

MINIREVIEW

Mechanism of wound healing in annelids**M Grdisa***Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Bijenicka 54, Croatia**Accepted September 15, 2010***Abstract**

All animals possess some type of tissue repair mechanism. In some species, the capacity to repair tissues is limited to the healing of wounds, but others possess a striking repair capability to replace the entire organs. It has been reported that some mechanisms, namely extracellular matrix remodeling, appear to occur in most repair processes. However, it remains unclear to what extent the process of wound healing is similar to organ regeneration.

Key Words: annelid; wound healing; mechanism

Introduction

The Phylum Annelid (13,000 living species) is a line of invertebrate life dating back 540 million years. Their elongated, segmented body plan and other body features have allowed annelids to specialize at burrowing through substrates, and to radiate into other ecological niches (class Polychaeta - segmented worms in marine environments; class Oligochaeta - in fresh water and on land; class Hirudina - leeches - parasites).

Animal species possess different capability to replace lost body parts through regeneration (Herlant-Meewis, 1964; Thouveny and Tassava, 1998; Brusca and Brusca, 2003). Some species such as Planaria, Hydra or starfish can readily regenerate lost organs or body parts. In contrast, other species, such as most vertebrates have limited regenerative capacities. But, all species have some capacity to heal wounds produced by external factors during their existence. Many researchers attempted to find the relationship between the process of wound healing and regeneration. There are some indications that these processes might differ at the cellular and molecular levels. In sea urchins has been suggested that healing of broken spines occurs by a morphallactic mechanism involving recruitment of differentiated cells, while regeneration of removed spines and pedicellaria occurs by an epimorphic process involving undifferentiated precursors (Dubois and Ameye, 2001). Also in zebra fish mutant (*dob-* devoid of blastema) was found that it failed to regenerate the

fin but the response in wound healing was normal (Whitehead *et al.*, 2005). Recently, it has been shown that both wound healing and regeneration in axolotl are dependent on epithelial/mesenchymal interactions, the formation of the wound epidermis, the restructuring of the extracellular matrix and other cellular/molecular events (Roy and Levesque, 2006). To see if the cellular mechanisms during wound healing and organ regeneration are similar, the sea cucumber *Holothuria glaberrima* was investigated (Miguel-Ruiz and Garcia-Ararras, 2007). Animals were lesioned and damaged tissue included muscle, nerve, water canal and dermis. The authors found that cellular events associated with wound healing correspond to those occurred during organ regeneration. These include: an increase in the number of spherule-containing cells, remodeling of extracellular matrix, formation of spindle-structures that signal dedifferentiation of muscle cells and intense cellular division. Thus, it is possible that regenerative limitations in some organisms are due to the absence of particular mechanisms associated with repair or the inability of activating the repair process in some tissues or stages.

Annelids: wound healing and regeneration

Annelids have a reputation for impressive regenerative abilities, but this ability varies widely. Many annelid worms are limited in their ability to regenerate anterior body parts, whereas posterior segment regeneration is much more common (Bely, 2006). In addition to their ability to regenerate body segments, annelids generally have a marked capacity for wound healing (Zoran and Martinez, 2009). The remarkable ability of some annelids to reconstruct their entire body require coordinated

Corresponding author:

Mira Grdisa
Division of Molecular Medicine,
Rudjer Boskovic Institute,
Bijenicka 54, 10 000 Zagreb, Croatia
E-mail: grdisa@irb.hr

activation of multiple developmental, regenerative and wound healing processes in response to injury.

On the other hand, leeches are incapable to regenerate lost segments, whereas they generate an effective response to injury by assembling an extracellular scaffold of proteins that facilitates the restoration of traumatized structures (Tettamanti *et al.*, 2004).

Wound healing in the earthworms could be used as a biomarker for assessing chemical toxicity. Wound healing in earthworms is continuing process from open to healed wound with different stages and activities (Cooper and Roch, 1986). It involves the inflammatory response and various cell types, including immunoactive macrophage-like celomocytes. Also the influence on cell membranes, cell division, energy production or use, synthesis of DNA or RNA and enzymatic pathways should be sufficient to interfere with the complex processes required to heal damaged tissue.

