An introduction to the regulation of wound healing by micromechanical forces

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

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.

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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 (Gustaffson et al, 2007) and reducing the wound area (Isago et al, 2003). In addition, NWPT induces granulation tissue formation (Jacobs et al,

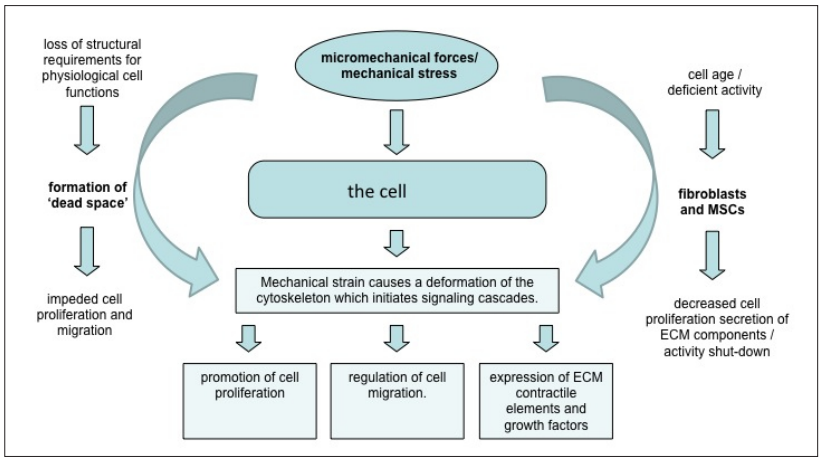

Figure 1. Overview on how micromechanical forces regulate cell behaviour by mechanotransduction pathways and in what way formation of 'dead space' and cellular changes affect wound healing.

2009; Zhou et al, 2013), cell proliferation (Scherer et al, 2009) and angiogenesis (Jacobs et al, 2009; Scherer et al, 2009; Zhou et al, 2013), as well as providing a moist wound environment. 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
- 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 (Raeber et al, 2008). Mechanical signals modulate almost all cell functions, including migration (Alenghat and Ingber, 2002; Raeber et 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, 2008) by a process called mechanotransduction (Alenghat and Ingber, 2002; *Figure 1*).

Other cellular structures, such as integrins (Katsumi et al, 2004) and the cytoskeleton itself (Ingber, 1997), also play a crucial role in mechanosensing; serving as sensors and actuators in cell migration (Raeber et 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

decreased surface scar tissue development (Sandbo and Dulin, 2011).

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 (Silver 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

"The transformation of fibroblasts into myofibroblasts plays an important role in promotion of wound contraction and healing by producing extracellular matrix components."

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 maintain intimate contact with the wound bed may help to avoid the creation of dead space. It 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. WUK

REFERENCES

Alenghat FJ, Ingber DE (2002) Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins. *Sci STKE PE* 2002(119): pe6

Atance J, Yost M, Carver W (2004) Influence of the extracellular matrix on the regulation of cardiac fibroblast behavior by mechanical stretch. *J Cell Physiol* 200(3): 377–86

Barrick B, Campbell EJ, Owen CA (1999) Leukocyte proteinases in wound healing: roles in physiologic and pathologic processes. *Wound Repair Regen* 7(6): 410–22

Borgquist O, Gustafsson L, Ingemansson R, Malmsjö M (2010) Micro- and macromechanical effects on the wound bed of negative pressure wound therapy using gauze and foam. *Ann Plast Surg* 64(6): 789–93

Culbertson EJ, Xing L, Wen Y, Franz MG (2011) Loss of mechanical strain impairs abdominal wall fibroblast proliferation, orientation and collagen contraction function. *Surgery* 150(3): 410–7

Cutting K, White R, Hoekstra H (2009) Topical silver-impregnated dressings and the importance of the dressing technology. *Int Wound J* 6(5): 396402

Erba P, Ogawa R, Ackermann M et al (2011) Angiogenesis in wounds treated by microdeformational wound therapy. *Ann Surg* 253(2): 402–9

