Asian Journal of Scientific Research

ISSN 1992-1454

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OPEN ACCESS Asian Journal of Scientific Research

ISSN 1992-1454
DOI: 10.3923/ajsr.2021.32.42

Research Article
Regeneration of the Posterior Segments in the Clam Worm *Platynereis dumerilii* (Audouin and Milne Edwards, 1833) (Polychaeta, Phyllodocida)

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Abstract

Background and Objective: Regeneration is characterized by the restoration of tissue components identical to those removed. The purpose of this study is to describe the origin of the totipotent cells and how they act as progenitors. Follow up the trans-differentiation cells to new cell types. **Material and Methods:** Specimens of *Platynereis dumerilii* were collected at 1-3 m depth from the Mediterranean Sea of Alexandria, Egypt in 2017. The posterior five segments with the pygidium are cut from clam worms with healthy body segments. The amputated worms are studied through light microscopy and TEM, starting at 12-24 hrs intervals to 2 weeks. **Results:** The hemocytes agglutinate and form a pseudo-clot at the wound site. The endoblast forms from the internal endoderm. The dedifferentiated cells form the true blastema during the first 5-6 days of post-amputation. The subsequent regenerating five segments are formed at front of the pygidium. After fifteen days of post-amputation, the transverse musculature thickens. The dermis is formed by the cells furthest from the longitudinal axis. **Conclusion:** If physicians could activate the human quiescent and permanent cells to follow the result of this study, limb regeneration could be possible. It is still an open question. This study provides a detailed description to help scientists extend researches on human regeneration.

Key words: Regeneration, totipotent cells, trans-differentiation cells, posterior five segments, hemocytes, endoblast, blastema

Citation: Gaber, I., 2021. Regeneration of the posterior segments in the clam worm *Platynereis dumerilii* (Audouin and Milne Edwards, 1833) (Polychaeta, Phyllodocida). Asian J. Sci. Res., 14: 32-42.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The ability to recover injured tissues after traumatic loss or amputation or an accident has attracted the attention of authors. Physicians exert a great effort to discover how to activate the human quiescent cells to make regeneration possible especially for extremities. In the twentieth century, concepts have been developed in experimental animals to explain how a part of an organ or tissue is recovered when lost. Regeneration is a characteristic of many invertebrates as annelid worms, *Hydra*, *Planaria* and the starfish. Some metazoans lost the ability to regenerate damaged parts due to unknown molecular and cellular bases. That is why many scientists study this biological event in various animal models to determine their nature and to finally able to apply knowledge to clinical practice. It is important to highlight the difference between repair and regeneration. Repair is a process that replaces damaged or lost tissue with another tissue but does not maintain its structural or functional identity. Repair is typical in adult vertebrates such as birds and mammals[1-3]. Regeneration, however, involves replacing lost tissue with another which retains the structure and functionality of the original. There is a great similarity between the genes involved in regeneration and repair, although the location of their expression and interactions differ in the two phenomena. Classically, it has been considered that regeneration may be due to two different mechanisms, morphallaxis and epimorphosis. Epimorphosis is the morphological and functional restoration of an anatomical structure lost in an adult organism through the formation of a blastema[4,5]. On the other hand, the term morphallaxis implies a drastic remodeling of pre-existing tissues so that part of the old tissues is transformed into those that have been lost. Thus, a complete organism is formed without a proliferative process, without the formation of a blastema proper[6,7]. When syllidian polychaetes are deprived of the back region they often fail in their total regeneration or may form small organisms consisting of little more than pygidium. At the front end, up to six or nine segments may be regenerated including the cephalic region. At the posterior extremity greater number of segments can achieve full regeneration. Regeneration in polychaetes is variable depending on the group or even the genus. The genus *Chaetopterus* can regenerate a complete worm from a single anterior segment[8,9]. The regeneration of the segregating segments is determined by the association of two different levels of the anterior-posterior differentiation gradient (newly formed pygidium-last segment) and an undifferentiated, very narrow zone (proliferation zone) persisting at the base of newly formed pygidium (intercross regeneration). Pygidial regeneration is ensured by the mitotic multiplication of the epidermal cells which line the wound after a fusion between the epidermis and the intestinal epithelium, correlatively and the mesodermal cells accumulate behind the scarred area[10,11]. The differentiation of pygidium is early and results from a redistribution of mesodermal cells within the blastema and the differentiation of the epidermal and mesodermal cells of the area corresponding to the future pygidium[12-14]. The normal regeneration of *Nereis diversicolor* consists of two stages: the regeneration of pygidium and the regeneration of the segregating segments. The study of the regeneration capacity of *Capitella teleta* revealed that this species is capable of surviving the loss of some segments. The histological study of the first stages of posterior regeneration in *Nereis diversicolor* shows that the epidermis and the intestinal epithelium close to the wound are respectively the sources of ectodermal and endodermal structures of the regeneration[15,16]. Moreover, this author concluded that regeneration in *N. diversicolor* takes place without the participation of totipotent and migratory cells but the epidermis, the coelomic epithelium the intestinal epithelium of the injured segment is the origin of ectoneural, mesodermal and endodermal structures, respectively. There is also news that *Lumbriculus variegatus* and the fire worm *Eurythoe complanata* have the power to regenerate in few days a minimum of three lost segments[17,18].

