Title
Controlled scar formation and animal husbandry method

International Patent Classification(s)
A01K 14/00 (2006.01) A61K 33/30 (2006.01)
A61D 7/00 (2006.01) A61K 38/43 (2006.01)
A61K 31/135 (2006.01) A61K 45/06 (2006.01)
A61K 33/00 (2006.01) A61P 43/00 (2006.01)
A61K 33/06 (2006.01)

Application No: 2006220394 Date of Filing: 2006.09.20

Priority Data

Number Date Country
2005905250 2005.09.20 AU

Publication Date: 2007.04.05
Publication Journal Date: 2007.04.05

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ABSTRACT

A method of inducing injury in an animal to a defined area beneath the surface of the skin. The method include the step of delivering an effective amount of a vasoconstrictor and a collagen cleaving agent to the defined area in sufficient amounts to cause an injury leading to scar formation. In particular this relates to the delivery of a collagenase and the vasoconstrictor adrenalin. This has a number of practical applications but in particular provides for a suitable alternative to mulesing of lambs.
COMPLETE SPECIFICATION
FOR A STANDARD PATENT
ORIGINAL

TO BE COMPLETED BY APPLICANT

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Invention Title: Controlled Scar Formation and Animal Husbandry Method

Details of Associated Provisional Application No. 2005905250 dated 20 September 2005
This invention relates to a method for the removal of hair from a mammal, and in one specific aspect the invention relates to removal of hair for animal husbandry purposes.

BACKGROUND OF THE INVENTION

Sheep raised for wool, such as merinos, have been bred to enhance wool production, and thus the more wool borne by the sheep the greater the economic return. Part of the breeding process has involved enhancing loose skin characteristics to increase the number of hair follicles and thus yield of wool. A side effect of not only the loose skin but also the greater production of wool is that the breech of a sheep where it is not appropriately maintained is readily subjected to urine staining, faecal soiling or dags. Excessive moisture in the skin folds also results in bacterial growth and an odour that is an attractant for the gravid blowfly female to lay eggs, resulting in an enhanced fly strike rate. Breech strike, as it is known is the primary form of blowfly strike accounting for more than 80% of all blowfly strikes.

While crutching of sheep at appropriate times of the year reduces the incidence of breech strike, a significant number of sheep still become struck in this region. An operation pioneered by J H W Mules was introduced in Australia in the 1930's to remove folds of skin in the breech and to reduce the amount of wool on the breech, hence the amount of faecal and urinary soiling of the region. The Mules operation has been widely adopted by Merino sheep producers. Approximately 20 million lambs are mulesed in Australia each year.

The original operation involved pinching a fold of skin on either side of the perineal area with Burdizzo pincers and cutting the fold off with a knife. This operation was considered, at the time, not to be painful because of the pressure of the pincers. Later the Mules operation was extended to remove skin from the tail, this was referred to as the 'Modified Mules operation.' The pincers and knife were replaced with blade shears to perform the operation. In Western Australia the mulesing contracting industry extended the area of skin removal in the crutch area as well as performing a total strip of the tail skin - the so called "Radical Mules Operation."
Apart from animal welfare concerns the Radical Mules Operation results in secondary problems such as a large wound area, increasing the chances of infection, secondary joint infections, wound contraction and distortion of tail and vulva. The longer-term problem of an increase in UV light induced skin cancer of the perineal region also became evident.

An alternative to the Mules operation is considered a high priority by the Merino sheep industry due to mounting consumer and animal activist pressure to improve animal welfare, but at present no such alternative exists.

Our earlier patent application published as WO03/056909 refers to the use of a collagen cleaving agent beneath the surface of the skin for removing hair from the breech of lambs, as an alternative to mulesing involving no open wound. WO03/056909 also refers to scar formation by the use of that procedure. It has since been found that the depilatory effect, permanent or otherwise whilst useful is perhaps not as important as the tightening effect of scar contraction. It is found that as the scar contracts the loose folds of the skin diminish thereby somewhat reducing the attractiveness of the breech to fly infestation.

