CUTANEOUS WOUND HEALING IN THE CAT: A MACROSCOPIC AND HISTOLOGIC DESCRIPTION AND COMPARISON WITH CUTANEOUS WOUND HEALING IN THE DOG

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CUTANEOUS WOUND HEALING IN THE CAT: A MACROSCOPIC AND HISTOLOGIC DESCRIPTION AND COMPARISON WITH CUTANEOUS WOUND HEALING IN THE DOG

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A Dissertation

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Doctor of Philosophy

Auburn, Alabama
May 10, 2006

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May 10, 2007
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DISSERTATION ABSTRACT

CUTANEOUS WOUND HEALING IN THE CAT: A MACROSCOPIC AND HISTOLOGIC DESCRIPTION AND COMPARISON WITH CUTANEOUS WOUND HEALING IN THE DOG

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Doctor of Philosophy, May 10, 2007
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202 Typed Pages

Directed by Ralph A. Henderson

Wound healing has been studied in a variety of human and animal models, and until fairly recently, the prevailing viewpoint has been that the basic processes of wound healing are the same between species. Our combined clinical experience, and recent reports of differences in wound healing between horses and ponies, led us to question whether or not there may be significant differences in the cutaneous wound healing of the cat, compared to the dog. We also had questions about the hitherto unexplored role of the underlying subcutaneous tissues in regard to cutaneous wound healing, both in the cat and the dog.

This study was undertaken first; to describe the first and second intention healing of cutaneous wounds in the cat and to compare cutaneous wound healing in the cat with that of the dog, and second; to learn more about the role of the subcutaneous tissues in their contribution to cutaneous wound healing. These objectives were met by macroscopic and histologic evaluation of experimentally created wounds along the dorsal midline of cats and dogs. We found significant macroscopic differences between cats and dogs in regard to wound granulation, contraction, epithelialization, and wound strength. Significant histologic differences between cat and dog wounds were seen in numbers of inflammatory cells and production and maturity of wound collagen. These findings have led us to conclude that cutaneous wounds in the cat heal more slowly than in the dog, and that cutaneous wound healing is associated with a more persistent inflammatory reaction to wounding and a less active proliferative phase in the cat.

ACKNOWLEDGEMENTS

The author would like to express his appreciation and thanks to Drs. Ralph Henderson, Steven Swaim, and Steven Kincaid, for directing this research and for their guidance and support during every phase of the work. Thanks are also due to Drs. James C. Wright and Eva A. Sartin for their help and advice with statistical analysis and histologic interpretation, respectively. Technical assistance with the project was provided by Drs. Michelle Goree, Ann Shower, and Lauren Spears, (all graduates of the Auburn University CVM Class of 2005), and the author's son Brian. Their invaluable help is gratefully acknowledged. Finally, the author expresses deepest thanks to his Lord and Savior, and to his wife, Sandra, and children: John, Brian, Steven, and David, for their unfailing love, encouragement, inspiration, and support during the entirety of this undertaking.

Style manual or journal used: Veterinary Surgery.

Computer software used: Microsoft Office Word 2003.
Open Office 2001.

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INTRODUCTION

Wound healing has been described as an orderly process that has been conceptually divided into three phases: the inflammatory phase first, followed by the proliferative phase, and finally the remodeling or maturation phase.[1] Historically it has been assumed that these processes are essentially the same in all animal species, perhaps differing only in their rates of occurrence and that those differences would be explainable on the basis of anatomic or metabolic variation.[2]

More recently, research has demonstrated that significant differences can exist in wound healing between animals, even within breeds or strains within a species, and that these differences may have their basis at the cellular and molecular level.[3,4] Our clinical observations have led us to hypothesize that the cat, although somewhat closely related to the dog, may have significant differences from the dog with regard to wound healing; this study was undertaken primarily to explore that hypothesis. Another motivation for this work involves the area of comparative medicine. With changing world demographics has come an increasing concern in human medicine regarding type II diabetes and its complications, particularly chronic wounds. This has in turn inspired an emphasis on wound healing research, and has some investigators questioning the validity of current animal models. We felt that studies that display the heterogeneity of wound

healing between species will advance wound healing research by fueling the search for models that will more accurately duplicate the conditions of chronic wound healing.

In the following investigation we have utilized classical macroscopic and histologic methods to describe wound healing in the cat, and to compare the cat to a better-known model, the dog. Our findings indicate that wound healing in the cat has significant qualitative and quantitative differences from the dog, and that these differences have significance for clinical practice and future research.

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I. METHODS IN WOUND HEALING RESEARCH: A REVIEW OF MACROSCOPIC AND HISTOLOGIC EVALUATION OF THE HEALING WOUND

This review of the literature deals with the evaluation of wound healing by measurement of changes in wound area and/or volume, strength of the healing tissues, measurement of wound perfusion, and histologic appearance of the healing tissues. Each method is discussed in terms of its relevance to wound healing, its principle and applications, and where appropriate, its limitations. Examples of advancements in our understanding of wound healing that were obtained by each method are also cited.

Part 1.1. Planimetry in the Evaluation of Wound Healing

1.1.1 Introduction

From ancient times men have evaluated the gross appearance of wounds, hoping to find some early indication of the final outcome – would the wound heal, or not?[5-7] Contraction and epithelialization, two processes central to the healing of open wounds, were originally assessed macroscopically and are still primarily evaluated this way, at least in the clinical setting. The outcome of contraction and epithelialization is a reduction in area of the open wound. Planimetry is the measurement of wound surface area, and is the means by which the processes of contraction and epithelialization can be quantified. By performing serial evaluations over time, planimetry also gives a measure of the *rate* of contraction and epithelialization of the wound, either separately or together as a measure of total healing rate.[8] Although usually considered primarily in the context of open wounds, planimetry can also be applied to the evaluation of ongoing contraction in previously open wounds that are now completely covered with new epithelium.[9-11]

1.1.2 Planimetric Methods

Traditional Planimetry – photographic, manual tracing, computerized and digital

A number of techniques are available to perform planimetry on open wounds. The photographic method involves photographing the wound at a predetermined distance from the surface at each evaluation. A millimeter ruler was included in the photograph for scaling. The area of the wound could then be manually determined with a gridded transparent plastic overlay of known grid size. In the manual tracing method, also called the acetate method the wound margins are traced directly onto a transparent plastic or acetate overlay. First, the overlay is placed directly onto the wound surface and held immobile, then the outline of the wound margins are traced with a fine-tip marking pen. Acetate planimetry can be used to differentiate and quantify contraction vs epithelialization. In order to do this, the margin between normal skin and the margin of the open wound are both traced; the outer margin is the epithelial border and measures wound contraction, while the area within the inner margin is the open wound area and represents total healing. The area between these margins represents the area of epithelialization.[8,12] The wound areas are determined by placing the tracing on a graph paper with squares of known area, and counting the squares. Another variation of acetate planimetry involves cutting out the margin of the tracing and weighing the piece of plastic, then comparing the weight to a known standard in mg/cm^2 for that particular plastic sheet.[13] In computerized planimetry, (eg, Sigma Scan, SPSS Science, Chicago,

IL) a tracing wand, connected to a computer, is used to trace over the wound outline. Software then converts this data to a calculated value for wound area. Digital planimetry is performed on a computer from digital photographs or prints of photographs that have been scanned into digital images, which are pixelized via computer software for computation of wound area. Digital planimetry requires calibration, which is accomplished by including a ruler or other object of known dimensions in the photograph of the wound.

The aforementioned planimetric methods each have advantages and disadvantages, but all have been shown to be accurate and repeatable enough to produce reliable measurements of wound area. Some comparisons have shown good agreement between methods, while others have shown systematic differences between methods. Thawer, et al[14] compared manual grid and computerized reading of acetate tracings of human leg ulcers; good agreement was demonstrated between methods in wounds up to 10 cm^2. Lagan and colleagues[15] conducted a four-way comparison in which acetate tracings and photographs were measured manually and digitized. They reported that manual readings of photos and tracings were less repeatable than digitizing, and that the variability was greater for tracings than for photographs. Acetate tracings also produced significantly larger readings (24% larger, $p = 0.019$) than photographs, regardless of whether the reading method was manual or digital. Thomas and Wysocki[16] reported similar results, with acetate tracings reporting wound area 20% larger than photographs. The author interprets the larger area of acetate tracings as being caused by wound distortion (spreading) from the inevitable pressure of holding the acetate flat on the wound. Other investigators[13] have also reported smaller areas for wound photographs

compared to tracings and concluded that acetate tracings may be more accurate, ie, that photographs may distort the actual size of the wound.[13,17]

Another concern regarding planimetry is the variation that may occur between different observers or even between repeated measures by the same observer. Comparison of three acetate tracing methods (manual and digital area calculation, and weighing the cut out plastic) confirmed that all are accurate.[13] The same observer should perform all tracings, as different observers may introduce variation in the final calculated wound area, regardless of which method is chosen to calculate the area from the plastic overlay tracing.[13] Thawer et al[14] compared manual acetate tracings and digital planimetry for human clinical and experimental animal wounds, both with multiple observers. Reliability of both methods was excellent, but digital planimetry proved somewhat superior for animal wounds, and for both types of wounds, the reliability increased when the average of three measurements was used. Precision also improved with averaging of three measurements. Agreement between the two methods was good for human wounds but poor for animal wounds, even with averaging. This lack of agreement in planimetric methods may be due to the difference in mobility between human and animal skin. Obviously this would not be a problem in a research setting, in which only one method would be used to obtain planimetric data. This study indicates the need for caution in comparing the results of studies that used different planimetric methods, particularly when the data are reported as absolute change in area rather than percent change.

Planimetry has also been used in combination with other techniques to evaluate the area of perfused wound. In one example, digital planimetry used in combination with

intravital fluorescence microscopy allowed the investigators to measure the perfused wound area while observing the effects of a vasodilator on wound healing in an ischemic wound model. [18]

Three-dimensional planimetry

With the exception of very superficial abrasions and stage I or II decubital ulcers, most open cutaneous wounds have measurable depth, and this component of the wound may in some cases be the most important practical measure of the progress of healing (eg, deep ulcers and open wounds over exposed bone often fit this category). With recognition of the importance of wound depth as a variable, researchers have developed 3-dimensional methods of wound assessment that allow measuring wound volume as a variable. One method uses gelatin molding (Jeltrate®, Dentsply Caulk International) of the wound. First, the wound is filled to the level of the surrounding skin with gelatin. The gelatin is then removed and weighed, and wound volume is calculated from the known density of the gelatin.[19] Comparing this volumetric method with standard 2-dimensional planimetry revealed that the former was able to detect differences between two wound treatments as early as day 7, while the latter did not show any difference until day 14. This finding is not surprising, as the production of granulation tissue must precede the onset of rapid reduction in wound area via contraction and epithelialization. More recently, advances in computer software have made it possible to combine digital photography and digital planimetry to produce a three-dimensional (rather than only

two-dimensional) representation of the wound (DigiSkin, RSI). This allows the quantification of wound volume without the need for relatively slow and cumbersome physical means of measurement.[20] Extensive testing of this new system and comparison with established wound measurement systems have not yet been published at the time of this writing.

Other combination wound evaluation techniques that utilize planimetry are the Kundin Wound Gauge and stereophotogrammetry. The former consists of a simple disposable plastic ruler that is used to measure length, width and depth of the wound and calculate volume. Stereophotogrammetry creates a topographical map of the wound much as a physical topographical map of land area and provides an area-by-area assessment of wound volume. In a clinical assessment in 82 human patients with leg ulcers, the Kundin Wound Gauge and standard digital planimetry demonstrated a high level of agreement with stereophotogrammetry (r = .99 and .98, respectively). Two other wound assessment protocols, the Healing Scale (measures perceptions of healing) and the Johnson Scale (measures wound characteristics) were included in the comparison. These methods did not show a high degree of agreement with the planimetric methods and were considered to have limited reliability.[21]

Studies have been performed to compare various two and three dimensional wound assessment techniques for accuracy, repeatability, and interobserver variation. Langemo et al reported on a comparison of 4 techniques: linear length and width measured with a millimeter ruler, planimetry, and stereophotogrammetric measurement of length and width, and area. Sixty-six evaluators (nurses and nursing students) evaluated 3 model wounds made from plaster of Paris, measuring each wound twice.

The lowest standard error of measurement was obtained with stereophotogrammetric area measurement, followed by ruler length and width, stereophotogrammetric length and width, and planimetry. Only stereophotogrammetric area measurement had a low enough interobserver variation to be useful for research purposes in multiple-observer studies. All methods showed low intraobserver variation, making them potentially useful for studies having only one observer.[22]

Limitations of planimetry

One weakness of all area-measuring methods of healing assessment is a systematic bias on the basis of wound size. In other words, given the same percentage of healing, wounds of larger initial size will report a larger absolute change in wound area than will smaller wounds. Conversely, when healing is reported as percent change in area from initial area, the healing of small wounds will tend to be overstated. Gilman[23] devised a calculation to report the linear healing to overcome this bias. His equation is the change in area divided by the average linear perimeter:

$$\text{Linear healing} = \frac{\text{initial area} - \text{final area}}{(\text{initial perimeter} + \text{final perimeter})/2}.$$

Gorin, et al[24] used the Gilman equation to compare the planimetric evaluation of healing for 40 venous stasis ulcers of 39 human patients. Regression analysis was performed to assess correlation between calculated healing rates and wound geometry.

When healing was expressed as area healed per day, there was a strong correlation between healing rate and initial wound area ($r = 0.8$; $p < 0.0001$), and between healing rate and perimeter ($r = 0.85$; $p < 0.0001$). When healing was expressed as linear healing per day, there was no correlation with initial area, perimeter, or wound width x length.

Omar, et al[25] also used the Gilman equation to evaluate a wound care product, Dermagraft®, (a dermal replacement product derived from human fibroblasts) on venous stasis ulcers. Area in cm^2 and linear healing both showed significant advantage for Dermagraft® compared to control; however, the difference between Dermagraft® and control was affected by how healing was reported. Dermagraft® was over 5 times better than control when healing rate was evaluated by area, but only 4 times better than control when evaluated by linear healing. Method of reporting made no practical difference in this evaluation, but in cases where the treatment and control are closer, a type I error might be made by reporting change in area. Given the prevalence of area-based reporting of results in wound healing research (80% of studies in one random sampling of 20 clinical trials[24]), this may partly explain the failure of some new treatments and products to live up to the expectations based on trial data.

1.1.3 Planimetry as a prognostic tool

The ability to make an early assessment of the rate of healing and time to final closure of wounds is very attractive. This allows more accurate prognostication of the final outcome and gives an early indication of which wounds require special intervention to assure complete healing. One reason for the importance of prediction based on early evaluation is that continued monitoring of chronic wounds to the final endpoint of complete closure is not always practical. This is particularly true in the clinical setting, where once the chronic wound is healing, the patient may be discharged to further care at the referring hospital or at home.

Kantor and Margolis[26] reported on the use of digital planimetry to predict healing of venous leg ulcers in 104 patients. Rate of healing over the first 4 weeks was a useful prognostic indicator of healing, but only when calculated as percent healing over time, and not when calculated as absolute change in wound area over time.

Tallman, et al[27] devised a novel measurement of healing, the mean-adjusted healing rate, as an early predictor of healing. The mean-adjusted healing rate is a calculation of healing rate that takes the mean of all prior healing rates from one measurement to the next. The calculation is performed in two steps. In the first step, planimetric measurements at each wound assessment are used in the Gilman equation shown in the prior section; however, the “final” and “initial” are the current and immediately prior measurements, respectively. This gives a sequential healing rate from

one assessment to the next. In the second step, all of the sequential healing rates are averaged. An example of a 3-week trial would look like this:

$$\text{Mean-adjusted rate} = \frac{\frac{(A^0 - A^1)}{(P^0+P^1)/2} + \frac{A^1 - A^2)}{(P^1+P^2)/2} + \frac{(A^2 - A^3)}{(P^2+P^3)/2}}{3}$$

where A^n = area at week^n , and P^n = perimeter at week^n

The authors employed their calculation in a 24-week clinical trial of 15 human patients with venous leg ulcers. They were able to accurately predict the time to complete healing as early as 3 weeks from the beginning of therapy.[27]

Mathematical modeling of wound healing is another approach that can be used to predict the rate and ultimate time of healing based on a few early measurements. A model has been developed,[28] applying a Gompertzian function to wound healing rates for human decubital ulcers. Wounds were photographed weekly and planimetric data was used to generate a nonlinear model to describe the rate of healing over time. Meta-analysis was performed on several large clinical studies in human chronic wound patients, in which different formulas were used to predict healing. Healing outcome was accurately predicted in approximately 75% of chronic wounds.[29] As of this writing, no comparable work exists to predict outcomes for veterinary patients with chronic wounds. Development of a similar algorithm for veterinary medicine is complicated by the small number of suitable patients, and the much greater variety of etiologies and complicating factors in veterinary wounds. Perhaps a multi-center study would be able to eventually amass the required amount of data.

Part 1.2. Tensiometry – the assessment of wound strength

1.2.1 Introduction

The measurement of wound strength has been used in the assessment of wound healing since the late 1920's.[30] Tensiometry is well suited to study wound healing because one test provides an overview of the success (or lack of success) of the complex interactions of events that constitute wound healing. This allows investigation into any facet of wound healing with the confidence that the overall effect on wound healing will not be missed, for example, by investigating effects on the "wrong" cell type or cytokine that happens not to be affected. The gain in wound strength is highly correlated to two key processes in wound healing, proliferation and remodeling or maturation;[31,32] this has been demonstrated for both first-intention[32-36] and second-intention healing.[37-41] Wound strength is a simple concept and easily measured. It generates immediate data that is reportable in objective interval format, favoring data analysis. Tensiometry does have certain limitations, however. It is a destructive test in nearly all methods; this not only limits its practicality to biopsies or terminal studies, but also renders the sample useless for other evaluation such as histology.[42] The requirement for biopsy samples of significant size also severely limits the use of tensiometry to evaluate wound healing in the clinical setting. Tensiometry is also not ideal for discrimination of changes in wounds during the early "lag" period before the rapid rise in wound strength begins at about post-wounding day 7.[35,43] Limitations notwithstanding, the measurement of wound strength remains a valuable method for assessment of healing.

1.2.2 Biomechanical Properties of Skin

Skin possesses certain biomechanical properties that influence its behavior under tensile testing. Skin is a viscoelastic material,[44,45] thus it has material properties of both viscous and elastic materials, which respond to stress in different ways. Stress is the internal force per unit area that is generated within a material in response to the application of an external load. Strain is the physical deformation of a material in response to stress. Viscous materials strain in a linear function with time when stress is applied. Elastic materials exhibit strain when under stress, but then return to their prior shape when the stress is removed. When placed under stress, skin exhibits the properties of *creep* and *stress relaxation*. If tension is placed on the skin and maintained, the skin will continue to deform – this is the property known as creep.[46] If instead the skin is loaded to a certain amount of strain and the length is held constant, the stress in the skin will decrease over time – this is stress relaxation.[46]

Skin tension over the body is not homogenous; instead, skin has lines of tension. This property of skin was first described by Dupytren in 1834, and the description was further refined by Langer in 1861.[47] The lines of skin tension on the body of a dog are parallel lines from dorsal to ventral.[48] Normal wrinkle lines of the body generally run parallel to the lines of tension.[47] Further investigation at the microscopic level has shown that the primary orientation of dermal collagen fibers is parallel to these lines of tension.[49] The tensile strength for unwounded skin and healed incisions oriented parallel and perpendicular to the wrinkle lines has been compared. Tensile strength of unwounded skin is significantly greater when the direction of pull is parallel to lines of

tension; in contrast, tensile strength is greater for healed incisions made perpendicular to lines of tension.[47] The relationship between tension at closure and tensile strength of the healed incision is discussed in section 2.4.

1.2.3 Tensile Testing

Breaking Strength vs Tensile Strength; Measurement of Skin Thickness

Tensiometry is technically defined as the measurement of tensile strength, although as applied to wounds the term more commonly refers to any measurement of wound strength. *Breaking strength* is the disruptive force that is required for the wound to fail, usually defined as the complete parting of the edges. *Tensile strength* is the breaking strength per unit of cross-sectional area; and is typically reported in kilograms or Newtons per square centimeter.[2] In this review we will use the terms "tensiometry" and "tensile testing" in the general sense as measures of wound breaking strength, and use "breaking strength" and "tensile strength" as defined above. Tensile strength can only be measured when wound cross-sectional area can be measured.[2] This requires an accurate measurement of skin thickness, a sometimes difficult task that may alter the wound, resulting in risk of significant error in measurement that may add a confounding factor to assessment of wound strength.[50] Skin thickness has been measured with a number of techniques including direct measurement with calipers[51] or micrometer

thickness testers,[52,53] radiography[54,55], and pulsed ultrasound.[56-59] Micrometer thickness testers such as those used in industrial applications are simple and accurate, at least on hard material; one that has been used in a tensiometric study[53] has an accuracy of ±0.015 mm. Little pressure (0.2N or less) is applied to the tissue when a thickness measurement is being made with this instrument. It is assumed that this pressure is light enough not to distort the measurement, but this has not been documented for skin. Ultrasonographic measurement of skin thickness has the advantage of being non-invasive; not only does it not damage a biopsy specimen, but it can also be used in living subjects. It possesses fairly good sensitivity, being able to "resolve" differences in skin thickness of 0.1mm.[58] In a comparison of ultrasound with micrometer and radiographic methods of skin thickness measurement in 16 human subjects, ultrasound measurement was found to have a reasonably high correlation ($r = 0.68$ to 0.75); all methods were sensitive enough to detect reduction in skin thickness resulting from a one-month steroid treatment.[52] An in vitro comparison between ultrasound and frozen section histology, reported an even higher correlation ($r = 0.99$, $p < 0.001$).[60]

The issue of tensile strength versus breaking strength in wound healing studies is somewhat controversial. Proponents of tensile strength claim that tensile strength is the more biomechanically correct measurement and point out that wound breaking strength is affected by tissue thickness and length of the incision.[2] Wound breaking strength is favored by other investigators as a more practical parameter for the surgeon, and note that differences in tissue thickness may be part of the difference in healing response and therefore should not be factored out of the wound strength evaluation.[50]

Types of Tensile Tests

Tensiometry has been classified into three types of tensile tests by Al-Sadi and Gourley.[61] The "strip in vitro"[62-65] method requires the wound to be oriented transversely in an excised strip of skin. One end of the strip is fixed and a distractive force is applied to the other end until the wound disrupts. In the "disruption-from-within"[66,67]method, the tissue containing the wound forms a portion of an airtight cavity; compressed air is pumped into the cavity and the bursting pressure in mm of Hg is recorded as the breaking strength. This method is best suited to wound strength measurements in hollow organs or body cavities. The "disruption-from-without"[61]method is similar in concept to the "strip in vitro" method, but the tissue containing the wound is left in situ.

Tensile Testing Instrumentation – "Strip in vitro" method

The first tensile testing of skin wounds in modern times used the "strip in vitro" method and was performed on a modified thread testing machine by Howes, et al[30] in 1929. A number of the early tensile testing instruments followed this basic concept. The skin sample was held by two clamps, one fixed and the other attached to a moveable apparatus that applied a linear distractive force across the wound. Some of these units used gravity to supply the required force; weights were added until the wound failed, with the breaking strength being recorded at that point.[2] Large incremental addition of

weight and no control over the rate of application of force were recognized as design weaknesses in these early instruments. Later units used reservoirs for weight into which water, sand, or lead shot could be poured, allowing a relatively slow, steady addition of force until wound failure.[2] The next major development was the replacement of the primitive weight system with a motorized screw apparatus and load cell; the rate of application of force could be controlled by the speed of the motor.[47] Data collection also improved; the earliest tensiometers only provided information on breaking strength, but with the addition of chart recorders[47] it became possible to produce a force/displacement curve, allowing stress, strain, and stiffness to be reported.

The modern tensiometer, exemplified by the Instron 5500 Series® materials testing system, is a sophisticated and versatile instrument. All of the set-up variables including preload, crosshead speed (extension rate) and maximum load can be set and retained in system memory so that exact conditions can be duplicated in each testing session. Specialized sample handling is now available including environmental chambers to more closely match *in vivo* temperature and moisture conditions. The system also includes proprietary application software that provides continuously updated information on load in Newtons, extension, stress, strain, and other parameters. This data is available in graphical format that is downloadable for publications or presentations.[68]

Tensile Testing Instrumentation – "Disruption-from-within" method

Other instruments have been developed to evaluate wound strength via the other methods such as "disruption-from-within". Most of the "disruption-from-within" devices were designed for tensile testing of healing in hollow viscera (for example, healing of anastomoses) or body cavities such as the abdominal wall.[69-74] The air insufflated positive pressure device, or AIPPD, developed by Meyers, et al in 1967,[66] is unusual in that it uses the disruption-from-within methodology but was designed to test excised skin samples. This instrument is categorized as "disruption-from-within" because the disruptive force comes from beneath the skin surface. The device consists of a cylindrical air pressure chamber with a 1 x 2 cm hole in the top. The skin sample is placed over this hole and secured in place with an o-ring. Air is pumped into the cylindrical chamber until the wound is disrupted, and the pressure (in psi or mm Hg) is recorded.

Tensile Testing Instrumentation – "Disruption-from-without" method

The "disruption-from-without" method was developed with the purpose of enabling tensile testing *in vivo*. The majority of instrumentation for this type of testing used hooks attached to either side of the healing wound;[61] tension was applied by a variety of means. Although wounds can be tested in a living animal, the test is destructive, therefore painful, and requires anesthesia for testing.

A different approach is the vacuum controlled wound chamber device (VCWCD). The device consists of a cylindrical glass vacuum chamber that is connected to a vacuum pump with appropriate control apparatus and vacuum gauge. An acrylic ring is affixed to the healed skin wound site with cyanoacrylate adhesive, and the ring is mounted on top of the glass cylinder, producing a sealed vacuum chamber. Vacuum is applied and the wound is monitored via video camera until the wound disrupts. The video images are digitalized and used to construct force/time disruption curves. Charles, et al[67] compared this device with the AIPPD and the Instron tensiometer; no significant difference was seen in their disruption curves. He found that for wounds tested at 2 days post-wounding, the VCWCD had a significantly lower standard error, indicating higher repeatability. The authors attributed this to the fact that the other methods required removal of the test specimen that was very friable and easily damaged, and cited this as an advantage of *in vivo* methods in general and the VCWCD in particular.

Other tensile testing instrumentation and methodologies

The same concept and technology of the VCWCD that was used by Charles, et al[67] was later modified by Gingrass, et al[75] in the first reported non-disruptive *in vivo* biomechanical test of linear incision wounds. In this study, paired incisions were made on the dorsum of experimental rats. One incision was tested by the disruptive method of Charles, while the other incision was tested with the same vacuum device but not to disruption. Video monitoring of the skin's deformation was synchronized with vacuum

pressure data, enabling the computer to generate a pressure/deformation curve and calculate wound stiffness in kilopascals. The authors cite several important advantages to their method, including:[75] 1) non-destructive nature of the test 2) true *in vivo* testing; the authors cited unpublished data stating that significant differences exist between *in vivo* and *ex vivo* samples 3) multiaxial stress applied to the wound vs uniaxial stress with linear tensiometry – the authors claimed the former to be more physiological 4) absence of tissue manipulation prior to testing, that could alter test results.

Hollander et al[51] employed a laser tensiometer to test the strength of scar tissue in a porcine open wound model. An exact description of the device and its use were not provided in the report.

1.2.4 Application of Tensile Testing to Wound Healing Research

Tensiometric investigation of normal wound healing physiology

Tensiometry has been employed to describe normal wound healing; such descriptive studies provide comparative data for experiments on pathologic wound healing and/or treatments to stimulate wound healing. Care must be taken when comparing data generated by different methods. For example, Al-Sadi and Gourley[61]

reported a 1.4 kg mean breaking strength for day 7 linear sutured wounds in dogs, using their *in vivo* tensiometer. Scardino, et al[76] reported a somewhat lower breaking strength (1.2 kg) *in vitro* at day 10 postwounding using the Instron tensiometer. Several factors may have contributed to the difference, including *in vitro* vs *in vivo* testing, difference in testing instrument, and different definition of wound failure.

One way to standardize tensile testing is to perform all tests in a single session, thus ensuring the same testing conditions for all samples. Unfortunately, this is not possible when different post-wounding times are compared. Paul, et al[77] examined the effect of freezing at – 80^{o}C on sutured wound breaking strength at 10 and 20 days post-wounding. The strength of fresh and frozen samples was almost the same. Frozen samples had a slight decline in strength compared to fresh samples; however, this difference was not statistically significant. Interestingly, a comparison of wounds taken from cranial and caudal positions along the dorsum also showed no difference in breaking strength at 14 and 28 days postwounding. This result is contrary to the reported effect of wound position on open wounds, in which a more cranial position is associated with increased rate of contraction and epithelialization.[78]

Although breaking strength is usually assessed within 1 to 4 weeks post-wounding, tensiometry can also be used to follow the course of healing over longer periods of time. Forrester, et al[79] followed wound strength for 150 days via tensiometry and histology and found that wound remodeling was still active 150 days post-wounding, although breaking strength and energy absorption remained well below pre-wounding levels. Normal data such as this are important for the study of chronic wound healing and wound healing in impaired states. In a guinea pig model that compared

normal and radiation-impaired healing, significant reduction in breaking strength of irradiated wounds was seen at 7 and 14 days post-wounding;[80,81] application of TGF-β ameliorated this effect.[80]

Some nutritional factors in wound strength

Collagen is a primary component of the gain in wound strength;[32] its synthesis, secretion, and cross-linking are vital processes in wound healing that are dependent on a number of enzyme systems, which in turn require vitamin C and Zn as cofactors.[82] Kaplan, et al,[32] examined the interaction of these factors in a rabbit full-thickness skin healing model and found that wound tissue levels of Zn, ascorbic acid, and hydroxyproline increased sharply by 5 days postwounding. These increases preceded a rapid gain in tensile strength on day 7, indicating that collagen secretion alone added little to the gain in tensile strength and that cross-linked collagen begins making a substantial contribution to wound strength beginning about day 7. The influence of other nutritional factors on wound healing has also been evaluated by tensile testing. Essential fatty acids (EFA), although necessary for normal dermatologic health, were shown not to be essential to normal wound breaking strength in a rat model of EFA deficiency.[65] In a similar study on riboflavin deficient rats, wound tensile strength was reduced by 63% compared to weight-matched controls.[83]

Evaluation of effects of surgical technique on wound healing via tensiometry

One common application of tensiometry is testing of the effects of various types of surgical techniques or devices on wound healing. For example, the type of instrument used to create a surgical incision might have an effect on the rate and strength of healing. Electrosurgical incision was compared with scalpel incision in a porcine model. Healing was determined to be delayed to at least 14 days postwounding by histological methods; however, no difference in wound tensile strength was demonstrable.[84] In another study, steel scalpel incisions were compared to CO_2 laser incisions at two wavelengths, 9.55 and 10.6 nm. Previous data in pigs had indicated that 9.55 nm laser produced less thermal damage due to a more specific targeting of collagen and therefore possibly resulting in a stronger incision, particularly in the early post-wounding period. Scalpel incisions were consistently stronger from 3 through 21 days post-wounding than either of the two laser incisions, which showed no differences.[85]

The CO_2 laser can be adjusted for continuous or pulsed delivery of energy. The latter offers the potential advantage of lower collateral heat damage and more rapid healing. This concept was evaluated by Sanders and Reinisch[86] in a rat model via tensiometry and histology. Pulsed laser incisions had significantly higher wound breaking strength than continuous wave laser incisions. This finding correlated to the histologic results: pulsed incisions had 118μm of lateral thermal damage compared to 333μm for continuous wave laser. Compared to scalpel incisions, pulsed laser produced a 1day delay in gain of wound strength, while continuous wave laser produced a 3.2 day

delay. Mison, et al,[53] evaluated the CO_2 laser for incision of skin flaps in dogs and compared this with scalpel incision via tensiometry and histology. Tensile strength of healed incisions was significantly greater ($p = 0.01$) for scalpel incisions ($0.49N/m^2$) than for laser incisions ($0.17N/m^2$). It should be noted that actual tensile strength was reported in this paper rather than the more common wound breaking strength. Taylor, et al,[87] compared the Nd:YAG laser and diode laser to scalpel incision in a rat model with a purpose-built computerized tensiometer. They also found that wound strength was higher in the scalpel group to 21 days postwounding, but no difference was found between the two laser groups.

