*Proceeding International Conference on Religion, Science and Education* (2022) **1**, 527-536

# Histological Structure of Regenerate Tail in Lizard (*Mabouya multifasciata* Kuhl) After Treatment Using Shark Cartilage Ointment

**Rakhmiyati[1,*], T Widiyani[1,2], A Budiharjo[1,2]**
[1]Department of Bioscience, Graduate Program, Sebelas Maret University
[2]Faculty of Mathematics and Natural Sciences, Sebelas Maret University
Jl. Ir Sutami No. 36A, Kentingan, Surakarta, 57126, Central Java, Indonesia.

**Corresponding author***
miarakhmiy@gmail.com

**Abstract:** Lizards are grouped into the order Sauria which has the characteristics of a scaly body, smooth, long tongue, long tail, and four legs. Lizards (*Mabouya multifasciata* Kuhl) will sever its tail as a self-protection mechanism. After the autotomy event, the tail regeneration process will be followed to replace the severed tail. Shark cartilage contains bioactive compounds that are beneficial for humans, including for the wound healing process. Shark cartilage extract has been used medicinally to treat pain and swelling, treat scars, and for the prevention of arthritis. The lizards were grouped into four groups before being treated using shark cartilage extract ointment. The formulation of shark cartilage extract ointment used in this study consisted of four types, that is control, 5%, 10%, and 15%. The purpose of this study was to determine the effect of shark cartilage extract ointment on the histological structure of the autotomy-set lizard regenerate tail. The method used is the paraffin method with hematoxylin-eosin staining. The results of this study showed that the 5% formulation of the ointment made the blastema layer thicker and perivertebral fat tissue more abundant.

**Keywords:** histology, lizard, shark cartilage.

## Introduction

Lizard (Mabouya multifasciata Kuhl) belongs to the Scincidae family that lives in litter, holes, and trees (Apriyanto *et al*., 2015). The name *Mabouya* was proposed by Fitzinger in 1826. The genus *Mabouya* is widely distributed in Africa, southwest Asia, Madagascar, Central, and South America, and the Caribbean (Greer and Broadley, 2000). The tail on the Mabouya multifasciata Kuhl serves for balance when moving (Etheridge, 1967, Daniels, 1983 in Jagnandan, 2014; Naya *et al*., 2007). In addition, almost 50% of body fat is stored in the tail. The tail of the lizard has several functions, including storing energy (Avery, 1970; Bustard, 1967; Congdon *et al*., 1974 in Jagnandan, 2014; Naya *et al*., 2007), reproduction, and sexual display (Vitt *et al*., 1977; Smyth, 1974 in Jagnandan, 2014) and social status (Naya *et al*., 2007).

Autotomy is the process of severing the tail if the animal is in a state of threat (Winchester, 1966 in Soesilo, 1992; Gilbert *et al*., 2013). The severed tail will move to attract the attention of predators, while the animal will run to protect itself from predators (Bustard, 1968 in Luthfi, 2002; Londono *et al*., 2017). This tail termination occurs at certain places in the tail that have a fracture plane often called an autotomy plane. (Bustard, 1968 in Soesilo, 1992; Delorme *et al*., 2012).

After the autotomy event, the next process will be followed by regeneration. Regeneration is a biological phenomenon in adult organisms to regenerates lost tissues, organs, or body parts (Patnaik *et al*., 2012; Narayanan, 2015). According to Soesilo (1992) and Alibardi (2017), the lizard tail regeneration process begins with wound healing, blastema formation followed by a differentiation phase, and then tail growth. Hutchins *et al*. (2016)


ISSN 2829-3355 | EISSN 2828-8467

stated that regeneration in lizard tails begins with wound restriction using clotting blood and extracellular matrix (ECM) remodeling, wound epithelial formation, growth, and differentiation of tail tissues including neuroependymal, cartilage, and myofibers.

A shark is a cartilaginous fish (*Elasmobranchii*) that can be consumed. All parts of the shark's endoskeleton are cartilage (Dean and Summers, 2006). Shark cartilage has several benefits, including controlling the growth and spread of tumor cells, cancer, helping to reduce bone pain, avoiding rheumatic diseases, strengthening and maintaining the bone function, relieving pain and gout, maintaining body health and vitality, and avoiding bone disorders. crooked back (Sulityowati *et al*., 2015; Dean and Summers, 2006). According to Martel-Pelletier (2015), shark cartilage can also treat osteoporosis and osteoarthritis because it contains chondroitin sulfate.

The components of bioactive compounds contained in shark cartilage are very important for the wound healing process (Bargahi and Rabbani-Chadegani, 2008). Angiogenesis is a physiological process that involves the growth of new blood vessels from pre-existing vessels. Angiogenesis is a normal process and is very important in growth and development, as well as wound healing (Narasimha *et al*., 2012).

The wound healing process is an evolutionary adaptation and a fundamental biological process that will result in one of two possibilities: scar formation or reparative regeneration (Jacyniak *et al*., 2017). The scar serves as a shield from the external environment and restores the homeostasis of the injured tissue. The initial stage of the wound healing process begins with a leukocyte response and then re-epithelialization through the wound epithelium. It is intended for scarless wound healing but is also necessary for subsequent tissue formation. According to Gilbert *et al*. (2013), the reepithelialization process is very important to maintain tissue homeostasis which will affect the tail regeneration process. Several main mechanisms involved in the wound healing process are re-epithelialization, cell proliferation, angiogenesis, extracellular matrix (ECM) deposition, and remodeling. (Jacyniak *et al*., 2017).

