The Role of Mast Cells in Fetal Wound Healing

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements for graduation *with distinction* in Biology in the undergraduate colleges of The Ohio State University

By

Melissa Meleski

The Ohio State University

March 2010

Project Advisor: Professor Traci A. Wilgus

Department of Pathology

Abstract:

Normally, in adults, wounds to the skin end in the formation of a scar. Scar tissue formation inhibits the normal functioning of skin and can lead to limited joint mobility, impaired growth, and wound dehiscence. This abnormal tissue often results from excessive inflammation at the wound site. Recently, studies have found that major differences exist in the healing process of adult and fetal skin, the most noteworthy of which is the lack of scar formation in fetal wounds. In mice, scarless healing occurs up until embryonic day sixteen (E16). One possible reason for the disparity in wound healing between adult and fetal skin could be the presence of different numbers of mast cells, a specific type of inflammatory cell. Toluidine blue was used to identify mast cells within both unwounded and wounded E15 and E18 skin. A significantly larger number of mast cells were found in unwounded E18 skin, which forms scars after injury, than were found in unwounded E15 skin. The mast cells in the unwounded E18 skin also possessed more granules than those found in the unwounded E15 skin. Similarly, a significantly larger number of mast cells were found in wounded E18 skin than were found in wounded E15 skin. The mast cells in the wounded E18 skin also released a larger number of granules indicating the presence of more activated mast cells. The results obtained in this study suggest a relationship between mast cell presence and the transition from scarless and fibrotic healing which takes place during development. These findings could lead to future studies which could develop new drugs and therapies that may help reduce or even eliminate scar formation in the future.

Introduction:

The largest organ in the body, the skin, is made up of two distinct layers, the outermost epidermis and the underlying dermis [1]. The skin has a natural mechanism by which it repairs itself, but this mechanism is not always completely effective. Each year approximately six million Americans alone suffer from chronic skin wounds which result from improper repair [2]. These wounds are caused by a variety of factors including diabetes, circulatory problems, and essential surgeries, but one attribute is commonly found among each of the individuals plagued with this affliction: the inevitable formation of scar tissue [3]. The problems that can arise from wound healing and the production of scar tissue are abundant. Scar tissue can potentially lead to limited mobility of the joints, impaired growth, and the loss of normal skin functions. Scar tissue is also weaker than normal skin which can lead to wound dehiscence [4]. Wound dehiscence is characterized by a premature bursting open of a wound along a surgical suture. This untimely exposure of the area beneath the wound oftentimes leads to infection and can cause an increase in overall patient recovery time [3]. Furthermore, the potential cosmetic consequences cannot be ignored. Scars have been found to cause both psychological and social problems later in patients' lives. Numerous studies have shown that even small scars can negatively impact a patient's quality of life [5].

Wound healing is typically defined as the body's natural process of regenerating dermal and epidermal tissues. Recently, studies have found that major differences exist in the healing process of adult and fetal skin. Ultimately though, the most significant disparity between the two is thought to be the lack of scar formation in fetal wounds resulting from a drastically reduced inflammatory response [6].

Adult wound healing is usually a three step process of inflammation, proliferation, and scar formation [3]. After an initial injury to the skin, hemostasis is the first phase of an individual's defense, hemostasis being the biological process which impedes bleeding. This is typically carried out within the human body by three consecutive processes. First and foremost, the cause of the bleeding event, a damaged blood vessel, must be addressed, typically by vasoconstriction, or a narrowing of the blood vessels as a result of muscular contraction with the vessel walls. Next, a 'platelet plug' is formed when platelets begin to adhere to the damaged endothelial layer of the vessel (the layer of cells which lines the interior surface of a blood vessel). Finally, a blood clot is formed when the inactive protein fibrinogen is converted into its active form fibrin. Fibrin is a protein involved in the clotting of blood. Once the bleeding has been adequately stopped, the inflammatory response occurs. The reaction typically serves to destroy the infectious agent and repair the injured tissues. Inflammation is normally beneficial during wound healing as it prevents infection, but too much inflammation can cause more tissue damage and excessive scarring. Next to occur is the proliferative phase which includes reepithelialization, angiogenesis, and collagen production. Reepithelialization can be defined as the restoration of the epithelium (the cells in the outermost layer of the skin), while angiogenesis is the formation of new blood vessels. Finally, a remodeling of the immature collagen matrix into scar tissue takes place. This procedure is performed by fibroblasts [3].

As stated previously, the fetal model of wound healing shows great dissimilarity from adult wound healing. In the first and second trimesters of development, fetal skin experiences an extremely fast healing time with little to no inflammation and, most importantly, no scarring [7]. The significance of the lack of inflammation on scarless healing cannot be understated; several additional studies have shown that by inducing inflammation in early fetal wounds through the

introduction of killed or live bacteria [8], chemical agents [9], or various inflammatory mediators (cytokines, growth factors, and prostaglandins) [10-12], one can stimulate scar formation in skin which would otherwise heal without a scar. Fetal wounds also demonstrate regeneration of skin appendages such as hair follicles and sebaceous glands which adult wounds do not. Additionally, a renewal of the dermal matrix occurs without scarring. Many early studies looking at fetal wound healing hypothesized that the warm, sterile nature of the uterine environment were integral for the scarless healing of fetal wounds. This misconception was later rectified through the study of developing opossums, which mature in a pouch instead of a uterine environment. It is now known that an amniotic fluid environment is nonessential for scarless healing [13]. Near the third trimester though, fetal skin starts to undergo fibrotic (adult-like) healing instead. In humans, scarless healing occurs until approximately twenty-two to twenty-four weeks of gestation. In mice, the conversion occurs at embryonic day sixteen (E16) [6]. The gestation period for a mouse is nineteen to twenty-one days. Evidenced by the transplantation of adult sheep skin or late gestation fetal lamb skin onto fetal lambs before wounding, once an individual has passed through this transition point and begun healing fibrotically, skin will continue to heal with a scar even if the repair takes place in a fetal environment. The early gestation fetal lambs with skin grafts from adult skin all produced scars upon wounding [14]. In our model of fetal wound healing, wounds in embryonic day fifteen (E15) mice heal without scars, while wounds in embryonic day eighteen (E18) mice heal with a scar (Figure 1) [15-17].