This study aims to follow up the regeneration process of the posterior five segments and the pygidium of *Platynereis dumerilii*. It describes definitely what happens during the post-amputated healing, how degeneration and dedifferentiation of all cellular categories close to the wound occur, how does blastema forms, steps of cellular activation, regenerative growth and finally segment differentiation.

MATERIALS AND METHODS

Sampling and identification of *Platynereis dumerilii*: Specimens of *Platynereis dumerilii* were collected at 1-3 m depth from the coastal water of Ras el Tin beach, Mediterranean Sea of Alexandria, Egypt from July-September, 2017. This clam worm belongs to Polychaeta, Nereididae, Phyllodocida. They live on rocks, crawling under algae or animals. Sometimes, they dig on sandy muddy benthos. Identification of the worm was according to Fischer *et al.*[19] and Thompson[20]. Description: beige to dark brown, with 2-4 cm length and of 30-40 segments, prostomium longer than wide, with a pair of antennal scars, black eyes in the trapezoidal arrangement, the former farthest from each other, palps as long as the width of the prostomium, palposthyl not obvious,

peristome near as wide as segment 1, with four scars of peristomial cirrus, everted pharynx with pectins in both pharyngeal rings, Areas I and II: no paragnates or pectins, area III: three lines of pectins, area IV: three diagonal lines of pectins, area VI: a transverse line of pectins, areas VII-VIII: three transverse lines of pectins, brown jaws with eight teeth, impaired biramous parapodia, with lobes, ligules and cirrus little distinguishable, anterior parapodia with three cylindrical lobes of equal size, the ventral as wide as twice the upper ones, cirriform cirrus, basally inserted and posterior parapodia with undifferentiated lobes.

Amputation procedures: Twenty juvenile clam worms with body segments that appear healthy, normal and relatively uniform in size and pigmentation are chosen from each collection. All 60 worms used for this experiment are 4 cm in length. Worms are transferred to containers by drawing them up quickly into a medicine dropper, along with a small amount of water. Cutting the posterior 5 segments with the pygidium is carried out, by placing a filter paper in the bottom of a petri dish. Moisten the paper to saturation with distilled water. Using the medicine dropper, the worm is transferred to the middle of the moist filter paper. Next, the dish is tilt to one side and excess water is withdrawn, leaving the worm about in the middle of the paper[21]. Wait until the worm is straightened and then position the razor blade above it to cut the posterior 5 segments with the pygidium. The blade edge was hold parallel to the dish but perpendicular to the long axis of the worm. Quickly the blade is pressed through the worm and is flushed against the paper, holding the blade down for a couple of seconds. The posterior part of the worm is separated with little or no bleeding. Containers were kept in the dark at room temperature 20°C. Zooplankton and algae were added to the seawater of the containers containing the amputated clam worms while regeneration experiments are in progress. All containers were provided with continuous aeration and the seawater is changed every other day. Using a dissecting microscope, repeated observations of amputated worms were carried out to follow up segment regeneration, starting at 12-24 hrs intervals and continuing for 2 weeks. Initial observations of the regeneration can often be done with worms still in their storage containers, thus minimizing handling. The appearance and relative length of blastema and regenerating segments are followed. Daily measures are made for the actual length (in mm) of each bud of new and old segments. Carefully lighting is adjusted to optimize the resolution of new segments.

Light microscopy: After one-day post-amputation till 15 days, amputated worms are relaxed in seawater with l-menthol. The posterior part of worms is isolated and fixed in Karnovsky's solution for 24 hrs. The material received baths of phosphate buffer and subsequently 70% alcohol, is maintained in this medium until its inclusion in histories[22,23]. After the inclusion routines, routine procedures of dehydration and staining in Hematoxylin and Eosin and toluidine Blue solution are performed. About 5 µm sections were obtained. Analyzes and photography are performed in an Olympus B Max-50 photomicroscope.

Transmission electron microscopy: Specimens after 1-15 days post-amputation are prepared for transmission electron microscopy. They are placed in primary fixative on a cold plate without primary anesthesia. Three fixation methods were employed, including phosphate-buffered glutaraldehyde, cacodylate buffered glutaraldehyde and seawater buffered glutaraldehyde. Osmication followed primary fixation using the same buffer or sodium bicarbonate. Stages of regeneration were fixed in 2.5% glutaraldehyde at 0.45 pm millipore-filtered natural seawater for 1 h at room temperature. This was followed by a rinse in 2.5% sodium bicarbonate (pH 7.2) and secondary fixation in 2% osmium tetroxide in 1.25% sodium bicarbonate for 1 h at room temperature. Following fixation, the tissue was rinsed in distilled water, dehydrated in ethanol, transferred to propylene oxide and embedded in Epon 812. For light microscopy, l-pm sections were stained with a mixture of methylene blue and Azur II. Thin sections were stained with 2% uranyl acetate followed by 2% lead citrate and viewed with a Zeiss EM9-S2 electron microscope[24].

