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# The Role of Physical Factors in Cell Differentiation, Tissue Repair and Regeneration

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## 1. Introduction

Physical factors may induce significant biological effects, therefore they can be applied in biomedical and biotechnological fields in order to drive and modulate biological processes. It is well known that both humoral and physical factors (in particular, but not limited to the mechanical ones) are necessary for maintaining tissue homeostasis. Both biochemical and physical factors can induce the cells to reprogram their functions to adapt dynamically to the environmental conditions.

It is evident, therefore, that the only way to approach functional tissue regeneration and repair is to supply combined humoral and physical stimuli in a dose- and time-dependent manner. For example, *in vitro* studies have shown that a biomimetic environment simulating pulsatile flow is an indispensable condition for the tissue engineering of functional trileaflet heart valves from human marrow stromal cells. Static controls show morphological alterations and weaker mechanical properties (Hoerstrup et al., 2002).

Studies on the role of physical factors in tissue repair and regeneration cover a very broad field that extends from investigations aimed at deepening our understanding of the physiological mechanisms of tissue repair and regeneration to biotech advances in tissue engineering, such as development of biocompatible scaffolds, 3D cell culture systems and bioreactors, which in the future must integrate the delivery of biochemical factors with the provision of physical stimuli that are equally necessary. In this chapter, far from providing a comprehensive overview of this field of studies, we introduce some issues concerning the application of physical factors in biomedicine and biotechnology and report the results of our research on the application of various physical stimuli (gravitational and mechanical stresses, laser radiation, electromagnetic fields (EMF)) for modulating cell commitment and differentiation, cell adhesion/migration, production and assembly of extracellular matrix (ECM) components, with the final aim of understanding when and how physical stimuli can be useful for promoting tissue repair and formation of functional tissue constructs. We also briefly mention how, in past centuries, the role of physical factors in biological processes has been understood and physical stimuli have been applied for therapeutic purposes.

## 2. Mechanical stresses

The importance of gravitational and mechanical factors in modulating biological processes has been known for a long time: from Galileo Galilei onwards, studies on functional adaptation of the skeleton demonstrated that bone loss or gain is related to the magnitude, direction and frequency of the stress acting upon the skeleton during application of loads (Galilei, 1632; Wolff, 1985; Rubin, 1985; Frost, 1988; Rubin, 1984; Ingber, 1998).

Within the body, cells are subject to mechanical stimulation, caused by blood circulation, ambulation, respiration, etc., which give rise to a variety of biochemical responses. It has been demonstrated that changes in inertial conditions, shear stress, stretching, etc. can strongly affect cell machinery. Cells may sense mechanical stresses through changes in the balance of forces that are transmitted across transmembrane adhesion receptors that link the cytoskeleton to the ECM and to other cells. These changes, in turn, alter the ECM mechanics, cell shape and cytoskeletal organization (Ingber, 1998, 1999). A great deal of information has revealed that the ECM is a highly dynamic and elastic structure which undergoes continuous remodelling, in particular during development, angiogenesis, wound healing and other tissue repair processes. The ECM interacts with cells to provide relevant microenvironmental information, biochemically through the release of stored soluble and insoluble factors, and physically through imposition of structural and mechanical constraints (Carson, 2004). On the other hand, mechanical stimuli modulate ECM homeostasis: mechanical forces strictly regulate the production of ECM proteins indirectly, by stimulating the release of paracrine growth factors, or directly, by triggering intracellular signalling pathways leading to the activation of genes involved in ECM turnover (Chiquet, 2003).

Mechanical stimuli affect cells through poorly understood mechanotransductive pathways that lead to changes in morphology and orientation, modulation of gene expression, reorganization of cell structures and intercellular communication through both secretion of soluble factors and direct intercellular contact (Maul et al., 2011; Kang et al., 2011; Park et al., 2006; Papachroni et al., 2009; Wall & Banes, 2005; Bacabac et al., 2010; Hughes-Fulford & Boonstra, 2010). Over the past decade, *in vitro* studies have indicated that the transduction of physical stimuli involves the ECM-integrin-cytoskeleton network and also calcium channels, guanosine triphosphatases (GTPases), adenylate cyclase, phospholipase C (PLC) and mitogen-activated protein kinases (MAPKs), all of which play important roles in early signaling (Rubin et al., 2006; Hoberg et al., 2005; Adachi et al., 2003; Mobasheri et al., 2005; Chiquet et al., 2009; Bacabac et al., 2010; Hughes-Fulford & Boonstra, 2010). It has been demonstrated that, in endothelial cells, different genetic programs leading to growth, differentiation and apoptosis can be mechanically switched. Cells grow when they spread, die when fully retracted, and differentiate into capillary tubes if maintained at a moderate degree of extension (Chen, 1997).

The *in vitro* application of mechanical stretch, simulating the mechanical load to whom heart cells are exposed *in vivo*, initiated in adherent cultures of neonatal cardiomyocytes morphological alterations similar to those occurring during *in vivo* heart growth (Vandenburg, 1996). Stem cell commitment, the process by which a cell chooses its fate, and differentiation, the resulting development of lineage-specific characteristics, have been

shown to be affected by cell shape (Roskelley, 1994; McBeath, 2004; Watt 1988; Spiegelman, 1983). Internal and external forces regulate cell shape and studies have shown that cell shape can control apoptosis, gene expression, and protein synthesis, in addition to stem cell fate (Chen, 1997; Thomas, 2002).

The cells belonging to tissues that resist the effects of gravity are particularly sensitive to mechanical and gravitational stimuli, which play a key role in the development and homeostasis of these tissues. Lack of gravitational and mechanical stresses leads to the formation of impaired tissues with lower mechanical properties and reduced function.