Wound healing in earthworms *Lumbricus terrestris* (Cikutovic *et al.*, 1999) was monitored after cutting of three-sided patch of integument of *L. terrestris* during 5 days, after exposure to variety chloride compounds. The authors found that both, concentration and duration of exposure significantly reduced wound healing. Similar finding was observed earlier (Cooper and Roch, 1992; Ville *et al.*, 1995). Suppression of healing could be the consequence of interfering chloride compounds with the membranes of macrophages and other cells that function in healing process. It was found (Ville *et al.*, 1995; Goven *et al.*, 1993, Giggelman *et al.*, 1998) that some organics (polychlorinated biphenyl, pentachlorophenol, chlordane) suppress phagocytosis in earthworm celomocytes, probably by affecting their cell membranes. Another chlorinated pesticide, lindane, was reported to reduce RNA synthesis in mammals (Lewis and Adams, 1985; Thomas and Faith, 1985), which could also interfere with tissue repair during wound healing. Cell division, which is also important in the wound healing process, might be affected with pollution in soil (Cikutovic *et al.*, 1993).

Heavy metals, such Cd^{2+} or Cu^{2+} may affect enzyme activity necessary for wound healing. It was shown (Chen *et al.*, 2001) that Cu^{2+} interferes with an enzymatic pathway in celomocytes leading to production of superoxide (O_2^-), which is responsible for killing phagocytosed microorganisms in many animal species. It has been shown that exposure to Cd^{2+} in mammals increased infections (Lawrence, 1985), inhibited RNA and DNA synthesis (Daun *et al.*, 1993) and ATP utilization (Graham *et al.*, 1975), whereas in *L. terrestris* celomocytes suppressed phagocytosis (Roy and Levesque, 2006).

Suppression of the healing process portends pathological effects in wildlife exposed to environmental toxicant if they are wounded during natural activities. Healing of lacerations, punctures and abrasions of the integument or digestive tract may be compromised, resulting in microbial infection or parasite infestation. Injured animals may be even more susceptible to pathogens if the chemicals that suppress the healing process also affect immunoactive cells responsible for phagocytizing and killing microorganisms.

During wound healing the extracellular matrix (ECM) is produced, as well as its structural component, collagen. Collagen also plays a major role in the modulation of several cell functions, including adhesion, migration, growth and differentiation (Hay, 1991; Birk and Zycband, 1994; Lim *et al.*, 1994). In addition, fibrillar collagens are also involved in numerous processes, including stabilization of tissue shape and form during both vertebrate development and tissue regeneration (Birk and Trelstad, 1984; Ingberg, 1994; Fraizer *et al.*, 1996; Kletsas *et al.*, 2000; Badylak, 2002). Collagen fibrils are present in both vertebrates and in lower invertebrates (Bradbury, 1958; Matsuda-Nakagava and Nicholls, 1991; Sicot *et al.*, 1997). Previously was demonstrated that *Hirudo medicinalis* (Annelida, Hirudinea) could be a very good animal model for investigation of tissue repair and wound healing (de Eguileor *et al.* 2001, 2004; Grimaldi *et al.* 2010). The body of leech has a simple organization. Tissue repair in leeches shows a high degree of similarity to wound healing in vertebrates in biochemical and structural-functional points of view. The wound healing process in leeches can be divided into three stages: inflammation, granulation tissue and scar tissue remodeling. Granulation stage in leeches is characterized by re-epithelization, angiogenesis and fibroplasias. During these steps occurs the formation of new epithelium, followed by the blood vessels and then by connective tissues (de Eguileor *et al.* 2004; Tettamanti *et al.* 2004) Reorganization of collagen was studying in leech wound healing (Tettamanti *et al.*, 2004). After surgical lesion of the leeches it was shown that newly synthesized collagen acting as an extracellular scaffold. It directs and organizes the outgrowth of new vessels and the migration of immune cells towards the tissue lesions. In these animals, the collagen fibrils generated during tissue regeneration, showed similarities to both the structural pattern of collagen bundles and assembly processes observed in several vertebrate systems (fish scales, amphibian skin, human cornea). Thus, leeches respond to surgical lesions with the same sequence of wound healing and tissue regeneration events as that described for vertebrate (Tettamanti *et al.*, 2005). It was found that the general architecture of leech collagen fibril organization and bundle orientation is identical with the structural pattern of collagen bundles observed in vertebrate cornea (Birk and Trelstad, 1984). Thus, it could be hypothesized that collagen structures, characterized by a striking structural complexity and multifunctional purposes, are anatomical system highly conserved throughout evolution. To support the observation that "Nature has followed economic and conservative strategies based on the conservation of a lot of molecules and related functions" (Ottaviani *et al.*, 2004), probably, evolution preserved the primitive models because of their excellent functional utility and effectiveness. The phases which could be involved in annelid wound healing is depicted on Figure 1.