According to Rosas-cruz *et al* (2020), tuber extract ointment *Solanum tuberosum* L. formulation 2% is very effective in accelerating the wound healing process in *Mus musculus* Balb/c. Alibardi and Meyer-Rochow (2021) explained that the regenerating tail tuatara has several functions, including as a place to store fat, regulate balance when moving, be more aggressive, especially in male tuatara. At the time of regeneration, the integumentary layer serves as a protection from microbes. Research on the effect of gecko saliva (*Gekko gecko* L.) on the healing process of garden lizard tail wounds (*Eutropis multifasciata* Kuhl) proved that gecko saliva can trigger angiogenesis during the wound healing process (Inayah *et al*., 2017).

## Materials and Methods

Thirty-six male lizards with average weight, body length, and total length along with their standard deviations are as follows: 48±13,35 grams, 10±0,75 cm, and 27±2,07 cm. Histology slides were made using the paraffin method with Hematoxylin-Eosin (HE) dye. While shark cartilage extract ointment is made using a mixture of vaseline album, liquid paraffin, and shark cartilage extract. All histology data were analyzed descriptively.

## Results and Discussion

The autotomy on the lizard's tail starts with the tearing of the skin and then the muscle will separate at the septum. After that, there will be a separation of fat tissue followed by the separation of the vertebrae in the autotomy plane. Wounds caused by autotomy events cause visible epidermis, muscle tissue, fat tissue, vertebrae, and spinal cord. According to Soesilo (1982), the surface of the wound will be covered by blood and damaged cells called crusts. The regeneration process begins with wound healing, blastema layer formation, differentiation, and morphogenesis (Soesilo, 1982). Wound healing begins with the

proliferation of epithelial cells present in the skin towards the wound surface area. Proliferative events occur 2 or 3 days after autotomy. During proliferation, these cells also divide by mitosis. At 4 and 5 days after autotomy, the wound surface on the lizard tail was completely covered by skin epitheliocity. The tissue beneath the epitheliocity layer is dedifferentiated and together with the epitheliocity will form a blastema. At 6 days after autotomy, the ependymal cells will proliferate. The spinal cord will appear first then followed by the redifferentiation of the tissues in the blastema.

In the longitudinal section of the lizard's tail 7 days after autotomy, the regenerating tail has not yet been formed. Tail with control ointment treatment (Figure 1. K) has not shown the formation of blastema tissue because the wound epithelium is still at the edges. Wound epithelium will migrate to the center of the wound and the tissue near the wound surface will increase and perform tissue dedifferentiation. This causes the spinal cord to come into direct contact with damaged tissue that is still degenerating (*degenerated stump tissue*). In this figure (1. K) It was also seen that the original tail muscle was under the skin's epidermal tissue and the original tail scales were seen protruding towards the middle so that the wound surface looked concave. Between the degenerated stump tissue and the original tail muscle tissue, there is no fat tissue like that in the lizard tail wound which was treated using shark cartilage extract ointment formulations of 5%, 10%, and 15% (Figure 1. P1, 1. P2 and 1. P3).

Treatment using shark cartilage extract ointment formulation 5% showed that very thick blastema tissue had formed and because the thickest layer was in the middle, it looked like the letter V. Fat tissue under the blastema layer was abundant and surrounded the spinal cord. (Figure 1. P1). Meanwhile, the histological structure of the lizard tail wound that was treated using a 10% formulation of shark cartilage extract ointment showed a thinner blastema layer and a flat surface. (Figure 1. P2), the same as the surface on the tail which was treated using a 5% ointment formulation (Figure 1. P1). Beneath the blastema layer is a large amount of fatty tissue. The most abundant fat tissue was found in lizard tails which were treated using a 10% ointment formulation.

Longitudinal cross-section of the tail wound of a lizard treated using a 15% formulation of shark cartilage extract ointment (Figure 1. P3) showed the thinnest blastema structure compared to the blastema structure in tail wounds treated using 5% and 10% ointment formulations. The blastema layer covers the entire wound surface and is visible at the tip (convex). This convex end is known as the apical cap. Around the apical cap is a layer of the blastema. This blastema layer is the thinnest when compared to the blastema layer on the wound surface of lizard tails treated using ointment formulations of 5%, and 10%. (Figure 1. P1, 1. P2).

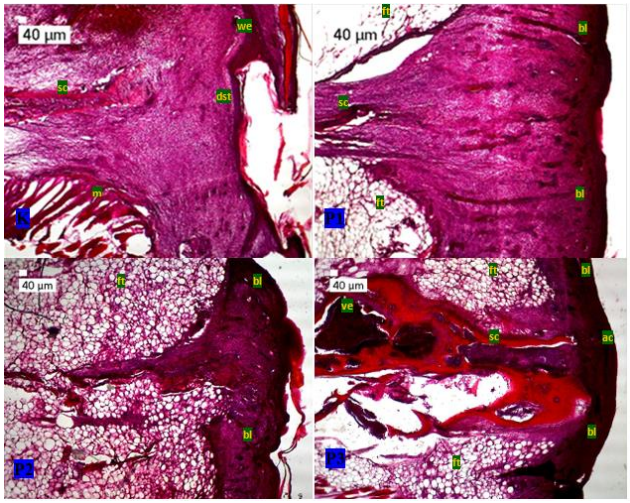


**Figure 1.** Histological structure of the regenerate tail in lizard (*Mabouya multifasciata* Kuhl) longitudinal section 7 days after autotomy.
Description: (**K**) control; (**P1**) 5%; (**P2**) 10%; (**P3**) 15%; **m**, *muscle*; **sc**, *spinal cord*; **we**, *wound epithelium*; **dst**, *degenerated stump tissue*; **ft**, ; *fat tissue*; **bl**, *blastema*; **ve**, *vertebra*; **ac**, *apical cap*.