RESULTS

Healing, degeneration and dedifferentiation: The healing starts with the migration of amoeboid hemocytes to the wound (Fig. 1a). These cells exhibit a spherical profile and display a nucleus with a large mass of heterochromatin and the cytoplasm contains numerous mitochondria, RER and abundant ribosomes with polysome formation. They have granular condensation with the formation of large and small vesicles. Phyllopods and pseudopods are present (Fig. 1b). Although the blood of *Platynereis dumerilii* does not contain fibrin and therefore cannot coagulate, at the amputation level, the hemocytes agglutinate and form a Plasmodial-like mass that isolates the tissues of the amputated segment from

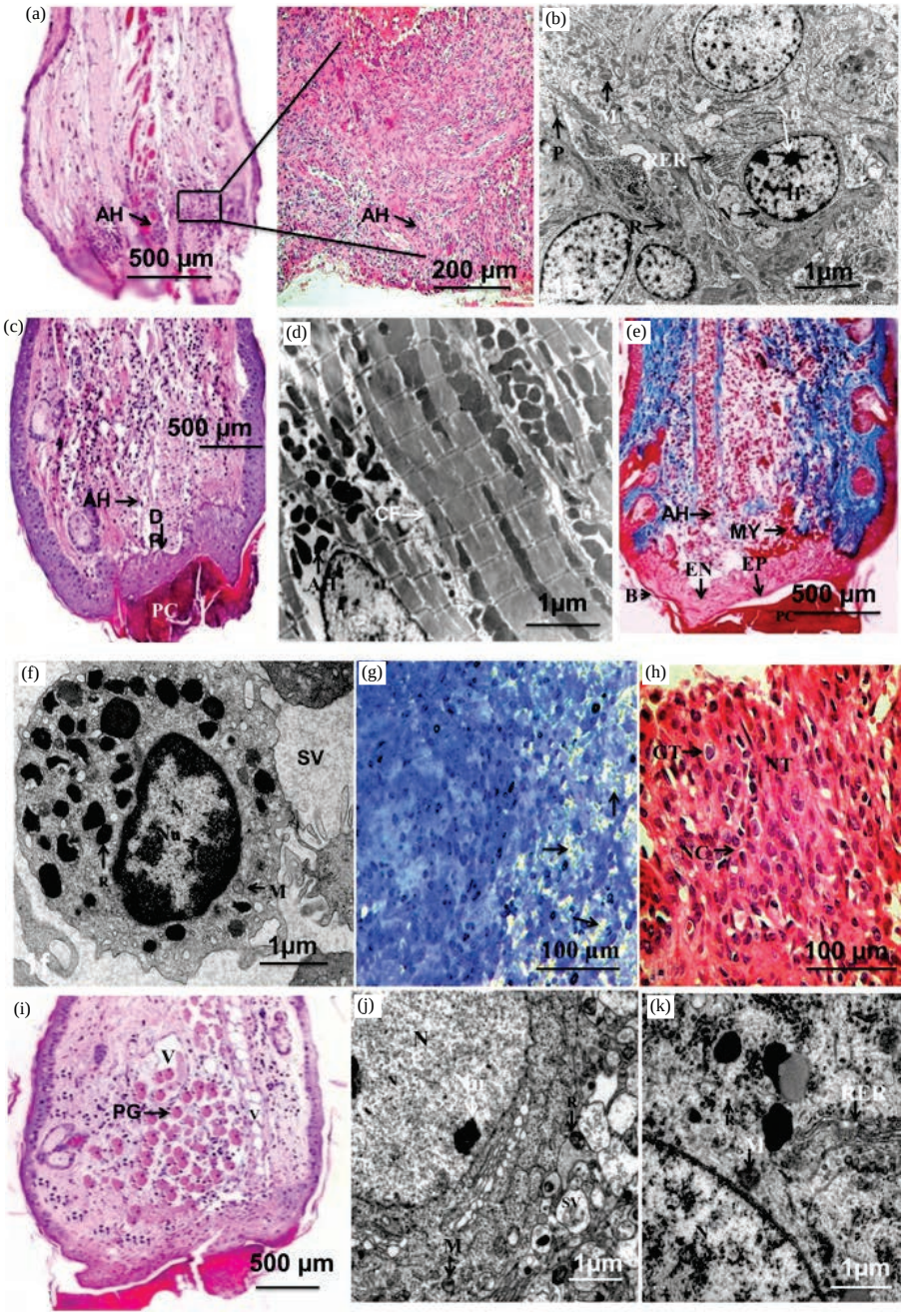

Fig. 1(a-k): Healing, degeneration and dedifferentiation of the posterior part of *Platynereis dumerilii*

