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Osteocyte pericellular and perilacunar matrices as markers of bone–implant mechanical integrity

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Abstract: To develop durable bone healing strategies through improved control of bone repair, it is of critical importance to understand the mechanisms of bone mechanical integrity when in contact with biomaterials and implants. Bone mechanical integrity is defined here as the adaptation of structural properties of remodeled bone in regard to an applied mechanical loading. Accordingly, the authors present why future investigations in bone repair and regeneration should emphasize on the matrix surrounding the osteocytes. Osteocytes are mechanosensitive cells considered as the orchestrators of bone remodeling, which is the biological process involved in bone homeostasis. These bone cells are trapped in an interconnected porous network, the lacunocanalicular network, which is embedded in a bone mineralized extracellular matrix. As a consequence of an applied mechanical loading, the bone deformation results in the deformation of this lacunocanalicular network inducing a shift in interstitial fluid pressure and velocity, thus resulting in osteocyte stimulation. The material environment surrounding each osteocyte, the so called perilacunar and pericellular matrices properties, define its mechanosensitivity. While this mechanical stimulation pathway is well known, the laws used to predict bone remodeling are based on strains developing at a tissue scale, suggesting that these strains are related to the shift in fluid pressure and velocity at the lacunocanalicular scale. While this relationship has been validated through observation in healthy bone, the fluid behavior at the bone-implant interface is more complex. The presence of the implant modifies fluid behavior, so that for the same strain at a tissue scale, the shift in fluid pressure and velocity will be different than in a healthy bone tissue. In that context, new markers for bone mechanical integrity, considering fluid behavior, have to be defined. The viewpoint exposed by the authors indicates that the properties of the pericellular and the perilacunar matrices have to be systematically investigated and used as structural markers of fluid behavior in the course of bone biomaterial development.

Introduction

Understanding how human cells respond to stimuli is of great importance for developing durable healing and tissue engineering strategies. Cellular activity is mainly investigated in the field of biology, and involves the strong multi-physic coupling between different biological, biomechanical, biochemical, or bioelectrical mechanisms. In a biological system that is made up of cells embedded in an extracellular matrix, the different elements interact together to coordinate cell activity and thus insure system viability. Bone tissue plays various roles within our body. Beyond its well-known mechanical function, bone is the main regulator for both phosphate and calcium. These two chemical species play a major role in organism homeostasis. Bone cells are thus sensitive to many kinds of stimuli, from hormonal to biomechanical stimuli.

As an example, it is well known that bone cells are sensitive to biomechanical stimuli that can tune bone properties and ensure biomechanical function (Turner, 1998). This viewpoint will mainly focus on this type of stimuli. This mechanosensitive nature is of great importance in the course of bone remodeling around implants (Li *et al.*, 2018). Among other factors, mechanical loading plays a

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major role in the risk for peri-implant osteolysis (Amirhosseini *et al.*, 2017; Goodman and Gallo, 2019). While mechanical loading is known to be involved in the stress shielding mechanism (Sumner, 2015), bone resorption can also be associated with a mechanically-induced inflammatory response (Amirhosseini *et al.*, 2017). The bone-implant system thus appears to promote the development of a complex mechanical environment in which specific tissue properties develop (Fraulob *et al.*, 2020; Le Cann *et al.*, 2020; Li *et al.*, 2018). Whether or not these properties are suitable for ensuring the mechanical integrity of the bone-implant system is still an open question. In order to provide some answers, it is important to first understand how bone ensures its own mechanical integrity.

What is bone mechanical integrity?

This paper defines bone mechanical integrity as the adaptation of bone structure in response to biomechanical loading that is experienced during life (Fig. 1). Bone mechanical behavior is closely associated with its structure at different scales (Zimmermann and Ritchie, 2015).

