**Identification of the Cell Thermal Microenvironment in Porcine Skin during
Laser Cauterization**

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THESIS

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I would like to dedicate this work to my parents who have always stood behind me during ups and downs in my life. This wouldn't have been possible without your love and support.

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LIST OF ABBREVIATIONS

ECM	Extracellular Matrix
GS	Ground Substance

SUMMARY

In order to minimize bleeding during surgical intervention, laser devices make it possible to produce local heat for cauterization and to reduce the blood loss at the site of the incision in the skin. However, the method is sometimes associated with local overheating and tissue partial vaporization. We present a 3D Finite Element Model (FEM) hierarchical model to simulate the effect of heat produced by different surgical laser devices on the skin. The model includes the macro-molecular, cell and tissue scales. The model follows the bio-heat equation where the transient heat diffusion includes heat conduction in the tissue, convection with the surrounding air, the metabolic heat and the heat source of the laser device. The laser heat flow propagating in the skin is assumed to exponentially decrease from the opening edge of the wound. A set of different lasers are tested on live porcine skin under general anesthesia. The skin morphology of harvested samples along the wound is digitally surveyed in light microscopy observations of the skin specimens that have been previously stained with an anti-swine antibody. The biological constituents of the skin tissue are modeled as separate continua of specific thermal properties taken from the literature in the presented preliminary model. The numerical model evaluates the temperature and the heat flux fields in the vicinity of the laser wound in porcine skin. Using an inverse procedure, these results are validated by comparison with antibody stained tissue images of the experimental samples in order to identify the temperature at which significant damage is experienced by skin cells.

CHAPTER 1

INTRODUCTION

Skin is the outer layer of the body of mammals that serves as a protective layer against the aggressions of the environment (pathogens, temperature, water) [2]. Skin is the organ that also preserves the body internal temperature and water [3]. Skin is also a storage layer for water and lipids. Skin breathes to some extent [4] as well. Skin is an organ able to sense heat and cold, touch, pressure, vibration and pain thanks to specific sensory receptors. Any surgical procedure will therefore cause a dramatic reaction in response to any inflicted damage. Thermal damage can therefore be imaged.

The two upper layer of the skin of human and most mammals are the epidermis and the dermis. The epidermis forms the outermost layer of the skin and is a protective barrier over the body's surface. The epidermis is composed of four sub-layers: the stratum corneum, the stratum granulosum, the stratum spinosum and the stratum basale where the keratinocytes originate [5]. The dermis, beneath the epidermis, is structurally divided into two areas: the papillary region and the reticular region. The dermis is a connective tissue that cushions the body from stress and strain. The dermis also contains hair follicles, sebaceous glands, apocrine glands, eccrine sweat glands in humans but not pigs, lymphatic capillaries, arterioles and venules. The dermis also contains sensory organs called pacinian corpuscle, meissner corpuscle, nociceptors and thermal receptors which sense pressure, touch, pain and heat, respectively. Below the dermis lies a deeper layer called hypodermis that is composed of adipose tissue, larger blood vessels

and nerves. The hypodermis ensures the attachment of the skin to the underlying bone and muscles structures as shown in Figure 1.

Figure 1. Detailed morphological analysis of the porcine skin constituents in a sample that has been treated with the Biolase iLase laser (Gingivectomy setting, CW, open beam) and stained via the Antibody method during Surgery 4BO.

During laser surgery, the blood vessels and nerves that are running through the dermis are cut and bleed. However the application of the high temperature with surgical devices

such as lasers cauterizes the tissue to stop the bleeding. The depth and the amplitude of the cauterization will depend on the energy absorbed by the skin which is related to the power and the pulse of the laser and to the duration of exposure. The heated tissue in the vicinity of the wound opening is unsuitable for immediate suture. The successful placement of subsequent stitches to close the wound will rely on the identification of the location of healthy tissue.

The presented study proposes an inverse procedure to characterize thermal damage resulting from the application of diverse surgical laser devices that are tested on live porcine skin under general anesthesia. First the skin sample preparation using antibody staining is presented. Second the skin morphology is surveyed in light microscopy observations of skin samples stained for antibody response. The explicit morphology is discretized in a 3D finite element model. The different skin constituents are modeled as separate continua of specific thermal properties. The thermal analysis follows the bio-heat equation where the transient heat diffusion includes the heat conduction term, the convection with the surrounding air, the metabolic heat and the heat source generated by the laser device. The laser heat flux is modeled by an exponential function that incorporates the geometry of surgery and the skin heat absorption properties. The thermal analysis is applied to a series of surgeries in order to evaluate the temperature elevation and the heat flux within the porcine skin specimens. Finally the regions of elevated temperature and significant thermal damage experienced by skin cells are validated through comparison with the histological antibody treatment images using an inverse procedure.

1.1 Sample preparation

Several histology analyses are performed on the harvest skin tissue: H&E (Hematoxiline and Eosine), MTT and Antibody staining. Antibody staining was found to be the most efficient to reveal the damage zone and the small skin constituents of which the contours are digitally digitized in the microscopy observations. The list of the names of the tested devices and their abbreviated notation throughout this work is given in Table 1.1.

Surgery Name	Laser Name
1	Dornier Holmium
2A	Lype FD-30-A
2C	Lype FD-30-C
3	Dornier Medilas D60
4BO	Biolase iLase Gingivectomy
4BG	Biolase iLase Gingivectomy with tip
4BF	Biolase iLase Frenectomy with tip
5	IPG DLR-200-AC diode laser
6	IPG ELR-1550-50 diode laser

TABLE I

SURGERY NAMES CORRESPONDING TO THE DIFFERENT LASERS USED

After surgery, porcine skin specimens of about 10 cm by 10 cm along the edge of the wound as shown in Figure 2. Small excerpts of nearly 5 mm by 5 mm from the skin large sample harvested along the edge of the wound were harvested from the pig. The small skin sample is

then finally sectioned using a microtome to prepare $0.7\text{ }\mu\text{m}$ thin skin slices for histology analyses using H&E, MTT and Antibody staining.

Figure 2. Subsequent cutting operations to prepare the thin slices skin samples for histology analysis

This tissue samples undergo a series of different processes that involves fixing, processing, embedding, sectioning and staining. Chemical fixatives are used to preserve this tissue, thus preventing them from degradation that can occur during staining process such as buffered Formalin is used [6]. The tissue is then processed to removes water and replaces it with the medium such as paraffin that solidifies the tissue to enable cutting in thin sections [7] of $0.7\text{ }\mu\text{m}$ thickness using a microtome. In this study, the tissue are stained with H&E staining, MTT and with anti-swine antibody. For the numerical model, the antibody staining used here that is based on the loss of antigenicity and do not use fluorescent tags, highlights best the skin

microstructure and damage. These microscopic observations of the stained tissue are therefore later digitized and are shown in Figure 1 where are seen the hair cavity, sebaceous glands, arrector pili muscle, arterioles, lymph capillaries, venules and nerves.

CHAPTER 2

MODEL DESCRIPTION

2.1 Multiscale Multiphysics Hierarchical Model of Skin

To represent the thermal properties of skin tissue, we propose a multiscale multiphysics hierarchical model for the different sub-layers of the epidermis and the dermis. The epidermis is made up of four different layers each having their own unique properties, whereas the dermis is a composite of fibrillar components such as collagen, elastic and reticular fibers and the ground substance consisting of water and glycosaminoglycans.

2.1.1 Epidermis

The epidermis is composed of four layers from the surface downwards called Stratum Corneum, Stratum Granulosum, Stratum Spinosum and Stratum Germinativum. Each layer is composed of close packed cells at different stage of differentiation immersed in very little ECM (extracellular matrix). The thickness of the layers are shown in the ???. At the attachment site of the stratum germinativum to the papillary region, stem cell keratinocytes are attached to the basal epithelium through the hemidesmosomes and desmosomes between cells. The basal keratinocytes progressively detach and undergo proliferation to move upwards and differentiate. At each stage of differentiation, the cells are attached by desmosomes. In our model, the different layers are represented by a single keratinocyte composed of a ellipsoidal nucleus and an ellipsoidal cytoplasmic body immersed in its ECM and carrying two desmosomes on its top and

bottom surfaces. The cell sizes in all the layers are measured from our microscopic observations show in ?? and given in Table II

	Germinativum	Spinousum	Granulosum	Corneum
Cell Cytoplasm(μm)	$9.3 \times 8.7 \times 9.3$	$10 \times 8.3 \times 10$	$13.5 \times 2.7 \times 13.5$	$21 \times 1.5 \times 21$
Nucleus (μm)	$6.0 \times 5.3 \times 6.0$	$5.8 \times 3.8 \times 5.8$	$7.7 \times 1.2 \times 7.7$	-
Desmoplakin (nm)	95.2×19.0	95.2×19.0	95.2×19.0	95.2×19.0
Cadherin (nm)	57.1×28.6	57.1×28.6	57.1×28.6	57.1×28.6

TABLE II

DIMENSIONS OF THE KERATINOCYTES AND CORNEOCYTES MEASURED IN MICROSCOPIC OBSERVATIONS FROM SURGERY 5 FOR THE HIERARCHICAL NUMERICAL MODEL. THE DESMOSOMES WERE MEASURED IN MICROSCOPIC OBSERVATIONS FROM [1].

