

Gravity, Mechanotransduction and Healing: How Mechanical Forces Promote Tissue Repair

Frederick H Silver^{1*} and Lisa L Silver²

¹Department of Pathology and Lab Medicine, Robert Wood Johnson Medical School, USA

²OptoVibronex LLC, USA

Article Information

Received date: Oct 26, 2017

Accepted date: Nov 10, 2017

Published date: Nov 14, 2017

*Corresponding author

Frederick H Silver, Department of Pathology and Laboratory Medicine, Robert Wood Johnson Medical School, Rutgers, the State University of New Jersey, Piscataway, NJ 08854, USA, Tel: +6104282173; Email(s): silverfr@rutgers.edu (or) fhsilver@hotmail.com

Distributed under Creative Commons CC-BY 4.0

Keywords Mechanotransduction; Collagen; Extracorporeal shock therapy; Pulsed electromagnetic waves; Low intensity vibrations; Vacuum assisted wound healing; Physical therapy; Cartilage; Bone; Skin; Scar; Tendon; Nerve; Cardiovascular tissue; Tissue engineering; Chronic wounds

Abstract

Gravity plays a central role in vertebrate development, evolution and repair and regeneration responses. Recent studies suggest that tissue repair may in part be stimulated by mechanical loading of tissues in a process by which addition of mechanical energy leads to changes in tissue metabolism by mechanotransduction. In this paper we summarize reported research studies on how mechanotransduction can stimulate repair of tissues to enable clinicians to promote healing of chronic wounds and assist in the facilitation of production of tissue engineered products.

External energy in the form of extracorporeal shock waves, low intensity vibrations, pulsed electromagnetic waves and vacuum assisted wound healing have been used to promote healing. Results of research studies on cells and tissues suggest that mechanical loading can down-regulate inflammation, modify natural immune responses during healing and is also associated with promoting angiogenesis. Using vibrational OCT, a non-destructive and non-invasive method to measure mechanical properties of tissues and implants, it is possible to evaluate the effects of mechanotransduction on repair responses. Further studies are needed to optimize the healing effects of mechanotransduction in bone, tendon, cartilage, nerve, myocardium, cardiovascular tissue, and skin.

Introduction

Gravity plays a central role in vertebrate development, evolution, and tissue repair and regeneration responses. In the presence of a gravitational field, muscular forces required for locomotion or for daily activities are increased [1]. External loading in a gravitational field leads to induction of pathways that modify cell division and protein synthesis [1]. As body mass increases during development, increased muscular forces are required to propel large vertebrates; this requires that the musculoskeleton as well as other tissues and organs be able to adapt by increasing the size of their functional units. In addition, the results of recent studies suggest that tissue regeneration and repair may in part be stimulated by external mechanical loading [2-7]. Application of external mechanical forces to cardiac tissue, cartilage, bone, muscle, nerve, tendon, skin and scar have been reported to promote tissue repair [2,8-16].

Tissues are primarily composed of Extracellular Matrix (ECM) containing collagen the most abundant protein found in the body [17,18]. They are found in four different classifications including: surface and internal lining structures, conduit and holding structures, dental and orthopaedic tissues and specialized organs [5]. All of these biological materials contain collagen and ECM and are candidates to promote integrin mediated mechanotransduction in regeneration and repair responses. The purpose of this paper is to review how external mechanical forces applied to tissues can positively influence regeneration and repair processes. This is an update of a previous literature review on mechanotransduction and the reader is directed to that paper for many references before 2003 [1].

What are Mechanosensing and Mechanotransduction?

Mechanosensing is used to describe the process by which cells sense mechanical forces while mechanotransduction is used to describe the processes by which internal and external forces influence the biochemical and genetic responses of cells and tissues [1]. Specifically these responses include stimulation of cell proliferation or apoptosis and synthesis and catabolism of components of the extracellular matrix.

Mechanotransduction enables a cell to sense and quickly adapt to mechanical forces and physical limitations [3]. It is also a process of sensing mechanical cues and evoking subsequent biochemical responses [19] and a means by which physical therapists can harness the mechanosensitivity of cells to potentiate cellular healing and tissue regeneration and repair [4].