It is well established that bone adapts its mass and architecture in accordance with the external mechanical loads applied and osteocytes, terminally differentiated cells of the osteoblastic lineage, may be considered “mechanosensory cells” (Vatsa et al., 2010). They are sensitive to both stretching and fluid flow. Mechanical stimulation of osteocytes induces intercellular signaling which results in the modulation of osteoblast and osteoclast activity (Chow et al., 1998; Turner et al., 1997). Interestingly, it has been shown that the stimulation of a single osteocyte activates many surrounding cells (Vatsa et al., 2007).

Osteoblastic differentiation can be induced by applying mechanical stress, for example by stretching the surface on which the cells are attached (Cavalcanti-Adam et al., 2002). Many studies revealed that the micromotions at the interface between bone and artificial scaffolds play a key role in scaffold integration: they can promote tissue differentiation or induce bone resorption (Prendergast et al., 1997; Carter et al., 1998; Buchler et al., 2003; Stadelmann et al., 2008; Jasty et al., 1997). In a recent paper on biomechanics of scaffolds for bone tissue engineering applications, it has been stated that in the development of a scaffold it is important to take in account not only the structural integrity but also the load transmitted to the cells via the scaffold deformation (Pioletti, 2011).

A recent review of studies which investigated the importance of loading in maintaining the balance of matrix turnover in the intervertebral disk, reported about the possible role of overloading in the initiation and progression of disc degeneration and proposed a physiological/beneficial loading range as a basis on which to design loading regimes for testing tissue constructs or favouring differentiation of stem cells towards “discogenic” cells for tissue engineering (Chan et al., 2011)

An overview of studies on the role of mechanical stimuli in chondrogenesis showed that uniaxial loading induces the upregulation of genes associated with a chondrogenic phenotype while multiaxial loading results in a broader pattern of chondrogenic gene upregulation, revealing that not only intensity, but also direction and other parameters which characterize the stimulation are relevant for the achievement of the final effect. The physiological multiaxial pattern of loading within articulating joints is so complex that currently, even with the most sophisticated bioreactors, it would be impossible to simulate the *in vivo* situation. Therefore, it has been suggested to use the body as an “*in vivo* bioreactor” (Grad et al., 2011).

Conditions of gravitational unloading, both real and modeled by a Random Positioning Machine (RPM), negatively affect cellular organization and ECM production in cartilage constructs, even if at different extent. (Stamenkovic et al., 2010).

Our group is conducting for several years research on the role of gravitational and mechanical stimuli in cell differentiation, tissue repair and regeneration, with particular attention to the remodelling phase.

Our studies demonstrated that gravitational unloading favours the differentiation of osteoclastic precursors (FLG 29.1 cells). After 72 hours exposure to conditions of microgravity, modelled by a RPM (angular velocity of rotation 60°/s), the cells showed a dramatic increase in apoptosis, but the viable ones showed osteoclastic-like morphology, cytoskeletal reorganization, significant changes in gene expression profile. The expression of the major osteoclastic markers Receptor Activator of Nuclear Factor Kappa-B (RANK) and Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) strongly increased and cells showed the ability to resorb bone (Fig. 1) (Monici et al., 2006).

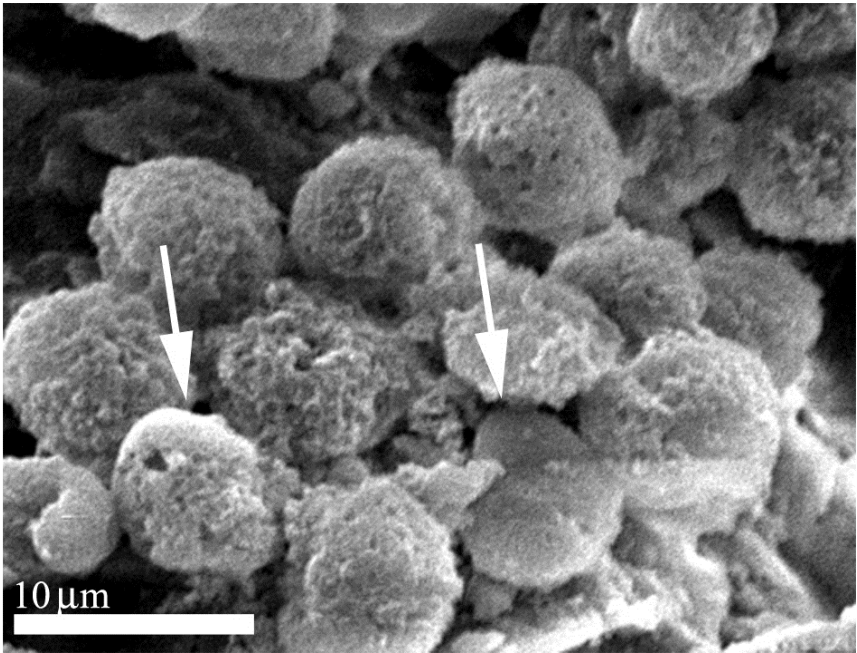


Fig. 1. Scanning electron microscopy of a bone slice exposed to FLG 29.1 cells cultured in modelled microgravity. Adherent cells on the bone surface can be observed. Arrows indicate the sealing zone.

Analysing the gene expression profile of human mesenchymal stem cells (HMSC) in loading conditions (3 hours exposure to 10xg in hyperfuge), we found overexpression of genes involved in osteoblastogenesis (*GLI1*, *NF1*, *MEN1*) and downregulation of genes involved in adipogenesis (*PPARγ*, *FABP4*) (Tab. 1) (Monici et al., 2008a).

Gene	Control	RPM	10 x g	Nd:YAG
FABP4	27	304	3	9
PPARG	12	587	11	14
GLI1	47	45	789	547
NF1	25	9	241	258
MEN1	48	25	158	258

Table 1. Gene expression profile in HMSCs.



