Clinics in Surgery

Research Article
Published: 16 Nov, 2016

Histological Findings from Controlled Application of a Thermal Plasma to Human Skin

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Abstract

Ionised gas or plasma is often described as the fourth state of matter since it can be produced from an electrically neutral gas which has been fully or partially ionised. The resulting mixture of free electrons, positively charged ions and un-ionized gas possess a rich chemistry and may be used to transport thermal energy. Plasma has thus found applications in such diverse fields as spacecraft propulsion, magnetic confinement fusion, silicon etching, and surface treatment and of course biomedicine.

We have developed a plasma generating device that can deliver a controlled depth epidermal and dermal burn injury as a function of energy delivery over a unit area and time of exposure. This is important as a therapeutic tool for treating skin lesions; a device to create burn wound healing models, for medicolegal understanding of burn depth and for the development of new wound care products. In our study plasma is delivered onto a human skin model in an experiment approved by the local ethics committee. Fresh abdominoplasty skin was marked according to protocol and treated with a controlled energy dose per unit area using the new plasma delivery system developed at the Surrey Space Centre at Surrey University. Skin samples are biopsied and immediately placed in formal saline before sectioning and staining.

The immediate histological changes of superficial burn injury in human skin can then be determined. The findings in this study show a reproducible depth of thermal injury as a function of energy delivery. Each 25 J/cm^2 increment up to 100 J/cm^2 causes a 0.5 mm depth of cutaneous thermal injury. In the 25 and 50 J/cm^2 injury the epidermis remains intact and appears as a 'first degree or superficial burn injury'.

Histologically the basal epidermal cells show characteristic oedematous morphology which we have called 'Frame Cells' because the morphology after superficial injury has not been previously recognized. There is a clear line of demarcation within the superficial dermis where the zone of coagulation meets the zone of stasis. In the 75 and 100 J/cm^2 the epidermis appears coagulated and has histologically separated from the dermis burn at the dermo-epidermoid junction. The line of dermal coagulative necrosis is clearly defined. Melanocytes are absent immediately after even the most superficial injury. Fibroblasts, vascular endothelial cells, epithelial cells, pigment cells, collagen and elastin are all examined using immunohistochemical stains.

Keywords: Plasma; Ionised gas; Thermal injury; 'Frame' cells; Burn depth; Dermis; Skin

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Received Date: *20 Sep 2016*

Accepted Date: *07 Nov 2016*

Published Date: *16 Nov 2016*

Citation:

Kamel D, Frame J, Frame JD, Harle T. Histological Findings from Controlled Application of a Thermal Plasma to Human Skin. Clin Surg. 2016; 1: 1176.

Introduction

Ionised gas or plasma is often described as the fourth state of matter since it can be produced from an electrically neutral gas which has been excited. This can be achieved by passing an electric current through the gas, or otherwise heating it sufficiently to remove some or all of the outer electrons thus creating a population of positively charged ions as well as un-ionized, electrically neutral, gas particles. This mixture of particles responds to electric and magnetic fields and possess a rich chemistry which is why plasmas have found applications in such diverse fields as spacecraft propulsion, magnetic confinement fusion, silicon etching, surface treatment and of course biomedicine.

Plasmas may be formed under a broad range of gas pressure conditions from 10^{-7} Pa, as found

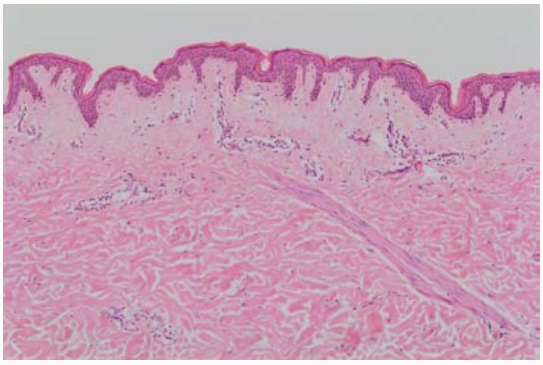

Figure 1: Haematoxylin and Eosin (H and E) stain of normal skin as control. There is a clear distinction between the dermis and epidermis. The epidermis shows keratin flaking from the surface. The dermo-epideroid junction is clearly defined and there are healthy rete pegs separating the papillary and reticular dermis.

in a geostationary satellite orbit and up to atmospheric pressure. High pressure plasmas are particularly well suited to biomedical applications since they may be created without the need for vacuum chambers and pumps and their generators can be made compact and easy to manipulate and apply to surfaces by hand or with the aid of a mechanical arm. The ability to control the atmospheric pressure plasmas chemistry and the emission of UV and thermal radiation has allowed application to the sterilization of surfaces against a range of bacteria and viruses, the accelerated healing of open wounds and the sterilisation of water.