Although bone is a complex material that presents heterogeneity at all length scales associated with mechanical properties (Bala *et al.*, 2012; Rho *et al.*, 1998; Rux *et al.*, 2022), the following investigation will focus only on the bone porous network. At the macroscale, two bone tissues can be distinguished in terms of their porosities: trabecular bone being porous, and cortical bone being compact ((Nawathe *et al.*, 2015), Fig. 1a). Interestingly, bone loading and load distribution follow the same pattern as bone mass distribution ((Nawathe *et al.*, 2015), Fig. 1e). Bone macroporosity is mainly made of vascular canals. In cortical bone, these canals account for less than 10% of the tissue volume with a diameter between 50 and 100 µm (Gauthier *et al.*, 2019, Fig. 1c). Vascular canals influence mechanical stress distribution within the tissue (Vaughan *et al.*, 2013, Fig. 1f). At a smaller scale, an interconnected network, called the lacunocanalicular network (LCN), is distributed

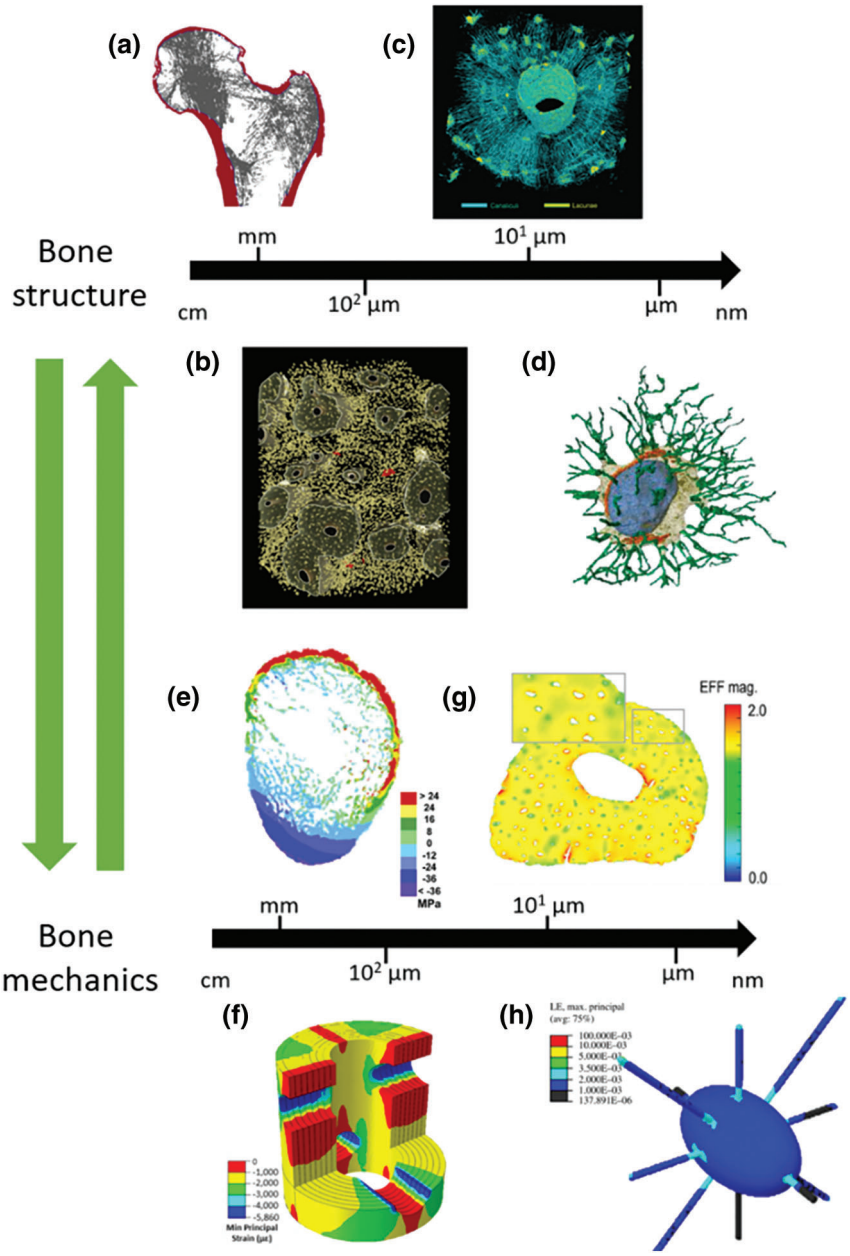

FIGURE 1. Illustration of bone mechanical integrity. At all length scale, bone structural elements are associated with the tissue mechanical response. a., projection of a proximal femur, with cortical bone in red, and trabecular bone in grey (reprinted from Nawathe *et al.* (2015)). 3D X-rays micrography reconstruction of human cortical bone with voxel size of 0.7 µm (b., reprinted from Gauthier *et al.* (2019)) and 0.28 µm (c., reprinted from Pacureanu *et al.* (2012)). d., 3D reconstruction of a lacuna through electronic microscopy based tomography (reprinted from Goggin *et al.* (2020)). From e. to h., stress (e., reprinted from Nawathe *et al.* (2015)) or strain maps (f., reprinted from Vaughan *et al.* (2013), g., reprinted from Hemmatian *et al.* (2021), and h., reprinted from Verbruggen *et al.* (2012)) at different bone length scales. For more accurate images, the readers are referred to the original articles.

