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MEETING REVIEW

Embracing the diversity of model systems to deconstruct the basis of regeneration and tissue repair

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ABSTRACT

The eighth EMBO conference in the series 'The Molecular and Cellular Basis of Regeneration and Tissue Repair' took place in Barcelona (Spain) in September 2022. A total of 173 researchers from across the globe shared their latest advances in deciphering the molecular and cellular basis of wound healing, tissue repair and regeneration, as well as their implications for future clinical applications. The conference showcased an ever-expanding diversity of model organisms used to identify mechanisms that promote regeneration. Over 25 species were discussed, ranging from invertebrates to humans. Here, we provide an overview of the exciting topics presented at the conference, highlighting novel discoveries in regeneration and perspectives for regenerative medicine.

KEY WORDS: Regeneration, Wound healing, Repair, Stem cells, Cellular plasticity

Introduction

A total of 173 researchers from around the globe gathered in Barcelona (Spain) in September 2022 to share advances in the understanding of tissue regeneration and repair at the eighth EMBO conference on 'The Molecular and Cellular Basis of Regeneration and Repair'. The meeting was organised by Catherina Becker (Dresden, Germany), Eric Röttinger (Nice, France), Maximina Yun (Dresden, Germany), Kenneth Poss (Durham, USA) and Yuval Rinkevich (München, Germany). The spirit of this meeting series has traditionally been to bring together researchers who use diverse model systems to share advances in identifying mechanisms governing regeneration and repair. The work presented in Barcelona covered an impressive suite of models across the animal kingdom ranging from highly regenerative organisms capable of whole-body regeneration (e.g. Cnidaria and Planaria) to less regenerative mammalian systems including mouse and human (Fig. 1). The breadth of systems lies at the heart of what makes this meeting unique and enables the elucidation of mechanisms underlying the regeneration of complex structures lost due to injury or disease. This is fundamental to the development of therapeutic strategies for regenerative medicine. Here, we present a collection of research advances shared at this vibrant meeting.

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Emerging model systems to study regeneration

In order to deconstruct the complexity of regeneration and its evolution, several emerging model systems were highlighted at the meeting, including species from *Spongia*, *Cnidaria*, *Acoelomorpha* and *Lophotrochozoa*. The sponge *Suberites domuncula* is becoming a useful genetic model to dissect mechanisms governing stem cell dynamics, regeneration and the ancestral function of evolutionarily conserved genes (Revilla-I-Domingo et al., 2018). Roger Revilla-I-Domingo (Vienna, Austria) reported on the identification of intermediate cell state trajectories of totipotent stem cells in whole-body regeneration of *S. domuncula* and suggested that Myc is an ancestral transcription factor controlling this transition. Moreover, he highlighted evolutionarily conserved gene regulatory networks (GRNs) controlling stem cell differentiation. Aldine Amiel (Nice, France) has identified two potential stem cell populations involved in whole-body regeneration of the Cnidaria Anthozoa *Nematostella vectensis*: fast cycling cells and quiescent label-retaining cells. She has been characterising their anatomical location, molecular signature, fate and cell-cycle re-entry during regeneration (Amiel et al., 2019 preprint). She also showed that a specific structure, the mesenteries, are essential for regeneration initiation. The acoel *Hofstenia miamia* (three-banded panther worm) and the flatworm *Macrostomum lignano* are also emerging as central models to elucidate the evolution of GRNs driving regeneration. Mansi Srivastava (Boston, USA) showed single-cell transcriptomic data revealing the transitional cell states of the pluripotent stem cells (neoblasts) underlying regeneration in *H. miamia* and has identified a distinct subset of cells that could represent a more undifferentiated state, in contrast to results from planarians (Hulett et al., 2022 preprint; Kimura et al., 2022). Eugen Berezikov (Groningen, Netherlands) highlighted *M. lignano* as an attractive experimental flatworm model for defining the differentiation of neoblasts during whole-body regeneration (Wudarski et al., 2020). Alejandro Sánchez Alvarado (Kansas City, USA) presented the adult snail *Pomacea canaliculata* as a system to study the regeneration of complex structures because their eyes can regenerate within 2 weeks. Finally, Natalie Grace Schulz (Chicago, USA) has been studying appendage regeneration in another mollusca species, *Octopus bimaculoides*. She described that complete regeneration of the octopus arm occurs through the formation of a blastemal structure and identified potential regeneration-specific genes.

