns specific to the regenerating limb of the Mexican axolotl	Flank skin outside of limb range, 4 mm skin punch	Day 0, 1, 3, 7	Microarray by Affymetrix GeneChips	Supplementary data of ref. 79 in NCBI: GSE37198_RAW.tar
Stewart *et al.* 2013 (axolotl)[80]	Comparative RNA-seq analysis in the unsequenced axolotl: The oncogene burst highlights early gene expression in the blastema	Right forelimbs at the mid-stylopod level	Hour 0, 3, 6, 12 and day 1, 3, 5, 7, 10, 14, 21, 28	*De novo* assembly of axolotl transcript, RNA-seq	Supplementary data of ref. 80 In NCBI: GSE34394_RAW.tar
Rao *et al.* 2009 (axolotl)[63]	Proteomic analysis of blastema formation in regenerating axolotl limbs	Bilateral hind limbs regenerating tissue and 1 mm of stump tissue	Day 0, 1, 4, 7	Proteomics by LC- MS/MS	Supplementary files of ref. 63: Table 2

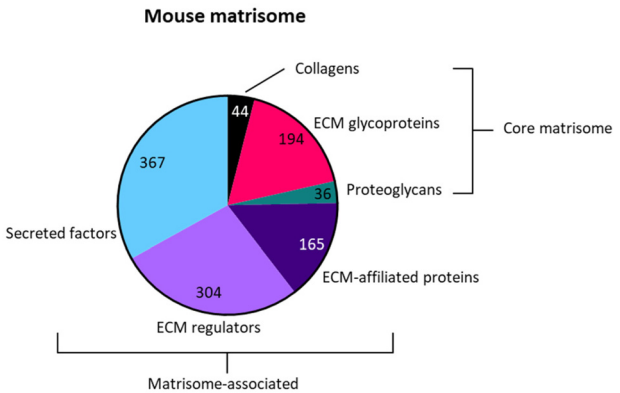

Fig. 5 Schematic representation of the subdivision of genes in the mouse matrisome.

Methods such as a colorimetric staining or ELISA allow for the basic quantification of GAGs in various sample types.[82] Methods that assess GAGs on the disaccharide level, *e.g.* RP-HPLC, are suited for identifying the various disaccharides but lose positional information in the chain. Raman spectroscopy may struggle to separate the signals of complex samples.[83] Mass spectrometry has made significant progress with novel ionisation techniques, but is not yet a standard method for GAG analysis.[84] Given the importance of GAGs in many biological processes, we performed an additional investigation into the presence of GAG related enzymes during the regenerative processes in axolotl and *Acomys*. Currently there are no methods available to directly assess GAG biosynthesis and modification. Instead, the expression of genes involved in GAG biosynthesis provides information on the regulation of GAGs during biological processes. A paper published in 2018 by Uijtdewilligen *et al.* provides a curated list of genes involved in proteoglycan/glycosaminoglycan metabolism,[85] dividing GAG-related genes into various categories based on their role in GAG homeostasis. The following four categories were selected for use in our comparison analysis: linkage region formation (8 genes), GAG chain polymerization (13 genes), GAG chain modification (32 genes), and GAG chain degradation (19 genes) (the complete gene list is available in Table S2†). Several of these genes were also present in the greater matrisome list as part of the ECM regulators. These were GAG chain modificators *sulfatase 1* and *sulfatase 2*, *heparanase* and *hyaluronidase 1*, *2* and *3*. The matrisome and GAG-related genes were extracted from each dataset. Any adaptions made to a dataset prior to gene extraction are covered in the results section for each publication. The next section focuses on the datasets that were compared.

5.3 Comparison of the matrisome and GAG components

The same biological processes (full-thickness skin wound regeneration or blastema-mediated regeneration) in *Acomys* and axolotl were compared at the transcriptome level. On the proteomic level this comparison could not be made due to absence of proteomics studies on blastema-mediated regeneration in *Acomys* and skin regeneration in axolotl. Although the processes are different, we propose that comparing skin regeneration and blastema on the proteomic level could still yield beneficial information. Thus, the matrisome and GAG-related genes of the following datasets were compared:

1. Transcriptomics of full-thickness skin wound regeneration in *Acomys* (Brant *et al.* 2019)[76] *versus* in axolotl (Monaghan *et al.* 2012).[79]

2. Transcriptomics of the (mammalian) blastema in *Acomys* (Gawriluk *et al.* 2016)[31] *versus* the (amphibian) blastema in axolotl (Stewart *et al.* 2013).[80]

3. Proteomics data of *Acomys* full-thickness skin wound healing (Yoon *et al.* 2020)[56,57] *versus* proteomics data of axolotl blastema-mediated limb regeneration (Rao *et al.* 2009).[63]

6. Results of the matrisome-GAG component analysis

In the following sections we describe the results of the matrisome and GAG gene comparisons. Each comparison is divided into three sections. Section 1 describes the preparation of the datasets in order to perform the comparison, section 2 highlights the matrisome components identified in the comparisons and section 3 focuses on the GAG-related components.

6.1. Comparison of the transcriptome profiles of regenerating full-thickness skin wounds on day 7 in *Acomys* (Brant *et al.* 2019)[76] *versus* axolotl (Monaghan *et al.* 2012)[79]

6.1.1 Preparation and matching of datasets. The work of Brant *et al.* (2019)[76] involved the transcriptomic profiling of full-thickness skin wounds with 8 mm diameter in *A. cahirinus*. Regenerating wounds at day 7 and 14 were compared to unwounded skin tissue (day 0). As the *Acomys* genome was unavailable at the time, these authors assembled a *de novo* transcriptome for *Acomys* which allowed the identification of differentially expressed genes expressed as fold changes compared to unwounded tissue with a p-value < 0.05. The data presented by the authors of Brant *et al.* (2019)[76] did not require any alterations and was ready for use. Monaghan *et al.* (2012)[79] studied the gene expression in full-thickness skin wounds of 4 mm diameter on axolotl flanks using *A. mexicanum* Affymetrix GeneChips (full microarray data can be found at the Gene Expression Omnibus (GSE37198)). Tissue was collected on day 0 (unwounded) and at days 1, 3 and 7 after wounding. The authors made a total of 16 comparisons. To identify differentially expressed genes on day 7, we ran our own expression analysis using the raw microarray data. Fold changes on day 7 (respective to unwounded tissue) were calculated using the software package Transcriptome Analysis Console (TAC 4). Significance was determined using Fisher's exact test for 2 × 2 contingency tables and genes with a p-value < 0.05 were identified (Table S3†).

The only matching timepoint in both publications was day 7 after skin injury. Descriptive studies on skin wound healing in *Acomys* and axolotl reported complete re-epithelialization within the first 3–5 days in both species.[46,79] Moreover, after wounding both animals exhibited a limited and low inflammatory response. After day 7 both animals start wound contraction, ECM deposition and new tissue formation.[67,86] Therefore, *Acomys* and axolotl should be in a similar regeneration stage on day 7 after wounding and a comparison at this time point may highlight the matrisome and GAG genes involved during early regeneration. Thus, only the datasets of day 7 in *Acomys* and axolotl were compared. First, differentially expressed matrisome and GAG-related genes were identified in *Acomys* (Table S4†) and axolotl (Table S5†). Finally, the differentially expressed matrisome and GAG-related genes that were present in both *Acomys* and axolotl were identified (Table S6†).

6.1.2 Expression of matrisome genes on day 7 of skin wound regeneration in *Acomys vs.* axolotl. The results of the matrisome matching process are visualized in Fig. 6. In *Acomys* 3921 genes were differentially expressed 7 days after skin wounding, of which 535 genes (13.6%) belonged to the matrisome. In axolotl 861 genes were differentially expressed on day 7 and 133 of these genes (15.5%) belonged to the matrisome. A total of 99 differentially expressed matrisome genes were identified between *Acomys* and axolotl.