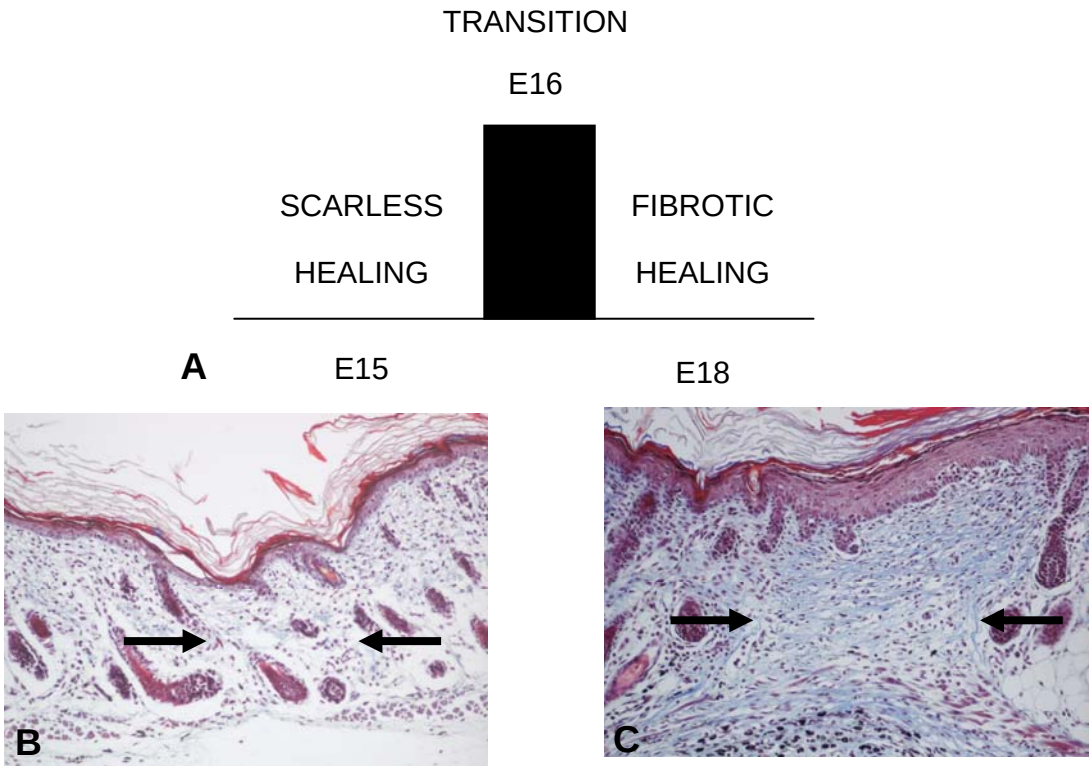

Figure 1. Differences in healing in fetal skin. The schematic in (A) illustrates the transition from scarless to fibrotic healing which occurs at day E16. Masson's trichrome-stained tissue sections, in which collagen is stained blue, illustrate the differences in scarless (B, E15) and fibrotic (C, E18) healing [17]. Arrows are used to highlight the margins of the wound bed/scar. Because inflammation is thought to be involved in the transition from scarless to fibrotic healing, we assessed the presence of mast cells in E15 and E18 wounds.

One specific reason for the disparity in wound healing between adult and fetal skin could be attributed to the presence of different numbers of mast cells, a specific type of inflammatory cell. It is believed that the only way a fetal wound can heal without a scar is if inflammation does not occur, but no one has studied the role mast cells specifically play in fetal wound healing. Almost immediately after injury occurs to adult skin, mast cells, which are present in the dermal layer of the skin and are also found near blood vessels, are activated. Once activated, they rapidly release their granules which include histamine molecules, protease molecules, cytokines, and growth factors [18]. These mast cell-derived mediators are what seem to control the

inflammatory response discussed above, and can affect restoration of the epithelium, angiogenesis, and collagen production/scar formation [18]. The granule contents recruit other inflammatory cells to the wound site including neutrophils and macrophages. While the exact role of neutrophils and macrophages in adult wound healing is not known, a function for these cells in scar formation of adult skin has been established by numerous studies [19-21]. It has also been established that fetal skin possesses significantly lower numbers of infiltrating inflammatory cells [6].While numerous studies have tried to assess the role of mast cells in the proliferative phase of fibrotic (adult) wound healing, inconsistent results seem to suggest that their function may be quite complex [22-25].

On a broad level, we wanted to determine through our research what link exists between the number of mast cells, both granulated and degranulated, in wounded skin and the severity of the eventual scar that forms.

Methods and Materials:

Fetal Surgery:

Female and male FVB mice were mated. After the discovery of a vaginal plug within the female, designated day zero, surgery was performed on the pregnant female after fifteen or eighteen days. In our mouse model of fetal wound healing, embryonic day 15 (E15) represents a time point at which scarless healing takes place and embryonic day 18 (E18) represents a time point at which fibrotic healing takes place. After preparing the abdomen for aseptic surgery, a midline laparotomy was performed under isoflurane anesthesia. Incisions were made in the uterine wall and amniotic sac overlying each fetus, and a full-thickness dermal wound, approximately 2mm in length, was made on the dorsum of the fetus using microsurgical scissors. One microliter of a 10% India ink solution was introduced subcutaneously into the wound site to allow visualization of the area after healing had occurred. The amniotic sac and uterine incision were closed using 6-0 nylon suture. After approximately four fetuses per dam were wounded in this manner, the muscle and skin layers of the pregnant mouse were sutured closed. Xylazine (3 mg/kg) was given subcutaneously for sedation and analgesia and to preclude cannibalism of the pups by the dam. Fetal skin was harvested at two, five, twelve, and twenty-four hours post-wounding to examine mast cells. The skin was fixed in formalin and paraffin embedded for subsequent histological analysis. Normal E15 and E18 skin was harvested as unwounded controls.

Toluidine blue Stains:

Paraffin embedded tissues were cut and subjected to toluidine blue staining which included deparaffinization, rehydration, exposure to the toluidine blue stain (0.2% toluidine blue in 0.7 N HCl) for two hours, dehydration, clearing in xylene and finally, cover-slipping. Toluidine blue staining was the primary method of mast cell identification used in this study. This stain functions by binding to the granules of mast cells and coloring them dark blue based on the pH of the solution. At a more acidic (lower) pH, toluidine blue binds more tightly to the mast cell granules [26].

Alcian Blue Stain:

Paraffin embedded tissues were cut and subjected to alcian blue/safranin staining which included deparaffinization, rehydration, exposure to the alcian blue/safranin stain for fifteen minutes, dehydration in butanol, clearing in xylene and finally, cover-slipping. Mast cells containing only biogenic amines, or immature mast cells, stained blue, while mast cells containing heparin, or mature mast cells, stained red [27].

