Aetiology of pressure ulcers

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October, 2007
BMTE 07.39

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Chapter 1

General information

1.1 Prevalence and costs of pressure ulcers

Pressure ulcers are localized areas of tissue necrosis that tend to develop when soft tissue is compressed between a bony prominence and an external surface for a prolonged period of time [58]. They occur in situations where people are subjected to sustained mechanical loads and are particularly common in subjects who are bedridden or wheelchair-bound. Prevalence figures remain very high; e.g. in a recent Dutch prevalence study it was shown that the prevalence of pressure ulcers was 15.4% in university hospitals, 11.8% in general hospitals, 18.3% in nursing homes and 8.4% in home care [47]. Especially spinal cord injury (SCI) patients are at risk for developing pressure ulcers, since prevalence rates for this group are much higher. For SCI patients, the occurrence of pressure ulcers is among the most common long-term secondary medical complications [50]. According to Dinsdale, the incidence of pressure ulcers in paraplegic and quadriplegic patients ranges from 25% to 85% [24]. In addition, Garber and Rintala found that a large part (56%) of the ulcers in SCI patients are grade IV pressure ulcers (most severe, see section 1.2) [27]. These ulcers usually start in deep tissues over bony prominences and are hard to detect before they reach the skin surface.

Besides the fact that pressure ulcers are very painful for patients themselves, they also represent a huge financial burden for health care in Western countries. In the Netherlands, more than 1% of the total health care budget is spent on prevention and treatment of pressure ulcers or the prolonged hospital stay once a pressure ulcer has developed. Here, pressure ulcers are the third costliest disorder, after cancer and cardiovascular diseases [55].

1.2 Classification systems

There are different systems to classify pressure ulcers. The system adopted by the European Pressure Ulcer Advisory Panel (EPUAP) consists of four grades in which each grade is defined by the anatomic limit of tissue damage, see table 1.1 [23] and figure 1.1. This classification system can be used to evaluate the anatomic depth of tissue
Chapter 1. General information

1.2. Classification systems

<table>
<thead>
<tr>
<th>Grade</th>
<th>Short description</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>Non-blanchable erythema of intact skin</td>
<td>A discolouration of the skin, warmth, oedema, induration or hardness may also be used as indicators, particularly on individuals with darker skin.</td>
</tr>
<tr>
<td>Grade II</td>
<td>Abrasion or blister</td>
<td>A partial loss in the thickness of the skin involving epidermis, dermis, or both.</td>
</tr>
<tr>
<td>Grade III</td>
<td>Superficial ulcer</td>
<td>A full loss in the thickness of the skin involving damage necrosis of subcutaneous tissue that may extend down to, but not through, underlying fascia.</td>
</tr>
<tr>
<td>Grade IV</td>
<td>Deep ulcer</td>
<td>An extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures with or without full loss in the thickness of the skin.</td>
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Classification systems are used to categorize wounds and injuries, but their usage in reverse order to monitor the healing process of an ulcer [49, 59]. This is not correct, as stage IV ulcers granulate progressively to a shallower depth, they do not replace the structural layers of muscle, subcutaneous fat, and dermis before they reepithelialize [49]. Furthermore, the inter- and intrarater reliability of the EPUAP classification system appeared both to be low for nurses. Misclassification of pressure ulcers can then lead to inadequate preventive and therapeutic measures and suboptimal nursing care [23]. Besides the fact that the system cannot be used for monitoring wound healing, it also focusses only on visible signs of skin or underlying tissues, so ulcers that initiate in deeper tissues, e.g. near bony prominences, are not recognized until they reach the skin and instantly become a grade IV ulcer [25]. Ankrom et al. also mentioned that the published literature did not offer consensus on how to describe, diagnose, or treat pressure-related deep tissue injury under intact skin [2]. Recently, the National Pressure Ulcer Advisory Panel (NPUAP) updated their classification system and also added deep tissue injury as a distinct pressure ulcer in this updated system [6].
1.3 Risk assessment

There are two major factors associated with the risk of developing pressure ulcers: the amount and duration of exposure to pressure and the ability of the tissue to tolerate the pressure [5], see figure 1.2. The primary cause of pressure ulcer development is the exposure to pressure: without a mechanical load no pressure ulcer will develop. However, the ability of the tissue to withstand mechanical loading determines whether or not a person will develop a pressure ulcer due to a certain loading. The exposure to pressure is influenced by the mobility, activity and sensory perception of a patient and tissue tolerance for pressure is influenced by intrinsic and extrinsic factors [5]. Intrinsic factors are related to the individual, e.g. age, smoking, incontinence, weight and body temperature. Extrinsic factors are related to the environment, e.g. contact surface and temperature and humidity of the environment.