A comparison of the **collagens** expressed in the two species revealed 11 matching genes. Among the top upregulated genes in axolotl were *Col11a1, Col7a1, Col28a1, Col4a5 and Col12a1.* In *Acomys*, the highest fold changes belonged to *Col12a1, Col24a1*, *Col5a2*, *Col5a1* and *Col7a1*. All genes were upregulated in axolotl but in *Acomys* four genes were downregulated: *Col4a5, Col4a6, Col17a1*, and *Col28a1*. Amongst the **glycoproteins** a total of 33 matches were found. The most upregulated genes in both species were tenascin C (*Tnc*), fibronectin (*Fn1*), laminin alpha 1 (*Lama1*), thrombospondin 1 and 2 (*Thbs1, Thbs2*), peroxidasin homolog (*Pxdn*) and collagen triple helix repeat containing 1 (*Cthrc1*). The genes with the most negative fold changes were adiponectin C1q collagen domain containing (*Adipoq*), spondin 2 (*Spon2*) and multimerin 1 (*Mmrn1*). Only four **proteoglycans** were present in both datasets, of which three were upregulated in both species: proteoglycan 4 (*Prg4*), serglycin (*Srgn*) and decorin (*Dcn*). Osteoglycin (*Ogn*) was downregulated in both axolotl and *Acomys*. Fifteen genes matched in the compartment of **ECM affiliated proteins**. Several of these genes were upregulated in both species, with the highest fold changes found in: C1q tumor necrosis factor related protein 1 (*C1qtnf1*), lectin galactose binding soluble 9 (*Lgals9*), syndecan 2 (*Sdc2*), complement component 1 q subcomponent B (*C1qb*), and C chain (*C1qc*). Only *C1qtnf2* was downregulated in both species. Notably, *Lgals8* and *Lgals2* were greatly downregulated in axolotl whereas their expression was barely affected in *Acomys*. Eleven **secreted factors** were identified in both datasets. Several genes were upregulated in both species, with the largest fold changes belonging to secreted frizzled-related protein 4 (*Sfrp4*), *Srfp2*, follistatin-like 1 (*Fstl1*) and angiopoietin-like 2 (*Angptl2*). Two genes were downregulated in both animals: chordin-like 1 (Chrdl1) and fibroblast growth factor 12 (*Fgf12*). A few notable differences in expression levels were present, the most notable being interleukin 1 beta (*Ilb*) which was the most upregulated gene in *Acomys* but showed a downregulation in axolotl. Among the **ECM regulators** 25 matches were found. The top upregulated genes in both species were matrix metallopeptidase 2 (*Mmp2*), *Mmp3*, *Mmp13*, procollagen lysine 2-oxoglutarate 5-dioxygenase 2 (*Plod2*), proprotein convertase subtilisin/kexin type 5 (*Pcsk5*), tissue inhibitor of metalloproteinase 1 (*Timp1*), peptidase domain containing associated with muscle regeneration 1

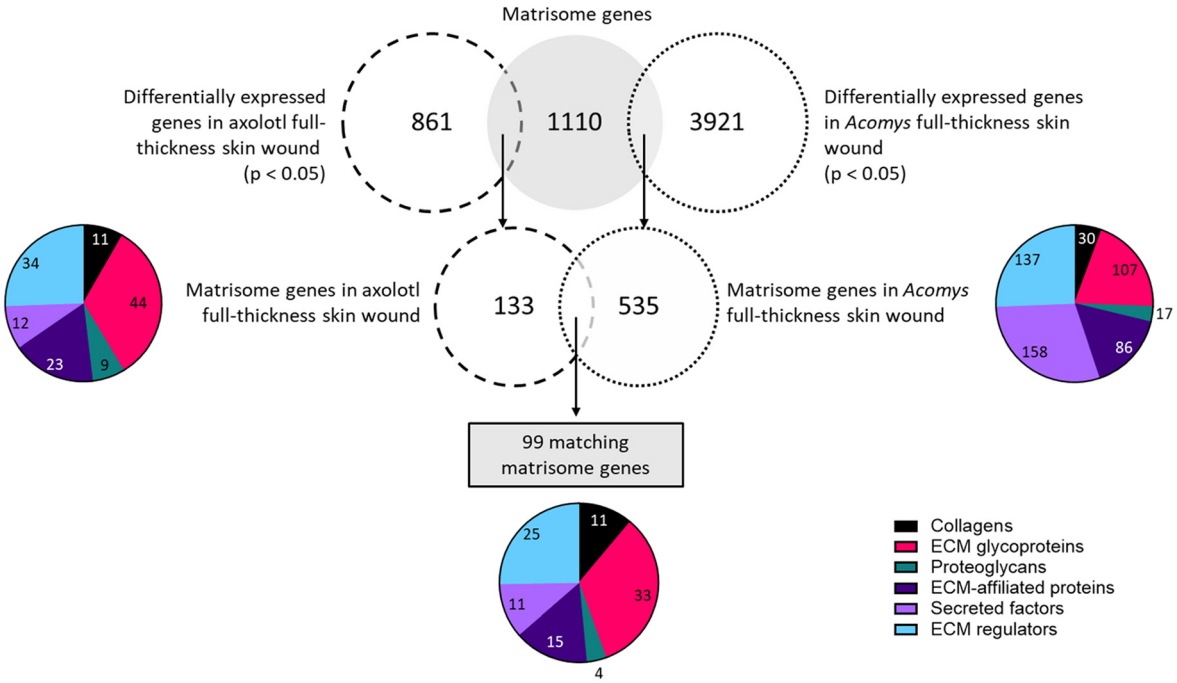

Fig. 6 Schematic representation of the identification of matrisome genes in full-thickness skin wounds. In axolotl 861 genes were differentially expressed as demonstrated by significant fold change ($p < 0.05$) on day 7 compared to day 0 (determined by our own analysis). Within this dataset 133 matrisome genes were identified. In *Acomys,* 3921 genes were differentially expressed on day 7 compared to day 0 ($p < 0.05$) and a total of 535 matrisome genes could be identified in this list. After comparing the matrisome genes of axolotl and *Acomys,* 99 matching matrisome genes were identified, representing all six matrisome categories.

(*Pamr1*) and lysyl oxidase-like 2 (*Loxl2*). Only one out of 25 genes was downregulated in axolotl: cathepsin S (*Ctss*). In *Acomys*, three out of 25 matching genes were downregulated: *Mmp28*, tolloid-like 1 (*Tll1*) and Kazal-type serine peptidase inhibitor domain 1 (*Kazald1*). Interestingly, *Kazald1* was the most upregulated gene in axolotl, but the most downregulated gene in *Acomys*.

In summary, of the 99 matrisome genes that were present in both species 77 genes (77%) had a similar expression pattern, where a gene was either upregulated (69 genes/70%) or downregulated (8 genes/8.1%) in both *Acomys* and axolotl. Twenty-two genes (22%) showed opposing expression patterns where a gene would be upregulated in one species but downregulated in the other species.

6.1.3 Description of GAG-related gene expression on day 7 of skin wound regeneration in axolotl *vs. Acomys*. Of all the differentially expressed genes in the axolotl dataset, only three GAG-related genes could be identified: these genes were all related to GAG chain modification. Conversely, all GAG gene categories were represented among the differentially expressed genes in the *Acomys* data, of which 30 genes were upregulated and five were downregulated.