It is desirable to control and reduce between sheep variability of the initial injury and scar formation.

For the purposes of this specification the word "comprising" means "including but not limited to", and the word "comprises" has a corresponding meaning. Also a reference within this specification to a document is not to be taken as an admission that the disclosure therein constitutes common general knowledge in Australia.

SUMMARY OF THE INVENTION

In a first aspect the invention might be said to reside in a method of inducing injury in an animal to a defined area beneath the surface of the skin by delivering an effective amount of a vasoconstrictor and a collagen cleaving agent to the defined area in sufficient amounts to cause an injury leading to scar formation.
In a second form of the first aspect the invention could be said to reside in a method of inducing injury in an animal to a defined area beneath the surface of the skin by delivering an effective amount of a vasoconstrictor and a tissue damage agent to the defined area in sufficient amounts to cause an injury leading to scar formation. The tissue damage agent preferably interferes with collagen structure subcutaneously, dermally or subdermally.

A specific form of the invention relates to an alternative to mulesing, wherein one or more injuries are induced in defined areas around the breech of a lamb so that on contraction of the scars there is a reduction in the degree to which skin is folded in the breech, and more preferably a tightening of the skin occurs.

The invention may also be said to relate to a preparation for scar formation comprising a collagen cleaving agent and a vasodilator.

The method may additionally lead to hair loss in the defined area, which hair loss may be more or less permanent, or at least result in reduced hair formation.

For a better understanding the invention will now be described by reference to examples and the drawings wherein:

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1  A midside model, 1 minute after treatment with adrenaline 0.1mg/ml.

Figure 2  Responses to collagenase treatment without adrenaline and with two concentrations of adrenaline, adrenaline pre-treatment at 0.1 mg/ml followed by collagenase treatment gave the most consistent results. Response scoring system. 1 = no response, 2 = minimal response (small areas of light bruising); 3 = reasonable response (reasonably dark bruising affecting the entire area treated evenly); 4 = good (dark even bruising evenly affecting the entire area treated); 5 = severe (oedema, hematoma or broken skin with associated dark bruising).
Figure 3  Three midside grids treated showing the enhancement of bruising caused by the addition of adrenaline to the working formulation.

5  Figure 4  Almost fully healed line scar several months after initial collagenase treatment of a 3x3 grid. Note the striations at the top and the effect of the collagenase running after treatment at the bottom causing extension of the line scar.

10  Figure 5  Shows three photographs of the same grid of one sheep injected with 0.1mg/ml adrenalin and 0.3% collagenase at the same time substantially with the method performed in example 2. The photographs are taken on day 1 of injection, two weeks following injection and five weeks following injection.