The formulation of shark cartilage extract ointment with different formulations (control, 5%, 10%, and 15%) used to treat lizard tail wounds after autotomy caused differences in histological structures. These differences include the amount of fat tissue and the thickness of the blastema layer. In addition, there are three types of shapes on the wound surface that is concave (Figure 1. K), flat (Figure 1. P1, 1. P2) and convex (Figure 1. P3).

According to Alibardi, 2010 in Fisher *et al*., 2012 this wound healing process will last for 10 days. Wounds caused by autotomy show the epidermis, spinal cord, adipose tissue, epaxial muscles, hypaxial muscles, caudal arteries, and vertebral bone (Gilbert *et al*., 2013). The wound healing stage begins with blood clotting, the exudate fluid dries up, and the formation of epidermal tissue. According to Cox 1969 in Soesilo, 1982 stated that wound closure occurs due to the process of mitotic proliferation of skin epitheliocytic cells. Epithelial tissue repair or reepithelialization occurs in the original tail epidermis starting from the edge. This process serves to re-establish homeostasis in the tissue and greatly determines the success of tail regeneration (Gilbert *et al*., 2013). The first blastema will form along with the process of epithelial formation.

Research conducted by Soesilo (1982) on the tail regenerate lizard (*Mabouya multifasciata* Kuhl) 6 days after autotomy showed that the tissue dedifferentiation process had occurred on the wound surface. This dedifferentiated tissue together with epitheliocytic cells will form a blastema that covers the wound surface. On green anoles (*Anolis carolinensis*) after the autotomy, it will be followed by reepithelialization so that the blood that comes out is not much and prevents tissue damage around the wound. After that, a blastema layer appears on the wound surface. This wound healing process occurs for 10 days after the autotomy (Nain *et al*., 2016; Alibardi, 2009).

On 18 days after autotomy Tail regeneration has appeared even though its growth has not been maximized. The base, middle, and ends of the tail of the regenerate treated using the control formulation ointment can be seen in figure 2. IA, 2. IB and 2. IC. The longitudinal section shows that the tail that has not undergone autotomy is composed of vertebrae, autotomy plane or fracture plane, and spinal cord, perivertebral fat tissue, muscles, and ventral processes. (Figure 2. IA, 2. IIA). This structure corresponds to that described by White (1925) in Soesilo, (1999).

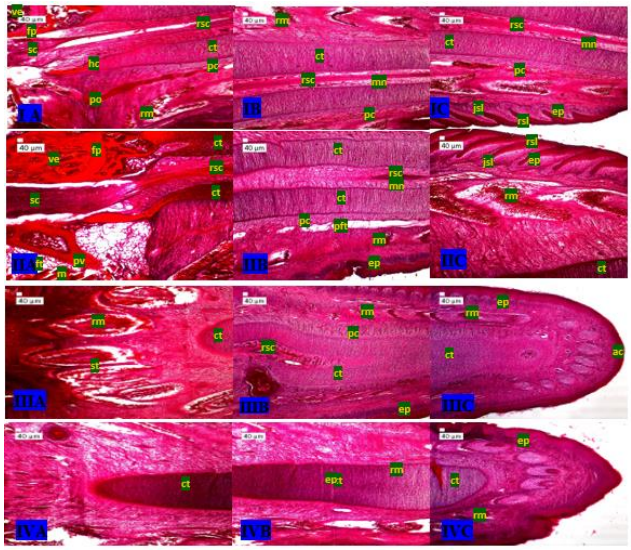


**Figure 2.** Histological structure of the regenerate tail in lizard (*Mabouya multifasciata* Kuhl) longitudinal section 18 days after autotomy. Description: (**I**) control; (**II**) 5%; (**III**) 10%; (**IV**) 15%; (**A**) anterior; (**B**) middle; (**C**) posterior; ; **ve**, *vertebra*; **fp**, *fracture plane*; **sc**, *spinal corde*; **hc**, *hypertrophic chondrocyte region*; **po**, *periosteum*; **rm**, *regenerated muscle*; **rsc**, *regenerated spinal cord*; **ct**, *cartilage tube*; **pc**, *perichondrium*; **mn**, *meninges*; **jsl**, *joints of scales*; **ep**,*epidermis*; **rsl**, *regenerated scales*; **ft**, *fat tissue*; **pv**, *processus ventral*; **m**, *muscle*; **pft**, *perivertebral fat tissue*; **st**, *septum*; **ac**, *apical cap*.