In clinical practice, these risk factors are used in risk assessment scales to identify patients at risk of developing pressure ulcers. Many different risk assessment scales exist, but the value of these scales in predicting the risk of pressure ulcer development remains questionable. For example, Schoonhoven et al. tested three commonly used scales (the Norton, Braden and Waterlow scales) and concluded that the use of the outcomes of these risk assessment scales to decide on preventive measures leads to ineffective and inefficient treatment for most patients [75]. Moreover, these scales cannot be used for SCI patients, since they are always classified as high risk patients. Still, they may stay free of ulcers for a long time, and suddenly develop an ulcer in a very short period.
Figure 1.2: Conceptual schema which accounts for the relative contributions of the duration and intensity of pressure and the tolerance for pressure.
Chapter 2

Mechanical loading

2.1 Pressure, shear and friction

Mechanical loading that can lead to the development of pressure ulcers not only consists of pressure; shear and friction are also mentioned to be important [24, 69]. It is believed that shear and friction mainly cause superficial ulcers, while pressure usually leads to deep tissue destruction, especially in soft tissues over bony prominences [13]. In practice however, it is difficult to have conditions of pure pressure, and impossible to develop pure shear [3]. For example, the application of pressure by means of a piston leads to a shear stress near the piston edge [4], and the absence of pressure during the application of shear will promote slip [3].

Not all kinds of mechanical load are equally damaging for biological tissues. Hydrostatic pressure does not have a large influence on the development of pressure ulcers, since divers can work at large depths without developing pressure ulcers [8, 53, 81]. Localized pressure on the other hand, causes deformation of the tissues and blockage of blood vessels, and is therefore far more damaging [20]. Furthermore, Nola and Vistnes found that, by applying pressure on skin and muscle tissue over bone in rats, that more ulceration appeared in the muscle layer than in the skin [54]. They therefore concluded that muscle was more sensitive to pressure than skin and subcutaneous tissue. Salcido et al. also observed that lesions occurred first in the muscle rather than in the skin when applying pressure to the trochanteric region of rats [74]. However, instead of being more sensitive to mechanical loading than skin, the muscle tissue in these studies could also be subjected to higher loadings than the superficial layers, see also section 2.2. This would also explain the earlier occurrence of ulceration in muscle tissue.

An applied shearing load does not lead to large tissue destruction, e.g. Bennett and Lee found that externally applied pressure was approximately twice as effective as applied shear in reducing pulsatile blood flow [3]. However, the combination of pressure and shear is very damaging, e.g. at a sufficiently high level of shear, the pressure necessary to produce occlusion was only half that when little shear was present [3]. Furthermore, Dinsdale found that friction also increases the susceptibility to skin ulceration, since the presence of friction decreased the amount of pressure necessary to produce ulcers in
paraplegic swine [24].

The amount and distribution of external pressure, shear and friction during sitting or lying is influenced by several factors. Firstly, the body configuration has a large influence on the applied mechanical loading [35]. In addition, the properties of the cushion or mattress material play an important role. Finally, the individual characteristics also affect the kind and distribution of mechanical loading. For example, in sitting on a hard, slick seat, paraplegic, geriatric, and ill subjects develop roughly three times the median shear load experienced by normal subjects in skin lateral to the ischial tuberosities [3]. Furthermore, by investigating the effects of seated posture and body orientation on the pressure distribution and shear forces acting at the body-seat interface, Hobson found that individuals with SCI have significantly higher maximum pressures than nondisabled subjects in all postures that were studied [35].

\section*{2.2 Internal stress state}

In clinical practice, the interface pressures between the body and the supporting surface are measured to assess the mechanical load that is applied to the body and thereby the risk of developing pressure ulcers. Interface pressures are however not a good measure to estimate the risk for deep pressure ulcers, since they do not provide information about the stress state in deeper tissue layers. For example, it was shown by numerical models [20, 31, 45, 46, 57, 80, 82, 86] and physical models [17, 67] that stresses and strains are not uniform within the tissues. Moreover, the nature of the external loading does not result in the same kind of loading within the tissues, which is illustrated in figure 2.1.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig2_1}
\caption{Schematic drawing of a uniaxial compression test. Solid and dotted lines show the undeformed and deformed configurations, respectively [56].}
\end{figure}

In this figure, a uniform axial compression is applied to an object. When small rectangular part the object is observed as in situation a, then the deformation of the small part is similar to the macroscopic deformation, and the object only experiences compression. However, if the small part is rotated as in situation b, then it experiences compression as well as shear. In fact, for each material point a configuration can be
found in which only compressive or tensile stresses and strains are present, as well as a configuration in which the maximum shear strain is reached. Thus, although the macroscopic deformation was compressive, it results in pressure and shear in the tissue [56]. Furthermore, since hydrostatic pressure is relatively harmless to biological tissues, the harmful stress is presumably a form of shear [20].

Several numerical studies in which soft tissues are compressed, showed that stresses and strains in the muscle layer adjacent to bony prominences are much higher than those near the surface [31, 45, 46, 57], see also figure 2.2. In addition, the mechanical state of the tissue can change over time [48]. Moreover, Linder-Ganz et al. showed that stresses and strains in muscle are less homogeneous than in fat, and that muscle tissue was also subjected to tensile stress where fat was not [46]. Furthermore, the study of Oomens et al. showed that the highest shear strains occurred in fat [57]. However, Sun et al. reported that the maximum shear stresses occurred in muscle tissue and within skin, and that they were significantly higher than in fat [80].