The amount of tension applied at wound closing has been considered to be a factor affecting the strength of healing; high closing tension has been reported to be a cause of delayed healing and possible wound dehiscence.[63] Tensiometry has been employed in a rat model to evaluate the effect of high closing tension on the strength of healing wounds.[43,63] Contrary to earlier belief and surgical dogma, beginning at 7 days postwounding and onward, wounds closed under high tension had higher tensile strength than wounds closed without tension.[43,63] This appears to be a demonstration of the biomechanical phenomenon of "tissue remodeling"[88] which is the principle of Wolff's law[89] expressed in soft tissue – stress leading to increased strength.[90] The relationship between wound tension and increasing tensile strength of healing has been further examined by Farahani, who has proposed the "coupled pendulums" hypothesis.[91] This hypothesis contends that repetitive mechanical strain downregulates acute wound inflammation[92] and stimulates fibroplasia.[93] The resulting increase in wound stiffness limits this effect,[94,95] causing a gradual shift from an acute inflammatory response to a

more chronic inflammatory response. This in turn inhibits contraction[96] and allows the wound to be once again exposed to greater extrinsic (tensile strain) forces. These forces induce expression of cytokines such as IL-1 and MMPs that stimulate remodeling,[97] leading to increased strength and stiffness. The shifting between intrinsic (wound contraction) and extrinsic (wound tension) forces has been compared to two coupled pendulums transferring energy to one another.[91]

Evaluation of wound healing stimulants by tensiometry

The current pandemic of type II diabetes and concurrent aging of the population throughout the industrialized world have brought an increase in chronic and nonhealing wounds. With that has come an increased interest in medications and other therapeutic agents to improve the speed and or strength of wound healing. Tensiometry has been and remains a standard technique for the evaluation of these many compounds.

One category of wound healing stimulants is natural products derived from various plants that are believed to have medicinal properties. Tensiometry is a rapid useful way to evaluate the ability of these products to stimulate the proliferative and remodeling events of wound healing, thereby producing stronger and more rapid first intention healing. A large number of such plant products that have been evaluated by tensiometry, including *Hamelia* patens,[98] *Aloe* vera,[99,100] *Hypericum* spp,[101,102] *Terminalia* spp,[103,104] and *Centella* asiatica.[105,106]

The effect of a pico-tesla electromagnetic field on healing on incisional wounds was investigated in a rat model.[107] The premise of the treatment is that very weak

electromagnetic fields may influence biologic systems including wound healing. Exposed sutured wounds demonstrated higher breaking strength than controls at 14 days; these results correlated with perfusion and histologic findings.

Part 1.3. Measures of Perfusion and Wound Healing

1.3.1. Introduction – the relationship between perfusion and wound healing

The evaluation of cutaneous perfusion is one of the oldest methods to assess wound healing and dates to ancient times when the inflammation associated with wounding was described in terms of *rubor, tumor, calor* and *dolor*, the first two being visual indications of perfusion and the third being a palpable indicator. These early changes relate to increased wound perfusion as a part of the inflammatory process that is the second major process in wound healing (after coagulation), often termed the *inflammatory phase* of wound healing. Perfusion increases in the acute post-wounding period in response to a large number of physical, cellular, and molecular signals, that all work to increase the delivery of the needed "materials" (oxygen, nutrients, cytokines) and "labor" (specific inflammatory and proliferative cells) to the wound.

1.3.2. Visual assessment of perfusion

Vital capillary microscopy can be used as a direct visual assessment of tissue perfusion. This method uses low magnification (10x to 100x) with a drop of oil placed

on the skin to reduce surface reflection of light.[108] The advantage of this method is that it allows regional capillary density and blood flow to be assessed in real time. Digital image analysis makes possible the quantification of flow. A modification of this method is the addition of intravenous fluorescein dye, which makes the images of the capillary loops much easier to see.

1.3.3. Pressure measurement as an estimate of perfusion

Direct measurement of capillary pressure

The direct measurement of capillary blood pressure as an estimate of cutaneous perfusion dates to the 1920's. In its earliest form, capillary pressure measurement was made in the capillary bed of the fingernail bed. A transparent membrane connected to a manometer was placed over the bed and capillaries were observed via vital microscopy while the pressure under the membrane was increased until capillary flow stopped. Pressure was gradually decreased again until flow resumed – this pressure was recorded as the capillary pressure. Although this method was too cumbersome and limited to be useful for clinical studies, it demonstrated the principle. Later, direct cannulation of capillaries was performed with glass micropipettes, allowing the first direct measurement of capillary pressures. The original design also required vital microscopy to observe the flow of blood into and out of the micropipette as the pressure was adjusted – the lowest pressure that stopped this to-and-fro motion was read as capillary

pressure. Advances in technology have since allowed continuous electronic monitoring of capillary pressure via conductance. The micropipette is filled with 2M saline solution, an excellent conductor. As blood enters the micropipette, conductance drops. This change in conductance is used to adjust the pressure in the micropipette. The pressure at which there is no further change in conductance is the capillary pressure.

Direct measurement of capillary pressure is accurate and has been used as a standard to verify other measurements of cutaneous blood flow. The disadvantages of the technique are its invasiveness and general impracticality for clinical studies.

1.3.4. Temperature as an estimate of perfusion

General comments

Skin temperature has been used from early times as a subjective measure of either inflammation, or cutaneous perfusion – *calor* of the four cardinal signs of inflammation. Even now, skin temperature assessment is used as an assessment parameter in subjective wound assessment scales.[109,110] Skin temperature can be used to assess either local or regional cutaneous perfusion; the former by thermometry or by radiometry, and the latter by thermographic imaging.[111] The surface temperature of skin, whether intact or wounded, is influenced by a number of factors both intrinsic and extrinsic, including not only the local perfusion (which is the primary factor), [112] but

also the ambient temperature, metabolic rate, and the thermal conductivity, which is influenced by the amount of overlying subcutaneous fat.[111,113]

Skin temperature and local cutaneous perfusion – thermometry and radiometry

Point contact thermometry involves the assessment of thermal clearance by cutaneous blood flow. Several variations of this method have been developed, including both invasive and noninvasive, constant power and isothermal, and steady-state and transient. One variant, the isothermal steady state arrangement, consists of a heating element surrounded by thermocouples placed in contact with the skin. The skin temperature is recorded first, then the heater is turned on and the skin temperature is raised by several degrees. Finally, it is turned off and the decline in skin temperature back to resting is recorded. The greater the cutaneous perfusion, the greater the rate of temperature decline. One disadvantage of all steady-state methods is that the rate of heat clearance by blood flow is also dependent on the thermal conductivity of the tissue, requiring an *in vivo* calibration of the instrument in order to report a quantitative evaluation of tissue blood flow in absolute terms (ml/100gm tissue/min). The noninvasive transient thermal clearance (NTTC) method, like steady-state methods also provides blood flow measurement in absolute terms. In this method, the temperature increase over time is measured in a metal plate in contact with the skin and otherwise thermally insulated from the surroundings. The initial rate of temperature increase is dependent on both heat conduction from the tissue, and convective heating from blood flow. As the plate approaches thermal equilibrium, the gradient between plate and skin

temperature becomes insignificant, and the only important contributor to plate heating is convection from skin blood flow.[114-116] The NTTC method has been compared with laser Doppler flowmetry and a high level of agreement ($r = 0.90$) was found, demonstrating its accuracy.[117] Unfortunately the NTTC can require 10 – 50 minutes to reach thermal equilibrium in normal skin and even longer with states of relative ischemia. A mathematical method was developed to derive the time constant of the exponential temperature increase without the need to know the final equilibrium temperature, thus reducing the measurement time to less than 10 minutes.[118]

Radiometric methods of perfusion measurement rely on the principle of Wien's displacement law, which states that the maximum energy dissipation from a body is dependent on the temperature of that body.[119] The peak radiation from mammals, with body temperature in the range of 37-39^{o}C is approximately10μm, the far-infrared range. Thus, an infrared radiometer with sensitivity in the 2-25μm range can be used to measure skin surface temperature. Thermography is a variation of radiometry in which infrared radiation from the skin is sensed by an indium antimonide or mercury cadmium telluride detector. The output converted to a visible light signal; this produces a "heat map" of the skin. As with any radiometric method, thermography is very sensitive to changes in ambient temperature relative to the animal's skin temperature, and requires a stabilization period of at least 15 minutes before obtaining a reading. Significant variation between the skin temperature and ambient temperature can influence the validity of readings via heat loss or gain.[111]

1.3.5. Radioisotope clearance studies

Radioactive isotopes have been used since the 1940's to measure cutaneous blood flow; the earliest studies used Kr^{85} (1944) and Na^{24} (1945), both injected intravenously. A limitation of intravenous injection is that it provides a qualitative rather than quantitative assessment of cutaneous perfusion. Subcutaneous injection of the radionuclide (1946) made a more quantitative assessment possible. The principle is that as radioactivity at the site of injection is monitored, the rate of decay is proportional to the local cutaneous and subcutaneous blood flow. The tissue blood flow, Q (expressed as ml/100g tissue/min) is estimated from the following equation, in which λ is the tissue/blood partition coefficient and $t_{1/2}$ is the half-life of the tracer.

$$Q_{(\text{ml/100gm tissue/min})} = \frac{100 \times \ln 2 \times \lambda}{t_{1/2}}$$

The effective half-life of the tracer is determined by biological and nuclear components; in most situations the biological component is more the influential determinant. The biological half-life is in turn highly dependent on the diffusibility of the tracer in the tissues.

Hydrophobic (lipophilic) radionuclides tend to be more diffusible, while hydrophilic radionuclides tend to be less so. Therefore, the hydrophilicity of the radionuclide chosen and the amount of subcutaneous fat will affect the clearance rate of

the tracer. The amount of subcutaneous fat varies widely between species and individuals, and also between sites on an animal. These factors complicate the ability to compare results across different studies when different tracers, animal subjects, or injection sites are used.

A variation of the radioisotope tracer clearance technique is the use of radiolabelled microspheres. Gadolinium^{153} microspheres (15μm) suspended in a solution of 10% dextran with 0.05% Tween 80 were injected into the left atrium; reference blood samples were taken from a femoral artery catheter. Radioactivity of the reference blood samples and tissue biopsies was measured by gamma counter and blood flow calculated from the following formula:[120]

$$BF_t = \frac{C_t \times 100 \times BF_r}{C_r}$$

where BF_t and C_t = tissue blood flow (in ml/min/100gm) and counts, respectively, and BF_r and C_r = reference (femoral artery) blood flow and counts, respectively. Using this method, the regional blood flow of several regions of the skin, and oral mucosa of the cat were measured and compared to tissue thickness to look for a relationship.[121] Blood flow ranged from 25 to 36 ml/min/100gm in the oral locations and only 4 to 5.5 ml/min/100gm in the skin. Rank order of epithelial thickness was compared to rank order of blood flow; the correlation coefficient $R = 0.87$ demonstrated significant ($p < 0.005$) positive correlation between epithelial thickness and blood flow.[121] While useful and accurate in an experimental setting, the microsphere method as used above is very labor intensive and is unlikely to find application in clinical wound healing studies.

1.3.6. Optical methods

Doppler Flowmetry

The Doppler Effect

All Doppler perfusion techniques rely on the Doppler effect, named for Christian Doppler, who discovered the effect and described it in 1842.[122] Briefly stated, the Doppler effect describes the shift in frequency that occurs in a waveform of energy as the source and its receiver change relative distance from each other. When the source and receiver are coming closer together, the wave compresses and the frequency therefore increases; when they are drawing farther apart, extension of the wave causes the frequency to decrease.[122] This effect is experienced by anyone standing near a train crossing as the locomotive goes past while sounding its horn. At the moment the locomotive passes the observer and goes from approaching to departing, the pitch of its horn drops as the frequency of the sound waves goes from being increased to being decreased. When applied to measurement of perfusion, the Doppler effect works in this way: an incident wave form of energy (sound or light) passes into living tissues, where some of it strikes moving blood cells. Most of the incident wave is scattered, but a small

portion (about 7 – 9%) of it is reflected back toward the source.[108] If the blood is in motion, the incident wave will be Doppler shifted, and a receiver located at or near the source can measure the change in frequency. The greater the frequency change, the higher the velocity of the moving blood.[123]

Laser light as it relates to Doppler flowmetry

Any wave-form of energy can experience the Doppler effect. The Doppler effect used in conjunction with ultrasound technology to produce another method of perfusion measurement will be described later. Laser light has certain inherent characteristics that make it attractive as the wave source for Doppler flowmetry. Laser light is monochromatic and convergent; a single wavelength of light that is in phase. This feature concentrates the energy of the beam, helping to maximize penetration into a semi-opaque object (tissue in this case) for a given energy density of the beam. This allows a useful level of tissue penetration to be achieved before cytotoxic energy levels are reached. The most commonly used laser light source for Doppler flowmetry is the red light He-Ne laser with an output wavelength of 632.8 nm.[117] Light at this wave length can penetrate from 0.6 to 1.5mm into skin, depending on tissue geometry.[124]

Principles of Laser Doppler flowmetry

Laser Doppler flowmetry, in its simplest form, employs a single laser beam of light that enters the skin, where it strikes both stationary and moving objects (mostly RBC). Much of that incident beam is scattered, but a portion of it is reflected back in the direction of the source. Some of this light will be reflected from moving RBC and therefore be Doppler-shifted in frequency as previously described. The magnitude of the Doppler shift is proportional to the velocity of the moving RBC.[123]

Comparisons Between Species

Manning, et al[125] compared the cutaneous blood flow of 9 animal species with single-point Doppler flowmetry. The species studied were mice, Sprague-Dawley rats, New Zealand White rabbits, cats, dogs, rhesus monkeys, Yorkshire pigs, Holstein cows, and horses. Eight cutaneous sites were scanned. Anatomical and other differences between species meant that only 4 of the sites were scanned on all animals. These sites were the buttocks, scapulohumeral area, thoracolumbar skin, and the ventral abdomen. Five scans were performed on each animal for each region and the readings averaged. The cats were consistently among the lowest in blood flow, velocity, and volume for all areas scanned. Cats were also statistically lowest of the 9 species in blood flow and velocity for the scapulohumeral skin, and lowest in blood volume for the thoracolumbar skin.

The study by Manning, et al[125] is a landmark in that it is the first and so far the only attempt to obtain standardized measures of cutaneous blood flow for purposes of

comparison among species. This information should be of tremendous importance to wound healing research due to the wide variety of animal models that were employed. An important limitation of in this study was the non-uniform use of pharmacologic agents for restraint. Six of the species (monkeys, pigs, cats, rabbits, rats, and mice) were given ketamine hydrochloride IM as the sole restraint agent; doses, however, were not reported. Cattle and horses were given xylazine IV, the former as a sole agent and the latter with butorphanol; again, doses were not reported. Dogs were not given any chemical restraint. The known effects on blood pressure and peripheral vascular resistance differ significantly between ketamine and xylazine. Ketamine is generally considered to increase cardiac output and systemic arterial pressure and to have variable effects on peripheral vascular resistance.[126] In one paper, ketamine anesthesia increased peripheral resistance in the rat compared to the conscious state. [127] In the dog, ketamine anesthesia induced a significant decrease in peripheral resistance (40%) and mean arterial pressure (23%).[128] Xylazine has cardiac depressant effects that cause a significant decline in cardiac output and resultant systemic hypotension with a reflex increase in peripheral resistance in several species including the dog and cat.[129-132] With differing cardiovascular effects, the 2 drugs probably have significantly different effects on cutaneous perfusion. Examination of the literature did not disclose any comparative studies on the effects of different drug protocols on measures of cutaneous perfusion. Thus, the exact extent of the effect of differing drug protocols is presently speculative. The effect of anesthetic protocol on cutaneous perfusion measurements for wound healing studies should be a fruitful area for research.

Laser Doppler Perfusion Imaging (LDPI)

Laser flowmetry is a useful technique for measuring blood flow velocity, but suffers from the limitation that it is a point measurement technique – it does not provide a "map" of perfusion over an area of interest. All single point techniques are subject to flow variations within the field of interest, particularly for repeated measurements. For example, the probe is placed on the skin at point "A" which is over a capillary, for a measurement at time 0. A week later at time 1, a second measurement is made, but the probe is placed slightly to the side of point "A" and there happens to be a branch of a direct cutaneous artery under the probe, causing an artifactual increase in perfusion measurement. Measurement at multiple points within the wound or other area of interest help to ameliorate such errors with the best case scenario being the ability to measure all points within the area of interest. This is what scanning and "full-field" techniques attempt to accomplish. Laser Doppler perfusion imaging is a scanning laser Doppler technique that utilizes a moving mirror to direct the laser light into the tissues (while receiving the Doppler-shifted reflection) in a back-and-forth pattern similar to a farmer ploughing rows across a field. The orientation of capillaries in tissue is highly convoluted with flow being more or less uniform in all possible directions with respect to the probe, and an average flow velocity of zero. Therefore, rather than measuring blood *flow velocity* (distance over time in a direction), the term *perfusion* is used with reference to scanning units because they measure all blood cells in motion, without

regard to the direction of motion (ie, *speed*, the vectorless measure of distance over time). Both Doppler *flow*metry and Doppler *perfusion* imaging imply that the *volume* of blood, as well as its velocity or speed, is being measured, when strictly speaking it is not. Some Doppler systems use the strength of the Doppler signal as an indication of the volume of blood in motion, and combine this with velocity to give a calculated measure of actual blood flow. This is somewhat unreliable in that other factors such as light scatter in stationary tissues will affect the strength of the Doppler signal, and many authors feel it is better to restrict the use of Doppler units to relative rather than absolute measures of perfusion.[133] Commercially produced LDPI units are available. These units can scan areas of the body up to 12 x 12 cm[134] with depth of penetration into the skin of 1 – 2 mm.[123] These newer scanning units have been employed in a number of wound healing studies to investigate wound healing, including evaluations of several products or therapies purported to be stimulants or aids to wound healing.[8,12,76,135,136] In some instances, LDPI demonstrated increased wound perfusion in the experimental group, in other cases no difference from control was seen. In general, good agreement was seen between the results of LDPI and other means of evaluation of wound healing such as histology and gross observation, confirming the connection between wound perfusion and healing (particularly the inflammatory phase).[135-138] In one study, the use of hyperbaric oxygen stimulated healing of open wounds without a concurrent increase in LDPI perfusion; this was explained as a positive response to diffused oxygen.[139] LDPI was also found useful in differentiating normally healing skin flaps from problematic flaps and was also able to accurately locate areas of necrosis and venous stasis.[134] Taken

together the results of these studies validate the use of LDPI as a tool for the evaluation of wound healing.

Laser Speckle

Early in the development of laser technology, investigators observed that when a beam of laser light was directed onto a matte surface such as a piece of paper, a highly contrasting grainy pattern of light would appear on the surface. This pattern was termed *laser speckle*. Laser speckle made focusing difficult and initially was treated as an annoying artifact to be minimized, but before long the phenomenon was being studied and applications were found. One early discovery was that when the target object moves, the speckle pattern that it produces changes and that the frequency pattern of the change is proportional to the velocity of motion. Stern in 1975[140] was the first to recognize the potential for application of the laser speckle phenomenon to moving blood. Laser speckle imaging has some great potential advantages over laser Doppler methods. It is a "full-field" technique, allowing assessment of perfusion in a region of interest similar to Doppler scanning. However, unlike scanning Doppler, laser speckle is virtually instantaneous, like a snapshot of the field of interest. This means that speckle is freed from the long data acquisition times of Doppler scanning. Also, these "snapshots" can be rapidly repeated and linked to produce a real-time "movie" of changing perfusion.

Laser speckle is newer than laser Doppler and is therefore not as well developed as a technology. Presently there are no commercially available laser speckle perfusion imagers. Laboratory studies have shown promise, however.

Optical Doppler Tomography

Optical Doppler tomography (ODT) is a further refinement and extension of the technology of optical coherence tomography (OCT). It combines the signals from OCT (which, as described earlier, enable the precise localization of vascular elements) with Doppler flowmetry. This combination of location and flow imaging gives a precise map of the perfusion and how much perfusion is coming from each location. A prototype unit was constructed and tested by measuring labial blood flow in human subjects. It detected and accurately mapped blood flow to a depth of approximately 3mm below the surface and was able to display the direction of blood flow as well as the velocity. The unit reports blood flow in real units of perfusion velocity (mm/sec) – an improvement over arbitrary perfusion units but still not the ideal of actual volume per unit time. The reported overall accuracy of velocity measurement (94.3%) was excellent when the ODT unit was tested against known flow velocities in a phantom flow model.[141] As of this writing, there are no reports of the use of ODT for perfusion measurement in wound healing. However, the technology has been used to measure perfusion in the coronary arteries,[142] optic nerve head,[143] lip,[141] and subcutaneous tumors treated with photodynamic therapy.[144] The latter two applications are closest to present use of

perfusion imaging in wound healing studies and hold some promise for the application of ODT to this area as well.

1.3.7. Other Methods of estimating perfusion

Doppler ultrasound perfusion measurements

Doppler ultrasonographic is the newest technology for perfusion measurement and is still in the developmental/research stage.[111] It works via the same Doppler principle as laser Doppler perfusion measurement methods, the difference being that sound rather than light is being Doppler shifted. Doppler ultrasound has been used for a number of years in the evaluation of flow through the heart and large vessels; this is done with a transducer in the 5 – 10 Mhz range. The wavelength of these transducers is much too long for blood flow measurements in the cutaneous microvasculature, so a probe of 70 – 90 MHz is required.[111] Another problem area is the fact that besides the moving blood in cutaneous capillaries, there are tissue movements of breathing and muscle twitching. The signal from this background "clutter noise" is usually several orders of magnitude greater than from the capillary blood flow.[145] Various filters are used to reduce this clutter noise in commercial diagnostic ultrasound units. Continuous wave, pulsed wave, color Doppler and power Doppler are all being investigated as for their potential in perfusion imaging.[145] Improving the signal strength to noise ratio seems to be the largest challenge of Doppler ultrasound technology at this time. If it can

be overcome, one advantage that ultrasound may offer over laser Doppler perfusion imaging is a greater depth of penetration into the tissues (sound waves vs light), so ultrasound may prove more useful for deep wounds such as deep decubital ulcers.

Transcutaneous oxygen tension measurement

The measurement of transcutaneous oxygen tension (Tc pO_2) differs in principle from the monitoring of transcutaneous hemoglobin saturation as is widely practiced in clinical anesthesia. In the latter method, commonly referred to as pulse oxymetry, two light-emitting diodes direct light into the tissues at 660nm (red) and 940nm (infrared).[146] The reason for two wavelengths is due to their differential absorbance by hemoglobin and oxyhemoglobin: absorbance by oxyhemoglobin is lower in the red spectrum, while absorbance by reduced hemoglobin is lower in the infrared spectrum.[146] Changes in the amount of blood in the tissue and the proportion of oxyhemoglobin alter the absorbance of the light and this change is read by an optical sensor that converts the input to an electric signal that is proportional to the percent of oxyhemoglobin.[147] In contrast, Tc pO_2 measures actual transcutaneous diffusion of oxygen by a Clark electrode. As oxygen diffuses from cutaneous capillaries and through the skin, it is reduced at the cathode on the skin surface. The electrical current thus produced will be proportional to the pO_2 in the tissues when reduction is rate limited by the rate of diffusion. From this description of the principle of operation, it becomes clear that besides capillary blood flow, Tc pO_2 is also proportional to skin thickness and metabolic rate, as these also affect transcutaneous oxygen diffusion.[111] The technique also suffers from other

limitations: non-repeatability of measurements between different electrodes,[148] and disagreement with other measures of cutaneous perfusion, particularly with areas of relative cutaneous ischemia, in which it is not uncommon for Tc pO_2 to report low and even zero readings when capillary blood flow can be demonstrated by other methods.[111]

Part 1.4. Histology in the evaluation of wound healing

1.4.1 Introduction

Light microscopic examination of tissue biopsies of the healing wound has been and remains a mainstay of wound healing research. Much can be learned from the examination of the wound tissue, even without the aid of immunohistochemistry or other special staining techniques. In this section we will discuss the methods used in our research and the rationale for doing so.

Hematoxylin and eosin is a general tissue stain that was developed in the late 1880's and 1890's and is still the primary stain for most examination of mammalian tissue, used to differentiate nuclei from cytoplasm and to allow various cells and tissues to be differentiated one from another. Although there are a number of variations, all consist of a basic dye (hematoxylin or equivalent) to stain nuclei and an acidic counterstain (eosin) to stain cytoplasm. Hematoxylin is a natural dye, a product of the logwood (*Caesalpina campechiana*). Eosin is tetrabromofluorescein, an acidic xanthine

dye. It is widely used as the red counterstain for hematoxylin where it serves the purpose of staining cytoplasm.[149]

Wound healing can be evaluated by microscopy qualitatively, semi-quantitatively, or quantitatively. Qualitative evaluation of healing involves descriptive histology and does not attempt to generate ordinal data by assigning scores for the parameter under observation. An example of a completely qualitative evaluation would be to observe whether an outcome (granulation tissue formation, infection, etc) were present or not in the examined tissue sample. Semi-quantitative evaluation assigns scores or ranks to the observed parameter and generates ordinal data: an example would be a score of 1-3 for collagen deposition in the wound based on a set of predetermined criteria. Quantitative evaluation assigns actual numerical values that come directly from the observation of the tissue, (ie, neutrophil count) and provide interval data. The last category is often the most difficult to obtain, so many wound healing studies use semi-quantitative data instead.

Stains for wound collagen evaluation

Masson's trichrome is a combination of hematoxylin, picric alcohol, acid fuchsin, and acetic aniline blue originally described by Masson in 1929, it is primarily used as a differentiating stain for collagen.[150] When stained with Masson's trichrome, collagen fibers have a deep blue to blue-green color and are well differentiated from muscle tissue, which stains red.

Sirius red is a strong anionic azo dye,[151] originally introduced as a substitute for acid fuchsin in Van Gieson's trichrome stain, because of the relatively rapid fading of the fuchsin after a few months.[152] The combination of Sirius red in a saturated aqueous solution of picric acid at pH 2.0 (ie, picrosirius red) is a highly selective stain for collagen fibrils.[153] Sirius red stains collagen fibers by the chemical bonding of sulfonic acid groups (four per dye molecule[153]) with basic groups in the collagen molecule. The dye molecules are elongated in shape, and bind to the collagen so that they are oriented parallel to the long axes of the collagen molecules. This parallel orientation of dye and collagen molecules enhances visualization in polarized light, intensifying the weak natural birefringence of the collagen molecules. Birefringence is the double refraction of light as it passes through a material that is anisotropic, causing the ray of incident light to split into two rays. The unique binding property of picrosirius red to fibrillar collagen has been used to examine the process of formation and maturation of collagen fibrils in wound healing.[154] During the first 4 to 6 days postwounding there is no detectable fibrillar collagen via picrosirius red staining. During the second week, small, thin fibers that exhibited a weak greenish to yellowish-green birefringence are seen. Beginning at about the end of the third week postwounding, thicker fibers with more intense, orange to red birefringence are visible. These visible changes in the thickness and color of the collagen fibers correspond to changes in the type of collagen being secreted into the ECM as wound healing progresses. Early in the proliferative phase, reticulin fibers of type III collagen, and newly secreted, immature type I collagen fibers constitute the bulk of ECM collagen. Later, as the type I collagen fibers mature, they become thicker by

increasing cross-linking with time, and their birefringence becomes more intense and changes to an orange to red color as the light is refracted to a greater degree.[154]

Evaluation of wound collagen deposition by ordinary light microscopy is best suited to semi-quantitative data reporting. Purely qualitative data is too subjective and lowers the power of statistical analysis of the parameter. Completely quantitative evaluation (counting the exact number of collagen fibers) is not practical for ordinary light microscopic evaluation of collagen. If purely quantitative data on collagen deposition is required, a biochemical or molecular study would probably be more desirable. Another approach would be the use of digital computerized histomorphometry, a technique that has been in use and development since the mid 1980's.[155] In this technique, the slide is examined and the area of interest is located, then a digital camera on the microscope is used to capture the image. Computer software is available that can analyze the image according to the shade and intensity of color and count the pixels of the color of interest that are present in the microscopic field. This technique has been used with a number of different tissues or cells for various analyses. Some example analyses include: bone,[156,157] neovascularization in cruciate ligament healing,[158] articular cartilage,[159] nuclear morphology of tumors,[160,161] and muscle glycogen storage disease.[162]

1.4.2 Histologic evaluation of wound inflammation and proliferation

The classical description of wound healing is divided into three phases; inflammatory, proliferative, and maturation or remodeling.[163] It is now understood that healing does not occur in discrete phases; instead, processes of inflammation, proliferation, and remodeling wax and wane, with considerable overlap.[164,165] Classical terminology may be still be used to describe periods of healing in which a particular process may predominate. The inflammatory phase of wound healing begins with the wound itself and the activation of the coagulation process. Histologic evidence of this earliest inflammatory response to wounding may be limited to edema and acute hemorrhage. Wounding damages blood vessels, exposing the subendothelium, which in turn activates platelets and the clotting cascade. Thrombin (factor IIa) has been demonstrated to have a number of stimulatory effects on wound healing. Application of thrombin to wounds has resulted in increased wound tensile strength,[166] promotion of wound contraction[167] and IL-6 production[168] by fibroblasts, macrophage chemotaxis, phagocytic activity and cytokine production,[169] wound angiogenesis,[170-172]and collagen deposition.[170] The end of the clotting cascade occurs when thrombin catalyzes the conversion of fibrinogen to fibrin, which is crosslinked by factor XIII and then binds to platelets to form the clot. This fibrin clot is the first or "provisional" extracellular matrix, necessary for migration of inflammatory and proliferative cells into the wound. Factor XIII deficiency results in deficient fibrin crosslinking and delayed wound healing.[164] Lack of fibrinogen causes deranged cellular migration into the wound resulting in incomplete wound granulation, epithelial hyperplasia, and decreased wound tensile strength.[173]

Platelets are the first cellular elements to arrive in the wound, where they are activated when exposed to subendothelial collagen resulting in adherence and aggregation.[174-176] Activated platelets release a wide variety of cytokines from their granules, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). These cytokines are in turn chemotactic for a number of inflammatory and proliferative cells that participate in wound healing; some of the most important of these include neutrophils, macrophages, mast cells, fibroblasts, endothelial cells, and keratinocytes.[165] Considering the many cytokines that platelets produce and release into the wound, one would think that platelets are essential to normal wound healing, however, this is not the case. Experimental thrombocytopenia (< 1% of normal count remaining) induced by injection of anti-platelet serum, had no significant effect on a variety of measures of healing including neutrophil cell counts, wound capillary and collagen content, reepithelialization, and levels of five primary wound healing cytokines. A significant reduction in macrophage and T-lymphocyte counts was noted, however. The authors concluded that platelets influence wound inflammation but do not significantly affect proliferative processes.[177]

Neutrophils are considered to be the first inflammatory cell to appear in the acute wound,[165] arriving within hours, their numbers peak in 2 – 4 days and then decline.[178,179] Neutrophils arrive in the wound in response to a wide variety of cellular signals, including proinflammatory products of the lipoxygenase pathway.[180]Neutrophils are

generally considered to play a primary role in immune surveillance and autolytic debridement, via phagocytosis and degranulation. More recent evidence indicates, however, that neutrophils play a much broader role in wound healing. Several examples include: Stimulation of angiogenesis via the expression of a large number of proangiogenic cytokines and other proangiogenic molecules including IL-8 and VEGF;[181] CD18-deficient mice (lacking normal neutrophil migration to wounds[182]) are deficient in neovascularization compared to wild-type mice.[183] Expression of TNF-α; PMNs serve as a major source of TNF-α during the early postwounding period, which serves roles in macrophage chemotaxis, and epithelialization.[184]

Macrophages arrive in the wound from blood monocytes and resident tissue macrophages soon after neutrophils in response to cytokine migratory signals from various sources: from the clot (including the entrapped platelets) comes fibrin, PDGF, thrombin and complement; from the damaged tissue, fibronectin and elastin, and nerve growth factor (NGF); from neutrophils, TNF-α and TGF-β among others. Newly-arriving wound macrophages proliferate and begin to synthesize and release cytokines in response to mitogenic and secretory signals such as TGF-β and PDGF.[185,186] Wound macrophages also recruit more monocytes to the wound via the release of MIP-1α.[187]Macrophage numbers peak later than neutrophils and they remain in the wound for a longer time period – days to weeks. Macrophages function in the wound as phagocytes, and also (and most importantly) as regulators of wound healing, via the large variety of cytokines that they produce and release into the wound milieu. The vital

role of the macrophage as regulator of wound healing was first demonstrated by Leibovich and Ross, who depleted macrophages in guinea pigs with anti-macrophage serum and observed significantly delayed wound healing.[188] Delayed wound healing in aged mice is responsive to local treatment with exogenous macrophages from young donor mice,[189] suggesting that an age related decline in macrophage function may be at least partly responsible for the reduced rate of wound healing seen in association with aging.[190-192]

The role of lymphocytes in wound healing has been examined. Besides the obvious role in inflammation and immune surveillance, lymphocytes appear to have a regulatory function in wound fibroplasia, possibly through the release of profibrotic cytokines such as bFGF, TGF-β, and connective tissue growth factor (CTGF).[193-197] In a mouse model, pre-wounding depletion of T-lymphocytes resulted in an impairment of proliferation in healing, with reduced tensile strength and wound hydroxyproline up to 4 weeks post-wounding.[198,199] Interestingly, when healing of normal and nude (athymic) mice was compared, wound collagen and tensile strength were higher for nude mice. When T-cells were fully depleted with anti-lymphocyte antibody, wound healing in normal mice was impaired, but completely unaffected in nude mice. Finally, repletion of T-cells to normal levels in nude mice resulted in a decline in wound strength and collagen deposition. The authors interpreted this data as an indication of a dual role for T-cells in wound healing: stimulatory to fibrosis in early healing, and inhibitory in later stages, possibly serving as a signal of completion of healing.[200] A later study that examined subpopulations of lymphocytes in relation to second intention healing. They found that total T-cell counts declined, but CD8+ T-suppressor cells and B-cells

increased as wounds approached closure, indicating a possible role for these subsets of lymphocytes in the downregulation of proliferation at the completion of healing.[201]

Although mast cells were first described over 125 years ago,[202] they remain incompletely understood. During most of the time since Paul Erhlich's original description, the study of mast cells has centered on their role in the immune response, particularly with regard to hypersensitivity reactions. Recently, investigators have described a number of roles of mast cells in wound healing, particularly in the modulation of the inflammatory response to wounding. Mast cells have been linked to both normal and pathologic wound healing, including chronic conditions such as chronic cutaneous ulcers, keloids, and hypertrophic scarring.[203] Mast cells appear to exert their positive effects on healing mainly by "piecemeal" degranulation, ie, the selective release of a portion of their stored granules. This is in contrast to the uncontrolled pathologic events that appear to be triggered by wholesale degranulation.[202] Mast cells modulate the inflammatory response by alteration of vascular permeability[204] and the expression of adhesion molecules on vascular endothelium,[204-207] neutrophils,[208]and macrophages,[209] thereby helping to regulate their extravasation in the acute postwounding period. Mast cells interact with a wide variety of inflammatory and other cells to regulate wound inflammation. Two examples are the stimulation of neutrophil chemotaxis via the release IL-8 in response to contact with activated T-lymphocytes[210] and neutrophil recruitment via the chemoattractant KC.[211] Mast cells are also activated by the release of substance P,[212,213] a neurotransmitter that is released from damaged nerve endings at the

time of wounding. With its involvement in so many complex interactions, it is clear that we still have much to learn about the mast cell, particularly in relation to wound healing.

