Analysis of mechanotransduction dynamics during combined mechanical stimulation and modulation of mechanotransduction cascade uncover hidden information within the signalling noise

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1. Background

Osteoporosis is a bone disease characterised by brittle bone and increased fracture incidence. The disease is globally a high burden on health systems which continues to increase with an aging society. There are limited treatments for osteoporosis with just two FDA approved pharmacological agents in the USA. Furthermore the drug discovery pipeline has limited success in producing novel and efficacious molecules. Osteoporosis arises due to changes in bone architecture, mineral density (BMD) and strength. These characteristics are believed to be affected by bio-mechanical stimulations. Such signals are sensed by bone cells in the bone remodelling unit and translated to cellular responses which ultimately maintain healthy bone. Recently, dual bio-mechanical stimulation with intermittent parathyroid hormone (PTH) treatment and mechanical stimulation were shown to increase BMD and bone formation in mice, thus suggesting a promising treatment for osteoporosis [1,2]. However, the exact regimes to induce potent therapeutic effects are yet uncharacterised. This is partly due to incomplete understanding of cellular and molecular mechanisms which sense and integrate the dual signals into a cellular response (i.e. mechanotransduction) which evolve into increased BMD, strength and growth at the tissue level.

This study examined the dual modulation of mechanical stimulation and variation in mechanotransduction activation dynamics of an osteoblast. The aim was to find fingerprints of mechanotransduction dynamics demonstrating a significant change which can be mapped to alteration in osteoblast (OB) responses. This was achieved using a 3D hybrid-multiscale model simulating mechanotransduction in OB and its interaction with the extracellular matrix (ECM), combined with a numerical analytical technique. The model and the analysis method predict that within the mechanotransduction signalling noise, there are unique events which can provide signatures indicating a shift in the system's dynamics due to modulation of the bio-mechanical stimulus. Furthermore, the study uncovered molecular interactions that can be potential drug targets. The model and the developed analysis scheme can be used as tools to assess if candidate drug-molecules can effectively replicate the perturbation to

steady state dynamics reported in this study.

2. Methodology

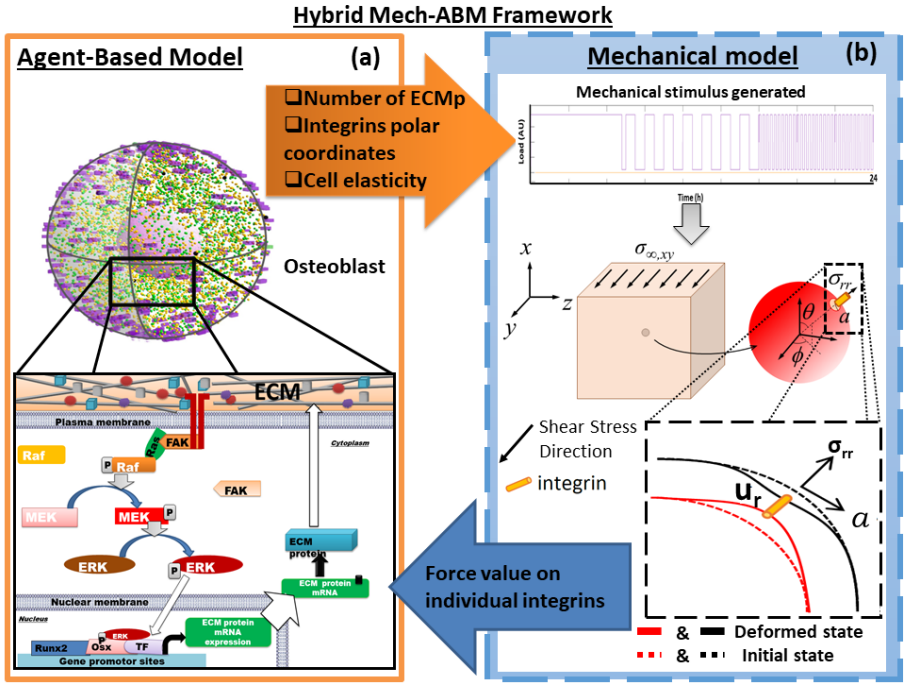

Figure 1 Representation of the Mech-ABM simulation framework (a) The ABM simulated intracellular events downstream of integrins in a spherical osteoblast. (b) The mechanical model simulated tissue biomechanics when various mechanical stimulations (shear stress) regimes were applied.

The mechanical-agent based model (Mech-ABM)

A hybrid mechanical-agent based model (Figure 1) was used to mimic the dynamic interplay between mechanical stimulation and intercellular response. The ABM simulated mechanotransduction, in an osteoblast, downstream of the integrins mechanoreceptors, which recruited the focal adhesion protein (FAK) and the Extracellular Regulated Kinase (ERK) signalling pathway and led to the synthesis and deposition of extracellular matrix (ECM) proteins. The mechanical model mimicked mechanical perturbation at the tissue level and the tissue biomechanics. Accumulated number of protein-agents over time (24 h) partitioned by their state variables (e.g. active/inactive and bound/unbound) was the primary output analysed from the Mech-ABM. Mechanical stimulations were examined by varying the magnitude (M = 100 and 10,000 μPa) and oscillation periods (P = 1000, 5000 and 20,000 s). The biochemical perturbation was achieved via modulating intercellular molecular interactions within the ERK pathway associated with protein activation cycle (i.e. feedback loops). Total number of simulations was 1800, with 15 repeats per condition. Manipulation of the 3D volume has no impact on overall intracellular reactions within an ABM framework as demonstrated by Rhodes et al 2016[3].