From the results of observations at 18 days after autotomy, a cartilaginous tube structure or cartilage was formed as a substitute for vertebrae. The regenerated tails were treated using a control ointment formulation and 5% showed the presence of regenerated scales (Figure 2. IC, 2. IIC) on the surface of the regenerate tail even though the scale structure is not perfect. The regenerated tail scales cover the entire surface down to the tip of the tail. Meanwhile, the regenerate tails which were treated with 10% and 15% ointment formulations on the surface have not yet appeared scales regenerated tail (Figure 2. IIIB, 2. IIIC, 2. IVB, 2. IVC). The higher the formulation of shark cartilage extract ointment, the scale primordia on the surface of the regenerate tail has not yet appeared.

According to research conducted by Soesilo (1982), the scales on the tail of the lizard (*Mabouya multifasciata* Kuhl) regenerate have started to form 14 days after autotomy. The beginning of the formation of these scales is marked by the protrusion of the epidermis towards the dermis. The structure of the regenerated tail scales is different from that of the original tail. The pattern

or layout of the scales on the original tail does not show a segmented structure and is arranged in an imbricate manner, while in the regenerated tail the scales are not arranged in an imbricate arrangement. According to Chang *et al*., 2009 scales on lizards are arranged overlapping one another and are asymmetrical. The arrangement of overlapping scales makes the position of the scales tilted towards the posterior and grows upwards to form an angle that eventually emerges to the surface skin (Landreneau, 2011). The surface of the scales is cornified so that the texture of the scales becomes stiff. Scales contain keratin $\alpha$ and $\beta$, where keratin $\alpha$ serves to reduce water evaporation, while keratin $\beta$ serves to make the skin stiff (Chang *et al*., 2009). Scales originate from the superficial layer of the epidermis of the skin and form associated joints (Rutland *et al*., 2019).

The color of the scales on the regenerate tail is paler than that of the original tail. The color difference between the original tail and the regenerated tail is due to the lack of melanophores in the regenerated tail (Soesilo, 1982; Wu *et al*., 2014). The results showed that the scales on the tail of the regenerate that were smeared with shark cartilage extract ointment were not pointed at the ends and were very close to each other. (Figure 2. IC, 2. IIC). Under the scales there is a thin layer of the epidermis, then under the epidermis, there is regenerating muscle tissue. The epidermis is a layer of squamous epithelium that continuously repairs. In the epidermis there is the stratum corneum, then under the stratum corneum lies the dermis layer which has collagen fibers and subcutaneous (Gilbert *et al*., 2013). According to Rutland *et al*. (2019), The scales on lizards consist of three layers, namely the basal layer (stratum germinativum) which is composed of a collection of cuboidal cells that produce the protein keratin. The intermediate layer (stratum granulosum) contains many lipids that serve as a permeable membrane in the skin. The stratum corneum is the outermost part of the skin that undergoes keratinization.

In the lower part of the epidermis, there is a regenerated tail muscle which is structurally different from the original tail muscle. When the tail is severed, 8 muscle bundles are visible. Soesilo (1982) said that in the tail of the lizard regenerate there are 16 muscle bundles. According to Fisher *et al*., 2012 the size of the muscle bundles in the tail of the regenerate is not uniform and irregular and the connective tissue is increasing in number. Observing the cross-section of the regenerate tail 18 days after autotomy, it will be seen that between the muscle bundles there is a septum as an insulator. One muscle bundle consists of several myotomes separated by the myoseptum which divides the muscle bundle into two parts. From the observations, it can be seen that the original tail muscle has a larger myotome size than the myotome in the regenerated tail (Figure 2. IIA, 2. IIC). The largest muscle bundle size was in the tail of the regenerate which was treated using a 5% ointment formulation. (Figure 2. IIC), In addition, the number of myotomes was the highest compared to the regenerated tails treated with control (0%), 10%, and 15%.

In the original tail, the muscle bundles were directly attached to the vertebral skeleton, while in the regenerated tail the muscles were not attached to the vertebral skeleton. In the regenerating tail, the vertebral skeleton is replaced by a cartilaginous tube as shown in the results of this research in Figure 2. IB, 2. IC, 2. IIB, 2. IIIB. The connective tissue in the regenerating tail is referred to as "*tendon-like*", connecting the radial septa with some fibrous tissue covering the perichondrium of the cartilaginous tube. The development of regenerating muscle starts from blastema cells which condense and differentiate into mononuclear myoblasts to form myomers. When distal myomers are formed, myoblasts from proximal myotomes elongate and align, and eventually coalesce to form multinucleated myofibers. Each myotome increases in size as new myoblasts differentiate and coalesce with existing fibers until successive myomers come into contact, forming myomeric segments.

At 18 days after autotomy, perivertebral fatty tissue had not yet formed. However, perivertebral fat tissue was only seen in the regenerated tails treated with a 5% formulation of TRIH ointment. (Figure 2. IIB). This tissue is found beneath the muscles surrounding the cartilaginous tube. From the histology image it can be seen that the

cartilaginous tube is surrounded by the perichondrium (Figure 2. IB, 2. IC, dan 2. IIIB). This makes the perichondrium directly adjacent to the cartilaginous tube. Within the cartilaginous tube are the meninges and the regenerated spinal cord. This is the same as the research conducted by Soesilo (1982) and Luthfi *et al.* (2003). In addition, in the cartilaginous tube, there are also ependymal cells (*ependymocyti*) and canalis centralis. According to Fisher *et al*., 2012 at this stage, the formation of mesenchymal and ependymal cells has begun.