These results, in agreement with those of other authors (Kaneuji et al., 2011; Wang et al., 2010; Searby et al., 2005) reveal that mechanical/gravitational stresses induce osteoblastic differentiation while gravitational unloading and loss of mechanical stress favour adipogenesis, osteoclastogenesis and bone resorption.

In cultures of fibroblasts exposed for 3 hours to hypergravity (10xg), we observed enhanced expression of collagen I and fibronectin (20% and 30% more than control, respectively), while chondrocytes exposed to the same treatment showed a marked increase in collagen II, aggrecan and Sox 9, a transcription factor which plays a key role in chondrogenesis. Therefore, after definition of optimal range of intensity and force direction, loading can be used to stimulate ECM production by cells of the connective tissues and to favour chondrogenesis.

A series of experiments we carried out with the aim of studying the effect of gravitational unloading on processes involved in tissue remodelling demonstrated that the loss of mechanical stress causes a dysregulation in laminin and fibronectin (FN) production by fibroblasts and endothelial cells (Fig. 2B) (Monici et al., 2011). In particular, FN forms a disordered and intricate network, reproducing the typical condition of fibrous scars. We hypothesized that the altered FN fibrillogenesis could be a cause of impaired ECM rebuilding and altered cell adhesion/migration and could contribute to the impairment of wound healing observed in microgravity (Midura & Androjna, 2006; Delp, 2008).

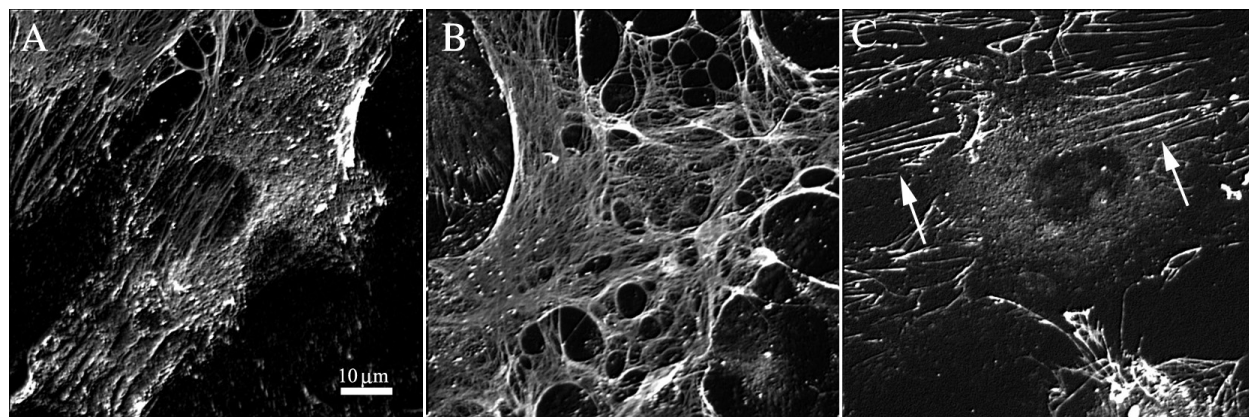


Fig. 2. FN expression in CVECs (analysed by immunofluorescence microscopy): A) control, B) exposed for 72 h to modelled microgravity and C) treated with pulsed Nd:YAG laser (1064 nm). In figure B a tight network of FN fibrils appears while in figure C the FN fibrils are parallel and ordered (see arrows).

Studying the behaviour of aortic endothelial cells cultured in micro- and hypergravity we found that the exposure to simulated microgravity conditions for 72 hours (angular velocity of rotation  $60^\circ/\text{s}$ ) caused a reduction in coronary venular endothelial cell (CVEC) number. Genomic analysis revealed that proapoptotic signals increased, while antiapoptotic and proliferation/survival genes were downregulated by the absence of gravity. Activation of apoptosis was accompanied by morphological changes, with mitochondrial disassembly and organelles/cytoplasmic NAD(P)H redistribution, as evidenced by autofluorescence analysis. Moreover, cells were not able to respond to angiogenic stimuli in terms of migration and proliferation (Morbidelli et al., 2005).

In contrast, after exposure to hypergravity (10xg), no significant changes were observed in cell morphology and energy metabolism. Cells remained adherent to the substrate, but integrin distribution was modified. Accordingly, the cytoskeletal network reorganized, documenting cell activation. There was a reduction in expression of genes controlling vasoconstriction and inflammation. Proapoptotic signals were downregulated. Overall, the results documented that hypergravity exposure maintained endothelial cell survival and function by activation of adaptive mechanisms. The behavior of cells derived from microcirculation was somewhat different, because the above described effects were associated with increased anaerobic metabolism and cell detachment from the substrate (Morbideilli et al., 2009). These findings demonstrate that gravitational/mechanical stress can strongly affect endothelial function and neoangiogenesis and the biological response could also depend on the different vascular districts.

### 3. Electromagnetic fields

It is said that in the first century AD an “electric fish” was used to cure headache. Paracelsus (1493-1542) studied the medical use of lodestone and, in the sixteenth century, Sir Kenelm Digby described the magnetic cure of wounds. At the end of the seventeenth century, Galvani, with his famous experiments on bioelectricity, opened the way for modern studies on physiological EMFs and the effects of external EMFs on the body.

Over the past forty years, important advances have been made in research on bioelectricity: differences in electrical potentials of plants, animals and humans have been measured (Burr, 1972), changes in voltage gradients have been correlated with morphogenetic events in plants and animals (McCaig et al., 2005), physiological currents have been found to be signals for key processes in development (Levin, 2007).