If the cellular mechanisms during wound healing are similar to those occurred during organ regeneration, these report could be expanded with additional data. Regeneration of annelids was studied

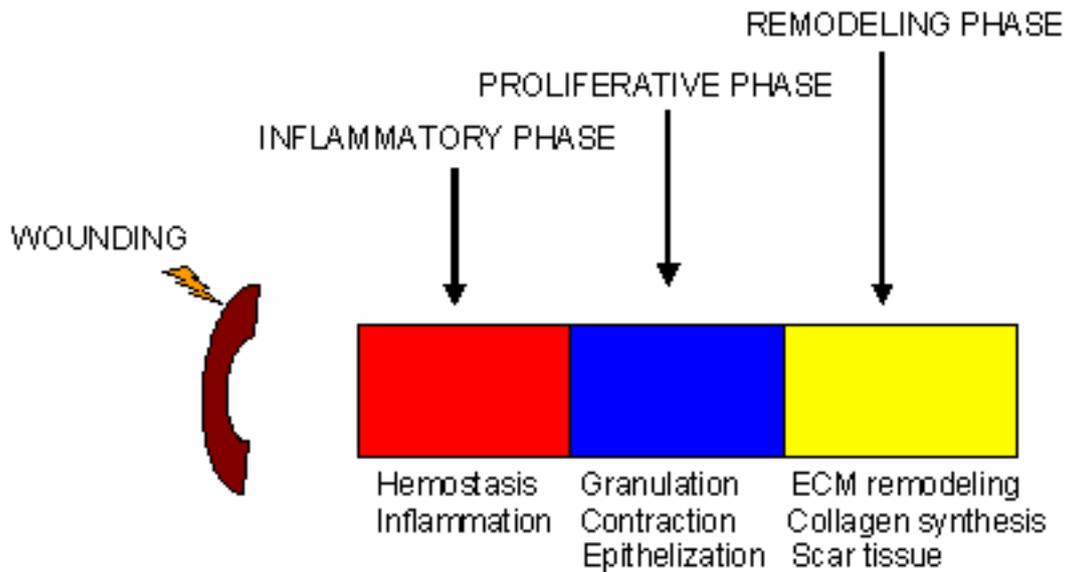


Fig. 1 Phase during wound healing in Annelids

in few groups, even at molecular level, with identification of the genes involved in the regeneration process (Bely and Sikes, 2010). In review Bely (2006) has described that the most annelids have the ability to regenerate posteriorly. The ability to regenerate anteriorly is common but less widespread. The molecular mechanisms for regeneration process have not been yet completed. Some annelids exhibit regenerative abilities very similar to planarians (Bode *et al.*, 1973; Newmark *et al.*, 2000), which can completely regenerate a new organism from small body fragment. However, the regeneration mechanisms are thought to be quite different between planarians and annelids. The planarians regenerate via totipotent stem cells (neoblasts) that are widely distributed throughout their bodies (Redien and Alvaro, 2004). The annelids regeneration is thought to occur primarily by cellular dedifferentiation and redifferentiation, without the contribution of totipotent cells (Thouveny and Tassava, 1998), but lately more date point on the involvement of stem cells (totipotent cells) in regeneration process (Grimaldi *et al.* 2008, 2010).

To better understanding the regeneration mechanisms in annelids, the genes that are expressed specifically during the course of regeneration, were identified on model animal *Enchytraeus japonensis* (Myohara *et al.*, 2006). Besides the known genes which play the roles in development (e.g genes for ECM, glutamine synthetase, NICE-5, glucosidase), the new genes Ejrup1-5 upregulated during regeneration, were isolated. After structural analyses of the products of these genes, a variety of putative functions that can be associated with their protein products were noticed. These functions include transportation and binding, transcriptional regulation, protein interaction and cell adhesion and perhaps some of them play

an important role in regeneration. Using in situ hybridization (Niv *et al.*, 2008), a strong expression of glutamine synthetase gene occurs in the blastemal regions of regenerating *E. japonensis*. Strong expression was detectable at the cell layer covering the wound and was found to persist in the epidermal cells during the formation and elongation of the blastema. Thus, according the results the authors suggested that *E. japonensis* glutamine synthetase may play roles in regeneration, nerve function, cell proliferation, nitrogenous waste excretion, macromolecule synthesis and gametogenesis.