Examples of the effects of mechanotransduction

A classic example of mechanotransduction is that of weight lifting. Not only does the lifter gain in muscle mass by direct loading of his or her skeletal muscle, but through stretching of the skin, the athlete gains additional skin to cover the expanded muscular tissue [17]. The carpenter's skin gains in thickness by repeatedly using a hammer that applies a compressive force to the epidermis that leads to thickening of the epidermis, the top layer of skin. It is well known that astronauts that reside in a gravity-free state for long periods of time experience resorption of collagen and mineral from their long bones [20]. Intimal hyperplasia as a result of mechanical mismatches at the interface between arteries and vascular implants as well as pressure induced fat necrosis around femoral closure sites are additional examples of tissue responses due to mechanotransduction [17].

What Biological Pathways are involved in Mechanotransduction?

Mechanotransduction involves several different pathways including: (1) direct stretching of collagen-cell surface integrin binding sites on the surface of all eukaryotic cells (integrin dependent processes); (2) direct deformation of gap junctions between cells containing calcium sensitive stretch receptors; (3) activation of ion channels in the cell membrane; and (4) activation of hormone and growth factor receptors [1] (see Table 1).

Integrin Mediated Mechanotransduction

Integrins are a family of cell surface embedded proteins that provide a link between the extracellular environment and intracellular elements [21,22]. Cells are believed to sense intrinsic mechanical properties of ECM by applying traction forces to the collagen fibers [22]. Collagen fibers of the ECM bind to transmembrane integrins which in turn span the cell membrane and connect to actin filaments within the cell.

The combination of integrins and actin filaments form Integrin Associated Complexes (IACs) that assemble into Focal Adhesions (FAs) [22]. FAs display a complex three dimensional organization containing three layers [23]. The vertical layer consists of a FA outer component containing integrin receptors. The intermediate layer couples the layer containing the integrin receptors and the inner layer is involved in force generation and mechanosensing [23].

Changes in these traction forces between the cells and the ECM are the mechanism by which cells respond to external mechanical signals [23]. However, since the moduli of collagen molecules in fibrils are between 4 and 7 GPa and that of cells is only in the kPa range, it is likely that cells respond to changes in strain rather than changes in the modulus or force [17]. In this manner shear strains applied to cells

that reside on the surface of the collagen fibers probably upregulate mechanochemical transduction. Tensional forces applied directly to cells bound to collagen fibers would result in cellular disruption and ultimately tissue necrosis.

Recent advances in understanding integrin mediated mechanotransduction are important to defining the relationship between external mechanical loading and the events that occur within the surrounding tissue [21,24]. This suggests that any interruption of integrin mediated binding to the surrounding ECM at the cell-tissue interface has consequences in terms of modifying mechanotransduction and the cellular response to surrounding tissues.

Focal adhesion maturation requires further integrin clustering, intracellular F-actin binding and reinforcement of linkages between actin and actomyosin. Activated integrins are coupled to F-actin through actin binding proteins including talin and vinculin. These molecules are involved in the "molecular clutch" that is responsible for cell movement as a result of actin flow inside the cell as F-actin is polymerized. This leads to force generation and propulsion of the cell body [24].

Gap Junctions, Ion Channels, Hormone and Growth Factor Receptor Mediated Mechanotransduction

The ability of gap junctions, ion channels, and hormone and growth factor receptors to affect mechanotransduction has been reported. Shear mechanotransduction in cardiac myocytes has been reported to activate gap junction hemichannels and alter Ca^{2+} signaling [11]. In bone cells, gap junctions, particularly connexin 43, is reported to influence the function of osteoblasts and osteocytes [25] (Table 2). Pressure sensitive ion channels have also been identified in *Escherichia Coli* [26], suggesting that ion channel permeability in a variety of cell types can be modified by applied forces. Stretch activation of ion channel results in conformational changes in proteins involved in focal adhesions. Lamins are an extended part of the LINC complex (Linker of Nucleoskeleton and Cytoskeleton) that structurally support the nucleus and enable forces to be transmitted from the extracellular matrix to cytoskeleton and nuclear interior [27].

Other molecules involved in mechanotransduction that have been studied include mechano growth factor that induces local protein synthesis and prevents apoptosis [16] and the endothelin system that is involved in bone matrix synthesis and mineralization [28].