Advances in understanding whole-body regeneration

Brigitte Galliot (Geneva, Switzerland) examined the apoptosis-Wnt-axis, which has previously been shown to control head regeneration in the freshwater *Hydra* polyp (Chera et al., 2009). In addition to the head activator Wnt3, she has identified the transcription factor Sp5 as a head inhibitor; Sp5 prevents the growth of additional heads during regeneration by repressing *wnt3* expression and Wnt pathway activation (Vogg et al., 2019). She has

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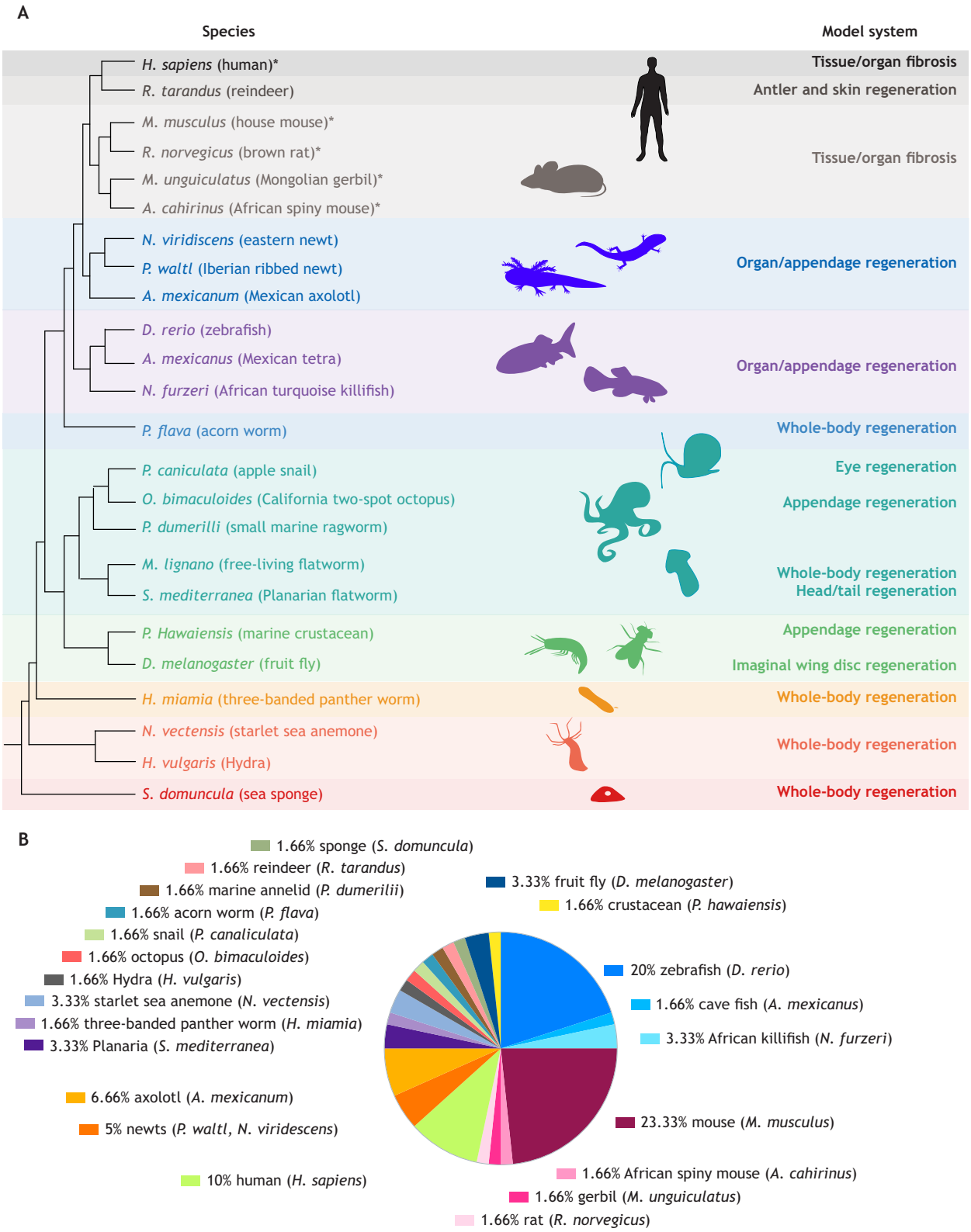

