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Review

Mechanical impairment on alveolar bone graft: A literature review

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1. Introduction

Cleft lip and palate represents the most common form of craniofacial malformation (1 in 700 newborns in Europe). Cleft lip and palate is usually closed within the first 2 years after birth. The alveolar cleft is left open so as not to impair on maxillary growth in the way that primary osteoplasty did (Robertson and Jolleys, 1968; Rehrmann et al., 1970). Secondary alveolar bone grafting has become well established since the original work of Boyne and Sands (1972). Cancellous iliac bone is most widely favored, but tibial shaft, mandible, rib, and calvaria have also been used.

This process is usually carried out between the ages of 9 and 12 years, in mixed dentition, before eruption of the canines, so a canine could erupt through the grafted site. More recently, other authors (Borstlap et al., 1990; Lilja et al., 2000; and Talmant et al., 2002) have advocated alveolar bone grafting before the eruption of maxillary lateral incisors, between the ages of 4 and 6 years. The graft allows a lateral incisor to erupt through the grafted bone. The authors reported better results in terms of residual bone height.

Management of residual alveolar cleft is essential for restoring union and stability of maxillary segments, allowing tooth eruption, closing the alveolar fistula, and giving support to the lip and nose (Witsenburg, 1985).

The peculiarity of cleft alveolar bone grafting lies in the particular geometry of the alveolar cleft, where cancellous bone is placed in between two cortical surfaces (Fig. 1). Despite the small volume of alveolar cleft (Feichtinger et al., 2008; Dissaux et al., 2016), a full reconstruction of alveolar bone cannot be achieved using only gingivoperiostoplasty. The neo-osteogenesis occurring in that case is different from fracture healing (Meyer et al., 2006) because two cortical bone surfaces are involved, and bone synthesis in this small alveolar cleft space cannot occur only through abrasion of cortical bone. Thus a bone graft should be carried out to restore adapted bone dimensions in the alveolar cleft.

During alveolar bone grafting the surgeon places iliac cancellous bone in a particular 3D space between two cortical bones, with smooth upper and lower layers (nasal floor and gingival mucosa), and applies a compression force on the graft. Subsequently, craniofacial and particularly alveolar bones are submitted to other forces exerted by teeth, muscles, and the tongue within the highly developed masticatory system.

This review paper aims to establish the extent of current knowledge on cancellous bone graft integration and the impact mechanical forces could have on it.

2. Cancellous bone graft: content and integration

Cancellous bone has a particular lamellar structure, called trabecular bone (Fig. 2), comprising cells (osteoblasts, osteoclasts, osteocytes) and extracellular matrix (organic and mineral fractions). The trabecular structure involves overlapping strips of collagen fibers, which lie in the same direction but are not organized in a cylindral way. Trabeculae are covered with a non-mineralized collagen layer incorporating active or dormant osteoblasts (Yaszemski et al., 1996). Free space between the trabeculae is filled by bone marrow, containing pluripotent bone mesenchymal stem cells. Despite its apparent immobility, bone tissue in this physiological environment is perpetually renewed under the influence of biological and mechanical factors (Goodmann and Aspenberg, 1993).

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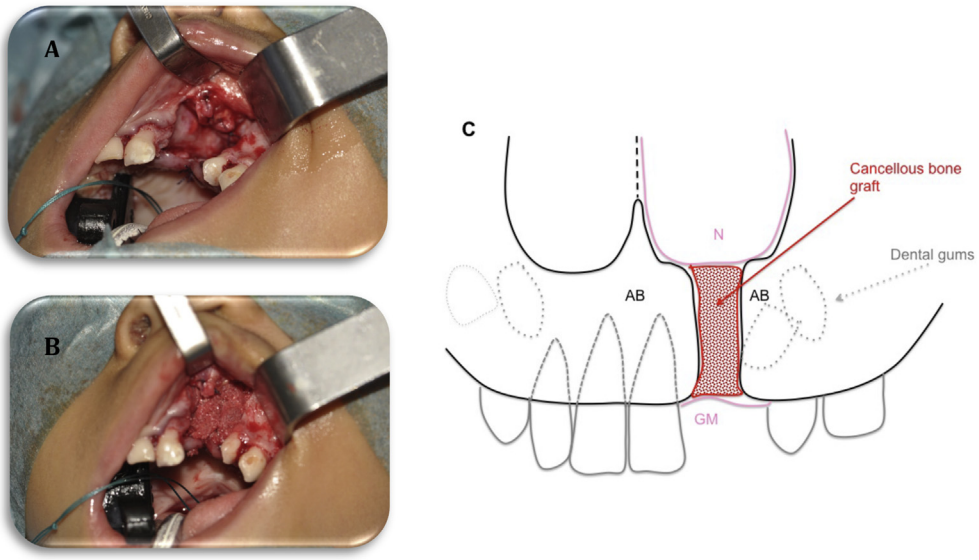

Fig. 1. *Illustration of bone graft*. Picture A: Status of the alveolar cleft before introducing cancellous bone graft. Nasal floor is reconstructed and sutured. Alveolar bone consists the 2 lateral borders and gingival mucosa the bottom layer. Picture B: Cancellous bone graft has been placed in between 2 cortical surfaces of alveolar bone; Picture is taken before gingival vestibular mucosa covers the site. Picture C: Schematic representation of bone graft placed between 2 cortical surfaces of alveolar bone (AB) and 2 smooth layers, Nasal floor (NF) at the top and gingival mucosa (GM) at the bottom. This is a 2-D picture. In 3-D, the anterior limit of the graft is gingival mucosa and the posterior limit palatal mucosa, so 2 other smooth structures.

