Aetiology of pressure ulcers

_A literature review_

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March 2003
BMTE03.10

Part I of MSc-thesis

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Chapter 1

Introduction

Problem definition: Pressure ulcers
Pressure ulcers are defined as "areas of localized damage to the skin and underlying tissue caused by pressure, shear, friction or a combination of these". They are also called pressure sores, bedsores, decubitus ulcers or just decubitus. The magnitude of the problem can be addressed by the prevalence, which is the number of patients with pressure ulcers as a percentage of the total number of patients in an institution at a certain moment. Recent Dutch prevalence measurements showed that approximately 13% of the patients in university hospitals has pressure ulcers, in 23% in general hospitals, 30% in nursing homes, and 17% in home care. The extra costs associated with decubitus in Dutch health care institutions are estimated to be approximately 450 million euros a year, due to lengthened hospitalization periods and expensive preventive materials.

These figures clearly indicate that pressure ulcers are a major problem in health care. It is generally accepted that prolonged external loads are the primary causative factors, but still, the exact pathway through which these loads lead to tissue damage, is unknown. This makes a good assessment of patients at risk and subsequent prevention difficult. Rather subjective risk scales like the Norton and Braden scales are used now to identify these patients. These scales are based on several risk factors, such as sensory perception, activity, mobility, malnutrition and incontinence, but the relative weight of these factors and the possible correlation between them, are unknown.

To improve the prevention, and hence decrease the prevalence, thorough research into the damage-producing pathways of pressure ulcers is needed. In 1942, Groth already differentiated between pressure ulcers that develop in the skin and progress towards deeper tissue layers, and those that initiate in deep tissues such as skeletal muscle, and subsequently spread to the surface. He called the latter the malignant form. Those deep ulcers mostly arise in muscle layers that overly bony prominences, as a result of sustained compression. This is in contrast to the superficial ulcers, which are primarily caused by shear stresses. Bours et al. (1999) developed a classification scheme that is commonly used in clinical institutions (table 1.1). Although the prevalence of stage I ulcers is highest, and these superficial pressure ulcers can easily be observed, the deep ulcers are much more difficult to detect and they develop much faster. Nola and Vistnes concluded in 1980 that muscle tissue was more sensitive to pressure than skin and underlying tissue. For the above stated reasons, the focus is on deep pressure ulcers in this study.
Table 1.1: Pressure ulcer classification scheme

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Discolouration of intact skin - light finger pressure applied to the skin does not alter colouration</td>
</tr>
<tr>
<td>II</td>
<td>Partial thickness skin loss or damage involving epidermis, and/or blister or shallow ulcer without undermining of adjacent tissue</td>
</tr>
<tr>
<td>III</td>
<td>Full thickness skin loss involving damage or necrosis of epidermis and/or dermis not extending to underlying bone, tendon or joint capsule</td>
</tr>
<tr>
<td>IV</td>
<td>Full thickness skin loss involving damage or necrosis of epidermis and/or dermis extending to underlying bone, tendon or joint</td>
</tr>
</tbody>
</table>

**Aim and outline**

The aim of this report is to give a general overview of the current knowledge on the aetiology of pressure ulcers. Because the focus is on deep ulcers, which start in skeletal muscle tissue, the next chapter describes some aspects of skeletal muscle that can be of importance for studying the aetiology of these ulcers. The third chapter gives a brief review of experimental and numerical literature concerning the possible causative factors of pressure ulcers. The conclusion is drawn in the last chapter, which contains a general outline for my future numerical study. This will be done in collaboration with others who perform experiments on a cellular level and animal experiments, to gain more insight into ischemia and cell deformation as causes of pressure ulcers.
Chapter 2

Skeletal muscle

Anatomy
Skeletal muscle tissue consists of muscle fibers and connective tissue, of which the latter constitutes only a small volume of the total muscle volume, as can be seen in figures 2.1 and 2.2.

Figure 2.1: Cross-section of human adult skeletal muscle: Staining with haematoxyline eosine shows the individual muscle fibers with their peripheral nuclei, separated from each other by the thin endomysium\textsuperscript{11}.