(a) LS amoeboid hemocytes migrate to the wound, (b) TEM cells with spherical profiles display a nucleus with a large mass of heterochromatin and the cytoplasm contains numerous mitochondria, RER and abundant ribosomes with polysome migrate to the wound, (c) LS the hemocytes agglutinate and form a Plasmodial-like mass of pseudo-clot. Densely packed and tightly compacted cells showing finger-like processes of the cell membrane and comprise a deeper plug, (d) TEM showing collagen fibers at the vicinity of the dermis, (e) LS the epiblast, endoblast cells and myoblast are the blastocystes of the blastema, (f) TEM cells in blastocyst have a fairly large nucleus with a very dense and large nucleolus. The cytoplasm is rich in mitochondria and free ribosomes but has small granular endoplasmic reticulum, (g) CS showing degeneration of tissues in contact with the environment: nervous tissue, muscles and blood vessels (indicated by arrows), (h) neuroglia cell's reaction to dyes differs from that of glial-nervous tissue, (i) LS at the level of the scar cap, the cleaning of cellular waste is performed by blood cells that phagocytize cell elements, nervous and muscular tissues. Blood cells that have participated in the process lysate. They lose first their typical granulations and huge vacuoles appear in the cytoplasm incomplete dislocation. This phenomenon takes place in conjunction with the arrival of the first nerve fibers which have regenerated, (j) TEM showing totipotent cells do not return to their embryonic cell status but retain their histological specificity. In other words, they undergo epimorphosis and recovery of the missing segments begins and (k) TEM growth of the blastema follows by dedifferentiated cells of neighboring tissues of the wound

the outside environment and prevents bleeding (pseudo-clot) (1-2 days Post-amputation). Densely packed and tightly compacted cells showing finger-like processes of the cell membrane and comprise a deeper plug (Fig. 1c). This fibrous plug is formed during the first hours of amputation. At the vicinity of the dermis, increased synthesis of collagen is detectable (Fig. 1d). The wound closes in two steps: the first is a superficial epithelium, of ectodermal origin, which becomes the undifferentiated or epiblast epidermis, A second layer formed by an internal endodermic epithelium which gives rise to endoblast. The epiblast, endoblast cells and myoblast (originated by the musculature of the place) are the blastocystes of the blastema (2-3 days Post-amputation) (Fig. 1e). Blastocystes have a fairly large nucleus with a very dense and large nucleolus. The cytoplasm is rich in mitochondria and free ribosomes but has small granular endoplasmic reticulum (Fig. 1f). Three days after amputation, the longitudinal muscles contract and the dermis with nervous tissue are protruding. Cells of the epidermis spread out and completely cover the wound. Degeneration of tissues that have been in contact with the environment starts immediately after the amputation, at the nervous tissue, muscles and blood vessels. Axial neurons degenerate first. The perikaryon closest to the wound becomes necrotic in the hours that follow the amputation (Fig. 1g). They disappear before the formation of scar tissue. At the level of the neuropile, these facts are reflected in the appearance of necrotic deficiency. There is also a neuroglia cells reaction whose affinity for dyes differs from that of glial-nervous tissue in good condition (Fig. 1h). The degeneration of the muscular system is quite reduced. The phenomenon is especially visible in the longitudinal musculature. The cells swell or split into spherical or oval masses. The anucleate fragments no longer take the dyes, they eventually disappear. The blood vessels are also altered. Organelles of pericytes disappear and then these cells and the endothelium dilate. The tissues of the end of the operating segment lose vascularization. At the level of the scar cap, the cleaning of cellular waste is performed by blood cells that phagocytize cell elements, nervous and muscular tissues (Fig. 1i). Blood cells lysate which already have participated in the process. They lose first their typical granulations and huge vacuoles appear in the cytoplasm incomplete dislocation. This phenomenon takes place in conjunction with the arrival of the first nerve fibers which have regenerated. Once the wound is closed, the lack of difference occurs (Fig. 1i). The undifferentiated totipotent cells do not return to their embryonic cell status but retain their histological specificity. In other words, they undergo epimorphosis and recovery of the missing segments begins. They are morphologically simplified but they conserve their functional specificity (Fig. 1j). Dedifferentiation is the process by which a specialized cell loses its morphological or/and physiological characters to resume an embryonic aspect. These cells are the only figured elements of regeneration recovery. In the segment of *Platynereis dumerilii* there are longitudinal, transverse and oblique muscles which are limited by a connective tissue sheath. Dedifferentiation occurs quickly in the regenerated segments and seems to concern all cellular categories close to the wound, except the epidermis. It is observed in the muscle tissue, below the zone of degeneration. The cells lose their characteristic oblique striated structure. This sarcolysis runs parallel with nervous tissue degeneration and that of vessels and capillaries. The fibrocytes of the connective tissue present myelin-like structures that interpret as the last stage of degeneration of cellular organelles. Neurons and glial cells close to the wound find an aspect of embryonic cells. At the end of the stump dedifferentiation, there is an identical morphology to all undifferentiated totipotent cells having participated in the formation of the scar plug. They are no longer distinguishable from each other. The only cells to emigrate from regions are amoeboid blood cells. Growth of the blastema follows by dedifferentiated cells of neighboring tissues of the wound (3-4 days Post-amputation) (Fig. 1k). Epidermal cells retain most of their characters. They remain identifiable throughout the healing process. During spreading, cellular links are altered. The basement membrane is not reappearing immediately but it is in contact with the blastema. During this first phase, mitosis is unobserved, neither in the epidermis nor in the regeneration blastema which begins to build.