Regenerative phenomenon has been explored also on the Echinoderms and many details are shown in the reviews (Candia-Carnevali, 2006; Kondo and Akasaka, 2010). Most explored model in echinoderm regeneration studies is the process of arm regeneration after lost following traumatic or self-induced amputation (Candia-Carnevali and Bonasoro, 1994, 2001; Bonasoro *et al.* 1995, 1998; Candia-Carnevali *et al.* 1995, 1998; Patruno *et al.* 2001) using the feather star (crinoid *Antedon mediterranea*). In this process new structures develop from migratory actively proliferating cells. Different type of cells is involved in regeneration, including those that are considered to be stem cells. During regeneration, coelomocytes from coelomic canal and amoebocytes from brachial nerve, migrate to the distal wound area and are involved in regenerative process. From migratory amoebocytes is formed a blastema. On the other hand, migratory coelomocytes contribute to regenerate the coelomic system. Cells proliferate at the blastema, coelomic canals and brachial nerve. Since the migrating cells differentiate into new structure of the arm, they are presumably undifferentiated multipotent stem cells. But the knowledge about stem cells in crinoids

would be further support with molecular analyses. Recently, similar results have been reported on the study of wound healing and arm regeneration in *Ophiderma longicaudum* and *Amphiura filiformis* (Biressi *et al.* 2010).

The earthworm provides a unique and valuable model to investigate the mechanism of regeneration because this process is rapid and its regeneration of a complete head and tail requires the reformation of various tissues and organs. To study the head and tail regeneration on annelid *Perionyx excavatus*, the expression pattern of three labial genes (Pex-la-01, Pex-lab02, Pex-lab03) was monitored (Cho *et al.*, 2009). The results indicated that these genes were expressed only in the head-regenerating tissues. Also, the authors found that the expression of Pex-lab01 and Pex-lab02 was up-regulated, and this indicated their involvement in wound healing and the blastema formation process during early head regeneration.

On the other hand, the leeches do not possess ability to regenerate segments posteriorly or anteriorly (Hyman, 1940). Only some leeches can wound heal (Le Gore *et al.*, 1971; Huguet and Molinas, 1996) and undergo limited nervous system repair (von Bernhardt and Muller, 1995), without any tissue or segment regeneration.

Data about wound healing in annelids are very obscure. The annelids have a big potential for regeneration of different body parts, because they produce unique and potent molecules. Probably, it is a reason that they are also investigated as a wound healing agent (Matausic-Pisl *et al.*, 2010).

The elucidation of the annelid regeneration mechanisms is thus expected to provide valuable information that may allow us in the future to explore strategies to enhance the regenerative capabilities in vertebrates.

The cellular events that occur during wound healing, namely the pattern of cell division, ECM remodeling, and muscle dedifferentiation are similar to those that take place during regeneration of complex structures in the animals that possess a high regeneration capacity. To date a few molecular studies have been carried out to the understanding of the mechanisms of annelid wound healing and regeneration. Additional studies should be conducted to shed more light on this subject.