Fischer JP, Nelson JA, Mirzabeigi MN et al (2012) Prophylactic muscle flaps in vascular surgery. *J Vasc Surg* 55(4):1081–6

Gustaffson R, Sjögren J, Ingemansson R (2007) Understanding topical negative pressure therapy. In: *EWMA position document: Topical Negative Pressure in Wound Management*. 2–4

Harris IR, Yee CE, Walters CE et al (1995) Cytokine and protease levels in healing and non-healing chronic venous leg ulcers. *Exp Dermatol* 4(6): 342–9

He C, Hughes MA, Cherry GW, Arnold F (1999) Effects of chronic wound fluid on the bioactivity of platelet-derived growth factor in serum-free medium and its direct effect on fibroblast growth. *Wound Repair Regen* 7(2): 97–105

Heit YI, Dastouri P, Helm DL et al (2012). Foam Pore Size is a critical interface parameter of suction-based wound healing devices. *Plast Reconstr Surg* 129(3): 589–97

Huang C, Holfeld J, Schaden W et al (2013) Mechanotherapy: revisiting physical therapy and recruiting mechanobiology for a new era in medicine. *Trends Mol Med* 19(9): 555–64

Huang S, Ingber DE (1999) The structural and mechanical complexity of cell-growth control. *Nat Cell Biol* 1(5): E131–8

Ingber DE (1997) Tensegrity: the architectural basis of cellular mechanotransduction. *Annu Rev Physiol* 59: 575–99

Ingber DE (2008) Tensegrity-based machanosensing from macro to micro. *Prog Biophys Mol Biol* 97(2–3): 163–79

Isago T, Nozaki M, Kikuchi Y et al (2003) Effects of different negative pressures on reduction of wounds in negative pressure wound dressings. *J Dermatol* 30(8): 569–601

Jackson WM, Nesti LJ, Tuan RS (2012) Clinical translation of wound healing therapies based on mesenchymal stem cells. *Stem Cell Trans Med* 1(1): 44–50

Jacobs S, Simhaee DA, Marsano A et al (2009) Efficacy and mechanisms of vacuum-assisted closure (VAC) therapy in promoting wound healing: a rodent model. *J Plast Reconstr Surg* 62(10): 1331–8

James TJ, Hughes MA, Cherry GW, Taylor PT (2003) Evidence of oxidative stress in chronic venous ulcers. *Wound Repair Regen* 11(3): 172–6

Kane BJ, Younan G, Helm D et al (2010) Controlled induction of distributed microdeformation in wounded tissue via a microchamber array dressing. *J Biomed Mater Res Part A* 95(2): 333–40

Kapetanaki MG, Mora AL, Rojas M (2013) Influence of age on wound healing and fibrosis. *J Pathol* 229(2): 310–22

Katsumi A, Orr AW, Tzima E, Schwartz MA (2004) Integrins in mechanotransduction. *J Biol Chem* 279(13): 12001–4

Kole TP, Tseng Y, Jiang I et al (2005). Intracellular mechanics of migrating fibroblasts. *Mol Biol Cell* 16(1): 328–38

Kung C (2005) A possible unifying principle for mechanosensation. *Nature* 436(7051): 647–54

Lancerotto L, Bayer LR, Orgill DP (2012) Mechanisms of action of microdeformational eound therapy. *Sem Cel Develop Biol* 23(9): 987–92

Lavery LA, Barnes SA, Keith MS et al (2008) Prediction of healing for postoperative diabetic foot wounds based on early wound area progression. *Diabetes Care* 31(1): 26–9

Lu F, Ogawa R, Nguyen DT et al (2011). Microdeformation of three-dimensional cultured fibroblasts induces gene expression and morphological changes. *Ann Plast Surg* 66(3): 296–300

Martinac B, Buechner M, Delcour AH et al (1987) Pressure-sensitive ion channel in Escherichia coli. *Proc Natl Acad Sci USA* 84(8): 2297–301

