



DELIVERING ULTRASOUND THROUGH A FINE SALINE MIST Effects on mice with diabetes mellitus

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- Nurse-led clinic for patients who self-harm ■ Acceptability of a honey dressing
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- Effectiveness of placental extract on chronic wounds
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- Educational intervention to manage treatment-related pain
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- Effects of ageing on healing ■ Raising the standard of leg ulcer care
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- Hyalofill-F plus compression for the treatment of chronic venous ulceration
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Effects of ultrasound delivered through a mist of saline to wounds in mice with diabetes mellitus

- **Objective:** To examine the effects of ultrasound administered through a fine mist of saline on surgically placed full-thickness excisional wounds in mice with experimental diabetes mellitus.
- **Method:** Fifty male CD-1 mice received alloxan monohydrate (100mg/kg), a drug known to induce diabetes mellitus. The animals received five ultrasound (n=27) or sham (n=23) treatments for 1.5 minutes, on alternate days, for 10 days, and were then sacrificed. Following sacrifice, each animal's wound was excised and the tissues prepared for qualitative and quantitative histological analysis.
- **Results:** No difference in wound-surface area was found between the groups after the treatment period. However, blinded assessment of tissue sections revealed significantly increased deposition of collagen and blood vessels in the granulation tissue of animals treated with ultrasound compared with those that received sham therapy.
- **Conclusion:** Ultrasound delivered through a fine mist of saline significantly altered the composition of newly formed granulation tissue in animals with experimental diabetes mellitus. Further research needs to be completed to determine other effects of this novel ultrasound therapy and to examine its clinical effectiveness.
- **Declaration of interest:** None.

ultrasound; wound healing; diabetes mellitus; granulation tissue

Therapeutic ultrasound, which delivers mechanical energy in the form of sound waves, is one of the most common modalities used by physiotherapists to treat musculoskeletal conditions, including chronic wounds.^{1,2} The ultrasound activates inflammatory cells, which leads to degranulation^{3,4} and the stimulation of phagocytosis,⁵ resulting respectively in the release of inflammatory mediators and the facilitation of wound debridement.^{6,7}

These chemical mediators include growth factors that have been shown to activate fibroblasts⁸ and lead to earlier accumulation of endothelial cells in treated tissues when compared with untreated ones.^{9,10} When administered to cultured fibroblasts, ultrasound can activate key intracellular processes such as collagen synthesis,¹¹⁻¹³ calcium influx,^{14,15} altered membrane permeability¹⁶ and stimulation of DNA synthesis.¹⁷ Animal studies have shown that ultrasonic energy can improve the organisation and strength of collagen deposited in the wound.¹⁸⁻²²

Therapeutic ultrasound accelerates closure of chronic wounds.²³⁻²⁷ Currently, it is administered to intact peri-ulcer skin using a conductive gel or via a water bath, in to which the wound is immersed.

In this study we used the prototype of a new product, the Mist Therapy Ultrasound (Celleration, Eden Prairie, Minnesota, USA), which delivers the mechanical energy of ultrasound through a fine mist spray (Fig 1), allowing the ultrasound to be

administered directly to the wound bed without contact with the ultrasound applicator, thereby minimising potential trauma to delicate capillary buds and emerging islands of epithelium.

This study aimed to determine whether this mode of ultrasound delivery affects the size and histological composition of full-thickness excisional wounds in mice with experimental diabetes mellitus.

Materials and methods

Animals

Adult (n=50) sexually mature male CD-1 mice (Charles River Laboratories, St Constant, Quebec, Canada), weighing between 25.0g and 35.0g, were used. They were cared for in an approved animal care facility (Canadian Council on Animal Care).

Diabetes induction

Following five days of acclimatisation, all animals were anaesthetised with an intraperitoneal injection of xylazine and acepromazine (0.03ml/10g of body weight) and given intravenous alloxan monohydrate (100mg/kg of body weight; Sigma-Aldrich Canada, Oakville, Ontario, Canada), as recommended by Mount²⁸, via the tail vein. The animals were anaesthetised beforehand to immobilise them and to ensure an accurate dosage.