During differentiation, the cells express different keratin proteins in their cytoplasm and lose their nuclei to form corneocytes in the stratum corneum. The keratin content in the cell cytoplasm therefore increases from 21.6% in the basal keratinocytes to 64.5 % in the corneocytes. On the other hand, the water content in the cell cytoplasm decreases from 70% in the basal keratinocytes to 27 % in the corneocytes [8]. In our model the keratin and the water content in the cell cytoplasm are linearly interpolated between the previously given values along the layer thickness. In each layers the respective lipid contents are given in Table III [9]. Among the lipids present, the neutral lipids are composed of six different lipids [10]. In the stratum germinativum, spinosum and granulosum, the ECM is assumed to be of the same composition

as the cell cytoplasms. In the stratum corneum the ECM is only composed of lipids [11] The detailed compositions of the keratinocyte cell cytoplasms and respective ECMs are given in the Table III. The density of the cytoplasm of each layer is calculated as follows,

$$\rho_{\text{cyt}}^p = \sum_{i=1}^{n_p} \frac{m_i \rho_i}{m_p} \quad (2.1)$$

where ρ_{cyt}^p is the density of layer p . m_i and ρ_i are the mass and density of the constituents of the layer p (water, keratin and lipid) [12]. m_p is the mass of the layer p . The density of the neutral lipids was calculated using the same weighted average formula applied to its lipidic constituents [13–16]. The thermal conductivities and the specific heats of the cytoplasms of the cells in layer p , k_{cyt}^p and c_{cyt}^p , were also calculated by a weighted average formula of the thermal conductivities of their respective constituents [17] [18] [19].

Stratum	Mass percentage(from dry weight)			
	Germinativum	Spinosum	Granulosum	Corneum
Water	70	55.559	41.5056	27
Keratin	21.6	36.01	50.0277	64.5
Keratohyalin			0.5662	
Total lipids	8.4	8.4	7.9	8.5

TABLE III

KERATINOCYTE CYTOPLASM COMPOSITIONS IN THE EPIDERMIS LAYERS

The cell nucleus is typically composed of densely packed chromatin fibers encapsulated by lamin membrane. Few physical and thermal information about cell nucleus are available in the literature. In our model, the basal keratinocyte density was calculated as the ratio of a mass of 39.9 pg [20] and a volume of 99.9 μm^3 measured on our observation. Note that the nucleus mass is value given for a liver cell that displays similar aspects and size as the basal keratinocytes in the stratum germinativum. For the other epidermal layers, their respective densities were linearly interpolated between 0.40 g/cm³ and 0.0.89 g/cm³ in the the corneum stratum where the nucleus has completely disintegrated and only the cytoplasm remains as follow,

$$\frac{\rho_i^n - \rho_{ge}^n}{\rho_c^n - \rho_{ge}^n} = \frac{\rho_i^{\text{cyt}} - \rho_{ge}^{\text{cyt}}}{\rho_c^{\text{cyt}} - \rho_{ge}^{\text{cyt}}} \quad (2.2)$$

where ρ_i^{cyt} , ρ_{ge}^{cyt} , ρ_c^{cyt} are the densities of the cytoplasm of the cells in the studied layer, the stratum germinativum and stratum corneum respectively given in table. ρ_i^n , ρ_{ge}^n , ρ_c^n are the densities of the nuclei of the cells in the studied layer, the stratum germinativum and stratum corneum respectively. The thermal conductivities and the specific heats of the nuclei of the cells in each epidermal layer p , k_n^p and c_n^p were calculated assuming that the thermal conductivities and the specific heats are proportionally increasing with the densities as follows,

$$k_n^p = \frac{\rho_n^p}{\rho_{\text{cyt}}^p} k_{\text{cyt}}^p \quad (2.3)$$

$$c_n^p = \frac{\rho_n^p}{\rho_{\text{cyt}}^p} c_{\text{cyt}}^p \quad (2.4)$$

The desmosomes are proteinous cell organelles composed of two plates of desmoplakin attached by cadherin. The size and volumes of the desmosomes elements were measured in microscopic observations. Knowing the density of a complete desmosome ρ_{c+d} [21], the mass ratio of the desmoplakin and cadherin m_d and m_c [21] and their measured volumes V_d and V_c , the respective density of cadherin and desmoplakin could be extracted when assuming a weighted average expression for the desmosome as follows:

$$\rho_{c+d} = \frac{V_c}{V_c + V_d} * \rho_c + \frac{V_d}{V_c + V_d} * \rho_d \quad (2.5)$$

Although desmosomes appears as spherical organelles, they were idealised as two cylindrical plates attached by a slender cylinder as shown in Figure 3 for volume partition efficiency in our preliminary model. The thermal conductivity and the specific heat of desmoplakin and cadherin were taken from the literature. [22] [23]

$$\rho_{c+d} = \frac{V_c}{V_c + V_d} * \rho_c + \frac{V_d}{V_c + V_d} * \rho_d \quad (2.6)$$

Additionally, the stratum germinativum contains about 4.6 % of merkel cells that are associated with the somatosensory nerve fibers and 5 to 10 % of melanocytes responsible for skin pigmentation [24] [25]. The stratum spinosum also harbours 1.8 % of langerhan cells that are part of the immune system [26]. Therefore keratinocytes represent up to 90 % of the epidermal cell population and are the main focus of our preliminary study.

	Germinativum	Spinousum	Granulosum	Corneum
ECM				
Density($\text{tonne}\mu\text{m}^{-3}$)	$0.9685 * 10^{-18}$	$0.9527 * 10^{-18}$	$0.9371 * 10^{-18}$	$0.9395 * 10^{-18}$
Thermal Conductivity ($\text{Ns}^{-1}\text{K}^{-1}$)	0.437	0.362	0.283	0.25
Specific Heat ($\text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$)	$3.579 * 10^{12}$	$3.279 * 10^{12}$	$2.9778 * 10^{12}$	$2.301 * 10^{12}$
Cytoplasm				
Density($\text{tonne}\mu\text{m}^{-3}$)	$0.9685 * 10^{-18}$	$0.9527 * 10^{-18}$	$0.9371 * 10^{-18}$	$0.8935 * 10^{-18}$
Thermal Conductivity ($\text{Ns}^{-1}\text{K}^{-1}$)	0.437	0.362	0.283	0.2076
Specific Heat ($\text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$)	$3.579 * 10^{12}$	$3.279 * 10^{12}$	$2.9778 * 10^{12}$	$2.686 * 10^{12}$
Nucleus				
Density($\text{tonne}\mu\text{m}^{-3}$)	$0.3994 * 10^{-18}$	$0.469 * 10^{-18}$	$0.609 * 10^{-18}$	-
Thermal Conductivity ($\text{Ns}^{-1}\text{K}^{-1}$)	0.1802	0.178	0.1839	-
Specific Heat ($\text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$)	$1.48 * 10^{12}$	$1.62 * 10^{12}$	$1.935 * 10^{12}$	-
Cadherin				
Density($\text{tonne}\mu\text{m}^{-3}$)	$1.18605 * 10^{-18}$	$1.18605 * 10^{-18}$	$1.18605 * 10^{-18}$	$1.18605 * 10^{-18}$
Thermal Conductivity ($\text{Ns}^{-1}\text{K}^{-1}$)	0.15	0.15	0.15	0.15
Specific Heat ($\text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$)	$1.483 * 10^{12}$	$1.483 * 10^{12}$	$1.483 * 10^{12}$	$1.483 * 10^{12}$
Desmoplakin				
Density($\text{tonne}\mu\text{m}^{-3}$)	$1.11776 * 10^{-18}$	$1.11776 * 10^{-18}$	$1.11776 * 10^{-18}$	$1.11776 * 10^{-18}$
Thermal Conductivity ($\text{Ns}^{-1}\text{K}^{-1}$)	0.15	0.15	0.15	0.15
Specific Heat ($\text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$)	$1.483 * 10^{12}$	$1.483 * 10^{12}$	$1.483 * 10^{12}$	$1.483 * 10^{12}$

TABLE IV

PHYSICAL AND THERMAL PROPERTIES OF THE KERATINOCYTE
CONSTITUENTS

Figure 3. Desmosomes represented in the numerical model by two desmoplakin circular plates joined by a cadherin cylinder interface

2.1.2 Dermis cell scale model

The dermis is a fibrous tissue composed of two regions: the papillary and the reticular regions. The fibers of the tissue are produced by dermal fibroblasts which represent the dominant cell population in the dermis [27]. In the case of skin injuries, additional cells such as plasma cells that are part of the immune system, histiocytes that are a type of macrophage cells and mast cells that are a type of monocytes can be punctually recruited for the tissue healing. Our study focus on fibroblasts immersed in their *in situ* micro environment. The dermis properties were evaluated on the basis of a hierarchical multiscale model of the *in situ* unit cell representation of the dermal tissue as shown in Figure 4.

