Harmonic resonance and entrainment of propagating chemical waves by external mechanical stimulation in BZ self-oscillating hydrogels

Article

Supplemental Material

Harmonic resonance and entrainment of propagating chemical waves by external mechanical stimulation in BZ self-oscillating hydrogels

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Smart polymer materials that are non-living yet exhibit complex "life-like" or biomimetic behaviours have been the focus of intensive research over the past decades, in the quest to broaden our understanding of how living systems function under nonequilibirum conditions. Discovery of how chemical and mechanical coupling can generate resonance and entrainment with other cells or external environment is an important research question. We prepared Belousov-Zhabotinsky (BZ) self-oscillating hydrogels which convert chemical energy to mechanical oscillation. By cyclically applying external mechanical stimulation to the BZ hydrogels, we found that when the oscillation of a gel sample entered into harmonic resonance with the applied oscillation during stimulation, the system kept a "memory" of the resonant oscillation period and maintained it post stimulation, demonstrating an entrainment effect. More surprisingly, by systematically varying the cycle length of the external stimulation, we revealed the discrete nature of the stimulation-induced resonance and entrainment behaviours in chemical oscillations of BZ hydrogels, i.e., the hydrogels slow down their oscillation periods to the harmonics of the cycle length of the external mechanical stimulation. Our theoretical model calculations suggest the important roles of the delayed mechanical response caused by reactant diffusion and solvent migration in affecting the chemomechanical coupling in active hydrogels and consequently synchronising their chemical oscillations with external mechanical oscillations.

Resonance | Entrainment | Chemo-mechanical coupling | Self-oscillating hydrogels

Synchronisation of oscillations is abundant in nature from physical, chemical to biological systems. Oscillations are also found in various biological systems and can operate at the molecular level (e.g. cardiac cell beating) and at the organism level (e.g. sleep–wake cycles). When two oscillating systems interact, their oscillations can be tuned to the same frequency with a certain phase difference (1). This kind of entrainment process serves a basis for synchronisation. From genes, body and cell physiology, to our daily routines, activities are influenced by the day-and-night cycle, e.g., transcription of genes, protein synthesis and repair of tissues are fundamentally entrained to the rhythm of the sunlight cycle. At the cellular level, synchronisation of electro-chemical oscillation can occur through interactions between single cells and with their environment. For example, it is well established that calcium wave across the heart generates the mechanical heartbeat as a single organ, i.e., individual heart cells synchronously contract in response to the local calcium concentration. However, Nitsan et al. recently found that external mechanical oscillation can also modify the calcium oscillation within the cell (2). Chemo-mechanical coupling as a form of cell-to-cell communication can thus be a key candidate to explain the robust heartbeat against perturbations.

As living systems such as hearts are fundamentally far from equilibrium, their functioning should naturally be subject to universal laws of non-equilibrium physics. Therefore, we can apply the concepts of non-equilibrium physics to study the physical-chemical forces underlying the rhythmic behaviour of living systems and reveal the fundamental principles behind them. We suggest that the stability and entrainment of the periodic behaviours in living systems emerge from the interaction between different thermodynamic forces, i.e., chemical-mechanical coupling, to produce stable synchronisation between cells.

Entrainment is defined as a temporal coupling process where one system with an inherent rhythm changes its rhythm in accordance with an external frequency. Entrainment can

Significance Statement

Synchronisation between cells plays a critical role in cell-to-cell communication. Although electrical and chemical communications was studied, mechanical communication is recently recognised as a form that affects chemical oscillation within cells; calcium oscillation of heart cells was altered by external mechanical oscillation. To study interplay between chemical and mechanical oscillations, we developed an experimental paradigm using smart polymer gels that exhibit chemical and mechanical oscillation synchronisation/entrainment to externally applied mechanical stimulation. This is the first study to demonstrate memory function necessary for 'reprogramming' the rate of inherent chemical oscillation by the external mechanical oscillation. Our finding paves a way of using smart active materials as chemical engine to produce mechanical force bridging active materials with biological discoveries in chemomechanical coupling.

T.G-H. designed and performed experiments, analysed data. Y.H. conceived the research question and coordinated the project. Z.W. performed theoretical modelling. T.M. R.Y. and N.V. provided crucial technical, instrumental and sample preparation support. T.G-H., Z.W. and Y.H. wrote the manuscript. All authors discussed results and contributed to conclusions.

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be induced by a variety of modalities, mechanical, chemical and electrical coupling between two systems. It was originally demonstrated using two pendulum clocks coupled through a wooden structure (1). Synchronisation in this system was achieved via mechanical vibrations through the wooden coupling bar. In physical chemical systems, aqueous drops containing Belousov-Zhabotinsky (BZ) solutions were shown to have a variety of synchronous regimes of chemical reaction, including in- and anti-phase oscillations, and stationary Turing patterns (3).