The fibers are composed of many myofibrils, which are repeated units of sarcomeres. The specific arrangement of actin- and myosin filaments that constitute these sarcomeres, gives skeletal muscle its characteristic cross-striated appearance. The muscle fibers contain multiple peripheral nuclei from the individual cells that are fused to form the fiber. The diameter of these fibers ranges from 10 to 100 $\mu$m\textsuperscript{11,18}, and they can span the whole length of the muscle or part of it, with a length of up to 30 cm\textsuperscript{10}. The individual fibers are separated from each other by a thin layer of connective tissue, the endomysium. Bundles of muscle fibers, fascicles, are surrounded by the perimysium, and the total muscle is surrounded by the epimysium, a thick connective tissue layer (figure 2.2).
Etiology of pressure ulcers
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**Blood supply**
Generally, one artery enters a skeletal muscle near its central part, and one or more veins exit there. The large arteries that branch from the main artery, run through the epimysium, smaller branches through the perimysium and the multiple capillaries run through the endomysium. There are several capillaries that supply one fiber of nutrients and oxygen (figure 2.2). In rest, the oxygen consumption of the muscles is only 20-30% of the total basal oxygen consumption, and the blood flow only constitutes 15% of the total cardiac output of the left ventricle, which is approximately 4 ml/min/100g. Oxygen diffuses from the capillaries to the cells across a distance of approximately 50 μm in rest. When the oxygen concentration in the tissue decreases, previously closed precapillary sphincters open, and the diffusion distance can decrease considerably to 1-5 μm as the result of this opening of many capillaries.

**Regeneration**
Beneath the endomysium, every muscle fiber is surrounded by a sarcolemma, which consists of the cell membrane and the basal membrane. Between these two, satellite cells (muscle precursor cells) reside that are responsible for the regenerative capacity of skeletal muscles. After necrosis of injured myofibers, inflammatory cells infiltrate and phagocytose the necrotic cells. Then, satellite cells proliferate, differentiate and fuse into multinucleated myotubes. These three processes are stimulated by the growth factors released by the inflammatory cells. Furthermore, a fast phagocytosis, an intact basement membrane and microvascularization accelerate muscle regeneration. When the microvascularization is not intact, and a large ischemic area is present, the satellite cells may die, and regeneration is impaired. As a result of continuous degeneration and
regeneration, the satellite cell pool can become exhausted, which also results in reduced regenerative capacity\textsuperscript{17}. These satellite cells can repair either total muscle fibers or just damaged parts of existing muscle fibers\textsuperscript{32}.
Chapter 3

Pressure ulcers

The European Pressure Ulcer Advisory Panel defines pressure ulcers as "areas of localized damage to the skin and underlying tissue caused by pressure, shear, friction or a combination of these". The microscopic changes seen after the application of pressure are edema, loss of cross-striations and myofibrils, hyalinization of fibers, neutrophilic infiltration and phagocytosis by neutrophils and macrophages.

Over the years, there have been many publications about experiments and models concerning the aetiology of pressure ulcers. Still, the precise damage mechanisms by which pressure ulcers develop are not fully understood. There are several hypotheses for the aetiology of pressure ulcers. The most widely accepted one is ischemia due to inhibited capillary perfusion. Reperfusion injury is also suggested as a process producing damage. Another hypothesis is that the change in interstitial pressure, interstitial fluid and lymph flow, and ion concentrations, causes a disturbed transport of nutrients and waste products, which leads to a disturbed metabolic equilibrium. Deformation of cells is also suggested to play a role in the development of pressure ulcers.

3.1 Compression

Experiments

Kosiak (1961) tried to determine the relation between microscopic changes in the muscle and constant as well as intermittent pressure on normal and denervated hamstring muscles of rats. The animals were sacrificed 24 hours after the end of the load application. He performed 80 experiments, and found that the application of 9.3 kPa (70 mmHg) for 2 hours produced moderate microscopic changes in the individual muscle fibers. Load applications up to 25 kPa (190 mmHg) for only 1 hour did not produce any changes. When the pressure was applied in 5 minute intervals, with 5 minutes of pressure relief between these load applications, the changes were considerably less than those resulting from constant loads. His results are summarized in line 4 from figure 3.1. Kosiak (1959) also determined a pressure versus time curve for actual skin ulceration in dogs, which is line 2 in this figure.

Reswick and Rogers (1976) accumulated data from patients by subjective comments from
clinicians about actual tissue breakdown occurrences, pressure measurements when signs of actual or possible tissue breakdown were present following known times of pressure application, and controlled pressure applications on volunteers for sufficient times to produce clinical signs of tissue breakdown. Over 980 observations led to the allowable-pressure versus time curve which is shown in figure 3.1.

Figure 3.1: Allowable-pressure versus time curves from different studies: In the area above the lines, tissue breakdown will occur.