Within seven days, alloxan monohydrate produces symptoms of diabetes mellitus, including hyperglycaemia, polyuria and polydipsia, and results

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References

- 1 Houghton, P.E., Campbell, K.E. Choosing an adjunctive therapy for the treatment of chronic wounds. *Ostomy/Wound Management* 1999; 45: 8, 43-52.
- 2 Johansen, F., Nyholm, A., Karlsmark, T. Ultrasound therapy in chronic leg ulceration: a meta analysis. *Wound Rep Reg* 1998; 6: 2, 121-126.
- 3 Crowell, J.A., Kusserow, B.K., Nyborg, W.L. Functional changes in white blood cells after microsonication. *Ultrasound Med Biol* 1997; 3: 185.



Fig 1. Mist Ultrasound Transfer — the device used to deliver the ultrasound

Table 1. Body weight and blood glucose measurements taken before and after treatments

Group	No.	Body weight (g)		Blood glucose (mmol/l)
		Surgery	Sacrifice	Surgery
Ultrasound	27	31.5 ± 3.0*	30.2 ± 3.1	31.2 ± 4.6
Control	23	31.2 ± 1.8*	29.3 ± 2.3	29.9 ± 8.5

Body weight and blood glucose levels were recorded in both animal groups (ultrasound and control) before treatment on the day of surgery and after treatment on the day of sacrifice

* A significant decrease in body weight was observed in both groups

4 Dyson, M., Luke, D.A. Induction of mast cell degranulation in skin by ultrasound. *IEEE Trans Ultrason Ferroelectr Freq Control* 1986; 33: 2, 194-201.

5 Fyfe, M.C., Chahl, L.A. Mast-cell degranulation and increased vascular permeability induced by 'therapeutic' ultrasound in the rat ankle joint. *Br J Exp Path* 1984; 65: 671-676.

6 Young, S., Dyson, M. Macrophage responsiveness to therapeutic ultrasound. *Ultrasound Med Biol* 1990; 16: 809-816.

7 Maxwell, L. Therapeutic ultrasound: its effects on the cellular and molecular mechanisms of inflammation and repair. *Physiother* 1992; 78: 6, 421-426.

8 Harvey, W., Dyson, M., Pond, J.B., Grahame, R. The stimulation of protein synthesis in human fibroblasts by therapeutic ultrasound. *Rheumatol Rehabil* 1974; 14: 237.

9 Young, S.R., Dyson, M. The effect of therapeutic ultrasound on angiogenesis. *Ultrasound Med Biol* 1990; 16: 3, 261-269.

in reduced collagen deposition, impaired growth-factor expression and slower healing rates than occur in other experimental models of diabetes.²⁸⁻³⁰

Surgery and recovery

About 24 hours after confirmation of hyperglycaemia, the animals were anaesthetised with an intraperitoneal injection of xylazine and acepromazine (0.03ml/10g of body weight). The area between the shoulder blades was shaved and cleaned with a topical antiseptic solution, following which a 1.5cm² square full-thickness excisional wound was made. Since wound-dressing materials are not well tolerated by animals, the wounds were left open for the duration of the study.

All animals received an analgesic (buprenorphine, 0.05mg/kg) following surgery for pain control.

Treatment

After the surgery, the diabetic animals were randomly assigned to receive either ultrasound (n=27) or sham treatment (n=23).

The ultrasound was administered on alternate days via a fine mist of prewarmed saline for 1.5 minutes, delivering approximately 15ml of saline. The animals had a total of five treatments over 10 days, which was delivered at a frequency 45kHz and an intensity of approximate 0.1Watt/cm². The ultrasound applicator was held perpendicularly, no more than 1.0cm away from the wound bed.

Animals receiving the sham treatment were treated in a similar manner for 1.5 minutes, when they received a similar volume of saline via an intravenous drip held at a perpendicular distance of 1.0m above the wound bed.

Determination of blood glucose and body weight

Body weights were recorded, using an electronic balance accurate to 1.0mg (ER-60A; Johns Scientific, Canada), at the time of surgery and after 10 days of sham or ultrasound treatment when the animals were sacrificed.