More recently atmospheric pressure plasmas have been considered for use as a cosmetic tool. A limited number of realisations can be found at present and are difficult to compare directly, due to their distinct operation principles, electrode arrangements and intended biological modes of action. Imagined as a heat source, plasma may be used to heat an area of the skin surface in order to create a controlled burn after which the skin heals. It is well known that the more superficial the injury the faster the wound heals and the less likely that hypertrophic scar, hair loss or variegated pigmentation will occur. Thin skin has little margin for error with thermal treatment.

Here we present a plasma generating device (4SM MK51) which delivers a controlled depth epidermal and dermal burn injury as a function of energy delivery over a unit area and time of exposure using an ergometrically constructed hand piece (Figure 2). The thermal energy produced by the plasma follows a Gaussian distribution around the application area and can be applied accurately to surface features as small as 1 mm^2. Unlike other plasma based cosmetic tools, which produce an oxidative burn similar to an electrical burn in air, the 4SM MK51 displaces the ambient air around the skin surface to produce less aggressive burning of the surface and to promote the diffusion of the energy to the below epidermis.

This new technology is important as a therapeutic dermatology tool, an investigative wound healing model and for the development of new wound care products [1]. The immediate histological changes in human superficial dermal and epidermal tissues after burn injury have not previously been defined in detail. However, it has long been accepted that after acute thermal injury there are three zones of injury. These were first described by Jackson in 1947 as the zone of coagulation (Zone 1- total cell death), the zone of stasis (Zone 2- potentially reversible cellular changes) and the zone of hyperaemia (Zone 3- inflammatory response to injury) [2]. Progressive burn

Figure 2: Ergometrically designed handpiece designed to deliver generated plasma.

depth injury over time is, in part, dependent upon the degree of circulatory flow, secondary sepsis and burn wound care. The ability to investigate and manipulate the detrimental secondary responses in a burn wound model is required to ameliorate the effects of infection, altered physiology and poor healing including scar development. A plasma delivery system has been designed and developed by Fourth State Medicine Ltd (Surrey Technology Centre, Surrey University) and we have used this tool to investigate the immediate histological effect on skin after delivering a controlled amount of plasma energy to a sheet of freshly excised abdominoplasty skin.

Anatomy of Skin

Human skin is a composite of two layers and being embryologically of both ectodermal and mesenchymal origin, they contain a variety of highly specialized cells and organs (Figure 1):

The epidermis

A multicell layer of continuously regenerating epithelial cells at the stratum germinativum layer, which progressively die as they are pushed to the skin's surface, eventually forming a non living outer keratin layer on the surface (the stratum corneum layer). They eventually desquamate as dead keratinocytes. The basal layer also contains pigmented cells of neuroectodermal origin (melanocytes), immune- surveillance cells (Langerhans cells) and other cells of unknown function. The Stratum Spinosum, Granulosum and Lucidium represent layers of epidermal apoptosis and morphologic differentiation. The epidermis transmits the passage of hair follicles, sebaceous secretions and sweat ducts. The function of the epidermis is temperature control, waterproofing, vitamin D production and as a barrier to infection, harmful effects of UV light, and trauma.

The dermis

consists of two layers:

Papillary dermis: The papillary dermis lies directly beneath the basal layer of epithelium. Projections of the basal layer extend into the dermis and the inter-digitating dermis constitutes the papillary layer. Here, collagen bundles are loosely arranged around elastin fibres. Fine blood vessels and sensory nerve endings branch underneath the epidermis.

Reticular dermis: The deeper reticular dermis contains collagen fibers, which are thicker and more densely packed and irregularly arranged. Elastin fibers are found in both the papillary and the reticular dermis, but they are more numerous in the reticular dermis. Cutaneous appendages, like sweat glands, originate from within the dermis. Hair follicles originate just deep to the dermis and are lined by a basal layer containing melanocytes as they project through the

Figure 3: Photo showing the application of the plasma to the sample. The waypoints used to control the speed can be seen as well as the demarcation between tracks which received differing exposure levels.