In *Acomys*, two genes involved with **linkage region preparation** were present: beta-1,4-galactosyltransferase 7 (*B4galt7*) and *B4galt2*, both were upregulated. Eleven genes related to **GAG chain polymerization** were found in *Acomys*, with the highest fold changes seen in hyaluronan synthase 1 (*Has1*), *Has2* and *Has3*. Only two genes were downregulated: exostoses (multiple)-like 1 (*Extl1*) and *Extl3*. In axolotl, three genes relating to **GAG chain modification** were found, these were all upregulated: carbohydrate sulfotransferase 2 (*Chst2*), *Chst11* and sulfatase 1 (*Sulf1*). In *Acomys* a total of 11 genes matched in this compartment, among which were the three genes identified in axolotl. Together with *Sulf2* these were amongst the most upregulated genes in *Acomys*. Only three genes were downregulated: sulfatase modifying factor 1 and 2 (*Sumf1*, *Sumf2*) and carbohydrate sulfotransferase 15 (*Chst15*). Finally, 11 genes involved with **GAG chain degradation** were present in *Acomys*. The most highly upregulated genes were glucuronidase beta (*Gusb*), hexosaminidase A (*Hexa*), arylsulfatase B and J (*Arsb, Arsj*). No genes were downregulated.

6.2 Comparison of the transcriptome profiles during blastemal regeneration in *Acomys* ear pinnae closure (Gawriluk *et al.* 2016)[31] *vs.* axolotl stylopod amputation (Stewart *et al.* 2013)[80]

6.2.1 Preparation and matching of the datasets. A transcriptomic study on blastema-mediated ear pinna regeneration in *Acomys* was performed by Gawriluk *et al.* (2016).[31] Full-thickness ear wounds of 4 mm diameter at days 5, 10, 15 and 20 were compared to unwounded ear tissue and analyzed by RNAseq using a *de novo* transcriptome assembly. EBseq was used to determine differentially expressed genes (false discovery rate < 0.05). Differentially expressed genes that matched

with the matrisome and GAG-related genes were extracted and are presented in ESI Table S7.† In the study of Stewart *et al.* (2013)[80] the researchers amputated forelimbs of axolotls; tissue from the amputation site was collected at 0 (healthy tissue control), 3, 6, and 12 hours and 1, 3, 5, 7, 10, 14, 21 and 28 days. Samples were subjected to RNAseq transcriptome analysis and data was generated as gene abundance using transcripts per million (TPM). Using the raw data containing TPM, we identified matches to the matrisome and GAG-related genes, then calculated a fold change for day 5, 10 and 14 ([TPM day *X*]/[TPM day 0]). Values of 0 < fold change > 1 were further converted to a negative number: −1/fold change. As the original paper pooled three biological samples for analysis to generate only one transcriptome per time point, it was not possible to test the acquired fold changes for statistical significance. Instead, we applied a threshold of at least 2-fold up or down regulation, thus excluding −2 < fold change > 2 from the dataset (full data available in Table S8†). To be included in our selection, a gene was required to pass the threshold for at least one time point.

In this comparison we focused only on the blastema stage. In axolotl this stage lasts approximately from day 3–4 up to day 21.[71] A similar timeframe is described by Gawriluk *et al.* (2016)[31] when investigating *Acomys* ear blastema. For this reason, we compare days 5, 10 and 14 in axolotl to days 5, 10 and 15 in *Acomys*. Later time points were excluded as they are not in the blastema stage in axolotl. The full dataset containing all matching matrisome and GAG-related genes of both species genes is available in the ESI (Table S9†).

6.2.2 Description of matrisome related genes in ear pinna blastema in *Acomys* vs. forelimb blastema of axolotl. The results of the matching process are visualized in Fig. 7. There were 14 735 differentially expressed genes identified in *Acomys* ear pinna of which 638 genes (4.3%) were part of the matrisome. In axolotl, a total of 11 928 genes were expressed in the regenerating limb. Following matching to the matrisome and application of the fold change threshold, 324 genes (2.7%) were extracted. Between *Acomys* and axolotl 278 matching matrisome genes were found.

Twenty-one genes that code for **collagen** proteins were present in both species during various points in the blastema formation. Of note, only three genes were upregulated in both models at all time points: *Col18a1*, *Col6a2* and *Col3a1*. One gene was downregulated on all time points: *Col22a1*. The remaining collagen genes showed opposite expression patterns in each species. For example, *Col4a6* was upregulated at all timepoints in axolotl but downregulated at all time points in *Acomys*; the reverse pattern was observed for *Col4a1*. In total 77 **glycoproteins** were identified in both species. Of the 17 genes that were upregulated in both species at all time points the top-upregulated genes were: collagen triple helix repeat containing 1 (*Cthrc1*), laminin alpha 1 (*Lama1*), tenascin C (*Tnc*), transforming growth factor beta induced (*Tgfbi*), thrombospondin 2 (*Thbs2*), peroxidasin homolog (*Pxdn*), fibronectin 1 (*Fn1*), and elastin microfibril interfacer 1 (*Emilin1*). A total of 10 genes were downregulated in both axolotl and *Acomys*, with the lowest fold changes seen in: adiponectin, C1Q and col-

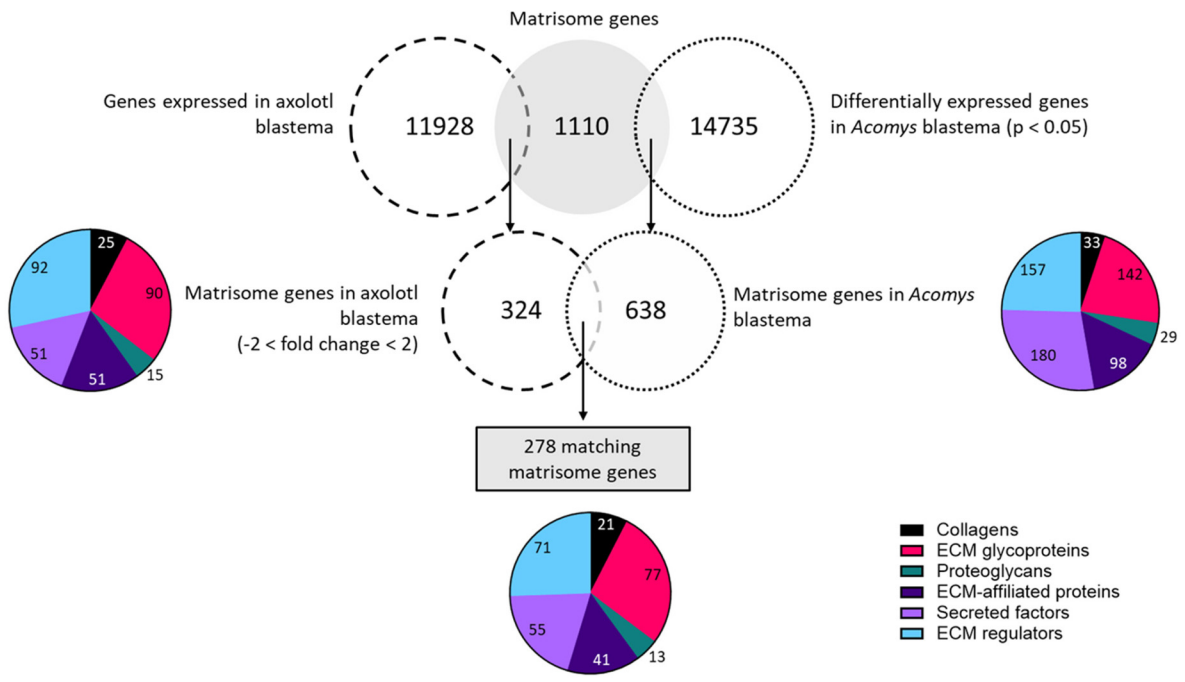

Fig. 7 Schematic representation of the identification of matrisome genes in axolotl limb blastema and *Acomys* ear blastema. In axolotl a total of 11 928 genes were expressed, of which 324 passed the threshold of −2 < fold change < 2 on day 5, 10 and 14 (differentially expressed genes could not be generated from this dataset). In *Acomys*, a total of 14 735 genes were differentially expressed ($p < 0.05$) in ear blastema. Among these were 638 matrisome genes. Between axolotl and *Acomys* 278 matching matrisome genes were identified and all matrisome categories were represented.