Mechanisms that Trigger Protein Synthesis and Cell Division

Phosphorelay pathways that support mechanotransduction can be activated not only by integrin binding to ECMs, but by direct stretching of the cell membranes, ion channels, intracellular junctions, and growth factor and hormone receptors. Activation of these moieties leads to changes in protein synthesis, altered gene expression and cell mitosis [1]. All these events occur at the cell-tissue interface and dictate rate, composition and extent of tissue that is deposited. Providing the correct boundary forces and stresses to equilibrate the cell-tissue interface is important to prevent cellular hyperplasia and excessive collagen deposition. When pressure is increased after insertion of a breast prosthesis, or after breast reduction surgery

Table 1: Tissue Components Involved in Mechanotransduction.

Component	Role	References
Integrins	Transmit forces from ECM to intracellular Actin	[21,23]
Gap Junctions	Upregulate Ca^{2+} signaling	[11,25]
Ion Channels	Pressure increases ion flow	[26]
Growth Factor and Hormone Receptors	Upregulate growth	[16,28]

Table 2: Cellular Components Involved in Mechanotransduction.

Cell	Component	Role	References
Bone	Endothelin System	Bone Matrix Synthesis	[28]
Bone	Connexin 43	Influence Osteoblasts	[25]
E Coli	Ion Channels	Pressure Increases Ion Channel Activity	[26]
Mycocyte	Gap Junction	Alter Ca ²⁺ Signaling	[11]
Mycocyte	Mechano Growth Factor	Induces Protein Synthesis	[16]

when breast tissue is excised and the tissue is sutured closed [29], interruption of the normal lines of tension within the breast lead to changes in mechanotransduction that result in the deposition of a collagenous capsule and scar tissue [5]. Pressure dressings applied to the skin are known to cause resorption of hypertrophic scar tissue [30] suggesting that pressure either from a dressing or from an implant pressing on host tissue can cause tissue catabolism.

Application of External Energy, Mechanotransduction and Healing

The application of external energy to tissues has been used to promote tissue repair and wound healing. Low energy shock waves have been reported to down regulate immune responses, reduce invasion of polymorphonuclear leukocytes, suppress production of proinflammatory cytokines and chemokines helping to promote wound healing [15] (Table 3). Extracorporeal Shock Wave Therapy (ESWT) has been reported to suppress the early proinflammatory immune response to a severe cutaneous burn injury [31], increase the levels of vascular endothelial growth factor [15], and modulate skin fibroblast recruitment and leucocyte infiltration for enhancing extended skin flap survival [32]. Macrophage exposure to low energy shock waves dampens the induction of the pro-inflammatory profile characterizing M1 macrophages and promotes the acquisition of an anti-inflammatory profile [33]. In addition, it has been reported that biophysical stimulation induces proliferation, differentiation and immunomodulation of mesenchymal stem cells during wound repair [14].

The application of tension on tissue appears to have positive effects on wound healing in a number of studies. Recent reviews suggest that negative pressure wound healing (that occurs by applying a vacuum to wounds and stretching wound tissues), physical therapy protocols, electromagnetic stimulation, dynamic fluid flow, low intensity vibrations, and extracorporeal shock wave therapies are treatments that promote healing through mechanotransduction [2,34]. The link between movement biomechanics, physical therapy, and subsequent

Table 3: Effects of External Energy on Tissues.

Effector	Effect	References
ESWT	Down regulates immune response	[15]
ESWT	Suppress early inflammation	[31]
ESWT	Modulate skin fibroblast recruitment	[32]
ESWT	Promotes macrophage anti-inflammatory profile	[33]
ESWT	Induces proliferation of MSC	[14]
Negative Pressure	Causes micro- and macro-deformation	[2]

Table 4: Conditions Used to Promote Healing via Mechanotransduction.

Method	Conditions	References
Cyclic Loading	100-2,000 microstrains	[41]
ESWT	Positive Pressure to 100 MPa Followed by Negative Pressure up to 20 MPa.	[9,12,40]
Low Intensity Ultra-Sound	30 Hz to 1.5 MHz	[36,37]
Negative Pressure Wound Healing	Vacuum 70 to 150 mm Hg	[2,35]

cellular and tissue mechanoadaptation is not well established; however, altered joint physiology has been shown to occur as a result of physical therapeutic intervention in osteoarthritis, anterior cruciate ligament reconstruction and total knee arthroplasty [4]. In addition, pulsed electromagnetic fields for promotion of tendon healing and ESWT for bone regeneration and chronic wound healing have gained acceptance [4]. Extracorporeal shock wave therapy has been used to reduce scar pain in burn patients after wound recovery [34]. Vacuum message and negative pressure induced wound healing have also been reviewed for their effects on healing [2,35]. Negative pressure wound healing is thought to occur by creating macro- and microdeformation of the wound surface [2].