within the bone matrix (Goggin *et al.*, 2020; Pacureanu *et al.*, 2012, Figs. 1c and 1d). This LCN is made of micrometer ellipsoidal lacunae that are spread within the tissue with a density higher than 20,000 mm^3 (Gauthier *et al.*, 2019, depicted in yellow in Fig. 1b). These lacunae are all connected together through canaliculi, 400 nm in diameter (Varga *et al.*, 2014; Yu *et al.*, 2020). It is estimated that one lacuna is connected to 58 canaliculi, on average, in human femoral diaphysis (Yu *et al.*, 2021). Even with their micrometer and nanometer scales, both lacunae and canaliculi play a role in the tissue mechanical response (Hemmatian *et al.*, 2021; Verbruggen *et al.*, 2012, Figs. 1g and 1h). Fig. 1 shows an overview of bone mechanical integrity, with a specific mechanical answer, in terms of stress distribution, being associated with bone structural organization at all length scales.

How does bone ensure its mechanical integrity?

Bone mechanical integrity is maintained through a balance between bone resorption and bone formation. The process ensuring this homeostasis is known as bone remodeling. Remodeling occurs to allow bone to adapt to its mechanical environment and to repair damaged tissue (Burr, 2002). Bone remodeling involves different types of cells:

- Osteoclasts are recruited to remove targeted tissues through an acidic dissolution of bone mineral and proteolytic digestion of organic matrix.
- Osteoblasts are recruited to deposit a new tissue by synthesizing an organic template for further nucleation and the growth of bone minerals.
- Osteocytes are former osteoblasts that have been trapped and embedded within the mineralized extracellular bone matrix. It has been estimated that 10% to 30% of osteoblasts become osteocytes (Franz-Odendaal *et al.*, 2006). They are believed to orchestrate bone remodeling through the regulation of both osteoclasts and osteoblasts (Robling and Bonewald, 2020). Osteocytes are able to sense a change in bone mechanical integrity. They can then secrete and send biochemical mediators towards the osteoclasts and osteoblasts and hence control bone remodeling. From the different signaling pathways of the osteocytes, their mechanosensitivity is determinant to ensure bone mechanical integrity (Cowin *et al.*, 1991; Delgado-Calle and Bellido, 2022; Palumbo and Ferretti, 2021). It is believed that abnormal mechanical stimulation explains the complex peri-implant bone organization (Gramanzini *et al.*, 2016; Li *et al.*, 2018; Okawara *et al.*, 2021).

How does the osteocyte sense a mechanical signal?

The structural organization of osteocytes within the bone matrix is of great importance to understanding their mechanical stimulation. *In vivo*, the connected osteocytes are trapped in the LCN. This porous network allows for the transport of interstitial fluid from the vascular canals to the cells. Within this porous network, the cells are surrounded by a glycoproteic pericellular matrix less than 100 nm in thickness (PCM) (You *et al.*, 2004), mainly made of perlecan (Thompson *et al.*, 2011), and are attached to the wall of the lacuna through tethering perlecan fibers (Bertacchini *et al.*, 2017; McNamara *et al.*, 2009) with an average spacing of 40 nm (You *et al.*, 2004). This leaves a space, the pericellular space, between the PCM and the lacunar wall, where interstitial fluids can flow from the vascular canals to the osteocytes. In summary, the osteocyte and its canaliculi are surrounded by a perilacunar matrix (PLM) around the porosity and a PCM between a bone mineralized matrix and the cell (Fig. 2). The lacunocanalicular network irrigates all the bone volume so that any tissue damage can be detected and processed by the cells. In addition to this complex micro- and nanostructural organization, the composition of these PCM and PLM also present some heterogeneity. As an example, it has recently been measured that there is a