Blood glucose was determined immediately before surgery. A blood glucose level of more than 11.0mmol/l was considered to represent the presence of diabetes mellitus (Table 1).

Determination of wound size

Each wound was photographed at surgery before the administration of either the ultrasound or sham treatment and again following eschar removal at the time of sacrifice. Wound-surface area was determined from the digital images using a previously validated computerised technique.³¹

Tissue processing and staining

Following sacrifice, each wound site was excised to a depth sufficient to include the newly formed granulation tissue, adjacent wound edge and periwound skin, and some of the underlying skeletal muscle. Tissue samples were fixed in 10% phosphate buffered solution (PBS) formalin and assigned a numerical code to conceal identification of either the sham or ultrasound treatment group.

Tissues were subsequently processed through graded alcohols, embedded in paraffin, sliced into sections 5µm thick, placed on microscope slides and stained using haematoxylin and eosin (H&E) stain (Sigma-Aldrich Canada, Oakville, Ontario, Canada) and a picro-Sirius Red Fast Green (SRFG) staining technique (Sigma-Aldrich). Haematoxylin and eosin bind to nuclei and cytoplasmic components respectively.³² Sirius Red binds to collagen and Fast Green to non-collagenous protein.³³

Analysis

Qualitative histological analysis

The cross-sections were examined by an experienced 'blind' assessor to identify differences between the histological appearance of tissues from the mice who had sham treatment and those treated with true ultrasound.

The collagenous material, indicated by the red stain, was examined for the presence of distinct fibres of collagen and for the organisation and density of the collagenous material. The presence and distribution of blood vessels in the granulation tissue were also assessed.

Quantitative histological analysis

• **Collagen deposition** Computerised image analysis (Northern Eclipse, Empix Imaging, Mississauga, Ontario) was used to obtain a colour densitometric measurement of the amount of red and green stain on sections of wounded tissues previously stained with the SRFG-dye technique. The ratio of the intensity of the red collagen stain over the intensity of the green protein stain was expressed as a collagen/non-collagenous protein ratio, as previously validated.³³⁻³⁶

Three measurements were recorded within a randomly selected 0.16 x 0.16µm region examined under 20X magnification:

- Newly formed granulation tissue immediately below the epidermis in the wound base
- Tissue adjacent to the wound edge
- Dermis of surrounding unwounded skin (Fig 2).
- **Blood vessels** Using cross-sections of the wound site previously stained using H&E, a 'blinded' assessor, using 40X magnification, counted the number of blood vessels in the 0.16 x 0.16µm region. regions sampled corresponded to those used to determine the collagen/non-collagenous protein ratio.

Data analysis

A two-tailed independent samples t-test (α=0.05) was used to identify changes in the body weights of animals in the two treatment groups over the experimental period. A two-tailed independent samples t-test (α=0.05) was also used to identify differences between the surface area of the wounds, the amount of collagen and the number of blood vessels present in the granulation tissue of animals in the two groups.

Results

Body weight and blood glucose

Body weights of the animals in the ultrasound and sham-treatment groups were similar at the start of the experiment. They decreased significantly during the experimental period, which is consistent with alloxan-induced diabetes.³⁰

Blood-glucose levels were the same in both groups, and were three times the level of that in mice without diabetes mellitus.

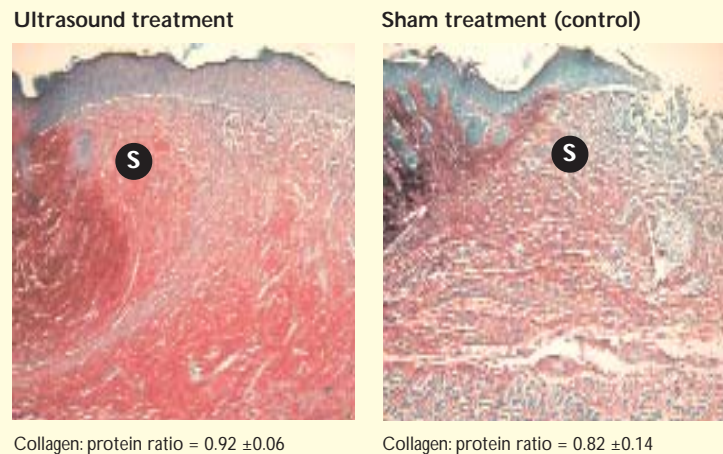
Wound size

Wound-surface area in both groups decreased over the experimental period, but there was no significant difference between the ultrasound group (0.30 ±0.26cm²) and the sham treatment group (0.30 ±0.17cm²) (Table 2).