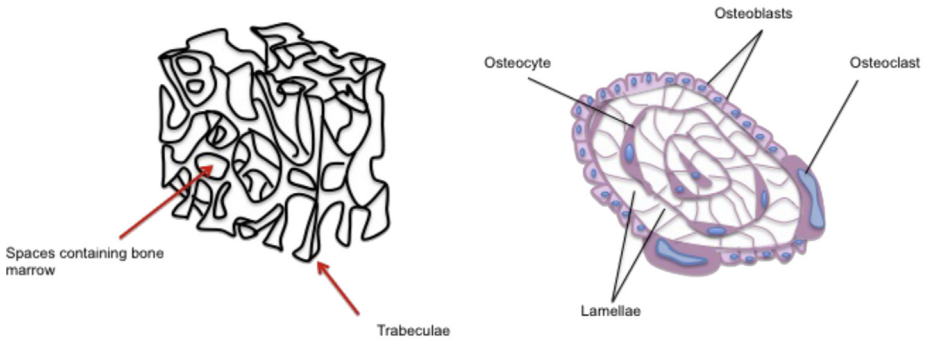

Fig. 2. Trabecular bone or cancellous bone.

Historically, the first clinical cancellous bone graft was performed in 1820 by Philips von Walter, who reconstructed part of a skull. Clinical use of bone graft has developed steadily, and is now an essential surgical technique. Despite its regular use in everyday surgical practice, the biology and mechanisms of bone graft integration are not well known. The bone graft provides the mineral fraction, the protein fraction, and morphogen signals essential for osteogenesis (Elima, 1993). Cancellous bone graft represents a biocompatible, osteogenic, osteoinductive, and osteoconductive material. Since 'no graft is an island' (Carmeliet, 2005), the major advantage of cancellous bone graft is its rapid revascularization (Kerwin et al., 1996), thanks to the porosity created by the trabeculae. Another major advantage of cancellous bone lies in its ability to adapt to the receptor site (Rosenthal and Buchman, 2003). Canady (Canady et al., 1993) suggested that only 10% of the grafted cells in bone graft survive after transplantation. According to Acocella (Acocella et al., 2010), an osteocyte cannot survive if it remains more than 0.1 mm away from a vessel.

Yano (Yano et al., 2000) found in his study on rabbits that bone formation was observed 3 weeks after cancellous allografting (60.2% of newly formed bone), and the woven bone underwent remodeling to mature trabeculae at 8 weeks (93% of newly formed bone). In human studies, complete cancellous bone graft revascularization takes at least 4 months, and transformation into trabecular bone 8 months.

Laird (Laird et al., 2003) also suggested that most of a bone graft is reduced and replaced by newly formed bone. Helms (Helms et al., 2007) carried out a literature review on the bone graft integration process after implantation. He reported several studies describing grafted cells becoming osteoblastic cells, or secreting factors that induced new osteogenesis. Beyond directly producing bone, these grafted cells would act by recruiting other cells to the grafted site via secretion of different factors.

According to these authors, it is usually accepted that a maximum amount of cancellous bone must be placed in the receptor site in order to maximize the amount of viable osteogenic cells. But the more bone that is placed into the receptor site, the higher the bone density, which could make it impermeable to blood supply. Precise data on bone density and the consequent degree of cell viability are not evident in the literature. Albert (Albert et al.,

2008) studied the influence of compaction force on the structure of bone graft, comparing different types and times of compaction application. He concluded that this process achieves better bone density using a cycle of 20 impactions in 90 s, but he did not compare the results of these impacted grafts in vivo after several weeks.

Finally, the exact biological process of bone grafting is not clear. Is it similar to the fracture healing process, with inflammatory, repairing, and remodeling phases? What is the role of the compression force during the process, in terms of density, porosity, cell viability, cell secretion, and graft result? What could be the impact of environmental forces on bone graft integration after its placement (for example, the masticatory system in alveolar cleft bone grafting)?

3. Mechanical influences on osteogenesis: history and examples

The influence of mechanical stimuli on the structure of bone has long been a topic of scientific interest. Mechanical forces were first identified as responsible for shaping the architecture of the skeleton in the nineteenth century by Von Meyer (1867) and Culmann (1866). Von Meyer identified arched trabecular patterns in a sagittally sectioned human first metatarsal and calcaneus. Culmann suggested that the patterns appeared to be aligned along principal stress lines produced by functional loading (Skedros and Brand, 2011). Some time later, Wolff (1892) first described the effect of external mechanical forces on the development of bone. He claimed that the shape of the bone is related to mechanical stress by Wolff's law of bone transformation. A century later, Ilizarov (1989) used these principles to develop limb distraction osteogenesis, followed by McCarthy et al. (1992), who considered craniofacial bone distraction. Other examples have demonstrated the influence of mechanical stress on bone caused by implant placement (Szmukler-Moncler et al., 1998; Brunski et al., 2000), dentofacial orthopedic stimulation of bone growth using orthodontic (Bonafe-Oliveira et al., 2003) or bone-anchorage appliances (De Clerck et al., 2010), physiological stimuli by the tongue in Pierre Robin sequence (Robin, 1923), or the masticatory environment.