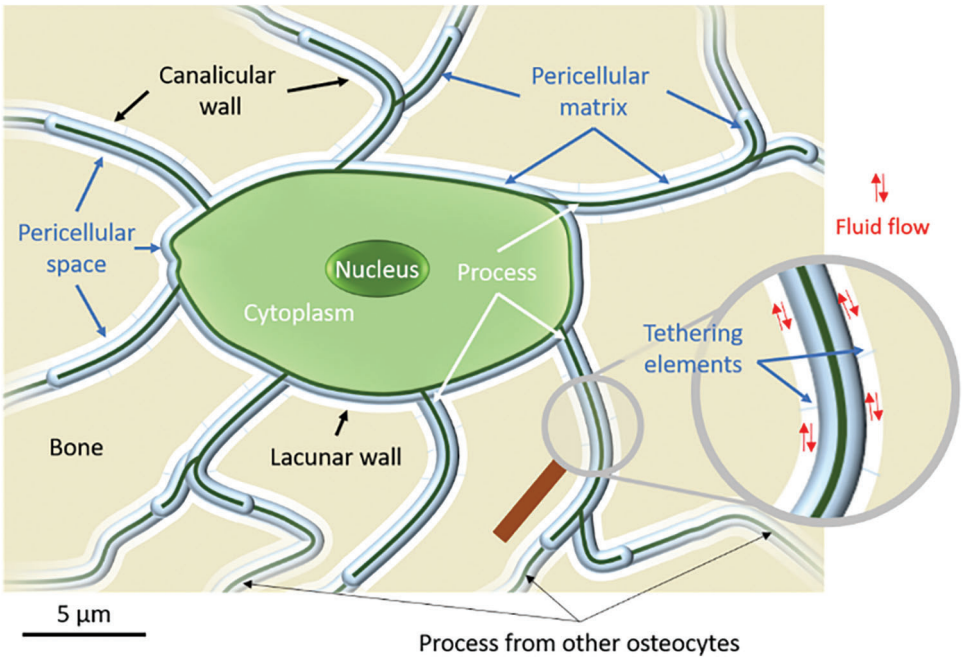

FIGURE 2. Schematic of an osteocyte structural organization within bone mineral matrix. Interstitial fluid flows through the pericellular space dragging the tethering elements. The elements of the perilacunar, pericellular, and cellular matrices are written in black, blue, and white, respectively.

decrease in elastic modulus with an increasing distance to the lacunar wall. Interestingly, the gradient magnitude is believed to be associated with the age of the osteocyte trapped within the studied lacuna (Rux *et al.*, 2022). Similarly, if the PCM is mainly composed of perlecan, other components could influence the fluid behavior within the LCN (Wang, 2018).

When bone is subjected to a mechanical loading, the whole lacunocanalicular network is deformed together with its surrounding mineralized matrix. Due to its particular organization within this network, osteocytes can experience the mechanical strain, or deformation, through different mechanisms.

The strain of the lacunae can directly be transmitted to the cell through hydrostatic pressure that can be up to the MPa (Cowin *et al.*, 2009; Gardinier *et al.*, 2010; Zhang *et al.*, 1998). It is known that a direct low pressure as low as 68 kPa applied on osteocytes induces their expression of bone remodeling mediators (Henstock *et al.*, 2013; Liu *et al.*, 2010). Due to their ellipsoidal morphological feature, lacunae play the role of strain concentrators within the tissue (Hemmatian *et al.*, 2021; Inglis, 1913). It has been measured that an apparent 0.2% deformation leads to a local deformation of up to 1.5% in the vicinity of a lacuna in an *in vitro* bovine bone (Nicolella *et al.*, 2006). This feature can increase the pressure developed within on lacuna and thus a compressive strain on the osteocyte.

The deformation of the lacunocanalicular network also induces pressure gradients within the porous canals, resulting in the flow of the interstitial fluid (Cowin, 1999; Lemaire *et al.*, 2011). Osteocytes are sensitive to fluid flow (Chen *et al.*, 2021; Tan *et al.*, 2007). The lacunae, through their ability to generate deformation concentrations, may locally modify the pressure gradient and thus the fluid velocity within this interconnected porous network. Pressure variations can also be induced by the variation of the canaliculi pericellular space during bone deformation. The induced fluid flow can drag the tethering elements attaching the PCM to the lacunar wall resulting in the deformation of the PCM and cell process (Wijeratne *et al.*, 2016; Yokoyama *et al.*, 2021). Hence, the properties of this PCM are a major factor involved in the mechanical stimulation of osteocytes (Thompson *et al.*, 2011; Wang *et al.*, 2014; Wijeratne *et al.*, 2016).