![Figure 2.2: Two examples of finite element models that show that stresses and strains are higher near bony prominences than near the surface. a) Deformed mesh of buttock model of Oomens et al. with von Mises stress [57]. b) Principle compressive strains in deep tissues under the ischial tuberosities of a healthy subject, modeled by Linder-Ganz et al. [46].](image)

The internal stress state in the tissues depends on several factors. Firstly, the cushion properties have an influence on the stresses and strains within the tissues [57, 67]. For example, Oomens et al. mentioned that a change in cushion properties mainly influences the shear strains in the fat layer [57]. Furthermore, Linder-Ganz and Gefen showed that a change in mattress stiffness led to larger changes in contact pressure and shear than in peak internal stresses [45]. Secondly, the mechanical properties of the tissues also affect the stress and strain distribution. For example, a higher Young’s modulus in the model of Chow and Odell resulted in smaller deformations and a more uniform stress distribution [20]. Mechanical properties of tissues can change when cell becomes damaged. It was found by Gefen et al. and Linder-Ganz and Gefen that damaged...
muscle tissue becomes stiffer [31, 45]. This increase in muscle stiffness leads to higher stresses in the injured muscle parts in the numerical model of Gefen et al., and these stresses were then transferred to adjacent tissue that was not yet injured [31]. Finally, the shape of a bony prominence also influences the internal stress state. This was shown by the physical buttock model of Candadai and Reddy, which consists of a PVC gel (soft tissue) molded around a wooden core (bone), in which two shapes of bone were tested [17]. Furthermore, a theoretical analysis of Gefen showed that the size of the injured muscle area and the time for injury onset depended on the radius of curvature of the bony prominence [30].

It is clear that the internal stress state is nonuniform in the tissues that are subjected to a mechanical load. However, the internal distribution of stresses and strains does not directly determine whether or not a pressure ulcer will develop at certain locations. This also depends upon the load threshold of the individual tissues.

2.3 Magnitude and duration of loading

Besides the mechanical loading of tissues, the time of exposure also plays an important role in the development of pressure ulcers. The influence of both factors was studied in animal models. In 1942, Groth applied pressure to the posterior ischi of rabbits, and found that an inverse relationship existed between the minimum pressure and minimum duration required to produce a pressure ulcer [33]. Husain confirmed the shape of this relationship by applying pressures of 100 to 600 mmHg to the legs of normal rats for 1 to 6 hours [37]. In 1959, Kosiak applied pressures ranging from 100 to 550 mmHg for periods of 1 to 12 hours to the femoral trochanter and ischial tuberosity of dogs. This also revealed an inverse relationship between the minimum pressure and time that lead to ulceration of tissue [39]. In 1981, Daniel et al. subjected the greater femoral trochanter of pigs to pressures of 30 to 1000 mmHg for periods of 2 to 18 hours, which also resulted in a hyperbolic pressure-time curve [21]. In 1976, a similar hyperbolic curve was produced for humans by Reswick and Rogers, who used comments of physicians, nurses and therapists, pressure measurements, and controlled tests on volunteers [70]. A large variation however exists between the threshold values found in different studies, see figure 2.3(a). This is due to diversity in experimental conditions, animal models, loading methods and locations of load application [77].

The shape of the curve was also determined by Sacks, who used dimensional analysis to determine a relationship between the required pressure to produce an ulcer, and the physical properties of the tissue, the blood flow through it, and the time of exposure [73]. This analysis showed that the pressure was a function of $t^{-4/3}$. If a linear relation was assumed between the pressure and $t^{-4/3}$, a fit of the equation to the data of Kosiak [39] and Reswick and Rogers [70] resulted in a good agreement for both experiments [73].
2.3. Magnitude and duration of loading

The hyperbolic pressure-time curve implies that loads that are applied for very short times would not lead to tissue damage. To investigate if this was true, Linder-Ganz et al. also applied varying loads for short durations and showed that high loads could lead to damage instantly [44]. The pressure-time curve as proposed previously is therefore probably not complete, and shows a more sigmoidal shape when short duration data are also included, see figure 2.3(b). This means that for short exposures (less than 1 hour) and also for long exposures (over 2 hours) the magnitude of the pressure is the most important factor for causing cell death; the exposure time has little or no effect. However, for the intermediate exposures (between 1 and 2 hours) pressure and time both are important factors [44].

The pressure-time relationship was also investigated for paraplegic animals. Groth [33] and Kosiak [40] studied the response of paraplegic animals when compared to normals. Both found no significant differences in susceptibility. Daniel et al. however noted that the animal model of Groth was probably experimented too soon following transection of the spinal cord, thereby precluding the tissue atrophy characteristic of the clinical condition [22]. Probably this also holds for the animal model of Kosiak. Daniel et al. therefore waited six weeks after transection of the spinal cords before using his paraplegic pigs. They found that the pressure-time curve for paraplegic pigs was also hyperbolic, but the magnitude of the variables was greatly reduced. Spinal-transected animals would therefore develop pressure sores at lower magnitudes of pressure and after a shorter time than normal animals. The difference between the results was attributed to tissue atrophy, which would lead to increased interface pressures between the support surface and the soft tissues overlying bony prominences [22].
Chapter 3

Damage pathways

Although it is known that sustained mechanical loading is the primary cause of pressure ulcers, the underlying pathways whereby mechanical loading leads to tissue breakdown are hardly understood. At the moment theories involve:

- Localized ischemia;
- Ischemia/reperfusion injury;
- Impaired lymphatic drainage;
- Sustained deformation of cells.

These four theories will be described in the following sections.