Fig. 1. Diversity of model systems presented at the eighth EMBO conference on 'The Molecular and Cellular Basis of Regeneration and Tissue Repair'. (A) Simplified phylogenetic tree depicting the breadth of animal model systems. (B) A breakdown of the percentages of talks with each model.

also identified a Wnt-controlled GRN that controls a transcriptional switch of cell identity during homeostasis and regeneration (Vogg et al., 2022). Alejandro Sánchez Alvarado (Kansas City, USA) addressed the molecular basis of adult tissue segmentation in the planarian flatworm *Schmidtea mediterranea*. He has defined molecular regulators of fission plane establishment and characterised transcriptional plasticity at a single-cell resolution in response to injury (Benham-Pyle et al., 2021). Also in planaria,

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Jakke Neiro (Oxford, UK) presented the identification of enhancer-like elements that allowed the prediction of active GRNs in neoblasts (Neiro et al., 2022).

Does regeneration re-deploy developmental programmes?

If and how developmental and regenerative programmes overlap is a long-lasting question in the regeneration field. Jessica Lehoczky (Boston, USA) showed that, despite being re-expressed during mouse digit tip regeneration, the developmental dorsoventral (DV) patterning genes *En1* and *Lmx1b* do not define the DV axis during regeneration (Johnson et al., 2022). Michalis Averof (Lyon, France) reported that, although original and regenerated legs are anatomically identical in the crustacean *Parahyle hawaiensis*, the transcriptional dynamics differ between development and regeneration (Almazán et al., 2022; Sinigaglia et al., 2022). Finally, Eric Röttinger (Nice, France) showed that developmental GRNs are partially redeployed during whole-body regeneration in the Cnidarian *Nematostella vectensis*, and that embryonic gene modules are rewired during regeneration. These modules are also interconnected with genes that display regeneration-specific expression dynamics (Johnston et al., 2021 preprint).

Turning back the clock: new insights into mechanisms of aging

The short-lived killifish displays a sharp decline in regenerative capacity upon aging, similar to mammals. Steven Bergmans (Leuven, Belgium) showed that optic nerve regeneration is complete in young adult animals, but partial or incomplete in aged killifish (Vanhunsel et al., 2022). He reported that changes in neuron-intrinsic and -extrinsic environmental factors, including the immune response and scarring, may underlie the reduced outgrowth and survival capacity of retinal ganglion cells in aged fish. Bergmans concluded that killifish resemble aged adult mammals and thus offer a model system to identify regulators of neuroregeneration. Ana Martin-Villalba (Heidelberg, Germany) has been exploring the mechanisms controlling stemness in the brain during aging, injury, and in disease. She presented recent findings from mice showing that quiescent neural stem cells (NSCs) and astrocytes share a similar transcriptome but differ in their methylome, which leads to silencing of neurogenic genes. Intriguingly, the stemness-methylome in astrocytes can be unlocked to enable generation of neuroblasts, offering a new direction to repair the diseased nervous system (Kremer et al., 2022 preprint). Moreover, she reported that interferon signalling controls stemness of NSCs, and that its manipulation in the aged but not young brain may be a potential target to improve stem cell homeostasis and repair (Carvajal Ibañez et al., 2023). During aging, senescent cells accumulate in tissues and contribute to the development of age-related diseases; however, cellular senescence can also play beneficial roles during development and tissue repair (Di Micco et al., 2021). Andy Yu (Dresden, Germany) showed that senescent cells in the regenerating Mexican axolotl (*Ambystoma mexicanum*) limb blastema mediate neighbouring progenitor cell expansion through non-cell autonomous Wnt signalling and are required for regeneration (Yu et al., 2022 preprint).

Bridging the gap: elucidating mechanisms driving regeneration of the nervous system

Certain species (including zebrafish and salamanders) possess an impressive capacity to regenerate the nervous system, which largely fails in humans (Joven et al., 2019; Lange and Brand, 2020). Hearing impairment is primarily caused by permanent loss of the mechanosensory receptors in the inner ear and their associated neurons. Unlike in humans, neurons regenerate well in the inner ear and lateral line organ in the zebrafish. Tatjana Piotrowksi (Kansas City, USA) showed that hair cell regeneration is enabled by the sequential activation of three distinct gene modules (Baek et al., 2022) and that injury-activated macrophages promote synaptogenesis during regenerative neurogenesis (Denans et al., 2022). Shawn Burgess (Bethesda, USA) presented work defining a GRN of Sox and Six transcription factors that directs cell identity during regeneration of the adult zebrafish inner ear (Jimenez et al., 2022).