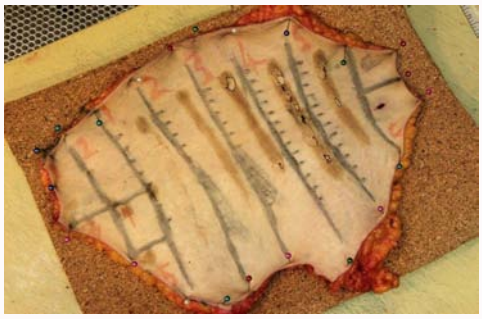

Figure 4: Photo showing the skin sample after exposure to the plasma. The number labels at the top of each vertical section corresponds to the time taken to traverse the 10 mm spaced waypoints.

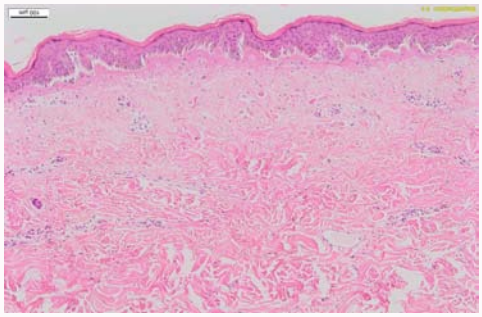

Figure 5: MP, H and E stain of skin treated with 1 second exposure of Plasma energy equivalent to 25 joules (LP and HP). The basal epithelial cells have become oedematous along the line of the basal epithelium. The oedematous cells are elongated and deformed perpendicular to the skin. There is a subtle colour change in the papillary dermis.

dermis to the surface. This layer contains sensory nerve endings, lymphatics, blood vessels and nerves. There are immune-privileged cells and epithelial stem cells at the level of the bulge in the hair follicle about the level of the erector pili muscle within the dermis.

The basement membrane is an important interface between the epidermis and papillary dermis, consisting of an intricately organized collection of intracellular, trans-membrane, and extracellular matrix proteins. The basement membrane zone has several main functions including acting as a permeability barrier, forming an adhesive interface between epithelial cells and the underlying dermis and

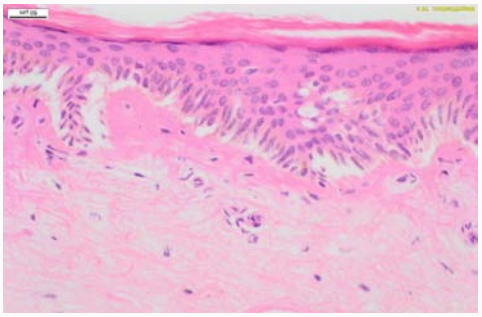

Figure 6: HP, H and E stain of skin treated with 1 second exposure of Plasma energy equivalent to 25 joules (LP and HP). The basal epithelial cells have become oedematous along the line of the basal epithelium. The oedematous cells are elongated and deformed perpendicular to the skin. There is a subtle colour change in the papillary dermis.

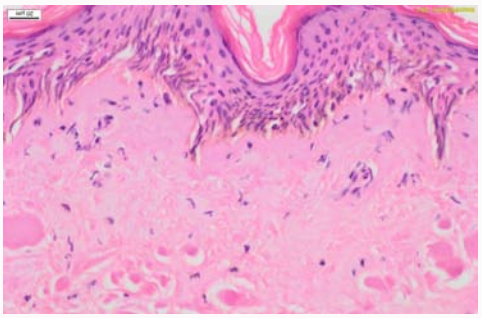

Figure 7: HP, H and E stain after a 2 second exposure. Injury extending into the superficial dermis.Oedema at the basal layer still obvious.

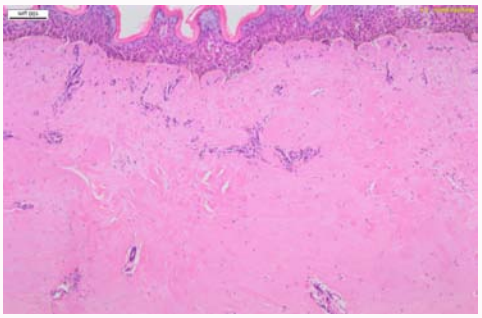

Figure 8: MP, H and E stain after a 3 second exposure. Marked flattening of rete pegs and persistence of elongation of 'frame cells'.