3.1 Localized ischemia

Ischemia is often considered to be the most important factor in the development of pressure ulcers [21, 39, 40]. When a mechanical load is applied to the body, blood vessels in the loaded tissue can collapse, thereby stopping blood flow and depriving the tissue of its oxygen and nutrients supply. In 1930, Landis studied the blood pressure within a capillary loop in healthy human fingernail beds using a microinjection technique, and found that an average pressure of 32 mmHg was sufficient to close the capillaries [43]. The validity of this value should however be questioned, since the capillaries were cannulated, which could have resulted in lower pressure readings as the bleeding vessels were not full or enclosed. Furthermore, capillary pressures can rise during mechanical loading due to autoregulation, in which case higher external pressures are needed to close the blood vessels [3, 81]. Finally, capillary closure depends on local pressure gradients across the vessel wall and not just on interface pressures [13]. Still, the capillary closing pressure found in Landis’ study is frequently used in clinical practice as a threshold for tissue damage, where care is taken to avoid interface pressures higher than 32 mmHg.

External pressures that are high enough to close blood vessels will lead to ischemia, which initiates a series of chemical and pH imbalances, accompanied by enhanced generation of injurious free radical species. The damage produced by short periods of ischemia
Chapter 3. Damage pathways

3.1. Localized ischemia

...tends to be reversible if the circulation is restored, but cells subjected to long episodes of ischemia become irreversibly damaged and die [71]. In general, muscle appears to be tolerant of ischemia for up to 4 hours, fat up to 13 hours, and skin up to 24 hours at normothermia [7]. Ceelen et al. developed a finite element model that describes deformation, diffusion of oxygen and damage in a cross-section of skeletal muscle tissue [18]. They investigated whether the cessation of oxygen consumption in dead cells could be beneficial for the remaining cells in the tissue, and if a change in mechanical properties upon cell death had an influence on damage development in other cells. The results showed that when cells stopped consuming oxygen after cell death, further cell death is delayed or even prevented. This effect is larger after more cells have died, because the amount of extra oxygen available for the other cells increases, and is more pronounced at lower compression levels, since at high compression levels there is already hardly any oxygen available, and therefore the little amount of extra oxygen hardly has any effect. A change in mechanical properties of cells upon cell death, in this study it was assumed that dead cells were less stiff, eventually led to delayed cell death at high compression levels. At low compression levels or when only a few cells had already died, no change of mechanical properties of dead cells resulted in slower cell death.

During ischemia, cells have to switch from aerobic to anaerobic metabolism due to the lack of oxygen. Anaerobic metabolism results in the generation of lactic acid which leads to a decrease in intracellular pH. The depletion of glucose and accumulation of lactic acid which decreases the intracellular pH both lead to cell death. For example, Gawlitta et al. showed that glucose deprivation and acidification in C2C12 murine myoblasts resulted in increased cell death [29].

![Figure 3.1: Schematic overview of the changes that occur during ischemia [71].](image-url)
Chapter 3. Damage pathways 3.2. Ischemia/reperfusion injury

Intracellular ion concentrations also change during ischemia. Due to the lack of ATP, the Na\(^+\)/K\(^+\) ion exchanges becomes inactive, which subsequently leads to the activation of the Na\(^+\)/H\(^+\) ion exchanger. When intracellular acidosis threatens, it pumps H\(^+\) out of the cell in exchange for Na\(^+\) to maintain proper intracellular pH. The increase in intracellular Na\(^+\) then leads to activation of the Na\(^+\)/Ca\(^{2+}\) ion exchanger, enhancing calcium entry. Since calcium pumps are ATP dependent and cannot pump the excess Ca\(^{2+}\) out of the cell anymore, calcium accumulates in the cells. High calcium concentrations in ischemic cells activate phospholipase A\(_2\), leading to degradation of membrane phospholipids and the consequent release of free fatty acids and lysophospholipids. These are both potent mediators of inflammation and lysophospholipids also act as detergents that solubilize cell membranes. The combination of electrolyte imbalance and increased permeability of the cell membrane causes cell swelling [71]. Figure 3.1 gives a schematic overview of the changes that occur during ischemia.

The rise in intracellular calcium concentration during ischemia was confirmed by Boffi et al. who examined intracellular calcium levels in mouse-derived C2C12 myotubes with inhibition of glycolytic and oxidative metabolism as ischemic condition [9]. On the other hand, Smith et al. found that the total tissue calcium contents of preischemic and end ischemic canine gracilis muscle were similar and started to increase after the ischemic period during reperfusion. They therefore concluded that calcium influx is a feature of reperfusion rather than the ischemic interval [76]. Furthermore, when the cell membrane ionic pumps become depressed, more ions enter the cells than leave them. This leads to osmosis of water into the cells and thus causes intracellular edema [34, 51].

Although ischemia will eventually lead to tissue death, it is probably not the only cause of pressure ulcers. One reason for this is that pressure ulcers can develop within two hours, whereas tissues can withstand ischemia for longer times. Furthermore, ischemia alone fails to explain why higher tissue pressures can create ulcers after a short period of ischemia, whereas lower pressures, which still create an ischemic state, need longer periods to cause the identical lesion [60].

3.2 Ischemia/reperfusion injury

Tissue can also become damaged when the blood flow in the tissue is restored after an ischemic period. Ischemia and reperfusion of skeletal muscle can trigger a series of deleterious phenomena in tissue, such as cell edema, increased permeability in the microcirculation, induction of the no-reflow phenomenon, free oxygen radical production, electrolytic changes in mitochondria, cytosolic calcium overload, and degradation of membrane phospholipids [32].