The cartilaginous tube structure in the regenerated tail is unsegmented, whereas in the original tail it is segmented (Lozito and Tuan, 2017). Another difference between the original caudal vertebrae and regenerated caudal vertebrae is that in the original caudal vertebrae there are dorsal processes, ventral processes, lateral processes, or transverse processes, prezygapophysis and postzygapophysis, whereas in the regenerated caudal vertebrae there are only cartilage tubes or cartilages. From Figure 2. IA and 2. IIA it can be seen that the type of vertebral joint in lizards is procelous (the convex intercentrum is on the posterior side of the centrum). This type of joint is by the statement of Soesilo (1982) and Kent (1987).

At the beginning of the lizard tail regeneration process, the wound healing process occurs. Giving glucosamine to the wound serves to prevent infection and inflammation or inflammation of the tissue around the wound. According to Hartomo *et al*. (2014) and Al-Saadi *et al* (2019) Glucosamine (GS) has anti-inflammatory activity and can prevent infection. After that, there was a proliferation of epithelial cells in the tail skin. The application of glucosamine (GS) and chondroitin sulfate (CS) can affect the process of epithelial cell proliferation. The results of this study indicate that in the 5% ointment formulation the rate of proliferation of epithelial cells runs faster than other ointment formulations so that the blastema layer formed is thicker. (Figure 1. P1). This is consistent with the statement that GS and CS can increase cell proliferation and increase cartilage length (Jerosch, 2011; Wolff, 2014; Al-Saadi *et al*, 2019; Lopes-senra *et al*., 2020; Sinclair *et al*., 2021). According to Hartomo *et al* (2014) There are four processes in cell proliferation, namely re-epithelialization, migration and proliferation of fibroblasts, angiogenesis (formation of new blood vessels), and formation of granulation tissue.

After the proliferative process, the blastema layer is formed. This regeneration process involves several types of cells for the formation of wound epithelium or what is known as the apical cap. From the wound epithelium, it gives a signal in the form of growth factors such as Transforming Growth Factor-Beta (TGF-β) and Fibroblast Growth Factor (FGF). This signaling response leads to the proliferation and migration of cells that make up the blastema (Sinclair *et al*., 2021; Ferreti *et al*., 2001).

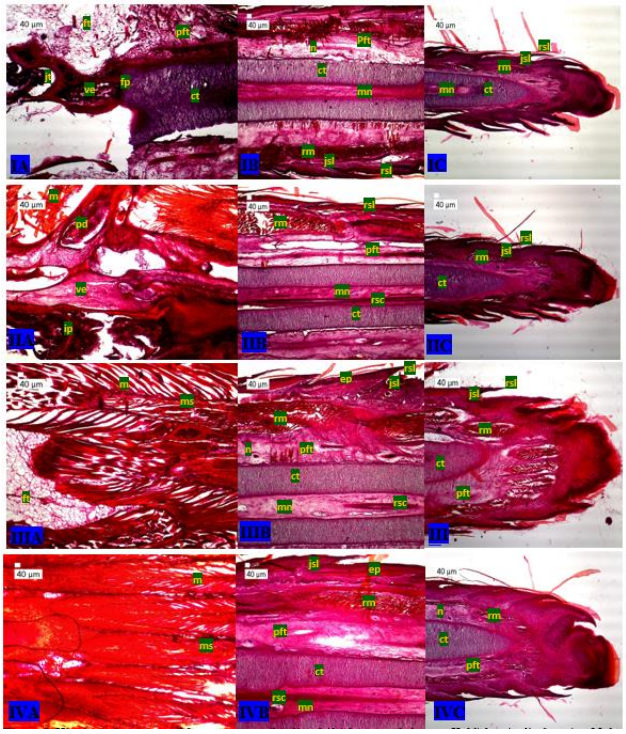

**Figure 3.** Histological structure of the regenerate tail in lizard (*Mabouya multifasciata* Kuhl) longitudinal section 35 days after autotomy. Caption: (**I**) control; (**II**) 5%; (**III**) 10%; (**IV**) 15%; (**A**) anterior; (**B**) middle; (**C**) posterior; **ft**, *fat tissue*; **ve**, *vertebra* ; **fp**, *fracture plane*; **ct**, *cartilage tube*; **jt**, *joints*; **pft**, *perivertebral fat tissue*; **ep**, *epidermis*; **n**, *nerves*; **mn**, *meninges*; **rm**, *regenerated muscle*; **jsl**, *joints of scales*; **rsl**, *regenerated scales*; **m**, *muscle*; **pd**, *processus dorsal*; **ip**, *intervertebral pads*; **rsc**, *regenerated spinal cord*; **ms**, *muscle segment*.

Ferreti *et al* (2001) added that FGF is essential for the regeneration of bone marrow, ependymal cells and mesen,chymal tissue. FGF also plays a role in the proliferation of nerve cells. After the

formation of blastema followed by the process of angiogenesis or vascularization. This is very necessary so that the oxygen needs of the new tissue are met. Soesilo (2002) said that if the angiogenesis process decreases, the wound healing process in lizards will be delayed.

