

Strain amplification and integrin based signaling in osteocytes

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Abstract

Recent morphological studies have suggested that osteocyte processes are directly attached at discrete locations along the canalicular wall by β_3 integrins at the apex of infrequent, previously unrecognized, canalicular projections. This discovery has led to a new paradigm for the initiation of intracellular signaling, which provides a possible long sought after molecular mechanism for the initiation of intracellular signaling in bone cells. The quantitative feasibility of this hypothesis is explored with a detailed theoretical model¹, which predicts that axial strains due to the sliding of actin microfilaments about the fixed integrin attachments are in order of magnitude larger than the radial strains in the previously proposed strain amplification theory^{4,5} and two orders of magnitude greater than whole tissue strains.

Keywords: Mechanotransduction, Bone Fluid Flow, Osteocyte Processes, Lacunar Canalicular Porosity, Bone Cell Integrins

Introduction

A fundamental paradox in bone biology is how osteocytes can experience sufficiently high cellular-level strains (>0.5%) needed for mechanical stimulation² when whole bone tissue strains *in vivo* are typically <0.2%³. Strain amplification models^{4,5} explain this paradox by showing that the load-induced fluid drag on tethering elements in the pericellular matrix can greatly amplify whole tissue strains when transmitted to the actin filament bundle in the cell process. However, the molecular elements still remain to be elucidated, because none of the likely molecules in the tethering complex surrounding osteocytes are known to initiate intracellular signaling.

Recent studies^{6,7} show that osteocyte processes directly attach to the canalicular wall at discrete, infrequent canalicular projections as shown in Figure 1 and that the adhesion molecules are β_3 integrins. Integrins are widely recognized to

initiate Ca^{+2} signaling in response to mechanical forces in various cells⁸ and co-localize with other membrane-associated proteins to form a mechano-receptor complex⁹. A model is developed to determine whether such attachment complexes would focally amplify strains to excite osteocytes.

Methods

Our idealized structural model for a local attachment complex is shown in Figure 2, which combines the new observations in Figure 1 with the basic model for tethering elements and the structure of the actin filament bundle in Han et al.⁵ obtained from the electron microscopic observations in You et al.¹⁰. Focal attachment complexes, consisting of canalicular projections, integrins and integrin intracellular anchoring proteins are considered to be rigid and thus treated as fixed supports.

The presence of rigid focal attachment complexes results in asymmetric loading of the osteocyte process and its cytoskeleton. Two mathematical idealizations are introduced to simplify the analysis, but retain the essential physics of the deformation for osteocyte processes. First, the central actin filament bundle with its fimbrin cross-links in the core of the osteocyte process is replaced by a homogenous cylindrical elastic structure that has the same size and overall radial elastic modulus as the original cross-linked structure. Second, the eleven transverse tethering elements and the

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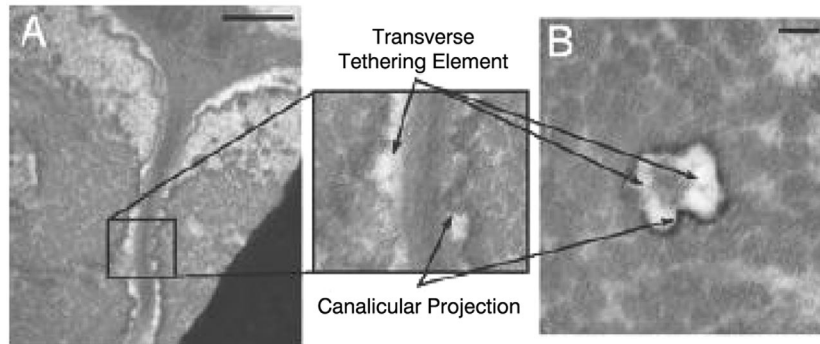


Figure 1. **A)** Transverse (bar=500nm) and **B)** longitudinal (bar=100nm) cross-section of TEM images showing canalicular projections coming into direct contact with the cell process and transverse tethering elements spanning the majority of the pericellular space between the cell process and canalicular wall.