After a relatively short ischemic period, the blood flow is temporarily higher than under normal circumstances. This increased blood flow after occlusion is called reactive hyperemia. It is a consequence of a local regulatory mechanism whereby the arterioles are dilated and the resistance to blood flow is reduced [51]. Ikebe et al. investigated the relationship between the duration of ischemia and the subsequent reperfusion blood flow in rats [38]. They found increased reperfusion blood flow after 90 minutes and
Chapter 3. Damage pathways  

3.2. Ischemia/reperfusion injury

3 hours of ischemia, but after 6 hours of ischemia there was no significant increase in postischemic blood flow. This is called the “no-reflow phenomenon” and is probably caused by cellular swelling, thrombosis, and white cell plugging in capillaries, which increases the resistance in the microcirculation [85]. The incidence of no flow depends on the severity of both the ischemic insult and the subsequent reperfusion injury [38].

During reperfusion oxygen is provided to the tissues, which combines with the free radical species generated during ischemia to form reactive oxygen species (ROS) [71]. When present in excess they damage the endothelium, attracting platelets and granulocytes, stimulating stasis of blood flow and thrombosis, further decreasing blood flow and thereby stimulating the development of tissue necrosis [36]. The superoxide anion (O$_2^-$) is produced principally by leaks in mitochondrial electron transport or as part of the inflammatory response, see figure 3.2. In the case of phagocytic inflammatory cells, activation of a plasma membrane oxidase produces O$_2^-$, which is then converted to hydrogen peroxide H$_2$O$_2$ and eventually to other ROS. H$_2$O$_2$ is also produced directly by a number of oxidases in cytoplasmic peroxisomes. By itself, H$_2$O$_2$ is not particularly injurious, but when produced in excess, it is converted to hydroxyl radicals (•OH). In neutrophils, myeloperoxidase transforms H$_2$O$_2$ to the potent radical hypochlorite (OCl$^-$), which is lethal for microorganisms and cells.

![Figure 3.2: Mechanisms by which reactive oxygen species are generated from molecular oxygen and then detoxified by cellular enzymes. CoQ = coenzyme Q; GPX = glutathione peroxidase; SOD = superoxide dismutase. [71].](image)

Hydroxyl radicals are formed by (1) the radiolysis of water, (2) the reaction of H$_2$O$_2$ with ferrous iron (Fenton reaction), and (3) the reaction of O$_2^-$ with H$_2$O$_2$ (Haber-Weiss reaction). The hydroxyl radical is the most reactive molecule, and there are several mechanisms by which it can damage macromolecules [71]:

- **Lipid peroxidation:** The hydroxyl radical removes a hydrogen atom from the unsaturated fatty acids of membrane phospholipids, a process that forms a free
lipid radical, which subsequently initiates a chain reaction. The destruction of the unsaturated fatty acids of phospholipids results in a loss of membrane integrity.

- **Protein interactions:** Hydroxyl radicals may also attack proteins. As a result of oxidative damage, proteins eventually undergo degradation.

- **DNA damage:** DNA is an important target of hydroxyl radicals. If the oxidative damage is sufficiently extensive, the cell dies.

The major enzymes that convert ROS to less reactive molecules are superoxide dismutase (SOD), catalase and glutathione peroxidase. SOD converts $O_2^-$ to $H_2O_2$ and $O_2$. Catalase in one of the two enzymes that complete the dissolution of $O_2$ by eliminating $H_2O_2$ and therefore its potential conversion to $\cdot OH$. Glutathione peroxidase (GPX) catalyzes the reduction of $H_2O_2$ and lipid peroxides in mitochondria and the cytosol, see figure 3.2. GPX uses reduced glutathione (GSH) as a cofactor, producing two molecules of oxidized glutathione (GSSG) for every molecule of $H_2O_2$ reduced to water. GSSG is reduced to GSH by glutathione reductase [71]. In tissues undergoing oxidative stress the amounts of these enzymes decrease, so during reperfusion the oxygen free radicals are not buffered, or are buffered to a lesser degree [36]. Furthermore, retinoids, the precursors of vitamin A, are lipid soluble and function as chain-breaking antioxidants. Vitamin C is water soluble and reacts directly with $O_2$, $\cdot OH$, and some products of lipid peroxidation. Vitamin E blocks free-radical chain reactions. Since it is fat soluble, it exerts its activity in lipid membranes, protecting them against lipid peroxidation [71]. Houwing et al. showed that pretreatment with vitamin E prevents damage caused by pressure applied to the femoral trochanters of pigs to a large extent. Vitamin E did not prevent oxidative stress during pressure application, but it did prevent the excess production of oxygen free radicals and hydrogen peroxide during reperfusion [36].