15  DETAILED DESCRIPTION OF THE INVENTION.

An injection of about 0.1ml of an aqueous solution of collagenase at a concentration in the order of 0.01% (w/v) or greater when introduced under the skin in the breech of a sheep has the effect of causing injury leading to scar formation and also leading to depilation. The collagenase spreads through the dermis from the point of injection, and is believed to disrupt the collagen network which is thought to be essential for hair follicle attachment in the skin, and also for normal hair growth processes. A subsurface injury forms in the defined area leading to bruising and eventually scar formation. The use additionally of the vasoconstrictor adrenalin is found to enhance the action of collagenase providing a more defined scar area, intensifying the bruising capacity of the collagenase, effects that are through to arise by means of controlling the spread of collagenase away from the injection site. The effect of adrenalin is found to coincide with the collagenase activity so that co-administration gives the desired control. A preferred form of the invention provides for co-delivery of the collagen cleaving enzyme and the vasoconstrictor.
A number of the family of matrix metalloproteinases are capable of cleaving collagen and therefore having the desired effect. These include MMP-3 (also known as stromelysin), but the preferred collagen-cleaving agent is crude collagenase, comprising a mixture of collagenases because these are cost effective. It will be understood that there are a large number of collagenases and matrix metalloproteinases available and that the invention is not restricted to any one particular collagenase or matrix metalloproteinase and therefore it is contemplated that most if not all of the available collagenases and matrix metalloproteinases can be used in the practice of the invention. The primary limitation on the selection of collagen cleaving agent is that it is able to weaken the adhesion that the connective tissue provides between the follicle and the surrounding dermis within a time period in which damage to the surrounding dermis is minimised. In a particularly preferred form of the invention the collagen cleaving enzyme is a mixture of at least two collagenases selected from the list of bacterial collagenases including Type IV, Type II, Type XI, Type I, Type VIII and Type V. These enzymes may be from Clostridium histolyticum and may be available commercially. The mixture of collagenases may also contain other proteases. It will be understood that the collagenases or matrix metalloproteinases may be altered proteins such as truncation, mutant or deletions. Additionally it will be understood that other tissue damage agent may also be effective at causing sufficient damage to give rise to scarring, the effects of these tissue damage agents can also be localised by the vasoconstrictor to give good control over the damage effected and thus provide an effective means of controlling the shape of the resulting scar. In a preferably form the tissue damage agent interferes with collagen structure subcutaneously, dermally or subdermally, and this may be achieved by cleavage of the collagen itself, but alternatively may otherwise interfere with the structure of tissue subcutaneously, dermally or subdermally which thereby indirectly interferes with the collagen structure. As a further alternative however the collagen structure may be maintained relatively intact and damage may be effected to other components of the tissue that results in the scarring.

In the case of collagenases or other matrix metalloproteinases, the enzyme(s) may also be used in conjunction with a source of divalent cations such as Zn\textsuperscript{2+} or Ca\textsuperscript{2+}. 
The collagen cleaving enzyme may be a protease, or a truncation, mutant or deletion thereof. However, there are a large number of available proteases and it is possible that there may be some proteases that are able to weaken the adhesion that the connective tissue provides between the follicle and the surrounding dermis within a time period in which damage to the surrounding dermis and to the hair fibre or follicle is minimised.

Experiments to date suggest that levels of enzyme(s) in the composition, at least for the crude collagenase preparation, may need to be greater than about 0.01% w/w. It is known that 0.001% appears not to be effective however with suitable delivery methods or using enzymes with higher specific activities such levels may still be effective. Empirical trials will readily determine an appropriate level of enzyme to be added for effective bruising and subsequent scar formation. The outcome of treatment to date has been subsurface bruising followed by scar formation over the defined area. The scar has subsequently contracted to form a line scar, with tightening of the skin. More importantly the skin tightening is effected without an open wound or unnecessary pain to the lamb.

Suitable vasoconstrictors may include but are not limited to vasoconstrictors referred to as α-adrenergic agonist. Such vasoconstrictors include adrafinil, adrenolone, amidephrine, apraclonidine, budralazine, clonidine, cyclopentamine, detomidine, dimetofrine, dipivefrin, ephe drine, epinephrine, fenoxazoline, guanabenz, guanfacine, hydroxyamphetamine, ibopamine, indanazoline, isometheptene, mephenetermine, metaraminol, methoxamine hydrochloride, methylhexaneamine, metizoline, midodrine, naphazoline, norepinephrine, norfenefrine, octodrine, octopamine, oxymetazoline, phenylephrine hydrochloride, phenylpropanolamine hydrochloride, phenylpropylmethylamine, pholedrine, propylhexedrine, pseudoephedrine, rilmenidine, synephrine, tetrhydrozoline, tiamenidine, tramazoline, tuaminoheptane, tymazoline, tyramine, and xylometazoline.