At the other extreme, mechanically passive states of the skeletal system due to zero gravity, functional immobilization, or post-operative bedfast have been shown to result in decreased bone formation and mineralization, as well as reduced protein synthesis (Rambaut and Goode, 1985).

Recent interpretations of Wolff's law have proposed that bone mass and architecture are governed by adaptive mechanisms that are sensitive to their mechanical environment (Duncan and Turner, 1995; Skerry, 2006). Recently, more and more studies have shown that physical forces applied to bone or progenitor cells enhance bone formation. This new concept of mechanotransduction is far from being fully understood.

4. Mechanostransduction

a) Definition

To meet the functional demands of its mechanical environment, the mass and geometry of bone are physically remodeled in a dynamic fashion (Wolff's law). Mechanostat theory is a refinement of Wolff's law, and proposes that bone adapts so that it can function mechanically as needed by detecting and responding to mechanical loads (Frost, 1987, 2003). Mechanotransduction is the process by which physical forces are converted into biochemical signals that are then integrated into cellular responses (Wang et al., 1993; Huang et al., 2004; Robling et al., 2006).

b) Mechanisms and pathways

We are only beginning to understand how bone cells sense their mechanical environment, and how these signals are translated into a cascade of biochemical events within the cell, and modify gene expression or recruitment of other cells.

The prevalent, widely accepted, hypothesis proposes that the osteocyte cell is responsible for mechanosensation (Thi et al., 2013). This hypothesis is supported by the finding that the targeted ablation of osteocytes in mice results in defective mechanotransduction and fragile bone with osteoblastic dysfunction (Tatsumi et al., 2007). Osteocytes comprise the majority of cells in bone tissue and are known as 'sensor cells', whereas osteoblasts and osteoclasts are 'effector cells' (Mi et al., 2005). Their specific anatomical location, encased within lacunae, enables them to 'sense' physiological loads.

Different levels must be considered when studying mechanical signals. Compressive loading of bone results in non-uniform strains macroscopically, creating small deformations throughout the calcified matrix. The associated volume and pressure differences within the interconnected canalicular network cause interstitial fluid flow, which imparts shear stresses to the osteocytes. At a cellular level, this conversion from strain to shear stress amplifies the stimuli received by cells (Cowin and Weinbaum, 1998), and osteocytes transduce these signals via different pathways: integrins, ion channels, cell membrane deformation, modification of cell oxygenation, and modification of the primary cilium.

Thus, mechanotransduction has been explored from a macro- and microscopic perspective, leading to two main lines of thinking: tensegrity theory and mechanosomes theory.

Donald Ingber (2003) first described the concept of tensegrity. This theory explains how a mechanical stress at a macroscopic level can influence the molecular structure and function of living cells. According to this concept, the whole cell — not just single, specialized mechanotransduction molecules — is believed to serve as a mechanotransducer. Central to this theory is the cytoskeleton system, which has the capacity to store energy in equilibrium between tensile and compressive forces. Thus it integrates the local signals and stored energy with other environmental mechanical inputs before eliciting a specific behavioral response by the cell. Cells and environment are closely related by focal adhesion complexes that anchor the cell to the extracellular membrane. These complexes, such as integrins, function as anchors for the cytoskeleton.

The mechanosomes theory was proposed by Palvalko (Pavalko et al., 2003) and considers mechanotransduction on a molecular level. In this theory, bone loading leads to deformation of the sensor cell membrane, which drives conformational changes in membrane proteins. Some of these membrane proteins are linked to a solid-state signaling scaffold that releases protein complexes called mechanosomes, which carry the mechanical information into the nucleus. In this way, 'bending bone ultimately bends genes' (Pavalko et al., 2003). Both of these approaches coexist and differ only in terms of level of study.

Mechanostransduction pathways have been increasingly studied over the last ten years. First the cell 'senses' the mechanical stimulus via a combination of focal adhesion complexes such as integrins, ion channels, cell deformation, cell oxygenation modifications, and receptors for different cytokines. After 'sensing' the mechanical stimulus, different pathways are involved in transmitting the signal to the nucleus, with the cell responding by producing a range of molecules. The pathways that translate the

mechanical stimulus into a biological signal are not clearly explained in the literature; many possibilities have been described and are probably all linked.

Integrins seem to play a major role in mechanotransduction by giving the cell the ability to interact with its environment (Schmidt et al., 1998). They induce enhanced phosphorylation of cytoskeletally anchored proteins such as mitogen-activated protein kinase (MAPK).

