An introduction to the regulation of wound healing by micromechanical forces

Mechanical forces influence cellular organisation and behaviour. Without mechanical stimuli, cells stop proliferating and migrating, undergo cell-cycle arrest and eventually die. Mechanical cues, therefore, have fundamental effects on wound healing. A literature review was conducted to explore the effects of micromechanical forces and tissue reactions at a microscopic level on wound healing, and how these forces may be harnessed in wound care. It is clear from research from a range of databases, chiefly on non-wound tissues, that micromechanical forces can have a significant influence on tissue growth and function. When applied to wound healing, it can be deduced that these forces alter cell proliferation and differentiation, and affect cytokine release and matrix protein secretion. In contrast to healing wounds, the structural requisites for the transduction of mechanical cues are lacking in chronic wounds. The absence of extracellular matrix and the accumulation of wound fluid can lead to the formation of ‘dead space’, across which mechanical stimuli cannot be transferred. It is suggested that application of micromechanical forces to chronic wounds — either by negative pressure wound therapy or specially designed dressings — will promote wound healing by induction of appropriate microdeformation and that further research is needed in this area.

KEY WORDS
- Chronic wounds
- Dead skin
- Mesenchymal stem cells
- Microdeformation
- Myofibroblasts
- Negative pressure wound therapy

It is widely acknowledged that exudate from non-healing wounds contains elevated levels of proteases, such as matrix metalloproteinases and polymorphonuclear elastase (Barrick et al, 1999; Trengove et al, 1999; Yager and Nwomeh, 1999). The excessive action of these proteases leads to considerable reduction in growth factors (He et al, 1999) and proteinase inhibitors; cleavage of matrix components, such as collagens, elastin and fibronectin, and, consequently, to the destruction of the extracellular matrix (ECM; Yager and Nwomeh, 1999).

In addition, markedly increased levels of pro-inflammatory cytokines are released by macrophages and granulocytes in non-healing wounds (Harris et al, 1995) and chronic wounds have comparatively higher concentrations of reactive oxygen and nitrogen species than acute wounds (James et al, 2003). As a consequence, these wounds are trapped in the inflammatory phase and often do not heal for months or even years.

It has been suggested that changing the mostly destructive state of chronic wounds to a more physiological wound milieu could re-establish the structural requisites for normal cell proliferation, migration and differentiation and would move the wound towards healing. A literature review was conducted to find and summarise relevant research on micromechanical forces and microdeformation related to wound healing. The following electronic databases were searched: CINHAL, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews, the Database of Abstracts of Reviews of Effects, Health Technology Assessments, National Health Service Economic Evaluation, Embase, Ovid Medline and PubMed.

SIGNIFICANCE OF MICROMECHANICAL FORCES FOR WOUND HEALING
Negative pressure wound therapy (NPWT) has been shown to effectively support healing by augmenting blood flow (Morykwas et al, 2001), decreasing oedema (Gustafsson et al, 2007) and reducing the wound area (Isago et al, 2003). In addition, NWPT induces granulation tissue formation (Jacobs et al,
NPWT may also influence the wound microenvironment by reducing inflammatory proteases (Moues et al, 2008) and decreasing bacterial load (Zhou et al, 2013). In terms of its mechanotherapeutic effects, NPWT induces two types of tissue deformation (Huang et al, 2013): macrodeformation: wound contraction and microdeformation: tissue reactions at microscopic level.

NPWT has, therefore, also been termed microdeformational wound therapy (MDWT) (Lancerotto et al, 2012; Huang et al, 2013).

Micromechanical forces inducing microdeformations on cellular level can stimulate cell proliferation and division (Saxena et al, 2004; Scherer et al, 2008; Lu et al, 2011); and this effect has been harnessed in medical fields. For example, plastic surgeons exploit tissue expansion to expand soft-tissue envelopes in reconstructive surgery; and orthopaedic surgeons and maxillofacial surgeons use distraction osteogenesis to lengthen bones (Saxena et al, 2004; Huang et al, 2013). Hence, it follows that mechanotherapy at tissue and cellular level, by application of micromechanical forces to the wound, will support wound healing.

**CELLS CAN RESPOND TO MECHANICAL SIGNALS**

The human body is constantly subjected to mechanical forces that directly affect cellular functions (Huang et al, 2013). Research in the field of mechanobiology has highlighted how mechanical forces regulate cellular organisation and behaviour. Mechanical stress applied in the 'non-traumatic' range causes changes in form and composition at the cellular level, until a suitable stress state is re-established (Raebet al, 2008). Mechanical signals modulate almost all cell functions, including migration (Alenghat and Ingber, 2002; Raebet al, 2008) and proliferation (Huang and Ingber, 1999; Atance et al, 2004; Saxena et al, 2004; Wells, 2008).

Martinac et al (1987) described the first mechanically responsive transmembrane-signaling protein in 1987. Transmembrane protein receptors allow cells to explore the biophysical state of their surroundings and to react to it (Kung, 2005; Ingber, 1997), also play a crucial role in mechanosensing; serving as sensors and actuators in cell migration (Raebet al, 2008).