In the dermal tissue, fibroblasts are mobile cells evolving in an extracellular matrix (ECM) that is formed of three types of fibers immersed in a gel like medium called the ground substance.

Figure 4. Hierarchical model of the Dermis that includes the macromolecular, cell and tissue scales

The ground substance appears as a clear region in Figure 5 that is composed mainly of water and only 0.22 % of glycosaminoglycons (GAG's) [28] including proteoglycans such as (5%) Heparin Sulfate, (30%) Chondroitin Sulfate, (11%) Dermaton Sulfate, (9%) Keratan Sulfate and non proteoglycan polysaccharides such as (45%) Hyaluron [29]. The GAG's density was calculated as a weighted average of the densities of its constituents given in Table V. [30–32]. The ground substance density is computed based on its GAG's and water contents. The ground substance thermal conductivity and specific heat were chosen equal to the properties of water due to its largely dominant content.

Constituent	Density(g/cm ³)
Hyaluron	0.115
Chondroitin Sulfate	1.34
Dermaton Sulfate	1.34
Keratan Sulfate	1.32
Heparin Sulfate	1.36
GAG	0.78795
Water	1.00
Ground Substance	0.9995

TABLE V

DENSITY OF GLYCOSAMINOGLYCANS IN THE DERMAL GROUND SUBSTANCE

The fibers present in ECM are decomposed of 48.5 % volume fraction of collagen fibers, 6.1 % volume fraction of elastic fibers [33] and 0.03 % volume fraction of reticular fibers. Note that the volume fraction of the reticular fibers made of type III collagen was determined knowing

the molecular weight ratio between elastin (65kDa) and type III collagen (300kDa) and their respective fibril volume ratio of nearly five fold lead to assume a similar mass for both fibers. The number of reticular fiber could then be expressed in terms of the number of elastic fibers knowing the volume ratio of the elastic fiber, the volume ratio of the reticular fiber of 0.03 could then be calculated. Due to their negligible percentage, the reticular fibers are not included in our preliminary model.

For the fibers, collagen fibers have an average diameters of $2.8\mu\text{m}$ [34] and are bundles of collagen fibrils of nearly $0.5\mu\text{m}$ [35]. Each fibril is composed of tropocollagen molecules of average diameter of 1.5nm and $300\mu\text{m}$ average length [36]. The density of the collagen fibers was taken equal to 1.33g/cm^3 [37]. The elastic fibers are composed of amorphous elastin connected by lysyl oxidase(LOX) encapsulated in a thin membrane made of fibulin knots containing tropoelastin molecule linked by fibrillin fibrils [38]. The average diameter of elastic fiber is $4.3\mu\text{m}$, elastic fibrils and microfibrils have diameters of $1.2\mu\text{m}$ and $0.6\mu\text{m}$ respectively [39] as shown in Figure 4. Elastic fibers provide skin elasticity and account for about 3% of the total ECM dry weight [40]. The calculated density of the elastic fibers as the weighted average of the densities of its constitutive amino acids [41] was found to be 1.35g/cm^3 . The thermal conductivity of the collagen fiber was taken equal to its value of $0.55\text{Wm}^{-1}\text{K}^{-1}$ near 38.8°C in sheep skin [42]. The thermal conductivity of the elastic fiber was calculated as the weighted average of the thermal conductivities of its constitutive amino acids near 38.8°C [41,43,44] and found to be equal to $0.0174\text{Wm}^{-1}\text{K}^{-1}$. The specific heat of collagen fibers was chosen equal to $0.2093 \times 10^{12}\text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$ near 38.8°C [45]. The specific heat of the elastic fibers was

taken equal to $3.764 \times 10^{12} \text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$ and $1.7998 \times 10^{12} \text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$ in the loose and dense tissues respectively due to its properties varying with the water content [46], here the ground substance content.

Fibroblast cells appeared in our microscopic observations as elongated irregular shapes containing an ellipsoidal nucleus. The cell morphology was digitally surveyed in our microscopic observations and their cytoplasm were modeled as spindle-shaped body encapsulating nucleus ellipsoid. The densities of the cell nucleus and cytoplasm were taken equal to $1\text{g}/\text{cm}^2$ and $1.04\text{g}/\text{cm}^2$ respectively [47]. Knowing the fibroblast thermal conductivity of $0.5\text{Wm}^{-1}\text{K}^{-1}$ [48], the thermal conductivities of the cell nucleus and the cytoplasm were calculated as positively correlated to the entire cell property and found to be equal to $0.5\text{Wm}^{-1}\text{K}^{-1}$ and $0.52\text{Wm}^{-1}\text{K}^{-1}$ respectively. The specific heats of the cell nucleus and the cytoplasm were calculated as positively correlated to the entire cell property and found to be equal to $3.9 \times 10^{12} \text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$ and $4.056 \times 10^{12} \text{N}\mu\text{ms}^{-1}\text{K}^{-1}\text{tonne}^{-1}$ respectively.

In our microscopic observations, two types of fibrous tissues were identified: either fibers intertwined in a loose organisation or fibers closely packed in a dense network. To determine the volume fraction of their respective constituents (fibers, ground substance and fibroblasts), series of five samples in each type of fibrous dermal tissues were digitally analysed. The morphologies of the different phases present in the microscopic observations of the selected samples shown in Table VI were digitally surveyed to compute their respective volume ratios. The size of the dermal unit cell samples at the cell scale was chosen to be equal to $82.2\mu\text{m}$. This value was calculated as one and a half times the average collagen fiber length [49, 50]. From Table VI,

the average contents of fibers and ground substance were found to be $56.12 \pm 5.7129\%$ and $43.8 \pm 5.7129\%$ respectively in the loose region. The average contents of fibers and ground substance were found to be $79.9 \pm 5.4736\%$ and $20.3 \pm 5.4736\%$ respectively in the dense region. For the *in situ* model at the cell scale, samples BF1 and BF4 were selected due to their specific contents close to the average properties of dense and loose tissues and higher resolution imaging.

Dense				Loose			
Sample	Fiber	GS	Fibroblast	Sample	Fiber	GS	Fibroblast
BF1	88	9.34	2.66	BF3	63.5	32.18	4.32
BF2	82.5	14.51	2.99	BF4	56.5	40.51	2.99
BG2	80	17.01	2.99	BG1	56	40.01	3.99
BG4	76	20.673	3.327	BG3	46	51.762	2.328
BO2	72	24.673	3.327	BO1	58.6	39.74	1.66

TABLE VI

MORPHOMETRIC MEASUREMENTS OF THE VOLUME FRACTIONS OF THE PHASES PRESENT IN DERMAL SKIN SAMPLES IN SELECTED DENSE AND LOOSE REGIONS FROM THE SURGERIES 4BF, 4BG AND 4BO.

2.1.3 Calculation of the effective densities, thermal conductivity and the Specific heat of the Epidermis and the Dermis tissues:

The epidermal and dermal effective properties were computed from the *in silico in situ* models of the constitutive tissue layers of the epidermis and the dermis previously described. For the epidermis, tissue unit cells of the stratum germinativum, stratum spinosum, stratum

Loose Unit Cell

(a)

(b)

(c)

Dense Unit Cell

(d)

(e)

(f)

Figure 5. Light Microscopy observations of the loose (a) and dense (d) dermal regions, finite element discretization of the loose (b) and dense (e) dermal regions and collagen and elastic fiber orientation in the loose (c) and dense (e) dermal regions.

granulosum and stratum corneum were modeled separately as shown in ???. Dense and loose tissue unit cells of the dermis were modeled separately as well as shown in Figure 7

Figure 6. Epidermal sublayers: (a) light microscopy observation, (b) single keratinocyte cell model, 3D thermal field of single keratinocyte in its ECM subjected to a laser surface flux (Biolase iLase Frenectomy) under steady-state condition (c) and under dynamic laser application of 1 second (d).