Angiogenesis is the formation of new blood vessels starting from existing blood vessels (old blood vessels) (Hartomo *et al*., 2014; Boba *et al*., 2017). The formation of new blood vessels is stimulated by growth factors such as Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), Transforming Growth Factor-Beta (TGF-β), Epithelial Growth Factor (EGF) (Hartomo *et al*., 2014). The interaction between growth factors and their receptors is what initiates angiogenesis. The role of GS and CS is to stimulate angiogenesis and help expand the new network of blood vessels (Borba *et al*., 2017).

At the age of 35 days of regenerating tathe il, the cartilage tube is in the process of maturation or calcification. Calcification starts from the inside and outside of the cartilage tube (Luthfi *et al*., 2003). Alibardi, 2010 in Fisher *et al*, 2012 said that the tail maturation process took place from the 25-60 days after autotomy.

At this time in each treatment (control, 5%, 10,% and 15%) scales appeared that covered the entire surface of the lizard regenerate tail. The structure of the scales was more clearly visible (stretched and pointed ends of the scales), in contrast to when they were 18 days after autotomy. The joints of the scales are visible because the epidermis is no longer protruding towards the dermis. The structure of these scales was the same in all regenerated tails smeared using the control formulation ointment, 5%, 10% and 15%. Under the scales, there is a layer of the epidermis, while at the bottom of the epidermis there is a regenerating tail muscle tissue. The thickness of the epidermis on the regenerate tail is almost the same as the original tail (Soesilo, 1982).

Muscle of original tail and regenerate tail (Figure 3. IIA. 3. IB, 3. IIB, 3. IIIB, 3. IVB) attached to different places. The original tail muscles will be attached directly to the cacaudalertebra via tendons or indirectly via connective tissue septa, whereas in the regenerated tail the muscles will be attached to the cartilaginous tube and bonded to each other. The size of the muscle bundle is getting bigger because the number of myotomes in it is increasing (Figure 3. IB, 3. IIB, 3. IIIB, 3. IVB).

Beneath the muscle tissue is the perivertebral fatty tissue. This tissue was found in all regenerate tails which were smeared using the control formulation ointment, 5%, 10% and, 15%. The cartilaginous tube in the regenerating tail is covered by perivertebral fat tissue. In this fat tissue, there is some nervous tissue. A neural network is present in all regenerate tails (Figure 3. IB, 3. IIB, 3. IIIB) except for regenerate tails which were treated with 15% ointment formulation. The higher the content of shark cartilage extract in the ointment causes the nervous tissue to not appear yet.

Inside the cartilaginous tube is the meninges layer and in the middle is the regenerated spinal cord. A In further development of the cartilaginous tube there will be fractures (*foramina*) whose layout or structure is not bilaterally symmetrical or unevenly spaced. This can be seen after 65 days post autotomy. *Foramina* or foramen serves as a gap for the entry of blood vessels into the cartilage tube (Fisher *et al*., 2012). Nain *et al* (2016) also said that the cartilaginous tube contains foramina that are irregularly spaced from one another. This irregular spacing of the foramina serves as an entry point for blood vessels.

Accordto ing Jerosh (2011) said that glucosamine and chondroitin sulfate are chondroprotective. Chondroprotective is an important component for the process of cartilage metabolism and stimulates the process of cartilage regeneration. Glucosamine (GS) plays an important role in the formation of hyaluronic acid. Chondroitin sulfate can stimulate chondrocyte metabolism, which leads to the synthesis of collagen and proteoglycans, which are components of cartilage. In addition, chondroitin sulfate (CS) can also stimulate the production of extracellular matrix (ECM) cartilage (Vasiliadis and Tsikopoulos, 2017).

Collagen synthesis by fibroblasts is stimulated by TGF-β. Fibroblasts will move actively from the tissue around the wound to the center of the wound, then proliferate and secrete substances in

the form of collagen, hyaluronic acid, elastin, fibronectin, and proteoglycans that are useful in tissue repair. (Hartomo *et al*., 2014). The combination of GS and CS can stimulate cartilage regeneration through mesenchymal cell chondrogenesis (Wolff, 2014).

The content of GS and CS compounds dissolved in the TRIH extract was influenced by the treatment at the time of making the TRIH extract. In addition, another factor that affects the glucosamine content is the length of maceration time. Zhang *et al*. (2018) explained that maceration takes a long time but the results are less efficient.

## Conclusions

Histological observation showed that at the age of 7 days of regeneration, the thickest blastema layer was found in the tail of the regenerate which was smeared with 5% ointment formulation. The tail of the 18-day-old regenerate which was smeared with 5% formulation of the ointment had already appeared perivertebral fatty tissue. Meanwhile, at the age of 35 days, histologically there was no difference between the tails smeared with the control formulation ointment, 5%, 10,% and 15%.

## References


Alibardi L .2009. *Morphological and Cellular Aspects of Tail and Limb Regeneration in Lizards: A Model System with Implications for Tissue Regeneration in Mammals*. In: Alibardi L. Regeneration in Reptiles and Its Position Among Vertebrates. Springer, United Status.

Alibardi L. 2017. FGFs Treatment on Amputated Lizard Limbs Stimulate the Regeneration of Long Bones, Opening New Avenues for Limb Regeneration in Amniotes: A Morphological Study. *Journal of Functional Morphology and Kinesiology* 2 (25): 1-17.

Albardi L and VB Meyer-Rochow. 2021. Regeneration in Reptiles Generally and the New Zealand Tuatara in Particular as a Model to Analyse Organ Regrowth in Amniotes: A Review. *Journal of Developmental Biology* 9 (36): 1-21.