lagen domain containing (*Adipoq*), tenascin XB (*Tnxb*), leucine-rich repeat LGI family member 1 (*Lgi1*) and gliomedin (*Gldn*). One gene, Tenascin N (*Tnn*), displayed a similar expression profile in axolotl and *Acomys*. This gene was downregulated on day 5, but positive fold changes were observed on day 10 and day 14/15. The remaining genes showed differences in expression patterns such as matrilin 4 (*Matn4*), cartilage oligomeric matrix protein (*Comp*), Fraser syndrome 1 homolog (*Fras1*) and nephronectin (*Npnt*), all of which were upregulated in axolotl but downregulated in *Acomys*. In contrast, matrilin 2 (*Matn2*) and connective tissue growth factor (*Ctgf*) were upregulated in *Acomys* but downregulated in axolotl. Among the **proteoglycan** genes, 13 matches were identified. Three of these genes were upregulated at all time points in both animals: proteoglycan 4 (*Prg4*), versican (*Vcan*) and serglycin (*Srgn*). Similar expression profiles were seen in osteoglycin (*Ogn*), which was downregulated on day 5 and 10 in both animals, a positive fold change was seen on day 14 in axolotl and on day 15 in *Acomys*. A contradicting profile was observed for neurocan (*Ncan*): this gene was highly upregulated on day 5 in axolotl after which fold changes < 2 were seen, whereas in *Acomys Ncan* was absent on day 5 but displayed high fold changes on day 10 and 15. Among the **ECM-affiliated** matrisome 41 genes matched between axolotl and *Acomys.* A total of 15 genes were upregulated in both animals: C-type lectin domain family 4 member e (*Clec4e*), lectin galactose binding soluble 1 (*Lgals1*), complement component q subcomponent alpha polypeptide (*C1qa*), plexin D1 and C1 (*Plxnd1*, *Plxnc1*) and syndecan 1 (*Sdc1*). Only four genes were downregulated in both animals and during all timepoints, of which the most downregulated genes were: C-type lectin domain family 3 member a (*Clec3a*) and family 2 member d (*Clec2d*). The remaining genes in this compartment had opposing expression patterns. For example, Fras1 related extracellular matrix protein 2 (*Frem2*) was highly upregulated in axolotl but downregulated in *Acomys.* Surfactant associated protein C (*Sftpc*) was highly upregulated in *Acomys* but downregulated in axolotl. A total of 55 genes were found in the **secreted factors** subset. Seventeen genes were upregulated in both species at all timepoints, with the highest fold changes observed in chemokine C–X–C motif ligand 14 (*Cxcl14*), multiple EGF-like-domains 11 (*Megf11*), platelet-derived growth factor C polypeptide (*Pdgfc*), wingless-related MMTV integration site 5A (*Wnt5a*), sonic hedgehog (*Shh*), transforming growth factor beta 3 (*Tgfb3*) and secreted frizzled-related protein 2 (*Sfrp2*). Three genes were downregulated in all datasets: chemokine C–X–C motif ligand 12 (*Cxcl12*), follistatin (*Fst*) and bone morphogenic protein 5 (*Bmp5*). The remaining genes often showed opposing expression patterns. Heparin-binding EGF-like growth factor (*Hbegf*) and chemokine C–C motif ligand 5 (*Ccl5*), were downregulated in axolotl but upregulated in *Acomys*. The other way around: leptin (*Lep*) and macrophage stimulating 1 (*Mst1*) were upregulated in axolotl and downregulated in *Acomys*. Finally, 71 **regulator genes** matched between *Acomys* and axolotl of which 40 genes were upregulated at all timepoints in both datasets. The highest fold changes belonged to several matrix metallopeptidases (*Mmp3*, *Mmp9, Mmp12, Mmp13, Mmp19*), transglutaminase 3 E polypeptide (*Tgm3*), tolloid-like 2 (*Tll2*), tissue inhibitor of metalloproteinase 1 (*Timp1*), serine (or cysteine) peptidase inhibitor clade E member 1 (*Serpine1*), and cathepsin Z (*Ctsz*). Only one gene, tissue inhibitor of metalloproteinase 3 (*Timp3*), was downregulated in both animals across all days. Once again, the remaining genes displayed opposite expression patterns. This was the case for Kazal-type serine peptidase inhibitor domain 1 (*Kazald1*) and coagulation factor XIII A1 subunit (*F13a1*), which were among the most upregulated genes in axolotl but displayed downregulations in *Acomys*. The opposite was also seen: cathepsin H and S (*Ctsh, Ctss*) were downregulated in axolotl but upregulated in *Acomys*.

To summarize this comparison demonstrated that many matrisome genes are present in the blastema of both *Acomys* and axolotl. A total of 278 matching matrisome genes were identified and 115 of these genes (41%) showed similar expression patterns, being either upregulated (96 genes/35%) or downregulated (19 genes/7%) in both species.

6.2.3 Presence of GAG-related genes ear pinnae blastema in *Acomys* compared to forelimb blastema of axolotl. In the category of **linkage region preparation** three genes matched between the two models and all were upregulated across all timepoints. In axolotl the highest expression was observed for beta-1,4-galactosyltransferase (*B4galt7*), in *Acomys* the most upregulated gene was beta-1,4-galactosyltransferase 2 (*B4galt2*). The remaining beta-1,3-glucuronyltransferase 3 (*B3gat3*), was upregulated in axolotl on day 5 after which the fold changes did not exceed the threshold. In *Acomys* a slight upregulation was seen for all timepoints. In the compartment of genes related to glycosaminoglycan **chain polymerization** nine genes matched between species. The highest fold changes in all timepoints, for both species, were seen in chondroitin sulfate synthase 1 (*Chsy1*), hyaluronan synthase 2 (*Has2*) and exostatin-like glycosyltransferase 3 (*Extl3*). In axolotl only one gene was downregulated: hyaluronan synthase 1 (*Has1*) on day 14. In *Acomys* this gene was also downregulated on day 10 and 15. A total of eight genes related to glycosaminoglycan **chain modification** were identified. Three genes were always upregulated in both animals: carbohydrate sulfotransferase 2 (*Chst2*), sulfatase 1 (*Sulf1*) and carbohydrate sulfotransferase 11 (*Chst11*). No negative fold changes were observed in axolotl and in *Acomys* only sulfatase modifying factor (*Sumf2*) was consistently downregulated. Opposing expression patterns were observed in heparan sulfate glucosamine 3-O-sulfotransferase 3B1 (*Hs3st3b1*): this gene was upregulated in axolotl but downregulated in *Acomys*. Lastly, 10 genes in the compartment of glycosaminoglycan **chain degradation** were matched and these mostly displayed varying expression patterns. A single gene, glucuronidase beta (*Gusb*), was upregulated in axolotl and *Acomys* on all timepoints. No genes were consistently downregulated in both animals. Arylsulfatase family member K (*Arsk*) was downregulated across all days in *Acomys* but upregulated in Axolotl. In axolotl *N*-sulfoglucosamine sulfohydrolase (*Sgsh*) was downregulated on day 5.