Figure 7. Dermis loose and dense regions: (a) tissue scale model including fibroblasts on elastic and collagen fibers immersed in ground substance, 3D thermal field of loose and dense regions subjected to a laser surface flux (Biolase iLase Frenectomy) under steady-state condition (b) and under dynamic laser application of 1 second (c).

The effective density of each tissue layer was calculated as a ratio between the effective mass and the effective volume of the considered unit cell.

Each layer thermal conductivity was calculated between both ends of the unit cells subjected to the considered laser surface heat flux on the left hand side and convective boundary condition related to the property of the immediate layer above and under the analysed layer. The convective coefficients of the epidermal layers were calculated based on the respective hydrous and lipid contents. The keratin content of the ECM was neglected in the calculation of the convective coefficient because of its solid nature. The convective coefficients of the dermal layers was chosen equal to the property of water because the fibers were solid phases as shown in `refDermis`. A static thermal analysis was applied to each tissue sublayers to calculate the effective thermal conductivity, k_i as follows:

$$k_i = Q_{\text{laser}}^s \frac{T_1 - T_0}{L} \quad (2.7)$$

where T_0 and T_1 are the nodal temperature average on the surfaces $l = 0$ and $l = L$ as shown in Figure 8 calculated as follows:

$$T_0 = \frac{\sum_{i=0}^{n_s 0} T_i^{S_0}}{n_s 0} \quad (2.8a)$$

$$T_1 = \frac{\sum_{i=0}^{n_s L} T_i^{S_L}}{n_s L} \quad (2.8b)$$

where $n_s 0$ and $n_s L$ are the number of nodes on surfaces S_0 and S_L respectively.

Figure 8. Calculation of the effective thermal conductivity of keratinocytes and fibroblasts in their respective ECMs

The specific heats of the epidermal and the dermal sublayers were calculated as the rise in temperature with time under the surface flux of the considered laser(surgery 4BF) in a dynamic thermal analysis of 1s in the middle of the tissue unit cell ($L/2$). As the specific heat was observed to gradually rise with time in a linear manner until 0.8 s, the studied time period spanned between 0.2 and 0.6 s. The effective tissue specific heat was then computed as follow:

$$c = \frac{Q_{\text{laser}}}{m} \frac{T_{L/2}^{t_2} - T_{L/2}^{t_1}}{t_2 - t_1} \quad (2.9a)$$

$$T_{L/2}^{t_2} = \frac{\sum_{i=0}^{n_{sL/2}} T_i^{t_1}}{n_{sL/2}} \quad (2.9b)$$

where $T_{L/2}^{t_1}$ and $T_{L/2}^{t_2}$ are the average temperature on plane $L/2$ as shown in the Figure 9.

The effective properties of the tissue unit cells are given in Table VII.

Figure 9. Calculation of effective specific heat of keratinocytes and fibroblasts in their respective ECMs

	Mass (tonne)	Volume (mm ³)	Density (tonne mm ⁻³)	Thermal Con- ductivity (N s ⁻¹ K ⁻¹)	Specific Heat (N mm) tonne ⁻¹ K ⁻¹)
Epidermis					
Stratum Corneum	4.25×10^{-16}	463.1	0.917×10^{-9}	0.44	1.78×10^9
Stratum Gran- ulosum	4.43×10^{-16}	485.8	0.912×10^{-9}	0.53	1.72×10^9
Stratum Spinosum	7.66×10^{-16}	840 .0	0.912×10^{-9}	0.63	1.65×10^8
Stratum Ger- minativum	6.46×10^{-16}	727.9	0.888×10^{-9}	0.74	1.68×10^8
Dermis					
Dense	1.07×10^{-13}	94596.51	1.131×10^{-9}	0.51	1.42×10^6
Loose	9.77×10^{-14}	94596.51	1.033×10^{-9}	0.89	1.55×10^6

TABLE VII

DENSITIES AND THERMAL PROPERTIES OF THE EPIDERMAL AND THE DERMAL CELLS IN THEIR RESPECTIVE ECMS.

2.2 Morphology digitization

The skin morphology is explicitly digitized from reflective light microscopy observations. The skin tissue layers are modeled as separate continua of specific thermal properties taken initially from the literature in the preliminary model. The contours of the four layers constituting the epidermis and the two upper layer of the dermis are surveyed using Plotdigitizer [51] as shown in Figure 10(c). A script in Python is then written using the digitized contours to feed the finite element code ABAQUS [52]. A skin layer of thickness $200\text{ }\mu\text{m}$ is constructed for a 3D thermal FEM analysis. The sample average height and width are noted H and L and are about 1 mm . For each surgery, the morphometric measurements of the successive layers are taken and given in Table VIII

Figure 10. (a) Light microscopic observations of antibody stained porcine skin sample, (b) contrast and color enhanced image of the same skin sample, (c) digitized morphology of the sample along the edge of the laser cut.

Thickness (μm)	Surgery Names								
	1	2a	2c	3	4BF	4BG	4BO	5	6
Height	1000	1000	1000	3194	3125	4464	3100	2643	3031
Epidermis									
Stratum corneum	10	6	6	10	10	14	10	15	15
Stratum granulosum	5	3	3	5	5	7	5	7	9
Stratum spinosum	30	10	10	38	30	40	39	28	26
Stratum germinativum	5	3	3	5	5	7	5	9	9
Dermis									
Papillary region	30	10	10	38	85	65	53	57	56
Reticular region	600	600	600	1889	1250	1897	1765	1429	1689
Hypodermis									
Adipose tissue	-	-	-	540×1008	1409	1804	1169	935	874

TABLE VIII

MORPHOMETRIC MEASUREMENTS OF THE SUCCESSIVE SKIN LAYERS IN THE PERFORMED LASER SURGERIES.

Figure 10 shows the successive steps necessary to enhance the microscopic observation before the morphology digitization. Figure 10(a) shows the antibody stained skin sample views under reflective light microscopy. The natural image is then photo-edited using Gimp to enhance the visibility of all the skin constituents shown in Figure 10(b). For this image, the hue factor and the color saturation level are increased. In this enhanced image the sample contours are digitized to build the FEM discretization of the model using ABAQUS as shown in Figure 10(c).

2.3 Thermal Modeling

2.3.1 Bio-heat equation in transient regime

The laser surgery is described by the Bio-Heat equation. In transient regime, the variation of heat flow within the skin is equal to the sum of the Fourier conductive heat flow, the convective heat flow between skin and air, the metabolic volume heat flow and the heat flow due to the laser source as follows:

$$\rho_{\text{skin}} c_{\text{skin}} V \frac{\partial T}{\partial t} = V \nabla (k_{\text{skin}} \nabla T) + h_{\text{air/skin}} A_{\text{conv}} (T_{\text{surface}} - T_{\text{air}}) + V Q_m^v + A_e Q_{\text{laser}}^{\text{se}} + A_d Q_{\text{laser}}^{\text{sd}} \quad (2.10)$$

where ρ_{skin} is the skin density. c_{skin} is the specific heat. k_{skin} is the skin conductivity and V is the sample volume. The skin medium is decomposed into a series of constituents detailed in Table IX immersed in a collagenic fibrilla matrix. The values of the densities and the thermal properties of the constituents and the epiderm and derm matrices are given in Table IX.

Skin constituents	Density (tonne mm ⁻³)	Specific heat (N mm tonne ⁻¹ K ⁻¹)	Conductivity (N s ⁻¹ K ⁻¹)	Reference
Epidermis	$1.2 * 10^{-9}$	$3.59 * 10^9$	0.21	[53] [54] [55] [56] [57]
Dermis	$1.2 * 10^{-9}$	$3.30 * 10^9$	0.37	[53] [54] [55] [56] [57]
Arteries	$1.13 * 10^{-9}$	$3.56 * 10^9$	0.414	
Veins	$1.0727 * 10^{-9}$	$3.63 * 10^9$	0.486	
Muscle	$1.2 * 10^{-9}$	$3.45 * 10^9$	0.30	[53] [54] [55] [56] [57]
Nerves	$1.2 * 10^{-9}$	$3.45 * 10^9$	0.30	[53] [54] [55] [56] [57]
Hair Cavity	$1.2 * 10^{-9}$	$3.45 * 10^9$	0.30	[53] [54] [55] [56] [57]
Adipose Tissue	$9 * 10^{-10}$	$3.33 * 10^9$	0.3075	[58] [59] [60]
Sebaceous Gland	$9 * 10^{-10}$	$2 * 10^9$	0.2	[61] [62]
Appocrine Sweat Gland	$9 * 10^{-10}$	$2 * 10^9$	0.2	[61] [62]
Lymph Vessels	$1.041 * 10^{-9}$	$3.68 * 10^9$	0.60	[63] [64]
Pacinian Corpus-cule	$1.2 * 10^{-9}$	$3.45 * 10^9$	0.30	
Meissner Corpus-cule	$1.2 * 10^{-9}$	$3.45 * 10^9$	0.30	

TABLE IX

THERMAL PROPERTIES OF THE DIFFERENT SKIN LAYERS.