Zebrafish can regenerate extensive damage to the CNS, including the forebrain (telencephalon), which involves injury-induced neurogenesis from resident progenitor cells, called ependymo-radial glia (ERG) (Becker and Becker, 2022). Using single-cell transcriptomics, Katharina Lust (Vienna, Austria) showed that the telencephalon of the axolotl also regenerates through neurogenesis from proliferating ERGs. Furthermore, regenerated neurons appear to re-establish afferent and efferent projections (Lust et al., 2022). Integration of new neurons into the existing circuitry is a prerequisite for recovery of function after CNS injury. Using vision-dependent social preference tests, quantitative optokinetic response assays and genetically encoded calcium sensors, Michael Brand (Dresden, Germany) provided strong evidence that retina regeneration in the zebrafish is functional on synaptic levels, which leads to an impressive recovery of monochromatic and colour vision to wild-type levels (Hammer et al., 2021). Catherina Becker (Dresden, Germany) has been exploring cellular interactions of the innate immune system with ERGs to activate regenerative neurogenesis in the zebrafish spinal cord. Upon injury, ERGs read Tumor necrosis factor alpha (TNFα), a cytokine derived from infiltrating macrophages, to promote regeneration of lost neurons via a Histone deacetylase 1 (Hdac1)-dependent mechanism (Cavone et al., 2021). This signalling mechanism is thought to be regeneration-specific because it has not been reported in the developing spinal cord. The development of therapeutic approaches targeting spinal cord ependymal cells inherently requires the existence of these cells in humans. Jean-Philippe Hugnot (Montpellier, France) showed that FOXJ+ PAX6+ SOX2+ ependymal cells, which exhibit stem cell-like properties in mice, persist throughout life in the human spinal cord. He concluded that this may open an opportunity to regenerate the spinal cord (Ripoll et al., 2022 preprint).

Zebrafish are not only capable of injury-induced neurogenesis but also show a remarkable capacity to regrow axonal connections after spinal cord injury (Tsata and Wehner, 2021). Valentina Cigliola (Durham, USA) reported on the identification of the Heparin-binding EGF-like growth factor (Hb-egf) as a molecular cue promoting axonal growth and neurogenesis in the zebrafish spinal cord. Interestingly, an enhancer element of the zebrafish *hb-egf* gene is also active in neonatal mice, which possess an elevated regenerative capacity for the CNS, but this enhancer is unresponsive in adult animals. A major factor limiting axonal regeneration in the mammalian spinal cord is the formation of fibrous scar tissue, which comprises excessive extracellular matrix (ECM) deposits. Daniel Wehner (Erlangen, Germany) showed that axon regeneration in the zebrafish is facilitated by a favourable composition of the injury ECM that is deprived of inhibitory factors. Moreover, he reported on the identification of ECM components that contribute to the differential capacity of mammalian and zebrafish axons to regrow across CNS lesions (Tsata et al., 2021; Kolb et al., 2022 preprint). In mammals, fibrous scarring is

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considered a roadblock to regeneration, yet ECM deposition is crucial for injury repair because interference with connective tissue formation prevents the sealing of wounds (Göritz et al., 2011). By tagging pre-existing ECM around the mouse mesothelium, Yuval Rinkevich (Munich, Germany) demonstrated that wound healing progresses by an early fibroblast and neutrophil-mediated transferral of pre-existing connective tissue in several mouse injury models (Fischer et al., 2022). These findings offer a window to potentially control scarring during wound repair.