An extensive discussion on electromagnetic effects from cell biology to medicine is presented in a recent review written by Funk et al. (Funk et al., 2009), where the coupling between physical mechanisms and cell biology is discussed in depth. In a nutshell, EMFs can cause polarization of bound charges, orientation of permanent dipoles (which results in topographical changes in molecules), drift and diffusion of conduction charges, ion bound or release from proteins, ion-channel or receptor redistribution, conformational changes of voltage-sensitive enzymes, modulation of binding kinetics, reorientation of membrane phospholipids and changes in activation kinetics of ion channels (Funk et al., 2009).

Endogenous EMFs in living tissues are generated by physiological activities, for example movements of the musculoskeletal system structures. Vibrations of human muscles induce mechanical strains and currents have been measured both during postural muscle activity (5–30 Hz) and walking (<10 Hz) (Antonsson & Mann, 1985). Muscle contractions induce in the underlying bone tissue EMFs which are important for maintaining bone mass. Bone cells are selectively sensitive to low frequencies, in particular those ranging from 15 to 30 Hz. In this narrow range of frequencies, fields as low as 0.01mV/cm affect the remodelling activity (McLeod & Rubin, 1993). It has been found that EM current densities produced by mechanical loading (e.g. 1Hz during walking) in bone lie in the range 0.1–1.0 mA/cm<sup>2</sup> (Lisi et al., 2006). Generally, physiological EMFs are characterized by extremely low frequencies (ELF), from 0 to 300 Hz, and have low intensity.

EMFs are widely used to treat musculoskeletal diseases and many studies indicated that the most effective devices use pulsed EMFs with frequencies from 1 to 100 Hz, which induce EF of the order of  $\mu\text{V}/\text{cm}$  (Pilla, 2002). Therefore, physiological effects may be induced by EMFs characterized by low frequencies (optimal range 8-60 Hz) and amplitudes  $\leq 1$  G (Funk et al., 2009).

It has been demonstrated that pulsed EMFs can increase osteoblastic differentiation and activity and, on the other hand, inhibit osteoclastogenesis, thus shifting the balance towards osteogenesis (Otter et al., 1998; Hartig et al., 2000; Chang et al., 2004).

Studies aimed at evaluating the possibility to apply EMFs to favour ligament healing and repair demonstrated that, after exposure to pulsed EMF, fibroblasts from calf anterior cruciate ligament increased migration speed and showed enhanced collagen I expression. On the contrary, static EMF had an inhibitory effect on wound healing, which was reversed by pulsed EMF (Chao et al., 2007).

EMFs can modulate cell proliferation. The literature indicates that both intensity and frequency of the EMF are important in determining the final effect. Kwee and Raskmark (Kwee & Raskmark, 1995) have found an increase in the proliferation of human fibroblasts exposed to 0.08 mT, while Kula and Drozd (Kula & Drozd, 1996) have shown inhibition of cell growth in murine fibroblasts exposed to 20 mT. Even trials carried out by exposing cultures of human lymphocytes have given different effects (increase, decrease or no effect in the proliferation) depending on the intensity of the applied EMF (Paile et al., 1995; Scarfi et al., 1999).

As regards frequency, many authors reported increases in proliferation of different cell types at 50 Hz frequency (Scarfi et al., 1991; Cossarizza et al., 1993).

Numerous studies have addressed the interaction between EMFs and calcium fluxes, because calcium is a principal regulator of several cellular processes. It is an activator of cyclic AMP, key molecule in triggering intracellular metabolic processes. It has been observed that the exposure to EMFs can modulate calcium concentration in a way which depends on cell type and field intensity. (Farndale, 1987; Waliczek, 1990).

The effects of EMFs on cell differentiation have been studied too. A progressive inhibition of enzyme activity and differentiation in MC-3T3 osteoblast-like cells, after exposure to 30 Hz EMF, was described by McLeod and Collazo (McLeod & Collazo, 2000). In HMSCs exposed to EMFs during chondrogenic differentiation, increase in collagen II and glycosaminoglycan (GAG)/DNA content was observed (Mayer-Wagner et al., 2010). Therefore EMFs might be a way to stimulate and maintain chondrogenesis of HMSCs and provide a new step in regenerative medicine regarding tissue engineering of cartilage.

In recent experiments aimed at studying the effects of EMFs on neuroblasts and understanding whether these effects can be useful in promoting tissue regeneration, we found that in neuroblasts (SHSY5Y human cell line derived from neuroblastoma) exposed to low frequency EMF (50 Hz; 2 mT, 3 hours) synaptophysin and TAU (microtubule-associated proteins) were overexpressed while Microtubule-Associated Protein 2 (MAP2) was downregulated. Synaptophysin participates in the formation of the channel for neurotransmitter release. TAU is associated with the protofilaments in neurites and MAP2 is a microtubule-associated protein found predominantly in the cell body. MAP2 function is



not required when the cell disassembles microtubules in the cell body to give rise to the formation of neurites, while TAU is required to add new subunits to microtubules which are forming in the neurites. Moreover, in the treated neuroblasts, we observed rearrangement of microtubules and actin microfilaments, with formation of cones and cytoplasmic extensions (Fig.3), and increase of neurofilaments, a marker of neurogenic differentiation (not yet published data). Therefore we hypothesize that EMFs can favour differentiation. The expression of synaptophysin, TAU and MAP2 returned to control values 24 h after exposure. Instead, the formation of neurites continued to progress even after 24 h, with the appearance of branched extensions. This means that the changes in protein expression are part of a complex biological response that, once triggered by exposure to the EMF, proceeds even after the cessation of the stimulus (Cerrato et al., in press)