Another class of vasoconstrictors that may be used are imidazolines. Imidazolines include naphazoline (2-(1 naphthylmethyl) imidazoline), oxymetazoline (2-(4-tert-butyl-2, 6-dimethyl -3-hydroxbenzyl)-2-imidazoline), tetrhydrozalone (2-(1, 2, 3, 4-tetrahydro-1-naphthyl)-2-imidazoline)), fenoxazoline (2-[(o-cumenyloxy)methyl]-2-imidazoline),
indanazoline (N-(2-imidazolin-2-yl)-N-(4-indanyl)amine), tramazoline (2-{(5, 6, 7, 8
tetrahydro-1-naphthyl)amino}-2-imidazoline, tymazoline (2-[(thymyloxy)-methyl]-2-
imidazoline), and xylometazoline (2-(4-tert-butyl-2, 6-dimethylbenzyl)-2-imidazoline).
The imidazoline may perhaps be selected from the group consisting of naphazoline,
oxymetazoline, tetrahydrozalone, fenoxazoline, indanazoline, tramazoline, tymazoline,
and xylometazoline.

Preferred vasoconstrictors are adrenalin and analogs or derivatives thereof particularly
noradrenalin, and in a specific form the vasoconstrictor is adrenalin.

To date the means by which the collagen-cleaving enzyme and vasoconstrictor have
been introduced in sheep is via injection by syringe. The needle in the above case is
inserted into the dermis delivering a volume of about 0.1ml. It will be understood that
the volume delivered and the method of delivery can be varied. The introduction of
active as effected in present trials, forces the collagen cleavage enzyme to spread
throughout the dermis, and thus the pressure of the volume that is introduced should assist with spreading the enzyme. Where only a minimal volume of some microlitres is
delivered without the application of pressure the interstitial fluids within the dermis might be expected to carry the enzyme from the site of introduction but perhaps not
distributing the enzyme to the same degree.

The site of delivery is preferably the dermis however it is anticipated that a
subcutaneous delivery may well also be effective, providing enough contact with collagen network of the area desired to be treated. It will be understood that the
method of delivery may be by breaching the skin (for example, by injection) or high
pressure aerosol (so called needleless injection) or by application of a cream or other dermatological carrier with properties allowing delivery of the enzyme through the protective layers of the skin, or perhaps by electrical co-migration techniques such as iontophoresis.

Where a needle of other point delivery system is used an array of delivery points is provided for, the array providing for delivery over the entire defined area. It will be
understood that there is some diffusion of active from the precise deposit of active, this may be 1mm to 10mm but more preferably between 2mm and 8mm and most preferably about 5mm from the point of deposit. This will to some extent be dictated by the action of the vasoconstrictor. Alternatively with a non point delivery system the shape of the area to which the active is applied will by and large determine the defined area. It will be understood that there will be some diffusion also with non-puncturing delivery systems.

The define area may take on a number of shapes, the simple square or rectangle will draw together a generally even amount of skin, a less regular shape may also be used, thus for example a triangle may be used to draw together a fillet shape of skin, alternatively a diamond or expanded diamond shape may be used if it is desired that the central potion of the area tighten more skin that at the ends of the area.

It will be understood that this method is ideally suited in the breech of a sheep as a replacement of the present practice of mulesing sheep.

There may be a number of delivery methods, other than via injection, and it is possible that delivery of the enzymes to their site of action may be by diffusion and thus might be achieved by simply applying active to the surface of the skin. However, it is preferred that the method includes the use of penetration enhancing means for assisting delivery of the enzyme(s) to their site of action. A number of means for achieving penetration enhancement might be used and these, include the employment of ultrasound, heat, pressure waves, iontophoresis or surfactants. Chemical penetration enhancers are also known and these might be used to assist penetration of the enzymes into the skin. Thus the enzymes may be used in a carrier which provides a low surface tension between the carrier and the skin so as to promote diffusion into the skin. Enhanced enzyme penetration could also be achieved using a combination of heat and chemical means.

The enzyme(s) may be applied topically in a composition comprising the enzyme(s) and a suitable non-toxic dermatologically acceptable carrier. Suitable carriers include water, ethanol, water/ethanol mixtures, oils such as paraffin oil, petroleum oil, mineral oil, silicone oil, fatty alcohols, glycerin, and soft white paraffin. The composition may be in any suitable form including as a solution, gel, lotion, cream, aerosol, water in oil or oil in
water emulsion. In one preferred form the carrier is soft white paraffin. In another preferred form from the carrier is water based.