It is hypothesized that the reperfusion phase after an ischemic period is more damaging to tissues than ischemia itself. Peirce et al. subjected rats to different numbers of ischemia/reperfusion (I/R) cycles (2 hours of ischemia and 0.5 hour of reperfusion), varied the duration of the ischemic insult, and compared ischemia-induced injury with I/R-injury [65]. They found that tissue injury increased with an increasing number of I/R cycles and duration of ischemia, and moreover that when the total extent of ischemia was constant, a greater number of reperfusion events during that period resulted in increased tissue damage. It was therefore concluded that the reperfusion phase of the I/R cycle is an important component of the total injury produced. Tsuji et al. subjected mice to two different loading protocols and used the functional capillary density as a measure for tissue damage [83]. The total duration of compression was equal for both groups, but the first group received four cycles of 2 hours compression and 1 hour release, while the second group was exposed to a continuous compression of 8 hours. The results showed that the cyclic compression-release procedure significantly decreased functional capillary density as compared to continuous compression, from which they concluded that repetition of the ischemia-reperfusion cycle was more damaging than a single prolonged ischemic insult, see figure 3.3(a). Furthermore, when applying pressure to the skin immediately above the greater femoral trochanters of pigs, Houwing et al.
found that early signs of damage in the muscles and subcutaneous tissue appeared only after a reperfusion time of one to two hours and not during the ischemic period [36].

Since I/R injury is more damaging than ischemia alone, it was investigated if gradual reperfusion was better than complete reperfusion at once. Durrani et al. isolated the left renal artery and vein of rats [26]. Microclamps were applied for 45 minutes and released at once in one group and gradually in another group. Although there were no significant differences between both groups in the malondialdehyde (MDA) and myeloperoxidase (MPA) levels, which are indicators of lipid peroxidation and polymorphonuclear infiltration, respectively, histopathologic scoring showed less tissue damage in the gradual reperfusion group when compared to the conventional reperfusion group. Ikebe et al. regulated the postischemic blood flow in rats by the administration of L-NMMA (nitric oxide synthase inhibitor) to investigate alterations in the viability and contractile function of the skeletal muscle [38]. They found that muscle viability of the L-NMMA-treated group was significantly better, which suggests that excessive blood flow during reperfusion deteriorates skeletal muscle contractile function. Unal et al. investigated the gradual reperfusion in a rat hind limb model and tried to elucidate its potential beneficial effect [84]. Their results showed that the MDA and MPO levels were significantly larger in the conventional reperfusion group than in the control and gradual reperfusion groups. There was no significant difference between the levels in the control and gradual reperfusion groups. Inflammatory cell infiltration and loss of striation of the muscle were also noticeably less in the gradual reperfusion group than those of the conventional reperfusion group.

**Figure 3.3:** Effects of reperfusion. a) Percentage of microcirculatory injury is higher after four ischemia-reperfusion cycles than after continuous compression with the same duration of ischemia [83]. b) The tissue damage of group II rats (conventional reperfusion) is significantly higher than the damage in group III rats (gradual reperfusion). Group I was a control group (without ischemia) [84].

### 3.3 Impaired lymphatic drainage

Ischemia and I/R injury cannot be the only factors contributing to the development of pressure ulcers. If oxygen would be the only factor, then all pressure intensities that cause capillary closure should produce ulceration in the same duration, which is
in contrast to the measured inverse relation between pressure and duration required to produce ulcers [66]. It is believed that the lymphatic system can also play an important role [42, 41, 52, 66]. The lymphatic system functions as an “overflow mechanism” to return excess proteins and excess fluid volume from the tissue spaces to the circulation. Therefore, it plays a central role in controlling: (1) the concentration of proteins in the interstitial fluids, (2) the volume of interstitial fluid, and (3) the interstitial fluid pressure [34].

During mechanical loading, the lymphatics can collapse, thereby obstructing lymph flow. This leads to an accumulation of waste products. Miller and Seale investigated the relation between the external pressure and lymph clearance in dogs [52]. They found a nonlinear rise in lymph flow with increasing pressure until a critical closing pressure was reached. At this point, the flow ceased. The increase in flow with increasing external pressure was believed to be caused by the increased translymphatic pressure drop at larger external pressures, which promotes increasing flow into the lymphatics. Therefore, as pressure increases, so does flow, until the external pressure collapses the vessel. At an applied pressure of 60 mmHg, some animals showed relatively large clearance levels indicating enhanced lymph flow. Others showed reduced lymph flow indicating the onset of vessel closure, which implied that the critical closing pressure might be at or near 60 mmHg, see figure 3.4.

Figure 3.4: Lymph flow per unit tissue volume as a function of applied pressure. Lymph flow increases with pressure until a critical closing pressure is reached. Some data at 60 mmHg show vessel closure and some show enhanced flow, indicating that the critical closing pressure may be at or near 60 mmHg [52].

It is also hypothesized that the application of mechanical loads impairs the contractility of the lymphatic vessels. Krouskop et al. postulated that hypoxia caused by external loading leads to damage of lymphatic smooth muscle which in turn results in the loss of lymph motility and impaired lymph flow [42, 41].

Another hypothesis implies that when interstitial fluid is squeezed out of a region, direct contact of cells (fibroblasts) may induce contact stresses. These may in turn cause contact inhibition or even rupture and interrupt collagen synthesis. Furthermore, if the external pressure is removed, the interstitial fluid pressure might become small enough.

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to cause capillary bursting, edema and lymphatic damage [66].

The lymphatic flow was also studied by means of mathematical models. For example, Reddy et al. offered a phenomenological theory to explain the formation of pressure ulcers [66]. They considered a cylinder of tissue and assumed that the flow in connective tissue can be described with Darcy’s law. They found that the product of the interstitial fluid pressure and time was constant for a reduction in interstitial fluid volume, which might lead to tissue damage. Since this inverse relationship is in correspondence with other studies in which the relationship between pressure intensity and load duration required to create ulcers was investigated, it was concluded that interstitial fluid flow could play a significant role in the mechanisms responsible for this inverse relationship, and therefore also in tissue breakdown.