Al-Saadi HM, K Lun-Pang, SI Nirwana, K Yong-Chin. 2019. Multifaceted Protective Role of Glucosamine against Osteoarthritis: Review of Its Molecular Mechanisms. *Scientia Pharmaceutica* 87 (34): 1-18.

Apriyanto P, AH Yanti, TR Setyawati. 2015. Keragaman Jenis Kadal Sub Ordo Sauria pada Tiga Tipe Hutan di Kecamatan Sungai Ambawang. *Protobiont* 4 (1): 108-114.

Avery RA. 1970. Utilization of caudal fat by hibernating common lizards, Lacerta vivipara. *Comp. Biochem. Physiol* 37: 119-121.

Bargahi A and A. Rabbani-Chadegani A. 2008. Angiogenic inhibitor protein fractions derived from shark cartilage. *Journal Biochemical Society* 28 (1): 15–21.

Borba FKSL, EVL Costa, VAB Polli, DS Coelho, M Maraschin, PF Dias and RA Nogueira. 2017. Pro-angiogenic Activity Assay of Chondroitin Sulfate and Glucosamine Sulfate on Vascular Network of Mouse and of Chick Embryo Chorioallantoic Membrane. *Journal of Glycobiology* 6 (3): 1-6.

Chang C, P Wu, RE Baker, PK Maini, L Alibardi, and CM Chuong. 2009. Reptile scale paradigm: Evo-Devo, pattern formation, and regeneration. *The International Journal of Developmental Biology* 53: 813-826.

Dean MN and AP Summers. 2006. Mineralized Cartilage in the skeleton of chondrichthyan fishes. *Zoology* 109 (2): 164-168.

Delorme SL, IM Lungu and MK Vickaryous. 2012. Scar-Free Wound Healing and Regeneration Following Tail Loss in the Leopard Gecko, Eublepharis macularius. *The Anatomical Record* 295 (10):1575–1595.

Ferreti P, F Zhang, LS Ruis, and JDW Clarke. 2001. FGF signalling and blastema growth during amphibian tail regeneration. *International Journal Development Biology* (45): 127-128.

Fisher RE, LA Geiger, LK Stroik, ED Hutchins, RM George, DF Denardo, K Kusumi, J Alan Rawls and J Wilson-Rawls. 2012. A Histological Comparison of the Original and Regenerated Tail in the Green Anole, Anolis carolinensis. *The Anatomical Record* 295 (10):1609–1619.

Gilbert RWD, MK Vickaryou,s and AM Viloria-Petit. 2013. Characterization of TGFb Signaling During Tail Regeneration in the Leopard Gecko (Eublepharis macularius). *Developmental Dynamics* 242:886–896.

Greer AE and DG Broadley. 2000. Six Characters of Systematic Importance In The Scincid Lizard Genus Mabuya. *Hamadryad* 25 (1): 1-12.

Hartomo BT, FG Firdaus. 2014. Pemanfaatan Biomaterial Kitosan Dalam Bidang Bedah Mulut. *Jurnal Kedokteran Gigi Universitas Baiturrahmah* 6 (1): 63-70.

Hutchins ED, Wilson-Rawls, and Kusumi. 2016. Innovations in Molecular Mechanisms and Tissue Engineering, Stem Cell Biology and Regenerative Medicine. Springer International Publishing Switzerland. Arizona State University, Phoenix. The USA. P:23-35.

Inayah N, NP Soesilo and R Pratiwi. 2017. Effect of Tokay Gecko (Gekko gecko LINNAEUS, 1758) Saliva on Angiogenesis During Wound Healing Phase of Autotomized Tail in Common Sun Skink (Eutropis multifasciata KUHL, 1820. *Jurnal Biologi Indonesia* 13 (2): 253-260.

Jacyniak K, RP McDonald, MK Vickaryous. 2017. Tail regeneration and other phenomena of wound healing and tissue restoration in lizards. *Journal of Experimental Biology* 220 (16): 2858-2869.

Jagnandan K, AP Rus,sell and TE Higham. 2014. Tail autotomy and subsequent regeneration alter the mechanics of locomotion in lizards. *The Journal of Experimental Biology* (217):3891-3897.

Jerosch J. 2011. Effects of Glucosamine and Chondroitin Sulfate on Cartilage Metabolism in OA: Outlook on Other Nutrient Partners Especially Omega-3 Fatty Acids. *International Journal of Rheumatology*. Page: 1-18.

Kent GC. 1987. *Comparative Anatomy of the Vertebrates*. Sixth edition. Times Mirror/ Mosby College Publishing. UThe nited States of America. p: 192.

Landreneau EB. 2011. *Scales and Scale-Like Structures*. Dissertation. College Station: Texas A&M University

Londono R, Wenzhong, Wang, RS Tuan, and TP Lozito. 2017. cartilage and Muscle cell Fate and Origins during lizard Tail regeneration. *Frontiers in Bioengineering and Biotechnology* 5 (70): 1-9.

Lopez-senra E, P Casal-Beiroa, M López-Álvarez, J Serra, P González, J Valcarcel, JA Vázquez, EF Burguera, FJ Blanco, and J Magalhães. 2020. Impact of Prevalence Ratios of Chondroitin Sulfate (CS)- 4 and -6 Isomers Derived from Marine Sources in Cell Proliferation and Chondrogenic Differentiation Processes. *Marine Drugs* 18 (94): 1-13.