Eosinophils have also been recently demonstrated to play a role similar to that of mast cells in their response to and production of cytokines, by which they participate in the regulation of inflammation.[214]In particular, eosinophils have been demonstrated in hamster[215] and rabbit[216] cutaneous healing models to be a source of TGF-α and TGF-β; the temporal orientation of the former followed by the latter may be important to the regulation of healing events. Furthermore, eosinophils in human chronic oral ulcers did not demonstrate activity for these cytokines,[217] supporting their role in the regulation of normal healing. EGF[218] and IL-4[219] both appear to normally suppress TGF-α release by eosinophils and IL-5[219,220] appears to be stimulatory. The relationship between EGF, interleukins, and eosinophils may give insight into the role of eosinophils in wound healing as a potentiator of wound inflammation. Eosinophils remain in the wound longer than heterophils,[221] and the depletion of eosinophils from the wound resulted in more rapid healing.[220]

Fibroblasts and capillary endothelial cells constitute the 2 primary cellular elements of granulation tissue and with the third major element, the extracellular matrix (ECM), make up the bulk of granulation tissue. New capillaries are formed within granulation tissue by budding from venules at the proliferating edge of granulation tissue.[222] This process begins as capillary endothelial cells traverse the basement

membrane and migrate into the ECM.[223] Interactions between capillary endothelial cells, fibroblasts, and the ECM are responsible for regulation of wound angiogenesis.[224] As healing progresses, the character of the ECM changes. The early fibrin clot, followed by the provisional ECM that is rich in Type IV collagen,[223] are stimulatory to capillary sprouting. Later, the highly organized collagenous ECM is inhibitory to sprouting.[224] An investigation of capillary bud migration revealed that new capillary buds migrate principally (60 – 80%) by aligning themselves along elastin fibers within the extracellular matrix.[225] Electrical polarity may also play a role in the orientation of migrating capillaries and fibroblasts. A recent study of endothelial cells grown in a weak electrical field showed that they tended to migrate toward the cathode, while fibroblasts migrated toward the anode.[226] Ionic concentration gradients may be the mechanism of the electrical effect; capillary endothelial cell migration and proliferation were stimulated by increasing Mg++ concentration *in vitro*.[227]

The following chapters detail the investigation of cutaneous wound healing in the cat and compare it with the dog, a better-known model. These investigations were undertaken with the goal of describing cutaneous wound healing in the cat, using several of the methodologies described in this chapter, in order to determine if wound healing in the cat differed significantly from other species. If wound healing in cats proved to be unique, further species-specific research would be advisable, and currently accepted

practices of surgery and wound management may have to be changed in light of new knowledge to deliver the optimum care to feline patients.

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II. CUTANEOUS WOUND HEALING IN THE CAT: A MACROSCOPIC DESCRIPTION AND COMPARISON TO THE DOG

Square, open cutaneous wounds created on the dorsal aspect of the thorax of cats and dogs were evaluated for 21 days for temporal and spatial development of granulation tissue, wound contraction, epithelialization, and total healing. To evaluate first intention healing, breaking strength of sutured linear cutaneous wounds was measured at 7 days post-wounding. Laser-Doppler perfusion imaging (LDPI) was used to measure cutaneous perfusion. Results – First intention healing: sutured wounds in cats were only half as strong as those in dogs at day 7 (0.406 vs 0.818 kg breaking strength). Second intention healing: cats produced significantly less granulation tissue than dogs, with a peripheral, rather than central distribution. Wound epithelialization and total wound healing (total reduction in open wound area from contraction and epithelialization) were greater for dogs than for cats over 21 days. Wound contraction on day 7 was greater for dogs, but not on day 14 or 21. Cutaneous perfusion was initially greater for dogs than for cats, but no differences were detected after day 7. Conclusions – Significant, previously unreported differences in cutaneous wound healing exist between cats and dogs. In general, cutaneous wounds in cats are slower to heal. Cats and dogs also appear to use different mechanisms of second intention healing. In cats wounds close mainly by contraction of the wound edges, whereas in dogs wounds close more from central pull, and epithelialization.

INTRODUCTION

Wound healing is an orderly process that has been conceptually divided into 3 continuous and overlapping phases; inflammation, proliferation, and maturation.[1] Because the phases of wound healing are identifiable in all species, and clinical observations generally indicate that wound healing follows the same basic pattern across broad taxonomic lines, there is an assumption that wound healing is a relatively homogeneous process across species lines. As evidence of this, we need only look to the presentation of wound healing in various human and veterinary surgical texts, or to the fact that nearly all of our historic knowledge of cutaneous wound healing has been derived from studies on relatively few species - rodent, pig, dog, horse, and human – and applied to all others. Recently, however, significant differences in second intention cutaneous healing between horses and ponies have been noted, demonstrating that considerable heterogeneity exists in wound healing.[2,3] These investigations were undertaken to clarify the nature of problem wound healing (exuberant granulation tissue, chronic wounds) in horses. Similarly, we have observed certain clinically significant wound healing problems, which appear to be more common in cats than in dogs. Others have also noted that certain wound healing problems such as indolent pocket wounds seem to be associated with feline patients.[4-6]

There are no published basic studies describing cutaneous wound healing in cats. Therefore our objectives were to describe the macroscopic features of cutaneous wound healing in the cat, and to compare these findings with cutaneous wound healing in the dog. We hypothesized**:** that there would be significant qualitative and quantitative differences in wound healing between cats and dogs**;** that the cat wounds would heal more slowly with a less exuberant inflammatory phase and with less early granulation tissue formation; and that these differences in wound healing might be due in part to differences in cutaneous blood supply between cats and dogs during the healing process.

MATERIALS AND METHODS

Animals

Six Beagle dogs and 6 mixed breed, shorthaired cats were studied. Young adult spayed females were used exclusively to eliminate experimental variation associated with age or estrus status. Animals were determined to be healthy based on the results of physical examination, complete blood count, and fecal parasitology. Animals were housed individually: dogs were kept in indoor runs with daily outdoor access and cats in large indoor cages. Commercial dry maintenance diets and water were offered *ad libitum*.

To acclimate the dogs and cats to the experimental protocol**,** 1 week before surgery a padded circumferential body bandage, that extended from the cranial thoracic to the caudal lumbar region, was placed on each animal. Side braces, constructed of padded aluminum splint rods, were placed on the dogs to prevent mutilation of the bandages while allowing greater freedom of vision and outdoor access through a dog door than would have been afforded by Elizabethan collars.

Anesthesia

On Day 0, animals were premedicated with acepromazine (0.02mg/kg, intramuscularly [IM]) and butorphanol tartrate (0.4mg/kg IM). Anesthesia was induced with ketamine (6mg/kg, intravenously [IV]) and diazepam (3mg/kg IV), and maintained with isoflurane (1.5%) in oxygen. Lactated Ringer's solution IV was administered at 11 mg/kg/hr during anesthesia. A single dose of ampicillin (22 mg/kg, IV) was administered preoperatively. Each animal was positioned in sternal recumbency and the dorsolateral area from the cranial aspect of the thorax to the lumbosacral junction was prepared for aseptic surgery. The same anesthetic and surgical preparation protocols were used for procedures on days 7, 14, and 21.

Wound creation

Using a sterile skin marker and millimeter ruler, 4 experimental wounds were marked on 1 side of the dorsal midline. Four matching wounds were marked on the opposite side of the dorsal midline in each animal (Fig 1); these wounds were used for another study. Assignment of experimental protocol to side was randomized. The cranial 3 wounds were full thickness skin defects in which all tissue down to and including the panniculus muscle was excised. The most cranial wound was a 2x2 cm square defect created with a #15 scalpel blade, and was used to evaluate second intention wound healing by planimetry, laser-Doppler perfusion imaging (LDPI), and, finally,

histologic evaluation. The 2 middle wounds, 1 cm diameter, were made with a disposable dermal biopsy punch. These wounds were used for a study involving the histologic evaluation of wound healing, the results of which will be reported in a subsequent paper. The caudal wound was a 3 cm craniocaudal linear wound created with a #15 blade. It was sutured with 5 simple interrupted sutures of 3-0 nylon, spaced and tied to approximate the skin edges without crushing them. This wound was used to evaluate first intention wound healing by LDPI, wound breaking strength, and histologic analysis. Because previous studies in rats had shown differential healing of cutaneous wounds based on their cranial-caudal location on the trunk,[7] the wounds were created in the same location on each animal regardless of differences in body size. The most cranial wound was located just caudal to the caudal border of the scapula and the most caudal wound was located just cranial to the ilial wing. The wounds were spaced far enough apart so that contraction in one wound would not cause interference with healing in adjacent wounds because of tension. This necessitated slightly closer wound spacing on the cats than on the dogs and small dogs were used to minimize this species difference.

Wounds were covered with a 3-layer padded bandage. The contact layer consisted of a non-adherent, semi-occlusive pad (Telfa® adhesive pad, Tyco HealthCare Kendall, Mansfield, MA) placed directly on the wound surface; these pads had adhesive edges to prevent them from shifting position. The secondary layer consisted of open weave cotton roll gauze (Sof-Band bulky bandage, Johnson & Johnson Medical, Ethicon Inc., Arlington, TX) applied in multiple layers and crossed over the shoulders and

between the forelimbs to prevent the bandage from slipping caudally. The tertiary layer consisted of 2 inch wide strips of white adhesive tape (Zonas, Johnson & Johnson) applied over the secondary layer.

After bandaging the animals were moved to a recovery ward for observation. Butorphanol tartrate (0.6 mg/kg IV every 6 hours) was administered to all animals during the initial 12 hours post-surgery. Each animal was evaluated for evidence of postoperative pain using the following criteria: elevated heart rate, panting, vocalization, restlessness, dullness or depression, inappetance, and sensitivity to touch at the surgical site. If ≥ 2 of these criteria were observed, butorphanol was administered for another 12 hours or until signs of pain abated.

Evaluation of Wound Healing

LDPI

Cutaneous perfusion in humans and some animal species has been measured by LDPI.[8] A probe containing a light source transmits laser light into the tissue, which contains stationary cells and moving red blood cells. Incident photons are randomly scattered when the laser light strikes the tissues. When the laser light strikes the moving red blood cells, the incident photons are Doppler-shifted. A portion of the scattered

incident light is reflected back into the probe head, where it is picked up by a fiber optic cable and transmitted to a photodetector, which converts the light into an electrical signal, measured in volts, and this is proportional to blood flow.[8]

After the cranial open wound was created and the caudal sutured wound was created and sutured, baseline perfusion in the wound bed of these wounds was measured by LDPI (LDPIwin 2, Lisca, Linköping, Sweden). Animals were placed in an oblique lateral position so that the imaging beam was directly perpendicular to the wound surface. The height of the imaging head above the wound surface was positioned according to manufacturers' recommendations to obtain minimum variation in repeat readings. Three consecutive readings at each wound site were recorded and averaged for statistical analysis. LDPI data was obtained again on days 7, 14, and 21, under the same anesthesia and positioning conditions described for initial measurements.

Planimetry

Planimetry was performed on days 0, 7, 14, and 21. Immediately after LDPI, the perimeter of the cranial square wounds was traced onto a sterile piece of clear acetate film with a fine-point indelible marking pen. The acetate was laid on the wound surface, smoothed, and held flat and immobile by an assistant while the tracing was made by the examiner wearing 2.5X loupes. Care was taken so that the pressure placed on the wound did not distort the wound edges during tracing. The examiner traced the wound margin at the border between normal skin and the wound and the outlined area was considered to be the total wound area.

Next, the examiner traced the margin at the leading edge of advancing epithelium. The area between these 2 margins was considered to be the area of epithelialization. The area within the margin of advancing epithelium was the area of open or unhealed wound. (Fig 2) The areas outlined on the acetate films were digitized using digital scanning software and hardware (Sigma Scan® Pro 5.0, SPSS Science, Chicago, IL) and the percent epithelialization, percent wound contraction, and percent total wound healing were calculated for each wound.

Percent epithelialization was calculated as

$$\%\ \text{epithelialization Day}_n = \frac{\text{area of epithelium Day}_n}{\text{total wound area Day}_n} \times 100$$

Percent wound contraction was calculated by

Step 1: $$\text{total wound on Day}_n \text{ as \% of original} = \frac{\text{total wound area Day}_n}{\text{original wound area (Day}_0\text{)}} \times 100$$

Step 2: $$\%\ \text{wound contraction Day}_n = 100 - \text{total wound on Day}_n \text{ as \% of original}$$

Percent total wound healing was calculated by

Step 1: $$\text{open wound Day}_n \text{ as \% of original} = \frac{\text{open wound area Day}_n}{\text{original wound area (Day}_0\text{)}} \times 100$$

Step 2: $$\%\ \text{total wound healing Day}_n = 100 - \text{open wound Day}_n \text{ as \% of original}$$

Observations during daily wound care

Animals were sedated (0.05 mg/kg butorphanol, 0.4 mg/kg ketamine, and 0.002 mg/kg medetomidine, IV) to facilitate bandage changes. This drug combination produced mild sedation for 5-10 minutes and animals were fully awake by the time they were returned to their cages or runs. Bandages were changed daily for the first 8 days, and then every other day until day 21; wounds were rebandaged as described above. All animals were administered amoxicillin prophylactically (20 mg/kg orally twice daily) throughout the study.

Information recorded from visual observations of the cranial open wounds at the time of each bandage change was: time in days until granulation tissue first appeared in the wound, and days to complete coverage and wound filling by granulation tissue. Days to complete coverage of the wound were determined as the 1st day that the entire bottom of the wound bed was covered with granulation tissue. Days to wound filling was determined as the 1st day that granulation tissue filled the wound bed level with the surrounding skin. The amount and character of any wound fluid in the bandage, and any appearance of infection or other abnormalities were noted.

Tensiometry

On day 7, the sutured wounds were harvested as a 2 cm wide x 4 cm long strip of skin, with the sutured wound centered transversely in the strip. The resulting defect was closed in 2 layers. The specimen was placed between saline (0.9% NaCl) solution

moistened gauze sponges and taken to the tensiometer (Instron Model 5542; Instron Corporation, Canton, MA) for immediate testing. (Note to editor: What I want to convey is that testing occurred immediately after sampling so that no drying or autolysis could have affected the results. After removing the sutures from the specimen, it was positioned in the jaws of the tensiometer with the suture line oriented perpendicular to the line of pull. Tension was applied to the wound by distraction of the upper jaw of the tensiometer at a constant crosshead speed of 50 mm/min, to a maximum force of 100N, or until failure, which was defined as complete parting of the healing wound edges. Peak tension (load in kg) was measured and recorded.

Data Analysis

Comparisons between groups (dogs, cats) were performed using the Wilcoxon rank sum test: median days to 1st appearance of granulation tissue, median days to wound coverage by granulation tissue, median days to complete wound filling by granulation tissue, and breaking strength of the sutured wounds at day 7. Repeated measures ANOVA was used for comparisons of perfusion, % epithelialization, % contraction, and % total wound healing (combined contraction and epithelialization) measured on days 0, 7, 14, and 21. For each comparison, differences between groups were considered significant at $P \leq .05$. All statistical analyses were conducted using SAS software (Proprietary software release version 8.2, SAS Institute, Cary, NC, USA).

RESULTS

LDPI

For sutured wounds (first intention healing), mean LDPI values (in volts) at day 0 for dogs (0.88) were significantly higher than in cats (0.30; $P = .005$). For open wounds (second intention healing), mean LDPI values for day 0 were not significantly different between dogs (2.11) and cats (0.75; $P = .08$). There were no significant temporal differences in LDPI values or percent change between cats and dogs on or after day 7 (Tables 2.1 and 2.2).

Planimetry (see Figs 2.3-2.5)

Percent Epithelialization.

On day 7, open wounds in 2 dogs had measurable epithelialization (mean, 3.2% of wound area; range, 0-14%) whereas none of the open wounds in cats had measurable epithelialization, however this difference was not significant ($P = .20$). By day 14, all wounds in dogs and cats had measurable epithelialization. Mean % epithelialization for dogs (44.7%; range, 32.1 - 70.4%) was significantly greater than in cats (mean, 13%; range, 1.3 - 22.2%; $P = .0007$) On day 21, 2 dogs had complete epithelialization of the open wound, whereas only 1 cat had > 50% epithelialization. Mean % epithelialization for dogs (89.4%; range, 59.8 - 100%) was significantly greater than in cats (mean, 34.4%; range, 26.5 - 55.0%; $P = .0001$).

Percent Wound Contraction

Mean % contraction on day 7 was significantly greater for dogs (41.2%; range, 34.3 - 51.2%; *P* = .0004) than for cats (18.2%; range, 13.2 - 33.8%). (Note to editor: these were reversed; I just un-reversed them) By day 14, mean % contraction for cats had nearly tripled to 53.0% (range, 34.3 - 69.4%) whereas contraction in dogs increased at a slower rate to 66.1% (range, 38.3 -81.0%). By day 21, mean % contraction for cats was similar to that for dogs (75.8% vs. 70.3%, respectively), however these differences between cats and dogs were not significant on either day 14 (*P* = .15) or 21 (*P* = 0.41).

Percent Total Healing

Mean % total healing on day 7 for cats (18.3%; range, 9.8 - 33.8%) was significantly less than dogs (43.1%; range, 32.8 - 52.4%; *P* = .0003). Likewise on day 14 (cats: 59.0%; range, 42.1 - 73.2%; dogs: 76.1%; range, 58.1 - 92.8%; P = .046) and day 21 (cats; 83.9%; range, 67.1 - 93.3%; dogs 97.6%; range, 80.6 - 100%; *P* = .036) mean % total healing for cats was significantly less than for dogs.

Observations (see Figs 2.3-2.5)

We observed significant differences between cats and dogs in the rate of granulation tissue formation, but not in the time to first appearance of granulation tissue. Median time to 1st observable granulation tissue was not significantly different for dogs (5 days) and cats (6 days; *P*= .14). Median time to coverage of the bottom of the cranial

open wound bed with granulation tissue was significantly shorter in dogs (7 days) compared with cats (18 days; $P = .0242$). In fact by day 21, only 5 cats had complete coverage of the bottom of the wound.

Filling of the open wound to skin level occurred by day 8 in 5 dogs, and by day 10 in the other dog (dog 6), yielding a median time for dogs of 8 days. In cats, the median time was 20 days, which was significantly longer than for dogs ($P = .0229$; Figs 2.3 – 2.5). Dog 6 appeared to be an outlier. Throughout the study her wound healing was seemingly not as rapid and robust as the other dogs. From data analysis dog 6 had the lowest % epithelialization, % contraction, and % total healing, and the lowest sutured wound breaking strength of all dogs. Granulation coverage of the bottom of the wound and wound filling were slowest of all dogs. All data from this dog was included in the statistical analyses. We also noted that her hair coat grew back more slowly than that of the other dogs. No other abnormalities were noted either on her pre-study general health screen or during the study. There was no apparent reason for her slower wound healing.

Tensiometry

On day 7, mean breaking strength of the sutured wounds in cats (0.406 kg; range, 0.280 - 0.751 kg) was significantly less than for dogs (0.818 kg; range, 0.399 - 1.235 kg; $P = .0292$). The sample from dog 4 was partially torn during preparation for testing and was excluded from the statistical analysis.

DISCUSSION

Most current knowledge of small animal wound healing has been based on observations in dogs, and from data extrapolated from rodent, porcine, and human studies. In our literature search, primary references to cat wound healing research related to ophthalmologic studies (primarily corneal healing).[9-12] A small number of more clinically oriented studies have used cats for investigation of healing in bone, airway epithelium, nerves, and in oral surgery.[13-17] We only identified 1 experimental study of cutaneous wound healing in cats; a comparison between adhesive polyurethane membrane and polypropylene sutures for skin closure.[18] From our (MWB, RAH, SFS) clinical experience we wondered if cats may have significant qualitative and quantitative differences in cutaneous wound healing when compared with dogs. These differences seemed to predispose cats to certain clinical wound healing problems. In particular, we have noted that large open wounds in cats appear to heal more slowly than do similar wounds in dogs, with noticeably less granulation tissue formation in the wound, particularly in the early stages of wound healing. A phenomenon which we have referred to as "false healing" of sutured cutaneous wounds has also been noted in cats more frequently than in dogs. In this phenomenon, the wound appears to be healed from superficial observation, but when the skin sutures are removed, there is complete dehiscence as soon as the animal stressed the wound through normal motion.

We hypothesized that there would be qualitative and quantitative differences in wound healing between cats and dogs; specifically, that cutaneous wounds in cats would heal more slowly with a less exuberant inflammatory phase and less early granulation

tissue formation. We also hypothesized that the differences in wound healing between cats and dogs might be in part due to differences in cutaneous blood supply during the healing process. The relationship between tissue perfusion and wound healing is well-established.[19,20] Previous investigations have reported interspecies differences in the cutaneous vascular supply of the trunk.[21] Dogs have a larger number of well-distributed cutaneous perforating vessels, whereas cats have a much smaller number of cutaneous perforators that are more widely distributed in 2 major lines along the trunk. Our LDPI data did not fully support our hypothesis. Although there were significant differences in baseline (day 0) perfusion between dogs and cats, these differences were not significant after day 0. One possible explanation for the lack of a significant difference may be the timing of subsequent perfusion measurements at days 7, 14, and 21. These times were chosen because the animals needed to be anesthetized for LDPI measurements, and the experimental protocol already called for anesthesia on these days for wound biopsies to be performed. Based on our wound observations where the greatest differences in granulation tissue formation and wound contraction occurred within the first 7 days, it may be that differences in cutaneous blood flow existed throughout much of the first 7 days, but were missed because our protocol did not include multiple perfusion measurements during this period. Other wound healing studies in dogs that used LDPI reported increased perfusion which peaked on the 5th day post-wounding and then declined.[22,23] A subsequent experiment in which daily LDPI measurements were performed would be required to address this issue.

Significant differences were noted in the healing of open wounds, where cats lagged behind dogs in % epithelialization and % total wound healing throughout the 21 days (Figs 2.3 – 2.5). Our results were somewhat different for % contraction, however, in that cat open wounds contracted more slowly for the first 7 days, but by day 14, although % contraction was still greater for dogs, the differences were not significant. By day 21, % wound contraction was actually slightly greater for cats, although again there was no significant difference between groups. One possible explanation for this finding is that cats may have a longer period during which the constituents of wound contraction (differentiated fibroblasts or myofibroblasts) appear in the wound, but once contraction begins it proceeds at a more rapid pace in cats than in dogs because of a greater contractile capacity of cat myofibroblasts. This may be because of an inherent property of fibroblasts as studies have shown that fibroblasts that appear early in the process of wound contraction do not contract as forcefully as those that appear later.[24] Another explanation for differential fibroblast contraction relates to the local wound environment. Experimental data in other species has demonstrated different contractile capability of fibroblasts based on their anatomic location of origin. It has been hypothesized that different local wound environments, for example, different species**,** can affect the contractile capability of wound fibroblasts.[3]

Another possibility is that total wound healing (contraction and epithelialization) proceeds at a more rapid rate in dogs than in cats. Because of more rapid epithelialization, there is a correspondingly greater reduction in area of exposed granulation tissue in the wound center, and with it, a reduction in the number of wound

myofibroblasts available to generate the centripedal force for wound contraction. The basis for this hypothesis has been established previously on the relationship between wound epithelialization and contraction. When epithelialization of wounds is complete, contraction ceases, because of a cAMP-mediated signaling pathway.[25] This phenomenon is also observed and used to clinical advantage in open wound treatment by application of free grafts or flaps to treat or prevent excessive wound contraction (contracture).[26] The authors (MWB, RAH, SFS) have noted clinically that the rate of contraction of open wounds often seems to slow near the end of healing as the wound becomes almost entirely epithelialized.

We also observed consistent differences between cats and dogs in the temporal and spatial distribution of granulation tissue within the wound (Figs 2.3 – 2.5). In dogs, granulation tissue always appeared first at the bottom of the wound, and covered the entire bottom surface of the wound by the 7th day. In cats, the amount of early granulation tissue was much less and the distribution was also different in that the granulation tissue first appeared at the cut skin edges and then spread to the central portion of the wound. We propose that this difference in the distribution of early granulation tissue may help explain the different theories of wound contraction that have been proposed in the past. The "picture frame" theory of wound contraction states that myofibroblasts located at the edges of an open wound are responsible for the centripedal forces that lead to wound contraction,[27] a model well represented by the cat. On the other hand, the "pull theory" of Abercrombie and coworkers states that fibroblasts throughout the granulation tissue generate the forces responsible for contraction, [28] a model

seemingly closer to observations of contraction in dogs, as dogs have an abundant early granulation bed in the center of the wound. More recently, it has been proposed that wound contraction occurs through a combination of these processes.[29] Our data indicate that although it may be a multifactorial process, wound contraction may rely on a primary mechanism which can differ between species.

Our results demonstrated a significant difference in breaking strength of sutured skin wounds at day 7 between cats and dogs, with mean wound breaking strength in dogs being double that in cats. This difference suggests that the production and maturation of wound collagen proceeds at a slower rate in cats than dogs.

To date, the veterinary medical literature has considered cutaneous wound healing to be virtually identical between cats and dogs.[30] We believe our results demonstrate that a different clinical approach to wound healing should be considered in cats. For example, since the day 7 breaking strength of sutured cutaneous wounds in cats was significantly less than in dogs, it would seemingly be advisable to leave skin sutures in place longer in cats than in dogs, and particularly so in locations where excessive motion or tension may be anticipated. Another alternative would be a buried tension relieving suture pattern that engages the dermis. Also, because it appears that open wounds in cats heal more slowly by second intention than in dogs, it may be rational to manage such wounds in a somewhat different manner by, for example, the more routine use of wound healing stimulants or earlier institution of reconstructive procedures such as skin grafts or flaps.

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Table 2.1. Second Intention Healing in Cats Compared with Dogs.

	Cats				Dogs			
	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day14	Day 21
LDPI (volts)	0.75	2.28	5.00	2.62	2.11	2.93	4.05	2.70
LDPI, mean % change from day 0		291.2	624.1	334.2		131.9	238.4	72.1
% epithelialization		0.0	13.0*	34.4*		3.2	44.7	89.4
% contraction		18.2*	53.0	75.8		41.2	66.1	70.3
% total healing		18.3*	59.0*	83.9*		43.1	76.1	97.6

* Indicates significant difference between cats and dogs on that day ($P \leq .05$, Repeated measures ANOVA)
LDPI = laser doppler perfusion imaging

Table 2.2. First Intention Healing in Cats Compared with Dogs.

	Cats		Dogs	
	Day 0	Day 7	Day 0	Day 7
LDPI (volts)	0.30*	0.18	0.88	0.56
LDPI, mean % change from day 0		-54.1		-27.2
Wound breaking strength (kg)		0.406*		0.818

* Indicates significant difference between cats and dogs on that day ($P \leq .05$, Wilcoxon rank-sum test)
LDPI = laser doppler perfusion imaging

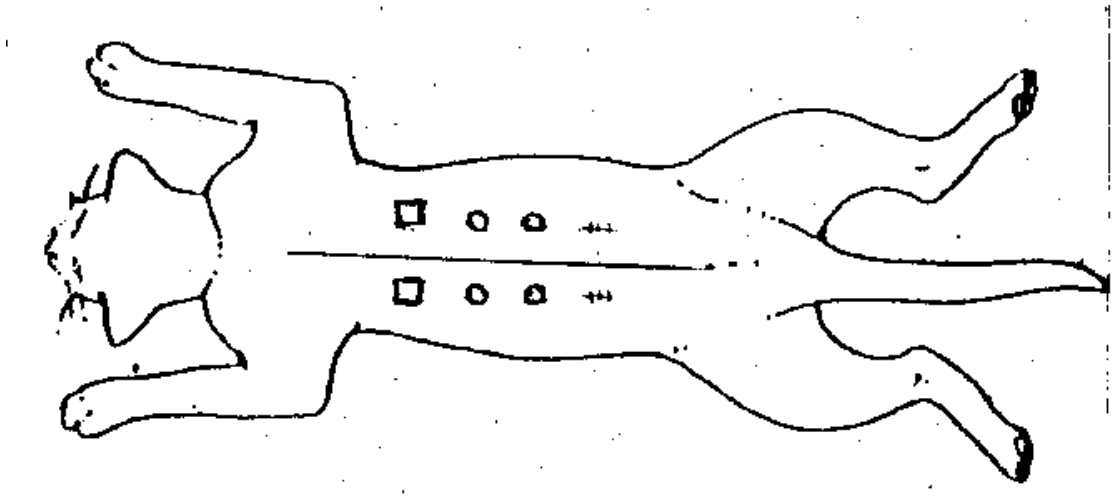

Fig 2.1. Drawing depicting the layout of wounds on the dorsum of a cat. Wounds on one side of the midline were used for this study – wounds on the other side of the midline were used for another study. Assignment of side was randomized. Layout of wounds on dogs was identical.