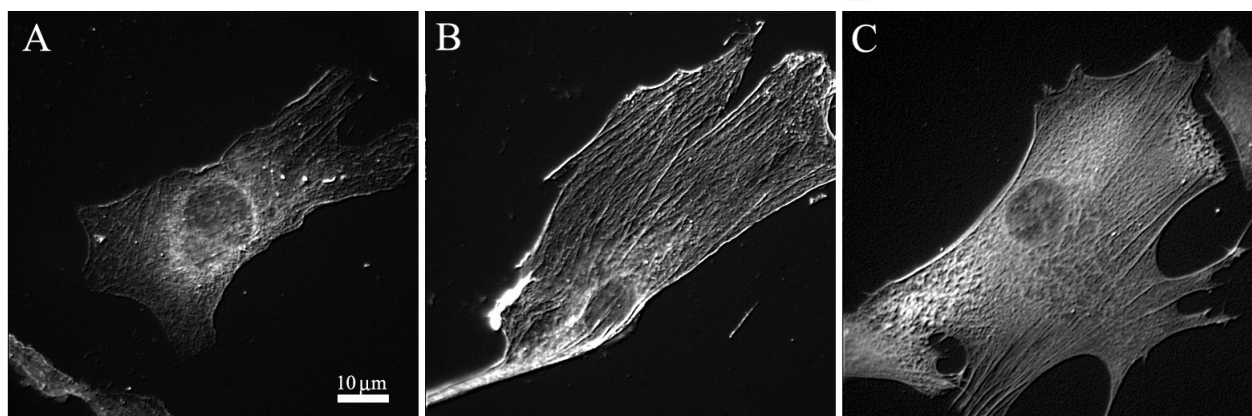


Fig. 3. Actin expression in SHSY5Y cells (analysed by immunofluorescence microscopy): control (A) and cells exposed to EMF (50 Hz, 2 mT, 3h) analysed immediately (B) and 24h (C) after the treatment. The formation of neurites can be observed in figures B and C.

Preliminary experiments on the effect of pulsed EMF on fibroblast behaviour in wound healing models showed, in agreement with other authors (Sunkari et al., 2011), that EMF can accelerate or slow the migration of fibroblasts, depending on the properties of the applied field (data not yet published). The possibility of modulating fibroblast migration during wound healing could be very interesting: in fact it might be useful to enhance the migration of fibroblasts to promote wound healing in chronic ulcers and, in general, in cases where healing is slow, while to inhibit the migration would be beneficial to prevent the formation of fibrous scars.

#### 4. Light

Ancient civilizations had learned that light can have effects on the tissues of the body: both Romans and Greeks widely used the exposure to sun for therapeutic purposes.

In ancient China, a ritual to attain immortality in use during the Tang dynasty (fifth century AD) prescribed exposure to the sun holding in the right hand a piece of green paper with the character representing the sun in red. Subsequently, the paper previously exposed to the sun should be soaked in water and eaten for "trapping" in the body the essence of the sun. At the turn of the nineteenth century and beginning of the twentieth, it was discovered the lethal effect of ultraviolet (UV) component of sunlight on microorganisms and the efficacy of

red light in preventing suppuration and scarring in patients with smallpox: the basis for the modern phototherapy were laid (Barnard & Morgan, 1903; Finsen, 1901; McDonagh, 2001). The extensive application of UV, visible, infrared (IR) radiation in biological and medical fields led to the development of suitable light sources. Actually, lasers are the latest and most advanced type of light source.

The advantages of lasers, compared to other sources, are the high intensity of radiation emitted, the directionality (which allows efficient coupling to optical fibers and focus), the monochromaticity (if needed) and, with pulsed lasers, the possibility to transfer large amounts of energy controlling the thermal effects.

Following the widespread clinical use, many studies have been conducted to investigate at the cellular and molecular level the mechanisms underlying the systemic effects produced by exposure to laser radiation. Propagation and absorption of radiation in a biological sample or in a tissue may produce photochemical, photothermal and photomechanical effects, which are able to induce a biological response (Jacques, 1992).

The actual laser systems, because of their versatility, are particularly suitable for application in biomedicine and biotechnology and the use of lasers to modulate biological and biotechnological processes has been proposed.

Hsu (Hsu et al., 2010) proved that endothelial cells pre-exposed to red-emitting laser (632 nm) and then seeded on a biomaterial surface (biomedical grade poly (carbonate) urethane) increase matrix secretion and are more resistant to flushing (greater cell retention on the graft) in comparison with non laser-exposed controls.

An overview on the state of the art in the photoengineering of bone repair showed that infrared (IR) radiation increases osteoblastic proliferation, collagen deposition and bone neoformation (Pinheiro & Gerbi, 2006)

A recent review in which the photoengineering of tissue repair in skeletal and cardiac muscles is discussed, reported that exposure to lasers with red/near infrared (NIR) emission is effective in favouring muscle repair: the application of He-Ne laser irradiation significantly enhanced muscle regeneration in rats, while Ga-Al-As laser radiation reduced muscle degeneration in the ischemia/reperfusion injury in skeletal leg muscle. Photoexposure also favoured proliferation of myogenic satellite cells. In mouse, rat, dog and pig ischemic heart models, phototherapy significantly reduced (50% - 70%) the formation of scar tissue after induction of myocardial infarction. Ventricular dilation was also reduced and ATP in the infarcted area increased (Oron, 2006).

In order to evaluate the effectiveness of light emitted by lasers and other sources in enhancing cell proliferation, Alghamdi and colleagues (Alghamdi et al., 2011) reviewed the literature in this specific field from 1923 to 2010. They concluded that light with wavelength ranging from 600 to 700 nm is helpful in enhancing the proliferation rate of various cell types, stem cells included. The increase in proliferation was generally associated with increased synthesis of ATP, RNA and DNA. The reviewed data indicated that the optimum value of energy density was between 0.5 and 0.4 J/cm<sup>2</sup>. The possibility to develop phototreatments aimed at favouring cell proliferation could be very useful in the production of vaccines and hybrid cell lines as well as in tissue engineering and regenerative medicine.