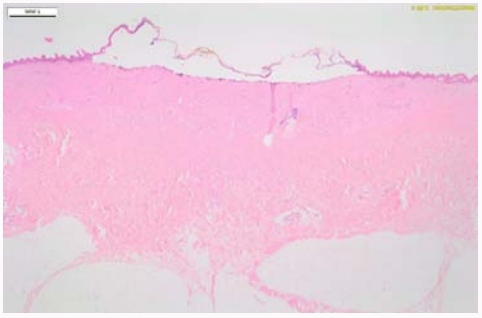

Figure 9: LP, H and E stain after a 4 second exposure. Separation of the layers of skin at the dermoepidermoid junction and mid to deep dermal injury. Loss of basal cell integrity and continuity.

controlling cellular differentiation. S100 stained cells, including Langerhans cells and melanocytes, exist within the basal epithelium

Table 1: Summary of the Micro Cellular effects of the plasma for varying plasma exposures/ energy depositions.

Plasma exposure time/Energy delivered	Micro-Cellular effect
1 second/25 joules	1. Oedematous elongation of the epithelial cells along the basement membrane, resembling flame figures which we have called 'Frame cells' 2. Coagulative changes within the superficial papillary dermis – enhanced staining with EVG. 3. IHC shows damage/loss of melanocytes highlighted with S100, however, melanocytes are retained around deeper hair follicles. 4. Depth of damage is 0.5 mm.
2 seconds/50 joules	1. The surface epidermis starts to detach from the basement membrane separating the dermis and epidermis (blister formation). 2. The coagulative tissue changes are more intense with EVG and the depth of damage is 1 mm. 3. IHC with S100 shows similar changes to that seen with 1 second application.
3 seconds/75 joules	1. The epidermis shows significant burn effect, with significant blister formation. 2. The coagulative tissue damage extends to a depth of 1.5mm. 3. IHC with S100 shows that the deep hair follicle melanocytes are still viable.
4 seconds/100 joules	1. The surface epidermis has completely sloughed off. 2. The coagulated tissue extends to just over 1.5 mm. 3. IHC with S100 shows that some of the deep hair follicle melanocytes are damaged.

and basement membrane.

Materials and Methods

A large sheet of lower abdominal skin and attached subcutaneous fat was excised during a standard Brazilian Abdominoplasty [3]. Ethics permission had been obtained prior to the start of the study (UK: NHS Health Research Authority Number 06/10/14). The skin was transferred immediately upon harvest to the Histopathology Department, Mid Essex Hospitals Trust, and Broomfield. A predetermined grid was drawn on the skin with permanent marker and appropriately labeled to monitor skin changes (Figure 3). The plasma was generated by a specially designed hand piece which was operated at a fixed power of 25 W for the duration of the study. This power was determined empirically through the use of a solid state calorimeter which allowed measurement of the energy imparted to a surface for plasma exposures of various time intervals. From these measurements a time averaged "effluent power" was calculated. For this study the plasma generator was operated under conditions where the effluent power was measured as 25 W.

The energy delivered to the skin was varied by increasing or decreasing the time taken to move the plasma along a series of tracks of fixed length. Thus the slower the plasma was moved along a track, the more energy was delivered to the skin and so on. The time taken to traverse the track was metered using a metronome and a series of way points marked at 10 mm intervals (Figure 4). The time, in seconds, between metronome beats was set and used to indicate to the clinician when to move from way-point to way-point. In this study, the metronome was set to 1, 2, 3, and 4 seconds between beats, which for fixed effluent power corresponds roughly to 25, 50, 75 and 100 J of energy deposited into the sample.

A control area of normal skin was also identified. Three histopathological blocks were taken from the visibly obvious, middle third of each treatment line and labeled for each time interval and the controls. The plasma was directed at the skin grid targets from a constant distance for intervals of 1 second, 2 seconds, 3 seconds and 4 seconds, with a separate control sample. Multiple skin biopsies from each treated area and the control area were embedded in paraffin blocks and then cut into 4μm sections using a microtome.