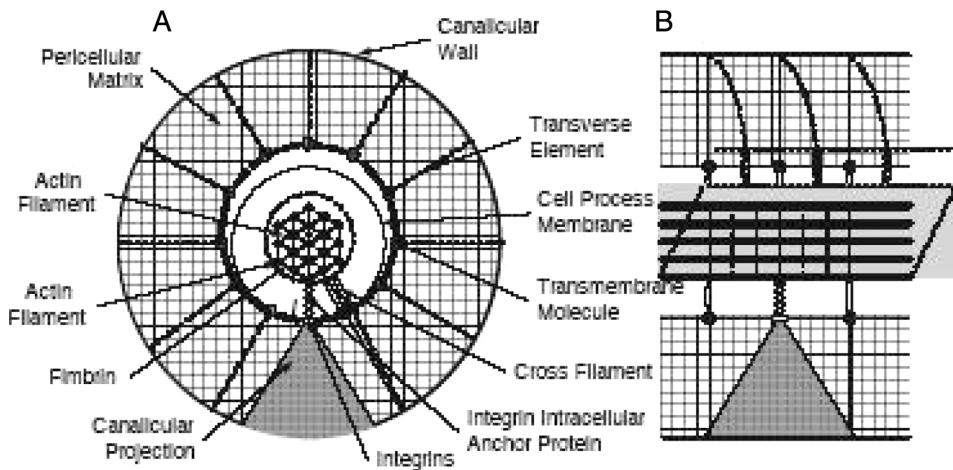


Figure 2. **A)** Transverse and **B)** longitudinal cross-section of the idealized structural model showing the direct attachment of an osteocyte process to a local canalicular projection via integrins. (Solid line – undeformed; dashed line – deformed process and tethering elements)¹.

focal attachment are mathematically assumed to act in the same cross-sectional plane when dealing with the overall radial force balance.

The radial (ϵ_r) and axial (ϵ_a) strains of the osteocyte process membrane in the vicinity of focal attachment complexes are shown in Figure 3.

Results

As shown in Figure 3, ϵ_a is approximately one order of magnitude larger than ϵ_r for the same tissue loading. ϵ_a is predicted to be about 6 percent at a physiological loading of 20 MPa at 1 Hz, nearly two orders of magnitude larger than the whole tissue strains. Similarly, small tissue strains of only 5 μ strain can be amplified to >1 percent at 40 Hz.

Discussion

Our mathematical model for the mechanical environment around focal attachment complexes predicts that these attachment complexes will dramatically and focally amplify cellular strains at these sites. Such high focal strain concentrations can provide a potential mechanism for osteocyte activation even at low amplitude but high frequency loading.

Our speculation about the underlying mechanism for cellular excitation is that integrin attachment sites are co-localized with mechanosensitive ion channels and the large axial strains at these sites provide a mechanism for the regulation of these stretch activated channels. Potential candidates include stretch-activated cation channels, hemichannels and the P2X₇ receptor.

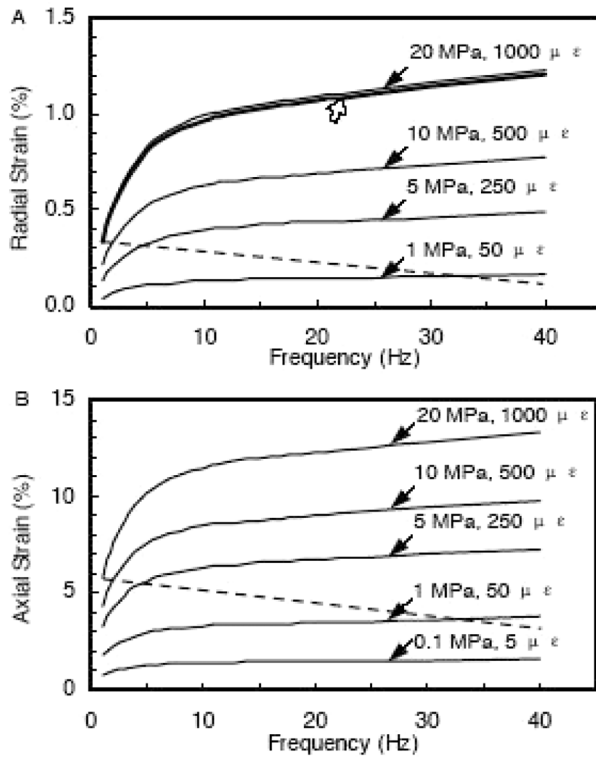


Figure 3. **A)** The radial strain ϵ_r for a tissue loading of 10 MPa is shown for comparison), and **B)** the axial strain ϵ_a as a function of loading frequency with tissue loading amplitude as a parameter¹.

Conclusion

Integrin attachments along osteocyte processes can induce a high focal axial strain concentration, which greatly amplify bone tissue strains and can provide a mechanism for osteocyte excitation.

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