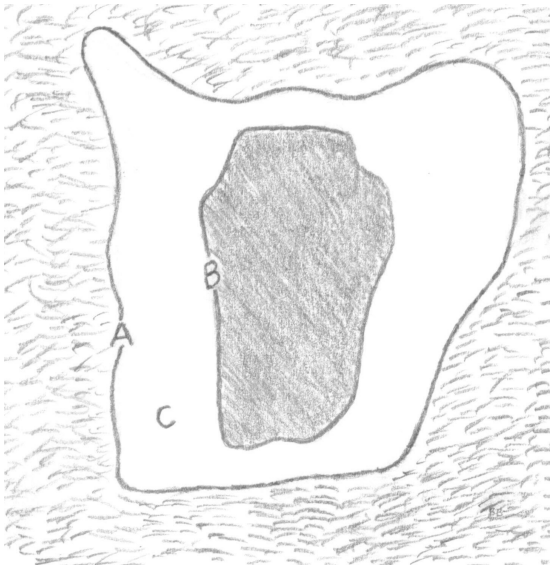

Fig 2.2. Drawing of an open square wound showing the wound margins traced on acetate. A = outer margin between normal unwounded skin and wound epithelium. The area within the margin of A is the total wound area. B = margin between wound epithelium and open wound. The area within the margin of B is the open wound area. C = area of wound epithelium (C = A – B).

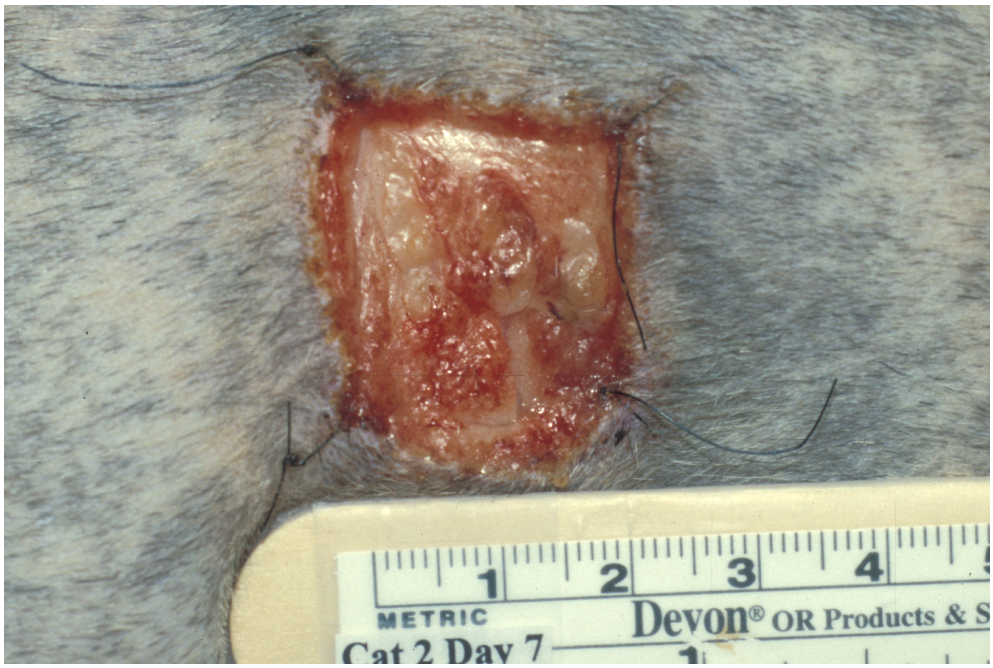

Figure 2.3a Representative cat wound day 7 postwounding.

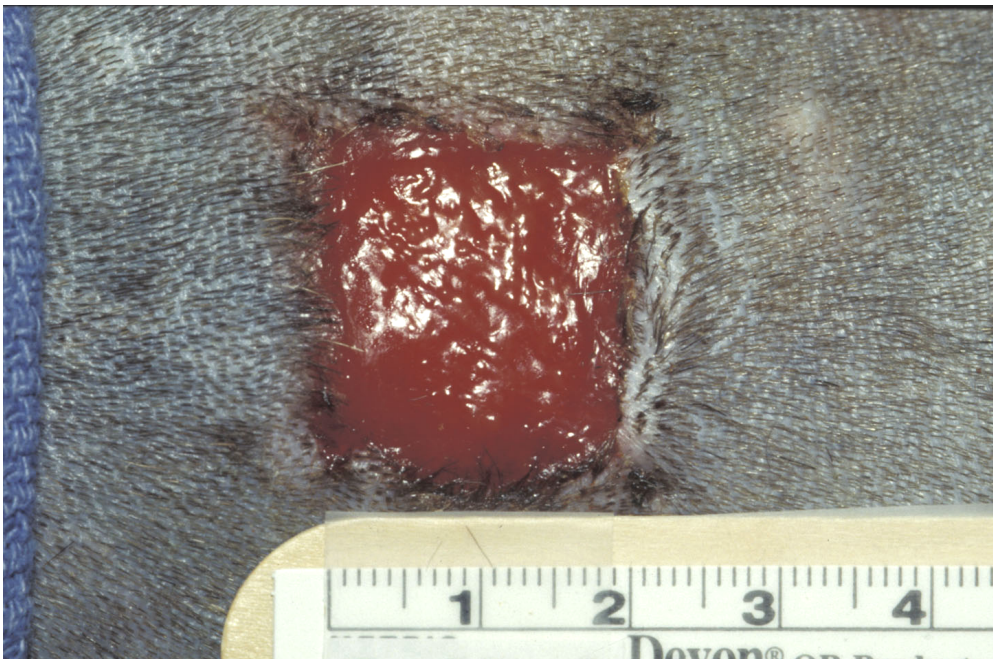

Figure 2.3b Representative dog wound day 7 postwounding.

Figure 2.4a Representative cat wound day 14 postwounding.

Figure 2.4b Representative dog wound day 14 postwounding.

Figure 2.5a Representative cat wound day 21 postwounding.

Figure 2.5b Representative dog wound day 21 postwounding.

III. COMPARATIVE ROLE OF THE SUBCUTANEOUS TISSUES IN CUTANEOUS WOUND HEALING IN THE DOG AND CAT

The objective of this study was to describe the contribution of the subcutaneous tissues to first and second intention cutaneous wound healing in the dog and cat and to compare these species for significant differences. Paired wounds were created on either side of the dorsal midline on 6 domestic shorthaired cats and 6 beagle dogs; the subcutaneous tissue was removed on one side and left intact on the other. Square open wounds of the dorsal thorax were monitored for 21 days for temporal and spatial development of granulation tissue, wound contraction, epithelialization, and total healing. Breaking strength of sutured linear wounds was measured at 7 days post-wounding. Laser-Doppler perfusion imaging (LDPI) was used to measure cutaneous perfusion. Results - First intention healing: Subcutaneous tissue removal produced no consistent effect on sutured wound strength at 7 days in dogs or cats (possibly a result of experimental design). Second intention healing: Removal of subcutaneous tissue reduced wound perfusion, granulation, contraction, epithelialization, and total healing. Granulation tissue formation and wound contraction were impacted to a significantly greater degree in cats than in dogs ($p<0.05$). Two of six dogs (33%) and no cats developed minor wound infections. Conclusions - reThe subcutaneous tissues make an important contribution to second intention cutaneous healing. Both dogs and cats experienced a delay in second intention healing with the removal of subcutaneous tissues, but while dogs had largely recovered from this setback by 21 days post-wounding, cats had not recovered. Veterinary surgeons should be aware of the significant contribution of subcutaneous tissue to cutaneous healing and of the possibility of delayed wound healing whenever performing extensive debridement or resection of subcutaneous tissues, particularly in feline patients. A higher risk for wound infections may also accompany extensive removal of subcutaneous tissues in the dog.

INTRODUCTION

Surgical reconstruction after removal of large volumes of tissue (e.g. for treatment of neoplasia, trauma, or infection) presents special challenges to the surgeon. Rapid uncomplicated wound healing, always a primary goal of surgery, is particularly desirable for large wounds or wounds over vital structures because failure of wound repair is potentially catastrophic. A limited number of reports,[1-4] and our clinical experience have indicated that in cases that require resection of large amounts of subcutaneous tissue, cats may be predisposed to certain types of large nonhealing wounds, including formation of indolent pocket wounds and "pseudo-healing" wounds.[1-4] It may be that the removal of large amounts of subcutaneous tissue necessitated in these excisions is itself a predisposing factor to problematic wound healing. These observations led us to review the role of the subcutaneous tissues in wound healing, and especially whether species differences exist.

Because nearly all reports of wound healing in small animals focus on cutaneous defects, we examined the role of the subcutaneous tissues in healing of sutured and open wounds. A 2nd objective was to determine if the subcutaneous tissues influence healing differently in dogs and cats. Our primary hypothesis, derived from clinical experience and the data obtained from a pilot project (unpublished), was that removal of the subcutaneous tissue would delay all aspects of open and closed wound healing. Our secondary hypothesis was that the removal of the subcutaneous tissue would cause a more pronounced effect in cats than in dogs.

MATERIALS AND METHODS

Animals

Six beagle dogs and 6 mixed breed, shorthaired cats were studied. We used only young adult spayed females, aged ~ 1-3 years, to eliminate variation from age or estrus status. Physical examination, complete blood count, and fecal flotation were performed to determine health status. All cats were FeLV and FIV negative. Animals were housed individually: dogs, in indoor runs with daily outdoor access and cats in large indoor cages. Commercial dry maintenance diets were fed and water was offered ad libitum. Animals were acclimated to wearing a padded circumferential body bandage that extended from the caudal cervical to the caudal lumbar region by applying the bandage starting 1 week before surgery. To prevent bandage mutilation and to allow greater freedom of vision and outdoor access through a dog door, side braces of padded aluminum splint rods[5] were added to the dog bandages.

Animal Preparation

Preoperative and operative procedures – on day 0, animals were administered acepromazine (0.02mg/kg, intramuscularly [IM]) and butorphanol tartrate (0.4mg/kg, IM) and anesthesia was induced with ketamine (6mg/kg, intravenously [IV]) and diazepam (3mg/kg, IV) and maintained with isoflurane (1.5%) in oxygen. Lactated Ringer's solution was administered (11 ml/kg/h, IV) during anesthesia. A single dose of ampicillin (22 mg/kg, IV) was administered preoperatively. Each animal was

positioned in sternal recumbency, and the dorsum prepared for aseptic surgery from the cranial aspect of the thorax to the sacrum. The same anesthetic and surgical preparation protocols were used for procedures on days 7, 14, and 21.

Wound Creation

Using a sterile skin marker and millimeter ruler, the locations of 8 experimental wounds, 4 on either side of the midline, were identified (Fig 2.1). On 1 side, wounds were full thickness skin defects in which all tissue including the panniculus muscle was excised. These wounds were designated as subcutis intact wounds. On the opposite side, similar skin defects were made, except that all subcutaneous tissue within the wound margins was excised to the level of the thoracodorsal or lumbodorsal fascia. These wounds were designated as the subcutis removed wounds.

Random drawing was used to assign treatment (subcutis intact versus subcutis removed) to the right or left side. The most cranial wound was a 2 cm × 2 cm square defect created with a #15 scalpel blade. This wound was used for laser-Doppler perfusion imaging (LDPI) and planimetric evaluation; a biopsy of wound tissue was performed at study end for histologic evaluation. Two middle circular wounds (1 cm in diameter), were made with a disposable dermal biopsy punch. Biopsy specimens were collected 7 and 14 days later (data not reported).

For open wounds with the subcutis removed, there was a tendency for the overlying skin to shift or slide over the underlying fascia. To prevent this, loosely applied tacking sutures of 3/0 nylon were placed in the corners of the square wounds

or at the dorsal, cranial, ventral, and caudal edges of circular wounds. Sutures were placed with small bites at the skin edge and in the underlying fascia to promote adhesion to the fascia, and were loosely tied using large suture loops. The effect was to restrict the skin from excessive sliding over the subcutis, thereby keeping the defects in the skin and subcutis aligned, while allowing the skin to remain freely moveable for contraction. Tacking sutures were removed at 7 days to further minimize opportunity for inhibition of wound contraction.

The caudal wounds were 3 cm cranio-caudal linear incisions created with a #15 blade. They were each closed with 5 full-thickness, simple interrupted skin sutures of 3/0 nylon, spaced and tied to approximate the skin edges without crushing them. On the "subcutis intact" side, a simple linear incision through the skin and panniculus was made and then closed as described above. On the "subcutis removed" side, the linear incision was made and then a 3 cm long × 1 cm wide strip of the underlying subcutaneous tissue, was removed down to the lumbodorsal fascia. The linear sutured wounds were used for LDPI, measurement of wound breaking strength, and histologic analysis.

Because previous studies have shown differential healing of cutaneous wounds based on their cranial-caudal position,[6] the wounds were located in the same region on each animal regardless of differences in body size; e.g. the most cranial wound was located just caudal to the caudal border of the scapula and the most caudal wound was located just cranial to the ilial wing. This necessitated slightly closer wound spacing on cats than on dogs; however, small dogs were chosen to

minimize this effect, and even on the cats, the wounds were spaced far enough apart so that contraction would not cause interference of adjacent wounds from tension.

Postoperative Wound Care

After surgery, the wounds were bandaged with a 3-layer padded bandage. The contact layer consisted of a nonadherent semiocclusive pad (Telfa® adhesive pad, Tyco HealthCare Kendall, Mansfield, MA) placed directly on the wound surface; these pads had adhesive edges to prevent displacement. The secondary layer consisted of open weave cotton roll gauze (Sof-Band bulky bandage, Johnson & Johnson, Arlington, TX), applied in multiple layers and criss-crossing the body cranial to the opposite forelimbs to prevent caudal displacement of the bandage. The tertiary layer consisted of strips of 2" wide white adhesive tape (Zonas, Johnson & Johnson). The length of the bandage and the cranial "suspenders" made it very secure. These features also made it unnecessary to apply the bandage tightly around the trunk, thereby minimizing or avoiding bandage-induced inhibition of wound contraction.

After bandaging, animals were moved to an anesthetic recovery ward where they were under continuous observation for postoperative pain during the next 18 hours. Opioid analgesia was administered postoperatively.

Evaluation Parameters

LDPI

LDPI was used to measure cutaneous perfusion in an earlier report that compared cutaneous wound healing in dogs and cats, which was conducted concurrently with this study.[7] After the wounds were made, baseline perfusion in the wound bed of the cranial square open wounds and the caudal sutured wounds were measured by LDPI (LDPIwin 2, Lisca, Linköping, Sweden). Animals were placed in an oblique lateral position so that the imaging beam was directly perpendicular to the wound surface. The height of the imaging head above the wound surface was positioned according to manufacturers' recommendations to obtain minimum variation in repeat readings (mean variation of repeat readings was $< \pm 10\%$ of the mean value) and 3 consecutive readings at each wound site were recorded. The mean of these readings was used as 1 data point for statistical analysis. LDPI data were obtained again on days 7, 14, and 21 postwounding, under the same conditions of anesthesia and positioning.

Planimetry

Planimetry was performed by direct wound tracings on days 0, 7, 14, and 21. Direct tracing planimetry is a standard method for the determination of wound surface area and produces highly repeatable measurements that correlate well with other techniques of wound area measurement such as photographic planimetry.[8-10] Immediately after LDPI, the perimeters of the cranial square wounds were traced

with a fine-point indelible marker on sterile, clear acetate held flat and immobile by an assistant. The same individual performed all wound tracings; 2.5× loupes were worn to increase precision. Avoiding pressure that would distort the wound edges, the border between normal skin and the wound, considered as the total wound area was traced 1st and then the leading edge of advancing epithelium (if present) was traced. The area between these 2 margins was considered the area of epithelialization whereas the area within the margin of advancing epithelium was the area of open or unhealed wound (Fig 2.2). The areas outlined on the acetate sheets were digitized using scanning software and hardware (Sigma Scan® Pro 5.0, SPSS Science, Chicago, IL) and the % epithelialization, % contraction, and % total wound healing were calculated for each wound according to the following formulas:

Percent epithelialization was calculated as:

$$\%\ \text{epithelialization Day}_n = \frac{\text{area of epithelium Day}_n}{\text{total wound area Day}_n} \times 100$$

Percent wound contraction was calculated by:

$$\text{Step 1: total wound on Day}_n \text{ as \% of original} = \frac{\text{total wound area Day}_n}{\text{original wound area (Day}_0\text{)}} \times 100$$

Step 2: % wound contraction Day_n = 100 - total wound on Day_n as % of original.

Percent total wound healing was calculated by

$$\text{Step 1: open wound Day}_n \text{ as \% of original} = \frac{\text{open wound area Day}_n}{\text{original wound area (Day}_0\text{)}} \times 100$$

Step 2: % total wound healing Day_n = 100 - open wound Day_n as % of original

Observations and Daily Care

Observations of the cranial open wounds were made by one individual and recorded at each bandage change, including 1st appearance of granulation tissue (defined as the 1st visible appearance of ≥ 1 mm diameter of red, glistening, irregular tissue anywhere on the wound surface), coverage of the bottom of the wound, and filling of the entire wound defect by granulation tissue, the amount and character of any wound fluid in the bandage, and any appearance of infection or other abnormalities. After data were acquired, the wounds were bandaged as described above. Bandages were changed daily for the 1st 8 days and then every other day until day 21. All animals were administered prophylactic amoxicillin (20 mg/kg orally every 12 hours) until day 21.

Tensiometry

On day 7, the linear sutured wounds were harvested as 2 cm wide × 4 cm long strips of skin, with the suture line extending transversely across the middle of the strip. The resulting defects were closed routinely in 2 layers. Each specimen was immediately placed between gauze sponges moistened with 0.9% NaCl solution and transported for tensiometry (Instron Model 5542; Instron Corporation, Canton, MA). After removal of the sutures, the specimen was positioned in the holding jaws of the tensiometer and tension was applied to the sample at a constant crosshead speed of 50mm/min to a maximum load of 100N or until failure, defined as the complete parting of the healing wound edges. Peak tension (load in kilograms) was measured and recorded for later analysis.

Data Analysis

Repeated measures ANOVA was used for comparisons of perfusion (LDPI), % epithelialization, % contraction, and % total wound healing (combined contraction and epithelialization) performed on days 0, 7, 14, and 21. Animals (cat, dog) were blocked by animal identification number, and the 2 treatments (subcutis intact versus subcutis removed) were tested as independent variables. Treatment-animal interaction was tested in 1 of 2 ways: by comparing results of within-treatment ANOVA between dogs and cats, or by calculating the percent change between treatments: ([subcutis intact - subcutis removed]/subcutis intact) × 100. This was calculated for each animal and ANOVA was performed on these data.

ANOVA was used to compare differences in granulation tissue formation (mean days to 1st observable granulation tissue and to cover and fill the wound with granulation tissue) between animals, and treatment groups. Individual animals in which wounds were not covered or filled with granulation tissue by day 21 were assigned a value of 22 days to calculate means and report a *P*- value. This method underestimates the actual clinical and statistical significance because none of the animals would have covered or filled the experimental wound in only 1 additional day.

Breaking strength of sutured wounds at day 7 was compared between animals (dogs, cats) and treatments (subcutaneous tissue intact versus removed) by use of a general linear model for ANOVA because of unequal numbers of observations between groups.

For all comparisons, differences between groups were considered significant at $P \leq .05$. All statistical analyses were conducted using SAS software (Proprietary software release version 8.2, SAS Institute, Cary, NC).

RESULTS

LDPI

For closed wounds, dogs had significantly greater cutaneous perfusion than cats on day 0 ($P = .006$) and day 7 ($P = .03$). There was a highly significant ($P = .0002$) reduction in cutaneous perfusion in dogs and cats by day 7, as wounds progressed into the repair stage. No significant difference ($P > .05$) was noted between treatment groups on either day, although the difference approached significance on day 0 ($P = .056$; Table 3.1)

Cutaneous perfusion of open wounds (subcutis intact and subcutis removed, combined) was higher in dogs than in cats at days 0 and 7, but not at days 14 and 21. Dogs and cats both had a highly significant time effect ($P = .0003$), with perfusion readings increasing until day 14 and then declining by day 21. Cutaneous perfusion in cats increased more slowly than in dogs over the 1st week, but then reached a higher peak value at day 14, and declined more slowly thereafter. Perfusion in cats on day 21 was still >3.5 times baseline (day 0), whereas in dogs perfusion had declined to near baseline by day 21. Cutaneous perfusion of open wounds in dogs and cats was also significantly reduced by removal of the subcutaneous tissues at day 7 ($P = .005$) and day 14 ($P = .02$). No significant treatment-animal interactions were identified (Table 3.2).

Planimetry

Epithelialization (Table 3.3):

Only 2 dogs and 2 cats had measurable epithelialization in any wounds by day 7, and no significant differences between dogs and cats or between treatment groups were identified. By day 14, a highly significant difference was seen between cats and dogs, with an overall (both treatments included) 10.5% epithelialization in cats compared with 38.1% in dogs ($P < .0001$). A significant difference also was noted between treatments ($P = .03$), with removal of the subcutis resulting in a 38% reduction in epithelialization in cats and 30% reduction in dogs. These effects continued to day 21, with highly significant animal ($P < .0001$) and treatment ($P = .0009$) effects: only 1 wound reached 50% epithelialization by day 21, whereas in dogs, epithelialization reached 50% in 10 wounds and ≥ 90% in 5 wounds. Removal of the subcutaneous tissue caused a significant reduction in epithelialization: 41.6% in cats and 31.6% in dogs. However, no significant treatment × animal interactions were identified. Figures 3.3 – 3.5 depict open wounds in dogs and cats at 7, 14, and 21 days.

Contraction (Table 3.4):

Mean contraction on day 7 for dogs was 41.2% with subcutis intact (range, 34.3-51.2%) and 7.5% with subcutis removed (range, -16.3 to 52.4%). Mean % contraction on day 7 for cats was 18.2% with subcutis tissue intact (range, 13.2-33.8%) and -2.6% with subcutis tissues removed (range, -41.7% to 24.2%). Note that

the negative number for contraction indicates that the mean wound size for cats with subcutis removed enlarged because of retraction of the wound edges from tissue relaxation during the 1st week. Three of 6 cats and 4 of 6 dogs had retraction of the wound edges in the subcutis removed side on day 7. There was a highly significant treatment effect on day 7 (P=.006) and a highly significant (P=.0004) difference between species was noted, but only when the subcutis was left intact. By day 14, contraction for all groups had markedly increased; for cats, 53% with subcutis intact (range, 34.3-69.4%) and 19.4% with subcutis removed (range, -14.5 % to 42.8%); contraction in dogs increased to 66.1% (range, 38.3-81.0%) with subcutis intact and 32.1% with subcutis removed (range, -1.29% to 61.5%). As at day 7, treatment effect was highly significant (P <.0001), but species effect just missed significance (P = .059).

By day 21, no significant differences existed in % contraction between cats and dogs when comparisons were made between animals within treatment group (cats 75.8% versus dogs 70.3% with subcutis intact; cats 53.9% versus dogs 63.5% with subcutis removed). However, a highly significant (P = .006) treatment effect was found for cats at day 21 when comparisons were made between treatments within animal group, indicating that removal of the subcutaneous tissue significantly affected wound healing in cats but not dogs at 21 days.

Total Healing (Table 3.5):

On day 7, mean total wound healing for cats was 18.3% (range, 9.8-33.8%) in the subcutis intact group and 0.2% in the subcutis removed group whereas for dogs

was 43.1% in the subcutis intact group (range, 32.8-52.4%)and 9.3% in the subcutis removed group. The difference between dogs and cats was significant at day 7 (P=.05), and the difference between treatment groups was highly significant (P= .006).

Mean total healing on day 14 was 59.0% in cats with subcutis intact and 25.8% with subcutis removed; the corresponding percentages for dogs were 76.1% and 56.7%, respectively. Species and treatment effects were both highly significant on day 14 (P= .001 and .0008, respectively). On day 21, mean total healing in cats was 83.9% with subcutis intact and 62.1% with subcutis removed whereas in dogs, it was 97.6% with subcutis intact and 83.4% with subcutis removed; once again, species and treatment effects were highly significant (P=.01 for both). No significant treatment × animal interactions were identified.

Observations (Figures 3.1 – 3.3)

There were significant differences between cats and dogs in time to 1st appearance and rate of production of granulation tissue. Mean time to 1st observable granulation tissue for all wounds was 4.5 days for dogs and 6.3 days for cats (P=.0004). Removal of the subcutis had no significant effect on time to 1st granulation tissue in either dogs or cats. Mean time to coverage of the bottom of the cranial open wound with granulation tissue was >11.7 days in dogs, and >19.2 days for cats (11 wounds in dogs and only 5 wounds in cats had complete coverage of the bottom of the wound by day 21). The open wound was filled with granulation tissue

to the level of the surrounding skin by day 21 in 10 dog wounds and 5 cat wounds. Species and treatment effects were highly significant (P=.005) for days to cover and days to fill the wound with granulation tissue.

Although we made no attempt to quantify wound fluid, we also noted subjectively that open wounds in dogs appeared to produce wound fluid in greater quantity and for a longer time compared with cats. The margins of dog wounds also appeared to be slightly but definitely more erythematous and edematous (as evidenced by a slight swelling of the skin edges) than those of cats.

As we reported previously,[4] dog 6 had slower wound healing than the other dogs, and had the lowest % contraction, epithelialization, and the lowest sutured wound breaking strength of all dogs. Dog 6 also had the longest time to cover and fill the cranial open wound with granulation tissue, and slower regrowth of her hair coat. No other abnormalities were noted either in her general health screen or during the study, and because we were not able to explain the reason for her slower wound healing, her data were not censored.

Two dogs developed infections in open wounds with the subcutis removed. A sanguinopurulent exudate was noted in the bandage, and the wounds (cranial 2 open wounds) were markedly edematous and erythematous. An odor characteristic of *Pseudomonas* spp. was noted. Enrofloxacin, (12.5mg/kg orally once daily) was administered when signs were noted on day 7 and continued for 10 days (dog 2). Marked improvement was noted within 24 hours and all signs of inflammation had resolved by the 10th day of treatment. Dog 7 developed similar signs on day 19 and

was treated similarly. Data from these dogs were not censored because the infections did not appear to have a significant effect on rate of healing.

Tensiometry (Table 3.1)

Data from 3 of 24 tensiometry samples were censored from the analysis because of variations caused by improper collection or handling of the specimens. Mean breaking strength of sutured wounds in cats on day 7 was 0.406 kg load in the subcutis intact group (range, 0.280-0.751) and 0.320 kg load in the subcutis removed group (range, 0.228- 0.447). Mean breaking strength for dogs was 0.818 kg load in the subcutis intact group (range, 0.399-1.235) and 0.675 kg load in the subcutis removed group (range, 0.510- 0.983). The difference between dogs and cats was highly significant ($P<.0001$); the effect of removal of the subcutaneous tissue was not significant ($P = .07$).

DISCUSSION

We found that the subcutaneous tissue as a whole plays an important role in cutaneous wound healing in dogs and cats; each wound healing variable we measured confirmed this observation. Our secondary hypothesis, that the negative impact on wound healing from removal of the subcutaneous tissue would be greater in the cat than in the dog, was only demonstrated conclusively with respect to 2nd intention healing.

The subcutis is the supporting layer of the skin, and provides protective cushioning to deeper structures while at the same time serving as a flexible attachment between the generally mobile skin and more fixed underlying fascia.[11] It is also the tissue through which cutaneous nerves and vessels must pass. The vascular supply to the skin is divided into 3 levels: (1) the superficial or subpapillary plexus, (2) the middle or cutaneous plexus, and (3) the deep, subdermal or subcutaneous plexus. All 3 levels are interconnected; the former 2 superficial levels receive their supply from the subcutaneous plexus, which is in turn supplied by direct cutaneous vessels. The panniculus carnosus, where present, is part of the subcutis, and the subcutaneous plexus lies both deep and superficial to it.[12] Because it consists of ~90% triglyceride by weight, the subcutis is usually regarded mainly as an adipose reservoir, however, it has been reported to be involved in other vital functions,[11] to which we now add wound healing.

Tissue Perfusion

Cutaneous perfusion of open wounds followed different time courses for cats and dogs. The following phenomena were observed: (1) wound perfusion in dogs was more than cats until day 7, then increased more rapidly in cats than dogs until day 14, after which there was no detectable difference in absolute perfusion; (2) perfusion decreased in open wounds of dogs and cats after day 14, but more rapidly in dogs than cats. By day 21, perfusion in cat wounds was still >3 times baseline (Day 0), whereas in dogs perfusion had decreased to near baseline level. We suggest that this more rapid rise to peak perfusion and more rapid decline after day 14 in dog wounds may be evidence of a more intense early inflammatory response to wounding in dogs resulting in more rapid healing. By day 21, wounds in 5 dogs were almost completely healed in contrast to cats where wounds were still actively healing at day 21 and therefore had a higher metabolic rate (and perfusion) than at baseline.

Removal of the subcutaneous tissue caused a significant reduction in perfusion of open wounds in dogs and cats. This finding is not surprising, given that the subdermal plexus originates in the subcutis and is supplied by perforating vessels that traverse the subcutis.[12] We also noted that on day 7, cat wounds with subcutis removed had a mean reduction in perfusion that was 62% more than in dogs. Similarly on day 14, cat wounds with subcutis removed had a 33% greater reduction in perfusion than those in dogs. Although there was not a significant treatment × animal interaction, clinically significant differences may exist but would require a larger sample size to demonstrate statistically. If true, this may suggest that greater

loss of cutaneous perfusion may at least be partly responsible for the greater inhibition of wound healing we observed with subcutis removal in cats compared with dogs. This differential effect on perfusion may be explained by differences in cutaneous angiosomes of dogs and cats;[13] dogs possess a greater density of collateral subcutaneous vessels, so that removal of a portion of subcutis may be reasonably expected to have a smaller overall effect on cutaneous perfusion in dogs than cats.

Cutaneous perfusion in closed wounds showed species differences but no treatment differences. We attribute this finding to the experimental design. The area of subcutaneous tissue removal for closed wounds was a relatively narrow strip, ~ 1 cm wide, centered beneath the incision. The LDPI scanner could only be set to scan a square region, so a scanning area 3 cm × 3 cm (the length of the incision) was used. So, the relatively small subcutaneous tissue defect coupled with the larger scanned area, meant that the LDPI scanner was detecting beyond the borders of subcutaneous tissue removal, and thus reading from regions with normal and impaired perfusion. Another possible explanation for lack of treatment effect is that removal of a 1 cm wide section of subcutis was insufficient to produce a measurable effect on perfusion.