The conditions reported in the literature for use of ESWT, low intensity vibration, low intensity pulsed ultrasound, dynamic hydraulic flow, and pulsed electromagnetic fields to promote healing vary from tissue to tissue [9,12,35-37] (Table 4).

For ESWT, Lee et al. [12] reported use of 12 sessions using 0.10 mj/mm² at a depth of 5 to 30 mm and frequency of 1 Hz to promote tendon healing. Notarnicole and Moretti [38] discuss use of pressures between 5 and 120 MPa in 5 ns followed by a pressure of negative 20 MPa for treatment of muscles, ligaments, and tendons at energy flux densities of between 0.08 nj/mm² and 0.6 nj/mm². Ramon et al. [9] report use of ESWT for myofascial pain syndrome and fibromyalgia at focused pressures of 10-100 MPa followed by negative pressures of 0.1 MPa to 1 MPa for focused periods of 10 to 100ns.

Other conditions reported for healing include applying cyclic microstrains of 100-2,000 to promote bone and fracture healing [39] and low intensity ultrasound at frequencies from 30 Hz [36] to 1.5 MHz [35] for bone repair. In the ultrasound study reported by Thompson et al. [36], the transducer had a radius of 11 cm and emitted 1.5 MHz ultrasound at a velocity of 1540 ms⁻¹.

Methods for Evaluation of the Repair Response as a Result of Mechanotransduction

Numerous tests have been used to elucidate mechanical properties of tissues and implants including tensile, compressive, shear, hydrostatic compression and three-point bending in one or more axial directions. The development of a non-destructive test that could be applied to tissues and materials *in vivo* would promote the analysis of the effect of external forces on the healing of tissues [40,41].

There are several new methods such as Magnetic Resonance Elastography (MRE), Ultrasound Elastography (UE), Optical Coherence Tomography (OCT), Optical Coherence Elastography (OCE) and OCT with vibrational analysis that are quite promising for measuring changes in the mechanical properties of tissues. However,

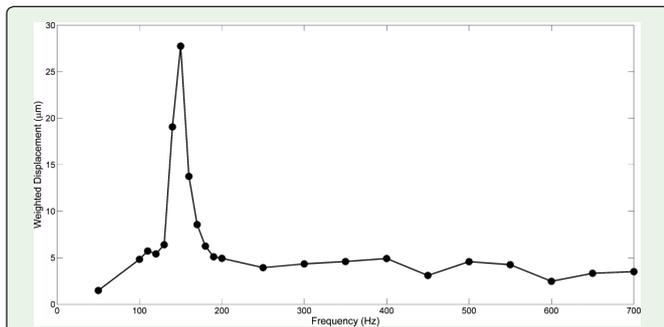


Figure 1: Plot of weighted displacement versus frequency for decellularized dermis. This figure illustrates how the maximum displacement of a sample is a function of vibrational frequency applied. The maximum displacement of the sample is used to determine the resonant frequency as discussed by Shah et al. [40-42]. The resonant frequency squared is related to the modulus.

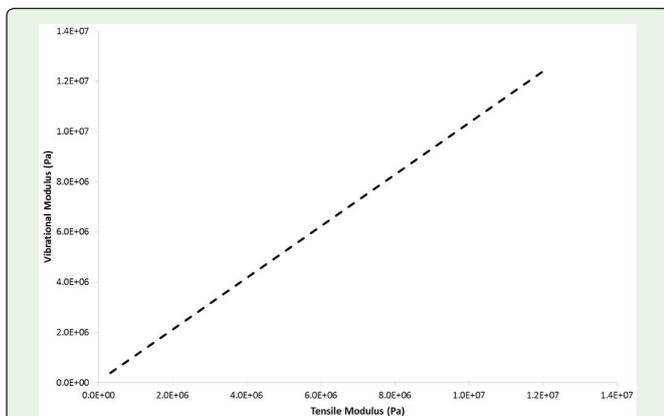


Figure 2: Calibration curve of modulus determined from vibrational studies and conventional tensile testing. This plot shows the modulus determined from a combination of vibrational stimulation and OCT is directly related to the modulus measured in a tensile incremental stress-strain test as reported previously [40-42].