Lozito TP and RS Tuan. 2017. Lizard Tail Regeneration As An Instructive Model of Enhanced Healing Capabilities In An Adult Amniote. *Connective Tissue Research* 58 (2): 145–154.

Luthfi MJ. 2002. Kalsifikasi Skeleton Aksial dan Kemampuan Autotomi Regenerat Ekor Kadal (Mabouya multifasciata Kuhl). ***Tesis***. Yogyakarta: Universitas Gadjah Mada.

Luthfi MJ, NP Soesilo, M Sagi. 2003. Kalsifikasi Skeleton Aksial pada Regenerat Ekor Kadal (Mabouya multifasciata Kuhl). *Biologi* 3 (1): 1-8.

Martel-Pelletier J, A Farran, E Montell, J Verges, JP Pelletier. 2015. Discrepancies in Composition and Biological Effects of Different Formulations of Chondroitin Sulfate. *Molecules* 20 (3): 4277-4289.

Nain Z, MDA Islam, SH Chowdhury, S Afroza, I Hussain. 2016. Current Understanding on Tail Regeneration in Green Anoles (*Anolis carolinensis*). *Cell Biology* 4 (2): 9-17.

Narasimha GA, A Janardhan, P Kumar, MR Pradeep, DS Gopal. 2012. Wound healing property of bioactive compound from actinomycetes. *Der Pharmacia Sinica* 3 (5):542-545.

Narayanan A. 2015. The Initiation and Progression of Tail Regeneration in Northern House Gecko Hemidactylus Flaviviridis at Role of Fibroblast Growth Factor 2 (Fgf2). *Journal Biochip and Tissue Chip* 5 (1): 1-7.

Naya DE, C Veloso, LP José, Muñoz and Francisco Bozinovic. 2007. Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comparative Biochemistry and Physiology* 146: 189–193.

Patnaik N, C Mahapatra, SK Dutta, PK Mahapatra. 2012. Role of Acid and Alkaline Phosphatase During Vitamin A Induced Abnormal Tail Regeneration in the Tadpoles of the Indian Tree Frog. *International Journal of Current Research* 4 (11): 115-120.

Rosas-cruz GP, CR Silva-Correa, AA Calderón-Peña, VE Villarreal-La Torre, CL Aspajo-Villalaz, JL Cruzado-Razco, JD Rosario-Chávarri, JC Rodríguez-Soto, OE Pretel-Sevillano, WAS Guarniz, AD González-Siccha. 2020. Wound Healing Activity of an Ointment from Solanum tuberosum L. "Tumbay Yellow Potato" on Mus musculus Balb/c. *Pharmacognosy Journal* 12 (6): 1268-1275.

Rutland CS, P Cigler and V Kubale. 2019. *Reptilian Skin and Its Special Histological Structures*. In: Rutland C and V Kubale. Veterinary Anatomy and Physiology. University of Nottingham, Great Britain.

Sinclair JW, DR Hoying, E Bresciani, DD Nogare, CD Needle, A Berger, W Wu, K Bishop, AG Elkahloun, A Chitnis, P Liu, and SM Burgess. 2021. The Warburg effect is necessary to promote glycosylation in the blastema during zebrafish tail regeneration. *Regenerative Medicine* 6 (55): 1-16.

Soesilo NP. 1982. *Regenerasi Ekor Kadal* (*Mabouya multifasciata* Kuhl) *Setelah Mengalami Autotomi*. Tesis. Yogyakarta: Universitas Gadjah Mada.

Soesilo NP. 1992. Proses Regenerasi Ekor Kadal (Mabouya multifasciata Kuhl). *Biologi* 1 (4): 169-175.

Soesilo NP. 1999. Peranan Lapisan Ependima Dalam Regenerasi Ekor Kadal (*Mabouya multifasciata* Kuhl). *Biologi* 2 (8): 419-450.

Soesilo NP. 2002. Pengaruh Regenerat Ekor Kadal (Mabouya multifasciata Kuhl.) Terhadap Angiogenesis. Berkala Ilmiah *Biologi* 2 (14): 833-843.

Sulityowati W, TA Indhira, A Arbai, Yatmasari. 2015. Glucosamine and Chondroitin Sulphate Content of Shark Cartilage (Prionace glauca) and its Potential as Anti-Aging Supplements. *International Journal of Chemical Technology Research* 8 (10): 163-168.

Vasiliadis HS and K Tsikopoulos, 2017. Glucosamine and chondroitin for the treatment of osteoarthritis. *World Journal of Orthopedics* 8 (1): 1-11.

Vitt LJ, JD Congdon, and NA Dickson. 1977. Adaptive Strategies and Energetics of Tail Autotomy in Lizards. *Ecology* 58 (2): 326-337.

Wolff RB. 2014. Glucosamine and chondroitin sulfate association increases tibial epiphyseal growth plate proliferation and bone formation in ovariectomized rats. *Clinics* 69 (12): 1-7.

Wu P, L Alibardi and CM Chuong. 2014. Regeneration of reptilian scales after wounding: neogenesis, regional difference, and molecular modules. *Regeneration* 1 (1): 15-26.

Zhang QW, LG Lin and WC Ye. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine* 13 (20): 1-26.