Histological Analysis

All tissue sections were stained as follows:

Haematoxylin and eosin (H+E)

This is a basic stain used on histological specimens. Haematoxylin stain is purple and delineates the cell nucleus whilst Eosin is pink and delineates the cell cytoplasm.

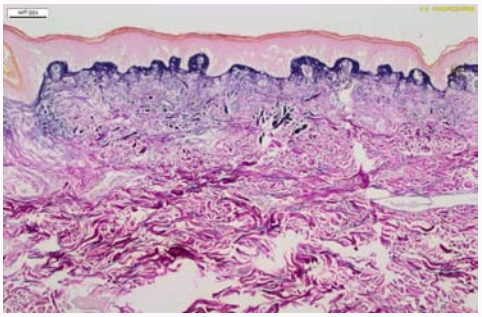

Figure 10: MP, EVG stain after 1 second exposure showing clearly demarcated injury with loss of collagen but retention of elastin stain extending into the reticular dermis, mild flattening of the rete pegs and superficial oedema at the basal layer.

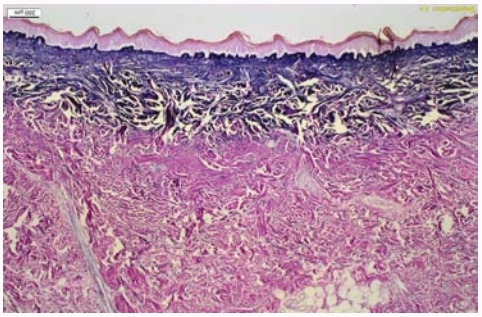

Figure 11: LP, EVG stain after 2 second exposure showing 1mm dermal injury with elastin positive staining and absent collagen extending into mid dermis.

Elastic von gieson (EVG)

This is a special stain for fibrous tissue, delineated collagen in red and elastin fibres, delineated in black or dark blue.

Periodic acid schiff (PAS)

This is a special stain for basement membranes, connective tissue, fibrous tissue, mucous and glycogen, delineated in red/pink; other types of tissue are delineated in blue.

Mason trichrome (MT)

This is a special stain for fibrous tissue, delineated in blue/green; other types of tissue are delineated in grey/red.

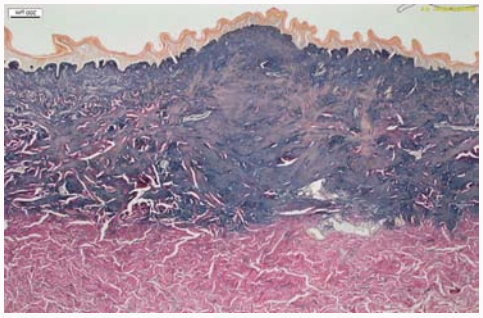

Figure 12: LP, EVG stain after 3 seconds exposure showing 1.5 mm dermal injury.

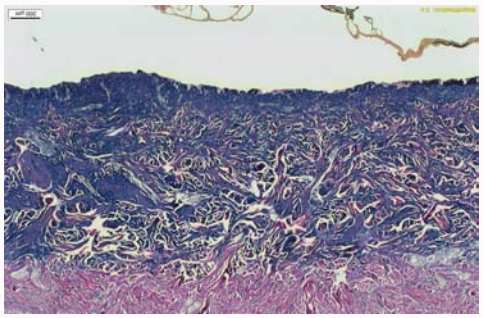

Figure 13: MP EVG stain after 4 seconds exposure showing separation of epidermis and 1.5mm of dermal injury.

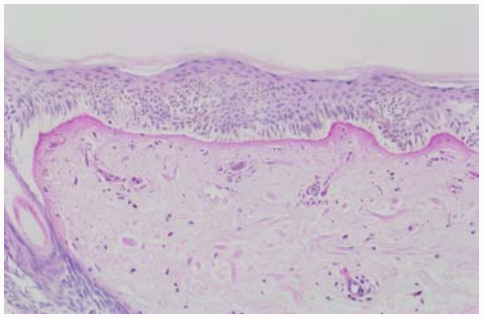

Figure 14: PAS stain for basement membrane and interstitial tissues at 1 second exposure. The membranes appear intact but with early oedema.