Quantification:

The number of mast cells present in unwounded skin samples was determined through the analysis of ten consecutive high powered fields in each skin sample. The area of the unwounded skin samples was determined as well by outlining the dermis using Axiovision software and data are expressed as the mean number of mast cells per mm^2 of dermis. The size of the mast cells found in unwounded skin was determined using Axiovision software. Ten random mast cells from each skin sample were outlined, with the software determining the size and integrated density of the mast cell from this outline. The average size per mast cell was then

calculated. The integrated density or the sum of the pixel densities per cell was also calculated. The integrated density value was used as a measure of mast cell granularity.

In wounded skin samples, the wound was identified and the number of mast cells and free granules in five high powered fields to both the left and right of the wound were counted. The number of free granules from each high powered field was added together and an average for each wound was calculated. Cells were only classified as mast cells if they were intact and possessed dark blue granules (due to toluidine blue staining).

Statistics:

Statistics were performed using student's t-test. P values less than 0.05 were considered statistically significant. The number of samples used for analysis is shown in Figure 2.

	Unwounded	2 Hour	5 Hour	12 Hour	24 Hour
E15 Samples	15	4	7	6	5
E18 Samples	6	6	5	3	7

Figure 2. Number of skin samples used for analysis. Differences in the number of skin samples used in unwounded skin and at each time point in wounded skin were the result of differences in the number of pregnant females, the number of pups produced per litter, and the number of pups which survived until the desired time point.

Results:

Mast Cell Characteristics in Unwounded Fetal Skin:

We decided to investigate the number of mast cells in fetal skin, and specifically unwounded fetal skin, to determine if the transition from scarless to fibrotic healing that occurs at day E16 in our mouse model of fetal wound healing could be attributed to the presence or absence of mast cells. Figure 3A depicts graphically a significant difference between the number of mast cells present in E15 unwounded skin, which does not scar upon wounding, and the number of mast cells present in E18 unwounded skin, which does scar upon wounding. This indicates that mast cells may in fact play a role in the transition from scarless to fibrotic wound healing that takes place during development. The mast cells found in E15 unwounded skin samples were generally smaller than mast cells found in E18 unwounded skin as shown in Figure 4A. Additionally, the mast cells found in unwounded E18 skin possessed a higher level of toluidine blue staining suggesting that the mast cells found in unwounded E18 skin were more granular than the mast cells found in unwounded E15 skin (Figure 4B). A photomicrograph showing the differences in size and granularity of two random mast cells from E18 and E15 unwounded skin respectively is shown in Figure 5. It is clear from the micrograph that the mast cells found in E15 unwounded skin were both smaller and less granular than those found in unwounded E18 skin. We also stained mast cells using Alcian blue/safranin stain to look at the maturity of mast cells found in unwounded E18 and E15 skin samples. Examples of staining results are shown in Figure 6. Mast cells found in E15 skin stained primarily blue, indicating that they were immature, while the mast cells found in E18 skin stained primarily red, indicating that they were mature. The smaller size, lower granularity, and immaturity of the mast cells found in unwounded E15 skin suggests that upon degranulation, these mast cells likely release fewer inflammatory mediators (cytokines,

prostaglandins, growth factors, etc.) which results in a drastically reduced inflammatory response. This diminished response resulting from the release of fewer inflammatory mediators may contribute to the scarless healing in E15 skin.

Mast Cell Number and Degranulation in Fetal Wounds:

Based on the encouraging results we found in unwounded fetal skin, we decided to look at the number of mast cells in wounded fetal skin. Figure 7 shows graphically that the number of mast cells found in E18 wounded skin was considerably higher than the number of mast cells found in E15 wounded skin at all four time points. The number of mast cells in E18 wounds increased between the 2 hr and 5 hr time points, suggesting that mast cells were recruited to the site of the wound immediately after wounding. The number of mast cells in E18 wounds decreased between the 5 hr and 12 hr time points, suggesting that the recently recruited mast cells had now been activated and released their granules into the skin. Using the toluidine blue staining method, after mast cells have released their granules (degranulated), the mast cells can no longer be identified because the stain only binds the granules inside of the cell, not the cell itself. The number of mast cells in E18 wounded skin remained fairly constant between the 12 hr and 24 hr time points, suggesting that the inflammatory response ended around this time. The number of mast cells found in E15 wounded skin was fairly steady throughout the time course. Once activated, mast cells must degranulate, and subsequently recruit other inflammatory cells into the wound site. For this reason, we also looked at the number of free granules (which are released by mast cells upon activation) in five high powered fields to the left and five high powered fields to the right of the wound as well. Figure 8 shows graphically that the number of granules found in E18 wounded skin was noticeably higher than the number of granules found in E15 wounded skin until the 24 hr time point. The number of free granules in E18 skin slightly

increased between the 2 hr and 5 hr time point, and increased more dramatically between the 5 hr and 12 hr time points. These increases in granule number suggest increased rates of degranulation. This assumption is supported by the data in Figure 7 which shows a decrease in intact mast cells between the 5 hr and 12 hr time point. It makes sense that the largest decrease in fully granulated mast cells would correspond with the largest increase in granule number. In order for the number of free granules to increase, mast cells must degranulate, decreasing the number of mast cells visible with toluidine blue. The number of free granules decreased drastically between the 12 hr and 24 hr time points in E18 wounds, once again suggesting an end to the mast cell response. The number of granules found in E15 wounded skin fluctuated throughout the study with a slight increase in the number of granules occurring at the 24 hr time point. This result may suggest that not only is the inflammatory response reduced in E15 wounded skin, but that the response time, or the time it takes for the mast cells to degranulate and recruit other inflammatory cells, is inhibited as well. Figure 9 shows representative toluidine blue stained E15 and E18 wounds. These sections illustrate visually the differences in mast cell number and activation between E15 and E18 wounds.

Ultimately, the results obtained in this study suggest that mast cells do play a role in the transition from scarless to fibrotic healing which takes place at day E16 in our mouse model of fetal wound healing. The differences in mast cell number, size, and granularity seen between unwounded E15 and E18 skin suggest that a higher number of mast cells which possess more granules (as in E18 skin) will ultimately result in an increased inflammatory response which may impact eventual scar formation upon wounding. The differences in degranulation between E15 and E18 wounds also indicates that the heightened inflammatory response seen in E18 skin may be caused by the activation of mast cells, which in turn recruit more inflammatory cells to the

wound site. An excess of inflammatory cells and mediators may influence the scar formation which takes place in E18 wounds and not E15 wounds.