Blastema formation, cellular activation, regenerative growth: Undifferentiated cells are blastocystes and dedifferentiated cells form the true blastema or regeneration bud during the first 5-6 days of post-amputation. The blastema cells are of local origin and proceed of the tissues adjacent to the surface of the wound. They are differentiated and become similar to the preceding healthy segments which are latent in the embryo. So, the first regenerating segment (the pygidium) is formed (7 days post-amputation) (Fig. 2a). The growth of the regenerate begins. Mitosis appears at the end of the 1st week of amputation when the nerve fibers invade the blastema and their currency is restored (Fig. 2b). The blood gaps are first visible and then the veins become individualized. The epidermis becomes thicker and appears stratified (Fig. 2c), but, gradually it is again simple. The cells first spread out become cubic and then palisade (Fig. 2d).

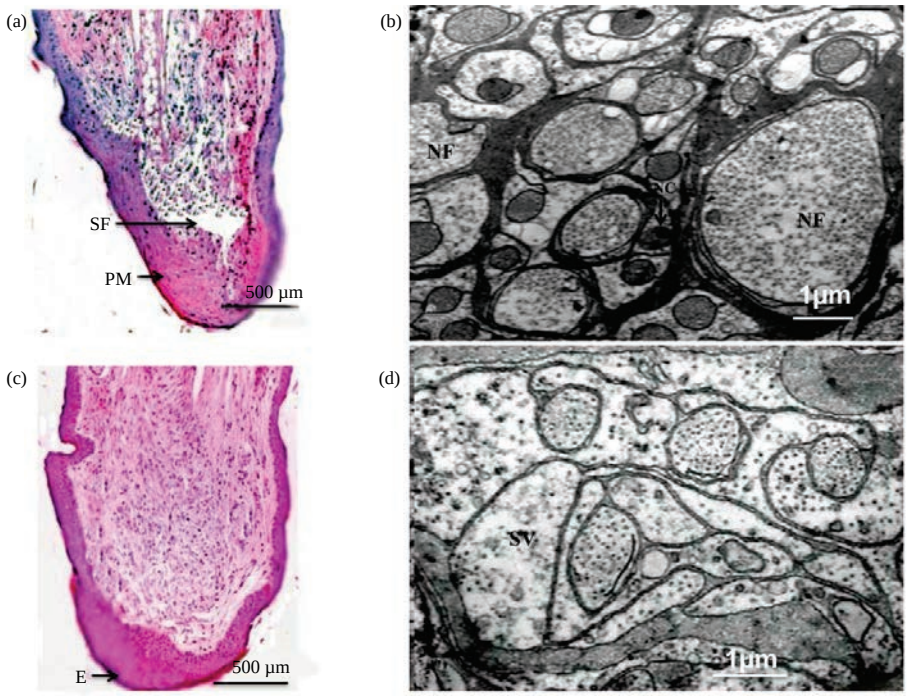

Fig. 2(a-d): Blastema formation, cellular activation, regenerative growth of the amputated segments of *Platynereis dumerilii*
(a) LS the blastema cells are of local origin and proceed of the tissues adjacent to the surface of the wound. They are differentiated and become similar to the preceding healthy segments which are latent in the embryo. So, the first regenerating segment (the pygidium) is formed, (b) TEM the growth of the regenerate begins. The nerve fibers invade the blastema and their currency is restored, (c) LS the epidermis becomes thicker and appears stratified and (d) TEM the epidermal cells gradually spread out become cubic and then palisade

Segment growth and histological differentiation: Eight to fourteen days of Post-amputation, mitosis continues but gradually becomes less and less frequent. The subsequent regenerating five segments are formed at front of the pygidium. The cells that have divided are differentiated. The first organs appear. This stage includes the stage of recovery of functioning. For some cells, the differentiation begins during the first week. Cells specialize in their situation in the blastema and their place relative to each other. The differentiation is done by concentric fields around the nervous tissue (Fig. 3a). The nervous system is the first to differentiate. Nerve fibers extend and penetrate the blastema, the ganglionic layer, formed by neuroblasts divide, then appears. The neuropile is built by the fibers emitted by the neurons. Amoebocytes infiltrated into the connective tissue near the wound (20 h after amputation) (Fig. 3b). Their nuclei have notches and their cytoplasm is filled with dense vesicles and collagen. Glial cells support nerve fibers to regenerate. They begin to divide and follow the progression of axons. During this process, they contain very large mitochondria that seem to divide. The axial nervous system, blood vessels and muscles differentiate together. At the level of the muscular system, it is the longitudinal musculature that develops first. Muscles result from the alignment in ribbons parallel to the nerve axis of fusiform myoblasts (Fig. 3c). Muscle cells differentiate in the extension of the muscular fields of the stump. The collagen sheath limits muscle cells to the exterior progress at the same time. Longitudinal extrinsic musculature appears shortly followed by transverse musculature. It is established slowly between the longitudinal musculature and the nervous tissue. It occupies initially only a small thickness. Muscles in Cross-section are still formed of myoblasts, whereas the cells of longitudinal musculature possess myofilaments. After fifteen days post-amputation, the transverse musculature thickens and mitosis is still observable. The dermis is formed by the cells furthest from the longitudinal axis. Dermal cells differentiate near the epidermis. Their cytoplasm splits into outer and inner plasmas. The pigment appears in the internal plasmas. The epidermis remains independent of other tissues (Fig. 3d). The basement membrane of epithelial cells appears at the time of differentiation. As morphogenesis proceeds, it folds to form drafts of cupping, then each of them individualizes invaginates itself by giving the suction and adherent rooms. This phenomenon is simultaneous with the appearance of the main blood vessels in the regeneration. The ectoderm, endodermis and the viscera are produced by