It should be realized that the data summarized in line 3 in figure 3.1 originate from different situations, and few controlled experiments. But the observed areas and measurements were all over bony prominences.

Another experimental allowable-pressure versus time curve was made by Daniel et al. (1981), who observed the skin of swines for ulcerations (line 1 in figure 3.1).

Numerical models
Mak et al. (1994) modelled the skin and the subcutaneous tissue with a biphasic poroelastic theory as a uniform layer. The fibrous network and the cells constitute a porous, elastically deformable solid, which was assumed to be fully saturated with an inviscid incompressible interstitial fluid. Friction between these two phases represents the viscoelastic behaviour of the tissue. Together with the volumetric fluid fraction, this friction characterizes the hydraulic permeability. The tissue layer is assumed to be homogeneous and of uniform thickness, and it is modelled on top of a rigid, impervious base representing bone. Following a load step (200 kPa), displacement and tissue hydrostatic pressure at the middle of the tissue layer under the center of the indenter, were determined in time.

It was found that the interstitial fluid pressure was generally lower than the applied pressure, and with increasing time, stresses were transferred to the solid, resulting in tissue compaction. This compaction may cause cellular damage, capillary occlusion or disturbed lymphatic drainage. By selecting a critical displacement above which pressure ulcers would develop, a pressure-time curve could be generated, which corresponded well with the experiments on humans by Reswick and Rodgers. However, Mak et al. made a lot of assumptions such as linear material elasticity, constant permeability, infinitesi-
mal deformation, uniform thickness and material homogeneity, and they did not model a specific geometry of the underlying bone, nor the different properties of the overlying distinct soft tissue layers.

Zhang et al. (1997) extended the model of Mak with a non-linear compaction-dependent permeability, and they included the effects of large deformations and a bony prominence. Again, the clinical results from Reswick and Rodgers could be explained well with this model, but they did not take into account the detailed non-homogeneous anisotropic microstructure of the soft tissues. They found that there was more compaction in the deeper tissue layers and that this compaction continued to increase even after the applied displacement reached its final value. This is in contrast to the interstitial pore pressure, which relaxed with time. These observations made Mak et al. and Zhang et al. suggest that tissue compaction rather than interstitial pore pressure is an important factor in the biological response of tissues subjected to prolonged loading. However, it is questionable whether this also applies to damage starting in skeletal muscle, because the major part of this tissue is cells, with very little extracellular matrix.

Todd et al. (1994) made a finite element analysis to compare numerical data to MRI data and to try to relate buttock/cushion interface pressures to internal pressures at the ischium. They generated the finite element geometry on the basis of nuclear magnetic resonance imaging for one male and one female, and modelled three distinct layers, namely bone, soft tissue and cushion. Although they assumed that the soft tissue was linear, isotropic and time-independent because of a lack of knowledge of the particular microstructure of the tissue, they found that the computational displacements were within 5% of their experimentally measured displacements with MRI. The computational minimum principal stresses differed approximately 50% with the interface pressures experimentally determined, while they should be analogous according to Todd et al. But the internal stresses are much higher than both these stresses, so it seems that there is no clear correlation between interface pressures and internal stresses.

Mak et al. as well as Zhang et al. and Todd did not specifically model a muscle layer, but used the same properties for the whole soft tissue layer beneath the skin. Oomens et al. (2002) did indeed model a distinct muscle layer. They made a finite element model of the human buttocks on a supporting cushion like Todd et al. did, to assess whether the interface pressure distribution is representative for the mechanical state of the soft tissues involved. The ischial tuberosity was modelled as a rigid body, and the overlying muscle, fat and skin as distinct layers. Both these three layers and the cushion were described with a non-linear Ogden material law.

Their results indicated that the stresses inside the tissue are higher than the interface stresses, and the highest normal stresses, and shear strains consistently appeared in the fat just underneath the bone and in the deeper muscle layer close to the bone. The bottleneck were the material properties for skin, muscle and fat. Therefore, parameter studies were performed, which led to differences in the shear strains in the fat layer, but hardly influenced the muscle layer. Data on the sensitivity of the various layers to mechanical loading is needed to draw conclusions about the aetiology of pressure ulcers, but this study again indicated that interface pressure alone does not reflect the internal mechanical state of the tissue.
3.2 Shear forces

Experiments
Dinsdale (1974) investigated the role of pressure and friction through the application of pressure with or without friction to the posterior iliac spines of swines in his study because the skin of these animals histologically resembles the human skin. He does not explain how he applied friction, so whether it was actually friction (with slip between skin and indenter) or shear (without slip), is not clear. The application site was observed for seven days, and it was characterized as normal, partial lesion, or full thickness lesion, which extends to the subcutaneous tissue. He concluded that friction increases the susceptibility to skin ulceration at pressures below 67 kPa (500 mmHg) in paraplegic swines. He also found that ulcers resulted with friction and a pressure as low as 6 kPa (45 mmHg), while 39 kPa (290 mmHg) was needed to produce ulcers when no friction was applied. He demonstrated with isotope clearance studies that friction did not further decrease the skin perfusion. His conclusion was that the observed difference in susceptibility to ulceration was not due to an ischemic mechanism.