Other major mechanical stimuli receptors are calcium-channel receptors (El Haj et al., 1999). One of the first responses to mechanical stimuli is fluid flow caused by calcium exchange between the extracellular and intracellular media. This exchange stimulates downstream pathways, leading to nitric oxide (NO) and prostaglandin E2 (PGE2) release (Rosa et al., 2015). NO and PGE2 are considered as potent anabolic regulators of bone growth. Inhibition of NO or PGE2 suppresses the osteogenic response to mechanical stimulation (Fox et al., 1996; Chow et al., 1998). NO stimulates bone formation by inhibiting osteoclast formation, and enhances secretion of PGE2 (Klein-Nulend et al., 2014). PGE2 seems to recruit and promote the differentiation of precursor bone cells (Tan et al., 2007; Dirckx et al., 2013). Van Griensven (Van Griensven et al., 2003) has also shown that cyclic longitudinal mechanical strain induces the secretion of NO.

Many authors also insist on the role played by bone morphogenic proteins (BMP), especially BMP-2 and BMP-4 (Huang and Ogawa, 2010). Mechanical stimuli enhance expression of BMP-2 and BMP-4 by osteoblastic cells (Rath et al., 2008) and by bone marrow stromal cells (BMSC) (Sumanasinghe et al., 2006; Kimelman-Bleich et al., 2011). Bone morphogenic proteins belong to the transforming growth factor beta family. There are 25 types of BMP. Studies on mechanotransduction only report on BMP-2 and 4. They play a major role in osteoblastic cell proliferation and differentiation (ten Dijke, 2006). First they bind to Ser-Thr kinase receptors (Kopf et al., 2012). This binding leads to phosphorylation of SMAD1 and/or SMAD5 (transcription factors) and activation of target genes induced in bone formation (Ryoo et al., 2006). The up-regulation of BMP-2 also results in the acetylation of RUNX2, leading to augmentation of its transactivational activity and inhibition of its degradation (Jeon et al., 2006). The expression of RUNX2 represents a molecular biomarker signaling pre-osteoblastic cell differentiation along the osteoblastic lineage, and continues to modulate bone formation by regulating the activity of differentiated osteoblasts (Ducy, 2000). Both RUNX2 and SMADs are essential for the synthesis of proteins important during osteogenesis, such as osteopontin, osteocalcin, collagen 1 or alkaline phosphatase Akp2. Moreover, BMPs activate other pathways, such as the MAPK and Akt/PKB pathways.

Mechanical stimuli potentiate the effect of BMP (Kimelman-Bleich et al., 2011; Kopf et al., 2012), but raise the question as to whether this only by increasing its expression or by using the same intracellular pathways, or by other mechanisms? It is probably a combination of several mechanisms and pathways, and it would need technical advances and much more research to really understand this interaction. Other strain-induced mechanotransduction pathways have been recognized. Parts of these are mediated via integrins or ion channels, but the all cascade from mechanical stimulus to cell answeris still not very clear. These include cyclic adenosine-mono-phosphate (cAMP), cyclic guanosine-mono-phosphate (cGMP) (El Haj et al., 1990), cfos (Kawata and Mikuni-Takagaki, 1998), inositol triphosphate (IP3) (Brighton et al., 1992), core binding factor alpha1 (cbfa1) (Jagodzinski et al., 2004), RhoA, and ROCKII (Arnsdorf et al., 2009). Dynamic compression has also been shown to up-regulate SOX-9 and TGF-β1 (Huang et al., 2005). Ivanosvska (Ivanovska et al., 2015) also reports the role of LINC (linker of nucleus and cytoskeleton complex), a protein that plays a role in the transduction of mechanical signals to the nucleus.

This list of different pathways is probably not exhaustive, and more will be discovered. It demostrates the complexity of the cell's response to mechanical stimulation and the difficulty of its complete comprehension. As Duncan and Turner (1995) said, there is 'no single mechanotransduction pathway'. Target genes of mechanotransduction are also numerous and not really well identified in the literature, except that for BMP (Id1) (Kopf et al., 2012).

These different pathways lead to the synthesis of proteins — markers of osteoblastic cell differentiation stages (Table 1). Those identified so far (Owen et al., 1990) are alkaline phosphatase (Jagodzinski et al., 2004), collagen 1 (Zaman et al., 1992), osteocalcine (Yoshikawa et al., 1997; Jagodzinski et al., 2004), osteonectine, osteopontine (Wozniak et al., 2000), and bone sialoprotein (BSP).

Mechanical stimuli can influence the cell's response, but it is important to understand that the cell is not a single entity; it belongs to a matrix that interacts with it through gap junctions (cadherins). Meyer (Meyer et al., 2006) insisted on the importance of considering mechanotransduction at a tissue level. The sensitivity of the response of the osteoblastic network was found to be much greater when the cells were part of a tissue than when they were evaluated as single entities.

The bony tissue integrates and amplifies a complex physical signal, such as a mechanical strain or functionally induced fluid flow, by transmitting the signal from the signal-detecting cells to the change-effecting cells. Bone cells are coupled functionally by gap junctions.

Table 1
Different markers of osteoblastic differentiation and Selective Genes involved in bone development.