References

- Badylak SF. The extracellular matrix as a scaffold for tissue reconstruction. *Sem. Cell Dev. Biol.* 13: 377-383, 2002.
- Bely AE. Distribution of segment regeneration ability in the Annelida. *Integr. & Comp. Biol.* 46: 508-518, 2006.
- Bely AE, Sikes JM. Latent regeneration abilities persist following recent evolutionary loss in asexual annelids. *Proc. Natl. Acad. Sci. USA* 107: 1464-1469, 2010.
- Biressi ACM, Zou T, Dupont S, Dahlberg C, Di Benedetto C, Bonasoro F, *et al.* Wound healing and arm regeneration in *Ophiderma longicaudum* and *Amphiura filiformis* (Ophiuroidea, Echinodermata): comparative morphogenesis and histogenesis. *Zoomorphology* 129: 1-19, 2010.
- Birk DE, Trelstad RL. Extracellular compartments in matrix morphogenesis: collagen fibril, bundle and lamellar formation by corneal fibroblasts. *J. Cell. Biol.* 99: 2024-2033, 1984.
- Birk DE, Zycband E. Assembly of the tendon extracellular matrix during development. *J. Anat.* 184: 457-463, 1994.
- Bode H, Berking S, David CN, Gierer A, Schaller H, Trenkner E. Quantitative analysis of cell types during growth and morphogenesis in *Hydra*. *Roux's Arch.* 171: 269-285, 1973.
- Bradbury S. A cytological and histochemical study of the connective tissue fiber of the leech, *Hirude medicinalis*. *Q. J. Microsc. Sci.* 99: 131-142, 1958.
- Brusca RC, Brusca GJ. *Invertebrates*. Sinaur Associates, Sunderland, MA, 2003.
- Carnevali MDC, Bonasoro F, Lucca E, Thorndyke MC. Pattern of cell proliferation in the early stages of arm regeneration in the feather star *Antedon mediterranea*. *J. Exp. Zool.* 272: 464-474, 1995.
- Carnevali MDC. Regeneration in Echinoderms: repair, regrowth, cloning. *Inv. Surv. J.* 3: 64-76, 2006.
- Carnevali MDC, Bonasoro F, Patruno M, Thorndyke MC. Cellular and molecular mechanisms of arm regeneration in crinoid echinoderms: the potential of arm explants. *Dev. Genes Evol.* 208: 421-430, 1998.
- Carnevali MDC, Galassi S, Bonasoro F, Patruno M, Thorndyke MC. Regenerative response and endocrine disruptors in crinoid echinoderms: arm regeneration in *Antedon mediterranea* after experimental exposure to polychlorinated biphenyls. *J. Exp. Biol.* 204: 835-842, 2001.
- Chen SC, Fitzpatrick LC, Goven AJ, Venables BJ, Cooper EL. Nitroblue tetrazolium dye reduction by earthworm *Lumbricus terrestris* coelomocytes: An enzyme assay for nonspecific immunotoxicity of xenobiotics. *Environ. Toxicol. Chem.* 10: 1037-1043, 2001.
- Cho AJ, Koh KS, Lee E, Park SC. Differential expression of three labial genes during earthworm regeneration. *Biosci. Biotechnol. Biochem.* 73: 2609-2614, 2009.
- Cikutovic MA, Fitzpatrick LC, Goven AJ, Venables BJ, Giggelman MA, Cooper EL. Wound healing in earthworm *Lumbricus terrestris*: A cellular-based biomarker for assessing sublethal chemical toxicity. *Bull. Environ. Contam. Toxicol.* 62: 508-514, 1999.
- Cikutovic MA, Fitzpatrick LC, Venables BJ, Goven AJ. Sperm count in earthworm (*Lumbricus terrestris*) as a biomarker for environmental toxicology: Effect of cadmium and chlordane. *Environ. Pollut.* 81: 123-125, 1993.
- Cooper EL, Roch P. Second-set allograft response in the earthworm *Lumbricus terrestris*: Kinetics and characteristics. *Transplantation* 41: 514-520, 1986.
- Cooper EL, Roch P. The capacities of earthworms to heal wounds and to destroy allografts are modified by polychlorinated biphenyls (PCBs). *J. Invertebr. Pathol.* 60: 59-63, 1992.
- Daum JR, Shepherd DM, Noelle RJ. Immunotoxicology of cadmium and mercury on