The densities and the thermal properties of most of the skin constituents are taken from our hierarchical model and from the literature given in Table IX. For the epidermis and dermis sublayers the values applied were calculated in the previous paragraphs 2.1.3. the values given in Table VII and for comparison with the literature. For the venules and arterioles, the densities and the thermal properties are calculated by a homogenisation approach that averages the densities and the thermal properties of blood flow within thin muscle tubes. The formula to calculate these properties weighs the muscle and the blood properties by the tube inner and outer radii, d_i and d_o respectively. The venule density is calculated as follows:

$$\rho_v = \left(\frac{d_i}{d_o}\right)\rho_b + \left(1 - \frac{d_i}{d_o}\right)\rho_m \quad (2.11)$$

where d_i and d_o are equal to 0.04mm and 0.044mm [65]. ρ_b is the blood density equal to $1.06 \times 10^{-9} \text{tonne mm}^{-3}$ and ρ_m is the muscle density given in the Table IX. The venule specific heat k and the thermal conductivity c are calculated using the same formula with a value of blood specific heat and thermal conductivity equal to $3.65 \times 10^9 \text{N mm tonne}^{-1} \text{K}^{-1}$ and 0.505 respectively [66]. The muscle specific heat and the thermal conductivity are given Table IX. The same approach is applied to calculate the density and thermal properties of the arterioles with inner and outer radii of 0.05mm and 0.09mm respectively [65].

The heat flow at the convective boundaries is expressed by:

$$q_{\text{convection}} = h_{(\text{air/skin})} * A_{\text{conv}} * (T_{\text{surface}} - T_{\text{air}}) \quad (2.12)$$

where A_{conv} designates the surface area where convection takes place between the skin layer and the surrounding air and $h_{\text{air/skin}}$ is the convection coefficient. The skin/air interface convection coefficient is taken from the literature and equal to $0.0057 \text{ N mm}^{-1} \text{ s}^{-1} \text{ K}^{-1}$ [67] [68].

The metabolic volumetric heat flow, Q_m^v , was defined as the volume heat necessary to balance the averaged convection heat loss between skin and air during a duration of 1 second as follows:

$$Q_m^v = \frac{\left(\frac{-1}{2} h_{\text{air/skin}} A (T_{\text{skin}} - T_{\text{air}}) \Delta t\right) \frac{1}{V}}{\Delta t} \quad (2.13)$$

$$Q_m^v = \frac{\left(\frac{-1}{2} 5.7 \text{ N m}^{-1} \text{ s}^{-1} \text{ K}^{-1} * 0.001 \text{ m} * 0.0002 \text{ m} (38.8^\circ - 25^\circ) 1 \text{ s}\right)}{1 \text{ s}} \frac{1}{0.001^2 \text{ m}^2 * 0.0002 \text{ m}} \quad (2.14)$$

$$Q_m^v = 0.03933 \text{ N m m}^{-2} \text{ s}^{-1} \quad (2.15)$$

On the surfaces in contact with the body, either the left or right edges and the bottom surfaces of the samples, the temperature is fixed to the body constant temperature of 38.8°C in the case of porcine tissue [69] as shown in Table XI. The following prescribed constant temperature boundary condition is then expressed by:

$$T = \vec{T} = 38.8^\circ \text{C} \quad (2.16)$$

2.3.2 Laser heat source model

On either the right or left surface of the sample, the laser is applied as boundary conditions and modeled by a prescribed surface heat flux $Q_{\text{laser}}^{\text{se}}$ and $Q_{\text{laser}}^{\text{sd}}$ on the epidermis and the dermis respectively. The laser heat flux expression includes the wound geometry, the angle of

application and the heat ratio that penetrates the sample by the coefficient ρ_{wound} , ρ_{angle} and ρ_{prop} respectively. For direct laser application ρ_{angle} is equal to 1. For the study of samples on a single side of the wound, ρ_{wound} is equal to 1 as shown in Figure 11.

Figure 11. Laser model including the wound geometry, the angle of application and the heat ratio that penetrates the sample.

The coefficient ρ_{prop} pertains to the amount of energy that penetrates into the skin depth in the transverse direction modeled. ρ_{prop} depends on the absorption coefficient μ which is function of the laser wave length and the skin layer (epidermis or dermis). ρ_{prop} is expressed by:

$$\rho_{\text{proportion}} = \frac{\int_0^L \frac{\partial P_{\text{exp}}(z)}{\partial z} dz}{\int_0^\infty \frac{\partial P_{\text{exp}}(z)}{\partial z} dz} = \frac{\int_0^L \mu \vec{P}_{\text{exp}} e^{-\mu z} dz}{\int_0^\infty \mu \vec{P}_{\text{exp}} e^{-\mu z} dz} = 1 - e^{-\mu L} \quad (2.17)$$

where $P_{\text{exp}}(z)$ is the volume heat flux described by Beer's law [70–72].

The laser surface heat flux is finally expressed by:

$$Q_{\text{laser}}^s = \rho_{\text{wound}} \rho_{\text{angle}} \rho_{\text{proportion}} (1 - R(\lambda)) P_{\text{exp}} \quad (2.18)$$

where R is the reflection [70] which is function of the laser wave length λ . The laser power or surface heat flux P_{exp} is experimentally determined as the ratio between the laser surface energy, $E_{\text{exp}}^{\text{laser}}$, in J cm^{-2} and the duration of the pulse, Δt_{pulse} that are measured in Professor Lacey's Lab as follows:

$$P_{\text{exp}} = \frac{E_{\text{exp}}^{\text{laser}}}{\Delta t_{\text{pulse}}} \quad (2.19)$$

The laser properties for all the surgeries are summarized in Table X.

2.3.3 Steady State Bio-Heat equation

In the case of a continuous laser, the heat source is modeled by the bio-heat equation in steady state regime. The simulation is static and no longer dynamic. The bio-heat equation becomes a Laplace equation as follows:

$$0 = V \nabla (k_{\text{skin}} \nabla T) + h_{\text{air/skin}} A_{\text{conv}} (T_{\text{surface}} - T_{\text{air}}) + V Q_{\text{m}}^v + A_e Q_{\text{laser}}^{\text{se}} + A_d Q_{\text{laser}}^{\text{sd}} \quad (2.20)$$

2.3.4 Simulation timeline

In Surgery 1, the dynamic and static boundary conditions were modeled as described in Figure 38 and Figure 12. The heat source surface flux of $12 \text{ milli W mm}^{-2}$ is applied on the

Laser Properties	Surgery								
	1	2a	2c	3	4BO	4BG	4BF	5	6
P_{exp} ($\text{N mm}^{-1} \text{s}^{-1}$)	12	4147.5	2500	951	15.1	5.5	6.4	13	3.3
$\delta t_{\text{pulse}}(\text{s})$	1	0.1	0.1	1	1	1	1	1	1
$\lambda(\mu\text{m})$	2.08	0.8052	0.9809	0.94	0.94	0.94	0.94	0.975	1.55
Skin Properties									
$L(\text{mm})$	0.99	1.06	0.69	2.1	5.5	3.27	3.59	1.89	2.95
R	0.04	0.577	0.488	0.504	0.504	0.504	0.504	0.49	0.065
Epidermis									
$\mu_e(\text{mm}^{-1})$	4.12	1.7	1.62	1.66	1.66	1.66	1.66	1.625	4
$\rho_{\text{proportion}}^e$	0.983	0.836	0.674	0.969	0.999	0.995	0.997	0.954	0.999
$Q_{\text{laser}}^{\text{se}}$ ($\text{N mm}^{-1} \text{s}^{-1}$)	11.32	1467.37	863.49	486.36	7.49	2.72	3.17	6.32	3.085
Dermis									
$\mu_d(\text{mm}^{-1})$	1.74	0.5	0.58	0.54	0.54	0.54	0.54	0.575	2.1
$\rho_{\text{proportion}}^d$	0.821	0.413	0.331	0.678	0.948	0.8292	0.856	0.663	0.997
$Q_{\text{laser}}^{\text{sd}}$ ($\text{N mm}^{-1} \text{s}^{-1}$)	9.4	724.39	423.68	319.95	7.10	2.26	2.72	4.39	3.08

TABLE X

LIST OF THE PROPERTIES OF THE DIFFERENT LASERS APPLIED DURING SURGERIES 1, 2A, 2C, 3, 4BO, 4BG, 4BF, 5 AND 6.