Markers genes	Alkaline phosphatase	Potential Ca^{2+} carrier, hydrolyse inhibitors of mineral deposition such as pyrophophatases
	Collagen Type I	Serves as scaffold of mineralization
	Bone sialoprotein	Nucleator of mineralization
	Osteopontin	Inhibits mineralization and promotes bone resorption
	Osteonectine	Inhibits mineralization
	Osteocalcine	Mediate hydroxyapatite deposition
Regulatory genes		
Transcription factors	RunX2	Required for osteogenic differentiation
	Twist	Positive regulator of osteoblast differentiation
	Msx2	Inhibits osteoblast differentiation
Growth factors/Receptors	FGF/FGF R	Stimulates proliferation and differentiation
	TGF/TGF R	Modulates bone remodeling
	BMP/BMP Recpetor	Increases Cbfa1/Runx2 expression and stimulate differentiation
	IGF	Stimulates cell proliferation, differentiation and matrix production.
	PDGF	Recruit progenitor cells

Mechanical stimuli exert their effect not only on bone cells but also on the collagen microarchitecture and the mineralization of the matrix (Wiesmann et al., 2001). Collagens are important stress-carrying proteins that are sensitive to the application of mechanical strains. Low strains lead to a straightening of collagen fibers, whereas higher strains cause molecular gliding within the fibrils, resulting in the disruption of the fibrillar organization and ultimately impeding the formation of hard tissue.

To sum up, a mechanical stimulus induces production of bone tissue thanks to the architecture of the cell (cytoskeleton, cell membrane and its components, intracellular molecules, and multiple intracellular pathways) as a component of the surrounding tissue, containing progenitor cells and effector cells with which the sensitive cell interacts. Within this tissue, stiffness and various components (cytokines, proteins) will play a direct regulatory role in the sensitivity of the cell to strain.

c) Influencing factors and controversiesInfluencing factors and controversies

Besides studying the different pathways of mechanotransduction, other authors report the influence of various factors on mechanotransduction, such as age, gender, nature, duration, intensity, type and frequency of mechanical stimulus application, cell geometry, and nature of the scaffold.

Age can affect the mechanotransduction process. Turner (Turner et al., 1995) compared mechanically induced bone formation in 9-month-old and 19-month-old rats. The degree of bone formation in the 19-month-old rats was one-sixteenth that exhibited in the younger ones. Nichols (Nichols et al., 2007) raised the question of a 'window of opportunity' during the development of peak bone mass, in which the bone is especially responsive to weight-bearing physical activity. Furthermore, during advancing age, remodeling rate increases and bone mass index decreases, resulting in a predisposition to bone fracture following mechanical stimulation such as minimal trauma (Seeman, 2008). In addition, gender affects bone mechanotransduction. Robling et al. (2007), studying the genetic regulation of mechanotransduction, could not identify well-defined regulator genes. He concluded that the genetic regulation of mechanotransduction was a complex process involving a high number of genes, but he highlighted that it was sex-specific. He reported that all male congenic mice exhibited significantly reduced mechano-responsiveness compared with controls, whilst the same comparison among females yielded no difference from the controls (Robling et al., 2007). Moreover, males suffer from larger bone mass reduction in both cortical and cancellous bone than females when they are subjected to limb unloading (David et al., 2006). Such age and gender-specific variations may be due to differences in the efficiency of mechanical sensors and sex hormone production and/or signaling.

Literature concerning mechanotransduction is extensive, with several kinds of mechanical stimuli being tested. Many in vivo and in vitro studies have shown that compressive forces have an anabolic effect on bone (Chao et al., 1998; Goodship et al., 1998; Rubin et al., 2001; Govender et al., 2002; MacKelvie et al., 2003; Gardner et al., 2006). Huang et al. (2005) reported dynamic compression up-regulated Sox-9 and TGF-β1, and induced bone formation. Others, such as Sumanasinghe et al. (2006), have shown that tensile strain up-regulates BMP-2, such that the strain alone could induce osteogenic differentiation. In bone tissue engineering, mechanical force types, such as linear straining or pressure loading, correlate most closely to physiological conditions and therefore are most widely used in connection with cultivating bone cells and generating bone-like tissue. However, the methods of strain application vary widely in terms of physical parameters such as strain intensity, frequency and duration, as well as substrate materials and geometry. Lancerotto and Orgill (2014) supported the hypothesis that cells not only detect mechanical stimulation, but also sense the intensity of the stimulation. Leading to a variable degree of cell stretch. Similarly, Rath et al. (2008) tested the hypothesis that compressive forces could exert an osteogenic effect on osteoblasts in a dose-dependent manner. In his in vitro study of pre-osteoblasts cultured on PCL (polycaprolactone) scaffolds, he showed that the exposure of cells to 10% of compressive strain (11.8 ± 0.42 kPa) resulted in a rapid induction of BMP-2, RunX2, SMAD-5 and enhanced further the expression of alkaline phosphatase (Akp2), collagen 1, osteocalcine, osteonectine, and osteopontine. But an exposure to 20% of compressive strain (30.96 ± 2.82 kPa) demonstrated no osteogenic response.