Levels of enzyme(s) in the composition for topical application may be between 0.05% and 30% w/w. In a preferred form using a crude collagenase preparation the levels of enzymes is between 0.1% and 10% w/w, and in one particularly preferred form the levels may be between 0.5% and 3%. The level of enzymes used may be determined in part by the type and the activity of the enzyme(s). Levels of enzyme(s) for subsurface delivery such as by injection may be considerably lower than that intended for topical application, and may be perhaps between 0.05% and 1%, preferably between 0.05 and 0.8% and more preferably between 0.1 and 0.5%. In particular the levels of activity of enzyme(s) may be between 500 and 4000 FALGPA hydrolysis units per milligram preferably between 1000 and 3000 and most preferably about 2000.

Levels of vasoconstrictor can be determined empirically but at least for adrenalin may range in from about 0.05 to 0.5 mg/ml.

Injection volumes may be between 0.02 and 0.2 ml per cm² of skin surface area, preferably between 0.05 and 0.15 ml and most preferably about 0.1ml.

It will be understood that this invention is applicable to a range of procedures especially in animal, and these may include tattooing of skin in dogs, horses, cats and other domestic animals. Scarring might be desired in for example branding of animals. There may also be human application for scar formation in cosmetic applications requiring skin tightening.

EXAMPLE 1
The Efficacy of Combining a Vasoconstriction Agent with Collagenase.

Previous experiments with collagenase resulted in considerable variation in scar formation in sheep. Doubling in concentration of collagenase from 0.5% to 1.0% did not achieve a reduction in between sheep variability in response to treatment.
Reduction in injection density and decreasing injection volume did reduce the amount of enzyme running away from the treated area.

This experiment was to see if a vasoconstriction agent used before collagenase treatment would reduce variation in response between sheep.

*Method*

The following enzyme solutions were prepared in saline:

10 Collagenase (C7926 Sigma Blend) 0.5% in saline, lot # 61K8620, Adrenaline Injection BP (AstraZeneca) 0.1 mg/ml.

Collagenase (C7926 Sigma Blend) 0.5% in saline, lot # 61K8620, Adrenaline Injection BP (AstraZeneca) 0.01 mg/ml.

20 Twenty mixed sex merino lambs (4-5 months of age) were treated. Each animal (n=20) had wool removed from the mid-side area on the left hand side only. This area was then clipped close to the skin with small animal clippers.

25 Each animal was secured on its side on a biopsy table with the left hand side facing upwards. Using a stencil and antiseptic marker spray, three grids of 3x3cm were marked on the midside of each animal, each grid consisted of 49 individual injection sites. Each grid covered an area of 9cm² with an injection point every 0.5cm².

Using a 1ml Ultra-Fine insulin syringe with a 29G needle, grid 1 was treated with collagenase 0.5% at 0.03mls per 0.5cm². After grid 1 was treated, grids 2 and 3 were treated with Adrenaline at 0.1ml per cm² (16 injections of 0.1mls over each grid). Again the time of the first and last injection for each grid was recorded as well as the time taken for the skin to 'blanch'. After grids 2 and 3 were treated with Adrenaline, grid 2 and then grid 3 were treated with collagenase 0.5% at 0.03mls per 0.5cm².
Treatment sites on each animal were followed and photographed throughout the healing process, regular measurements of grid size were taken to determine the rate and amount of shrinkage of the treated area over time.

5 Results

Almost immediately after the adrenaline solution was injected into the skin the skin colour noticeably turned white ('blanching'). This occurs because the adrenaline causes the small capillaries near the surface of the skin to constrict so less blood flows nearer to the surface of the skin. Bruising occurs due to collagenase action degrading capillaries in the dermis. Bruising was often noted to occur around the borders of the grid first and then begin to occur inside the grid after approximately 30 mins.