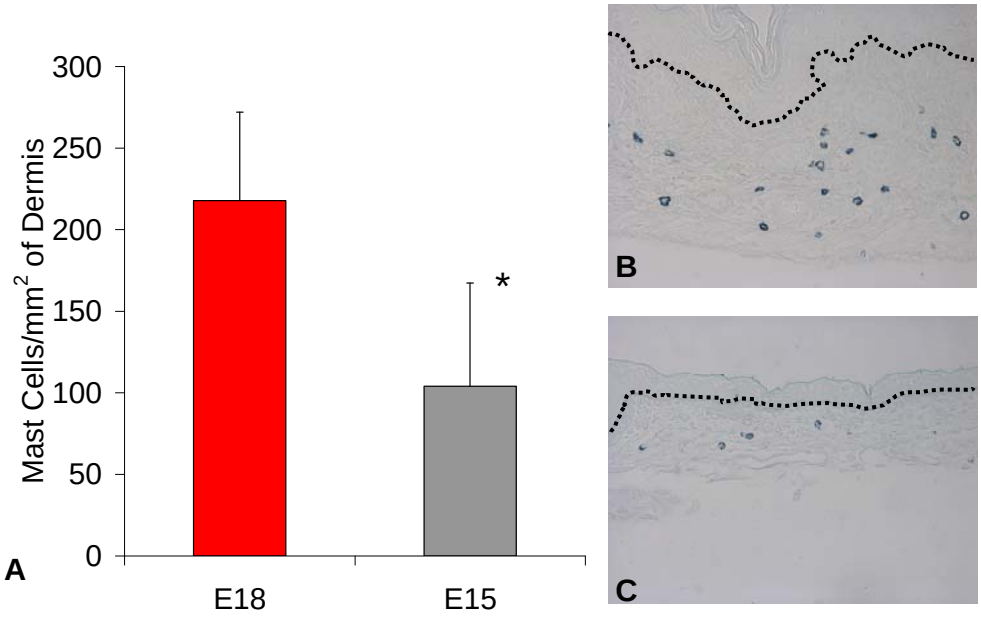

Figure 3. Comparison of mast cell number between E18 and E15 unwounded skin. The number of mast cells/mm^2 of dermis in unwounded skin was determined using toluidine blue stain. The number of mast cells in ten consecutive high powered fields was counted for each skin sample. The bars represent the mean number of mast cells per mm^2 of skin ± SD. (* p value < 0.001) Representative photomicrographs of E18 (B) and E15 (C) skin demonstrate typical toluidine blue staining of mast cells. The dotted lines represent the dermal-epidermal junction.

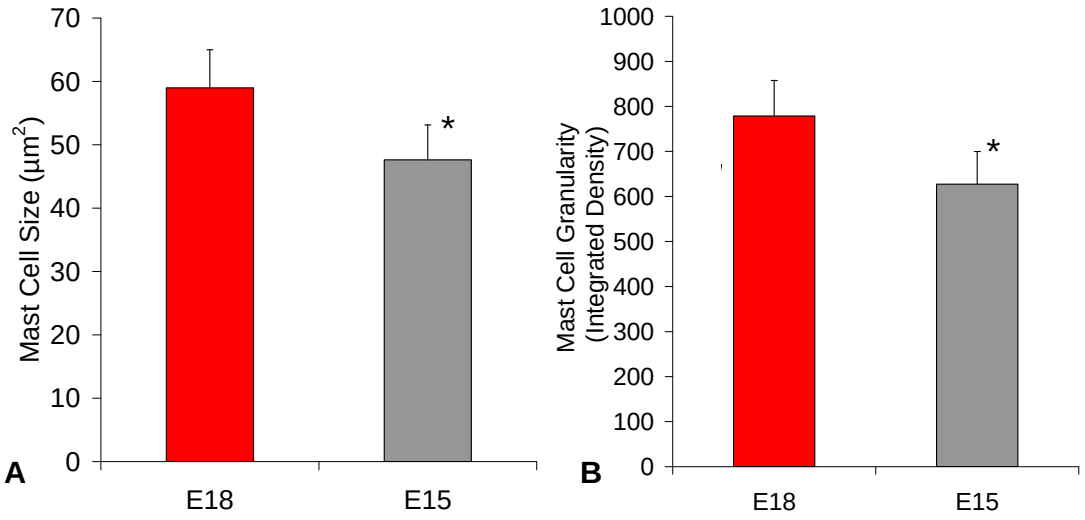

Figure 4. Comparison of the size and granularity of mast cells found in E18 and E15 unwounded skin. Both the mast cell size (A) and integrated densities (B) were determined using toluidine blue stain. Ten random mast cells per skin sample were outlined using Axiovision software to determine the average size and integrated density of the cells. Integrated density measurements were used to estimate the intensity of granule staining per cell. The bars in Graph A represent the mean size of mast cells ± SD. (* p value of 0.002), while the bars in Graph B represent the mean integrated density of mast cells in E18 and E15 skin ± SD. (* p value of 0.002)

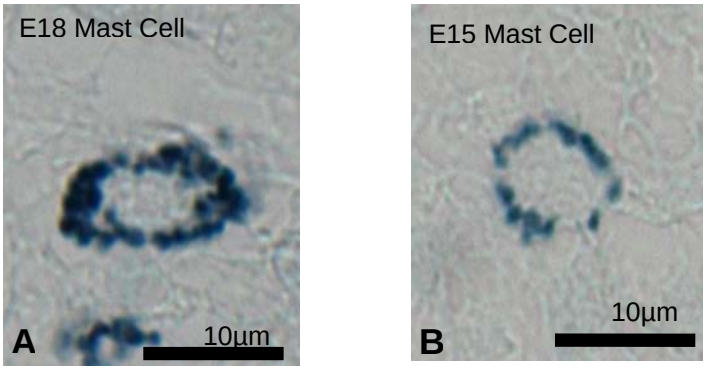

Figure 5. Representative photomicrographs of E18 and E15 skin. A is a representative photomicrograph of a mast cell found in unwounded E18 skin stained with toluidine blue. B is a representative photomicrograph of a mast cell found in unwounded E15 skin stained with toluidine blue. Note the smaller size and lower number of granules in the E15 mast cell.