The deformation of an osteocyte and its related processes could stimulate intra-cellular mechanosensors, such as integrin or ion channels (Qin *et al.*, 2020), which then induce the secretion of bone remodeling mediators such as nitric oxide (Tan *et al.*, 2007), calcium ions (Lewis *et al.*, 2017), or sclerostin (Nishiyama *et al.*, 2014). These mediators are then transported through interstitial fluids to the osteoblasts, osteoclasts, and other biological actors of bone remodeling that are located in the vascular porosities. Such solute transport is also related to LCN mechanical stimulation (Fan *et al.*, 2016), with for example bigger molecules being transported only under mechanical stimulation. The diffusion of different chemical species involved in bone metabolism, and more generally in our organism metabolism, is largely influenced by the properties of the PCM (Wang, 2018).

These results highlight the importance of fluid behavior (i.e., fluid pressure in the lacunae and fluid velocity in the canaliculi) surrounding the osteocytes and their processes on the mechanotransduction pathway of bone tissue. Osteocyte-based bone remodeling is activated through a shift in such fluid pressure and velocity.

What is the marker of bone mechanical integrity?

Mechanical loading applied at the organ scale induces mechanical strains at the tissue level that subsequently produces the development of hydrostatic pressure and pressure gradient-induced fluid flow within the LCN (Palumbo and Ferretti, 2021; Zhang *et al.*, 1998). This pathway defines how osteocytes sense a mechanical stimulation and hence regulate bone mechanical integrity through tissue remodeling.

Such a pathway has led some scientists to develop a theoretical model of bone remodeling based on tissue deformation. In that context, major progress has been made by Frost who developed the mechanostat mechanism theory (Frost, 1987). In this theory, Frost defined different thresholds, known as minimum effective strains (MES), as the strains developed at a tissue scale below which bone resorption occurs (MES for bone remodeling, MESr), and above which bone formation occurs (MES for bone modeling, MESm). This also implies that there is a range of deformations, between MESr and MESm, in which bone structure remains the same (Frost, 1983).

This strain-based principle of bone remodeling is attractive, because bone tissue strains can be routinely estimated using numerical tools such as finite element modeling (Hemmatian *et al.*, 2021; van Rietbergen *et al.*, 2003; Werner *et al.*, 2019). Bone tissue strain has hence been used to predict the course of bone remodeling (McNamara and Prendergast, 2007; Schulte *et al.*, 2013), and can thus be considered as a marker of bone mechanical integrity.

What about the bone-implant system mechanical integrity?

While this strain-based theory has also been widely investigated in the context of peri-implant tissue remodeling (Huiskes *et al.*, 1987; Mirulla *et al.*, 2021), its relevance is not obvious.

Considering an osteocyte mechanical stimulation pathway, the strain-based theory could indirectly suggest that strains at a tissue scale promote fluid movement within the LCN. All the tissue elements on which strain is calculated are thus considered as equivalent in terms of fluid behavior. Nevertheless, in the vicinity of an implant, fluid behavior is different from what may occur in the bulk (Fahlgren *et al.*, 2010), with a direct incidence on tissue remodeling, and hence on the bone-implant system mechanical integrity. The shift in fluid behavior will be different between the bone surrounding the implant and in the bulk due to tissue strain.

This means that there is a need for additional markers to help understand strain-induced fluid behavior close to the bone-implant interface.

Osteocyte perilacunar and pericellular matrices as markers of bone-implant system mechanical integrity

The viewpoint exposed by the authors is that PCM and PLM properties can be considered as suitable markers, and that these matrices have to be systematically investigated to

validate the efficiency of future bone implants. There is increasing evidence that PCM and PLM properties and remodeling are associated with bone mechanical function (Dole *et al.*, 2017; Milovanovic and Busse, 2019; van Tol *et al.*, 2020b). Interestingly, it has recently been shown that a mechanical loading (*in vitro* and *in vivo*) influences the turnover of this PCM (Pei *et al.*, 2021). Such results further support the need to have a better understanding the roles of both PCM and PLM on bone mechanosensitivy.