Wound Granulation

Perhaps the most striking differences between dogs and cats were seen in wound granulation. Removal of the subcutaneous tissue had a markedly inhibiting effect on the production of granulation tissue in cat wounds. When the subcutis was

removed, none of the cats produced enough granulation tissue to cover the bottom of the wound cavity by day 21 whereas in contrast, wounds in 5 dogs were covered in a median of 17 days (mean >16.5 days). Similar differences were noted in the time to fill the wound with granulation tissue. Because granulation tissue is the primary source of fibroblasts and the myofibroblasts responsible for wound contraction,[14] inhibition of granulation because of subcutis removal is a plausible explanation for the observed inhibition of wound contraction. Previously, we reported that the origin of granulation tissue differs between the dog (primarily from the wound center) and the cat (primarily from the periphery of the wound).[4] This finding would seem to indicate that removal of the central subcutaneous tissue should have a greater effect on granulation in dogs than cats, yet the opposite occurred. One possible explanation is that the effect on granulation was secondary to effects on perfusion. Another possibility is that some process relating to wound granulation, probably occurring during the early inflammatory stage of healing, was affected more in cats. Perhaps, histologic investigation will yield a more complete explanation.

Wound Contraction

Planimetry results (for epithelialization, contraction, total healing of open wounds) were also significantly affected by removal of the subcutaneous tissue in both dogs and cats. Significant animal × treatment interactions were identified for contraction. By day 21, wounds in dogs but not in cats had recovered from the effect

of removal of the subcutis. One possible explanation for the reduced wound contraction in cats was that slightly closer spacing of wounds in cats than dogs (because of body size) may have interfered with wound contraction in cats. If true, wound tension should have caused cat wounds to have elongated in a cranio-caudal direction, but no such elongation was noted. In fact, although very slight elongation occurred in some wounds in dogs and cats, it was only along the normal (dorsoventral) lines of trunk skin tension. We contend that the most likely reason for the observed difference in wound contraction between dogs and cats relates to the production and function of granulation tissue. The slower and less abundant production of granulation tissue observed in cats correlates well with their reduced wound contraction.

Wound Infection

A presumptive diagnosis of wound infection was made in 2 dogs based on the presence of erythema, swelling, purulent exudate, and the "moldy tortilla" odor characteristic of *Pseudomonas* spp. growth. Enrofloxacin was administered and the condition resolved rapidly and without complications in both dogs. Some may fault our approach because microbial culture and susceptibility testing was not obtained before initiating treatment; however, we believe our approach was justified on the basis of the following considerations: (1) the short time course of this experiment demanded that rapid action be taken or risk interference with healing and invalidation of data; (2) to have allowed the condition to follow a natural course would have been

indefensible; and (3) rational empirical antibiotic therapy is an effective and proven strategy, and the antibiotic selection and dosing met the standard of practice.

We found it interesting that both infections occurred in dogs, and both on the side with the subcutaneous tissue removed. Although the number is small, we considered this finding to be potentially noteworthy because it may indicate that removal of large amounts of subcutaneous tissue could predispose to wound infection. Further work on the immunologic role of the subcutaneous tissues in wound healing is indicated.

Our subjective observations that open wounds in dogs produced more wound fluid and were slightly more erythematous and swollen than wounds in cats may be interpreted as indicating that the dog has a more intense early inflammatory response to wounding than the cat; however, this hypothesis awaits histologic confirmation.

Wound Strength

We found that removal of the subcutaneous tissues produced no statistically significant effect ($P = .078$) on sutured wound strength. In animals with paired observations, 3 of 5 cats and 3 of 4 dogs had lower 7 day sutured wound strength when subcutis was removed, whereas 2 of 5 cats and 1 of 4 dogs actually had higher wound strength with the subcutis removed. Thus, even though mean 7 day sutured wound strength was reduced by 22.3% in cats, and by 17.4% in dogs when the

subcutaneous tissue was removed, this difference was not statistically significant. The lack of a consistent treatment effect on sutured wound strength may indicate that the subcutaneous tissue plays a relatively minor role in first intention healing compared to second intention healing. However, this explanation seems unlikely, given the highly significant role that we observed in 2nd intention healing and the fact that 1st and 2nd intention healing are governed by the same underlying processes and production of collagen is an essential aspect of both. Another explanation is that our experimental design may have masked a true difference. As we described earlier, the 1 cm wide strip of subcutaneous tissue we removed may have been too narrow to consistently affect perfusion, and this observation may also apply to other aspects of 1st intention healing. Also, the loss of the data form 3 tensiometry samples (2 dogs, 1 cat) caused by improper sample handling may have contributed to our failure to demonstrate a significant effect of subcutis removal on 1st intention healing. Further investigation, focused on clarifying the role of the subcutaneous tissue in 1st intention healing, is indicated.

In conclusion, to our knowledge, this report represents the 1st study to investigate the direct contribution of the subcutaneous tissue to cutaneous wound healing in any species. Although it was surmised that the subcutaneous tissue was linked to cutaneous healing through provision of blood supply, no prior work has directly demonstrated the importance of the subcutaneous tissue in wound granulation and 2nd intention healing. The practical implication for surgical practice, particularly for wound debridement and reconstructive surgery, is that subcutaneous tissue may not be as "expendable" as it has traditionally been thought, as suggested by

recommendations for liberal debridement.[15] Our findings support a more conservative approach to subcutaneous excision when possible,[16] especially in potentially problematic closures where open wound management may become necessary, or when wound healing is already known to be impaired. Methods of dealing with the subcutaneous defects such as drains, walking sutures, or wound healing stimulants for open wounds, may be more important decisions than previously thought when large amounts of subcutaneous tissue must be removed.

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	Cats		Dogs	
	Subcutis intact	Subcutis removed	Subcutis intact	Subcutis removed
LDPI, volts	0.18[a]	0.29[a]	0.56[b]	0.45[b]
Wound breaking strength, kg	0.41[c]	0.32[c]	0.82[d]	0.67[d]

Table 3.1. First intention healing, Day 7, cats vs dogs. Different superscript indicates significant difference among means within row ($p \leq 0.05$, Wilcoxon rank-sum test).

	Cats				Dogs			
	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
LDPI, volts, subcutis intact	0.75[a]	2.27[a]	5.00[a]	2.62[a]	2.11[a]	2.92[a]	4.05[a]	2.70[a]
LDPI, volts; subcutis removed	0.80[a]	1.11[b]	2.83[b]	2.94[a]	1.17[a]	2.00[b]	2.72[b]	1.61[a]
LDPI, volts; overall	0.77[*]	1.69[*]	3.91	2.78	1.64	2.46	3.39	2.16

Table 3.2. LDPI, open wounds; cats vs dogs and subcutis intact vs subcutis removed. Different superscript indicates significant difference among treatments on that day. *Indicates significant difference between cats and dogs on that day ($p \leq 0.05$, repeated measures ANOVA).

% epithelialization				
	rx	day 7	day 14	day 21
cats	s	0.0[a]	13.0[a]	34.4[a]

	n	2.4[a]	8.1[b]	20.1[b]
dogs	s	3.2[a]	44.7[c]	89.4[c]
	n	2.1[a]	31.5[d]	61.2[d]

Table 3.3. Percent epithelialization, open wounds; cats vs dogs and subcutis removed vs subcutis intact. Within each day, different superscript indicates significant difference among means ($p \leq 0.05$, repeated measures ANOVA).

% contraction				
	rx	day 7	day 14	day 21
cats	s	18.25[a]	53.0[a]	75.8[a]
	n	-2.6[c]	19.4[b]	53.9[b]
dogs	s	41.2[b]	66.1[a]	70.3[a]
	n	7.5[c]	32.1[b]	63.5[a]

Table 3.4. Percent contraction, open wounds; cats vs dogs and subcutis removed vs subcutis intact. Within each day, different superscript indicates significant difference among means ($p \leq 0.05$, repeated measures ANOVA).

% total wound healing				
	rx	day 7	day 14	day 21
cats	s	18.3[a]	59.0[a]	83.9[a]
	n	0.2[b]	25.8[b]	62.1[b]

dogs	s	43.1[a]	76.1[c]	97.6[c]
	n	9.3[b]	56.7[d]	83.4[d]

Table 3.5. Percent total wound healing, open wounds; cats vs dogs and subcutis removed vs subcutis intact. Within each day, different superscript indicates significant difference among means ($p \leq 0.05$, repeated measures ANOVA).

Granulation tissue formation, mean time in days					
	Rx	first gran.	wound covered	wound filled	
Cats	s	6.2[a]	>17.2[a]	>19.7[a]	(5/6 covered and filled)
	n	6.5[a]	>21.0[b]	>21.0[b]	(0/6 covered or filled)
	all	6.3[a]	>19.1[c]	>20.3[c]	(5/12 covered and filled)
Dogs	s	4.3[b]	6.8[d]	7.5[d]	(6/6 covered and filled)
	n	4.6[b]	>16.5[e]	>20.0[e]	(5/6 covered, 4/6 filled)
	all	4.5[b]	>11.7[f]	>13.7[f]	(11/12 covered, 10/12 filled)

Table 3.6. Granulation tissue formation in open wounds, cats vs dogs and subcutis removed vs subcutis intact. Within each measurement, differing superscript indicates significant difference among means ($p \leq 0.05$, ANOVA).

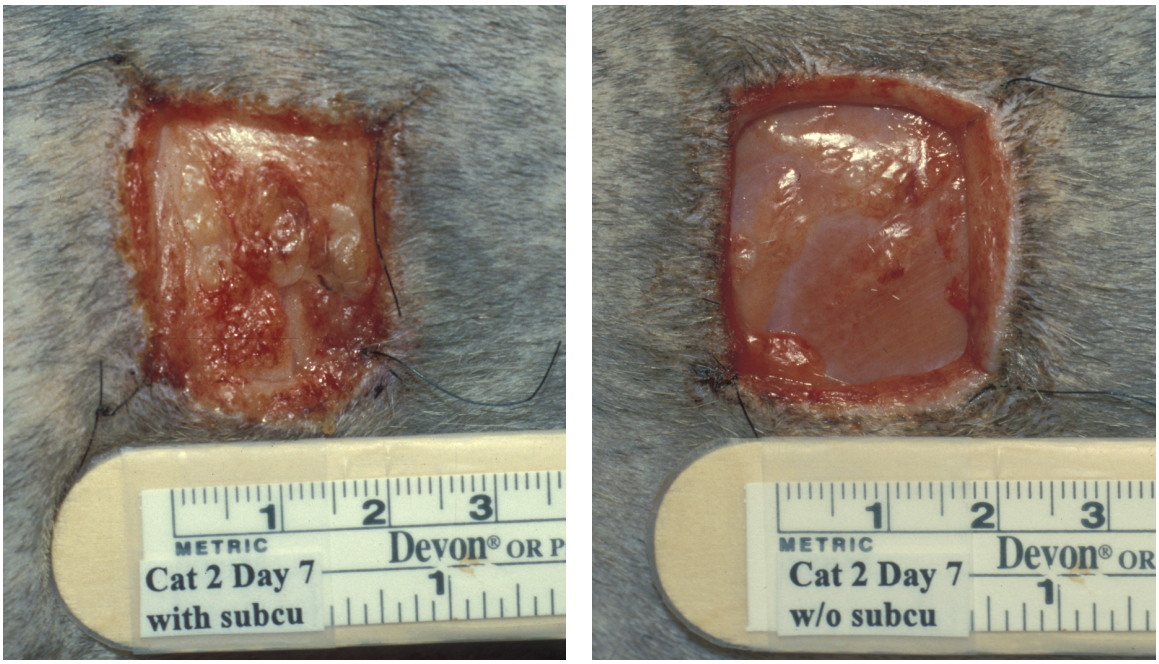

Figure 3.1.1 a,b. Cat 2, Day 7 postwounding; subcutis intact and removed.

Figure 3.1.1 c,d. Cat 4, Day 7 postwounding; subcutis intact and removed.

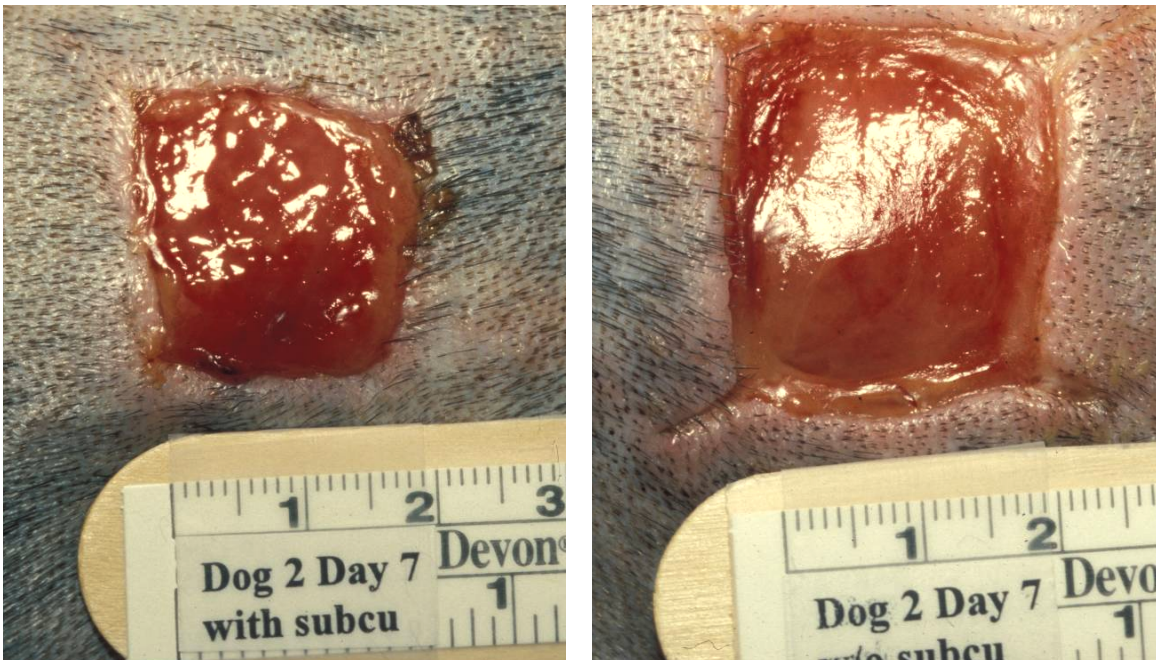

Figure 3.1.2 a,b. Dog 2, Day 7 postwounding; subcutis intact and removed.

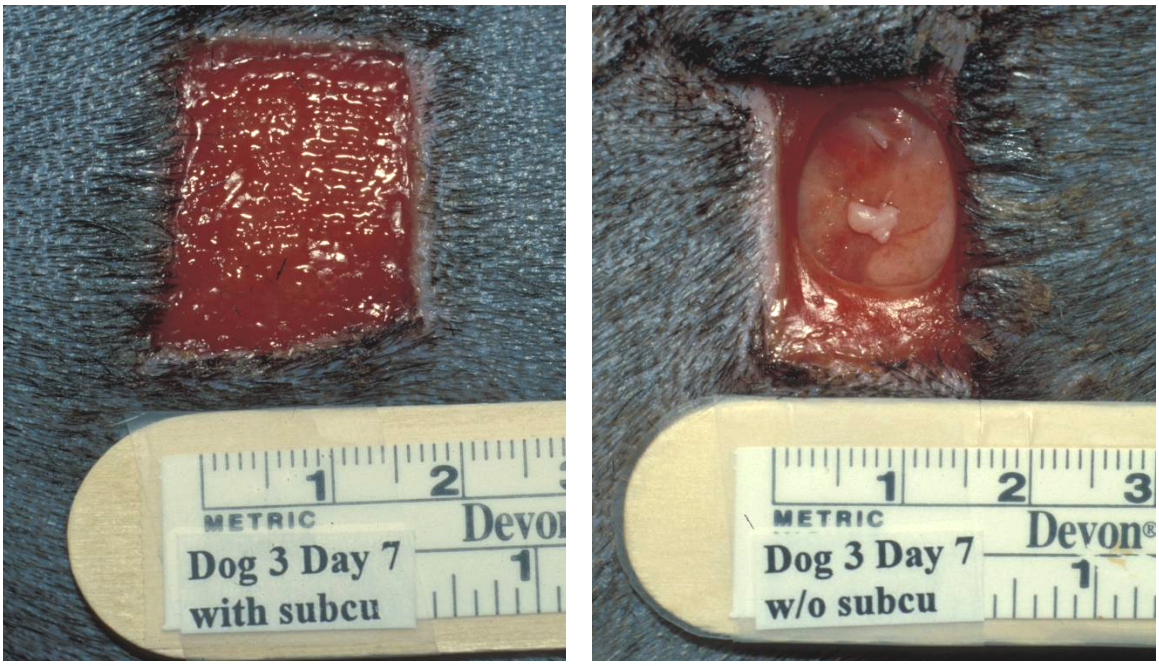

Figure 3.1.2 c,d. Dog 3, Day 7 postwounding, subcutis intact and removed.

Figure 3.1.2 e,f. Dog 6, Day 7 postwounding, subcutis intact and removed.

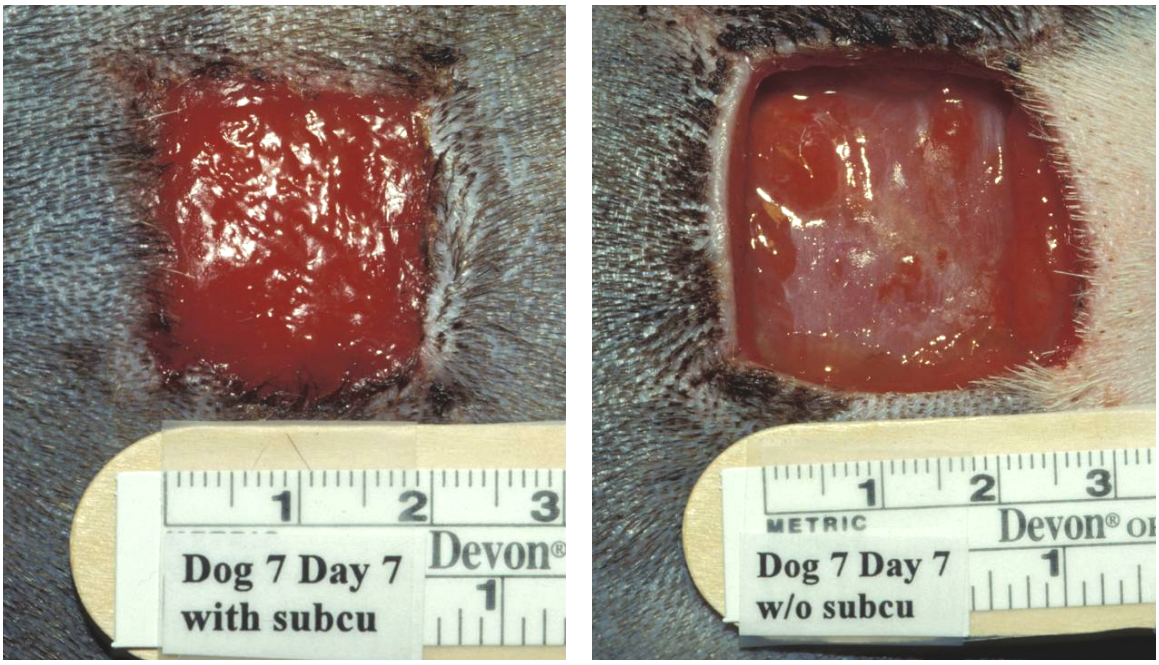

Figure 3.1.2 g,h. Dog 7, Day 7 postwounding, subcutis intact and removed.

Figure 3.2.1 a,b. Cat 2, Day 14 postwounding; subcutis intact and removed.

Figure 3.2.1 c,d. Cat 4, Day 14 postwounding; subcutis intact and removed.

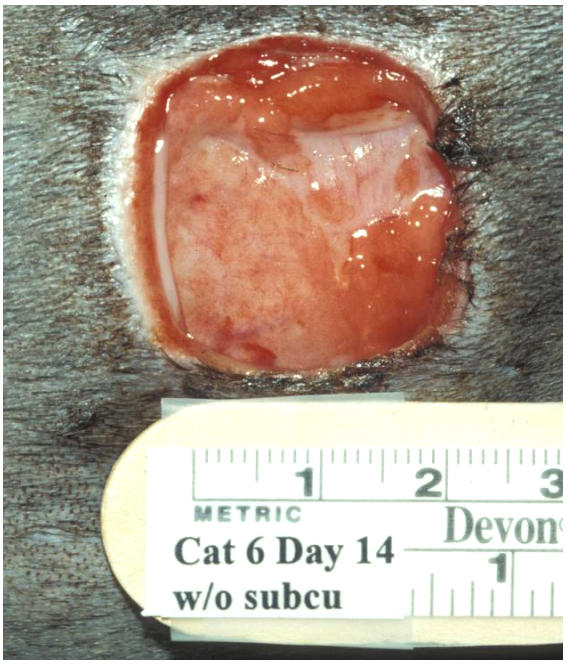

Figure 3.2.1 e,f. Cat 6, Day 14 postwounding; subcutis intact and removed.

Figure 3.2.1 g,h. Cat 7, Day 14 postwounding; subcutis intact and removed.

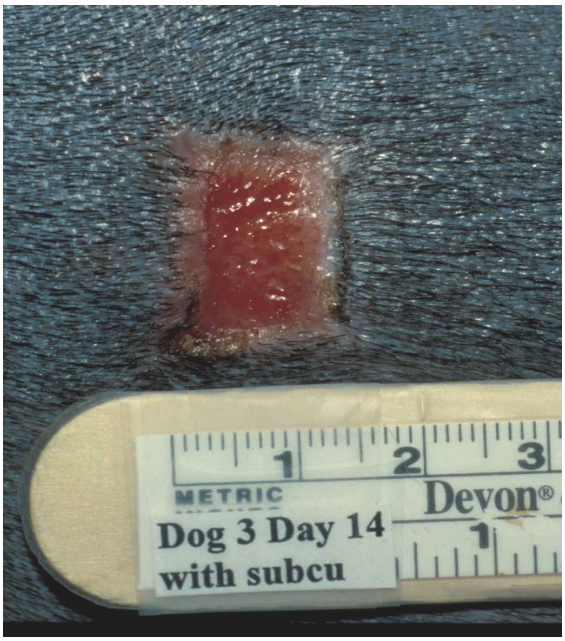

Figure 3.2.2 a,b. Dog 3, Day 14 postwounding; subcutis intact and removed.

Figure 3.2.2 c,d. Dog 4, Day 14 postwounding; subcutis intact and removed.

Figure 3.2.2 e,f. Dog 6, Day 14 postwounding; subcutis intact and removed.

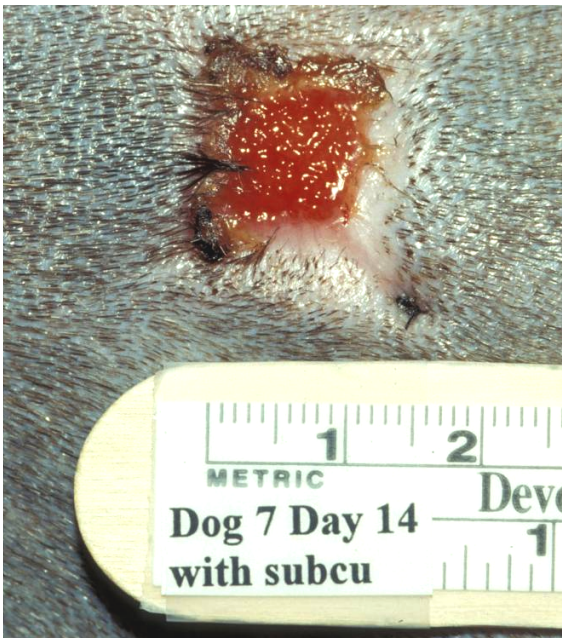

Figure 3.2.2 g,h. Dog 7, Day 14 postwounding; subcutis intact and removed.

Figure 3.3.1 a,b. Cat 2, Day 21 postwounding; subcutis intact and removed.

Figure 3.3.1 c,d. Cat 3, Day 21 postwounding; subcutis intact and removed.

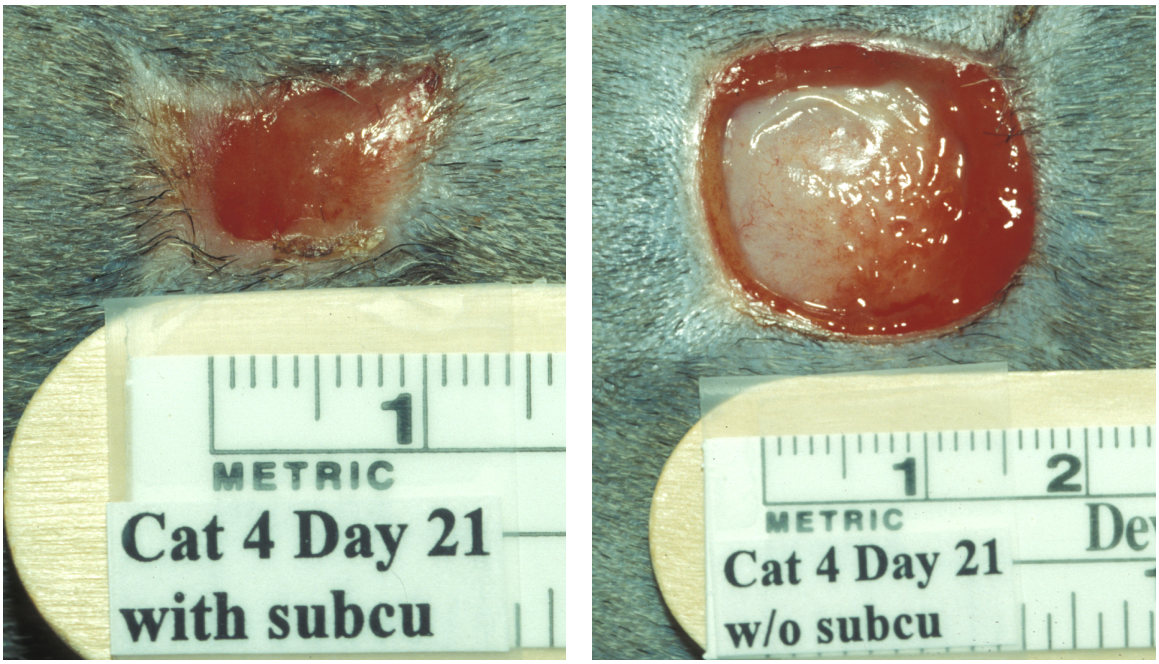

Figure 3.3.1 e,f. Cat 4, Day 21 postwounding; subcutis intact and removed.

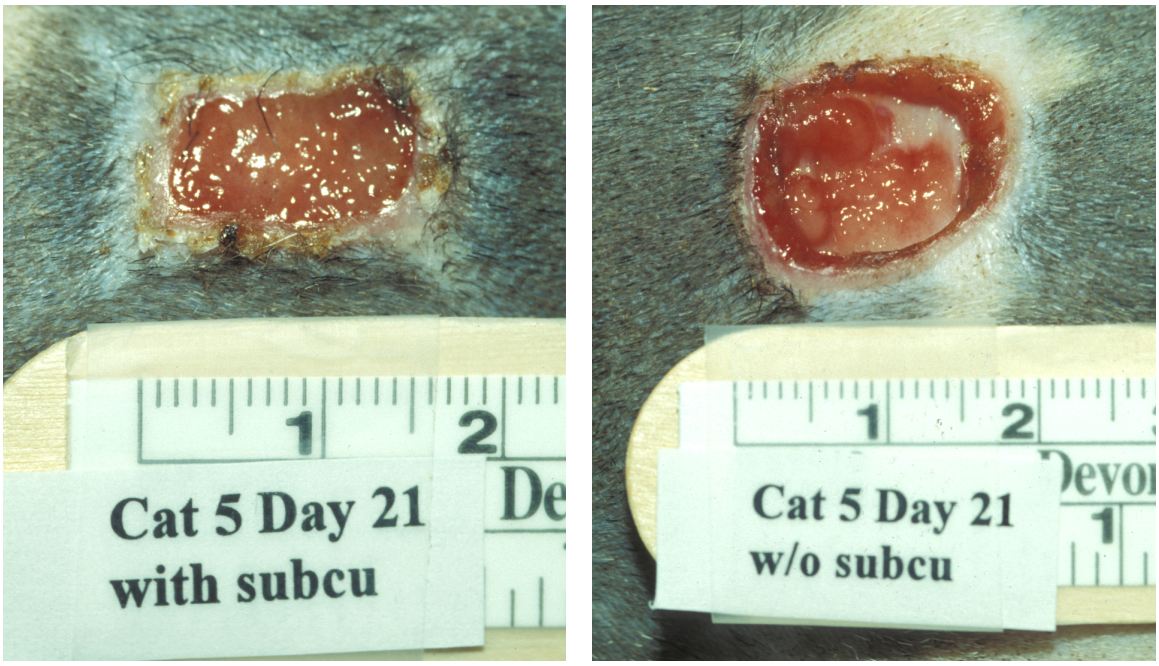

Figure 3.3.1 g,h. Cat 5, Day 21 postwounding; subcutis intact and removed.

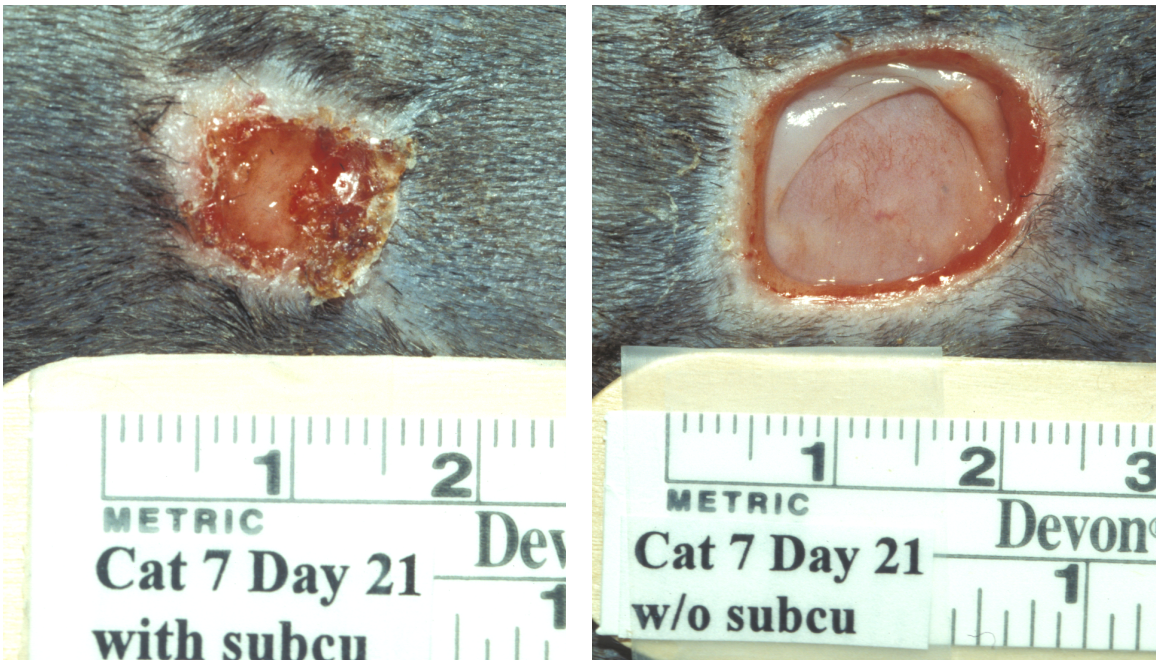

Figure 3.3.1 i,j. Cat 7, Day 21 postwounding; subcutis intact and removed.