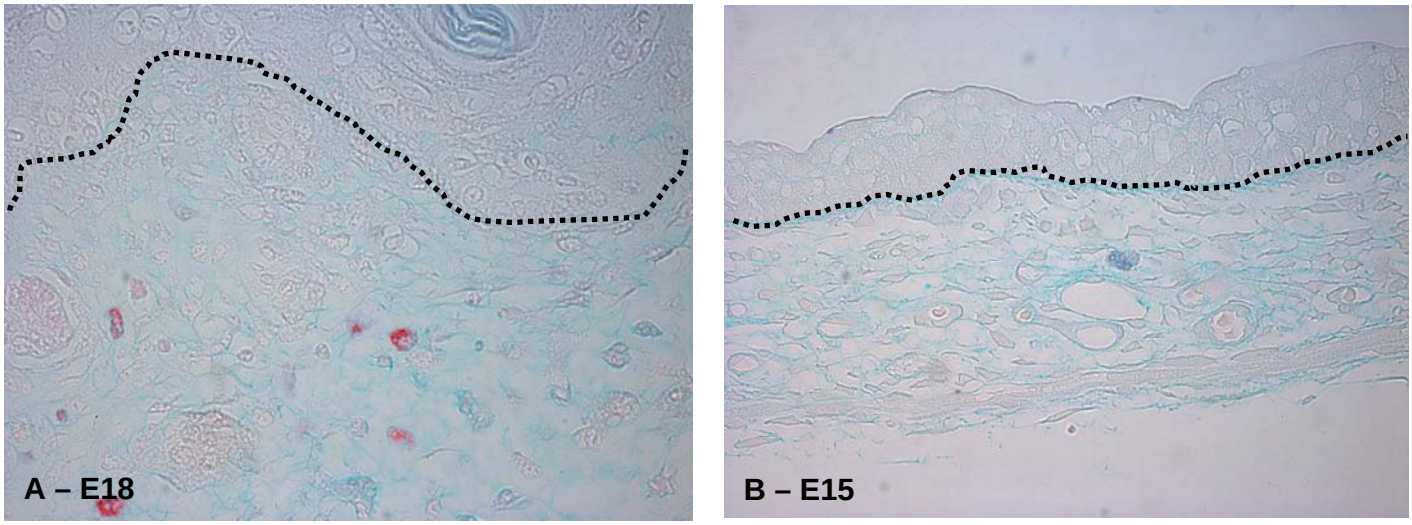

Figure 6. Comparison of mast cell maturity in E18 and E15 skin. Representative photomicrographs of E18 (A) and E15 (B) skin demonstrate alcian blue-safranin staining of mast cells. Red stained cells are considered to be mature mast cells, while blue stained cells are considered to be immature mast cells. The dotted lines represent the dermal-epidermal junction.

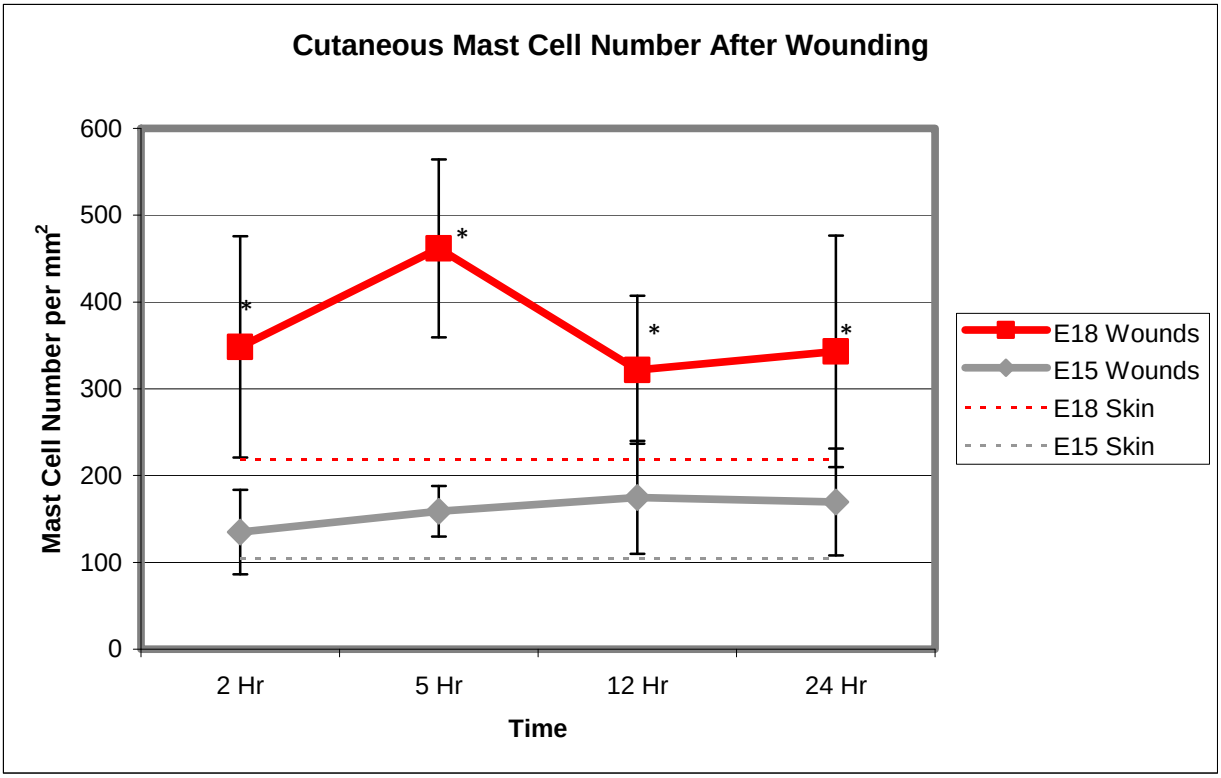

Figure 7. Comparison of mast cell number between E18 and E15 wounds over time. The number of mast cells per mm^2 of skin was determined using toluidine blue stain. The number of mast cells in five consecutive high powered fields to the left and five consecutive high powered fields to the right of the wound was counted. Only intact, granulated mast cells were counted. The data points represent the mean number of mast cells ± SD. (*p value < 0.04).