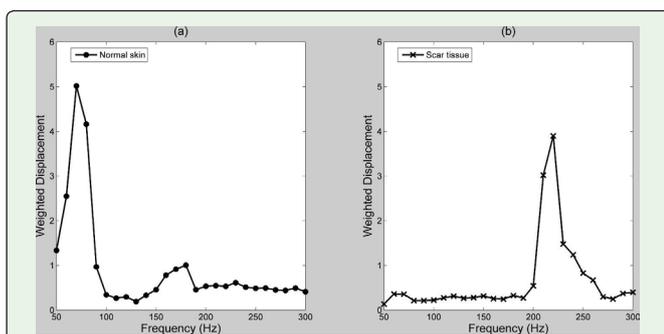


Figure 3: Vibrational OCT measurements on human skin and scar tissue. This figure shows the weighted displacement versus frequency for normal skin (left) and scar tissue (right). The resonant frequencies were 70 Hz (normal skin) and 220 Hz (scar tissue). Note the small peaks in normal skin at about 180 Hz and in scar at about 70 Hz represent collagen and elastic fiber contributions, respectively. In this manner the modulus of each component of a tissue can be measured at the same time [40-42]. In scar tissue the elastic tissue contribution to the modulus is lower than that measured in normal skin due to the lack of elastic tissue that is deposited during wound repair. If the normal skin is stretched further by stretching the skin the collagen peak is enlarged (data not shown).

for these techniques to provide relevant information about the effects of mechanical forces on healing, they must consider the non-linear behavior, strain-rate dependence and volumetric effects that occur during mechanical loading of tissues [40,41].

Recently, we have reported the use of vibrational OCT to visualize and measure the mechanical properties of tissues [40-42]. Results of previous studies suggest that wound healing in skin and tendon is accompanied by an increase in tissue modulus (resistance to stretching) and ultimate tensile strength [43,44]. Using vibrational OCT the modulus of tissue can be measured non-invasive and non-destructively *in vivo* [40-42]. The experimental parameter measured using this technique is the resonant frequency of tissue which is converted into the modulus [40-42] (Figure 1). The method gives an elastic modulus that is calibrated based on measurements made by comparing uniaxial tensile measurements and those made using vibrational OCT on the same substrate [41,42] (Figure 2). There is approximately a one to one correlation between the moduli measured using both of these techniques [41,42]. Using vibrational OCT it is possible to evaluate the properties of normal skin and scar tissue [45] (see Figure 3). The ability to measure increases in the mechanical properties of tissues associated with mechanical stimulation of wound healing is an important aspect of optimizing mechanotransduction in each tissue type.

Conclusion

Mechanotransduction appears to be a powerful tool by which vertebrates have adapted to life in a gravitational field. While compressive forces or a lack of tension appear to lead to tissue destruction, tensile forces appear to up-regulate mechanotransduction. At least one mechanism involves direct stretching of cell membrane components termed integrins that bind specifically to collagen fibers of the ECM. Additional cellular components involved in mechanotransduction include cell membranes, gap junctions, ion channels and hormone and growth factor receptors. The latter are also activated by stretching.

External forces generated by EWST, low intensity vibrations, ultrasound, fluid flow and electromagnetic fields appear to be able to up-regulate mechanotransduction and lead to wound and tissue repair as well as promote cell division and protein synthesis *in vivo*. The harnessing of these methods to promote healing of chronic non-healing wounds and in the preparation of cell seeded tissue engineered materials will require further research and careful optimization of the effects of each type of mechanical stimulation.