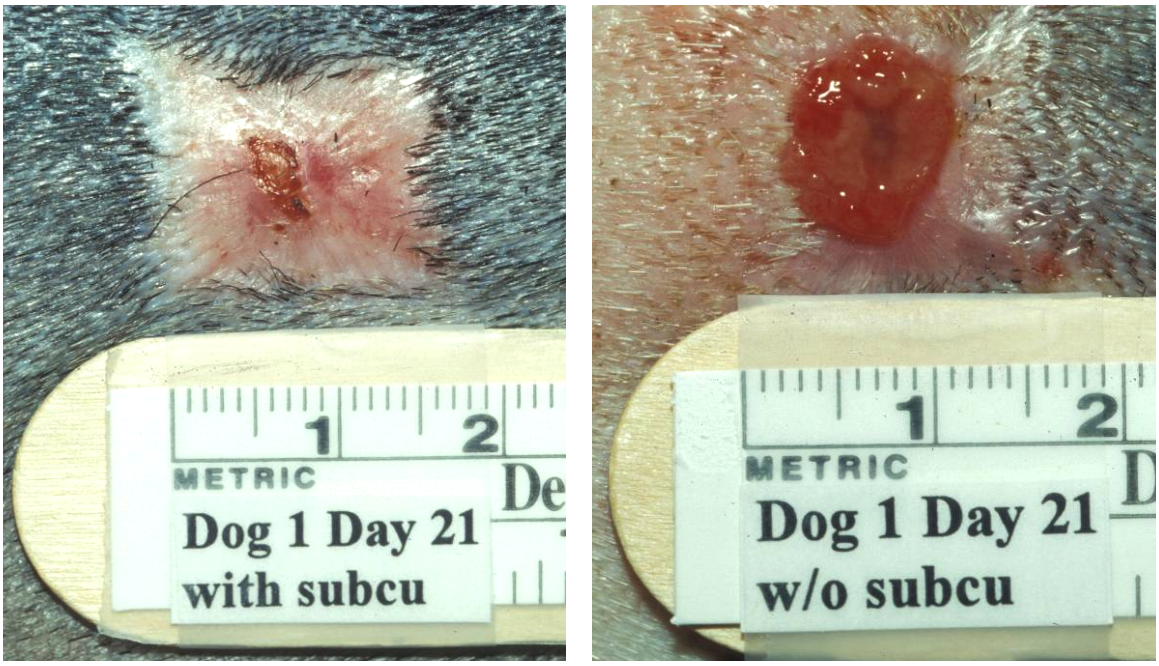

Figure 3.3.2 a,b. Dog 1, Day 21 postwounding; subcutis intact and removed.

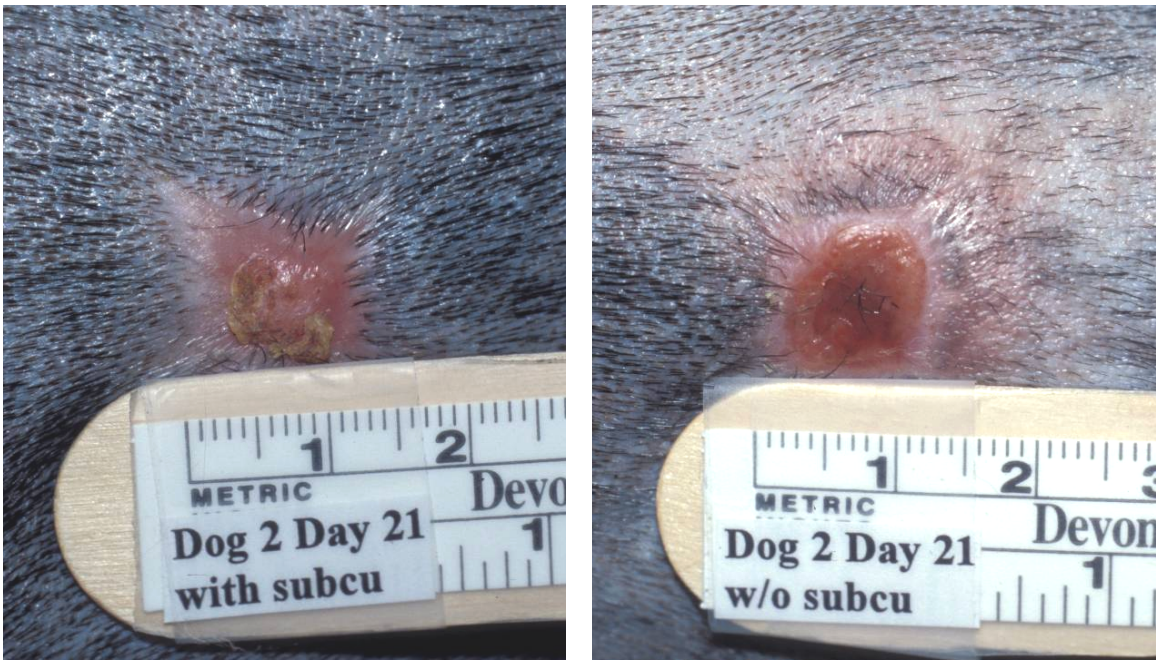

Figure 3.3.2 c,d. Dog 2, Day 21 postwounding; subcutis intact and removed.

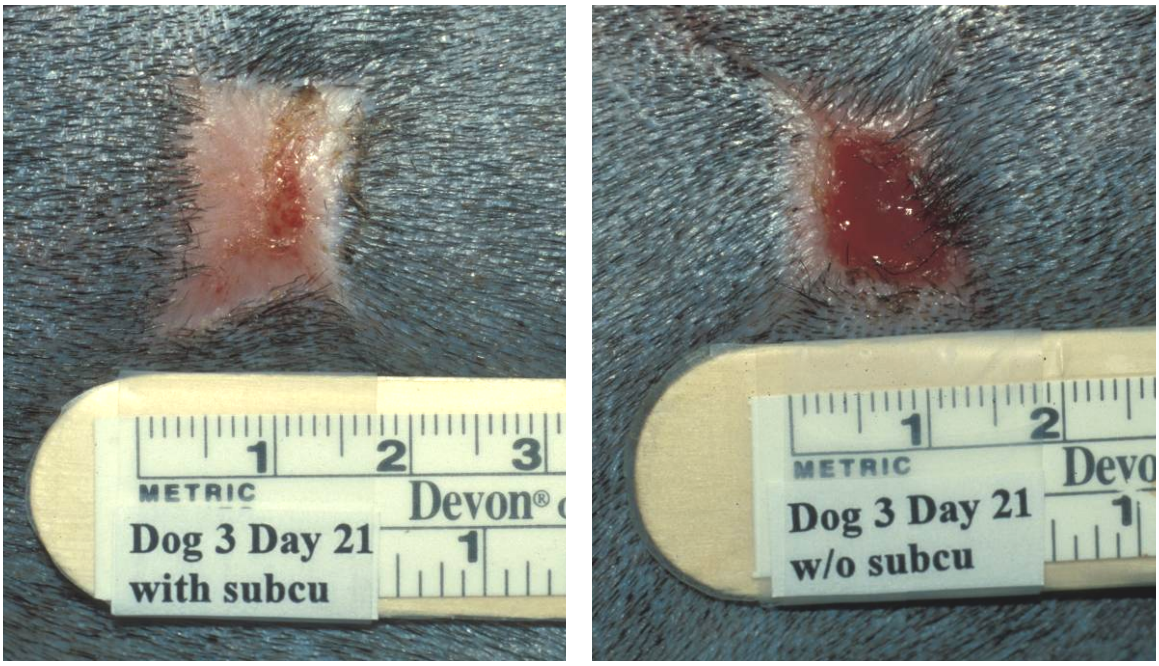

Figure 3.3.2 e,f. Dog 3, Day 21 postwounding; subcutis intact and removed.

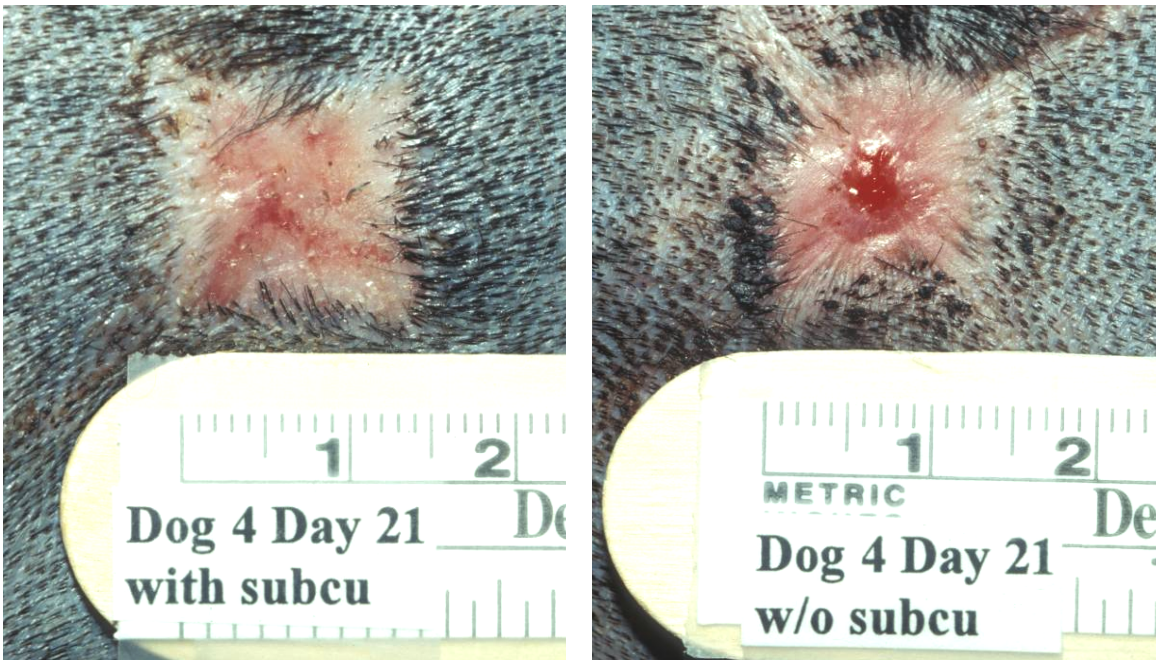

Figure 3.3.2 g,h. Dog 4, Day 21 postwounding; subcutis intact and removed.

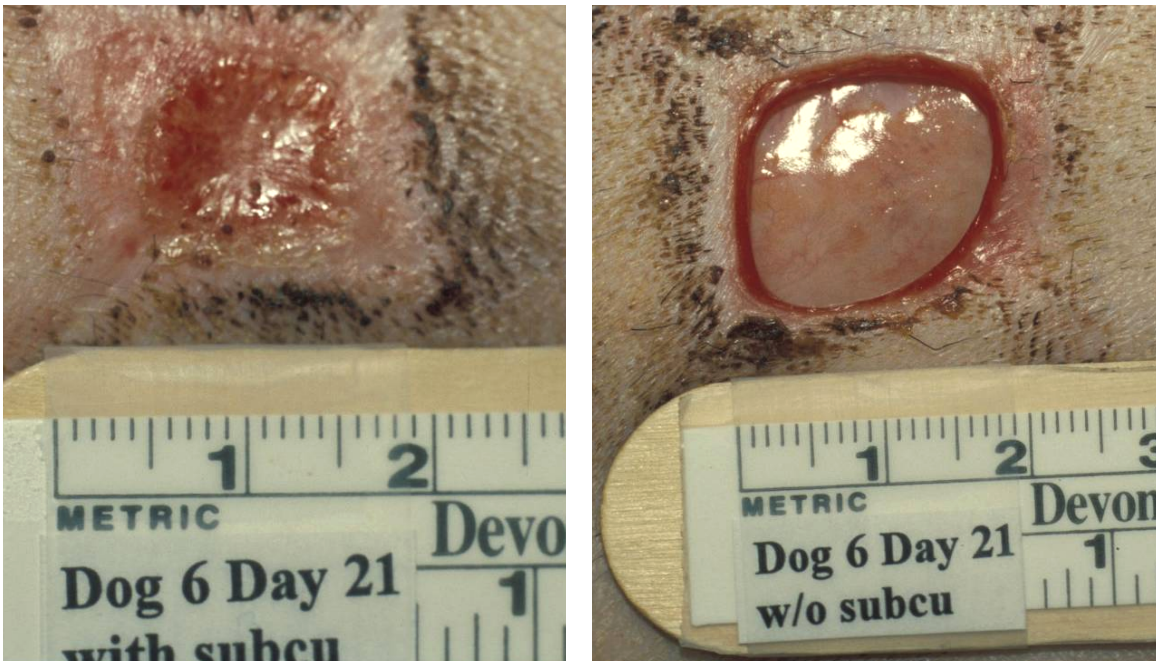

Figure 3.3.2 i,j. Dog 6, Day 21 postwounding; subcutis intact and removed.

Figure 3.3.2 k,l. Dog 7, Day 21 postwounding; subcutis intact and removed.

IV. HISTOLOGIC EXAMINATION OF NORMAL AND DELAYED CUTANEOUS WOUND HEALING IN THE CAT AND COMPARISON TO THE DOG.

Paired sets of open and closed wounds were created along the dorsal midline of cats and dogs; one set of wounds had the subcutis left intact while the subcutis was removed in the other set of wounds. Biopsies were taken from these wounds at 7, 14, and 21 days post-wounding and stained with H&E, Masson's trichrome, and picrosirius red stains. Histologic examination included histomorphometry on inflammatory cells and elements of granulation tissue including capillaries, fibroblasts, and collagen. Wounds in cats displayed a more chronic inflammatory reaction and less abundant wound fibrosis compared to wounds in dogs.

INTRODUCTION

In previous papers we reported our findings regarding macroscopic observations of the cutaneous wound healing of cats compared with that of dogs. As an ongoing part of that investigation, we now report on the results of histologic examination of biopsies taken during the aforementioned study. The work we now report on was undertaken with three main goals in mind.

The first goal was to provide a histologic description of cutaneous wound healing in the cat as a unique species. The literature contains several descriptions of feline cutaneous histology; however we are not aware of any histologic descriptions of feline cutaneous wound healing in the veterinary literature.

The second goal was to learn more about the causes of problem wound healing in cats. Our clinical impression is that wound healing in cats can have particular problems that are not seen in healing wounds in dogs, including chronic nonhealing axillary wounds termed indolent pocket wounds, and failure of primary intention healing that we have termed “pseudo-healing”. The third goal for this study was to learn more about perturbed wound healing in the cat in order to better treat these and other clinical conditions of abnormal healing. Our fourth goal was to gather histologic evidence to corroborate our earlier macroscopic findings, and prove or disprove our hypotheses about feline cutaneous wound healing that were derived from these findings. Our hypothesis was that we would also find histologic evidence of differences between cats and dogs in the cellular events of wound healing (inflammation, proliferation, and maturation) that would help to explain the previously reported macroscopic differences. Specifically, we hypothesized that the cat would have a less severe inflammatory

reaction, ie, fewer inflammatory cells would be seen in cat wounds than seen in dog wounds. This difference would result in a less active proliferative phase, ie, fewer capillaries and less fibroplasia in the cat. We further hypothesized that removal of the subcutaneous tissues, which we demonstrated earlier to result in delayed wound healing, would produce similar histologic evidence of reduction in proliferation in both cat and dog wounds, and that this effect would be more pronounced for wounds in the cat.

Materials and Methods

Wound creation

Four matching wounds were created with one of each pair on either side of the dorsal midline in the dogs and cats. They were bandaged for protection during healing, according to a previously published protocol. The cranial 3 pair of wounds were open wounds. The most cranial wounds were 2 x 2 cm square defects created with a #15 scalpel blade, and were used to evaluate second intention wound healing by histologic evaluation at 21 days post-wounding. The 2 middle pairs of wounds, 1 cm diameter, were made with a disposable dermal biopsy punch, and were used for histologic evaluation of wound healing at 7 and 14 days post-wounding. The caudal wounds were 3 cm craniocaudal linear wounds created with a #15 blade. They were sutured with 5 simple interrupted sutures of 3-0 nylon, spaced and tied to approximate the skin edges without crushing them. These wounds were used to evaluate first intention wound

healing by histologic examination at 7 days post-wounding. Because previous studies in rats had shown differential healing of cutaneous wounds based on their cranial-caudal location on the trunk, the wounds were created in the same location on each animal regardless of differences in body size. The most cranial pair of wounds was located just caudal to the caudal border of the scapula and the most caudal wounds were located just cranial to the ilial wing. The 2 middle pairs of wounds were spaced equidistant between these. The wounds were spaced far enough apart so that contraction in one wound would not cause interference with healing in adjacent wounds because of tension. This necessitated slightly closer wound spacing on the cats than on the dogs, and small dogs were used to minimize this species difference. The tissues that were excised in the creation of each of the wounds were retained as the baseline (day 0) samples.

Matched wounds were used to provide two wound treatments in each experimental subject. In the first treatment, open wounds were created by excision of all tissues down to and including the panniculus muscle, and closed wounds were created by simple linear incision down through the level of the panniculus. These wounds were designated the “subcutis intact” wounds and represented the control group. In the second treatment, open wounds were created by excision of all tissues down to the level of the dorsal fascia. Closed wounds were created by excision of a small strip consisting of all tissue deep to the dermis down to the level of the dorsal fascia. The dimensions were 1 cm wide x 3 cm long (the length of the incision), with the 1 cm width of the defect centered under the sutured incision.

Tissue Processing and Examination

Tissue samples for histologic evaluation were immediately fixed in 10% neutral buffered formalin solution and mounted in paraffin blocks, from which sections were cut and mounted on slides. One slide from each block was stained with hematoxylin and eosin stain for overall evaluation of healing including cell counts and scores. One slide was stained with Masson's trichrome stain and another with picrosirius red stain, both for evaluation of wound collagen content.

All identifying information associated with each block (ie, individual animal ID number, species, nature of wound whether open or closed, and number of days postwounding) was recorded in a key; this information was not revealed to the primary examiner (MWB) until after the histologic evaluation was completed. The order of viewing of the slides was selected via random drawing.

Slides were examined by the primary author using standard bright field microscopy (BH-2, Olympus Corp., Tokyo, Japan). Examination was performed sequentially at low power (40x), then at intermediate power (100x and 200x), and finally at high power (400x) for counting and scoring specific parameters.

During the examination process, regular slide consultations were held with a veterinary histologist (SAK) and histopathologist (EAS) to ensure accuracy and also to provide continuity with previously published studies.

A previously published histologic scoring system, used by Swaim and Sartin in several wound healing studies,was used without modification, to record the results of our histologic evaluation. In addition to this ordinal scoring system, an additional

evaluation system that produced interval data was also used. In the interval data system, the total number of each type of cell or structure of interest (eg, neutrophils, capillary buds) in a representative high power field (hpf) was counted and recorded. The actual count was then used as the numerical score, unless the number exceeded 100/hpf, in which case the count was truncated at 100. The purpose of count truncation was to reduce the statistical impact of a single section that might have an extremely high count, in an otherwise more moderate group of sections; we questioned whether there was additional biologic significance of, for example, 200 vs 100 neutrophils/hpf.

For collagen evaluation, two scoring systems were used. (see Figs 4.1 – 4.5) The first was the same scoring system used by Swaim, et al in several prior studies. In this scoring system, random distribution of collagen in a pattern typical of normal dermis was considered normal and given a score of 0. Scant accumulation of collagen bundles, causing slight separation of fibroblasts was considered a mild increase in collagen and assigned a score of 1. Somewhat dense collagen bundles between fibroblasts was considered a moderate increase in collagen and given a score of 2, while extensive separation of fibroblasts by dense collagen was considered a marked increase and assigned a score of 3. The second scoring system was a modification of that system, having a range of from 0 to 4. The modified scoring system was introduced to potentially gain greater sensitivity in discrimination between levels of wound collagen production, particularly when collagen production was extensive. In the modified system, a score of 0 or 1 used the same definition as for the original system. However, a score of 2 (moderate) was redefined as a background field of mild collagen accumulation as described above, having scattered focal areas of fibroblasts separated by

dense collagen bundles. Marked collagen accumulation (score 3) was defined as having regionally extensive separation of fibroblasts by dense collagen bundles, but with some areas of scant collagen bundles also visible. A score of 4 (very marked) was defined as pervasive dense collagen bundles separating fibroblasts, with no areas of scant collagen bundles.

Sections through the healing wounds demonstrated a large variation in the proportion of total area that consisted of active healing response, either inflammatory or proliferative. On some slides the sections would consist almost completely of granulation tissue, whereas in others there was only a tiny area of granulation tissue, at the edge of the section in some cases or in the center in other sections that appeared to be (and were later confirmed to be) sections from sutured wounds. With such wide variation in the amount of granulation tissue on the slides, it was at times difficult to determine what defined a "representative area of healing." In general we considered that the most actively proliferating area located at the advancing edge of granulation tissue would be considered representative; if epithelialization was noted over the surface of the granulation tissue, we considered the area of granulation tissue just beneath the advancing edge of epithelium to be most representative of healing.

Likewise, in evaluation of the inflammatory response, there was wide variation on most sections. If we were able to identify a visible focus of inflammation for which we could reliably state that the cause of inflammation was not directly connected to wound healing (examples: suture tracks and hair – see below), we did not consider this area to be representative of the inflammatory response in wound healing, and did not count inflammatory cells from these areas. On the other hand, if no immediate

inflammatory cause was seen, we selected the area of the section with the most active inflammation as being representative of wound healing inflammation. Typically these areas were very close to, and often were in the exact same field as the one used to evaluate proliferation; however, if, for example, an area of higher concentration of inflammatory cells were nearby an area of higher capillary density, the field would be moved to read each count.

Data Analysis

All cell count data was tested for normality of distribution; normally distributed counts (interval data) are reported as means. Non-normally distributed count data, and all scores, were analyzed as rank (ordinal) data; medians and ranges are reported for ordinal data. A mixed model repeated measures analysis of variance was used to compare interval data (counts) and ordinal data (ranked counts and scores). The model was used to test for significant effects on cell counts and histologic scores due to species (all cat wounds vs all dog wounds), time (day 7 vs day 14 vs day 21), treatment (subcutis intact vs subcutis removed), and type of wound (open vs closed wounds, available for day 7 only). The Tukey-Kramer test was applied to all comparisons, with differences between groups considered to be significant at $P \leq 0.05$. A commercial statistical software program was used to perform all analyses (SAS, proprietary software release version 9.1, SAS Institute, Cary, NC USA).

Results

Descriptive histopathology – subjective measures of cat and dog wounds

All of the day 0 sections were devoid of any inflammatory or proliferative response, and accordingly, all were assigned a score of 0 for each of the evaluated parameters. The day 0 sections appeared at random throughout the slides as they were examined, and as with all the slides, their identifying information was not revealed until the key was opened, however, it was easy to assume that these were day 0 sections due to the lack of histopathologic change. Since no area of active inflammation or proliferation was seen in any of these sections, we did not perform cell counts on the normal areas of dermis, as the intent of taking day 0 samples was to provide a basis for subjective comparison between healing and non-healing skin, and not to accumulate data on inflammatory cell counts for normal skin.

The first subjective impression of the healing (granulation) tissue of dogs and cats was not one of differences, but rather one of similarity. Although the amount of granulation tissue on individual sections varied quite widely, our first observation on low power (40X total magnification) scan was how similar all granulation tissue looked, regardless of time of sampling, type of wound, or species of origin. Only upon closer examination of the tissue, often only with the cell counts, did differences become visible. The significance of this observation to us is that low power scanning may not yield sufficient information to differentiate subtle histologic differences that may correlate to clinically important differences between species, so that higher power

evaluation and even quantitative evaluation (cell counts) may be required to uncover the histologic basis of interspecies differences in wound healing.

Subjective evaluation of the H & E sections resulted in two interesting observations of apparent differences between dogs and cats. Certain sections contained moderate to large numbers of hair elements embedded in the granulation tissue (Fig 4.9), with the hairs cut at various angles from perpendicular to nearly parallel to the hair. Not surprisingly, there was always a fairly intense inflammatory reaction immediately surrounding these hair elements; however, away from the hairs, the granulation tissue did not appear to be inordinately infiltrated with inflammatory cells and was in fact usually relatively "quiet" with respect to inflammation. We (MWB, EAS, SAK) made the judgment at the time to intentionally ignore these hair-containing areas with respect to cell counts and scores, as we felt that they represented reaction to buried keratin rather than any aspect of normal wound healing, and all counts were made in "normal" areas away from the hair-infiltrated ones. The interesting observation was made after opening the slide "key" that all of these wounds were from cats. The cause of this phenomenon is not known; we suspect it may be related to the way that granulation tissue seems to propagate from the edges of the wound rather than throughout the entire open wound area. Its significance is speculative at this time.

Another observation was actually made grossly at biopsy, but confirmed when seen again during histopathologic examination. Certain of the sections had areas of granulation tissue that appeared to be "floating" above the subcutis and not connected to it in any way (Fig 4.10). We suspected (and later confirmed with the opening of the key) that these were biopsies of open wounds in cats, in which the granulation tissue that

proliferated from the wound edges often did not adhere to the deeper tissues, particularly when the subcutis had been removed at the creation of the wound.

Inflammatory cells and edema – measures of acute inflammatory response

When cat wounds from all days and all treatments were grouped together and compared to all dog wounds, the neutrophil count for cat wounds (median 47; range 0 - 100) was significantly greater ($p = .001$) than for dog wounds (median 4.5; range 0 – 100). This difference was also reflected in the mean neutrophil scores for all cat wounds (mean score 2.5) compared to all dog wounds (mean score 1.2); scores in cat wounds were significantly greater ($p = 0.003$). Similar differences between cat wounds and dog wounds were also seen for macrophages, eosinophils, and mast cells. The mean macrophage count for all cat wounds (47.7 ± 6.5) was significantly greater ($p = 0.01$) than the mean macrophage count for all dog wounds (19.8 ± 6.5). The difference in macrophage count was noted at each of the three biopsy intervals, and was also reflected in a significant difference in median macrophage score between dog (median score, 3) and cat (median score, 2) wounds ($p = 0.02$).

The median eosinophil count for all cat wounds was significantly greater for cat wounds than for dog wounds (8 vs 3; $p = 0.02$); differences in median eosinophil scores between cats and dogs just missed statistical significance ($p = 0.06$).

Mean mast cell count for all cat wounds (mean 4.0) was significantly greater ($p = 0.02$) than for all dog wounds (mean 1.1). Evaluation of mast cells using the previously

published scoring system for mast cells, also recognized significantly greater ($p = 0.002$) median scores for mast cells in cat wounds (1) than for dog wounds (0). No significant differences between cat wounds and dog wounds were detected for lymphocytes or plasma cells, using either total cell counts or the scoring system.

Further histologic evidence of a difference in the inflammatory response to wounding between cats and dogs was noted in the edema score. Median edema scores for all cat wounds (1) were significantly ($p = 0.04$) higher than those from dog wounds (0.5). Scores for acute and chronic hemorrhage, and necrosis were not significantly different ($p > 0.05$) between wounds in cats and wounds in dogs.

Capillaries, collagen and fibroblasts – measures of proliferation

Capillary counts for all cat wounds had a significantly higher mean than dog wounds (20.6 vs 14.9, $p = 0.03$). Evaluation of capillaries using the previously published scoring system did not reveal any significant differences ($p = 0.11$). Evaluation of collagen density yielded mixed results depending on the scoring system. The previously published scoring system did not show a significant difference between cats and dogs ($p = 0.35$), but the expanded four-point scale revealed slightly less deposition of collagen in cat wounds compared to those of dogs. Using the four-point scale, even though the median score for both was 3, the distribution of collagen scores for all cat wounds was lower than for dog wounds; this difference was statistically significant ($p = 0.05$).

Using the same four-point scoring system for collagen, a highly significant increase in collagen density over time was also noted for all wounds ($p < 0.0001$), and a

species x time effect was also noted, with collagen density in cat wounds significantly lower than for dog wounds at day 21 but not at day 7 or 14. The counts and scores for fibroblasts were not significantly different between wounds in cats and wounds in dogs; this was because in any representative 400X field in which active proliferation was occurring, the fibroblast count and score received the maximum value.

Day 7 – comparisons of open and closed wounds

The biopsies on day 7 allowed us the opportunity to compare wound healing between cats and dogs in open and closed wounds, and to look for two-way interactions – species by type of wound (open or closed) and treatment (subcutis intact or removed).

Open wounds in cats with the subcutis removed had a median macrophage score of 3; closed wounds in cats with subcutis removed had a median macrophage score of 1.5, a significant difference ($p = 0.03$). All open wounds (subcutis intact and removed) in cats also demonstrated a significantly ($p = 0.01$) higher edema score than did closed wounds (1 vs 0); there was no significant difference in dog wounds in this regard.

Collagen production was highly affected by the status of wound closure; for all animals (cats and dogs combined), median collagen score in open wounds (2.0) was significantly higher ($p < 0.0001$) than for all closed wounds (1.0). The lowest median collagen scores were seen in cats with closed wounds; the highest in dogs and cats with open wounds. Capillaries, fibroblasts and extracellular matrix make are the principal components of granulation tissue; therefore we expected and observed that the mean

number of capillaries was also lower in closed wounds (6.9) and higher in open wounds (16.8); this effect of wound type was also highly significant ($p < 0.0001$).

Effects of removal of the subcutis

We also noted an effect on fibroblasts from removal of the subcutis in cats. Fibroblast numbers on day 7 were significantly lower in closed wounds with subcutis removed compared to open wounds with subcutis intact; no difference was seen between subcutis removed and intact when comparisons were made within wound type; eg, closed wounds, subcutis intact vs closed wounds, subcutis removed.

We observed significant differences in mast cell numbers in two areas. Mean number of mast cells in all closed wounds (cats and dogs combined) with the subcutis intact was significantly higher (5.5) than in all open wounds with the subcutis intact (1.6); this difference was statistically significant ($p = 0.03$). Also, when comparing subcutis intact vs removed in closed wounds in cats, we observed a significantly larger mast cell count with the subcutis intact (9.2 vs 3.0); this difference did not apply to wounds in dogs.

Discussion

Most of the indices of acute inflammation that we evaluated, including counts and/or scores for neutrophils, macrophages, mast cells, and eosinophils, were higher in the cutaneous wounds of cats than those of dogs. This finding initially came as somewhat of a surprise, as we were expecting the dogs to have a more "robust" inflammatory response to wounding, which we assumed would be manifested as higher histologic scores for all measures of inflammation. However, based on the results of this study, the "robustness" or quality of the inflammatory response is at least as much an issue of timing as it is of degree or amount.

Neutrophils are the first leukocytes to arrive at the site of a cutaneous wound. Serial histologic evaluation of wounds in rodents and humans demonstrated neutrophil influx into wounds within hours, peaking at 2 – 4 days postwounding and declining thereafter. Regarding our post-wounding neutrophil counts and scores, we observed that cats had substantially higher median counts and scores than dogs. This phenomenon was observed throughout the experimental period, as demonstrated by a significant species x day interaction; ie, neutrophil score had significantly dropped in dog wounds by the end of the third week post-wounding, but had not significantly declined in cat wounds. This result is in general agreement with the findings of Wilmink, et al, in their comparison of wound healing of the horse and pony. Their results indicated that neutrophil counts from proximal hindlimb wounds were initially higher in ponies, but declined more rapidly than in horses, so that by 2 weeks post-wounding the counts in horses were higher and remained so for the remainder of the 6 week experiment. Our

results differed somewhat in that the neutrophil counts in cats were higher at all observations (days 7, 14, and 21). This may reflect an earlier peak and more rapid decline in the inflammatory response of the dog; in retrospect, a different experimental protocol with additional biopsies at days 3 and 5 post-wounding, may have been more useful in answering this question. Differences in wound management between our experiment and Wilmink, et al, may also have influenced the course of the inflammatory response and thus account for the slower decline in neutrophil counts seen in the equine.

Wound macrophages are derived from blood monocytes and precursor cells resident in the local tissues; they constitute the secondary responders in wounds and are considered the "conductors" of wound healing because of the large variety of cytokines that they produce and release. We found that macrophage counts and scores were significantly higher in cat wounds than in dog wounds. This finding agrees with our finding of higher neutrophil counts, which secrete a variety of chemokines that are the stimulus for macrophage migration into the wound. While the macrophage is often considered primarily as a regulator of wound healing events, particularly with regard to initiation and propagation of proliferative processes,we believe that our findings serve to underscore the importance of the inflammatory role of the macrophage, and the complexity of the interrelationship between macrophage and neutrophil with respect to regulation of the inflammatory phase of healing – more macrophages do *not* automatically lead to more wound fibrosis.