Figure 12. Example of boundary conditions applied in the model for Surgery 1

right side. Note: the heat source flux of the Holmium laser is set to the average value of the experimental measurements as a laser is continuous and generates a heat surface energy of 912 to 1583 milli J cm^{-2} . A dynamic model of 1 s is also tested.

In surgery 2A, the sample is subjected to a heat source surface flux of 4147.5 milli W mm^{-2} on the right side for a pulse of 0.1sec as shown in Figure 39.

The sample in surgery 2c is subjected to a heat source surface flux of 2500 milli W mm^{-2} on the right side for 0.1sec as shown in Figure 40.

In Surgery 3, the boundary conditions are described in Figure 41. The laser heat surface flux is 951 milli W mm^{-2} which leads to the surface heat flux of $Q_{laser}^{se} (\text{N mm}^{-1} \text{ s}^{-1}) = 16.16$ in the epidermis and $Q_{laser}^{sd} (\text{N mm}^{-1} \text{ s}^{-1}) = 11.31$ in the dermis and are applied on the sample right side. The static simulation leads to very high temperature. Therefore an additional dynamic

simulation of 0.1 second is performed. The time of the dynamic simulation is identified so that the temperature range remains consistent with the temperatures observed in the other surgeries causing similar heat burns shown in the histology study.

In Surgeries 4BO, BG, BF, 5 and 6 the lasers are continuous and the models are static. For surgery 4BF, it was observed that the fixed temperature boundary condition applied on the bottom edge of the sample influenced the propagation of the heat flow within the tissue. Additionally part of the bottom sample edge seemed to have been torn. Therefore for the simulation of this surgery, the fixed temperature boundary condition was applied along a distance on the bottom edge that provided a heat flux profile that seemed to match the burn delimitation in the histological observation in Figure 44.

Laser name	sim type	Cycle	Convection	Fixed temp	Q_{laser}^s
Holmium Laser	Dyn	1	T	SL	SR
		2	T+SRp	SL	SRp
	Stat	1	T+SRp	SL	SRp
Lype-FD-A Laser	Dyn	1	T	SL+B	SR
Lype-FD-C Laser	Dyn	1	T	SL+B	SR
Dormier Laser	Dyn	1	T	SL	SR
	Stat	1	T	SL	SR
4BO	Stat	1	T+SLp	B+SR	SL
4BG	Stat	1	T+SLp	B+SR	SL
4BF	Stat	1	T+SLp	B+SR	SL
5	Stat	1	T	B+SL	SR
6	Stat	1	T	B+SL	SR

TABLE XI

LIST OF THE BOUNDARY CONDITIONS APPLIED DURING THE SURGERIES. T DESIGNATES TOP, B BOTTOM, SR SIDERIGHT, SL DESIGNATES SIDELEFT, SRP IS SIDERIGHT(PARTIAL).

CHAPTER 3

RESULTS

Dynamic analysis: Figure 47(a) and (b) show the temperature field after 1 second and 2 second respectively for the thermal dynamic simulation of the Medilas D Holmium laser effect on porcine skin in Surgery 1. The boundary conditions are given in Figure 38. Figure 48(a) and (b) show the the heat flux magnitude field after 1 second and 2 second respectively for the thermal dynamic simulation of the Holmium laser effect on porcine skin in Surgery 1. In Figure 13(a) the region of significant thermal damage is visible by Antibody staining and in agreement with the zones where the temperature ranges from 50.3°C to 44.5°C (green) and 44.5°C to 42°C (cyan) in the FEM model. In Figure 48(b) the heat flux of higher magnitude appears localized near the region of heat application. The heated zone is about 231 μm deep and the secondary heated zone about 192 μm deep in the experiment.

3.1 Temperature and heat flux profiles for Surgery 1

Static Analysis: Figure 14(b) and (c) shows the resulting temperature field and the heat flux magnitude field respectively for the static Bio-Heat analysis of the Holmium laser on porcine skin. In Figure 14(b) the zones of significant thermal damage that are visible by antibody staining shown in Figure 14(b) corresponds the region where the temperature ranges from 45.9°C to 44.1°C (dark purple) and 44.1°C to 42.4°C (light pinkish purple) in the model. In Figure 14(c) the heat flux of higher magnitude appears localized near the same region where the

Figure 13. Identification of the temperature threshold in the damage areas in Surgery 1
Dynamic: (a) histology, (b) temperature field, (c) heat flux.

heat is applied. The highly damaged zone is about $231\text{ }\mu\text{m}$ deep and the secondary damaged zone about $192\text{ }\mu\text{m}$ deep in the experiment.

3.2 Temperature and heat flux profiles for Surgery 1

3.3 Surgery 2A

Figure 15(a) and (b) present the resulting temperature field and the heat flux magnitude field respectively for the dynamic Bio-Heat simulation of the Lype Surgilas Diode Laser System A on porcine skin for 0.1 second. In Figure 15(b) the zones of significant thermal damage shown in antibody staining shown in Figure 15(a) corresponds to the zones where the temperature ranges from 844.1°C to 441.5°C (red-yellow-green) and 441.5°C to 240.1°C (cyan) in the model. The region in grey above 120°C , a temperature at which tissue vaporizes, shows the region where the tissue is burnt. In Figure 15(c) the heat flux of higher magnitude appears localized near the

Figure 14. Identification of the temperature threshold in the damage areas in Surgery 1 steady-state: (a) histology, (b) temperature field, (c) heat flux

region of heat application. The highly damaged zone is about 214 μm deep and the secondary damaged zone about 107 μm deep in the antibody histology.

3.4 Surgery 2C

Figure 16(b) and (c) present the resulting temperature field and the heat flux magnitude field respectively for the dynamic Bio-Heat simulation of the Lype Surgilas Diode Laser System C on porcine skin for 0.1 second. In Figure 16(b) the zones of significant thermal damage visible by antibody staining shown in Figure 16(b) corresponds to the zones where the temperature ranges from 476.5°C to 257.7°C (red-yellow-green) and 257.7°C to 148.2°C (cyan) in the model. The region above 120°C, a temperature at which tissue vaporizes, shows the region where the tissue has been burnt beside the tissue showed. In Figure 16(c) the heat flux of higher magnitude

Figure 15. Identification of the temperature threshold in the damage areas in Surgery 2A dynamic after 0.1 s: (a) histology, (b) temperature field, (c) heat flux

appears localized near the region of heat application. The highly damaged zone is about $231\mu\text{m}$ deep and the secondary damaged zone about $115\mu\text{m}$ deep in the antibody histology.

3.5 Surgery 3

Dynamic Analysis: Figure 17(b) and (c) present the resulting temperature field and the heat flux magnitude field respectively for the dynamic Bio-Heat simulation of the Dormier Medtech Medilas D Flexipulse LPS laser on porcine skin for 0.1 second. In Figure 17(b), the zones of significant thermal damage visible by antibody staining shown in Figure 17(b) corresponds to the zones where the temperature ranges from 90.1°C to 65.0°C (red-yellow-green) and 65.0°C to 51.9°C (cyan) in the model. In Figure 17(c) the heat flux of higher magnitude appears localized near the region of heat application. The highly damaged zone is about $437\mu\text{m}$ deep and the secondary damaged zone about $285\mu\text{m}$ deep in the antibody histology.

Figure 16. Identification of the temperature threshold in the damage areas in Surgery 2C dynamic after 0.1 s: (a) histology, (b) temperature field, (c) heat flux

Figure 17. Identification of the temperature threshold in the damage areas in Surgery 3 dynamic after 0.1 s ($P_{\text{exp}} = 95.1 \text{ W cm}^{-2}$): (a) histology, (b) temperature field, (c) heat flux

Static Analysis: Figure 18 (b) and (c) show the resulting flux field and the temperature magnitude field respectively for the Static Bio-Heat simulation of the Dormier Medtech Medilas D Flexipulse LPS laser on porcine skin. In Figure 18(b) the zones of significant thermal damage is located in the upper skin layer with temperature ranges from 479.6°C to 369.4°C (yellow and red). The static model represents a permanent regime simulation with continuous heat application. The transient model shown in Figure 18 where the heat is progressively applied display a heated region which is more in agreement with the histology staining. The dynamic model may be more relevant for the modeling of this laser in particular if the heat was progressively applied down in the wound and more heat was applied for longer time at the bottom of the wound. In Figure 18(c) the heat flux of higher magnitude above 200mWmm^{-2} in steady-state condition appears localized in regions that are consistent with the thermal damage observed in antibody staining image shown in Figure 18(b). For this laser the experimental measure of P_{exp} of 95Wcm^{-2} is very high. The simulation is also run for a surface heat flux P_{exp} of 9.5Wcm^{-2} of which the results are given in Figure 19 and seems more reasonable.