Getting to the 'heart' of regeneration

Unlike humans, zebrafish and salamanders retain the ability to regenerate their heart after resection or cryoinjury, which model myocardial infarction (Ross Stewart et al., 2022). As heart regeneration in these systems occurs mainly via the proliferation of cardiomyocytes (CM), stimulating their proliferation has become a focal point of the field. Kazu Kikuchi (Osaka, Japan) showed that the transcription factor *klf1* is required for CM proliferation in the regenerating zebrafish heart (Sugimoto et al., 2017; Ogawa et al., 2021). Cardiac-specific knockout of *klf1* impairs CM dedifferentiation and proliferation, whereas cardiac-specific *klf1* overexpression causes hyperplasia even in uninjured hearts. Notably, he demonstrated that adenoviral delivery of *klf1* to adult mouse hearts post-infarction boosts CM proliferation, reduces scar size and improves functional recovery. Rita Alonaizon (Oxford, UK) presented her findings comparing injury-responses between regenerative surface and non-regenerative cave-dwelling morphs of *Astyanax mexicanus* using single-cell transcriptomics. She reported that surface fish exhibit higher levels of glucose metabolism than cave fish and blocking glucose metabolism inhibited heart regeneration in surface fish. As increased fatty acid oxidation dependency correlates with reduced heart repair clinically, she highlighted that understanding the metabolic changes required to boost regeneration will be key going forward. Olaf Bergmann (Dresden, Germany) presented on the development and use of a human induced pluripotent stem cell (iPSC)-derived CM proliferation screening platform to identify CM proliferation-inducing compounds. His group identified the alpha-adrenergic receptor agonist clonidine as a promising candidate that can increase proliferation in human iPSC-derived CM *in vitro* and cell cycle activity in neonatal mice *in vivo* (Murganti et al., 2022). Although CM are thought to be the main contributors to heart regeneration, Elif Eroglu (Stockholm, Sweden) uncovered the contribution of CLDN6+ epicardial cells to heart regeneration in the newt (*Pleurodeles waltl*) (Eroglu et al., 2022). She showed that tight junctions mediate epicardial activation and subsequent differentiation into CM. Eroglu posited that targeting the epicardium may be a strategy to enhance heart repair in patients.

Moving towards a deeper mechanistic understanding of musculoskeletal regeneration

Musculoskeletal diseases and injuries are prevalent and encompass a broad range of tissues including muscle, bone and connective tissues, such as tendons. Intramuscular adipose tissue formation is correlated with decreased muscle function in aging and disease. Daniel Kopinke (Gainesville, USA) demonstrated that fibro-adipogenic progenitors (FAPs) give rise to intramuscular fat (IMAT) following muscle injury. Desert hedgehog (Dhh) secreted from endothelial and Schwann cells inhibits FAP differentiation into IMAT (Kopinke et al., 2017; Norris et al., 2022 preprint). Dhh knockout mice exhibit decreased IMAT formation and impaired muscle regeneration. Intriguingly, they also display enhanced IMAT formation following sciatic nerve injury. Thus, Hedgehog signalling may be an effective target to control IMAT formation in neuromuscular diseases characterised by pathological IMAT infiltration. Andras Simon (Stockholm, Sweden) has been examining miRNA-mediated regulation of muscle dedifferentiation and blastemal cell formation in newt limb regeneration and highlighted the unique positioning of miRNAs throughout the newt genome (Elewa et al., 2017; Brown et al., 2022 preprint). He showed that cellular dedifferentiation and blastema formation are coupled with decreased transcription/translation, and that miR-10b modulates translational recovery in dedifferentiating cells by controlling ribosomal gene expression.

Stefano Di Talia (Durham, USA) has been deciphering signalling dynamics during *de novo* osteoblast regeneration following zebrafish scale removal (De Simone et al., 2021). He showed that osteoblast hypertrophy and growth during scale regeneration occurs via the propagation of ERK activity waves across the regenerating scale. Functional perturbation of these waves during regeneration impairs the rate of scale growth, altogether identifying a dynamic signalling mechanism that directs regenerating tissues and appendages to the appropriate size. Kenneth Poss (Durham, USA) has been exploring the role of genetic enhancer elements in signalling control during regeneration (Sun et al., 2022). He not only showed evidence for the presence of regeneration-specific enhancers that direct expression of key genes even in distant tissues upon injury, but also reported on the identification of silencing enhancers that repress gene expression unfavourable for regeneration. Finally, focusing on tendons, Stephanie Tsai (Boston, USA) demonstrated that the adult zebrafish tendon can fully regenerate. Unlike their mammalian counterparts, endogenous zebrafish tendon cells activate upon injury and are the main cell source of regeneration following a full transection injury, opening up new doors for using the adult zebrafish tendon to uncover mechanisms required for regeneration.

Scratching the surface: mechanisms of epithelial wound healing and repair

Several talks revealed novel insights into stem cell dynamics and the role of mechanical forces during epithelial homeostasis and repair. Pantelis Rompolas (Philadelphia, USA) examined the heterogeneity and functional organisation of corneal stem cells in the mouse limbus of the eye. He showed that actively cycling inner limbal stem cells contribute to epithelial homeostasis and migrate inwards, whereas stem cells located in the outer limbus are quiescent but can be induced to participate in corneal injury repair and regeneration. Further, Rompolas demonstrated that the centripetal flow of corneal progenitors is independent of their differentiation state and is regulated by Notch signalling at the limbal niche. Using laser ablation to study epithelial wound repair in the *Drosophila* embryo, Gordana Scepanovic (Toronto, Canada) showed that inhibition of mTORC1-dependent autophagy drives rapid embryonic wound healing. Yanlan Mao (London, UK) highlighted the role of mechanical forces driving wound repair in the *Drosophila* wing and demonstrated that increased tissue fluidity promotes more efficient wound closure (Tetley et al., 2019).