References

1. Silver FH and Siperko LM. Mechanosensing and Mechanochemical Transduction. *Crit Rev Biomed Eng.* 2003; 31: 255-331.
2. Saxena V, Huang C-W, Huang S, Eichbaum Q, Ingber D, Orgill D, Vacuum assisted closure: microdeformations of wounds and cell proliferation. *Plast Reconstr Surg.* 2004; 114: 1086-1096.
3. Rosineczuk J, Taradaj J, Dymarek R, Sopol M. Mechanoregulation of wound healing and skin homeostasis. *BioMed Res Int.* 2016; 2016: 1-13.
4. Ng TL, Kersh ME, Kilbreath S, Tate HK. Establishing the basis for mechanobiology based physical therapy. *Front Physiol.* 2017; 8: 303.
5. Silver FH. *Mechanosensing and Mechanochemical Transduction in Extracellular Matrix.* Springer. 2006.

6. Najrana T and Sanchez-Esteban J. Mechanotransduction as an adaptation to gravity. *Front Pediatr.* 2016; 4: 140.
7. Silver FH. A matter of gravity-mechanotransduction: how mechanical forces influence biological materials. *Material Sci & Eng Int J.* 2017; 1: 00012.
8. Cheng JH, Wang CJ. Biological mechanism of shockwave in bone. *Int J Surg.* 2015; 24: 143-146.
9. Ramon S, Gleitz M, Hernandez L, Romero LD. Update of efficacy of extracorporeal shockwave treatment for myofascial pain syndrome and fibromyalgia. *Int J Surg.* 2015; 24: 201-206.
10. Klein-Nulend J, Bakker AD, Bacabac RG, Varsa A, Wanbaum S. Mechanosensation and transduction in osteocytes. *Bone.* 2013; 54: 182-190.
11. Kim JC, Son MJ, Wang J, Woo SH. Regulation of cardiac Ca²⁺ and ion channel by shear mechanotransduction. *Arch Pharm Res.* 2017; 40: 783-795.
12. Lee JY, Yoon K, Park CH, Lee JS, Seo KH, Park YS, et al. Long-term outcome and factors affecting prognosis of extracorporeal shock wave therapy for chronic refractory Achilles tendinopathy. *Ann Rehabil Med.* 2017; 41: 42-50.
13. Ogawa R, Hsu CK. Mechanobiological dysregulation of the epidermis and dermis in skin disorders and in degeneration. *J Cell Mol Med.* 2013; 17: 817-822.
14. Vigano M, Sansone V, d'Agostino MC, Romeo P, Orfei CP, Girolamo L. Mesenchymal stem cells as therapeutic target of biophysical stimulation for the treatment of musculoskeletal disorders. *J Ortho Surg Res.* 2016; 11: 163.
15. Hausner T, Nogradi A. The use of shock waves in peripheral nerve regeneration: new perspectives. *Int Rev Neurobiology.* 2013; 109: 85-97.
16. Goldspink G, Yang SY. Effects of activity on growth factor expression. *Int J Sport Nutr Exerc Metab.* 2001; 11, 21-27.
17. Silver FH and DeVore D. Materials science and mechanics of collagenous tissue. *Res Rev J Mat Sci.* 2017; 5: 38-47.
18. Silver FH. *Biological Materials: Structure, Mechanical Properties and Modeling of Soft Tissues.* NYU Press: New York. 1987.
19. Wang J, Zhang Y, Zhang N, Wang C, Herrier T, Quinfeng L. An updated review of mechanotransduction in skin disorders: transcriptional regulators, ion channels and microRNAs. *Cell Mol Life Sci.* 2015; 72: 2091-2106.
20. Bloomfield SA, Martinez DA, Boudreaux RD, Mantri AV. Microgravity Stress: bone and connective tissue. *Compr Physiol.* 2016; 6: 645-686.
21. Sun Z, Shengzan SG, Fassler R. Integrin-mediated mechanotransduction. *J Cell Biol.* 2016; 214: 445-456.
22. Jansen KA, Atherton PM, Ballestrem C. Mechanotransduction at the cell-matrix interface. *Semin Cell Dev Biol.* 2017; 71: 75-83.
23. Ringer P, Colo G, Fässler R, Grasshoff C. Sensing the mechano-chemical properties of the extracellular matrix. 2017.
24. Case LB and Waterman CM. Integration of actin dynamics and cell adhesion by a three dimensional, mechanosensitive molecular clutch. *Nat Cell Biol.* 2015; 17: 953-963.