- B-lymphocytes: I. Effects on lymphocytes function. *Int. J. Immunopharmacol.* 15: 383-394, 1993.
- De Eguileor M, Grimaldi A, Tettamanti G, Ferrarese R, Congiu T, Protasoni M, *et al.* *Hirudo medicinalis*: a new model system for testing activators and inhibitors of angiogenesis. *Angiogenesis* 4: 299-313, 2001.
- De Eguileor M, Tettamanti G, Grimaldi A, Perletti G, Congiu T, Rinaldi L, *et al.* *Hirudo medicinalis*: avascular tissues for clear-cut angiogenesis studies? *Curr. Pharm. Des.* 10: 1979-1988, 2004.
- Dubois P, Ameys L. Regeneration of spines and pedicellariae in echinoderms: A review. *Microsc. Res. Tech.* 55: 427-437, 2001.
- Fraizer K, Williams S, Kothapalli D, Klapper H, Grotendorst GR. Stimulation of fibroblast cell growth, matrix production and granulation tissue formation by connective tissue growth factor. *J. Invest. Dermatol.* 107: 404-411, 1996.
- Giggleman M, Fitzpatrick LC, Goven AJ, Venables BJ. Effect of pentachlorophenol (PCP) on survival of earthworms *Lumbricus terrestris* and on phagocytosis by their immunoreactive coelomocytes. *Environ. Toxicol. Chem.* 17: 2391-2394, 1998.
- Goven AJ, Eyambe G, Fitzpatrick LC, Venables BJ. Cellular biomarkers for measuring toxicity of xenobiotics: Effects of polychlorinated biphenyls on an earthworm *Lumbricus terrestris* coelomocytes. *Environ. Toxicol. Chem.* 12: 863-870, 1993.
- Graham JA, Gardner DE, Waters MD, Cogffin DL. Effect of trace metals on phagocytosis of alveolar macrophages. *Infect. Immun.* 11: 1278-1283, 1975.
- Grimaldi A, Bianchi C, Greco G, Tettamanti G, Noonan DM, Valvassori R, *et al.* *In vivo* isolation and characterization of stem cells with diverse phenotypes using growth factor impregnated biomatrices. *PLoS ONE* 3(4):e1910. doi:10.1371, 2008.
- Grimaldi A, Banfi S, Bianchi C, Greco G, Tettamanti G, Noonan DM, *et al.* The leech: a novel invertebrate model for studying muscle regeneration and diseases. *Curr. Pharm. Des.* 16: 968-977, 2010.
- Hay E. Collagen and other matrix glycoproteins in embryogenesis. In: Hay E (ed), *Cell biology of extracellular matrix*, Plenum Publishing, New York, NY, pp 419-462, 1991.
- Herlant-Meewis H. Regeneration in annelids. *Adv. Morphog.* 4: 155-215, 1964.
- Huguet G, Molinas M. Myofibroblast-like cells and wound contact in leech wound healing. *J. Exp. Zool.* 275: 308-316, 1996.
- Hyman LH. Aspects of regeneration in annelids. *Am. Nat.* 74: 513-527, 1940.
- Ingberg D. Cellular tensegrity: exploring how mechanical changes in the cytoskeleton regulate cell growth, migration and tissue pattern during morphogenesis. *Int. Rev. Cytol.* 150: 173-224, 1994.
- Kletsas D, Pratsinis H, Zervolea I, Handris P, Sevaslidou E, Ottaviani E, *et al.* Fibroblast responses to exogenous and autocrine growth factors relevant to tissue repair. The effect of aging. *Ann. NY Acad. Sci.* 908: 155-166, 2000.
- Kondo M, Akasaka K. Regeneration in crinoids. *Dev. Growth Differ.* 52: 57-68, 2010.
- Lawrence DA. Immunotoxicity of heavy metals. In: Dean JH, Luster MI, Munson AE, Amos H (eds), *Immunotoxicology and immunopharmacology*, Raven Press, New York, NY, pp 341-353, 1985.
- Le Gore RS, Sparks AK. Repair of body wall incision in the rhynchobdellid leech *Pisiccol salmositica*. *J. Invertebr. Pathol.* 18: 40-45, 1971.
- Lewis JG, Adams DO. The mononuclear phagocytic system and its interactions with xenobiotics. In: Dean JH, Luster MI, Munson AE, Amos H (eds), *Immunotoxicology and immunopharmacology*, Raven Press, New York, NY, pp 23-44, 1985.
- Lim A, Greenspan DS, Smith BD. Expression of $\alpha 2$ type I collagen in W8 cells increases cell adhesion and decreases colony formation in soft agar. *Matrix Biol.* 14: 21-30, 1994.
- Masuda-Nakagawa LM, Nicholls JG. Extracellular matrix molecules in development and regeneration of the leech CNS. *Philos. Trans. R. Soc. Lond.* 331: 323-335, 1991.
- Matausic-Pisl M, Cupic H, Kasuba V, Mikecin AM, Grdisa M. Tissue extract from *Eisenia foetida* as a wound-healing agent. *Eur. Rev. Med. Pharmacol. Sci.* 14: 177-184, 2010.
- Miguel-Ruiz JES, Garcia-Ararras JE. Common cellular events occur during wound healing and organ regeneration in the sea cucumber *Holothuria glaberrima*. *BMC Dev. Biol.* 7: 115-134, 2007.
- Myohara M, Niva CC, Lee JM. Molecular approach to annelid regeneration: cDNA subtraction cloning reveals various novel genes that are upregulated during the large-scale regeneration of the oligochaete, *Enchytraeus japonensis*. *Dev. Dyn.* 235: 2051-2070, 2006.
- Newmark PA, Sanchez Alvarado A. Bromodeoxyuridine specifically labels the regenerative stem cells of planarians. *Dev. Biol.* 220: 142-153, 2000.
- Niv CC, Lee JM, Myohara M. Glutamine synthetase gene expression during the regeneration of the annelid *Enchytraeus japonensis*. *Dev. Genes evol.* 218: 39-46, 2008.
- Ottaviani E, Franchini A, Kletsas D. Platel-derived growth factor and transforming growth factor- β in invertebrate immune and neuroendocrine interactions: another sign of conservation in evolution. *Comp. Biochem. Physiol.* 129C: 295-306, 2001.
- Patrino M, Thorndyke MC, Carnevali MDC, Bonasoro F, Beesley PW. Growth factors, heatshock proteins and regeneration in echinoderms. *J. Exp. Biol.* 843-848, 2001.
- Reddien PW, Alvarado AS. Fundamentals of planarian regeneration. *Ann. Rev. Cell Dev. Biol.* 20: 725-757, 2004.
- Roy S, Levesque M. Limb regeneration in Axolotl: is it superhealing? *TSW Dev. Embryol.* 1(S1): 12-15, 2006.