In a review concerning the literature from 1960 to 2008 on the use of laser treatments for the improvement of tissue repair, the authors stated that, despite the difficulty in comparing results obtained with different laser sources, treatment protocols and experimental models, the majority of the reviewed reports clearly indicated that laser irradiation (the most frequently used is red/NIR radiation) speeds up tissue repair (da Silva et al., 2010).

In order to evaluate the possibility to use laser treatments as a tool to stimulate cell differentiation processes and cell functions involved in tissue repair, we studied the effect of NIR-emitting lasers on HMSCs, endothelial cells and cells of connective tissues. We found that in HMSCs treated with NIR pulses emitted by a high power Nd: YAG laser (1064 nm wavelength, 200  $\mu$ s pulse duration, 10 Hz repetition rate, 458.65 mJ/cm<sup>2</sup> energy fluence, 73 sec exposure) genes involved in osteoblastogenesis (*GLI1*, *NF1*, *MEN1*) appeared upregulated while *PPAR $\gamma$* , which is a major marker of adipogenesis, and *FABP4* were downregulated, suggesting that the treatment can favour osteoblastogenesis and inhibit adipogenesis (Tab. 1). Interestingly the results obtained with laser treatment are very close to those obtained by exposure of HMSCs to hypergravity (10xg) (Monici et al., 2008b).

Moreover, the Nd:YAG laser pulses resulted effective in enhancing the production of ECM molecules, such as collagen I, collagen II, aggrecan and FN in cultures of connective tissue cells. A similar increase in ECM molecules was found when the cells were exposed to hypergravity (10xg) (Monici et al., 2008b, Basile et al., 2009).

The results of experiments in which we compared the effects of gravitational loading (10xg) and laser pulses on cells belonging to tissues with antigravitational function are consistent with the hypothesis that, using suitable laser pulses, it is possible to induce transient ECM rearrangements in the cell microenvironment (cell niche) which, in turn, act as indirect “photomechanical” stimuli on the cells (Rossi et al., 2010).

Experiments carried out on fibroblasts and endothelial cells, which are responsible for ECM production and neoangiogenesis in the remodelling phase of wound healing, demonstrated that NIR pulses not only increase (30%), FN production, but also favour the ordered assembly of FN fibrils in fibrillogenesis (Fig. 2C) (Monici et al., 2011). This effect is interesting because it could improve the quality of the neoformed ECM: in fact, FN fibrils act as a template for the formation of collagen fibers and strongly affect ECM properties (Shi et al., 2010). Moreover, we observed that NIR laser pulses can also favour the formation of ordered monolayer of endothelial cells (Monici et al., 2011). This effect could be of consequence in neoangiogenesis. Finally, data obtained with preliminary experiments carried out in our laboratory show that pulsed NIR radiation enhances the production of inflammation cytokines (not yet published data). The treatment could thus have the effect of accelerating the transition from inflammatory to the remodelling phase in tissue repair.

Advanced laser systems allow to apply more complex treatment protocols, to try to potentiate or to exploit synergistically the effects produced by emissions with different characteristics.

After exposure of myoblasts to a Multiwave locked System (MLS) laser, emitting at 808 and 905 nm (continuous/interrupted and pulsed mode, respectively) we observed increased activity of enzymes involved in cellular energy metabolism and enhanced expression of MyoD (Vignali et al., 2011), an early marker of muscle differentiation. These results provide an interesting premise for the future application of Multiwave systems to favour muscle tissue repair.

Physical stimulus	Parameters	Experimental model	Effects	Author
MECHANICAL STRESS				
Mechanical stress lengthening	Lengthening of the substratum on which cells adhered (5 mm/day resulting in a 94-110% increase in 4 days	Neonatal rat cardiomyocytes	Cardiomyocytes organized into parallel arrays, increased binucleation and hypertrophy.	Vandenburg, 1996
Mechanical stress topographical stimulation	Micropatterned substrates with ECM-coated adhesive islands	Human and bovine capillary endothelial cells	Modulation of growth, differentiation and apoptosis	Chen, 1997
Mechanical stress loading	150N, 1 Hz	Female rats	Increased bone formation	Chow et al., 1998
Mechanical stress stretching	2% biaxial strain applied cyclically, 0.25 Hz.	MC3T3-E1 osteoblast-like cells	Reorganization of the cytoskeleton and increased expression of alpha(v)- beta3 integrin receptor	Cavalcanti-Adam et al., 2002
Mechanical stress	Mechanical perturbation using a glass microneedle	MC3T3-E1 osteoblastic cell	Changes in intracellular Ca <sup>2+</sup> concentration and actin microfilament organization	Adachi et al., 2003
Mechanical stress stretching	Cyclic stretching, 5% to 15% elongation, 0.3 to 1 Hz	Fibroblasts	Production of ECM components (in particular tenascin-C)	Chiquet et al., 2003
Mechanical stress stretching	30 cycles of uniaxial stretching, 1 Hz, 4000 µε	Human osteoblast-like osteosarcoma cell line MG-63 and primary human osteoblasts	Increased proliferation rates and activation of PLC beta 2 expression	Hoberg et al., 2005
Gravitational stress microgravity	Microgravity modelled by RPM, angular velocity of rotation 60°/s, 72 h	Coronary venular endothelial cells	Decreased cell number, increased proapoptotic signals and down regulation of antiapoptotic and proliferation/survival genes	Morbidelli et al., 2005
Mechanical stress	Different mechanical stresses fluid flow, strain, shear and combinations of them	Connective tissue cells	Activation of mechanotransduction pathways, Ca <sup>2+</sup> signalling, stretch-activated channels, voltage-activated channels	Wall and Banes., 2005 <b>Review on</b> Early responses to mechanical loading in connective tissue cells