Signal transformation, subordination theory and detection of events

Due to the inherent stochasticity of the system and therefore the ensuing temporal

variations in the output (fluctuating signals), the signal was transformed, via a filter (equation 1 and 2)

$$\overline{y_i}(t) = \widehat{A}_{\Delta_T} y_i(t) \xrightarrow{\Delta_T \to \infty} \overline{Y_i}(t), \tag{1}$$

$$\widehat{A}_T = \frac{1}{\Delta_T} \int_t^{t+\Delta_T} (\cdot)\, dt'. \tag{2}$$

A numerical method utilising subordination theory was used to disentangle random process into their respective parent process and directing process in order to analyse the latter in terms of patterns of recurrence of events [4]. This was via applying a subordination process separating natural time and the physical time (the macroscopic and experimentally observable time). A moving average filter with a Hilbert transformation was applied twice, thus determining signal time series and amplitude as positive defined time series with enhanced prominent peaks and dumped valleys close to the zero axis, fig. 2. the peaks are detected as events $\mathcal{E}$, if the signal minus one standard deviation of the fluctuations drops after each peak, pn(t), below the corresponding preceding peak value:

$$\mathcal{E} = \{p_n(t_1), \exists\, t_2 \mid y_i'''(t_2) - \sigma_i(t_2) < y_i'''(t_1)\} \tag{3}$$

The probability density obtained by normalizing the integral distribution to 1 was expressed as a waiting time distribution (WTD). WTDs between peaks or the time extent of peaks are considered. a Kernel Density Estimator (KDE) defined as:

$$\mathrm{KDE(t)} = \tfrac{1}{w} \int_t^{t+w} K(t-\tau)(\cdot)\, d\tau \text{ such that } \int_{-\infty}^{\infty} K(t)\, dt = 1 \tag{4}$$

w is the support of the operator. Due to the stochasticity within the model and the applied mechanical stimulation, the critical events would be gamma distributed, and characterize by multimodality. The WTD of integrins, the ERK pathway, and RUNX2 were illustrated as these are the primary factors involved in osteogenesis.

3. Results

The WTD of many classes of molecules presented multimodality which highlights the presence of preferred intermediate times (IT) in the occurrence of events. Changes in the activation cycle times (i.e. feedback loops dynamics) of molecules in the ERK signalling pathway showed variations in WTDs of individual protein molecules. For example, under a 1000 s time period mechanical perturbation, the WTD of Rapidly Accelerated Fibrosarcoma (RAF) bound to MAPK/ERK Kinase (MEK) was characterized by a bimodal distribution for low values of active MEK dissociation times, while it became unimodal at larger value of the same parameter. Many of the mRNAs WTDs were affected by ageing, a variation of the shape from a bimodal to trimodal distribution, showing the emergence of a new preferred intermediate times between the occurrences of event. Changes in the WTDs shape are proved to be significant by local error estimation of the distribution.

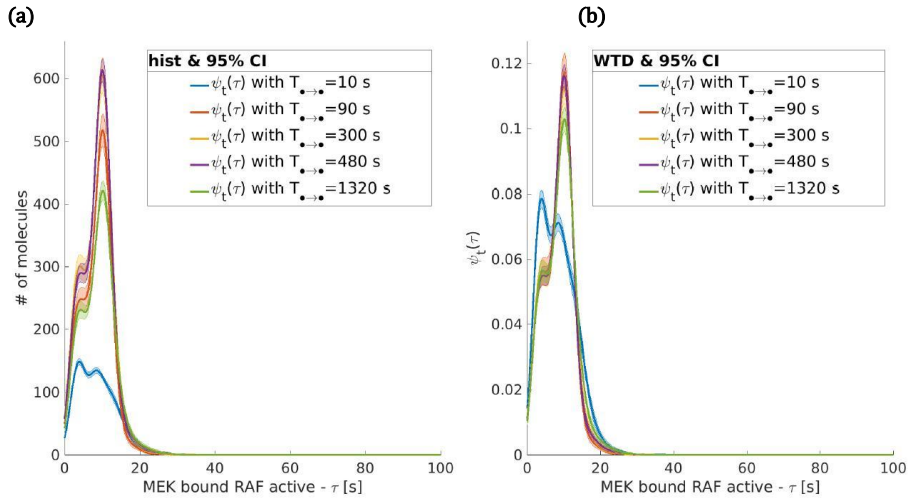

Figure 2 Impact of modulating the feedback loops of the ERK signalling pathway on mechanotransduction dynamics. (a) The variation in active MEK protein magnitude with manipulation of activation cycle time at MEK protein level ($T_{\bullet\to\bullet}$, in s). (b) Illustrates the impact as waiting time distribution (WTD). Applied oscillatory mechanical stimulation (P) = 1000s and magnitude M = 10000 µPa .

Variation in the modes implies variations in the dispersion times between chained events of the molecular network. Therefore, the WTD of a specific molecule is a dynamic fingerprint which can be used as a signature for pathological conditions, helping to design drugs capable of improving target selectivity and maximizing the interaction with targets and antagonizing chained reactions.

4. Conclusion

We observed large fluctuations enclosing information hidden in the noise which is beyond the dynamic variations of molecular baselines. These were observed as alterations in WTD of activated proteins. Therefore, WTD of each molecule are a signature of the system's dynamics. Differently from more traditional approaches, where noise has been adopted as a measure to quantify the error around the expected values of time dependent signals, in our study, the abrupt fluctuations departing from the trend have been explored and analysed, thus hidden information characteristic to each class of molecule and of the process dynamics were extracted. These forecast candidate protein-protein interactions, such as RAF -MEK, and MEK-ERK, thus modulation of scaffold proteins as innovative targets to modulate mechanotransduction to develop innovative therapies for osteoporosis.

5. References

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