Numerical models
Zhang et al. (1994) used a laser Doppler flowmeter to measure the flow within the skin to a depth of 0.5 to 1.0 mm in 5 healthy volunteers during the application of pressures. With an indenter, the normal pressure was increased stepwise from 2.5 to 13 kPa, with increasing shear force (without slip between indenter and skin surface) from 0.2 to 2.5 N during each pressure increment. The experimental results indicated that the reduction in skin blood flow was nearly proportional to the increase of the resultant of the applied normal and shear forces, which made them conclude that shear and normal forces have nearly the same effect on blood flow in the skin and underlying tissue. They made a theoretical model in which the skin and underlying tissue were modelled as linear, uniform and isotropic materials, to analyse the internal stress distribution in response to point shear and normal forces. The conclusions they draw from these analyses were that normal forces influence the stresses mainly in the deeper tissue, while shear forces mainly affect the superficial tissue. However, Chang et al. mention that shear forces are much higher within the deep tissue than at the surface, and that they are very critical because they cut off a large area of their vascular supply.

3.3 Ischemia

Compression and shear forces may cause capillary closure, which leads to hypoxia, eventually resulting in necrosis. Landis (1930) measured the pressure in an open arterial capillary in the finger, and found that it was 4.26 kPa (32 mmHg). Since then, this pressure has been used as the capillary closing pressure, which is often cited in the literature. External pressures above this capillary closing pressure were thought to produce ischemia of the skin, and when applied for an extended period of time, to cause pressure ulcers. But it is known now that the pressure inside capillaries is different in different parts of the body and depends on the general condition of the patient.
More recent measurements showed an average arterial capillary pressure of 6.26 kPa (47 mmHg). Moreover, self-regulation of blood vessels prevents them to occlude when a constant high external pressure is exerted, at least when it is below the diastolic pressure.

Blaisdell (2002) in his review of skeletal muscle ischemia and reperfusion, reports that skeletal muscle is tolerant of ischemia up to 4 hours, while skin changes are still reversible after up to 24 hours of ischemia. This is in agreement with the fact that muscle tissue is metabolically more active than skin tissue. Moreover, higher pressures are needed to stop the blood perfusion in muscle than in skin tissue, but still, muscle tissue is more susceptible to develop pressure ulcers at a certain pressure level than skin tissue.

Experiments
To test the difference in sensitivity to pressure between skin and muscle and skin alone, Nola et al. (1980) studied the occurrence of pressure lesions 3-4 days after they applied a pressure of approximately 14 kPa (100 mmHg) with syringes used as air pistons, for 6 hours at 4 consecutive days to the tibia or the greater trochanter, with or without an overlying muscle flap. Although the occurrence of skin ulceration decreased when muscle tissue was present between the bone and the indenter, there was edema of the skin and underlying muscle, increased cellularity, vacuolization and muscle fiber necrosis in almost every animal. They concluded that muscle tissue is more sensitive to pressure than skin and subcutaneous tissue, although they did not know the pressures inside the different tissue layers.

Measuring the transcutaneous oxygen tension and skin blood flow during the application of increasing compressive weights to the trochanter of healthy volunteers, Xakellis et al. (1991) found that the blood flow continued to decrease with increasing compression when the transcutaneous oxygen tension had already reached zero. This observation suggests that pressures lower than the capillary occluding pressure may already produce tissue anoxia. Moreover, the change in oxygen tension with increased compression accelerated above a certain threshold pressure, while the change in blood flow showed a linear decline as compression increased.

Despite a few limitations on the instrumentation and the fact that this study focussed on the skin instead of muscle tissue, the results of this study suggest that it might be important to take the oxygen tension into account when modelling the influence of pressure on tissues, and not just the blood flow.