Qualitative histological analysis

The granulation tissue of mice in the ultrasound group appeared to be different to that of mice in the sham group. In the former, the collagenous tissue

Fig 2. Collagen:protein ratio



A significantly (p<0.05) higher collagen-protein ratio was measured in the superficial (S) region of the granulation tissue of animals with diabetes mellitus treated with ultrasound than in the controls

Table 2. Wound sizes in the ultrasound and control groups

Group	No.	Wound size (surface area, cm ²)	
		Mean ±standard deviation	Mean ± standard deviation
		Surgery	Sacrifice
Ultrasound	27	1.53 ±0.41	0.30 ±0.26
Control	23	1.51 ±0.33	0.30 ±0.17

appeared to be densely associated, although distinct collagen fibres could not be identified (Fig 2). Also, there was a distinct transition between the wound bed and the neighbouring uninjured tissue at the wound edge (Fig 2). In contrast, distinct, thick, coarse collagen fibres appeared throughout the granulation tissue and at the wound edge of the animals who had received sham treatment (Fig 2).

In tissue sections taken from animals treated with ultrasound, areas of blood vessels were consistently noted in the granulation tissue and hypodermis. These highly vascularised areas were often found in the granulation tissue at the wound edge and were frequently associated with several empty vacuoles (Fig 3). Fewer, less obvious, blood vessels and vacuoles were noted throughout the granulation tissue and hypodermis of the animals that had undergone sham treatment (Fig 3).

Quantitative histological analysis

• **Collagen deposition** The ratios of red-staining collagen fibres to green-staining proteinous tissue

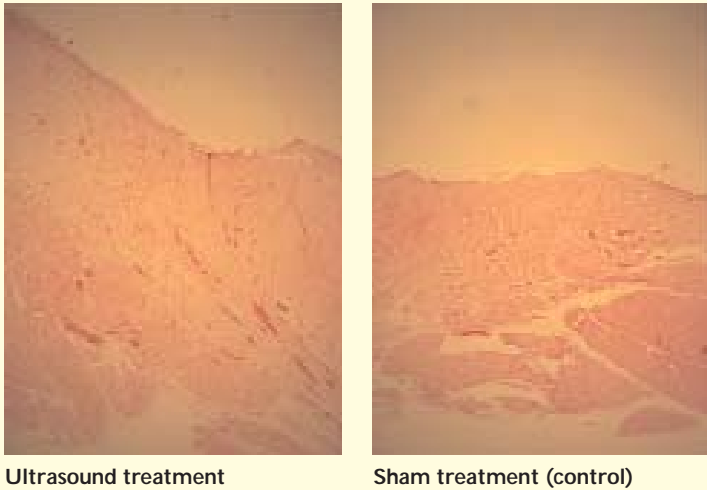
10 Young, S.R., Dyson, M. Effect of therapeutic ultrasound on the healing of full-thickness excised skin lesions. *Ultrasonics* 1990; 28: 3, 175-180.

11 Webster, D.F., Harvey, W., Dyson, M., Pond, J.B. The *in vitro* stimulation of collagen synthesis in human fibroblasts by ultrasound induced cavitation. *Ultrasonics* 1980; 16: 33.

12 Ramirez, A., Schwane, J., McFarland, C., Starcher, B. The effect of ultrasound on collagen synthesis and fibroblast proliferation *in vitro*. *Med Sci Sports Exerc* 1997; 29: 3, 326-332.

13 Webster, D.F., Pond, J.B., Dyson, M., Harvey, W. The role of cavitation in the *in vitro* stimulation of protein synthesis in human fibroblasts by ultrasound. *Ultrasound Med Biol* 1978; 4: 343-351.