Physical stimulus	Parameters	Experimental model	Effects	Author
Gravitational stress microgravity	Microgravity modelled by RPM, angular velocity of rotation 60°/s, 72 h exposure	Osteoclastic precursors	Increased apoptosis, osteoclast-like morphology, cytoskeleton reorganization, overexpression of osteoclastic markers	Monici et al., 2006
Mechanical stress stretching	Cyclic stretching 0.5 Hz , magnitude 8% (8 % deformation of cell-seeded silicone substrate)	Ligament fibroblasts	Enhanced cell proliferation and collagen production	Park et al., 2006;
Mechanical stress	Mechanical load, strain, pressure, different parameters	Osteoblasts, osteoclasts, osteocytes and cells of the vasculature	Many effects are reported	Rubin et al., 2006 <b>Review</b> on Molecular pathways mediating mechanical signalling in bone
Mechanical stress	Mechanical stimulation by microneedles	Osteocytes	Upregulation of NO production in the stimulated cell and in the surrounding osteocytes	Vatsa et al., 2007
Gravitational stress hypergravity	5 periods of 10 min at 10 x g spaced with 10 min recovery periods at 1 x g	Human mesenchymal stem cells	Overexpression of genes involved in osteoblastogenesis	Monici et al., 2008a
Mechanical stress compression	Compression 0.5 Mpa, sinusoidal micromotion 100 µm, 1 Hz	Bone implant	Activation of bone resorption	Stadelmann et al., 2008
Gravitational stress hypergravity	5 periods of 10 min at 10 x g spaced with 10 min recovery periods at 1 x g	Human fetal fibroblast and human chondrocytes	Increased production of ECM molecules	Basile et al., 2009
Gravitational stress hypergravity	5 periods of 10 min at 10 x g spaced with 10 min recovery periods at 1 x g	Coronary venular endothelial cells	Activation of adaptive mechanisms, increased anaerobic metabolism, detachment from the substrate	Morbidelli et al.,2009
Mechanical stress compression	Compressive strain of 5% to 20% elongation, 0.15 to1 Hz, 1 to 12 h/day hydrostatic pressure 0.1 to 10 Mpa, 0.25 to 1 Hz	Intervertebral disc (IVD) and stem cells	Possible role of loading to favour differentiation of stem cells toward “discogenic” phenotype	Chan et al., 2011 <b>Review</b> on The effects of loading on IVD and stem cells

Physical stimulus	Parameters	Experimental model	Effects	Author
Mechanical stress loading	Uniaxial and multiaxial, hydrostatic pressure 7 to 10 Mpa, average tension, 3.8% radial and 2.1% circumferential tensile strains, compression 0.5 to 7.7 MPa	Chondrogenic cells	Upregulation of genes normally associated with a chondrogenic phenotype with uniaxial loading, upregulation of a broader pattern of chondrogenic genes with multiaxial loading	Grad et al., 2011 <b>Review</b> on Chondrogenic cell response to mechanical stimulation in vitro
Mechanical stress	Cyclic strain, 1Hz, 10 % strain of 3D culture + ultrasound 1.0 MHz and 30 mW/cm2	MC3T3-E1 pre-osteoblasts	Acceleration of matrix maturation	Kang et al., 2011
Mechanical stress	Cyclic stretch 5%, 1Hz; cyclic pressure 120/80mmHg, 1Hz; shear stress 10 dynes/cm2.	Mesenchymal stem cells	Increased smooth muscle cells expression with cyclic stretch and endothelial cells expression with cyclic pressure, and laminar shear stress	Maul et al., 2011
Gravitational stress microgravity	Microgravity modelled by RPM, angular velocity of rotation 60°/s, 72 h	Fibroblast and endothelial cells	Disregulation in laminin and fibronectin production	Monici et al., 2011
ELECTROMAGNETIC FIELDS				
EMF	Many different parameters and treatment protocols	Many different experimental models	Many effects are reported	Funk., 2009 <b>Review</b> on Electromagnetic effects from cell biology to medicine
ELFEMF	15 to 30 Hz, 0.01 mV/cm	Bone cells	Affect remodelling activity	McLeod & Rubin, 1993
Pulsed EF	100V external voltage, 16 Hz, EF across cell membrane 6 kV/m (estimated by computer simulation)	Osteoblast-like primary cells	Increased proliferation, enhancement of alkaline phosphatase activity, enhanced synthesis and secretion of ECM proteins	Hartig et al., 2000
Pulsed EMF	15 Hz, 0.1 mT, EF 2 mV/cm	Osteoblast-like primary cells	Increased proliferation, OPG upregulation and RANKL downregulation	Chang et al., 2004