Integrins are directly engaged in cell migration through actinomyosin-controlled cell contraction and integrin-mediated binding and release of extracellular matrix ligands necessary for cell movement. Research shows that stretched cells proliferate (Atance et al, 2004; Wells, 2008) in vitro, but cells without mechanical stress assume spherical shape, go into cell-cycle arrest and die by apoptosis (Huang and Ingber, 1999). Saxena et al (2007) investigated these effects more closely in vivo, employing the rat ear model to investigate the biological response of soft tissue to forces. Their gene expression studies demonstrated that the hypoxia pathway might be an important modulator of cellular reactions to mechanical stress (Saxena et al, 2007).

Moreover, fibroblasts react to MDWT with increased proliferation and expression of collagen type I (COL1A1), alpha smooth muscle actin (α-SMA), basic fibroblast growth factor (bFGF) and transforming growth factor beta (TGF-β1) (Lu et al, 2011). α-SMA is rapidly incorporated into actin stress fibres, which results in an increased capacity of myofibroblasts to generate contractile forces (Sandbo and Dulin, 2011). Myofibroblasts are modified fibroblasts, which contract wounds and result in

![Figure 1. Overview on how micromechanical forces regulate cell behaviour by mechanotransduction pathways and in what way formation of 'dead space' and cellular changes affect wound healing.](image-url)
In chronic wounds, higher numbers of myofibroblasts have been observed compared to healing wounds, despite the lack of sufficient wound closure (Schwarz et al, 2013). An in vitro investigation showed that fibroblast migration and proliferation were decreased in a non-healing wound group, compared to a healing wound group (Schwarz et al, 2013). This finding seems unexpected because myofibroblasts are mostly linked to the late proliferative phase (Reinke and Sorg, 2012). The transformation of fibroblasts into myofibroblasts plays an important role in promotion of wound contraction and healing by producing ECM components (such as collagen). In physiological wound repair, this happens in the late proliferative phase. However, the chronic wound by definition is stuck in the inflammatory phase, where no deposition of ECM takes place. It could be that fibroblasts accumulate, become myofibroblasts, but cannot comply with their physiological task due to the inflammatory environment (everything is destroyed as soon as it's been build) and the intracellular changes (senescence, reduced synthesis).

Aging significantly enhances the stiffness of fibroblasts, owing to a shift in the degree of actin polymerisation favouring the filamentous form (Schulze et al, 2010). This results in both an age-associated loss of cell flexibility and an impairment of cell motility. It is also known that aged fibroblasts have a decreased proliferation rate. In addition, in vitro experiments have demonstrated that senescent fibroblasts isolated from chronic wounds exhibit a reduced migratory capacity after stimulation with mesenchymal stem cells (Rodriguez-Menocal et al, 2012).

Bone marrow-derived mesenchymal stem cells of patients with chronic wounds also exhibit defects in provoking fibroblast migration, although it is not known why. However, it is known that mesenchymal stem cells from older donors possess a distinctly different morphology compared to young people, show signs of oxidative damage, and DNA-methylation changes affect cell differentiation and decrease proliferation in vitro (Kapetanaki et al, 2013).

As mesenchymal stem cells can modify their activities and functions depending on the biomolecular context (Jackson et al, 2012), it is not surprising that mesenchymal stem cells exhibit mechanosensitivity. Observations suggest that cytoskeleton structure and cell tension are vital regulators of mesenchymal stem cells survival, self-renewal and differentiation (Wang and Li, 2010; Sun and Fu, 2013).

Mechanotherapy for induction of cell proliferation

Wound microdeformations induce cellular proliferation (Lancerotto et al, 2012) and migration (Borgquist et al, 2010). Such mechanical strain can be applied by NPWT using highly porous interface materials (Borgquist et al, 2010). During NPWT application, small tissue blebs (termed ‘tissue mushrooms’) form and extend into the pores of the dressing. These tissue mushrooms convey the shearing strains at the wound-dressing interface (Borgquist et al, 2010). NPWT also effectively removes fluid, which may exert additive mechanical strain owing to hydrostatic pressure gradients and fluid shear forces (Lu et al, 2011).

A wide variety of molecular responses to NPWT have been observed, such as changes in ion concentration and permeability of membrane ion channels, release of second messengers, stimulation of molecular pathways, and alterations in gene expression (Siller and Siperko, 2003; Borgquist et al, 2010). A study by Saxena et al (2004) showed that most elements stretched by NPWT underwent deformations of 5–20% strain. This is comparable to in vitro strain levels, which were found to endorse cell proliferation.

Lu et al (2011) demonstrated an increase in fibroblast proliferation and gene expression of COL1A1, α-SMA, bFGF, and TGFβ1 by NPWT. Lavery et al (2008) showed that under NPWT, the wound area was 2.5 times more likely to be decreased within one week in comparison to standard moist wound therapy. In addition, NPWT induces neovascularisation; most likely by a combination of direct effects of mechanical forces on pre-existing blood vessels and the establishment of hypoxia and vascular endothelial growth factor gradients (Erba et al, 2011).