Fig 3. Comparison of granulation tissue in ultrasound and sham treatment (control) groups



A larger number of blood vessels, indicated by the arrows, and empty vacuoles (V) were observed in the granulation tissue of mice with diabetes mellitus treated with ultrasound compared with the controls

14 al-Karmi, A.M., Dinno, M.A., Stoltz, D.A. et al. Calcium and the effects of ultrasound on frog skin. *Ultrasound Med Biol* 1994; 20: 1, 73-81.

15 Mortimer, A.J., Dyson, M. The effect of therapeutic ultrasound on calcium uptake in fibroblasts. *Ultrasound Med Biol* 1988; 14: 6, 499-506.

16 Dinno, M.A., Dyson, M., Young, S.R. et al. The significance of membrane changes in the safe and effective use of therapeutic and diagnostic ultrasound. *Phys Med Biol* 1989; 34: 11, 1543-1552.

17 DeDeyne, P., K-Volders, M. *In vitro* effects of therapeutic ultrasound on the nucleus of human fibroblasts. *Phys Ther* 1995; 75: 629-634.

18 Jackson, B.A., Schwane, J.A., Starcher, B.C. Effect of ultrasound therapy on the repair of Achilles tendon injuries in rats. *Med Sci Sports Exerc* 1989; 23: 2, 171-176.

19 el-Batouty, M., el-Gindy, M., el-Shawaf, I. et al. Comparative evaluation of the effects of ultrasonic and ultraviolet irradiation on tissue regeneration. *Scand J Rheumatol* 1986; 15: 4, 381-386.

determined in selected regions of granulation tissue were significantly greater in tissues taken from animals treated with ultrasound (0.92 ± 0.06) than with sham treatment (0.82 ± 0.14). This suggests that the effects of sound energy increase the relative amount of collagen deposited in granulation tissue (Fig 2).

- **Blood vessels** Significantly more blood vessels ($p < 0.05$) were present in the granulation tissue of animals treated with ultrasound (41.3 ± 23.0) than with sham treatment (25.7 ± 20.3) (Fig 3).

Discussion

The present study is the first to examine the effect of ultrasound delivered through a fine mist of saline on the size and histological composition of wounds surgically placed in an impaired model of healing.

Ultrasound administered directly to the wound bed through a fine mist of saline did not accelerate wound closure. However, it did produce a significant increase in the relative amount of collagen and a significantly greater number of blood vessels in granulation tissue at the wound site in mice given ultrasound, when compared with the sham treatment group.

Increases in collagen deposition seen in the present study may, in part, be due to ultrasound-induced intracellular mechanisms previously documented in *in vitro* studies using cultured fibroblasts^{3-9,11-17} and in previous animal studies.^{10,18-22}

Increased collagen deposition has been reported following ultrasound treatment of the wound bed and the intact peri-ulcer skin of excisional wounds created in pigs.²¹

Dyson and Pond reported that administration of ultrasound to open wounds in the ears of rabbits resulted in morphological changes in fibroblasts.²² They suggested that ultrasound stimulated the molecular processes of fibroblasts responsible for collagen synthesis.

In vitro studies have suggested that ultrasound can act directly on fibroblastic cells to stimulate DNA synthesis and fibroblast proliferation.^{12,17} In addition, calcium-ion fluxes respond to changes in membrane permeability^{14,15} by acting as chemical signals, or as second messengers, controlling the cell's enzymatic activity and stimulating the increased synthesis and then secretion of proteins.^{7,13} Harvey et al. reported that cultured human skin and muscle fibroblasts synthesised more protein than unsonicated controls.⁸ Similarly, Webster et al. found an increase in both collagen and general protein synthesis in human embryonic fibroblasts following exposure to ultrasound.^{11,13}

In the present study tissues treated with ultrasound had highly vascularised areas within the newly formed granulation tissue. These qualitative observations were consistent with quantitative measures of the number of blood vessels identified in wounds subjected to ultrasound.