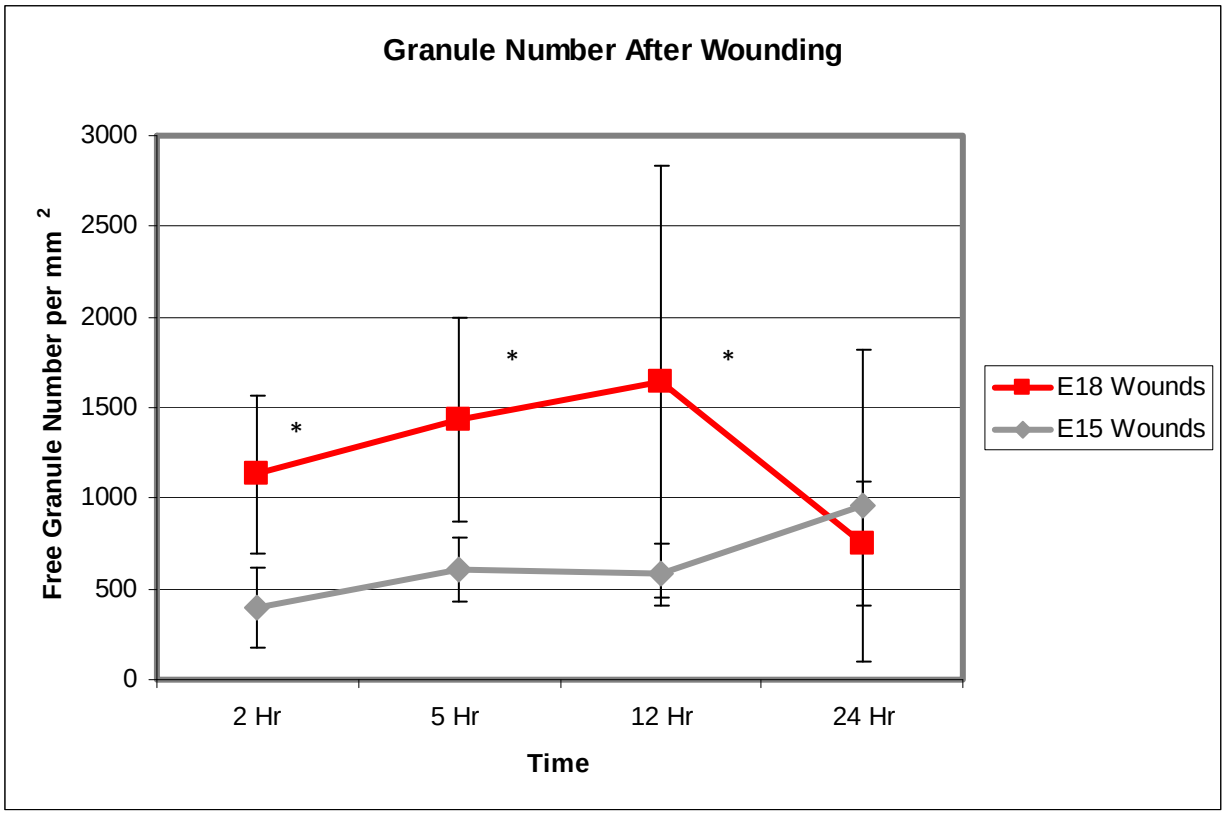

Figure 8. Comparison of granule number between E18 and E15 wounds over time. The number of free granules per mm^2 of skin was determined using toluidine blue stain. Counting the number of free granules near a wound is one way of measuring mast cell degranulation. The number of free granules in five consecutive high powered fields to the left and five consecutive high powered fields to the right of the wound was counted. Granules were only classified as free if they were not associated with an intact mast cell. The data points represent the mean number of granules ± SD. (* p value < 0.03).

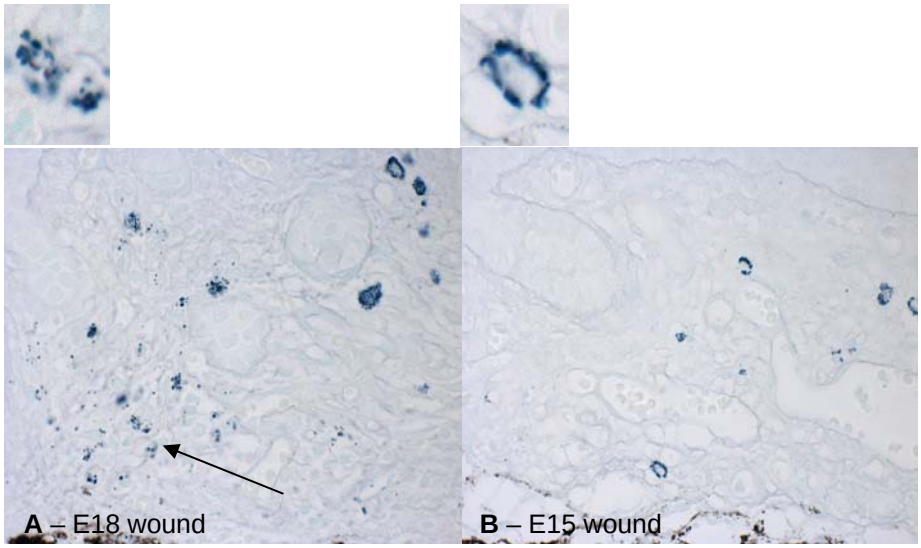

Figure 9. Differences in mast cell number/degranulation in fetal wounds. Toluidine blue-stained tissue sections were used to determine the number of mast cells in E18 and E15 wounds 12 hours post-wounding. Significantly more mast cells were found in E18 wounds (A) compared to E15 wounds (B). There was also evidence of increased mast cell degranulation in E18 wounds, but not in E15 wounds. The arrow (A) is pointing to an area with abundant free granules.

Discussion:

Inflammation is an integral step in the adult wound healing process as it destroys any infectious agents in order to prevent infection and also stimulates the replacement of injured host tissue. While inflammation is necessary to prevent infection and repair damaged tissues, too much inflammation can be detrimental as well. Excessive inflammation can lead to delays in wound closure, additional tissue damage, and excessive scar formation [3]. Because mast cells possess a wide assortment of pro-inflammatory and growth-promoting mediators, such as histamine, leukotrienes, prostaglandins, proteases, and cytokines, it has repeatedly been hypothesized that they are important factor for wound healing [28-30]. Studies have demonstrated a role for mast cells in cutaneous wound healing; however, their exact function is largely unknown [18, 23, 31-33]. Mast cells release the aforementioned mediators upon activation (by an injury to the skin) and recruit inflammatory cells to the wound site. Previously published studies have shown that mast cell-deficient mice show decreased amounts of certain inflammatory cells, most notably neutrophils [22, 23]. Mast cell-deficient mice possess a spontaneous genetic mutation in the C-Kit gene causing them to lack mast cells. Dr. Karsten Weller of Germany used mast cell-deficient mice in her research [23]. She demonstrated that a lack of mast cells can lead to impaired wound closure during the first six days of healing, reduced edema, and confirmed other published studies, including one performed by Dr. Eric Egozi, which showed decreased recruitment of neutrophils to the wounded area [22]. Neither of these studies, however, looked at the impact of using mast cell deficient mice on either the size or severity of scar formation.