Numerical models
Chang et al. (1999) developed a model to predict the onset of pressure ulcers, based on the assumption that ischemia of the skin, resulting in pressure ulcers, can be expected when the oxygen tension is below 1.3 kPa. They included the contribution of shear due to friction in the form of a friction coefficient of the skin, which could be adjusted to the amount of sweating. Previous publications were used to determine a formula from which a pressure-time relationship could be calculated for different friction coefficients.

Sacks (1989) performed a dimensional analysis to come to a pressure versus time curve for the onset of pressure ulcers. It was based upon the assumption that there is a definable pressure that will initiate a pressure sore, and that this pressure depends primarily upon the physical properties of the tissue, the blood flow through it and the duration of the loading. The result was the linear relationship \( p = a + bt^{-\frac{4}{3}} \), with pressure \( p \), applic-
tion time \( t \), and \( a \) and \( b \) being universal constants that are the same for all experiments and contain a lumped elastic modulus for the loaded tissue, as well as tissue density and local blood flow before loading. He fitted this to experimental data from Kosiak\(^{20}\) and Reswick and Rodgers\(^{33}\) (figure 3.1). A further simplification Sacks made was the assumption that the elastic modulus, the tissue density and the local blood flow before loading were the same for each subject tested. Nevertheless, he found a good agreement between his linear equation and the experimental results from Kosiak\(^ {20}\) and Reswick and Rodgers\(^ {33}\). However, in both these models, local geometry and load distribution were not taken into account.

### 3.4 Ischemia-reperfusion injury

Upon relieving the pressure, reperfusion should occur. However, due to the disturbed energy supply during ischemia, calcium ions may have accumulated intracellularly because of impaired functioning of ion pumps. This may lead to irreversible contraction of endothelial smooth muscle cells, which makes reperfusion impossible\(^ {26}\).

But reperfusion itself is also known to have deleterious effects. It is thought to produce more oxygen free radicals than can be scavenged, which causes oxidative stress in the tissue. This in turn stimulates endothelial cells to recruit leukocytes, causing inflammation. There are a lot of hypotheses regarding the subsequent pathways of reperfusion injury, among which are endothelial cell swelling associated with the recruitment of monocytes and macrophages, reduction in arteriolar diameter, permeability changes in postcapillary venules due to leukocyte adhesion, and increased flow reduction in the microcirculation\(^ {30}\).

The various mechanisms through which reperfusion may induce injury are investigated in several different studies. Among these are up-regulated collagen degrading enzymes\(^ {34}\), recruited leukocytes producing high levels of oxygen free radicals\(^ {35}\), elevated intracellular calcium levels\(^ {37}\), mediation through neutrophils and the complement membrane attack complex\(^ {22}\) and reduction of radical scavenging enzymes\(^ {12}\).

**Experiments**

Peirce et al. (2000) subjected the skin of unanesthetized rats to cyclic ischemia-reperfusion cycles, consisting of 2 hours of ischemia and 1 hour of reperfusion, using clinically relevant pressures of 6.7 kPa (50 mmHg). They analyzed the skin blood flow, the area of necrosis, the transcutaneous oxygen tension and the average leukocyte extravasation and skin thickness within 12 hours after cessation of the ischemia-reperfusion cycles. Their conclusion was that a larger number of cycles, a longer period of ischemia, and a higher frequency of the cycles, exacerbate the skin damage. Moreover, more reperfusion while keeping the total period of ischemia the same, leads to more damage, and repeated ischemia-reperfusion cycles were more damaging to the skin than ischemia alone.

Peirce\(^ {30}\) compressed only the skin, while Grisotto et al. (2000) performed experiments on rat skeletal muscle. They concluded that injury due to free oxygen radicals is more obvious after reperfusion than after ischemia alone.

Houwing et al. (2000) also showed the damaging effects of free oxygen radicals. They did not observe any damage in muscles immediately after cessation of pressure application, but two hours later histopathological specimens showed increasing invasion of
granulocytes. During the reperfusion period, the usual buffering of oxygen free radicals is diminished, and their production is elevated due to inflammation. As a result, they damage the vascular endothelium, which attracts platelets and granulocytes, and stimulates stasis of blood flow and thrombosis, leading to a further decrease in blood flow. These phenomena may contribute to the development of tissue necrosis\textsuperscript{16}.

Another observation by Houwing et al.\textsuperscript{16} was histological damage more distal to the pressure application area in addition to directly under this area. This spreading of the damage followed the same pattern as the arterial blood supply, indicating further that tissue (re-)perfusion plays an important role in the aetiology of pressure ulcers. If the ischemia-reperfusion injury is indeed an important contributor to muscle damage, the question arises why frequent turning of patients seems to help in preventing the development of pressure ulcers\textsuperscript{15}.