Young and Dyson also found a significant increase in the number of blood vessels in wounds treated with 0.75MHz ultrasound compared with sham treatment.⁹

Ultrasound-induced angiogenesis may be mediated through the direct effects of sound energy on endothelial cells, or effected indirectly by the release of many angiogenic factors from cells in the area (okay?). For example, macrophages play a critical role in stimulating angiogenesis, and produce and release potent angiogenic factors such as fibroblast growth factor and tumour necrosis factor-alpha.³⁷ Mast cells also release heparin, which is thought to facilitate endothelial cell migration and angiogenesis.³⁷

Dyson and colleagues showed that ultrasound applied to either macrophages or mast cells can alter membrane permeability and stimulate their ability to form and/or liberate stored chemical mediators.^{4,6}

Ultrasound-induced release of endogenous stores of growth factors would be advantageous for people with diabetes, in whom healing is impaired. Reduced expression of growth factors[?] and receptors has been well documented in diabetes mellitus.^{38,39}

While exogenous administration of platelet-derived growth factor (PDGF) can accelerate healing of chronic wounds in humans^{40,41} and experimental wounds in diabetic animals,⁴² this intervention is expensive.

Preliminary research conducted by the present ►

investigators revealed that transforming growth factor β (TGF β) was co-localised with collagen in the wounds of animals that had been treated with ultrasound.⁴³

Further research is needed to determine whether administration of ultrasound can increase the quantity of endogenous growth factors, such as PDGF and TGF, and/or their receptors, in the wounds of animals with diabetes mellitus.

The mechanism of wound closure in loose-skinned animals, such as those used in this study, is different to that in the tight, adherent skin of humans. In the former, wounds close primarily by the process of contraction, whereas in humans, re-epithelialisation plays a much greater role.

However, the changes in collagen deposition and angiogenesis documented in this study may not have affected the mechanisms underlying wound contraction.

The similar wound-closure rates in this study contrast with the work of Dyson and Smalley,⁴⁴ who reported that administration of traditional ultrasound within 72 hours of injury could be used to promote wound contraction. These discrepancies may be due to alterations in the timeline of ultrasound administration, or may represent differences in the cellular responses to ultrasound when administered through a fine mist spray.

We, like other authors,⁴⁵⁻⁴⁸ felt it important to use an impaired healing model when studying the ability of a novel treatment modality to accelerate the healing process.

We have previously reported significantly lower collagen levels in both wounded and unwounded regions of skin taken from alloxan-induced diabetic mice, compared with normal non-diabetic mice.³⁴

In addition, collagen deposition was delayed in the granulation tissue of the alloxan-treated diabetic animals following wounding, and there was a strong correlation between lower or delayed collagen deposition and impaired wound closure.³⁴

Lower collagen and impaired wound closure are both consistent with cutaneous changes associated with diabetes mellitus secondary to alloxan monohydrate.^{28,30,32,49-55}

Diabetes mellitus is associated with decreases in the production of collagen and the rate at which proline is incorporated into collagen. Healing takes longer in wounds that require collagen formation.^{50,51} In addition, the hydroxyproline content in the polytetrafluoroethylene wound cylinders was significantly less in rats injected with alloxan monohydrate than in the non-injected controls.²⁸

Smith et al. reported pathological changes in the epidermis and granulation tissue of mice five days following injection of alloxan monohydrate.⁴⁵

Therefore, in the present study, although the seven-day period between the injection of alloxan

monohydrate and wounding may not be long enough to generate complications of diabetes mellitus, such as atherosclerosis, neuropathy and retinopathy, it was sufficient to interfere with the cellular processes associated with the deposition of new granulation tissue.

Each animal had a single wound located in an identical region between the shoulder blades to ensure a consistent blood supply between wounds, and to eliminate the potential for overflow of chemical mediators, which may occur in a multiple-wound model.

Collagen deposition was measured by obtaining a colour densitometric measurement of the amount of red and green stain on sections of wounded tissues previously stained via the SRFG-dye technique using computerised image analysis.