Physical stimulus	Parameters	Experimental model	Effects	Author
EF	Static and pulsing direct current (DC) EFs	Calf anterior cruciate ligament (ACL) fibroblasts	Increased migration speed and enhanced collagen I expression with pulsing direct current (DC) EF, inhibition in wound healing with static direct current (DC) EF	Chao et al., 2007
EMF	50 Hz, 25 to 180 $\mu$ T	Human fibroblasts	Increased proliferation	Kwee & Raskmark, 1995
ELFMF	50 Hz, 0.020 T	Murine fibroblasts	Inhibition of cell growth	Kula & Drozd, 1996
EMF	50 Hz sinusoidal MFs intensities: 30 $\mu$ T, 300 $\mu$ T, and 1 mT	Human lymphocytes	No effect on proliferation	Paile et al., 1995
EMF	50 Hz sinusoidal MF intensities: 1.0, 0.75, 0.5, 0.25, 0.05 mT exposure 72 h	Human lymphocytes	Slight decrease of cell proliferation at the intensities tested	Scarfi et al., 1999
MF	Sinusoidal 60 Hz, 44 $\mu$ T	Rat thymic lymphocytes	Modulation of calcium concentration	Walleczek, 1990
EMF	30 Hz, 1.8-mT	MC-3T3 osteoblast-like cells	Progressive inhibition of enzyme activity and differentiation	McLeod & Collazo, 2000
ELFEMF	15Hz, 5mT	Human mesenchymal stem cells (hMSCs)	Increase of collagen II and glycosaminoglycan (GAG)/DNA content during chondrogenic differentiation	Mayer-Wagner et al., 2010
EMF	50 Hz; 2 mT, 3 hours exposure	SHSY5Y neuroblast model	Promotion of neurogenic differentiation	Cerrato et al., 2011
EMF	1 GHz, power density of exposure area 5 nW/cm <sup>2</sup>	Human fibroblasts	Activation of fibroblast migration	Sunkari et al., 2011
LIGHT				
Light He-Ne and Ga-Al-As laser	Various treatment protocols and instrumental parameters	Injured muscles in rat Ischemic leg muscles Ischemic heart model of mouse, rat, dog and pig Myogenic satellite cells (SC)	Enhanced muscle regeneration; reduced muscle degeneration; reduction in scar tissue formation after induction of myocardial infarction (MI) and in ventricular dilatation. Increment of ATP in the infarcted area; MAPK/ERK activation	Oron, 2006 <b>Review</b> on Photoengineering of tissue repair in skeletal and cardiac muscles

Physical stimulus	Parameters	Experimental model	Effects	Author
Light IR radiation	Various protocols and parameters	Bone cells and tissues	Increment of osteoblastic proliferation, collagen deposition and bone neoformation	Pinheiro & Gerbi, 2006 <b>review</b> state of the art on photoengineering of bone repair using laser therapy
Light High power Nd: YAG laser	$\lambda$ 1064 nm, 200 $\mu$ s pulse duration, 10 Hz repetition rate, 458.65 mJ/cm <sup>2</sup> energy fluence, 73 sec exposure	Human mesenchymal stem cells	Upregulation of genes involved in osteoblastogenesis, downregulation of genes involved in adipogenesis	Monici et al., 2008a
Light High power Nd: YAG laser	$\lambda$ 1064 nm, 200 $\mu$ s pulse duration, 10 Hz repetition rate, 458.65 mJ/cm <sup>2</sup> energy fluence, 73 sec exposure	Human fetal fibroblasts and human chondrocytes	Increased production of ECM molecules	Monici et al., 2008b
Light	Different laser sources and treatment protocols	Different experimental models	Promotion of tissue repair	Da Silva et al., 2010 <b>Review</b> on Lasertherapy in tissue repair processes
Light He-Ne laser	$\lambda$ 632.8 nm, average energy on sample 1.18 J/cm <sup>2</sup>	Endothelial cells pre-exposed to laser and then seeded on a biomaterial surface	Increase in ECM secretion and increased resistance to flushing	Hsu et al, 2010
Light Red radiation	$\lambda$ from 600 to 700 nm energy density 0.4 - 0.5 J/cm <sup>2</sup>	Various cell types	Increase in proliferation associated with enhanced synthesis of ATP, RNA and DNA	Alghamdi et al., 2011 <b>Review</b> on The use LLLT for enhancing cell proliferation
Light High power Nd: YAG laser	$\lambda$ 1064 nm, 200 $\mu$ s pulse duration, 10 Hz repetition rate, 458.65 mJ/cm <sup>2</sup> energy fluence, 73 sec exposure	Fibroblasts and endothelial cells	Increased production of fibronectin and ordered assembly of FN fibrils in fibrillogenesis	Monici et al., 2011
Light MLS laser	$\lambda$ 808 and 905 nm, 1500 Hz	Myoblasts	Enhanced expression of MyoD and increased activity of enzymes involved in cellular energy metabolism	Vignali et al., 2011

Table 1. Based on the studies cited in this chapter, the table lists physical factors, treatment parameters applied, experimental models used and observed effects.



## 5. Conclusion

Over the past twenty years, studies on molecular and cellular mechanisms that underlie biological responses evoked by physical stimuli have made great progress. However, in this fascinating field of study, many problems still remain and their solution will require further advances in our knowledge.

The results of our studies are a further, albeit modest, contribution to a large body of literature that shows how physical stimuli can be effective in modulating cellular functions and the production of ECM. It is obvious that the development and standardization of technologies for delivering appropriate physical stimuli, strictly controlled with regard to the intensity, frequency and timing of exposure, is a prerequisite for making progress in tissue engineering.

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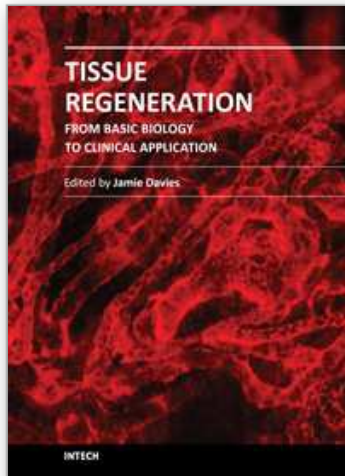
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When most types of human tissue are damaged, they repair themselves by forming a scar - a mechanically strong 'patch' that restores structural integrity to the tissue without restoring physiological function. Much better, for a patient, would be like-for-like replacement of damaged tissue with something functionally equivalent: there is currently an intense international research effort focused on this goal. This timely book addresses key topics in tissue regeneration in a sequence of linked chapters, each written by world experts; understanding normal healing; sources of, and methods of using, stem cells; construction and use of scaffolds; and modelling and assessment of regeneration. The book is intended for an audience consisting of advanced students, and research and medical professionals.

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