6.3 Comparison of the proteomic profiles of skin regeneration in *Acomys* (Rao *et al.* 2009)[63] *vs.* blastema-mediated limb regeneration in axolotl (Yoon *et al.* 2020)[56,57]

In this section a comparison was performed of skin regeneration (absence of blastema) and limb regeneration (blastema mediated) on the proteomic level. The previous comparisons of transcriptome profiles each generated an extensive list of matrisome-related genes that were present in both species. However, up or downregulation on the transcriptional level does not necessarily translate to an increase or decrease in protein abundance, thus investigating regeneration at the proteomic level is important.

Comparing the matrisome-related proteins from the proteomics studies on *Acomys* skin regeneration (Yoon *et al.* 2020[56,57]) and axolotl limb regeneration (Rao *et al.* 2009[63]) revealed only four proteins that were present in both species. The data from Yoon does not provide a fold change for the variations in protein abundance. To be able to compare trends a simple fold change for the Yoon data was calculated: fold change = [protein QV on day *X*]/[protein QV on day 0]. Following this, values of 0 > fold change < 1 indicate a decrease in the protein and these values were converted to negative fold changes by calculating −1/fold change. Values of fold change >1 indicate an increase in protein abundance, but this difference could not be statistically tested for a differential increase or decrease. Data are represented in Table 4.

Of the matrisome **collagens**, only collagen type XII alpha 1 (*Col12a1*) and *Col1a1* were found in both species. In *Acomys* the abundance of *Col12a1* was lower on day 3 compared to day 0, but abundance had risen on day 5 and 7 as demonstrated by a positive fold change. On the other hand, *Col12a1* abundance was increased in axolotl on day 1 and 4 but protein levels dropped with a negative fold change observed on day 7. The protein form of *Col1a1* was again less abundant in *Acomys* on day 3, but the protein abundance increased with positive fold changes observed on day 5 and 7. In axolotl the abundance of *Col1a1* was always increased. Among the **ECM glycoproteins**, only fibrinogen beta and gamma chain (*Fgb* and *Fgg*) matched between the datasets. No negative fold changes were observed in either animal. In *Acomys* the protein abundance of both *Fgb* and *Fgg* remained steady over all days, whereas in axolotl the fold change for both *Fgb* and *Fgg* was highest on day 1 with a decrease in protein abundance over the following days.

To conclude, only four matching matrisome proteins were identified and no matches were found among the GAG specific lists. This could be the result of comparing two distinct biological processes. While the axolotl hind limb regeneration is mediated by the blastema, the process of full-thickness skin regeneration in the spiny mouse has not been hallmarked as a blastema-mediated process.[46] The blastema is a very specialized tissue, thus the proteins present during this process may not be present during skin regeneration in *Acomys*. An alternative explanation is that sample preparation methods resulted in biased protein sets in each species. A recent study that discusses methods to obtain ECM molecules from tissue samples states that the sample extraction method should be optimized to also obtain insoluble ECM,[87] which was not done for the publications of Yoon *et al.* (2020)[56,57] and Rao *et al.* (2009).[63] Proteins from *Acomys* tissue were extracted using a buffer made with Tris-Cl, NaCl, ethylenediaminetetraacetic acid (EDTA), phosphatase inhibitors and protease inhibitors. Soluble proteins were then separated from undissolved tissue using centrifugation. Tissue derived from axolotl was homogenized in a lysis buffer containing urea and dithiothreitol (DTT) followed by further peptide extraction from cell lysates using triethylphosphine, iodoethanol and finally trypsin digestion. To determine the engagement of the matrisome on the proteomics scale it will be necessary to conduct carefully designed experiments that are tailored to the analysis of ECM.

7. Discussion

Identifying key regulatory factors of regeneration is crucial to develop biomaterials that are capable of inducing regeneration in systems that respond to wounding with fibrosis. Research on *Acomys* demonstrates that regeneration in mammals is possible. Here, we focused on the extracellular matrix (ECM) by performing a direct comparison of the matrisome components present during two distinct regenerative processes in two regenerative species. These were blastema formation and skin wound healing in axolotl and *Acomys.* This approach

Table 4 Proteins identified in both axolotl and *Acomys*. Data represent a fold change compared to day 0

Collagens		*Acomys* (fold change)			Axolotl (fold change)		
Gene symbol	Prot. description	Day 3	Day 5	Day 7	Day 1	Day 4	Day 7
Col12a1	Collagen alpha-1(XII) chain	−1.29	1.90	1.80	1.32	1.05	−1.12
Col1a1	Collagen alpha-1(1) chain	−1.32	1.87	1.62	1.43	1.44	1.92
ECM glycoproteins		***Acomys*** **(fold change)**			**Axolotl (fold change)**		
Gene symbol	Prot. description	Day 3	Day 5	Day 7	Day 1	Day 4	Day 7
Fgb	Fibrinogen beta chain	1.83	1.97	2.10	3.39	1.63	1.14
Fgg	Fibrinogen gamma chain	1.13	1.21	1.36	4.64	2.17	1.24

identified the common ECM-related denominators in the regenerative wound healing processes that occur in both species. Neither axolotl nor *Acomys* can be directly compared to humans due to obvious differences in their (skin) biology. However, the identification of ECM-related factors that were present in both an amphibian and mammal support the existence of shared drivers of regeneration that exceed the differences in species. We propose that these matrisome factors play a vital role in regeneration and that specific matrisome factors should be incorporated into novel biomaterials to improve skin healing in non-regenerative species.[27,88]

Recent research provides evidence that regeneration can be induced using only a small selection of components. In axolotl the process of innervation is essential to achieve limb regeneration. In the absence of a nerve, the application of a combination of fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs) was enough to rescue limb regeneration.[89] Frogs (*Xenopus laevis*) lose the ability to replace amputated hind limbs through blastema after metamorphosis. Instead, adult individuals resolve amputated limbs through the formation of a cartilaginous spike. Murugan and colleagues reported on the ability of a functionalized device that induced hindlimb regeneration in adult *Xenopus leavis.*[90] A wearable dome-shaped bioreactor ('biodome') constructed of a silicone sleeve and containing a silk-based hydrogel loaded with five drugs was attached to the amputation site during the first 24 hours. Animals were followed for 18 months, during which functional hindlimbs regenerated that resembled wild-type hindlimbs. The five drugs in question had been selected for their individual pro-regenerative effects. 1,4-Dihydrophenonthrolin-4-ene-3 carboxylic acid (1,4-DPCA), an inhibitor of prolyl 4-hydroxylase, is an enzyme involved in collagen formation.[91] Brain-derived neurotrophic factor (BDNF) is associated with neuron development and axon regeneration.[92,93] Growth hormone (GH) has many functions and a recent study on the side effects of GH as an anti-aging therapeutic identified its role as an inhibitor of TGF-β1-mediated myofibroblast activation.[94] Resolvin D5, an oxidized lipid mediator, has anti-inflammatory properties by mediating immune cell behavior and recent work indicated this agent has a direct role during re-epithelialization.[95,96] Finally, retinoic acid (a vitamin A metabolite) has been found in regenerating zebrafish tissues[97] and it was shown to enhance the production of ECM components during wound healing.[98] In another study, full-thickness skin wounds of 12 mm diameter were inflicted on the backs of fetal sheep.[99] At this wound size and gestational age (day 79 of 140–147 days) fetal sheep are not able to regenerate the skin and instead a fibrotic scar forms.[33] However, the regeneration of full-thickness skin wounds in fetal sheep was induced using type I collagen scaffolds functionalized with heparin, vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2).[99] After postnatal analysis, there was an increase in the skin surface area, a reduction in myofibroblast numbers and evidence of hair follicle formation. In the regenerating ear tissue of *Acomys* sustained ERK activity was identified as a crucial pro-regenerative factor.[100] The application of ERK activators (FGF2 and neuregulin-1) to *Mus* ear punch wounds induced a pro-regenerative matrisome profile that was characterized by the presence of matrix metalloproteinase 9 and fibronectin 1 and in addition hair follicle neogenesis was stimulated. Together these studies emphasize the feasibility of driving a biological system towards regeneration using only a few selected components.