3.6 Surgery 4

Simulations for the Biolase iLase setting Gingivectomy without tip (Surgery 4 BO) are given in Figure 20 (b) and (c). The morphology of BO has been digitized in Figure 33. In Figure 20(a) the zones of significant thermal damage visible by antibody staining corresponds to the zones where the temperature ranges from 63.1°C to 55.0°C (red-yellow) and 55.0°C to 48.9°C (green) in the model. The highly damaged zone is about $833\mu\text{m}$ deep and the secondary damaged zone about $833\mu\text{m}$ deep in the antibody histology.

Figure 18. Identification of the temperature threshold in the damage areas in Surgery 3 steady-state ($P_{\text{exp}} = 95.1 \text{ W cm}^{-2}$): (a) histology, (b) temperature field, (c) heat flux

Figure 19. Identification of the temperature threshold in the damage areas in Surgery 3 steady-state ($P_{\text{exp}} = 9.51 \text{ W cm}^{-2}$): (a) histology, (b) temperature field, (c) heat flux

Figure 20. Identification of the temperature threshold in the damage areas in Surgery 4BO steady-state: (a) histology, (b) temperature field, (c) heat flux

Simulations for the Biolase iLase setting Gingivectomy with tip (Surgery 4 BG) are given in Figure 21 (b) and (c). The morphology of BG has been digitized in Figure 33. In Figure 21(a) the zones of significant thermal damage visible by antibody staining corresponds to the zones where the temperature ranges from 51.3°C to 47.1°C (red-yellow) and 47.1°C to 45.0°C (green) in the model. The highly damaged zone is about 333 μm deep and the secondary damaged zone about 400 μm deep in the antibody histology.

Simulations for the Biolase iLase setting Gingivectomy with tip (Surgery 4 BF) are given in Figure 22 (b) and (c). The morphology of BF has been digitized in Figure 35. In Figure 22(a) the zones of significant thermal damage visible by antibody staining corresponds to the zones where the temperature ranges from 71.4°C to 60.5°C (red-orange) and 60.5°C to 55.1°C (orange-yellow) in the model. The highly damaged zone is about 978.03 μm deep and the secondary damaged zone about 707.9 μm deep in the antibody histology. The most heated region appeared

Figure 21. Identification of the temperature threshold in the damage areas in Surgery 4BG steady-state: (a) histology, (b) temperature field, (c) heat flux

in the histological observation further inside the large indent on the left side of the sample. In the simulation the most heated region does not penetrate as deep in the sample. However, we note that the upper tip of the indent resemble a lymph vessel and we know that lymph vessel appeared usually more heated than the surrounding tissue. It is conceivable to assume that this presumed lymph vessel had exploded in the indent and had stored more heat prior to explosion that had diffused in the surrounding tissue. This scenario could partially explain the difference between the simulation and the experiment.

3.6.1 Surgery 5

Simulations for the IPG DLR-200-AC diode laser (Surgery 5) are given in the Figure 23 (b) and (c). The morphology has been digitized in Figure 36. In Figure 23(a) the zones of significant thermal damage visible by antibody staining corresponds to the zones where the

Figure 22. Identification of the temperature threshold in the damage areas in Surgery 4BF steady-state: (a) histology, (b) temperature field, (c) heat flux

temperature ranges from 54.82 $^{\circ}\text{C}$ to 50.82 $^{\circ}\text{C}$ (red-orange) and 50.82 $^{\circ}\text{C}$ to 46.81 $^{\circ}\text{C}$ (yello-green) in the model. The highly damaged zone is about 129.82 μm deep and the secondary damaged zone about 148.9 μm deep in the antibody histology.

3.6.2 Surgery 6

Simulations for the IPG ELR-1550-50 diode laser(Surgery 6) are given in the Figure 24 (b) and (c). The morphology has been digitized in Figure 37. In Figure 24(a) the zones of significant thermal damage visible by antibody staining corresponds to the zones where the temperature ranges from 76.259 $^{\circ}\text{C}$ to 67.6 $^{\circ}\text{C}$ (red-orange) and 67.6 $^{\circ}\text{C}$ to 57.529 $^{\circ}\text{C}$ (yello-green) in the model. The highly damaged zone is about 157.37 μm deep and the secondary damaged zone about 16.882 μm deep in the antibody histology.

Figure 23. Identification of the temperature threshold in the damage areas in Surgery 5 steady-state: (a) histology, (b) temperature field, (c) heat flux

Figure 24. Identification of the temperature threshold in the damage areas in Surgery 6 steady-state: (a) histology, (b) temperature field, (c) heat flux

For all the laser surgeries, Table XII shows the temperature of the damaged region and its width in the simulations and experiment.

Laser	Regime	Experimental Damage		Numerical Zone
		Colour	Width	Temperature
1	S	Purple	231	55.9 – 48.7
		Brown	192	48.7 – 45.7
	D	Purple	231	50.3 – 44.5
		Brown	192	44.5 – 42.0
2A	D	Purple	214	558.3 – 70
		Brown	107	70 – 39.2
2C	D	Purple	231	498 – 48
		Brown	115	48 – 40.5
3 (9.5Wcm ⁻²) (95.1Wcm ⁻²) (95.1Wcm ⁻²)	S	Purple	437	52.7 – 49.3
		khaki	285	49.3 – 45.8
	S	Purple	437	479.6 – 369.4
		khaki	285	369.4 – 259.2
	D(0.1s)	Purple	437	91.1 – 78.1
		khaki	285	78.1 – 65.0
4BO	S	Purple	833	63.1 – 55.0
		Khaki	833	55.0 – 48.9
4BG	S	Purple	333	51.3 – 47.1
		Khaki	400	47.1 – 45.0
4BF	S	Purple	383.6	71.4 – 60.5
		Khaki	978.03	60.5 – 55.1
		Green	707.9	55.1 – 47.0
5	S	Purple	129.82	54.82-50.82
		Khakee	148.9	50.82-46.81
6	S	Khakee	157.37	76.26-67.60
		Green	169.882	67.6-57.53

TABLE XII

THERMAL FIELD IDENTIFICATION IN THE REGIONS TREATED BY LASER AND DEPTH MEASUREMENTS OF THE THERMAL DAMAGE.

CHAPTER 4

DISCUSSION AND CONCLUSION

The experimental results show that the localised heated regions in porcine skin observed in the numerical model is in agreement with the antibody staining observations of the skin samples harvested during surgery. These results appear to show adequate magnitude of the heat source and location of application of the different boundary conditions. When comparing the numerical results with the antibody staining observations, the skin damaged tissue seems to be localized in regions where the temperature is at least $50 - 55^{\circ}\text{C}$. The heat rapidly dissipates when the laser application is removed.

Figures Figure 25 and Figure 26 shows the thermal fields in two finite element models that either uses epidermal and dermal properties from the literature (a) or properties calculated by the previously described hierarchical approach. In both surgeries the hierarchical model shows a larger temperature span and a higher temperature region of dimensions resembling the microscopic observation. However despite varying properties of the skin constituents with higher temperatures [71], the presented preliminary model applies single value skin properties throughout the simulations. The orientation of the collagen and elastic fibers play a role in the anisotropic response upon mechanical loading and could also play a role in redistributing the temperature diffusion throughout the tissues modeled in our unit cell model.

As stated in 2.1.2 two distinct tissue regions were detected in the dermis. Simulations including dense and loose region were therefore developed. Even further away from the site of

Figure 25. Porcine skin sample for surgery 5: (a) antibody staining observation, temperature fields (b) calculated using epidermal and dermal property values from literature (c) calculated using hierarchical models for the epidermal and dermal tissues.

Figure 26. Porcine skin sample for surgery 6: (a) antibody staining observation, temperature fields (b) calculated using epidermal and dermal property values from literature (c) calculated using hierarchical models for the epidermal and dermal tissues.

laser application alternating loose and dense regions near large skin constituents such as hair follicle seems to naturally present in the dermal tissue. However, near the site of kllaser application the tissue observed is usually dense, which indicates a possible effect of heat application in vaporising part of the water content of the fibrous tissue on one hand and contributing to possible crosslinks between collagen fibers, a large macro molecule, on the other hand. Therefore a series of simulation with dense or loose tissue near the site of laser application were conducted to appreciate the heat flux redistribution in a loose tissue that becomes progressively dense. In Figures Figure 27 and Figure 28 we observe that the progressive densification of the dermal fibrous tissue tends to slow down the heat flux and acts like a barrier to further propogation.