The process of fetal wound healing differs greatly from that of adult wound healing simply because little to no inflammation occurs in fetal wounds and, subsequently, scar formation does

not take place. The transition from scarless to fibrotic healing occurs at day E16 in our mouse model of fetal wound healing and between the second and third trimesters in human fetuses [6]. The role of mast cells in inflammation in adult wound healing have been extensively studied, but their role in fetal wound healing is not known. For this reason, we decided to investigate the number of mast cells in both unwounded and wounded fetal skin in order to determine if mast cells contribute to the diminished inflammation and scarring seen in E15 fetal skin. We quantified the number of mast cells present in both unwounded and wounded E15 skin (which heals scarlessly) and E18 skin (which heals fibrotically).

We hypothesized that mast cells may play a role in the transition from scarless to fibrotic healing which takes place in fetal skin. Our data did show a significantly larger number of mast cells in unwounded E18 skin than in unwounded E15 skin. Additionally, those mast cells found in the unwounded E18 skin were larger and more granular than those found in the E15 unwounded skin. These results suggest that not only are there more mast cells in unwounded E18 skin, but that the mast cells found in E18 skin possess more granules, indicating that they likely possess more inflammatory mediators. After degranulation, these mediators recruit inflammatory cells into the wound site. A larger number of free granules and mast cells were found in E18 wounds than were found in E15 wounds. The larger number of granules being released by mast cells in wounded E18 skin, compared to wounded E15 skin, provides a possible explanation for why E18 wounds heal with a scar and E15 skin heals scarlessly, because mediators released by mast cells stimulate fibroblasts. Ultimately, these results suggest that mast cells do in fact play a role in the transition between scarless and fibrotic healing.

Our findings are supported by numerous published studies, some of which show increased numbers of mast cells in hypertrophic scars [25, 34, 35]. Hypertrophic scars are raised fibrous

lesions that are common after thermal injuries and other injuries that involve the deep dermis. Hypertrophic scars are more severe than normal scars [34]. The data from our study suggests that the increased number of mast cells in E18 wounds (as opposed to E15 wounds) contributes to scar formation which takes place in E18 skin (and not in E15 skin); thus, a study which shows that a larger number of mast cells are found in more severe scars supports our findings. Our results are also supported by a study showing reduction in hypertrophic scar formation in red Duroc pigs treated with the mast cell stabilizer ketotifen before wounding [25]. The drug ketotifen 'stabilized' the mast cells, preventing them from degranulating even when the skin was wounded. When the mast cells in this species of pig were chemically inhibited from degranulating, scar formation was significantly reduced. These results support our findings that mast cells contribute to scar formation as without mast cell activation, as in the case of E15 wounds, scar formation does not occur. Interestingly, several mediators released by mast cells can induce scar formation when introduced into fetal wounds [10, 11, 36]. These mediators include TGF- β1 (transforming growth factor – β1), PDGF (platelet-derived growth factor), FGF-2 (fibroblast growth factor-2), and IL-6 (interleukin-6) [37-39]. Our results show an increase in mast cell number and activation in E18 skin when compared to E15 skin, suggesting that they may contribute to scar formation. The next step in our research is to actually prove that mast cells are important for scar formation in fetal skin.

A preliminary study was performed in our laboratory involving the injection of lysates of cultured mast cells into E15 wounds to see if mast cells produce factors that induce scar formation. Initial results have been encouraging as after seven days, trichrome-stained tissue sections showed a disruption in the scarless wound healing process. E15 control wounds injected with PBS instead of lysates showed the regeneration of hair follicles consistent with scarless

wound healing, while E15 wounds injected with lysates showed an almost complete loss of hair follicles. Mediators released by mast cells stimulate fibroblasts to produce collagen and scar tissue[27, 39] . Because the release of these mediators in E15 wounds is severely diminished, it is likely that less fibroblast stimulation occurs, allowing the wounds to heal without a scar.

Other future studies which could be performed based on these data are innumerable. Studies investigating what exactly is causing the mast cells to accumulate in E18 wounds (cytokines, growth factors, etc.), examining which factors (granular contents) released by mast cells contribute to scar formation (histamine, chymase, tryptase, etc.), or testing the effect of chemical inhibition of mast cell degranulation all could be performed. Alternatively, the employment of mast cell-deficient mice to look at scar formation in E18 or adult mice could provide similar information to chemical inhibition. A reduction in scar formation with either inhibitory drugs or mast cell-deficient mice would provide basic proof as to whether mast cells are involved in scarring.

We have shown through the results of this study that mast cells seem to play an important role in scar formation in fetal wound healing. Future studies should be done to clarify the role of mast cells in scar formation as they could potentially lead to new information regarding the exact mechanism of scar formation. Additionally, the information could be used to generate new drugs and therapies that may help to reduce or even eliminate scar formation in the future. Applying this information to an even broader scale, fibrotic diseases which affect the internal organs of the human body could also potentially be treated by extrapolating the data discovered through this work.

Literature Cited:

1. Kanitakis, J., *Anatomy, histology and immunohistochemistry of normal human skin.* Eur J Dermatol, 2002. **12**(4): p. 390-9; quiz 400-1.
2. De, S.K., E.D. Reis, and M.D. Kerstein, *Wound treatment with human skin equivalent.* J Am Podiatr Med Assoc, 2002. **92**(1): p. 19-23.
3. Singer, A.J. and R.A. Clark, *Cutaneous wound healing.* N Engl J Med, 1999. **341**(10): p. 738-46.
4. Schmid, P., et al., *Enhanced expression of transforming growth factor-beta type I and type II receptors in wound granulation tissue and hypertrophic scar.* Am J Pathol, 1998. **152**(2): p. 485-93.
5. Ginsburg, I.H., *The psychosocial impact of skin disease. An overview.* Dermatol Clin, 1996. **14**(3): p. 473-84.
6. Wilgus, T.A., *Regenerative healing in fetal skin: a review of the literature.* Ostomy Wound Manage, 2007. **53**(6): p. 16-31; quiz 32-3.
7. Burrington, J.D., *Wound healing in the fetal lamb.* J Pediatr Surg, 1971. **6**(5): p. 523-8.
8. Frantz, F.W., et al., *Biology of fetal repair: the presence of bacteria in fetal wounds induces an adult-like healing response.* J Pediatr Surg, 1993. **28**(3): p. 428-33; discussion 433-4.
9. Kumta, S., et al., *Acute inflammation in foetal and adult sheep: the response to subcutaneous injection of turpentine and carrageenan.* Br J Plast Surg, 1994. **47**(5): p. 360-8.
10. Liechty, K.W., N.S. Adzick, and T.M. Crombleholme, *Diminished interleukin 6 (IL-6) production during scarless human fetal wound repair.* Cytokine, 2000. **12**(6): p. 671-6.
11. Stelnicki, E.J., et al., *A new in vivo model for the study of fetal wound healing.* Ann Plast Surg, 1997. **39**(4): p. 374-80.
12. Adolph, V.R., et al., *The potential role of the lymphocyte in fetal wound healing.* J Pediatr Surg, 1993. **28**(10): p. 1316-20.
13. Armstrong, J.R. and M.W. Ferguson, *Ontogeny of the skin and the transition from scar-free to scarring phenotype during wound healing in the pouch young of a marsupial, Monodelphis domestica.* Dev Biol, 1995. **169**(1): p. 242-60.
14. Morykwas, M.J., et al., *Cellular inflammation of fetal excisional wounds: effects of amniotic fluid exclusion.* Inflammation, 1991. **15**(3): p. 173-80.
15. Wilgus, T.A., et al., *The impact of cyclooxygenase-2 mediated inflammation on scarless fetal wound healing.* Am J Pathol, 2004. **165**(3): p. 753-61.
16. Wilgus, T.A., et al., *Regulation of scar formation by vascular endothelial growth factor.* Lab Invest, 2008. **88**(6): p. 579-90.
17. Wilgus, T.A., et al., *Hydrogen peroxide disrupts scarless fetal wound repair.* Wound Repair Regen, 2005. **13**(5): p. 513-9.
18. Artuc, M., et al., *Mast cells and their mediators in cutaneous wound healing--active participants or innocent bystanders?* Exp Dermatol, 1999. **8**(1): p. 1-16.
19. Martin, P., et al., *Wound healing in the PU.1 null mouse--tissue repair is not dependent on inflammatory cells.* Curr Biol, 2003. **13**(13): p. 1122-8.
20. Wilgus, T.A., et al., *Reduction of scar formation in full-thickness wounds with topical celecoxib treatment.* Wound Repair Regen, 2003. **11**(1): p. 25-34.

21. Dovi, J.V., L.K. He, and L.A. DiPietro, *Accelerated wound closure in neutrophil-depleted mice.* J Leukoc Biol, 2003. **73**(4): p. 448-55.
22. Egozi, E.I., et al., *Mast cells modulate the inflammatory but not the proliferative response in healing wounds.* Wound Repair Regen, 2003. **11**(1): p. 46-54.
23. Weller, K., et al., *Mast cells are required for normal healing of skin wounds in mice.* FASEB J, 2006. **20**(13): p. 2366-8.
24. Iba, Y., et al., *Possible involvement of mast cells in collagen remodeling in the late phase of cutaneous wound healing in mice.* Int Immunopharmacol, 2004. **4**(14): p. 1873-80.
25. Gallant-Behm, C.L., K.A. Hildebrand, and D.A. Hart, *The mast cell stabilizer ketotifen prevents development of excessive skin wound contraction and fibrosis in red Duroc pigs.* Wound Repair Regen, 2008. **16**(2): p. 226-33.
26. Todd, M.M., J.W. Lee, and V.J. Marks, *Rapid toluidine blue stain for Mohs' micrographic surgery.* Dermatol Surg, 2005. **31**(2): p. 244-5.
27. Tsai, M., et al., *Induction of mast cell proliferation, maturation, and heparin synthesis by the rat c-kit ligand, stem cell factor.* Proc Natl Acad Sci U S A, 1991. **88**(14): p. 6382-6.
28. Gordon, J.R., P.R. Burd, and S.J. Galli, *Mast cells as a source of multifunctional cytokines.* Immunol Today, 1990. **11**(12): p. 458-64.
29. Biedermann, T., et al., *Mast cells control neutrophil recruitment during T cell-mediated delayed-type hypersensitivity reactions through tumor necrosis factor and macrophage inflammatory protein 2.* J Exp Med, 2000. **192**(10): p. 1441-52.
30. Moller, A., et al., *Human mast cells produce IL-8.* J Immunol, 1993. **151**(6): p. 3261-6.
31. Hebda, P.A., M.A. Collins, and M.D. Tharp, *Mast cell and myofibroblast in wound healing.* Dermatol Clin, 1993. **11**(4): p. 685-96.
32. Trautmann, A., et al., *Mast cell involvement in normal human skin wound healing: expression of monocyte chemoattractant protein-1 is correlated with recruitment of mast cells which synthesize interleukin-4 in vivo.* J Pathol, 2000. **190**(1): p. 100-6.
33. Noli, C. and A. Miolo, *The mast cell in wound healing.* Vet Dermatol, 2001. **12**(6): p. 303-13.
34. Harunari, N., et al., *Histology of the thick scar on the female, red Duroc pig: final similarities to human hypertrophic scar.* Burns, 2006. **32**(6): p. 669-77.
35. Kischer, C.W., H. Bunce, 3rd, and M.R. Shetlah, *Mast cell analyses in hypertrophic scars, hypertrophic scars treated with pressure and mature scars.* J Invest Dermatol, 1978. **70**(6): p. 355-7.
36. Haynes, J.H., et al., *Platelet-Derived Growth-Factor Induces Fetal Wound Fibrosis.* Journal of Pediatric Surgery, 1994. **29**(11): p. 1405-1408.
37. Liao, C.H., et al., *Cardiac mast cells cause atrial fibrillation through PDGF-A-mediated fibrosis in pressure-overloaded mouse hearts.* J Clin Invest. **120**(1): p. 242-53.
38. Reed, J.A., A.P. Albino, and N.S. McNutt, *Human cutaneous mast cells express basic fibroblast growth factor.* Lab Invest, 1995. **72**(2): p. 215-22.
39. Kendall, J.C., et al., *Promotion of mouse fibroblast proliferation by IgE-dependent activation of mouse mast cells: role for mast cell tumor necrosis factor-alpha and transforming growth factor-beta 1.* J Allergy Clin Immunol, 1997. **99**(1 Pt 1): p. 113-23.