The ratio of the intensity of the red collagen stain over the intensity of the green protein stain was expressed as a collagen:non-collagenous protein ratio. This method is based on the selective binding of Sirius Red to collagen, read at an optical density of 540nm, and Fast Green to non-collagenous proteins, read at an optical density of 600nm.³⁶ Houghton et al. showed there was a high correlation ($r=0.98$) between Sirius Red absorbance spectra and hydroxyproline (OH-proline) amino acid content.³⁶

Determination of the OH-proline amino acid content is a commonly used and well-recognised measure of collagen content. A high correlation ($r=0.98$) was also found between the optical density of Fast Green stain and leucine content.³⁶ This technique has been shown to be effective in the quantification of collagen in small tissue samples.^{34,35}

Using a ratio helps to eliminate the measurement error that can arise from uneven illumination or shading on the image, and potential differences in the intensity of the stains, which may occur when staining a large number of slides at different time periods.

Conclusion

Use of relatively low intensity, low frequency ultrasound delivered through a fine mist resulted in increased collagen production and an increase in the number of blood vessels in the granulation tissue of animals with diabetes mellitus. These stimulatory effects on collagen synthesis and angiogenesis are similar to those documented previously in studies in which ultrasound was administered via traditional application techniques.

Additional experimental research to further characterise the cellular and physiological effects of this new ultrasound therapy is warranted, and clinical research to examine the effects of this new therapy on chronic wounds, including diabetic wounds, will be of interest. ■

20 Turner, S., Powell, E., Ng, C. The effect of ultrasound on the healing of repaired cockerel tendon: is collagen cross-linkage a factor? *J Hand Surg* 1989; 14B: 4, 428-433.

21 Byl, N.N., McKenzie, A.L., Wong, T. et al. Incisional wound healing: a controlled study of low and high dose ultrasound. *J Orthop Sports Phys Ther* 1993; 18: 5, 619-628.

22 Dyson, M., Pond, J.B. The effect of pulsed ultrasound on tissue regeneration. *Physiother* 1970; 56: 4, 136-142.

23 Nussbaum, E., Biemann, I., Mustard, B. Comparison of ultrasound/ultraviolet-C and laser for treatment of pressure ulcers in patients with spinal cord injury. *Phys Ther* 1994; 74: 812-823.

24 Dyson, M., Franks, C., Suckling, J. Stimulation of healing of varicose ulcers by ultrasound. *Ultrasonics* 1976; 14: 5, 232-236.

25 Callam, M., Harper, D., Dale, J. et al. A controlled trial of weekly ultrasound therapy in chronic leg ulceration. *Lancet* 1987; 2: 204-206.

26 Roche, C., West, J. A controlled trial investigating the effect of ultrasound on venous ulcers referred from general practitioners. *Physiotherapy* 1984; 70: 475-477.

27 McDiarmid, T., Burns, P., Lewith, G. et al. Ultrasound and the treatment of pressure sores. *Physiother* 1985; 71: 66-70.

28 Mount, L.E. The effects of graded doses of alloxan on the blood sugar in the mouse. *J Physiol (London)* 1951; 115: 2, 52-53.

29 Covington, D.S., Xue, H., Pizzini, R. et al. Streptozotocin and alloxan are comparable agents in the diabetic model of impaired wound healing. *Diabetes Res* 1993; 23: 47-53.

30 Mohanam, S., Bose, S.M. Influence of streptozotocin and alloxan-induced diabetes in the metabolism of glycosaminoglycans. *Acta Diabetol Lat* 1984; 21: 3, 203-210.

31 Thawer, H.A., Houghton, P.E., Keast, D.H. et al. A comparison of computer-assisted and manual wound size measurement. *Ostomy/Wound Management* 2002; 48: 10, 46-53. ▶