Modulating the immune system to promote regeneration

The innate and adaptive immune system plays a central role in directing favourable regenerative outcomes. Several talks focused on dissecting immune mechanisms governing regeneration and a new model for regeneration in the immune system. Kerstin Bartscherer (Osnabrück, Germany) showed that the presence of immune cells

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may contribute to scar-free ear-hole closure in the African Spiny mouse (*Acomys cahirinus*), which occurs asymmetrically in a proximodistal (P-D) manner. By comparing transcriptional profiles along the P-D axis between the *Acomys* ear and that of the non-regenerative Mongolian gerbil (*Meriones unguiculatus*), she has identified spatial differences and *Acomys*-specific upregulation of immune system-related gene signatures following injury, suggesting that differential immune cell infiltration dynamics direct regenerative repair. Interestingly, upregulation of similar immune signatures also occurs in the injured *Acomys* heart, suggesting a potentially conserved mechanism across organs. Elodie Labit (Calgary, Canada) reported that immunosuppressive interactions between fibroblasts and immune cells direct scar-free regeneration of deer antler velvet skin (Sinha et al. 2022). João Cardeira-da-Silva (Bad Nauheim, Germany) has examined the role of antigen presentation and T-cells during zebrafish heart regeneration. He showed that *cd74a/cd74b* double mutants exhibit defective T-cell dynamics associated with defective regeneration after injury. Furthermore, CD4+ T-helper cells associate with activated endocardium in the border zone, suggesting a link between these two cell types during the regenerative response and/or in the direct or indirect activation of T-helper cells. Finally, Maximina Yun (Dresden, Germany) showed that the axolotl can regenerate its entire thymus. She demonstrated that *midkine* (*mdk*; also known as *neurite growth-promoting factor 2*) is required for regeneration post-thymectomy and provided evidence that epidermal keratinocytes may contribute to regeneration.

Looking forward: translating advances in basic science into clinical outcomes

Understanding the factors that drive and prevent regeneration opens up opportunities for therapeutic targeting in a variety of disease and injury contexts. Elizabeth Bradbury (London, UK) has been developing an inducible gene therapy to alleviate the inhibitory effect of the scar-associated chondroitin sulphate proteoglycans in the injured rat spinal cord, which restores skilled hand function in rats with spinal cord injury, in part by preventing chronic inflammation (Burnside et al., 2018; Francos-Quijorna et al., 2022). Catherine Wilson (Cambridge, UK) showed that overexpression of Myc and Cyclin T1 can promote CM proliferation in the adult mammalian heart (Bywater et al., 2020). She has been exploring the potential of direct injection of modified mRNA to induce CM proliferation in the infarcted mouse heart for therapeutic use. Moving into humans, Michaele De Luca (Modena, Italy) presented progress on the regeneration of skin lesions in patients with genetic disorders. Using gene-corrected autologous keratinocytes, De Luca showed that an entire, fully functional epidermis could be regenerated in a 7-year-old child suffering from life-threatening epidermolyis bullosa (Hirsch et al., 2017; Kueckelhaus et al., 2021). Molly Stevens (London, UK) showcased a wide range of bioengineering platforms her lab has developed for molecular delivery to cells and tissue-engineered scaffolds. She discussed how they can each be tuned to promote regeneration or modulate stem cell differentiation (Armstrong et al., 2020). Finally, the philosopher Lucie Laplane (Paris, France) described a framework to categorise stem cells based on features required for the acquisition and maintenance of stemness. These principles can be readily applied to regenerative systems and will be important considerations when designing clinical strategies (Laplane and Solary, 2019).

Conclusion

In summary, the diversity of model systems paired with recent advances in gene editing and single-cell -omics technologies has enabled the deep interrogation of the molecular and cellular basis of tissue repair and regeneration, including the ability to tackle long-standing questions in the field. The findings presented provide hope for future therapeutic approaches in humans. Great things can be expected!

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Competing interests

The authors declare no competing or financial interests.

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