In a later study, Reddy and Patel developed a mathematical model to simulate lymph flow through the terminal lymphatics under different physiological conditions [68]. Blood pressure and protein concentration in the capillary formed the upstream boundary conditions and the pressure in the adjacent contractile lymphatic was taken as the downstream boundary condition. Results showed that the flow increased with increasing stiffness of the terminal lymphatic vessel wall, increasing stiffness of the anchoring filaments, and increasing hydraulic conductivity of the terminal lymphatic vessel wall and blood capillary wall. Also, the flow through the terminal lymphatics increased during edema, which was simulated by increasing the capillary pressure. Furthermore, the results showed that the flow through the terminal lymphatics is due to periodic fluctuations in interstitial fluid pressure and due to the suction mechanisms of the adjacent contractile lymphatics.

3.4 Sustained deformation of cells

Recently, it was hypothesized that sustained deformation of cells could also be a cause of pressure ulcers [12, 13, 11, 72]. Cell deformation influences local membrane stresses, volume changes, and the cytoskeleton organization, which may be involved in early cell damage [12, 11].

The role of deformation in the development of tissue damage was investigated by several researchers. Peeters et al. developed a single cell loading device to monitor the biomechanical response of skeletal muscle cells under sustained compression [61]. During compression the volume of the cell remained constant, but the surface area of the cell increased, which was probably caused by stretching of the cell membrane. In a later study, Peeters et al. also found that the deformation of the cells was anisotropic, since the cells deformed from an elliptical to a more circular shape [62]. It was suggested that this anisotropic deformation was due to the preferred orientation of actin filaments. With this loading device, the mechanical and failure properties of attached muscle cells were also determined [63], as well as the viscoelastic properties of the cells [64]. Bouten et al. developed a three-dimensional in vitro model system, consisting of muscle cells seeded and cultured in agarose [12]. Cylindrical constructs cultured up to day 12 were subjected to 20% gross strain for periods of 1, 2, 4, 12, and 24 h. For all straining periods the
total percentage of cell damage appeared to be significantly higher in strained constructs than in unstrained controls. In addition, Breuls et al. developed a compression device to simultaneously compress tissue engineered muscle constructs with circular, impermeable glass indenters [14]. Results showed that dead cells were highly localized below the indenter and that higher strains led to earlier damage initiation. Furthermore, Gawlitta et al. exposed tissue engineered muscle constructs to different combinations of hypoxia and deformation, and found that deformation had decremental effects on tissue viability, but that hypoxia had no additional effect within 22 hours [28].

The effect of deformation on tissue damage was also studied in animal models. Bosboom et al. investigated the location of damage development by applying compressive loading to the tibialis anterior muscle of rats [10]. They reported that besides histology, also MRI can be used to find locations of damage, since increased signal intensities in T2-weighted MR images correlated with damage determined by histological examination. Stekelenburg et al. also examined muscle damage after compressive loading of rat tibialis anterior muscle with T2-weighted MRI in combination with histological examination [78]. In contrast to the study of Bosboom et al. [10], the loading procedure in this study also took place in the MR scanner, so the exact indentation of the muscle could be observed, see figure 3.5.

![Figure 3.5: Transversal T2-weighted-sum images taken before loading (A), during loading (B), and immediately after unloading (C). The TA muscle is indicated by the white line in A [78].](image)

In the muscle underneath the indenter a necrotic region was observed, which was assumed to be caused by the large deformation that pulled the muscle fibers apart. It was also noted that T2 values in slices distal to the indenter were higher than in slices proximal to the indenter. This was probably due to a different perfusion status of the tissue, since the large indentation presumably led to the collapse of blood vessels, whereas in the tissue proximal to the indenter the perfusion was hardly affected during loading. The difference in amount of affected volume largely disappeared during the first hour after unloading, which indicates that the effect of ischemia was reversible within the muscle tissue. However, it was also noted that ischemia might accelerate or increase tissue damage. In a later study, Stekelenburg et al. studied muscle damage in rat tibialis anterior muscle during ischemia by applying a cuff around the thigh, and during ischemia and deformation using an indenter applied to the muscle. This revealed that ischemia alone only led to reversible damage, whereas the combination of ischemia
and deformation was far more damaging [79].

Breuls et al. developed a multilevel finite element model to investigate local cell deformations in engineered tissue constructs, subjected to macroscopic loads [16]. The results showed that there were large differences in strain energy density within the microstructure at one macroscopic point, indicating that individual cells can experience deformations which highly exceed the macroscopic deformation. Therefore, it should be questioned whether cell damage due to deformation can be evaluated based on macroscopic deformations. Breuls et al. also used this multilevel finite element approach to model the development of tissue damage in muscle tissue which is compressed over a bony prominence [15]. In this study, a damage law was derived from in vitro experiments with tissue engineered skeletal muscle constructs to evaluate tissue damage [14]. It was reported that muscle damage started at locations deep within the muscle tissue layer, where shear strains were largest. A parameter study also showed that a lower cell stiffness resulted in an increased area of tissue damage and a faster damage growth. Furthermore, related to the animal experiments of Stekelenburg et al., Ceelen et al. developed dedicated two-dimensional finite element models of the hind limbs of rats used in the experiments to investigate the strain state of the muscle. The results of these models show a correlation between the location of damage and local strain [19], see figure 3.6.