Before we elucidate on the matrisome components that were extracted in our study the limitations of our study should be addressed. A single study which compares differentially expressed genes in human partial-thickness skin wounds to axolotl full-thickness skin wounds, using pre-existing datasets, is available. This study was seeking for genes that were upregulated in humans and downregulated in axolotl, and *vice versa.*[101] This work identified genes involved in collagen formation, biosynthesis and modification, as well as ECM organization, ECM-receptor interactions and connective tissue development. To date, no transcriptomic or proteomic studies have been published regarding healing of adult human skin following a full-thickness skin wound. Despite the widely acknowledged caveats of extrapolating animal data to humans, comparing datasets obtained from regenerating animal models may be useful to gain knowledge that can be applied to human wound healing. We found a compelling overlap between the matrisome transcriptomes of axolotl and *Acomys.* This suggests there is a possibility of shared ECM/matrisome based regenerative mechanisms between amphibians and mammals that could potentially also be present, albeit inactive, in humans.[102,103] While there was a clear overlap found between the datasets, a direct comparison of numerical data was challenging due to differences in sample harvesting, experimental methodologies and data analyses. Each step from sample extraction to data normalization can affect the final dataset.[104–106] Therefore, the expression level of one gene cannot be directly compared between two datasets. For these reasons, our analysis will only show general trends of gene expression. Our approach also leaves out the effects of enzyme activity on protein synthesis, since an enzyme's gene expression does not need to be increased for the enzyme activity to be increased. An example of this may be found in the hyaluronan synthase genes, which were identified in only one comparison, even though hyaluronic acid is known to have positive effects on regeneration.[107] Second, different publications use different sets of temporal data points which did not always coincide. In particular, the early timepoints of blastema formation (day 0–4) have not been characterized in *Acomys*. Comparing only the later time points means early contributors to regeneration are left unexplored. Third, the age range of the animals used in the experiments should be briefly considered. The publications focusing on axolotl all used juvenile animals of 7–11 cm in length.[63,79,80] Axolotls reach sexual maturity after 9 to 12 months, when the animal is on average 15 cm long.[89,108] The publications on *Acomys* used animals of varying ages; 6 weeks – 6 months,[76] 6 months only,[31] or 'sexually mature'.[56,57] Both male and female *Acomys*

reach sexually maturity at 2–3 months of age,[44] thus the majority of the individuals in the experiments would have been mature. Comparing the life phases of axolotl and *Acomys* is negligible as axolotls naturally retain their juvenile characteristics even after obtaining sexual maturity. Lastly, the proteomic analyses of the incorporated papers are limited to the soluble fractions of the ECM. For example, only four proteins were found in the proteomics datasets and two of these (**fibrinogen beta (*Fgb*) and gamma chain (*Fgg*)**) are known soluble proteins. Fibrinogens have a well-known role in the healing response: after conversion to fibrin it assembles into a temporary support network. Fibrinogen-deficient mice have highlighted this role in wound stabilization and matrix organization.[102] Although many important ECM proteins are soluble, identification of insoluble components of the ECM remains elusive.

Our analysis found multiple matrisome related genes that had similar expression patterns in both models and these genes could therefore potentially be essential to regeneration. In this discussion we will focus solely on the genes that were upregulated in both skin regeneration and blastema, as these could be candidates to be included in pro-regenerative biomaterials.

Glycoproteins are essential molecules in the ECM and are known to be involved in wound healing.[109] The widespread functions of glycoproteins explain the presence of many highly upregulated glycoproteins during the regenerative processes. Glycoproteins that were highly upregulated in both axolotl and *Acomys* during skin regeneration and in the blastema were: **tenascin C, fibronectin, laminin alpha 1, thrombospondin 1 and 2, peroxidasin homolog and collagen triple helix repeat containing 1**. In general, tenascins are involved with cell adhesion modulation; thereby influencing cell proliferation and migration.[110,111] **Tenascin C** is activated after injury in axolotl and is deposited in the wound margins.[67] Due to its anti-adhesive behavior and promotion of a softer matrix,[112] Tenascin C is thought to lead to proliferation and migration of keratinocytes, fibroblasts, and endothelial cells.[113] Tenascin C has been indicated as a component of a "transitional matrix" in regeneration-competent species such as newts and zebrafish.[114–116] In humans, tenascin C is highly expressed during wound healing, inflammation and injury. A study using mice demonstrated that tenascin C polypeptides increased type I collagen expression, contributing to ECM strength in young skin.[117] **Fibronectin 1**, another component of the transitional matrix, was found in both *Acomys* and axolotl during skin regeneration and blastema-formation. Fibronectin is crosslinked to the fibrin matrix during hemostasis and enables fibroblast migration.[118] Mouse full-thickness skin wounds sealed with a combination of plasma fibrinogen and fibronectin demonstrated enhanced wound healing.[119] Similarly, pre-coating fibronectin on full-thickness skin wounds in mice before the application of autologous basal cells improved wound healing.[120] Furthermore, it has been shown that fibronectin regulates collagen assembly.[121,122] An in depth review on the role of fibronectin and its potential in regeneration was recently published.[123] **Laminin alpha 1**, a protein that interacts with fibronectin, was present and upregulated in all datasets. Laminin alpha 1 encodes the alpha I chain of the trimeric laminin protein and is a major component of the basement membrane, with an important role in re-epithelization and angiogenesis during wound healing.[124] Laminins are also important in growth factor regulation: the heparin-binding domains on laminin can bind growth factors and fibrin matrices functionalized with laminin improved wound healing in diabetic mice.[125] Another consistently upregulated gene during regeneration was the relatively unknown **peroxidasin homolog**. A study into the role of peroxidasin in the ECM demonstrated that human dermal fibroblasts treated with TGF-β1 to induce myofibroblasts secrete peroxidasin to the ECM where it may co-localize with fibronectin in thick bundles.[126] Peroxidasin may also contribute to ECM stabilization *via* tyrosine crosslinking of proteins[127] and it was identified as a catalyst in type IV collagen crosslinking through sulfilimine.[128,129] Inhibition of peroxidasin reduced endothelial cell attachment and growth, and without peroxidasin the organization of type IV collagen, fibronectin and laminin into fibrillar networks was diminished.[130] The potential role of peroxidasin in ECM formation coupled with its consistent presence in the regenerating matrix indicates this peroxidasin may be an important player in wound healing. The consistent presence of both fibrin(ogen) and fibronectin, as well as their associated proteins, demonstrates their importance during regeneration in regenerative and non-regenerative systems alike. **Thrombospondin 1**, a large trimeric glycoprotein secreted by a plethora of cell types, has been implicated in many (patho)physiological processes.[131] Researchers demonstrated that the inhibition of thrombospondin 1 with antisense oligonucleotides in full-thickness skin wounds of mice markedly impaired the wound healing process.[132] Recently thrombospondin 1 has been implicated in fibrillar collagen organization through its inhibitory effect on lysyl oxidase, which is an enzyme responsible for collagen crosslinking in the ECM.[133] Thrombospondin 1 also indirectly inhibited myofibroblast differentiation through its effects on the organization of collagenous ECM.[133] **Thrombospondin 2**, like thrombospondin 1, is involved in ECM organization and it is secreted primarily by fibroblasts and smooth muscle cells.[134] In thrombospondin 2 knock-out mice the skin displayed disorganized collagen fibers, contrasting the parallel orientated fibers found in wild-type mice.[135] Additionally, the researchers demonstrated that dermal fibroblasts obtained from these mice produced more matrix metalloproteinase 2, indicating thrombospondin 2 may affect cell–matrix interactions. Taken together, the increased expression levels of thrombospondin 1 and 2 during regeneration underline the impact of ECM organization. **Collagen triple helix repeat containing 1 (*Cthrc1*)** is a secreted glycosylated protein that is usually present in embryonic and neonatal tissues. *Cthrc1* has shown the ability to inhibit type I and III collagen synthesis.[136,137] In skin wounds, it has been localized at sites of collagen deposition and in myofibroblast clusters.[137] Moreover, *Cthrc1* regulates and inhibits TGF-β1 production

which may be a potential anti-fibrotic treatment in the skin.[138,139] The consistent upregulation of Cthrc1 during regeneration is indicative of a need for controlled collagen synthesis.