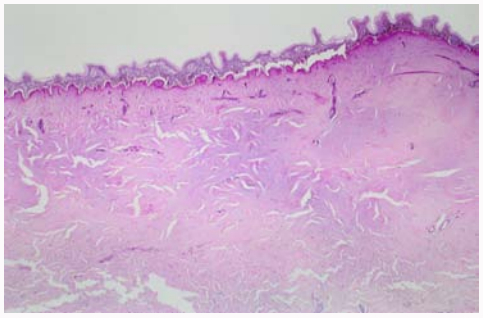

Figure 15: PAS stain at 3 seconds exposure showing blue discolouration and disruptive oedema into the superficial dermis.

Immunohistochemical staining (IHC), f or S100 and cytokeratin

IHC is a biochemical assay that takes advantage of chemical reactions to locate and visualize the interaction between antibodies and their target antigen on sample tissue. These reactions are visualised using chromogen and the most commonly used is DAB (3,3'-diaminobenzidine), which is brown. S100 is used to identify cutaneous melanocytes, nerve endings and Langerhans cells. Cytokeratin is a marker for epithelial cells.

Results

The histological findings are demonstrated in Figures 5 – 21 and described in detail Table 1.

Discussion

Plasma has been a very important source of energy in space research for years and the development of a terrestrial application had 2 objectives. The first was to build a plasma delivery system and the second was to apply the technology for use on human skin to give safe and reproducible results. Fourth State medicine developed patented technology into a functional delivery system previously tested on cadaveric porcine skin. The technology subsequently received ethical

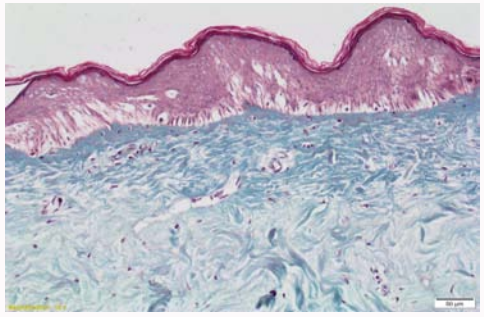

Figure 16: MP, MT stain for fibrous tissue at 1 second showing slight retraction of the collagen in the reticular dermis, frame cell oedema of basal cells and a normal intradermal collagen structure beneath.

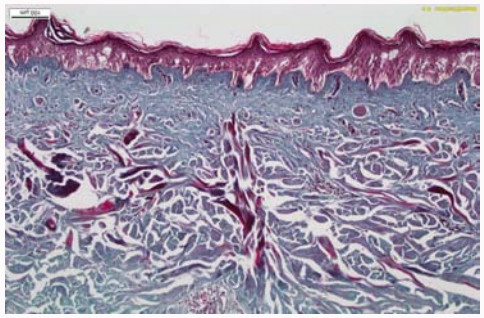

Figure 17: LP, MT stain after 2 second exposure. The superficial dermal fibrous composition is losing the normal laminar architecture down to 0.5mm.

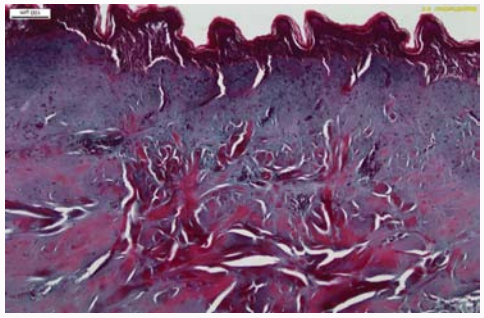

Figure 18: MP, MT stain after 3 second exposure showing a clearly demarcated area 1mm into the dermis. The deeper dermis retains its normal uninjured wavy appearance.

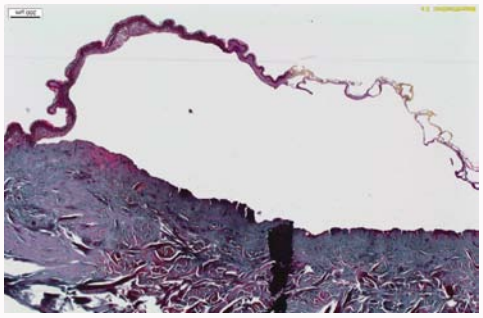

Figure 19: MP, MT stain after 4 seconds exposure showing 1.5 mm dermal injury and epidermal sloughing. The deep dermal laminate pattern shows no injury.