### 3.5 Interstitial fluid and lymph flow

Normal cell functioning depends on normal metabolism, a sufficient supply of nutrients through the blood, and elimination of waste products essentially through the lymph. Any disturbance of this metabolic equilibrium stresses the cell, and may eventually lead to cell damage and death.

The fact that pressures higher than the capillary closing pressure do not always produce pressure ulcers, made Reddy\textsuperscript{31} suggest that in addition to a disturbed microcirculation, lymph circulation and interstitial transport processes also play an important role. His hypothesis on the role of lymphatics in pressure ulcer formation is that tissue pressure can damage or directly block the lymphatics, leading to impaired absorption of lymph from the interstitium. Hormone release in response to capillary occlusion may also result in impaired lymph flow, due to dysfunction of the lymphatic smooth muscles. The consequence of an inhibited lymph flow is accumulation of metabolic waste products, proteins and enzymes, which leads to tissue necrosis. He cites results of Miller and Seale\textsuperscript{27} who found that the lymphatic clearance of a radioactive particle was inhibited when the externally applied pressure exceeded 8-9.3 kPa (60-70 mmHg).

Ryan (1990) also mentions the lymphatics as a system that is very sensitive to pressure and shear, and that may cause lymphoedema when it can not function properly. This oedema leads to a higher interstitial pressure, which worsens the damage.

#### Numerical models

With a very simple model for interstitial fluid flux calculations, Reddy\textsuperscript{31} observed a similarity between the pressure-duration relationship in experimental production of pressure ulcers and the inverse relationship between pressure intensity and load duration required to squeeze a certain part of the interstitial fluid volume out of a pressurized volume into non-pressurized surrounding volume. This led him to suggest that the slow viscous flow of interstitial fluid may play a significant role in tissue breakdown. He hypothesized that this occurs through contact stresses between cells after the removal of a large volume of interstitial fluid, and the bursting of capillaries and subsequent interstitial flooding and protein transfer into the surrounding tissues after load removal. Especially, in addition to the previously mentioned impaired lymph flow, this can result in tissue necrosis\textsuperscript{31}. 14
However, the relative contribution of the impaired lymph flow to the aetiology of pressure ulcers that start in muscle tissue, is questionable since only 5% of the blood flow in skeletal muscle is filtrated, so the volume flow of lymph is really very small compared to that of blood\textsuperscript{19}. Nevertheless, if this lymph flow is an important way through which skeletal muscle has to get rid of its waste products, it can be relevant if it is impaired in the development of pressure ulcers.

### 3.6 Cellular deformation

It has been suggested that cell deformation is an important aetiologic factor in the development of pressure ulcers\textsuperscript{3,6}. It triggers local membrane stresses, volume changes and cytoskeletal reorganization, which may be involved in cell damage\textsuperscript{5}.

**Experiments**

Bouten et al. (2001) compressed constructs of cells in agarose, with a final cell concentration small enough to exclude interactions between adjacent cells. They determined a deformation index, the ratio between the cell dimension in the direction of the load application and the cell dimension perpendicular to this direction. They found a decrease in deformation index with strain till a certain strain value at which membrane buckling occurred, which might eventually lead to membrane rupture. In addition, they counted the number of viable and dead cells to assess cell damage. The percentage of damaged cells, assessed through markers which might indicate both necrosis and apoptosis, was higher in strained than in unstrained constructs, and it increased with time of compression.

These damage experiments were performed with a construct strain of 20%, corresponding to a stress of approximately 4.3 kPa in the agarose\textsuperscript{5}, which is often said to be the capillary closing pressure. However, since the levels of oxygen and nutrient supply were similar in strained and unstrained constructs, the fact that the number of viable cells was significantly higher in unstrained than in strained constructs, supports the hypothesis that cell deformation is an important factor in cellular damage\textsuperscript{5}. In the study by Bouten et al.\textsuperscript{5} there was no extracellular matrix which probably affects the integrity of the cytoskeleton, and through this, the local deformability of the cells.

**Numerical models**

Guilak and Mow (2000) developed a biphasic finite element model to study stresses and strains in the microenvironment of chondrocytes modelled as biphasic spheres in a biphasic extracellular matrix. The results of compression of the macroscale, were used to define boundary conditions for the microscale. They found that the magnitude of stresses and strains in the microenvironment of the cell may be several times higher than in the extracellular matrix. Moreover, cell and tissue morphology and mechanical properties were found to influence the biomechanical environment of the cells. An important assumption they made was that the cells did not influence the mechanical behavior of the macroscopic tissue.