Figure 3.6: Left and middle: maximum shear strains in finite element models of Ceelen et al. based on two experiments of Stekelenburg et al. Locations of damage (x) in MR images are superimposed on the image. Right: number of damaged and undamaged pixels against maximum shear strain. [19]
Chapter 4

Discussion

4.1 Summary

Pressure ulcers are caused by sustained mechanical loading; without a mechanical loading no pressure ulcer will develop. External loading of the tissues leads to a nonuniform internal stress and strain state. This internal mechanical state depends on the magnitude of the applied loading, type of external loading (pressure, shear, friction), and the mechanical and geometrical properties of the tissues. In addition, the time of exposure to mechanical loading also plays an important role in the development of tissue damage. On the other hand, the ability of the tissue to withstand mechanical loading determines whether a certain loading will lead to the development of an ulcer or not.

Both the mechanical loading and patient susceptibility are influenced by intrinsic and extrinsic factors. Intrinsic factors are related to the individual, e.g. with age, mobility, weight, posture, incontinence and nutritional state. Extrinsic factors are related to the environment, e.g. with nursing, the contact surface, nutrition, and the temperature and humidity of the environment. In clinical practice, these risk factors are used in risk assessment scales to identify patients at risk of developing pressure ulcers. However, the value of these scales remains questionable. Moreover, SCI patients are always identified as high risk patients, while they may stay free of ulcers for a long time, and suddenly develop an ulcer in a very short period. Risk assessment scales can therefore not be used in this group of patients.

The underlying pathways whereby mechanical loading leads to tissue breakdown are hardly understood. At the moment theories involve:

- **Localized ischemia**

  Mechanical loading can lead to the collapse of blood vessels, thereby stopping blood flow and depriving the tissue of its oxygen and nutrients supply. Furthermore, ischemia initiates a series of chemical and pH imbalances. In general, muscle tissue appears to be tolerant of ischemia for 4 hours, fat up to 13 hours, and skin up to 24 hours [7].
4.2 Focus questions for our research

At this moment, by combining the results of the animal model of Stekelenburg et al. [78, 79] with dedicated finite element models [19], it is already studied whether there is a correlation between local tissue deformation and damage. Since the local tissue deformation is important for the development of tissue damage, it would also be interesting to investigate how the internal mechanical state of tissues depends on variations in mechanical and/or geometrical properties of tissues among individuals. Furthermore, it will be studied how the internal mechanical state is influenced by changes in material and geometrical properties as a result of disease.

It is also known that a certain mechanical loading will not lead to the same amount of damage in each individual, i.e. some individuals are more susceptible to develop pressure ulcers than others. Therefore, if a certain relation between local tissue deformation and damage can be derived from the animal experiments, it is important to investigate whether such a relation is the same for each animal or not. If it is similar, then the local tissue deformation probably is the main trigger for the development of tissue damage, and thus the susceptibility of an individual to develop a pressure ulcer is mainly a mechanical issue. Otherwise, also other factors play a role in this process, e.g. lack of tissue repair, bad tissue perfusion, and altered metabolism. It would therefore be interesting to compare the relations between tissue deformation and damage of subjects with a similar (patho)physiological state, and also to compare these results with a group of subjects with a different (patho)physiological state.
The overall goal of this project is to develop a biosensor system on the basis of biochemical markers to detect deep tissue injury at an early stage. To achieve this goal, it is important to know at which moment it is necessary to adopt preventive measures to prevent deep tissue injury, and at which moment it is too late. This is illustrated in figure 4.1. In situation a, the amount of tissue damage $D$ increases until the load is removed at time $t_a$. At this moment, there is no significant tissue damage yet ($D < D_{\text{min}}$) and the tissue will return to its original status. In situation b, there is much more damage ($D_{\text{min}} < D < D_{\text{max}}$) and preventive measures are needed to enable the damaged tissue to heal again. In situation c, the amount or degree of damage has passed a critical value ($D > D_{\text{max}}$), after which it is expected that tissue damage will continue to increase even if the load is removed. Probably, it is not easy to detect tissue damage by visual inspection before it has reached damage criterion $D_{\text{max}}$. A biosensor system should therefore be able to detect tissue damage in the range $D_{\text{min}} < D < D_{\text{max}}$.

An important question for our research is therefore: what are the amounts or kinds of skeletal muscle damage corresponding with $D_{\text{min}}$ and $D_{\text{max}}$?

Furthermore, biochemical markers have to be chosen that are released upon skeletal muscle damage. With respect to these markers, it should be investigated how specific they are for skeletal muscle damage and also how large the minimal amount of damage is that can be detected with these markers. A theoretical model may also be developed to gain more insight into the kinetics of these biomarkers in injured skeletal muscle.

![Figure 4.1](image-url) **Figure 4.1:** Illustration of how tissue damage is expected to change in time due to mechanical loading. Below a certain damage criterion $D_{\text{min}}$, the damage is not considered to be significant. Above damage criterion $D_{\text{max}}$ tissue damage is expected to increase even if the load is removed. Between these two criteria, preventive measures can probably lead to healing of the tissue.
Bibliography