- Sicot FX, Exposito JY, Masselot M, Garrone R, Deutsch J, Gaill F. Cloning of an annelid fibrillar-collagen gene and phylogenetic analysis of vertebrate and invertebrate collagen. *Eur. J. Biochem.* 246: 50-58, 1997.
- Tettamanti G, Grimaldi A, Rinaldi L, Arnaboldi F, Congiu T, Valvassori R, *et al.* The multifunctional role of fibroblasts during wound healing in *Hirudo medicinalis* (Annelida, Hirudinea). *Biol. Cell* 96: 443-455, 2004.
- Tettamanti G, Grimaldi A, Rinaldi L, Congiu T, Perletti G, Raspanti M, *et al.* Collagen reorganization in leech wound healing. *Biol. Cell* 97: 557-568, 2005.
- Thomas PT, Faith RE. Adult and perinatal immunotoxicity induced by halogenated aromatic hydrocarbons. In: Dean JH, Luster MI, Munson AE, Amos H (eds), *Immunotoxicology and immunopharmacology*, Raven Press, New York, NY, pp 305-313, 1985.
- Thouveny Y, Tassava RA. Regeneration through phylogenesis. In: Ferretti P, Geraudie J (eds), *Cellular and molecular basis of regeneration: from invertebrates to humans*. John Wiley & Sons, New York, pp 9-43, 1998.
- Ville P, Roch P, Cooper EL, Masson P, Narbone J-F. PCBs increase molecular-related activities (lysozyme antibacterial, hemolysis, proteases) but inhibit macrophage-related functions (phagocytosis, wound healing) in earthworms. *J. Invertebr. Pathol.* 65: 217-224, 1995.
- von Bernhardt R, Muller KJ. Repair of the central nervous system: lessons from lesions in leeches. *J. Neurobiol.* 27: 353-366, 1995.
- Whitehead GG, Makino S, Lien C-L, Keating MT. fgf20 is essential for initiating zebrafish fin regeneration. *Science* 310: 1957-1960, 2005.
- Zoran MJ, Martinez VG. *Lumbriculus variegatus* and the need for speed: A model system for rapid escape, regeneration and asexual reproduction. In: *Annelids in Modern Biol.* Shain DH (ed), John Wiley & Sons Inc. New York, N Y, pp 185-204, 2009.