Wu and Herzog (2000) first modelled cartilage as a macroscopically homogenized mate-
rial with material properties depending on the cell and matrix properties and the relative amount of cells. Second, similar to Guilak and Mow\textsuperscript{13}, the results of the macroscopic finite element simulation were used as boundary conditions for a microscopic submodel. Herein, the cell and surrounding matrix were both modelled as biphasic materials, to obtain the mechanical behavior of the cells. The chondrocyte deformations were up to 3-4 times as large as the imposed macroscopic deformation, and those cell deformations were highly location dependent in the simulated unconfined compression.

Although these models describe stresses and strains on the cellular level, they are for cartilage, in which the extracellular matrix constitutes a much larger part than in muscle, and there is no feedback from the cellular level to the macroscopic level. Breuls et al. (2002) predicted local cell deformations in engineered tissue constructs with a multilevel finite element approach. In contrast to the models from Guilak and Mow\textsuperscript{13} and Wu and Herzog\textsuperscript{40}, this multilevel approach determines the macroscopic constitutive behavior from the microscopic mechanical response, so no macroscopic constitutive law has to be assumed. A macroscopic finite element mesh was made of the 2D transversal cross-section of the tissue construct, and to each macroscopic integration point, a microscopic representative volume element (RVE) was assigned.

Two different microstructures were defined, one in which spherical cells were randomly distributed, and one with polygonal myofiber cross-sections and a thin layer of extracellular matrix between them. The behaviour of cells and extracellular matrix was modelled as isotropic compressible neo-Hookean. The macroscopic deformations and stresses are assumed to equal the averaged microscopic quantities in the RVE assigned to the macroscopic integration point. An external macroscopic compression was applied, which produced large differences in individual cell deformations that could highly exceed the macroscopic deformation. Apart from this, the experienced strain in the cells was found to be strongly influenced by the microscopic architecture, which indicated that indirect mechanical interaction between neighboring cells affects their deformation.

Breuls (2003) extended his multilevel model to include a damage law. When the strain energy density exceeded a certain cell tolerance, a cumulative damage parameter was updated. When this damage parameter reached a threshold value, that particular cell died. He constructed local damage curves that showed the amount of expected dead cells in time for several external macroscopic loads. Shape and position of this curve are affected by the microstructural constitutive properties and the tissue tolerance. The bandwidth of these curves, from 0 to 100% dead cells, is related to the heterogeneity of the microscopic RVE. Breuls\textsuperscript{7} stated that a critical damage curve will run somewhere within this bandwidth, according to the amount of dead cells that initiates irreversible damage.
Chapter 4

Conclusion

Based upon a review of the literature, Defloor (1999) formulated a conceptual scheme for the risk of pressure ulcers, in which he postulated that tissue tolerance determines the duration and intensity of the applied forces needed to produce pressure ulcers. He splits this tissue tolerance in a tolerance for pressure and one for changes in oxygen concentration. This tolerance for oxygen is important because compression can cause capillaries to collapse with ischemia and oxygen deficit as a result. Pressure tolerance determines how much pressure, and resulting deformation, a tissue can withstand before it will be damaged irreversibly. Because it is hypothesized in this report that cell deformation is directly related to the development of pressure ulcers, the scheme in figure 4.1 contains tissue tolerance for deformation instead of tissue tolerance for pressure as Defloor suggested.

Figure 4.1: Conceptual scheme for the aetiologic factors for pressure ulcers as investigated in this report: Compressive forces can produce ischemia, and subsequently an oxygen deficiency may occur, which can lead to cell death, depending on the tolerance for oxygen. Compressive forces also deform cells, and, depending on their tolerance for deformation, this may lead to cell death. Eventually, when a certain percentage of cells is dead, irreversible damage occurs, and pressure ulcers may develop. (partly adapted from Defloor)

These two damage pathways, ischemia and cell deformation, are likely to be involved in the aetiology of pressure ulcers. Ischemia has been thought to play a crucial role since
the first studies on the aetiology of pressure ulcers. However, it is most unlikely that ischemia alone results in pressure ulcers, because skeletal muscles are able to survive 3 to 4 hours of ischemia while pressure ulcers can develop much faster. As Reddy said, if oxygen would be the only factor, all pressure intensities in excess of the capillary closing pressure should produce ulceration in the same amount of time. Since this is not true according to the various pressure versus time curves shown in figure 3.1, other factors will contribute to the development of pressure ulcers.