Within the physiological window defined by Frost, strains do not promote either bone formation or resorption (Frost, 1987). Within this physiological window, the strain-based shift in fluid pressure or velocity is not high enough to induce bone remodeling. The current viewpoint thus considers that PCM and PLM reach specific properties while bone tissue lies within this physiological strain window. In other words, the limits of this physiological window are defined by PCM and PLM properties.

Considering this viewpoint, and due to different fluid behavior, the physiological window close to an implant should thus be different from the bulk. Investigating both PCM and PLM properties is thus necessary to define the physiological range during which bone remodeling does not occur.

It is known that the fluid behavior within the LCN depends on the distance to vascular canals in cortical bone (van Tol *et al.*, 2020a) and to canaliculi interconnectivity (Bortel *et al.*, 2021). Accordingly, it has been observed that both lacunar and canalicular morphological parameters depend on their location between the vascular canal and the cement line of an osteon (Gauthier *et al.*, 2019; Repp *et al.*, 2017).

This viewpoint is also interesting when considering the chemoregulator role of osteocytes within our body (Bonewald, 2017). The properties of the LCN do not depend only on bone mechanical integrity. Osteocytes also act as regulators for both calcium and phosphate metabolism to maintain systemic mineral homeostasis in physiological conditions (Cheng and Hulley, 2010; Delgado-Calle and Bellido, 2022; Horner, 2004). During specific adaptation cases, for example during lactation, changes in lacunar morphology have already been highlighted (Qing *et al.*, 2012), and are associated with a decrease in the effective elastic properties of bone tissue (Kaya *et al.*, 2017). It is known that their activity is partly regulated through hormonal pathways. For example, parathyroid hormone (PTH) is very important in osteocyte functions, and hence in bone homeostasis (Bellido *et al.*, 2013). PTH inhibits sclerostin expression that hence prevents the osteoblast from synthetizing new bone. Nevertheless, sclerostin can be expressed by bone cells through mechanical loading and independently of PTH (Spatz *et al.*, 2015). Furthermore, it is known that osteocyte apoptosis can be induced through mechanical loading (Hughes and Petit, 2010; Nakao *et al.*, 2021). Such results highlight that osteocytes need to be in a quiescent state, or equilibrium state, and within the physiological strain window, even when considering their chemical regulator role.

In contrast to peri-implant bone, the variations in PLM properties are not related to a modification in fluid behavior (i.e., fluid pressure and velocity), but instead to a metabolic need for calcium or phosphate. Nevertheless, with different PCM and PLM properties, the strain-induced shift in fluid pressure and velocity necessary to activate osteocyte–based bone remodeling is also different. As for peri-implant bone, the physiological strain window evolves with PCM and PLM properties. This may explain the decrease in bone mechanical properties in the case of lactation. As the external mechanical loading remains the same, the strain at the tissue level does not evolve. However, with different limits in the physiological window, bone remodeling is not activated at the same strain magnitude. This results in the development of a different structural organization, and thus a different result in mechanical integrity.

This viewpoint highlights that the consideration of bone remodeling as just a result of strains at the tissue level may not be accurate enough to cover different abnormal cases. While such remodeling is true in healthy bone, it might not be accurate in the vicinity of an implant, where fluid behavior is unknown, or in the case of a biological pathology or aging, which can induce an evolution in PCM and PLM properties. According to the present hypothesis, the strain-based shift in fluid pressure and velocity is the real determinant in bone remodeling and mechanical integrity. Hence, in addition to tissue strain, PCM and PLM properties have to be considered as major features involved in this fluid behavior strain-based shift.

To better understand this relationship between strain and fluids, further investigations of the LCN has to be performed considering their precise location in regard to the vascular network, and how this acts as the main fluid reservoir. Are there particular patterns in PCM and PLM distribution properties in relation to the distance to a vascular canal? Is there a difference between trabecular or cortical LCN? Those questions remain unanswered. Similarly, there is no data on PCM and PLM properties in the vicinity of an implant. Furthermore, while bone implant efficiency is currently defined as its capacity to induce suitable strain in the peri-implant bone, major efforts have to be made regarding the implant's ability to influence interstitial fluid behavior in its surrounding LCN.

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