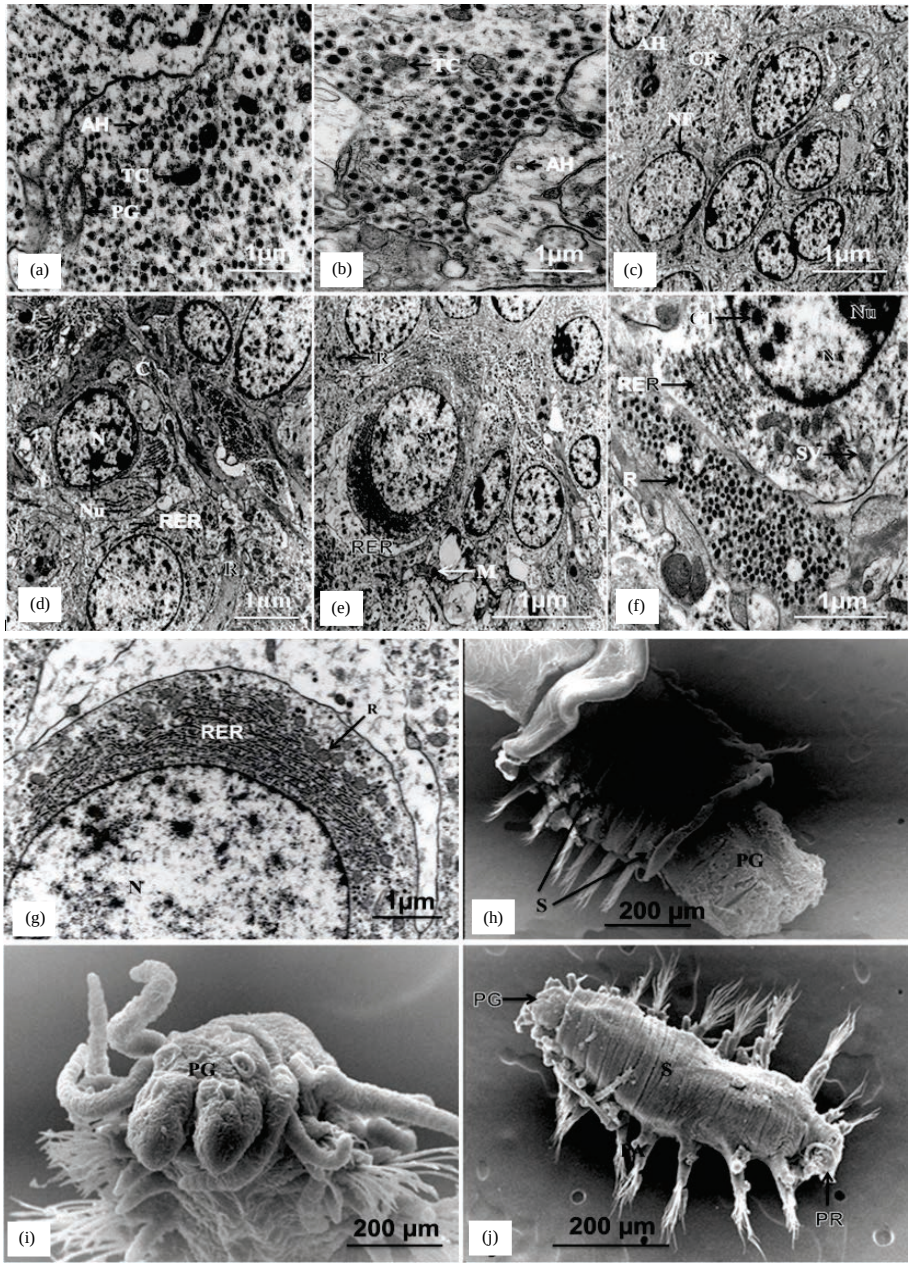

Fig. 3(a-j): Segment growth and histological differentiation

(a) Cells specialize in their situation in the blastema and their place relative to each other. The differentiation is done by concentric fields around the nervous tissue, (b) TEM amebocytes infiltrated into the connective tissue near the wound, (c) TEM the axial nervous system, blood vessels and muscles differentiate together. Muscles result from the alignment in ribbons parallel to the nerve axis of fusiform myoblasts, (d) The dermis is formed by the cells furthest from the longitudinal axis. Their cytoplasm splits into outer and inner plasmas. The pigment appears in the internal plasmas. The epidermis remains independent of other tissues, (e) TEM the endodermal epithelium is relatively undifferentiated and is rich in ribosomes and mitochondria, (f) TEM the neoblasts are spindle-shaped cells that lack a clear central zone and with a nucleus that contains chromatin accumulations. The rough endoplasmic reticulum is dispersed but ribosomes and mitochondria abound. The Golgi apparatus is rarely observed and shows no signs of secretory activity, but centrioles are sometimes seen with the associated vesicular body, (g) TEM mesodermic cells are granulocytic, (h) SEM regeneration of pygidium, (i) SEM differentiation of pygidium and the regenerated five segments took place and (j) SEM in the aquaria some experimented clam worms did epitoky and larvae are formed, AH: Amoeboid hemocyte, B: Blastema, BV: Blood vessel, CA: Chromatin accumulation, CF: Collagen fiber, CT: Connective tissue, C: Cytoplasm, DP: Deeper plug, D: Dermis, E: Epidermis, EN: Endoblast, EP: Epiblast, GT: Glio-nervous tissue, H: Heterochromatin, MA: Mesodermic cell, M: Mitochondrion, M: Muscle, MY: Myoblast, NF: Nerve fiber, NT: Nervous tissue, NC: Neuroglia cell, N: Nucleus, Nu: Nucleolus, PA: Parapodium, PG: Phagocyte, PT: Pigment, P: Polysome, PR: Prostomium, PC: Pseudo-clot, PM: Pygidium, R: Ribosome, RER: Rough endoplasmic reticulum, SF: Segmentation furrow, S: Segments, TC: Totipotent cell, V: Vacuole, SV: Secretory vesicle

blastocystes that come from the de-differentiation of the corresponding layers in the place of the amputee. The mesoderm can be derived from undifferentiated cells from the animal intact. The epidermal epithelium consists of supporting cells, basal cells and secretory cells. It also includes sensory capsules and nerve endings. The basal cells are small but they have Golgi apparatus and a highly developed rugged endoplasmic reticulum which form the secretion of the glandular cells. The endodermal epithelium is relatively