The porosity of the polyurethane foam chosen for NPWT plays a significant role in mechanotherapy during wound healing. Larger pore sizes induce

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bigger wound bed deformations, resulting in thicker granulation tissue and greater induction of contractile myofibroblasts (Heit et al, 2012). Interestingly, microdeformations of the wound bed surface have been observed when wounds were treated with foam or gauze at atmospheric pressure, most likely because the sponge struts of the foam and the threads of the gauze cause imprints in the underlying wound bed tissue even without the application of NPWT (Saxena et al, 2004; Borgquist et al, 2010).

However, if close contact of the dressing to the tissue was not achieved, no significant granulation was observed (Saxena et al, 2004). This is in accordance with the clinical demand to ensure a close association of the applied dressing to the wound bed (Cutting et al, 2009) to avoid the formation of ‘dead space’ (Wiegand and White, 2013). To test this hypothesis, microfabrication techniques have been used to develop adhering dressings capable of inducing controlled and distributed tissue microdeformation (Kane et al, 2010).

Microfabrication designates the construction process of miniature structures at micrometer scales and smaller. Initially, they were used for fabrication of electronic circuits. Nowadays these methods are also used in cell biology and microbiology to build systems and structures at micron or submicron scales that make it possible to manipulate individual cells and their immediate extracellular environments.

In addition, it has been found that stimulation of the hypoxia pathway using interference RNA — a natural mechanism for silencing gene expression — or other gene therapy methods mimic the effects of mechanical forces. This pathway has been shown to be part of the biological responses to tissue deformation and micromechanical strains (Saxena et al, 2007). These findings might enable the design of clinical therapies, which rely on pharmaceutical intervention, rather than exposing the patient to mechanical treatments that can have negative effects, such as wound separation and wound pain (Wiegand and White, 2013).

THE MECHANICAL ROLE OF THE ECM
The ECM is a dynamic, mobile and multifunctional regulator of cellular behaviour (Schwarz and Bischofs, 2005; Susilo et al, 2010; Huang et al, 2013). Cells require a number of factors for tissue organisation and maintenance. These include biochemical signals, such as cytokines and ECM proteins; topographical information, such as cell orientation and ECM fibre organisation; and mechanical qualities, such as fibre elasticity and substrate stiffness.

Fibroblasts are known to stiffen their cytoplasm while migrating into a wound (Kole et al, 2005). Hence, cells may use actively generated internal forces to explore the environment termed ‘active mechanosensing’. This enables them to steer through the ECM according to its mechanical resistance, a process called ‘mechanotaxis’ (Schwarz and Bischofs, 2005). Fibroblasts also prefer to migrate towards more rigid or strained substrates (Schwarz and Bischofs, 2005). This phenomenon is most likely necessary because soluble growth factors and attachment to ECM proteins, although indispensable, are not enough to stimulate cell proliferation (Huang and Ingber, 1999), and progression through the cell cycle needs a suitable physical context to respond to these two chemical stimuli (Saxena et al, 2004). In chronic wounds, these structural requirements are often lacking owing to the degradation of the ECM. Hence, the scaffold on which cells normally stretch and proliferate is absent.

The term ‘dead space’ describes a void within a viscus, or between the wound bed and its dressing or tissue flaps (Cutting et al, 2009), formed by the absence of ECM and accumulation of wound fluid. Where dead space exists, micromechanical stimuli cannot be transferred. Wound failure in laparotomy has been linked with early fascial separation forming a dead space.

Culbertson et al (2011) suggested that the decreased tension causes the loss of stimulatory mechanical signals necessary for fibroblast proliferation, alignment and contractile function. As a consequence, this often leads to hernia formation (Culbertson et al, 2011). In accordance, prophylactic muscle flaps in vascular surgery, which are specifically aimed to avoid the creation of dead space, have been shown to improve healing outcomes (Fischer et al, 2012). In addition, neutrophils and macrophages cannot invade this area and, therefore, microbial contamination may proceed to wound infection (Wiegand and White, 2013).

CONCLUSION
Mechanical forces are able to affect wound healing through changes in cell proliferation rates,
cell differentiation, release of cytokines, and stimulation of matrix protein secretion. Cells isolated from chronic wounds of older patients demonstrate distinct alterations in phenotype and behaviour (e.g. mesenchymal stem cells fail to induce cell migration, and fibroblasts do not proliferate or secrete new ECM components). This might be owing to the absence of structural requirements for propagation of mechanical cues in chronic wounds as a result of ‘dead space’ forming. There is, therefore, a clinical indication for wound-related research in this area. In simple practical terms, closely adhering dressings that help to avoid the creation of dead space can also be assumed that application of micromechanical forces to chronic wounds in vivo, either by NPWT or specially designed dressings, will promote wound healing by induction of appropriate microdeformation.

REFERENCES