In vivo studies on distraction osteogenesis have also observed up-regulation of osteocalcine during lengthening after osteotomy (Lammens et al., 1998) and down-regulation of osteocalcine at high strains (Meyer et al., 1999). Frost, in his 'mechanostat theory' of bone adaptation to strain had already defined different windows of bone stimulation (Fig. 3). Some researchers claim that Frost's theory is a qualitative, theoretical construction of several hypotheses, and that the precise threshold limits that control bone remodeling remain unknown. One important point to understand is that the intensity of the strain at the tissue level is different from that at the cellular level. Studies have found that strains experienced by bone tissues due to everyday activity range from 0.1% to 0.35% (Duncan and Turner, 1995). Strains above this range (but below the yield point) lead to bone strengthening, whilst sub-physiological strains lead to bone resorption. Concerning the cellular level, the conversion from strain to shear stress amplifies the stimulus received by cells, so the osteocytes sense shear stresses in the order of 1–3 Pa

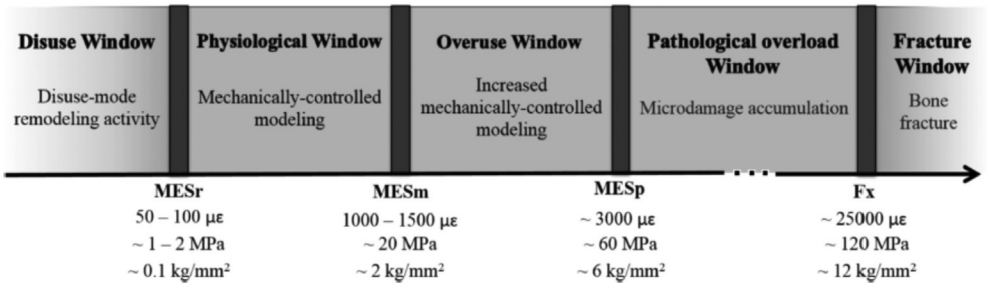

Fig. 3. *Mechanical usage window used by Frost's "mechanostat" theory of bone adaptation to strain (adapted from* Duncan and Turner, 1995 and Frost, 1987). The horizontal arrow at the bottom shows the typical minimum effective strain (MES) levels and the set point values for bone's thresholds and ultimate strength – microstrain (με), stress (MPa) and unit-load (kg/mm^3).

(2.1–9 Pa) (Cowin and Weinbaum, 1998). A recent study developed by Verbruggen (Verbruggen et al., 2014) showed that individual osteocytes might be subjected to a maximum shear stress stimulus of approximately 11 Pa. Finally, Van Griensven et al. (2009) argued that, during in vitro experiments, strain amplitudes are chosen depending on the utilized cell types: bone marrow stromal cells, adipose-derived mesenchymal stem cells (ADSC) or pre-osteoblastic cells.

Another major influencing factor taken into account in the literature is the frequency of strain application. Should the application of mechanical strain be continued or discontinued? It is commonly suggested since Jacobs' work (Jacobs et al., 1998) that bone needs 'time off' from mechanical loading. Mechanical loading presents a potent osteogenic stimulus, but bone cells desensitize rapidly to mechanical stimulation. Resensitization must occur before the cells can transduce future mechanical signals effectively (Robling et al., 2002). Consequently, it has been suggested that cyclical or intermittent loading, which provides bone with regular 'time off' periods, may be more effective than continuous loading in inducing the bone formation needed to promote the growth and repair of the skeleton (Saxon et al., 2005). Other studies follow the same hypothesis — that cyclic mechanical stimulation enhances osteoblastic proliferation and differentiation (Visconti et al., 2004; Gabbay et al., 2006). Hsieh and Turner (2001) showed that the frequency of mechanical stimulation could have an effect on bone formation. In vitro, an optimal frequency of 1 hz has been reported by Kaspar et al. (2000) on ADSC, BMSC, or pre-osteoblastic cells. Thus, many studies consider oscillatory strain to lead to more efficient bone formation because it is closer to physiological conditions. However, Alzahrani et al. (2014), in his review on distraction, found inconsistent results when loading frequency was changed. Moreover, Bonafe-Oliveira et al. (2003) argued that intermittent forces in orthodontic treatment were not clearly superior, or necessary.

It is generally believed that repairing of alveolar bone during orthodontic movement occurs after a decrease in force. Bonafe-Oliveira et al. (2003) provided evidence that the application of continuous forces produced concomitant bone resorption and formation at the pressure areas in rat molars. Liu et al. (2010) also compared the efficiency of continuous and intermittent forces on bone formation in rabbits. The aim of the study was to compare the effectiveness of intermittent (distractor) and continuous (spring) forces on calvarial sutures. Continuous forces produced significantly greater overall suture separation (1.3 mm) than intermittent forces (0.8 mm) over a period of 29 days. Nevertheless, bone formation seemed to approach a limit under continuous forces (Fig. 4). The limit of this study is that the experiment was stopped before the expansion potential of the device applying continuous forces had been reached.

Considering the application of strain, one last influencing factor is the time of application. Van Griensven et al. (2009) performed a 2D experiment in vitro on BMSC and ADSC, and insisted that the period of strain application should be over an hour to have an effect on bone formation. The application of strain for 60 min was more effective on the synthesis of collagen-1, osteocalcine, osteopontine, and BMP-4 (analysed by RT-PCR) than a for a duration of 15 min. Yang et al. (2010) showed that a long-duration, continuous pattern of strain application (6 h) induced the expression of BMP-2 and Run-X2 of ADSC, in contrast to a short-duration pattern (17 min every day for 10 consecutive days).