In our study, mast cells were significantly more abundant in the healing wounds of cats compared to those of dogs, and this relatively greater abundance of mast cells in the cat wounds may partially explain their slower course of healing. Mast cells are

multifunctional directors of wound healing, particularly in the early inflammatory stage. Mast cells are stimulated to degranulate by vascular and neurologic signals that are directly associated with wounding. Vascular signals include cytokines, IgE, and complement, while the neurologic signals include the release of NGF and substance P from damaged and/or stimulated nerve endings. These tissue signals in turn stimulate mast cells to release a number of wound healing cytokines that stimulate or regulate many of the early inflammatory events in the wound. One event in which mast cell degranulation plays a critical role is the recruitment of leukocytes into the wound. Mast cells products including leukotrienes, proteases, and cytokines, (particularly IL-8), are chemotactic signals for neutrophils, eosinophils, and basophils. In addition, mast cells play a direct role in the extravasation of leukocytes into the wound via release of TNF, histamine, and other mediators that increase the expression of adhesion molecules on vascular endothelium and also directly stimulate leukocyte adhesion to vessel walls. The significance of this to wound healing relates to the timing of mast cell appearance and disappearance from the wound and its relation to healing. In the normally healing wound, mast cells are believed to play a beneficial role in the early stages as mentioned above; a “get in – get done – get out” scenario. In chronic or problematic wound healing, investigators have reported the histologic persistence of abnormally large numbers of mast cells in the wound. It has been hypothesized that these higher reported mast cell numbers are due to greater histologic visibility, which is a result of reduced degranulation. The implication is that abundant mast cells after the early inflammatory phase may be a sign of reduced availability of mast cell granule contents in the wound. Our results showed approximately twice the number of mast cells in feline wounds as in

canine wounds. Although every reasonable effort was made to ensure accurate and complete identification of all mast cells, it is possible that mast cells may have been somewhat underrepresented in our cell counts. Some mast cells can be difficult to identify, particularly those that are degranulated, or those with poorly staining granules, a relatively common feature of feline mast cells that can complicate their identification. The effect on this study is that there may have been a systematic underreporting of feline mast cells, so that the differences between cat and dog may be even larger than we suspect.

Eosinophils were also more abundant in the wounds of cats compared to wounds in dogs. The eosinophil has long been considered to play an important role in allergic and parasitic disease, but its role in wound healing has not been thoroughly investigated and thus remains incompletely understood. Prior studies have demonstrated several functional links between eosinophils and wound healing; eosinophils produce and release known wound healing cytokines during healing, including TGF-α and TBF-β. Histologic examination of healing rabbit skin has shown that eosinophils arrive in the wound concurrently with heterophils. Unlike heterophils, which virtually disappear from the wound by postwounding day 21, eosinophil numbers continued to increase through day 21, suggesting a possible long-term regulatory function in wound healing that extends well beyond the inflammatory phase, at least in the rabbit. It is not known at this time if the kinetics of eosinophil counts are comparable between the rabbit and the dog and cat, but if so, the persistence of greater numbers of eosinophils in cat wounds may be functionally related to their slower rate of healing compared to dog wounds.

Eosinophils have also been linked to mast cells in a number of acute and chronic

inflammatory states including wound healing. In light of this fact it is tempting to speculate that the higher eosinophil counts in cat wounds may also be related to the higher mast cell numbers that were observed; however, at this time it is unknown whether the observed mast cell-eosinophil correlation is causal or not.

Our evaluation of measures of wound proliferation (fibroblasts, capillaries, and collagen) yielded mixed results that were not entirely what we would have predicted. For example, contrary to expectation, capillary numbers were actually higher overall in cat wounds than in dog wounds. At first this finding seemed contradictory to our earlier macroscopic observations, in which granulation tissue in cat wounds was lower in volume and paler in color than in dog wounds, thus seeming to indicate less proliferation in cat wounds. However, the collagen scores, which were lower for cats at day 21, do confirm our macroscopic findings. We contend that the higher capillary scores in cat wounds are indicative of a less mature (less fibrotic) granulation tissue than we saw in the dogs. In other words, cat wounds were still actively proliferating throughout the 21 day experimental period as evidenced by higher capillary counts and less collagen. In the dogs, initial healing and proliferation were largely completed before day 21, by which time wounds were progressing into remodeling, i.e. there was an initial increase in capillaries followed by regression of their numbers as the wounds moved more rapidly to the maturation stage than in cats. Fibroblast counts and scores were comparable between cats and dogs throughout the experiment. We attribute this finding mainly to the scoring and counting system; we truncated counts at 100 cells/hpf, and in almost every field with active proliferation fibroblast counts exceeded that number.

Examining our results for the day 7 comparisons between closed and open

wounds, some of findings were fairly predictable while others were more interesting. Not surprisingly, fibroblast numbers, collagen production, and capillary numbers were lower in closed wounds than in open wounds; this no doubt reflects the much greater amount of granulation tissue production in open wounds. Removal of the subcutis also had a negative effect on fibroblast numbers, but only in cats. Wounds in dogs showed no significant difference in fibroblast number from removal of the subcutis. This histological finding confirms our macroscopic observation regarding the effect of subcutis removal on wound granulation. Subcutis removal delayed wound granulation in both dogs and cats, but the effect was much more marked in cats; 5 of 6 dog wounds were covered by granulation tissue in an average of 16 days, but none of the cat wounds were even close to being covered with granulation tissue by day 21 postwounding.

Some of the other results for Day 7 wounds are more puzzling; for example, why did we see larger numbers of mast cells in closed wounds than in open wounds? One might reason that closed wounds would have less inflammation than open wounds, resulting in lower mast cell numbers. Perhaps at 7 days postwounding the immediately adjacent dermis continues to be an important source of wound mast cells, so that removal of the overlying dermis with open wounds removed a significant supply of mast cells and resulted in lower numbers in the wound. Whatever the reason for their lower numbers, one wonders if there might be a functional connection between the timing and height of peak wound mast cell numbers and the speed of wound healing. In other words, are higher mast cell numbers in the early postwounding period related in some way to their more rapid and uneventful healing, while persistence of mast cells into the second and third weeks postwounding are a cause of delayed healing? This may be the

case; removal of the subcutis in cats was associated with a 3X decline in wound mast cell numbers and a profound negative impact on healed wound strength, whereas in dogs, subcutis removal produced no significant impact on mast cell numbers or healed wound strength.

We also noted that, based on edema and macrophage scores, cats showed greater histologic inflammation in open wounds than closed wounds, whereas dogs did not. Our earlier macroscopic observations of wound fluid production, redness, and swelling during the first 3 - 5 days postwounding seemed to indicate that the dog wounds were more inflamed than the cat wounds, and that open wounds in both species were more inflamed than closed wounds. Combining our histologic findings with our gross observations, we hypothesize that the peak of inflammation in dog wounds is earlier than seen in cat wounds and is largely resolved by the 7th day postwounding. By then, both open and closed wounds in dogs are moving into out of inflammation and into proliferation. However, cats, having weaker inflammation in the very early postwounding period, are still dealing with the additional challenge of inflammation of the open wound beyond the 7th day.

Based on our macroscopic observations, we expected to see more pronounced histologic effects on wound healing from removal of the subcutaneous tissues. Instead, we saw little histologic evidence of effects from removal of the subcutis, other than decreased numbers of mast cells and fibroblasts in cat wounds, (the latter only in a subset). Our assumption is that the lower fibroblast numbers are probably the result of removal of precursors located in the subcutis (adipocytes, fibrocytes, and unspecified mesenchymal cells) that differentiate into wound fibroblasts. It is puzzling to us that

vascularity of the wound (capillary numbers) did not also appear to suffer with subcutis removal, as we would have expected. Lower mast cell numbers in wounds with the subcutis removed was, again, only seen in cat wounds, and is also somewhat surprising, in that mast cells are traditionally considered to derive principally from dermal sources. Further investigation into the source of wound mast cells in the cat is clearly indicated. It may be that removal of the subcutis causes a purely quantitative change in granulation tissue; ie, a lesser amount, but the cellular composition is the same as for normal wounds.

As with any experiment, this study had some limitations and/or areas that are deserving of criticism. One potential for criticism was our decision to truncate the cell counts at 100/hpf. This may have led to otherwise unnecessary transformation of interval data into ordinal data, somewhat reducing the power of our statistical tests in the parameters thus affected. Potentially more importantly, we may have missed significant biological differences between cats and dogs, particularly in cell types that are very abundant (fibroblasts). Perhaps a flow cytometric study of acute wound fluid would have been a better method to assess fibroblast numbers than the histomorphometric method that we used.

Given the promising nature of our findings so far, two obvious avenues (molecular studies and an acute wound study of postwounding days 1 - 5) should be undertaken without delay. In addition, some old ground should be re-traced; in particular, the findings regarding removal of the subcutaneous tissues from under sutured wounds were less than satisfactory and probably due to the design of the surgical wounds. Repeating this portion of the experiment is more than an academic exercise; on

the contrary, our clinical experience (RAH, SFS, MWB) with problematic wound healing in cats points to a deficiency of subcutis as a potentially leading cause of "pseudo-healing," and this point needs to be clearly proved or disproved due to its important implications for surgical practice in the feline, and perhaps other species as well.

This study is, to our knowledge, the first reported direct histologic comparison of cutaneous wound healing of the cat and dog to be conducted under controlled conditions. The authors believe that it provides us with one more piece of the puzzle to help explain why some species (and perhaps also why some individuals within a species) tend to have rapid uncomplicated wound healing, while others tend more toward a chronic, indolent course of healing. In summary, we feel that the primary significance and value of this study is that it reinforces the value of comparative wound healing studies, not only in the cat, but in general. Normal uncomplicated wound healing is a predictable, well ordered sequence of events that involves the same cellular and molecular elements, even across broad species lines. However, the way that these elements interact with each other and are influenced by factors intrinsic and extrinsic to the wound environment appears to have significant differences between species. These important qualitative and quantitative differences in wound healing should continue to be both acknowledged and investigated, for the benefits that this knowledge will bring to surgical practice and the management of both normal and problematic wounds in the cat.

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Table 4.1: Inflammatory cells, second-intention healing: between-day comparisons of cat and dog wounds, Days 7 – 21.

		Neutrophils		Eosinophils		Lymphocytes		Plasma cells		Macrophages		Mast cells	
		Count	Score	Count	Score	Count	Score	Count	Score	Count	Score	Count	Score
Day 7	Cats	52 ^{b}	3 ^{a}	7.5 ^{a}	1 ^{a}	22.1 ^{a}	2 ^{a}	4.3 ^{a}	0.5 ^{a}	58.6 ^{a}	3 ^{a}	3.3 ^{abc}	0 ^{ab}
	Dogs	14 ^{ab}	2 ^{a}	3 ^{a}	1 ^{a}	14.1 ^{a}	1 ^{a}	4.6 ^{a}	0.5 ^{a}	24.8 ^{abc}	2 ^{ab}	0.9 ^{c}	0 ^{b}
	All												
Day 14	Cats	71.5 ^{b}	3 ^{a}	9 ^{a}	1 ^{a}	17.8 ^{a}	2 ^{a}	0.8 ^{a}	1 ^{a}	49.2 ^{ab}	3 ^{ab}	3.7 ^{ab}	1 ^{a}
	Dogs	10 ^{ab}	1.5 ^{ab}	2.5 ^{a}	0.5 ^{a}	11.5 ^{a}	2 ^{a}	0.4 ^{a}	0 ^{a}	13.4 ^{c}	1.5 ^{b}	1.2 ^{bc}	0 ^{b}
	All												
Day 21	Cats	51 ^{b}	2.5 ^{a}	8 ^{a}	1 ^{a}	12.9 ^{a}	1.5 ^{a}	0.4 ^{a}	0 ^{a}	35.3 ^{abc}	2 ^{ab}	5.1 ^{a}	1 ^{a}
	Dogs	1.5 ^{a}	0 ^{b}	2 ^{a}	0 ^{a}	16.5 ^{a}	2 ^{a}	0.3 ^{a}	0 ^{a}	21.4 ^{bc}	2 ^{ab}	1.3 ^{bc}	0 ^{ab}
	All												
Overall (Days 7-21)	Cats	57.5 *	3 *	8 *	1	17.6	2	3.8	0.5	47.7 *	3 *	4.0 *	1 *
	Dogs	4.5	1	3	1	14.0	2	3.2	0	19.9	2	1.1	0

Notes: 1) For comparisons within a column, a different superscript letter indicates a significant difference at $p \leq 0.05$.
2) For overall comparisons (Days 7-21) between cats and dogs, * signifies a significant difference at $p \leq 0.05$.
3) Counts for neutrophils and eosinophils were not normally distributed; therefore measures of central tendency for these and all other ordinal data (scores) are reported as median values. Counts for lymphocytes, plasma cells, macrophages, and mast cells are reported as means.

Table 4.2: Inflammatory cells, Day 7: three-way comparisons (dog vs cat, open vs closed, subcutis intact vs removed)

			Neutrophils		Eosinophils		Lymphocytes		Plasma cells		Macrophages		Mast cells	
			Count	Score	Count	Score	Count	Score	Count	Score	Count	Score	Count	Score
Cats	Open wounds	Subcutis intact	50 [a]	3 [a]	7.5 [a]	1 [a]	19.5 [a]	2 [a]	5.2 [a]	1 [a]	54.3 [a]	2.5 [ab]	2.7 [b]	0 [a]
		Subcutis removed	63 [a]	3 [a]	6.5 [a]	1 [a]	24.7 [a]	2 [a]	3.3 [a]	0 [a]	62.8 [a]	3 [a]	3.8 [ab]	0.5 [a]
	Closed wounds	Subcutis intact	36 [a]	3 [ab]	12 [a]	2 [a]	16.8 [a]	2 [a]	4.2 [a]	0 [a]	48.2 [a]	3 [ab]	9.2 [a]	2 [a]
		Subcutis removed	11.5 [a]	1.5 [ab]	2.5 [a]	0.5 [a]	19.3 [a]	2 [a]	2.7 [a]	0.5 [a]	30.0 [a]	1.5 [b]	3.0 [b]	0.5 [a]
Dogs	Open wounds	Subcutis intact	12 [a]	1.5 [ab]	3 [a]	1 [a]	13.7 [a]	1 [a]	5.8 [a]	1 [a]	27.7 [a]	2.5 [ab]	0.5 [b]	0 [a]
		Subcutis removed	14 [a]	2 [ab]	3 [a]	1 [a]	14.5 [a]	1 [a]	3.3 [a]	0 [a]	21.8 [a]	2 [ab]	1.3 [b]	0 [a]
	Closed wounds	Subcutis intact	4.5 [a]	1 [b]	3.5 [a]	0.5 [a]	19.5 [a]	2 [a]	5.0 [a]	1 [a]	31.0 [a]	1.5 [ab]	1.8 [b]	0 [a]
		Subcutis removed	10 [a]	1.5 [ab]	2 [a]	0.5 [a]	16.0 [a]	2 [a]	2.3 [a]	0 [a]	48.3 [a]	3 [ab]	1.3 [b]	0 [a]

Notes: 1) For comparisons within a column, a different superscript letter indicates a significant difference at $p \leq 0.05$.
2) Counts for neutrophils and eosinophils were not normally distributed; therefore measures of central tendency for these and all other ordinal data (scores) are reported as median values. Counts for lymphocytes, plasma cells, macrophages, and mast cells are reported as means.

Table 4.3: Second-intention healing: Edema, hemorrhage, necrosis, fibroblasts, capillaries, and collagen. Between-day comparisons of dog and cat wound, Days 7-21.

		Edema	Acute hemorrhage	Chronic hemorrhage	Necrosis	Fibroblasts		Capillaries		Collagen 3	Collagen 4
						Count	Score	Count	Score		
Day 7	Cats	1 [a]	2 [a]	0 [ab]	1 [ab]	100 [b]	3 [a]	19.3 [a]	2 [a]	2 [b]	2 [c]
	Dogs	1 [ab]	2 [a]	0 [a]	1 [a]	100 [b]	3 [b]	14.4 [a]	2 [a]	2 [b]	2 [c]
	All	1 [a]	2 [a]	0 [a]	1 [ab]	70 [a]	3 [b]	16.9 [a]	2 [a]	2 [b]	2 [c]
Day 14	Cats	1 [ab]	1 [a]	0 [ab]	1 [ab]	100 [b]	3 [b]	20.8 [a]	2 [a]	3 [a]	3 [b]
	Dogs	0.5 [b]	1.5 [a]	0 [ab]	1 [ab]	100 [b]	3 [b]	15.2 [a]	2 [a]	3 [a]	3 [b]
	All	1 [ab]	1 [ab]	0 [a]	1 [ab]	100 [b]	3 [b]	18.0 [a]	2 [a]	3 [a]	3 [b]
Day 21	Cats	1 [ab]	1 [a]	0 [ab]	1 [ab]	100 [b]	3 [b]	21.8 [a]	2 [a]	3 [a]	3 [b]
	Dogs	0 [b]	1 [a]	0 [b]	1 [b]	100 [b]	3 [b]	15.2 [a]	2 [a]	3 [a]	4 [a]
	All	1 [b]	1 [b]	0 [a]	1 [ab]	100 [b]	3 [b]	18.5 [a]	2 [a]	3 [a]	3.5 [a]
Overall (Days 7-21)	Cats	1 *	1	0	1	100	3	20.6 *	2	3	3 *
	Dogs	0.5	1.5	0	1	100	3	14.9	2	3	3

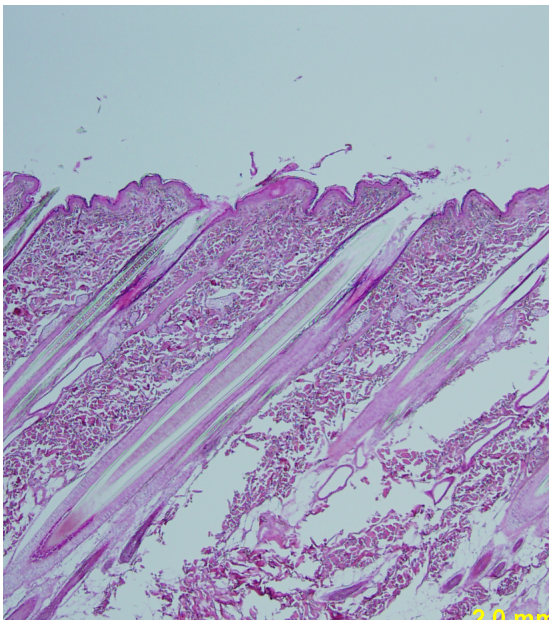
Notes: 1) For between-day comparisons within a column, a different superscript letter indicates a significant difference at $p \leq 0.05$.
2) For overall comparisons (Days 7-21) between cats and dogs, * signifies a significant difference at $p \leq 0.05$.
3) Counts for fibroblasts were not normally distributed; therefore values for these and all other ordinal data (scores) are reported as median values. Counts for capillaries are reported as means.

Table 4.4: Edema, hemorrhage, necrosis, fibroblasts, capillaries, and collagen Day 7: three-way comparisons (dog vs cat, open vs closed, subcutis intact vs removed)

			Edema	Acute hemorr-hage	Chronic hemorr-hage	Necrosis	Fibroblasts		Capillaries		Collagen 3	Collagen 4
							Count	Score	Count	Score		
Cats	Open wounds	Subcutis intact	1 ^{b}	2 ^{a}	0 ^{a}	1 ^{a}	100 ^{b}	3 ^{a}	23.7 ^{a}	2 ^{a}	2 ^{a}	2 ^{a}
		Subcutis removed	1 ^{b}	1.5 ^{a}	0 ^{a}	1 ^{a}	92.5 ^{ab}	3 ^{a}	15.0 ^{ab}	2 ^{ab}	1.5 ^{a}	1.5 ^{abc}
	Closed wounds	Subcutis intact	0 ^{a}	1 ^{a}	0 ^{a}	0 ^{a}	51 ^{ab}	3 ^{a}	6.8 ^{b}	1 ^{b}	1 ^{a}	0 ^{c}
		Subcutis removed	0 ^{a}	1.5 ^{a}	0 ^{a}	0 ^{a}	37 ^{a}	3 ^{a}	3.2 ^{b}	1 ^{b}	1 ^{a}	1 ^{c}
Dogs	Open wounds	Subcutis intact	1 ^{ab}	2 ^{a}	0 ^{a}	1 ^{a}	100 ^{ab}	3 ^{a}	14 ^{ab}	2 ^{ab}	2 ^{a}	2 ^{ab}
		Subcutis removed	0 ^{ab}	1.5 ^{a}	0 ^{a}	1 ^{a}	100 ^{ab}	3 ^{a}	14.8 ^{ab}	2 ^{ab}	2 ^{a}	1.5 ^{abc}
	Closed wounds	Subcutis intact	0 ^{ab}	1.5 ^{a}	0 ^{a}	1 ^{a}	78 ^{ab}	3 ^{a}	9.8 ^{b}	1.5 ^{ab}	2 ^{a}	1 ^{bc}
		Subcutis removed	0 ^{ab}	1.5 ^{a}	0 ^{a}	1 ^{a}	50 ^{ab}	3 ^{a}	7.8 ^{b}	1.5 ^{ab}	1.5 ^{a}	1 ^{bc}

Notes: 1) For between-day comparisons within a column, a different superscript letter indicates a significant difference at $p \leq 0.05$.

2) Counts for fibroblasts were not normally distributed; therefore median values for these and all other ordinal data (scores) is reported as median values. Counts for capillaries are reported as means.



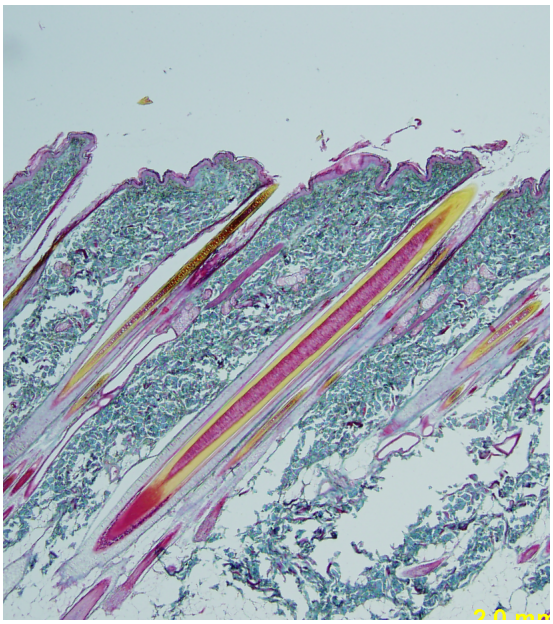
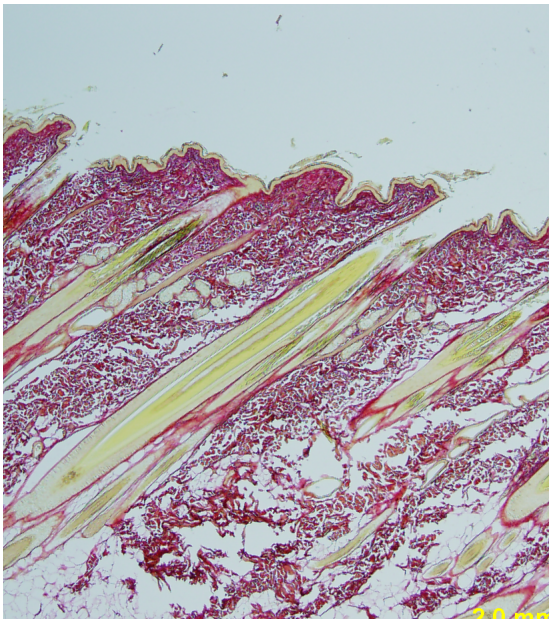


Figure 4.1a Normal canine skin. Hematoxylin and eosin stain, 40x.

Figure 4.1b Normal canine skin. Masson's trichrome stain, 40x.



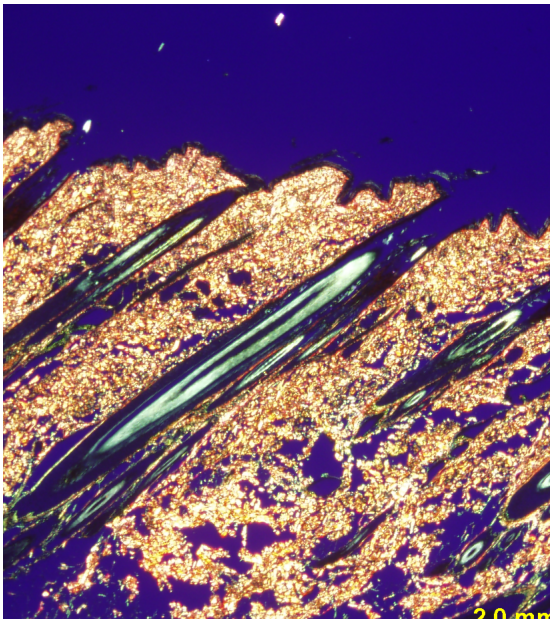
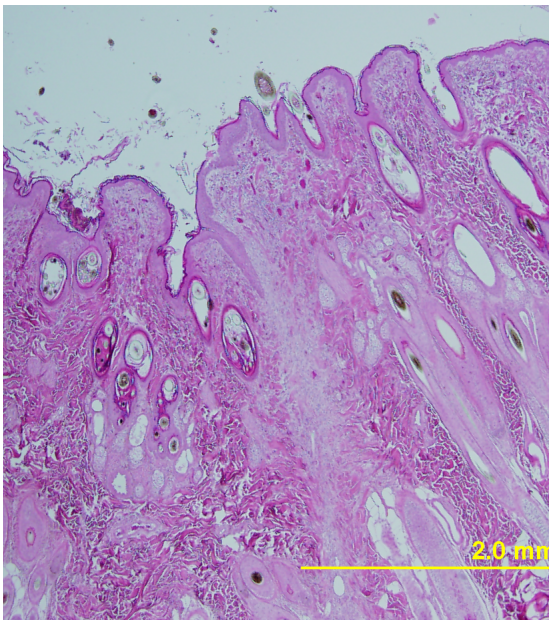


Figure 4.1c Normal canine skin. Picrosirius red stain, 40x.

Figure 4.1d Normal canine skin. Picrosirius red stain, polarized light, 40x.



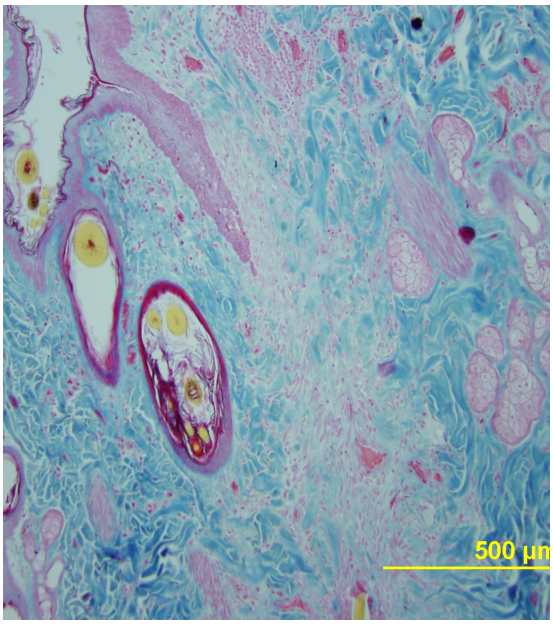
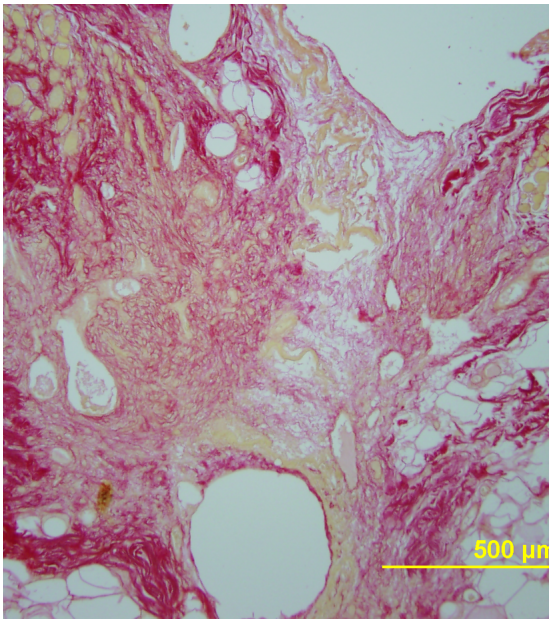


Figure 4.2a Healing wound, collagen score 1. Hematoxylin and eosin stain, 40x.

Figure 4.2b Healing wound, collagen score 1. Masson's trichrome stain, 100x.



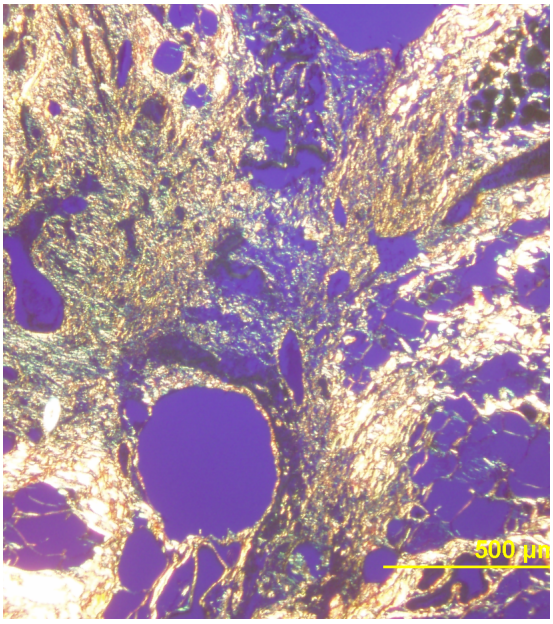
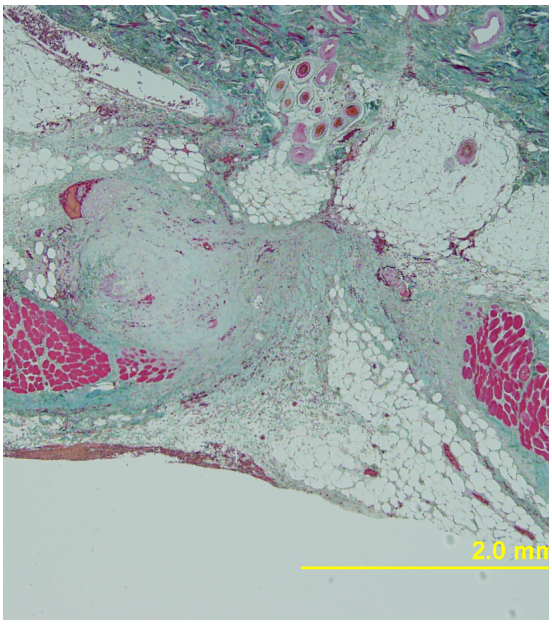


Figure 4.2d Healing wound, collagen score 1. Picrosirius red, polarized light, 100x.

Figure 4.2c Healing wound, collagen score 1. Picrosirius red, 100x.