undifferentiated and is rich in ribosomes and mitochondria (Fig. 3e). This epithelium lacks basal and glandular cells. Immediately after the amputation, the epithelium detaches from its basement membrane and the endodermic cells enlarge and become blasts in the dermis. Mesodermal regenerative cells are neoblasts or undifferentiated migratory cells and myoblasts or dedifferentiated muscle fibers. Ultrastructurally, the neoblasts are spindle-shaped cells that lack a clear central zone and with a nucleus that contains chromatin accumulations. The rough endoplasmic reticulum is dispersed but ribosomes and mitochondria abound. The Golgi apparatus is rarely observed and shows no signs of secretory activity but centrioles are sometimes seen with the associated vesicular body (Fig. 3f). At the beginning of the pre-regeneration all the mesodermal cells, except muscle cells, participate in the formation of the scar cap. These mesodermic cells are granulocytic type cells coming from epidermal somatopleure cells, which can penetrate in the musculature (conjunctive cells) or cell liquid (granulocytes or vacuolar amebocytes) (Fig. 3g). The terminal structure of the pygidium appears then the formation of new segments begins in front of the pygidium. (Fig. 3 h-i). In the aquaria some experimented clam worms did epitoky and larvae are formed (Fig. 3j).

DISCUSSION

This study dealt with the regeneration of posterior segments of *Platynereis dumerilii*. After amputation of the five posterior segments with the pygidium, several layers of amoeboid hemocytes cover the wound completely and the wound is reinforced with collagen. The epithelial cells deform and spread over the entire surface of the scar. Some amoeboid hemocytes degenerate when the nerve fibers arrive at the level of the scar cap. Migration of the amoeboid hemocytes to the wound stops before the formation of the blastema. The dedifferentiation of the tissues of *Platynereis dumerilii* segments is related to the cessation of normal blood flow. As in all cases of regeneration, before dividing, blastema cells are activated. The multiplication of blastema cells is related to the nervous and blood systems. The connective tissue cells (fibrocytes) may have an old origin of fibrocytes or amoeboid hemocytes. The different stages of regeneration in this study are related to the evolution of the nervous and blood systems. The study of the mechanisms of regeneration and its regulations should take this into account as well the possible role of neurosecretory centers. Finally, note that the boundaries between the phases are not free. Regeneration is a continuous phenomenon.