Adrenaline concentration was reflected in the degree of blanching seen in the skin. 0.1 mg/ml gave a good even white blanching of the grid area compared to 0.01 mg/ml adrenaline, which produced a slightly lighter shade of blanching. The effect of adrenaline 0.1mg/ml can be seen in Figure 1.

Adrenaline pre-treatment of the skin greatly reduced the variation in response to treatment that we had seen previously (see figure 2). Control grids, which received collagenase only treatment, showed typically variable response to treatment seen previously with only one animal showing a good response. The grids, that were pre-treated with adrenaline at 0.01mg/ml, showed less variation than the control grids. Grids pretreated with adrenaline 0.1mg/ml showed greatly reduced variation in response and in some sheep, that had a very poor response to the control, the higher adrenaline concentration, produced and adequate response, see Figure 3.

Conclusion

Results from this experiment have lead to a significant improvement in collagenase treatment of skin to achieve skin damage and associated healing and skin contraction. Treatment of skin with a vasoconstricting agent before collagenase treatment greatly enhances the effect of collagenase degradation in the dermis and significantly reduces variation seen in response to treatment between sheep.
A further trial also found that the same collagenase at 0.3% also gave satisfactory effect when used in conjunction with prior injection of 0.1mg/ml adrenaline.

EXAMPLE 2

5 Use of collagenase and adrenaline in single solution delivery

Methods

A single grid on the right hand midside of each of eight animal was treated, the grid was the standard midside model which consisted of a 3x3 grid with 0.5cm spacing of injections (49 injections), each injection side received 0.03mls solution, the whole grid received a total of 1.47 mls solutions.

The following enzyme solution was prepared:
Collagenase 0.5% and adrenaline 0.1 mg/ml in 0.9% saline.

The collagenase and adrenaline solution was prepared and stored on ice away from light until use. The procedure for treatment of each animal was the same as previously. Each lamb was secured to the biopsy table with the right hand side facing upwards; the midside area was prepared for treatment by close shaving of the wool with electric clippers. One grid was marked onto the midside using a pre prepared stencil and antiseptic marker spray. Using a 1ml Ultra-Fine insulin syringe the grid was treated with collagenase and adrenaline solution, 0.03mls per 0.5cm.

Results

Response 24hrs after treatment showed that most animals had responded with a dark, even and consistent bruise over the entire treated area. Running of the enzyme below the treated area was seen on most animals. Running of the enzyme below the treat area most often occured down wrinkles in the skin with bruising localised to the tops of the wrinkles. Some partially affected areas on several sheep were still noticed but this was only seen on the sheep with a high wrinkle score. Several sheep responded with very dark bruising over the treated area and in one sheep this resulted in disruption of the epidermis within 48 hrs after treatment. Of the eight grids treated, seven of the grids healed to form linear scars. These linear scars ranged in length but all were very light
colours scars that were not raised form the skin. All of the line scars healed with striations at the top however not all grids healed with striations at the bottom, due to the running of the enzyme below the treated area. (figure 4).

Further trials with a collagenase concentration of 0.3% when co-administered with adrenaline (0.1mg/ml) gave a less satisfactory result when compared to collagenase used at 0.5%.

Various features of the invention have been particularly shown and described in connection with the exemplified embodiment of the invention, however, it must be understood that these particular arrangements merely illustrate and that the invention is not limited thereto and can include various modifications falling within the spirit and scope of the invention.
CLAIMS DEFINING THE INVENTION ARE AS Follows:

1. A method of inducing injury in an animal to a defined area beneath the surface of the skin by delivering an effective amount of a vasoconstrictor and a collagen cleaving agent to the defined area in sufficient amounts to cause an injury leading to scar formation.

2. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in claim 1 wherein the vasoconstrictor is used in an amount sufficient to keep the collagen cleavage agent activity within the defined area.

3. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in either or claim 1 or claim 2 wherein administration is selected from the group consisting of i) breaching the skin with a sharp item such as a needle and injecting, ii) a high pressure aerosol technique, ii) application of a cream or other dermatological carrier and iv) an electrical co-migration technique.

4. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in any one of the preceding claims wherein the collagen cleavage agent is a matrix metalloproteinase.

5. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in claim 4 wherein the collagen cleavage agent is a collagenase.

6. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in either of claims 4 or 5 the method further including administration of a divalent cation.

7. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in either claim 5 or claim 6 when read through claim 5 wherein the collagenase is administered by breaching the skin and is present in an aqueous solution in at between 0.05 and 1% with enzyme activity levels of 200 and 4000 FALGPA hydrolysis units per milligram.
8. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in either claim 5 or claim 6 when read through claim 5 wherein the collagenase is administered by breaching the skin and is present in an aqueous solution in at between 0.05 and 0.8% with enzyme activity levels of 1000 and 3000 FALGPA hydrolysis units per milligram.

9. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in any one of the preceding claims wherein the vasoconstrictor is adrenaline or an analog or derivative thereof.

10. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in claim 9 wherein the vasoconstrictor is adrenalin.

11. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in claim 10 wherein adrenalin is administered by breaching the skin and is used in concentrations of between 0.05 and 0.5 mg/ml.

12. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in any one of claims 7, 8 and 11 wherein collagenase and adrenalin are co-administered in a volume of 0.02 and 0.2ml is delivered per cm² of skin surface.

13. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in any one of claims 1 to 6, 9, and 10 wherein the collagen cleaving agent and the vasoconstrictor are co-administered.

14. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in any one of the preceding claims wherein the collagen cleaving agent and the vasoconstrictor are administered as a plurality of sites within the defined area.
15. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in any one of the preceding claims wherein the animal is a non-human mammal.

16. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in any one of the preceding claims wherein the animal is ovine and the defined area is around the breach so that on contraction of the scar there is a reduction in the degree to which skin is folded in the breach.

17. A composition comprising a tissue damage agent and a vasoconstrictor at concentrations suitable for inducing scar formation on delivery beneath the surface of the skin of an animal.

18. The composition as in claim 17 wherein the tissue damage agent interferes with collagen structure subcutaneously, dermally or subdermally.

19. The composition of claim 18 comprising a collagen cleaving agent and a vasoconstrictor.

20. The composition of claim 19 comprising collagenase and adrenaline.

21. The composition of claim 20 additionally comprising divalent cations.

22. The composition of claim 21 wherein the divalent cations are either Zn^{2+} or Ca^{2+}.

23. The composition of any one of claims 20 to 22 wherein adrenalin is present in concentrations of between 0.05 and 0.5 mg/ml.
24. The composition of any one of claims 20 to 23 wherein collagenase is present at between 0.05 and 1% with enzyme activity levels of 200 and 4000 FALGPA hydrolysis units per milligram.

25. The composition of any one of claims 20 to 24 wherein collagenase is present at between 0.05 and 0.8% with enzyme activity levels of 1000 and 3000 FALGPA hydrolysis units per milligram.

26. A method of inducing injury in an animal to a defined area beneath the surface of the skin by delivering an effective amount of a vasoconstrictor and a tissue damage agent to the defined area in sufficient amounts to cause an injury leading to scar formation.

27. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in claim 26 wherein the vasoconstrictor is used in an amount sufficient to keep the collagen cleavage agent activity within the defined area.

28. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in either or claim 26 or claim 27 wherein administration is selected from the group consisting of i) breaching the skin with a sharp item such as a needle and injecting, ii) a high pressure aerosol technique, ii) application of a cream or other dermatological carrier and iv) an electrical co-migration technique.

29. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in any one of claims 26 to 28 wherein the vasoconstrictor is adrenaline or an analog or derivative thereof.

30. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in claim 29 wherein the vasoconstrictor is adrenalin.

31. The method of inducing injury in an animal to a defined area beneath the surface of the skin as in claim 30 wherein adrenalin is administered by breaching the skin and is used in concentrations of between 0.05 and 0.5 mg/ml.