Several genes involved in matrix degradation were upregulated in all transcriptomics datasets: **matrix metalloproteinase 3**, **matrix metalloproteinase 13** and **tissue inhibitor of metallopeptidase 1** (***Timp1***). Matrix remodeling is essential in wound healing, as demonstrated by the total of 14 differentially expressed MMPs that were found during healing of human split-thickness skin wounds, which do not scar.[140] It has been described that **matrix metalloproteinase 3** degrades both type I and III collagen.[67] Research in regenerating axolotl digits showed that the presence of matrix metalloproteinases was essential to effective digit regeneration.[141] **Matrix metalloproteinase 13** is capable of cleaving collagen type I, II, and III and various other fibrillar ECM components, thereby modulating fibroblast-matrix interactions and it is even upregulated in fetal wounds.[142] ***Timp1*** can inhibit all matrix metalloproteinase family members.[143] It has been shown that increased levels of this gene were implicated as a poor outcome marker for increased fibrosis in burn patients and persistence of foot ulcers in diabetic patients.[144] On the other hand, a study performed in newts indicated that increased levels of *Timp1* during regeneration is required to maintain optimal concentrations of matrix metalloproteinases.[145]

Regarding proteoglycans associated with the matrisome, **proteoglycan 4** and **serglycin** were present and upregulated in all datasets. Proteoglycans consist of a core protein and one or more covalently attached glycosaminoglycan (GAG) side chains. They make up a major part of the ECM and have a range of purposes. **Proteoglycan 4**, also known as lubricin, contains the GAGs chondroitin sulfate and keratan sulfate. The biosynthesis of keratan sulfate was highly increased in the regenerating spinal cord of *Acomys* and the expression of one related biosynthesis gene, beta-1,3-N-acetylglucosaminyltransferase 7 (*B3gnt7*), was identified as an axon growth enhancer.[47] Proteoglycan 4 is more formally associated with blastema formation because of its role in cartilage-bone protection.[146] In humans, proteoglycan 4 is associated with the regeneration of cartilage tissue between joint surfaces.[147] A recent study elaborated on the anti-fibrotic properties of proteoglycan 4 by demonstrating the ability of this proteoglycan to decrease synovial fibroblast activation *in vitro* and reduce fibrosis *in vivo.*[148] Its presence during skin regeneration in both *Acomys* and axolotl underscores the importance of proteoglycan 4 to regeneration in general. This finding is supported by the results of Krawetz *et al.* (2022), who demonstrated proteoglycan 4 is essential in ear wound healing in mice.[149] They showed that proteoglycan 4 modulates macrophage polarization, increases vascularization and promotes cartilage regeneration. The proteoglycan **serglycin** was upregulated during both blastema and skin regeneration. Serglycin is a well-known intracellular proteoglycan and one of its main roles is the regulation of inflammatory mediators in the granules of mast cells.[150] After secretion serglycin may act as a vehicle for the extracellular delivery of the molecules inside granules, such as cytokines, or act as a scavenger in the ECM.[151] Thus, serglycin may have a role as an inflammation mediator. Moreover, serglycin is upregulated under the influence of TGF-β1 during epithelial–mesenchymal transition, this transition is an important process during wound healing.[152] Serglycin is densely packed with various GAGs, leading to a high density of GAG-binding proteins, and one of these associated GAGs is heparin.[151] Heparin is most commonly associated with anticoagulation and anti-inflammation and has widespread use in therapeutic applications.[153] For example, treatment of chronic ulcers with low molecular weight heparin resulted in increased numbers of healed patients and reduced wound recurrence rates.[154,155] These findings illustrate the beneficial effects of heparin on the healing of chronic wounds.

Considering the upregulation of various proteoglycans, it is to be expected that several genes related to the linkage and modification of GAG chains were also upregulated during regeneration: **carbohydrate sulfotransferase 2**, **carbohydrate sulfotransferase 11** and **sulfatase 1**. Carbohydrate sulfotransferases are responsible for the transfer of sulfate groups onto carbohydrates. Our analysis returned two carbohydrate sulfotransferase genes that were always upregulated during regeneration. **Carbohydrate sulfotransferase 2**, also known as *N*-acetylglucosamine 6-*O*-sulfotransferase or GlcNAc6ST1, is associated with the biosynthesis of keratan sulfate.[156] This enzyme may play a direct role in the inflammatory response as it is a part of L-selectin ligand synthesis: these ligands are responsible for leukocyte rolling and capture in high endothelial venules.[157,158] **Carbohydrate sulfotransferase 11**, or chondroitin-4-sulfotransferase 1, is an enzyme involved in the biosynthesis of chondroitin sulfate.[159] In Costello syndrome, where overexpression of the HRAS oncogene leads to a reduction in carbohydrate sulfotransferase 11, skin fibroblasts do not produce elastic fibers. Forced expression of carbohydrate sulfotransferase 11 rescued the elastic fiber production.[160] Chondroitin sulfate itself was shown to improve palatal wound healing by promoting fibroblast adhesion and proliferation.[161] **Sulfatase 1** is an extracellular enzyme that removes 6-*O*-sulfates from heparan sulfate and augments Wnt/β-catenin signaling. Following wounding of the corneal epithelium in mice sulfatase 1 was found in the wound edges and knockdown of sulfatase 1 led to a decrease in cell migration and delayed wound healing.[162] Similarly, during hind limb regeneration in *Xenopus laevis* high levels of sulfatase 1 were present during the first three days in the blastema, which demonstrates its the importance in regeneration.[163] Moreover, it was found that sulfatase 1 is upregulated during mouse embryonic skin development and is likely involved in cellular signaling.[85] Taken together, the three enzymes that were found in the datasets regulate sulfation patterns of GAGs, which in turn modulates the bioactivity. The GAG heparan sulfate (HS) binds many proteins, such as growth factors, that reside in the ECM and the interaction of HS-binding proteins and receptors is modulated by HS proteoglycans.[164,165] Changes in sulfation patterns of GAGs may thus have far-reaching effects in modulating cell behavior.