It has been hypothesized more recently that sustained deformation of muscle cells causes severe damage to the cells, which may contribute to the development of pressure ulcers. Bouten et al. (2001) and Breuls (2003) indeed found that compression of cells in culture led to increasing amounts of dead cells with time of pressure. Breuls (2003) applied a multilevel finite element approach to predict damage evolution due to local cell deformations in tissue-engineered constructs. He used a multilevel model because tissue damage starts at the cellular level and is related to the local mechanical condition experienced by a cell. Moreover, changes in the microstructure affect the macroscopic constitutive behavior. He found that the threshold curve, which describes at what combinations of pressure and time damage occurs, is different in each macroscopic point in the tissue. Furthermore, the individual cells in the microstructure experience different mechanical conditions, so different cells die after different times of compression.

Obviously, these results are influenced by the damage law he used, which should be more extensively validated with experiments. However, they revealed that studying the pressure ulcer aetiology on a cellular level is indeed important because the stresses and strains experienced by the cells cannot easily be related to the macroscopic ones. Moreover, various cells or macroscopic areas may have different tolerances for pressure. When one cell died according to the damage law, it was made 5 times less stiff. What exactly happens with the material properties of the dying cell is a difficult subject. They may already change gradually before the cell is irreversibly damaged, which will influence the stresses and strains in the neighboring cells. So, in addition to the changing cellular properties when a cell is damaged, interaction between cells can also be taken into account to improve the existing model.

The scheme presented in figure 4.1, shows ischemia as another important factor in the aetiology of pressure ulcers. To get to know something more about the relative contribution of ischemia and cell deformation to the development of pressure ulcers, ischemia can also be studied with a multilevel finite element approach. In this way, the oxygen supply to individual cells can be taken into account. This is dependent of the blood flow through the capillaries, which is influenced by the compression. But because Xakellis et al. found that the transcutaneous oxygen tension responded differently to increased compressive load than the capillary blood flow in the skin, it might be important to include oxygen diffusion in addition to perfusion.

Like cell deformation, ischemia may be a very local process, because not every capillary will be compressed in the same amount, and therefore, some cells may still receive enough oxygen while others already die because of a shortage of oxygen. Again, a damage law is needed that predicts which oxygen amount in a cell is too low and after what time this will lead to cell death. Bader (1990) showed that repeated loading produced a smaller reduction in tissue oxygen level after several cycles. He explained this with the stress induced release of vasodilators and the anoxia in the tissue, which may cause
reactive hyperaemia. These autoregulatory mechanisms, such as vasodilatation or the earlier mentioned opening of precapillary sphincters, are complicating factors for a model.

After implementing both damage laws separately (figure 4.1), the predicted damage evolutions can be compared. Together with experimental results, this may give some indications in the relative contribution of both factors in pressure ulcer development. The percentage of cells that can die before irreversible tissue damage occurs, is important to relate this model to macroscopic experimental studies.

It would also be interesting for both ischemia and cell deformation, to make the model three- instead of two-dimensional, because capillaries run parallel to the long muscle fibers, which is perpendicular to the currently modelled cross-section. It was found that damage extends in a pattern corresponding to the arterial supply. Bosboom (2001) also found that the observed damage in rats was more extended in longitudinal than in transverse direction. These are two interesting observations that can only be studied in a three-dimensional model. Another adjustment to the current multilevel model is the adaptation of the micro- and macroscopic meshes to more realistic ones, which makes it more suitable for comparisons with experiments.

Following the simulations of ischemia and cell deformation, what happens when the external load is relieved might be studied because of possible muscle repair and the proposed reperfusion injury. A difficulty is that there is no general agreement in the literature on which phenomenon of reperfusion is most damaging. For example, increased permeability of the microcirculation may cause tissue edema which results in a longer diffusion distance for oxygen from capillary to cell. Induction of the no-reflow phenomenon, which is also proposed as a consequence of reperfusion, would lead to longer periods of oxygen deprivation. The possibility of cells to return to their unstrained state, might play a protective role though.

In the end, integration of more and more sophisticated experiments and more comprehensive numerical modelling will lead to a better understanding of blood supply and cell deformation in compressed skeletal muscles. To gain insight into the aetiology of deep pressure ulcers in this way, might help to come to a better prevention to decrease the occurrence of these ulcers.
Bibliography