Figure 27. Porcine skin sample from Surgery 5: heat flux distribution for (a) loose reticular region and (b) partially dense reticular region.

Figure 28. Porcine skin sample from Surgery 6: heat flux distribution for (a) loose reticular region and (b) partially dense reticular region.

The presented finite element model made it possible to study the effects of temperature elevation on the skin tissue morphology and identify the temperature in the damage regions. The FEM model could make it possible to perform *in silico in situ* experiments for other types of skins such as human skin exposed to virtual laser treatments. For instance numerical model of infrared CO₂ laser have been tested to study the temperature distribution in human skin [72] in 2D. Our 3D model offers to compensate for the 2D geometrical limitations that cannot model skin constituents besides the epidermis and the dermis.

A 3D FEM model was developed in this work to primarily study the effect of laser application on porcine skin tissue. The model includes the skin microstructure from the tens of micron level

to the tissue scale and include different constituents such as apocrine glands, nerves, blood and lymph vessels. The numerical results to identify the temperatures produced by surgical laser devices and measure the damage regions validated by the experiments. The model provide some improvements compare to the literature data on skin properties. Finally the model introduced insights about possible change in the fibrous structure induced by laser application.

APPENDIX

MODEL GEOMETRY

Images of the porcine skin samples harvested for each surgery are presented below. The skin samples were treated by antibody staining. An image color enhancement of the microscopic observations renders the different skin constituents more visible to be later discretised to build the finite element model of the thermal treatment applied during surgery on porcine skin.

Figure 29. Porcine skin sample from surgery 1 (a) antibody staining observation, (b) discretised morphology in the FEM model.

APPENDIX (Continued)

Figure 30. Porcine skin sample from Surgery 2A: (a) antibody staining observation, (b) discretised morphology in the FEM model.

Figure 31. Porcine skin sample from Surgery 2C: (a) antibody staining observation, (b) discretised morphology in the FEM model.

APPENDIX (Continued)

Figure 32. Porcine skin sample from Surgery 3: (a) antibody staining observation, (b) discretised morphology in the FEM model.

Figure 33. Porcine skin sample from Surgery 4BO: (a) antibody staining observation, (b) discretised morphology in the FEM model.

APPENDIX (Continued)

Figure 34. Porcine skin sample from Surgery 4BG: (a) antibody staining observation, (b) discretised morphology in the FEM model.

APPENDIX (Continued)

Figure 35. Porcine skin sample from Surgery 4BF: (a) antibody staining observation, (b) discretised morphology in the FEM model.

APPENDIX (Continued)

Figure 36. Porcine skin sample from Surgery 5: (a) antibody staining observation, (b) discretised morphology in the FEM model.

APPENDIX (Continued)

Figure 37. Porcine skin sample from Surgery 6: (a) antibody staining observation, (b) discretised morphology in the FEM model.

APPENDIX

BOUNDARY CONDITIONS

The boundary conditions including the initial body temperature, the convection with the surrounding air and the laser heat application with their timeline of application are given below. The static boundary conditions are identical to the dynamic boundary condition but applied instantaneously.

Figure 38. Boundary conditions applied for the preliminary model of Surgery 1: (a) location of the boundary conditions applied in static and dynamic simulations, (b) timeline of the laser application in dynamic simulation.

APPENDIX (Continued)

Figure 39. Boundary conditions applied for the preliminary model of Surgery 2A: (a) location of the boundary conditions applied in dynamic simulations, (b) timeline of the laser application in dynamic simulation.

APPENDIX (Continued)

Figure 40. Boundary conditions applied for the preliminary model of Surgery 2C: (a) location of the boundary conditions applied in dynamic simulations, (b) timeline of the laser application in dynamic simulation.

APPENDIX (Continued)

Figure 41. Boundary conditions applied for the preliminary model of Surgery 3: (a) location of the boundary conditions applied in static and dynamic simulations, (b) timeline of the laser application in dynamic simulation.

APPENDIX (Continued)

Figure 42. Boundary conditions applied for the preliminary model of Surgery 4BO, location of the boundary conditions applied in static simulations.

Figure 43. Boundary conditions applied for the preliminary model of Surgery 4BG, location of the boundary conditions applied in static simulations.

APPENDIX (Continued)

Figure 44. Boundary conditions applied for the preliminary model of Surgery 4BF, location of the boundary conditions applied in static simulations.

APPENDIX (Continued)

Figure 45. Boundary conditions applied for the preliminary model of Surgery 5, location of the boundary conditions applied in static simulations.

Figure 46. Boundary conditions applied for the preliminary model of Surgery 6, location of the boundary conditions applied in static simulations.

APPENDIX

RESULTS

The FEM model includes the material thermal properties, initial boundary conditions and the thermal load. The numerical results of all the surgeries performed are given below:

Figure 47. Dynamic Bio-Heat simulation of the Dormier Medilas Holmium laser on porcine skin during Surgery 1: temperature field (a) after 1 second, (b) after 2 second.

APPENDIX (Continued)

Figure 48. Dynamic Bio-Heat simulation of the Dormier Medilas Holmium laser on porcine skin during Surgery 1: magnitude of the heat flux field (a) after 1 second, (b) after 2 second.

APPENDIX (Continued)

Figure 49. Static Bio-Heat simulation of the Dormier Medilas Holmium laser on porcine skin during Surgery 1: (a) Temperature field, (b) magnitude of the heat flux field within the tissue.

Figure 50. Dynamic Bio-Heat simulation of the Lype Surgilas Diode Laser System A on porcine skin during Surgery 2A after 0,1 second: (a) temperature field, (b) magnitude of the heat flux field within the tissue.

APPENDIX (Continued)

Figure 51. Dynamic Bio-Heat simulation of the Lype Surgilas Diode Laser System C on porcine skin during Surgery 2C after 0,1 second: (a) temperature field, (b) magnitude of the heat flux field within the tissue.

APPENDIX (Continued)

Figure 52. Static Bio-Heat simulation of the Dormier Medtech Medilas D Flexipulse LPS laser on porcine skin during Surgery 3: (a) temperature field, (b) magnitude of the heat flux field within the tissue.

Figure 53. Dynamic Bio-Heat simulation of the Dormier Medtech Medilas D Flexipulse LPS laser on porcine skin during Surgery 3 after 1 second: (a) temperature field, (b) magnitude of the heat flux field within the tissue.

APPENDIX (Continued)

Figure 54. Static Bio-Heat simulation of the Biolase iLase gingivectomy laser on porcine skin during Surgery 4BO: (a) temperature field, (b) magnitude of the heat flux field within the tissue.

APPENDIX (Continued)

Figure 55. Static Bio-Heat simulation of the Biolase iLase gingivectomy laser on porcine skin during Surgery 4BG: (a) temperature field, (b) magnitude of the heat flux field within the tissue.

APPENDIX (Continued)

Figure 56. Static Bio-Heat simulation of the Biolase iLase gingivectomy laser on porcine skin during Surgery 4BF: (a) temperature field, (b) magnitude of the heat flux field within the tissue.

Figure 57. Static Bio-Heat simulation of the IPG DLR-200-AC diode laser on porcine skin during Surgery 5: (a) temperature field, (b) magnitude of the heat flux field within the tissue.

APPENDIX (Continued)

Figure 58. Static Bio-Heat simulation of the PG ELR-1550-50 diode laser on porcine skin during Surgery 6: (a) temperature field, (b) magnitude of the heat flux field within the tissue.

APPENDIX

DETAILED MORPHOLOGY STUDY

Figure 59. BO Details1.

APPENDIX (Continued)

Figure 60. BO Details2.

APPENDIX (Continued)

Figure 61. BO Details3.

APPENDIX (Continued)

Figure 62. BO Details4.

APPENDIX (Continued)

Figure 63. BO Details5.

APPENDIX (Continued)

Figure 64. BG Details1.

APPENDIX (Continued)

Figure 65. BG Details2.

APPENDIX (Continued)

Figure 66. BG Details3.

APPENDIX (Continued)

Figure 67. BG Details4.

Figure 68. BG Details5.

APPENDIX (Continued)

Figure 69. BF Details1.

APPENDIX (Continued)

Figure 70. BF Details2.

APPENDIX (Continued)

Figure 71. BF Details3.

APPENDIX (Continued)

Figure 72. BF Details4.

APPENDIX (Continued)

Figure 73. BF Details5.

APPENDIX (Continued)

Figure 74. S5 Details1.

APPENDIX (Continued)

Figure 75. S5 Details2.

APPENDIX (Continued)

Figure 76. S5 Details3.

APPENDIX (Continued)

Figure 77. S5 Details4.

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