Lastly, two collagens were identified in the proteomics datasets. These collagens, **type I collagen type alpha 1 chain** and **type XII collagen alpha 1 chain**, are important during regeneration albeit in different ways. As part of the core matrisome, collagens have a great influence on the overall structure of the ECM and cell behavior. **Type I collagen**, a member of the fibrillar collagens, contributes to over 90% of the collagen content in skin ECM.[166] Scar tissue consists of a dense collagenous matrix organized in thick parallel orientated bundles, in contrast to healthy dermis where the collagen is organized into a basket weave-like pattern.[7] In regenerating *Acomys* skin, type I collagen was loosely organized and less abundant as shown through trichrome staining, resembling undamaged skin.[51] To achieve skin regeneration, the architecture of type I collagen should match that of unwounded skin, which requires guiding the deposition and remodeling of type I collagen during wound healing. **Type XII collagen** is a member of the Fibril Associated Collagens with Interrupted Triple helices (FACIT). This collagen has binding sites for type I collagen, to assist in type I collagen packing, and GAGs (such as heparan sulfate).[167,168] Additionally, type XII collagen helps to maintain the structure of the ECM and responds to mechanical stretch by absorbing stress. Type XII collagen is expressed in the dermis during skin development in humans, however, after birth it is found only in the papillary dermis and surrounding hair follicles.[169] Following skin injury, the scarring *Mus musculus* displays significant increases in type XII collagen compared to *Acomys*, which indicates that an abundance of type XII collagen could also be detrimental to a regenerative matrix.[51,55] Similarly, timing the deposition of type I and XII collagen has shown to play a key role in skin regeneration in the axolotl.[103]

In summary, our analysis of matrisome factors revealed several genes that are upregulated during regeneration in both axolotl and *Acomys*. In some cases, they encode proteins which are known to have a positive influence on wound healing in mammals or are in some way related to the structural organization of the ECM. The fact that fibrotic skin is characterized by the thick parallel organization of type I collagen while in undamaged skin a basket-weave pattern is seen suggests that a regenerative biomaterial should focus on re-establishing the normal ECM architecture and organization (Fig. 8).

Several of the components identified in our analysis may be considered for use in a regenerative biomaterial. The incorporation of **fibronectin** in a biomaterial would aid in the regulation of collagen fiber assembly. Several other components can be considered to strengthen the matrix organizing properties, such as **thrombospondin 1 and/or 2**, as well as **peroxidasin homolog**. Growth factors are an integral part of regeneration, and their capture should be facilitated. This may be achieved through the incorporation of molecules that have growth factor binding abilities, such as **laminin alpha 1**, or various **proteoglycans** which may bind growth factors through their GAG chains. The glycoprotein **tenascin c** is known for its positive influence on wound healing by enhancing cell migration. Its constant presence in our datasets supports the inclusion of tenascin c in a pro-regenerative biomaterial. The proteoglycans **serglycin** and **proteoglycan 4** were an unexpected finding in our dataset, as their exact roles in skin wound healing are not well known. This warrants a more detailed investigation into the influence of the various proteoglycans on wound healing to determine which would be the most appropriate for incorporation in a pro-regenerative scaffold. Proteoglycan-associated GAGs, such as heparin in serglycin and chondroitin sulfate or keratan sulfate in proteoglycan 4, have shown potential in promoting wound healing and should be considered for incorporation into the pro-regenerative biomaterial. Phan *et al.* (2021) showed that heparan sulfates, together with fibroblast growth factor signaling, are required to provide the correct positional information during axolotl limb regeneration.[170]

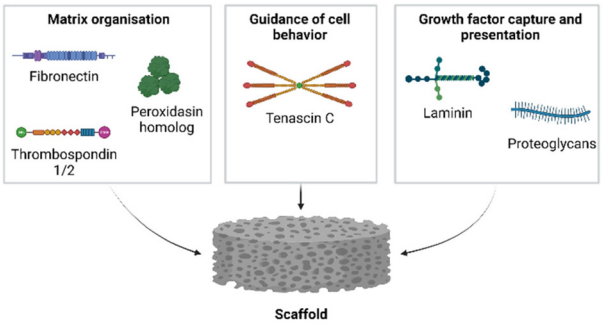

Fig. 8 Components to be considered for incorporation in pro-regenerative biomaterials. Created with BioRender.com.

There is a myriad of options available for the development and production of pro-regenerative biomaterials. Such materials may contain natural or synthetic components, or a combination of both. The application of natural-derived components is widespread. For example, decellularized tissues maintain the native tissue architecture and many of the signaling factors.[171,172] A wide range of decellularized skin substitutes from human and animal sources are commercially available and have been used in clinical practice.[173] Decellularized human nail beds have been used to stimulate bone regeneration in rats.[174,175] These examples establish the ability of the native ECM to promote regenerative processes. In addition to naturally derived components, chemically or enzymatically produced components may be considered. Standardized processes greatly decrease variations between batches and the low immunogenicity makes synthetic compounds an interesting alternative.[176] Besides the incorporation of proteinaceous compounds in biomaterials, carbohydrates – and GAGs in particular – encompass a class of molecules with diverse biological functions. Unlike proteins, they are more difficult to analyze due to the non-template driven biosynthesis and extensive structural heterogeneity of these long polysaccharides. In the context of biomaterial development their multitude of functions make them important to consider. GAGs are known for their role in organizing the extracellular matrix and for their ability to bind and present bioactive molecules, protecting

these from proteolytic degradation, and regulating growth factor gradients.[177] Many examples of the exploitation of such functions can be found in the literature, from hyaluronan injectables[178] to collagen-based skin constructs containing dermatan sulfate and heparin.[179] However, one GAG may have various domains in the sulfation pattern and its structure can vary during development and (patho)physiology. To avoid the use of heterogenous natural GAGs with potential batch-to-batch variations, novel strategies have been developed to obtain more defined preparations. One approach includes the use of degradation-resistant carboxymethyl-dextran sulfates: these heparan sulfate mimetics are capable of binding and protecting growth factors already present in the wound matrix, thereby allowing the tissue to regenerate.[180] These ReGeneraTing Agents (RGTA®) have shown great potential during *in vivo* studies and were clinically used to treat ulcers.[181,182] The basic research into defined saccharides offers an original approach to their application in biomaterials.[183] In addition, chemo-enzymatic synthesis of well-characterized oligosaccharides with specific biological functions opens a whole new avenue in the field of regenerative medicine.[184–186]

Future efforts should be directed towards functionality-based studies in order to elucidate the function of the matrisome components identified here in the regenerating matrix. This may be aided by improving the proteomic analysis of insoluble matrisome components. For example, the insoluble ECM pellet can be digested to allow proteomic analysis of insoluble matrisome components.[87] This can be achieved by digesting insoluble ECM pellets with hydroxylamine[187] or with a photocleavable linker such as 4-hexylphenylazosulfonate.[188,189] Both approaches yield a solubilized ECM fraction compatible with proteomic analysis pipelines and are relatively easy to implement. Efforts are already being made to improve the proteomic coverage of the matrisome. A universal tool titled 'MatrisomeDB' is available to all researchers, and it facilitates the reuse of proteomic datasets with a focus on the matrisome.[190] To obtain robust insights in the trends in gene and protein expression patterns observed in both species, it would require a united effort to analyze all data *via* identical methods and with matching timepoints in the regenerative process. Furthermore, the investigation of early timepoints in *Acomys* is highly recommended to provide a detailed image of the processes that occur during the first phases of regeneration.

8. Conclusions

With the increasing knowledge on the complexity of regenerative environments, the next generation of biomaterials should focus on the rational incorporation of various pro-regenerative matrix molecules. By applying such knowledge to the development of biomaterials we open new avenues towards improving wound healing and obtaining regeneration in humans.

Author contributions

Conceptualization, T. H. v. K. and W. F. D.; formal analysis N. A. M. and M. G.; methodology, N. A. M. and M. G.; funding acquisition, W. F. D.; project administration, W. F. D.; supervision, W. F. D. and T. H. v. K.; visualization, M. G. and N. A. M.; writing – original draft, N. A. M. and M. G., J. V.; writing – review and editing, W. F. D., T. H. v. K., G. T., M. V., I. M. A., H. J., J. V., M. G. and N. A. M.

Conflicts of interest

There are no conflicts to declare.

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