The responses of cells to a particular timing of strain application can also differ, depending on the type of cell. ADSC respond to the application of strain after 6 h by changing their orientation and starting a bone formation process, whereas BMSC only react after 48 h (Grottkau et al., 2013).

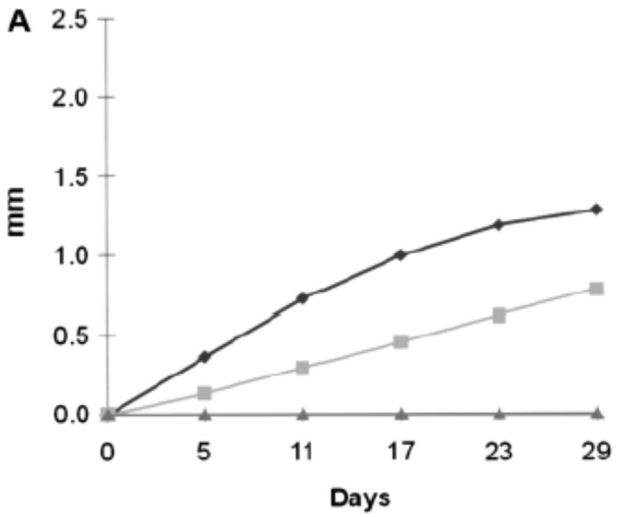

Fig. 4. *Comparison of the efficiency of continuous and intermittent forces on bone formation on rabbits* (Liu et al., 2010). Longitudinal radiographic changes suture widths between continuous and intermittent forces. Continuous forces produced significantly greater overall suture separation (1.3 mm) than intermittent forces (0.8 mm) over a period of 29 days, but they seem to have a limit in the bone formation (the slope of the curve concerning continuous forces decreases). The rates of sutural separation decelerated over time with continuous forces and remained constant with intermittent forces.

This last concept leads us to think that the characteristics of the cell, as well as the type of strain, need to be taken into account. Thus, the shape of the cell could influence its differentiation, by modulating its interaction with its scaffold (via the actinomyosin system). Geometric features that increase cell contractility over a constant spread area favor osteogenesis over adipogenesis, independently of solubility factors (Kilian et al., 2010). If cell shape has such an impact, it is because cell interacts with the environment, so the characteristics of the scaffold may also have a major influence, especially its stiffness (Fig. 5). Cell fate can be influenced by several mechanical properties, including elasticity, geometry of the matrix, and adhesion to the scaffold, which ultimately impact on cytoskeletal tension.

Engler et al. (2006) showed that the rigidity of polyacrylamide gel substrates critically influenced the differentiation of MSCs (mesenchymal stem cells). On a matrix with osteoid-like stiffness (40 kPa), MSCs became osteogenic, whereas on a softer matrix (10 kPa), MSCs became myogenic. The same study showed that pharmacological inhibition of myosin-II contractility blocked lineage specification. The expression of bone markers was the highest when MSCs were cultured on the stiffest gels (Young moduli around 100 kPa — similar to those measured for non-mineralized bone). Yim (Yim et al., 2005) found equivalent results and added that the geometry of the matrix, as well as its stiffness, also played a

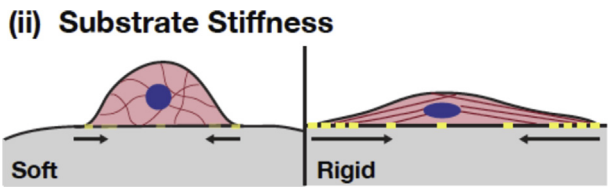

Fig. 5. *Illustration by* Hung et al. (2013) *about the influence of medium stiffness*. Substrate rigidity influences cell adhesion, spreading and differentiation patterns. Soft surfaces provide low resistance, decreased focal adhesions (yellow) strength and reduced cytoskeletal organization relative to more rigid surfaces. This leads to changes in nuclear shape and gene expression.

role. However, it should be noted that while induction media containing traditional osteogenic biochemical factors can direct cells towards bone lineage if the cell is cultured on a scaffold with optimal ranges of stiffness, a scaffold with the right stiffness only cannot guide undifferentiated cells towards a particular lineage.

Rosa et al. (2015) also reported on the role of the scaffold in vitro, including pore size, pore interconnectivity, and total porosity, which could directly influence cell behavior and cell exposition to mechanical load. These authors insist on the importance of a 3D scaffold used in vitro, rather than a 2D scaffold. Once again the literature focuses more on in vitro studies — with new bone engineering in mind — than on understanding what really happens in vivo.

d) Level of study

Particularly noteworthy about the literature on mechanical loading and osteogenesis is the number of in vitro studies (Table 2), and the extreme variability of materials and methods used. They mostly focus on newly synthesized bone engineering, using scaffold enriched with cells and growth factors. Most of the authors analyze the effects of mechanical loading on different types of cell — ADSC, BMSC, periodontal cells, pre-osteoblastic cells — included in 2D or 3D scaffolds. A few of them study the impact of strain in vivo, mostly on rats (site calvaria), using distraction or orthodontic devices, but no article could be found on the impact of mechanical loading on bone graft integration.