Regeneration is the normal response of the organisms to restore lost structures through a process of cell multiplication and differentiation[25]. The regenerative capacity is highly developed in Phylum Annelida[26-29]. These animals can regenerate easily the anterior and posterior region of the body after being cut or damaged. If the amputation is cephalic the number of segments regenerated is typical for each species. The regeneration process has two important characteristics. Firstly, the ability to produce the blastema, a structure specialized which directs the formation of the lost organ[30,31]. Secondly, the memory, since only the missing part is always regenerated, the cells that are going to start regeneration know somehow what level has amputation occurred. Regeneration begins with the blastema formation, which is related to significant flexibility in the process of cell differentiation. This structure is composed of cells that accumulate at the site of the wound and that is totipotent, that is, they are capable of giving rise to all types of tissues, such as the primitive cells of the embryo[32]. After the wound is closed, these undifferentiated cells divide to give rise to more cells and then begin to specialize to generate the necessary structures: integument, muscle or a complete segment. What distinguishes the different regenerating polychaetes is the origin of these blastema cells. In some cases, there are pre-existing stem cells that are recruited to the site of the amputation. As in planarians these cells are distributed throughout the body and constitute up to 20% of the total organism. In other cases as in salamander, there are muscle cells close to the wound which, in a surprising deconstructive process of reverse, dedifferentiate, acquire the potential of stem cells and regenerate tissues[33]. But for the regeneration to be effective, the cells must know what they must rebuild, not only were to make muscle for example but also what muscle. This information could be present in the amputation environment (in nearby cells that, in some way, would transmit to the regenerators) or in their stem cells or dedifferentiated, by some molecular memory mechanism. The authors wish to find an approach to promote regeneration in humans to insert stem cells in the wound. The problem is that we humans do not have a large amount of totipotent stem cells spread throughout the body such as planarians[34]. There

are adult stem cells that are responsible for tissue renewal. These cells are in small numbers and are not easily recruitable when a wound occurs. They have a capacity of limited differentiation not being able to regenerate all types of tissue but only a subset. Therefore, a line of research consists of isolating adult stem cells of a patient, cultivate and expand them in the laboratory and then differentiate them to specific tissues using specific mixtures of differentiation factors. This necrotic action of the nervous system on the scar cap has already been reported in the polychaetes, *Eisenia fetida* do not regenerate segments in the absence of a nerve chain or if growth is inhibited do not regenerate, the scar cap remains in place. The newly formed integument starts to change color with that species characteristic morphology. The cleaning of cellular debris is done by phagocytosis. This property of amoebocytes, widespread in polychaetes, has been demonstrated by Chipman[35]. In amphibians, the denervation, done respecting blood flow, slows the establishment blastema and that it cannot grow but there is neither dedifferentiation nor regression of the *Salamanders extremities*. These authors consider the nervous system as an activator of mitosis. It would therefore be responsible for the growth of the blastema. Miyamoto *et al*.[36] showed the direct neurotrophic action of the nerve on the regeneration of *Lamellibrachia satsuma*. He reversibly disorganized the arrangement of microtubules in neurons by colchicine. This alkaloid does not stop neurosecretions but they cannot be transported anymore. Regeneration is disturbed and it is slower. The nervous axis pushes cells back to the periphery so that what is left of amoebocytes are found in the outermost position. This observation was also made on Octopus by Matzner *et al*.[37]. This implies the possibility of redifferentiation of a cell type (amoebocyte) into another (fibrocyte) after dedifferentiation[38], based on morphological criteria, has already made this assumption for blood cells of *Sepia officinalis*. Two hypotheses have been put forward: two cell types derive from a common precursor or else the leucocyte evolves into a fibrocyte. Works on the embryogenesis of the cuttlefish are few. Tressler *et al*.[39] followed the normal histogenesis of *Sepia officinalis* and *Sepia pharaonis* arm. They signal that the nervous axis appears early. These authors attach especially to the description of the formation of the different parts of the brachial muscular system. The sequence of events is equivalent to our observations during morphogenesis. Regeneration of the arms of *Sepia officinalis* is a complex phenomenon that is made by epimorphosis for the epidermis (from neighboring cells and without dedifferentiation) and by morphallaxis for other tissues. This study shows that regeneration requires a supply of cellular material. Only the amoebocytes emigrate during the 1st days (healing). But when this migration stops, the number of cells that will form the blastema continues to increase without mitosis. There is a local cellular reorganization. At neighborhood of the wound, cells that are too damaged degenerate and are eliminated. The others dedifferentiate, they lose their own characters and become a source of regenerative cells.

CONCLUSION

The healing process after posterior segment amputation starts with the migration of amoeboid hemocytes to the wound to form a Pseudo-clot. The new cell types which come from totipotent cells or De-differentiation cells act as progenitors. The dedifferentiated cells form the true blastema. The newly formed cells which act in the regeneration process may arise from trans-differentiation, which undergo Re-differentiation into new cell line with a different function and type.

SIGNIFICANT STATEMENT

This study concludes that totipotent cells act as progenitors during regeneration of the posterior segments and hemolymph agglutinate and form a pseudo-clot at the wound site. The endoblast forms from the internal endoderm. This study will help the researcher to uncover the critical areas of regeneration that many researchers were not able to explore. Thus a new theory on regenerative capability may be arrived at.

ACKNOWLEDGMENT

I would like to thank Alexandria University, Faculty of Science, Department of Zoology for supporting this article. Ethical clearance for this study was obtained from the Alexandria University ethics committee. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

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