Morykwas MJ, Faler BJ, Pearce DJ, Argenta LC (2001). Effects of varying levels of subatmosphereic pressure on the rate of granulation tissue formation in experimental wounds in swine. *Ann Plast Surg* 47(5): 547–51

Moues CM, van Toorenenbergen AW, Heule F et al (2008) The role of topical negative pressure in wound repair: expression of biochemical markers in wound fluid during wound healing. Wound Repair Regen 16(4): 488–94

Raeber GP, Mayer J, Hubbel JA (2008) Part I: A novel in-vitro system for simultaneous mechanical stimulation and time-lapse microscopy in 3D. *Biomech Model Mechanobiol* 7(3): 203–14

Reinke JM, Sorg H (2012) Wound repair and regeneration. *Eur Surg Res* 49(1): 35–43

Rodriguez-Menocal L, Salgado M, Ford D, van Badiavas E (2012) Stimulation of skin and wound fibroblast migration by mesenchymal stem cells derived from normal donors and chronic wound patients. *Stem Cell Trans Med* 1(3): 221–9

Sandbo N, Dulin N (2011) Actin cytoskeleton in myofibroblast differentiation: ultrastructure defining form and driving function. *Transl Res* 158(4): 181–96

Saxena V, Hwang CW, Huang S et al (2004) Vacuum-assisted closure: microdeformations of wounds and cell proliferation. *Plast Reconstr Surg* 114(5): 1086–96

Saxena V, Orgill D, Kohane I (2007) A set of genes previously implicated in the hypoxia response might be an important modulator in the rat ear tissue response to mechanical stretch. *BMG Genomics* 8: 430

Scherer SS, Pietramaggiori G, Mathews JC, Orgill DP (2009) Short periodic applications of the vacuum-assisted closure device cause an extended tissue response in the diabetic mouse model. *Plast Reconstr Surg* 124(5): 1458–65

Scherer SS, Pietramaggiori G, Mathews JC et al (2008) The mechanism of action of the vacuum-assisted closure device. *Plast Reconstr Surg* 122(3): 786–97

Schulze C, Wetzel F, Kueper T et al (2010) Stiffening of human skin fibroblasts with age. *Biophys J* 99(8): 2434–42

Schwarz F, Jennewein M, Bubel M et al (2013) Soft tissue fibroblasts from well healing and chronic human wounds show different rates of myofibroblasts in vitro. *Mol Biol Rep* 40(2): 1721–33

Schwarz US, Bischofs IB (2005) Physical deterninants of cell organization in soft media. *Med Eng Phys* 27(9): 763–72

Silver FH, Siperko LM (2003) Mechanosensing and mechanochemical transduction: how is mechanical energy sensed and converted into chemical energy in an extracellular matrix? *Crit Rev Biomed Eng* 31(4): 255–331

Sun Y, Fu J (2013). Mechanobiology: a new frontier for human pluripotent stem cells. *Integr Biol (Camb)* 5(3): 450–7

Susilo ME, Roeder BA, Voytik-Harbin SL, Kokini K, Nauman EA (2010). Development of a three-dimensional unit cell to model the micromechanical response of a collagen-based extracellular matrix. Acta Biomat 6: 1471–86

Trengove NJ, Stacey MC, Macauley S et al (1999). Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. *Wound Rep Reg* 7: 442–52

Wang JHC, Li B (2010). Mechanics rules cell biology. *Sports Med Artheros Rehabil Ther Technol* 2: 16010

Wells RG (2008). The role of matrix stiffness in regulating cell behavior. *Hepatology* 47: 1394–400

Wiegand C, White R (2013). Microdeformation in wound healing. *Wound Rep Reg* 21: 793–9

Yager DR, Nwomeh BC (1999). The proteolytic environment of the chronic wounds. *Wound Rep Reg* 7: 433–41

Zhou M. Yu A, Wu G, Xia C, Hu X, Qi B (2012). Role of different negative pressure values in the proves of infected wounds healing treated by vacuum-assisted closure: an experimental study. *Int Wound J* 10: 508–15