The evident lack of in vivo studies reflects the complexity of the osteogenesis process, and the problems in understanding it; for example, multiple pathways need to be taken into account. Before using newly synthetized bone or newly enriched matrix, it is important to experience step by step and in vivo the characteristics of autologous bone. Not only should cells and growth factors be taken into account, but also the environment of the bone tissue, including acellular and cellular parts, inflammatory processes, and neo-angiogenesis, all of which play a role in bone graft integration and neo-osteogenesis.

e) Angiogenesis

Bone graft integration requires cells, growth factors, and nutrients, so it needs a blood supply. 'No graft is an island' (Carmeliet, 2005) and so angiogenesis is vital. Its key role raises the question of the influence of mechanical loading on angiogenesis. In vivo examples of the impact of mechanical load on angiogenesis are mentioned by Lancerotto and Orgill (2014), for example in tissue expansion and scar compression therapy. In tissue expansion, normalization of the dermis can take up to 2 years, whereas vascular proliferation can be seen in just a few hours. In contrast to this positive effect, tension on scar tissue during scar compression often has a negative impact.

Mechanical stimulation can affect blood vessels at multiple levels. Tension transmitted through the extracellular matrix and focal adhesions can induce multiplication of endothelial cells and guide the sprouting of new capillaries. In addition, stretching of tissues can result in alterations to the blood flow, causing temporary ischemia that activates the HIF-1a pathway in nearby cells, which by releasing VEGF stimulates the proliferation of endothelial cells and formation of a richer vessel network. Bhatt et al. (2007) suggested that osteoblasts responded to mechanical stimulus by increasing matrix production. The induction of metalloproteinases after mechanical strain is believed to enhance the attraction and penetration of blood vessels (Reich et al., 2005).

More recent research (Maul et al., 2011) examined in vitro differentiation of mesenchymal stem cells into smooth muscle and endothelial cells. Three forces — cyclic stretch, cyclic pressure, and laminar shear stress — were applied independently to mimic several vascular physiological conditions. This experiment demonstrated that mechanical stimulation has significant effects on morphology, cell density, and differentiation in rat MSCs towards vascular lineage cells, and that these effects are dependent on the type, magnitude, and frequency of the applied stimulation.

5. Conclusion

Because of its shape and its nature as a malformation, alveolar bone graft in cleft lip and palate patients raises a new challenge in the comprehension of bone graft integration.

Autologous bone graft used for decades has demonstrated its effectiveness, but its mechanisms and characteristics of integration remain unclear. The literature reports that the majority of the graft is replaced by newly formed bone, but no studies have really traced the destiny of the cells involved, or of other constituents of the graft. The complexity of bone grafting lies in the fact that it involves an array of different cells (osteoblastic, endothelial, inflammatory cells…), with a scaffold having different proportions of components depending on age, gender, site of harvesting, and other individual differences.

The addition of a mechanical strain at the time of the placement of the graft could result in a change in density in the case of alveolar cleft grafting, but it is not the only modification. Applying a mechanical stimulus could lead to the liberation and secretion of growth factors, which could act directly on the vital cells. Even if the extent of these vital cells in the bone graft is low, it could be sufficient to enhance a whole process of osteogenesis, particularly after mechanical stimulation. The variety of studies on, and controversies about, mechanotransduction reflect the fact that we are only at the beginning of understanding what really happens when

Table 2
In Vitro/In Vivo studies. Most of studies on the influence of mechanical stimuli on bone formation are realized in vitro.

	os	cells	Force type
Shen, 2014		Periodonta cells	Cyclic shear
Yang et al. (2010)		ASCs	Cyclic stress
Grottkau et al. (2013)		BMSCs et ASCs	Cyclic stress
Rath et al. (2008)		Pré-OB	Compression
Maul et al. (2011)		MSCs	Cyclic compression
Bonafe-Oliveira et al. (2003)	Rats maxilla		Cyclic compression
Hsieh and Turner (2001)	Ulna diaphysis rats	C3H	Cyclic compression
Kimelman-Bleich et al. (2011)		OB	Cyclic compression
Kopf et al. (2012)		MSC	Cyclic compression
Klein-Nulend et al., 1987	Rats calvaria		Intermittent Compression
Jacobs et al., 1998			Continuous or Oscillatory compression
Van Griensven et al. (2009)		BMSC and ADSC	Cyclic longitudinal strain
Liu et al. (2010)	Rabbits calvaria		Continuous and intermittent longitudinal strain

the surgeon carries out a bone graft. Thus many authors relate numerous in vitro studies, whist the complexity of the process makes in vivo experiments difficult to realize and analyze.

One solution could be to study one influencing factor, such as strain application, while introducing the graft, in a reproducible experimental manipulation — applying a very well-defined, increasing strain on a bone graft during its placement in the site in vivo, and studying the result over time, could be one example. Focusing the study on only one variable parameter, while the levels of other variables parameters are fixed, could be the way to obtain one clinical answer without needing a full understanding of all the mechanotransduction pathways.

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