25. Buo AM, Stains JP. Gap junction regulation of signal transduction in bone cells. *FEBS Lett.* 2014; 588: 1315-1321.
26. Martinac B, Buechner M, Delcour AH, Adler J, Kung C. Pressure-sensitive ion channel in *Escherichia coli*. *Proc Natl Acad Sci U S A.* 1987; 84: 2297-2301.
27. Isermann P, Lammerding J. Nuclear mechanics and mechanotransduction in health and disease. *Curr Biol.* 2013; 23: 1113-1121.
28. Kristianto J, Johnson MG, Afzal R, Blank RD. Endothelin signaling in bone. *Endocrinol Metab Clin North Am.* 2017; 46: 51-62.
29. Silver FH, Pins GD, Rizvi A, Olson RM, D'Aguillo A. Silicone gel-filled breast implants: Is local inflammation associated with fat necrosis? *The Breast Journal.* 1995; 1; 17-21.
30. Tejjam S, Zhang J, Travis TE, Carney BS, Alkhalil A, Moffatt LT, et al. Compression therapy affects collagen type balance in hypertrophic scar. *J Surg Res.* 2016; 201: 299-305.
31. Davis TA, Stojadinovic A, Anan K, Amare M, Naik S, Peoples GE, et al. Extracorporeal shock wave therapy suppresses the early proinflammatory immune response to a severe cutaneous burn. *Int Wound J.* 2009; 6: 11-21.
32. Kuo YR, Wang CJ, Wang FS, Yang K, Chang YC, Wang CJ. Extracorporeal shock wave treatment modulates skin fibroblast recruitment and leukocyte infiltration for enhancing extended skin flap survival. *Wound Repair Regen.* 2009; 17: 80-87.
33. Sukubo NG, Tibalt S, Respizzi S, Locati M. Effect of shock waves on macrophages: A possible role in tissue regeneration and remodeling. *Int J Surg.* 2015; 24: 124-130.
34. Cho YS, Joo SY, Cui H, Cho SR, Yim H, Seo CH. Effect of extracorporeal shockwave therapy on scar pain in burn patients. *Medicine.* 2016; 95: e4575.
35. Harrison A, Lin S, Pounder N, Mikuni-Takagak Y. Mode & mechanism of low intensity pulsed ultrasound (LIPUS) in fracture repair. *Ultrasonics.* 2016; 70: 45-52.
36. Thompson WR, Scott A, Loghmani MT, Ward SR, Warden SJ. Understanding mechanobiology: physical therapists as a force in mechanotherapy and musculoskeletal regenerative rehabilitation. *Phys Ther.* 2016; 96: 560-569.
37. Hu M, Cheng J, Qin YX. Dynamic hydraulic flow stimulation on mitigation of trabecular bone loss in a functional disuse model. *Bone.* 2012; 51: 819-825.
38. Notarnicola A, Moretti B. The biological effects of extracorporeal shock wave therapy (ESWT) on tendon tissue. *Muscles Ligaments Tendons J.* 2012; 2: 33-37.
39. Mavcic B, Antolic V. Optimal mechanical environment of the healing bone fracture/osteotomy. *Int Orthop.* 2012; 36: 689-695.
40. Shah RG and Silver FH. Viscoelastic behavior of tissues and implant materials: Estimation of the elastic modulus and viscous contribution using optical coherence tomography and vibrational analysis. *J Biomed Tech Res.* 2017; 3: 105-109.
41. Shah R, Pierce MC, Silver FH. A method for non-destructive mechanical testing of tissues and implants. *J Biomed Mat Res A.* 2017; 105: 15-22.
42. Shah R, DeVore D, Pierce MG, Silver FH. Morphomechanics of dermis-A method for non-destructive testing of collagenous tissues. *Skin Res Tech.* 2017; 23: 399-406.
43. Doillon CJ, Dunn MG, Bender E, Silver FH. Collagen Fiber Formation in repair tissue: Development of Wound Strength and Toughness. *Coll Relat Res.* 1985; 5: 481-492.
44. Goldstein JD, Tria AJ, Zawadsky JP, Kato YP, Christiansen D, Silver FH. Development of a Reconstituted Collagen Tendon Prosthesis. *J Bone Joint Surg Am.* 1989; 71: 1183-1191.
45. Shah RG, Silver FH. Vibrational analysis of extracellular matrix scaffolds: comparison of skin, dermis, cartilage, subchondral bone using OCT and vibrational analysis. *Adv Tissue Eng Regen Med.* 2017; 2: 00048.