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- 32 Sheehan, D.C., Hrapchak, B.B. *Theory and Practice of Histo-technology* (2nd edn.) Mosby, 1980.
- 33 Junqueira, L.C., Bignolas, G., Brentani, R.R. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J* 1979; 11: 4, 447-455.
- 34 Thawer, H.A., Houghton, P.E. Effects of electrical stimulation on the histological properties of wounds in diabetic mice. *Wound Rep Reg* 2001; 9: 2, 107-115.
- 35 Thawer, H.A., Houghton, P.E. Effects of laser irradiation on the growth and development of fetal mouse limbs in an *in vitro* model. *Lasers Surg Med* 1999; 24: 4, 285-295.
- 36 Houghton, P.E., Keefer, K.A., Diegelman, R.F., Krummel, T.M. A simple method to assess the relative amount of collagen deposition in wounded fetal mouse limbs. *Wound Rep Reg* 1996; 4: 489-495.
- 37 Waldorf, J., Doughty, D. *Wound healing physiology*. In: Bryant, R.A. (ed). *Acute and Chronic Wounds* (2nd edn). Mosby, 2001.
- 38 Beer, H.D., Longaker, M.T., Werner, S. Reduced expression of PDGF and PDGF receptors during impaired wound healing. *J Invest Dermatol* 1997; 109: 132-138.
- 39 Doxey, D.L., Ng, M.C., Dill, R.E., Iacopino, A.M. Platelet-derived growth factor levels in wounds of diabetic rats. *Life Sci* 1995; 57: 11, 1111-1123.
- 40 Robson, M.C., Phillips, L.G., Thomason, A. et al. Platelet-derived growth factor BB for the treatment of chronic pressure ulcers. *Lancet* 1992; 339: 23-25.
- 41 Steed, D.L. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. *The Diabetic Ulcer Group Study. J Vacs Surg* 1995; 21: 1, 71-78.
- 42 Grotendorst, G.R., Martin, G.R., Pencev, D. et al. Stimulation of granulation tissue formation by platelet-derived growth factor in normal and diabetic rats. *J Clin Invest* 1985; 76: 2323-2329.
- 43 Thawer, H.A., Houghton, P.E. Effects of ultrasound mist therapy (UMT) on wound size and histological composition in mice with experimental diabetes. Paper presented at the 15th Annual Symposium on Advanced Wound Care, 11th Annual Medical Research Forum on Wound Repair, April 2002, Baltimore.
- 44 Dyson, M., Smalley, D. Effects of ultrasound on wound contraction. In: Millner, R., Corbet, U. (eds). *Ultrasound Interactions in Biology and Medicine*. Plenum Press, 1983.
- 45 Smith, J., Romansky, N., Vomero, J., Davis, R.H. The effect of electrical stimulation on wound healing in diabetic mice. *J Am Podiatr Assoc* 1984; 74: 71-75.
- 46 Goodson, W.H., Hunt, T.K. Studies of wound healing in experimental diabetes mellitus. *J Surg Res* 1977; 22: 221-227.
- 47 Arquilla, E.R., Weringer, E.J., Nakajo, M. Wound healing: a model for the study of diabetic angiopathy. *Diabetes* 1976; 25: Suppl. 2, 811-819.
- 48 Taskan, I., Ozyazgan, I., Tercan, M. et al. A comparative study of the effect of ultrasound and electrical stimulation on wound healing in rats. *Plast Reconstr Surg* 1997; 100: 4, 966-972.
- 49 Diabetes Control and Complications Trial Research Group. The effect of long-term intensified insulin treatment on the development of microvascular complications in diabetes mellitus. *N Engl J Med* 1993; 329: 977-986.
- 50 Goodson, W.H., Hunt T.K. Wound healing in experimental diabetes: importance of early surgical therapy. *Surg Forum* 1978; 29: 95-98.
- 51 Carrico, T.J., Mehrhof, A., Cohen, I.K. Biology of wound healing. *Surg Clin North Am* 1984; 64: 721-733.
- 52 Morain, D., Colen, L. Wound healing in diabetes mellitus. *Clin Plast Surg* 1990; 17: 493-501.
- 53 Rosenthal, S., Lerner, B., DiBiase, F., Enquist, I.F. Relation of strength to composition in diabetic wounds. *Surg Gynecol Obstet* 1962; 115: 437-442.
- 54 Goodson, W.H., Hunt, T.K. Wound healing and the diabetic patient. *Surg Gynecol Obstet* 1979; 149: 600-608.
- 55 Thawer, H.A., Houghton, P.E., Kloth, L.C., Butryn, A. Effects of electrical stimulation on wound closure in mice with experimental diabetes mellitus. *Wounds* 2000; 12: 6, 159-169.