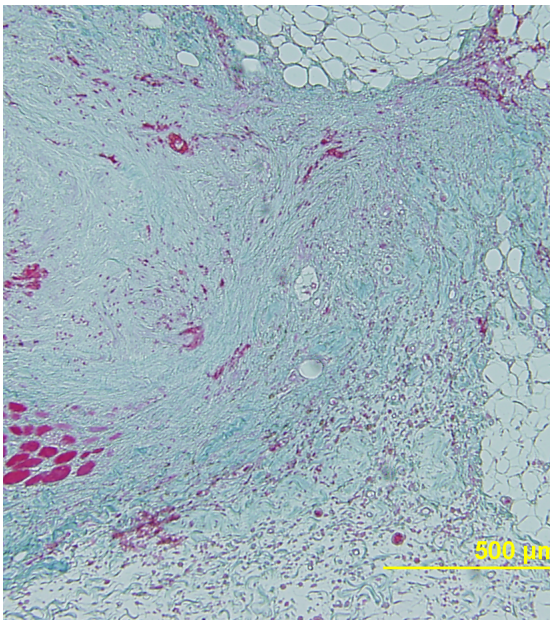
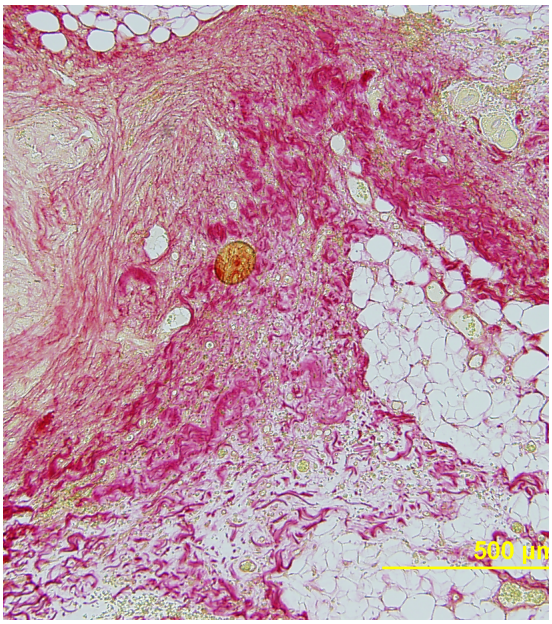


Figure 4.3a Healing skin, collagen score 2. Masson's trichrome stain, 40x.

Figure 4.3b Healing skin, collagen score 2. Masson's trichrome stain, 100x.



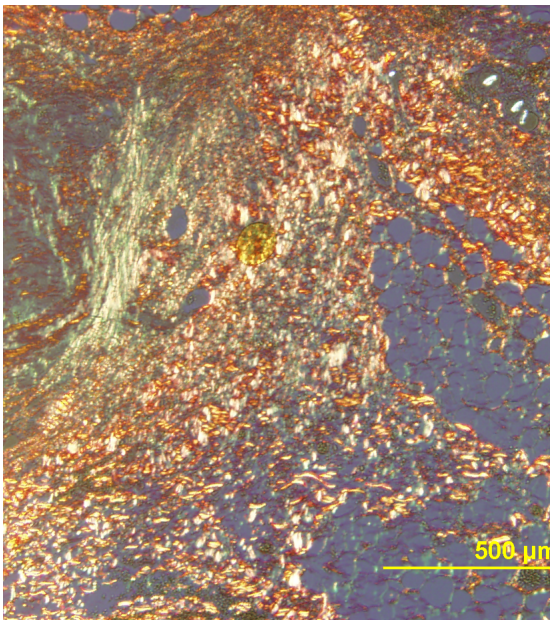
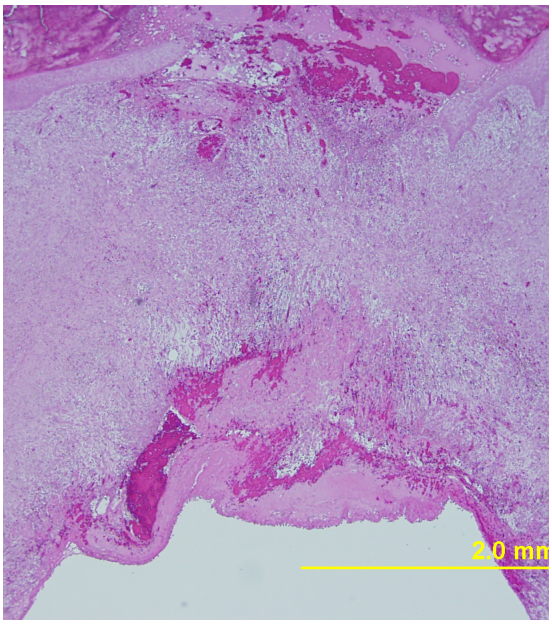


Figure 4.3c Healing skin, collagen score 2. Picrosirius red, 100x.

Figure 4.3d Healing skin, collagen score 2. Picrosirius red, polarized light, 100x.



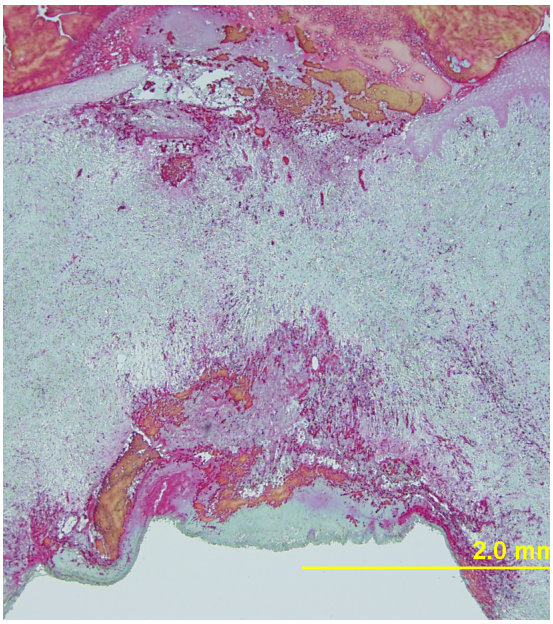
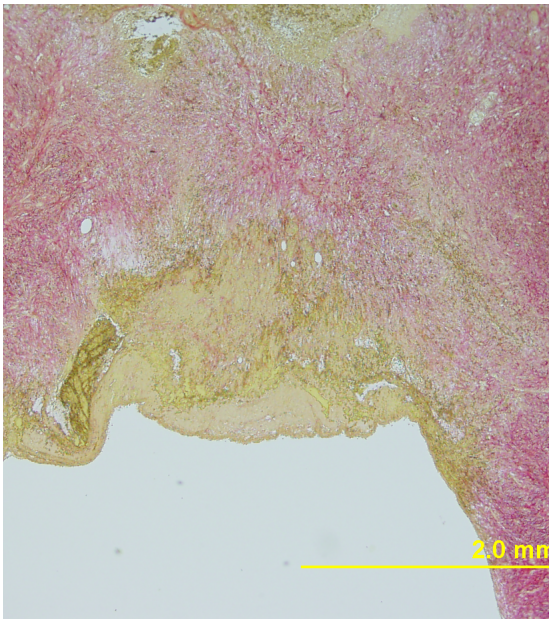


Figure 4.4a Healing skin, collagen score 3. Hematoxylin and eosin stain, 40x.

Figure 4.4b Healing skin, collagen score 3. Masson's trichrome stain, 40x.



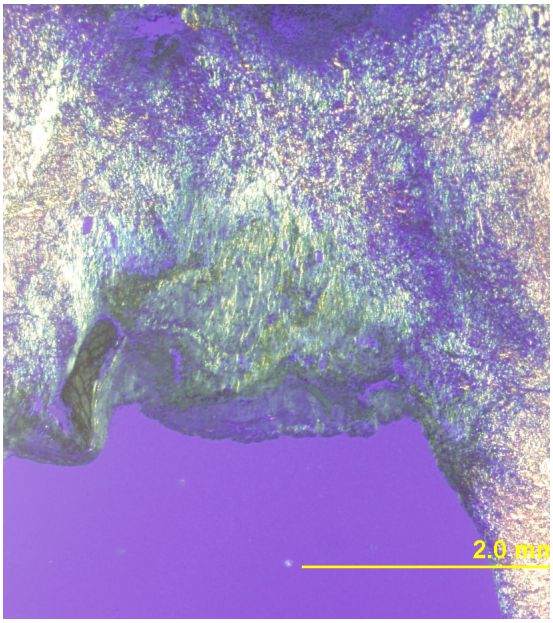
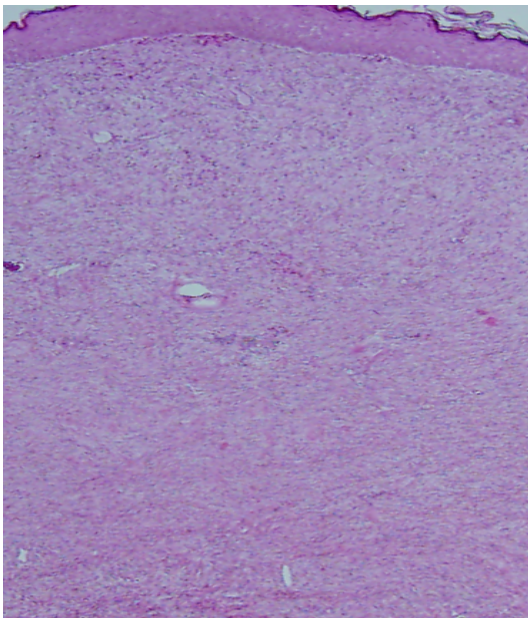


Figure 4.4c Healing skin, collagen score 3. Picrosirius red, 40x.

Figure 4.4d Healing skin, collagen score 3. Picrosiuius red, polarized light, 40x.



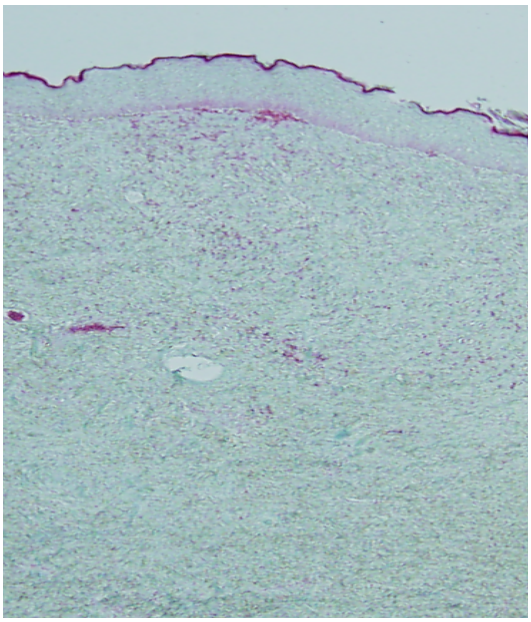
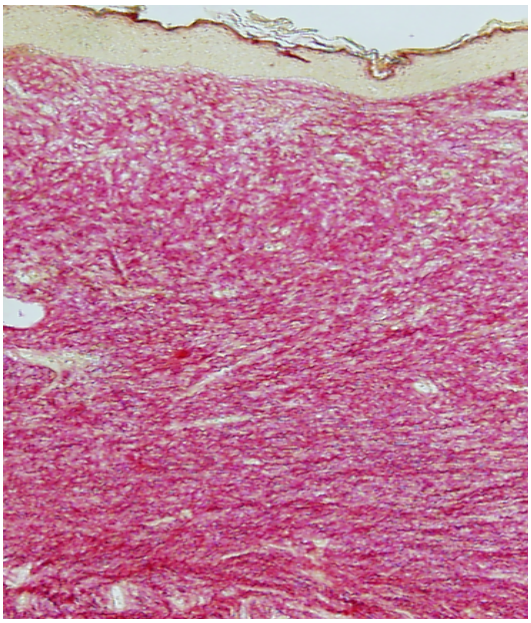


Figure 4.5a Healing skin, collagen score 4. Hematoxylin and eosin stain, 40x.

Figure 4.5b Healing skin, collagen score 4. Masson's trichrome stain, 40x.



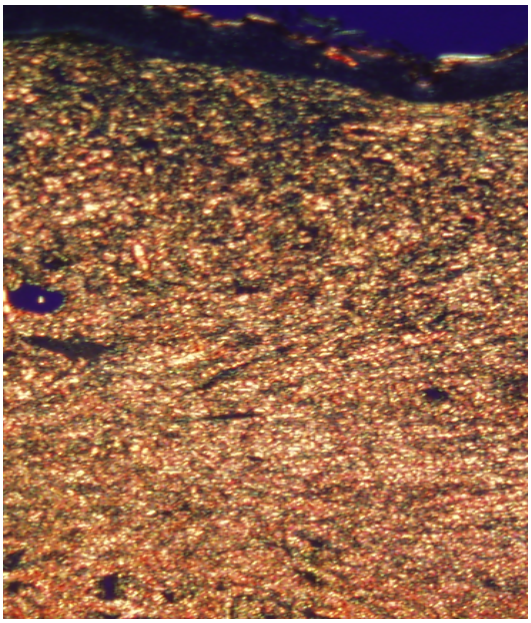


Figure 4.5c Healing skin, collagen score 4. Picrosirius red, 40x.

Figure 4.5d Healing skin, collagen score 4. Picrosirius red, polarized light, 40x.

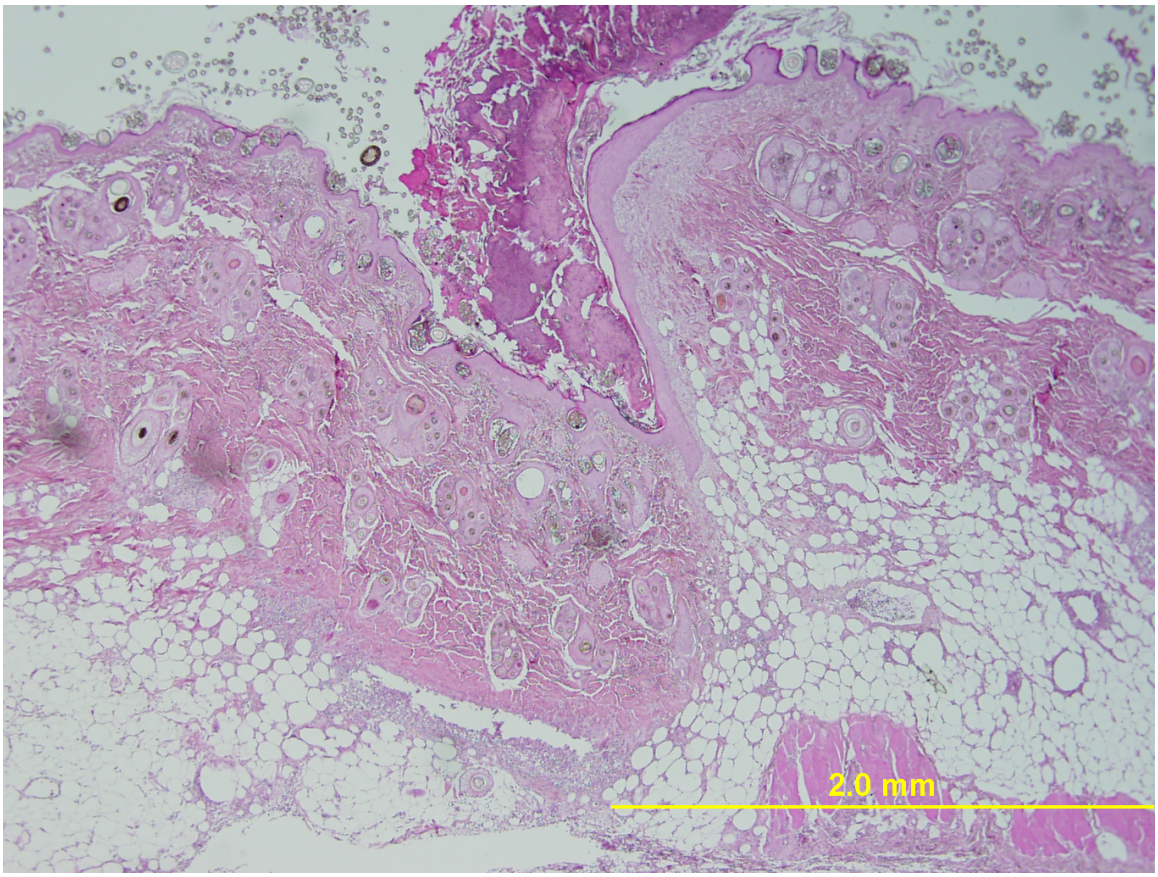


Figure 4.6 Feline first intention healing, 7 days postwounding

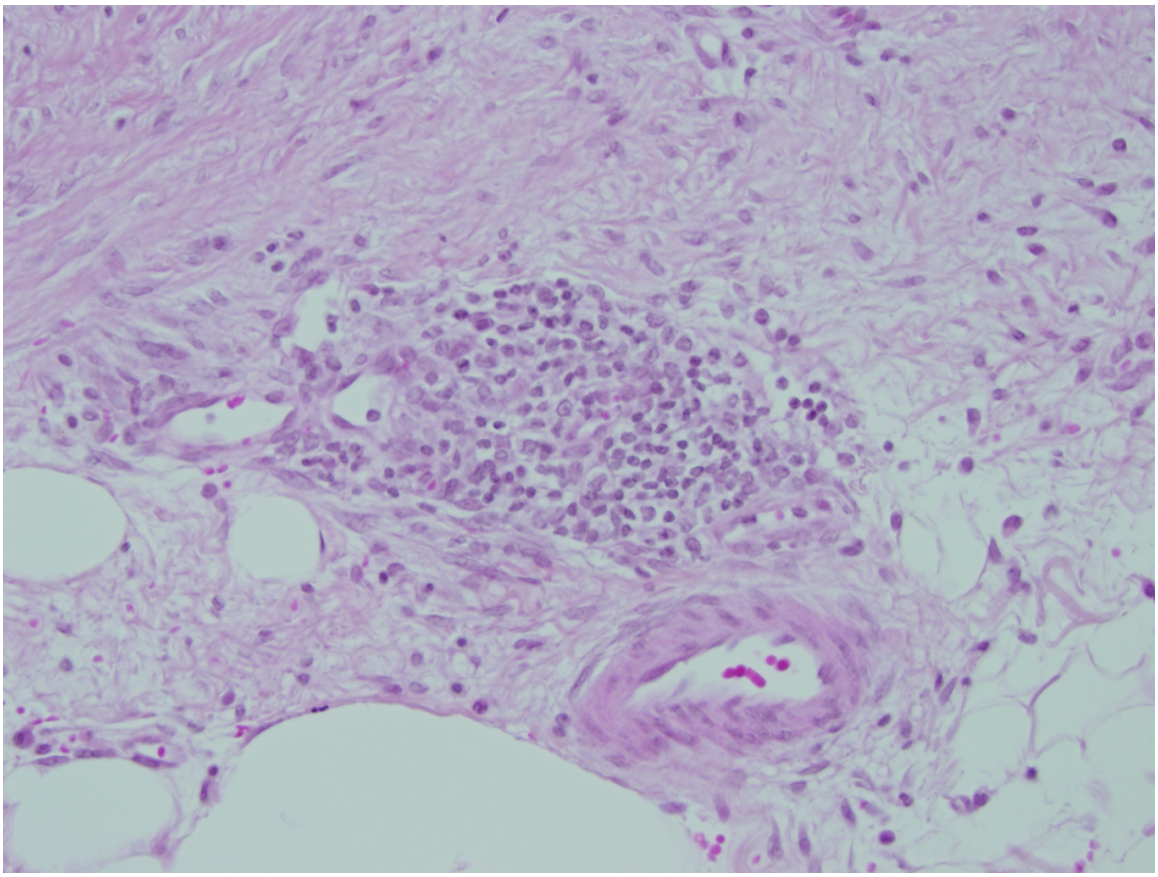


Figure 4.7 Dense macrophage accumulation; feline second intention healing, 21 days postwounding.

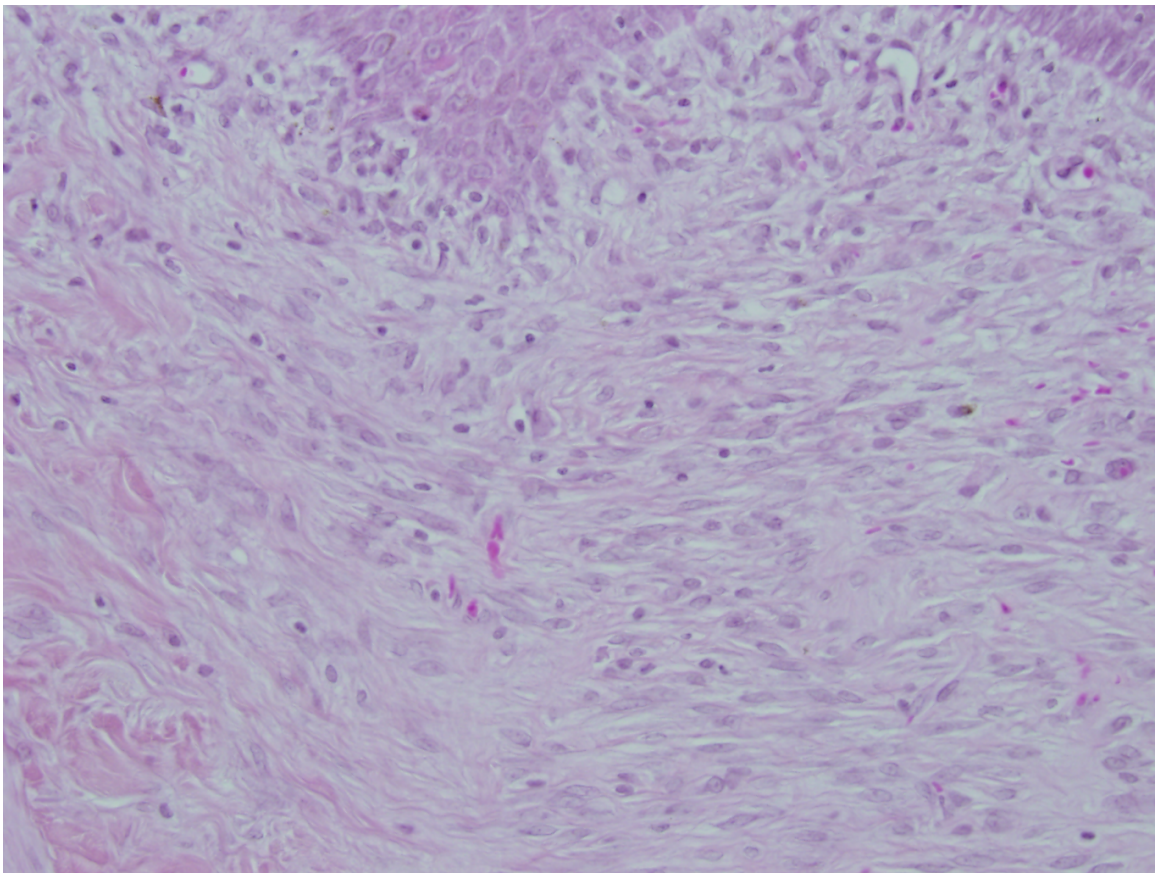


Figure 4.8 Abundant mast cells; feline second intention healing, 21 days postwounding. 400x

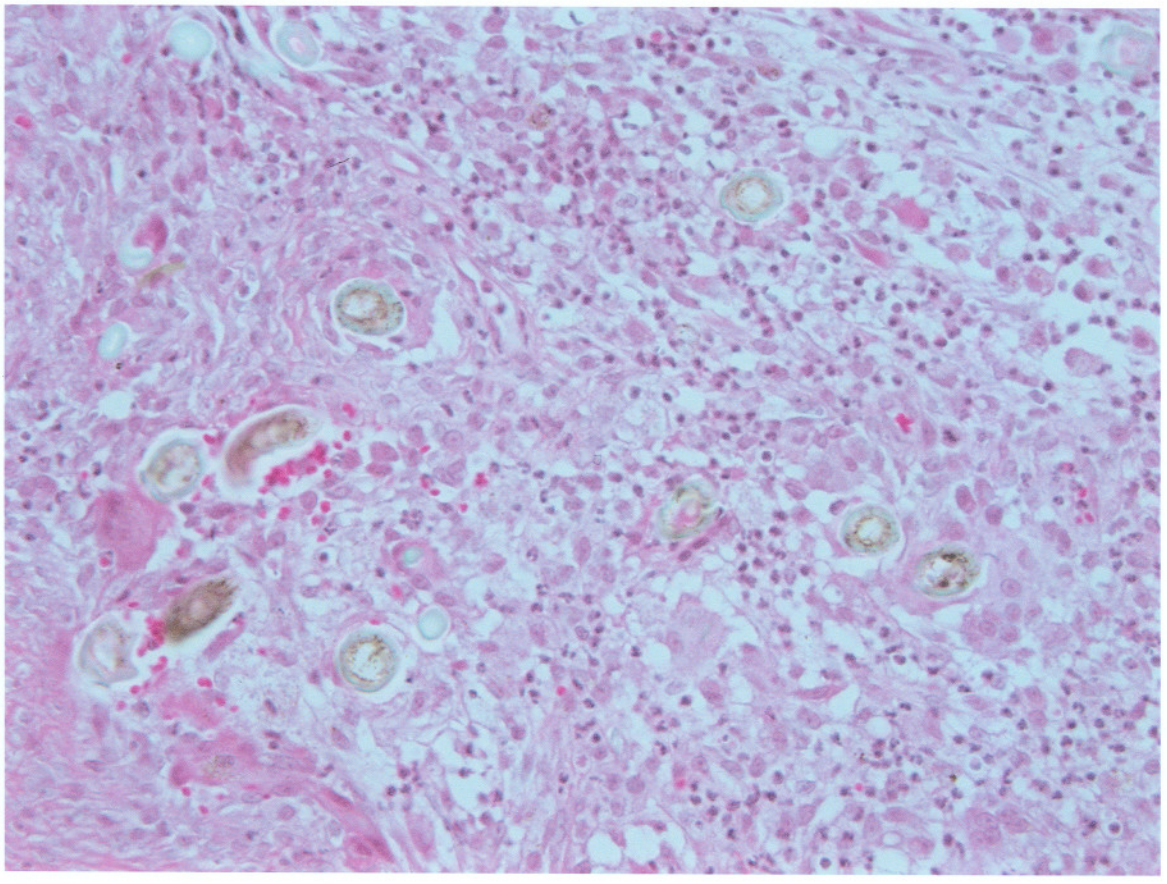


Figure 4.9 Feline second intention healing; inflammatory reaction to hair, 14 days postwounding. 400x

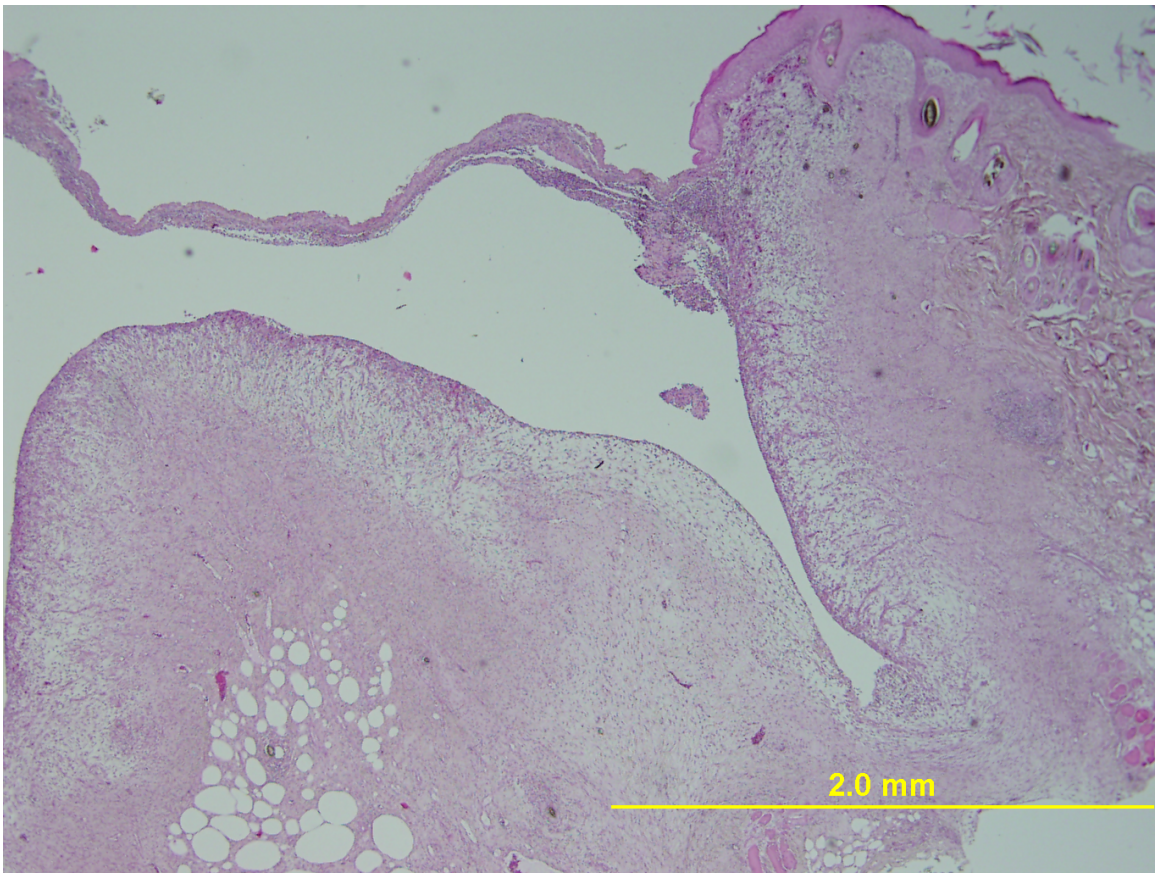


Figure 4.10 Feline second intention healing, 21 days postwounding, granulation tissue not attatched. 40x

V. CONCLUSIONS

In the first portion of our investigation, our primary goal was to give a macroscopic description of feline wound healing, and to compare wound healing in the cat with that of a better-known model, the dog. Based on our clinical impressions, we believed that there would be significant differences between the two species and we did observe significant differences between the cat and the dog in both first and second intention healing. These specific differences and our discussion of them appear at length in the first paper; however, three statements will serve to summarize our findings: 1) Wound strength for first intention healing in cats at 7 days postwounding is significantly less than for dogs. 2) Compared to dogs, second intention healing in cats is associated with less gross evidence of inflammation (less redness, swelling, and exudate) during the first 3 – 5 days postwounding, slower production of granulation tissue, and slower contraction and epithelialization. 3) Cutaneous perfusion was not identified as a causal factor to explain differential rates of healing.

The second part of our study investigated the contribution of the subcutaneous tissues to cutaneous wound healing. We compared the healing of cutaneous defects with

that of combined cutaneous-subcutaneous defects. We observed that the removal of the subcutaneous tissues caused a marked reduction in the rate of production of granulation tissue, and in the rate of wound contraction and epithelialization, and further noted that these consequences were more striking in wounds in cats than in dogs. We also found a significant effect on wound perfusion from removal of the subcutaneous tissue. Based on these observations we concluded that the subcutaneous tissue plays a highly significant role in cutaneous wound healing, and that this is probably due to its contribution to cutaneous perfusion and as a supply of cellular precursors for granulation tissue.

The purpose of the third part of our study of cutaneous wound healing in the cat was to provide a histologic description and comparison with the dog. The primary histologic findings were that wounds in cats are associated with a more persistent inflammatory response than seen in dogs. We also observed that granulation tissue in the wounds of cats was characterized by lesser amount and density of collagen deposition. The overall histologic impression of feline wound healing in comparison to the canine is that in cats, a persistent inflammatory phase leads to a relative delay in the onset and progression of the proliferative phase.

We believe that the principal significance of our findings is that for the first time: 1) A macroscopic and histologic description of first and second intention cutaneous wound healing in the cat has been reported. 2) The contribution of the subcutaneous tissues to cutaneous wound healing has been described. This observation may lead to a greater focus in research and therapeutics on the subcutis as it relates to delayed healing and other chronic wound conditions. 3) The cat has been identified as a unique species with regard to wound healing, having significant differences in rate and strength of

healing compared to the dog. Prior to these results, it has been generally assumed that no significant differences in wound healing were present between the two species. This opens up two important issues, one for veterinary medicine, and one for the field of comparative medicine and physiology. In veterinary medicine, the significance of our findings is that we may no longer assume that all of the treatment products and protocols that have been applied to dogs' wounds as the standard of care are also optimal for cats' wounds. Regarding comparative medicine, as can be noted in the review of the literature, there are many animal models that have been used and are being used to help solve problems in human wound healing. At least some of the difficulty in finding answers to problems in human wound healing relates to the limitations of these animal models, in particular, that in most of the animal models, wound healing is much more rapid than in the human systems that the model is supposed to reflect. There is certainly a perception among human wound researchers that a better model of chronic wound healing is needed. Perhaps the cat will find some role in this area of comparative medical research.