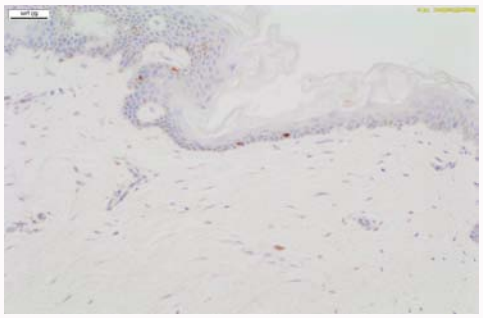

Figure 20: HP, S100 immuno stain showing the normal distribution of pigment producing melanocytes at the dermo-epidermal junction. Langerhans cells, intradermal nerve cells and cells of non- specific origin will also give a positive stain. These are important cells in immunorecognition. Melanocytes are also present along the dermo-epidermal junction that follows along the depths of the hair follicle. These positive staining cells occupy only about 4% of the basal cell content.

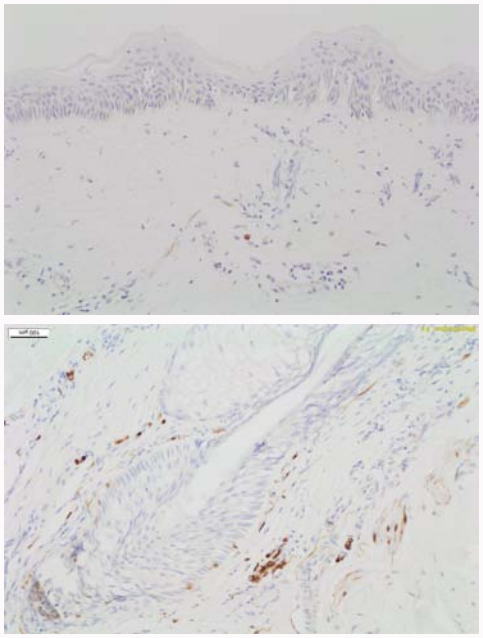

Figure 21: MP, S100 stain showing complete loss of S100 positive stain at the dermo-epidermoid junction after 1 second of exposure.

committee approval to test on human skin with a view to further understanding the effects on living cells. The results of this study clearly demonstrate that Plasma can be safely delivered in fractionated doses to cause a standard depth injury. Even after 4 seconds of contact the depth of injury did not cause a full thickness injury. At minimal contact time of 1 second the injury is barely into the papillary dermis and interesting changes in cellular morphology with oedema of basal cells was noticed. Specific stains showed progressive injury into the reticular dermis with a relationship of 0.5 mm of depth for each 1 second of exposure to 25 Joules.

The clinical applications of this technology are considerable as both a research tool for basic wound healing experiments but also in clinical care from topical skin rejuvenation to treatment of diabetic leg ulcers, hypertrophic scars and superficial skin lesions. Plasma delivery has a role in biofilm treatment and infection prophylaxis and the tool clearly has commercial applications.

Conclusion

This study demonstrates a series of standard depth burn injuries in human skin from delivery of 25, 50, 75 and 100 J of energy. On analysis, for every second of treatment by the plasma generator for the first 3 seconds, the depth of damage increased by 0.5 mm (depth of damage (mm) = T (s) X 0.5).

The thermal energy produced by the plasma follows a Gaussian distribution around the application area and can be applied accurately to surface features as small as 1 mm^2. Unlike other plasma based cosmetic tools, which produce an oxidative burn, similar to an electrical burn in air, the 4SM MK51 displaces the ambient air around the skin surface to prevent aggressive burning of the surface and to promote the diffusion of the energy to the superficial dermis. This allows the body to maintain the outer layer of the skin while the subsurface trauma is allowed to heal and regenerate new elastin, collagen and epidermis.

This histological study has identified a tool that can create skin burn wounds of predictable depth. This enables further opportunities for in-vitro and in-vivo injury studies. There are also potential dermatologic applications within the NHS but also within the aesthetic sector.

Acknowledgement

Fourth State Medicine, Surrey, for providing the technology and equipment for this study.

The Histopathology Department at Broomfield Hospital, Chelmsford, for access to their facilities.

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