In the quest to broaden our understanding of how living systems function and how life could have emerged, smart polymer materials that are non-living yet exhibit complex "life-like" or biomimetic behaviours have been the focus of intensive research over the past few decades (4–7). One branch of such smart materials are the extensively studied BZ self-oscillating hydrogels (8) that are capable of exhibiting a rich variety of physical-chemical and biomimetic behaviours (9–12) and show great promise as potential soft actuators, drug delivery systems and other applications (13, 14). In these hydrogels, the key reactant of BZ reaction, ruthenium complexes, are covalently bound to the polymer chains as pendant groups, which act as the catalyst in the redox oscillation. Consequently all periodic redox changes of these groups lead to rises and falls in polymer charge density, which in turn induces excess counterion migration and osmotic pressure changes, and prompts water to enter or leave the polymer network, making it swell or deswell. The spontaneous periodic swelling-deswelling of BZ hydrogels, known as chemomechanical self-oscillation, is reminiscent of the rhythmic beating of cardiac cells.

BZ gels are fundamentally active and autonomous materials where chemical oscillations are coupled with mechanical responses. Such chemomechanical coupling is primarily driven by the chemical reaction, because alterations in the chemical environment are required for the hydrogel to undergo volume changes (see, e.g., Sasaki *et al.*(15)).

However, the function of 'reprogramming' chemical oscillations in heart cells by external mechanical oscillation (2) was not studied, i.e., entrained frequency should relax to the original oscillation frequency. The concept of reprogramming should be tested against; the heart cells or self-oscillating gels should be reprogrammed again and again with different frequencies. In this study, we explore the potential functions of entrainment and reprogramming (relaxation process), manipulating chemical oscillations in BZ hydrogels by cyclically applying external mechanical stimulation.

We found that in addition to synchronisation and entrainment, the inherent oscillation of a BZ gel could enter a distinct resonant frequency during stimulation. After which the system kept a "memory" of the resonant oscillation period, and maintained it post-stimulation, before relaxing back to its original frequency. More surprisingly, we observed that resonance and entrainment behaviours are embedded into the self-oscillations in a discrete nature, i.e., hydrogels slow down their oscillation periods to the harmonics of the cycle length of the external mechanical oscillation. Our numerical calculations based on a theoretical framework for describing the chemomechanical oscillations in BZ gels (16–18) suggest that these experimental observations can be partly related to the diffusion of reactants and poroelastic effects due to solvent migration.

To the best of our knowledge, this is a first study demonstrating that internal chemical oscillations in physical chemical systems can be truly "reprogrammed" by applying external mechanical stimulation. Such reprogramming can be realised not only during the stimulation via synchronisation, but more promisingly also post stimulation via sustained 'entrainment', leading to the relaxation process.

Results and Discussion of BZ hydorogel experiments

To perform a systematic study on reprogramming BZ hydrogels via mechanical stimulation, a custom-built rig was used to compress samples cyclically in a pulsatile manner (see Fig. S2 and S3 in the SI for full illustration and details). All experiments lasted six hours and consisted of three phases. In the first hour chemomechanical oscillation of the gel was allowed to emerge and proceed at its natural period. Then for the next three hours external mechanical stimulation was repeatedly applied at various cycle lengths (CLs). In each cycle, gel pieces were compressed by 25-35% volume for one minute, then released for CL-1 minutes. This pulsatile stimulation waveform was found to be the most optimal pattern, with various cycle lengths between $2-20$ minutes, chosen according to the hydrogel's natural oscillation period T_{nat}, to achieve around $1{=}T_{\mathrm{nat}}/CL$ or higher/lower ratios. Finally after stimulations ended, observation was still continued for further two hours to assess any long-term and sustained changes in the hydrogel's oscillation due to external stimuli.

BZ hydrogels were cut to thin, long quasi-1D geometry which allowed the emergence of propagating chemomechanical waves. The ruthenium catalyst concentration was kept constant in the gel, while four different compositions C1–C4 were mixed for the catalyst-free BZ solution, which contained the reactants sodium bromate, malonic acid and nitric acid in different concentrations (see Table S1 in the supplementary information document for details). These C1–C4 compositions all yielded different T_{nat} hydrogel oscillation periods, which are listed in Table S2 in the SI (and were obtained from 6-hour non-stimulated reference measurements as shown in Figure S1), along with the corresponding chosen CL cycle lenghts. Since redox changes of the ruthenium catalyst corresponded to clear red/green colour changes in the gel, chemomechanical oscillation as well as mechanical compressions could be reliably followed via time lapse imaging and pixel analysis, as per established methods (16).

Time series of resonance at fundamental frequency, 1/n and n harmonics. We note that for consistency the term "oscillation period" (T) is used for the inherent chemomechanical oscillation of BZ gels, whereas the external mechanical compressing oscillator is referred to as having a "cycle length" (CL); the unstimulated natural oscillation period is denoted as T_{nat}, while $T_{\mathrm{stimulation}}$ is the altered period that can be measured during the application of the external mechanical compressions. Resonant oscillation could occur if, due to the interaction between the gel's inherent and the externally applied oscillations, $T_{\mathrm{stimulation}}$ became synchronised to CL, while accompanied by increases in oscillation amplitude A. In addition, we specifically defined 'entrainment' as resonant adjustment to the forcing oscillator, where crucially the period adjustment was sustained, i.e., maintained at least for a while, even after the forcing oscillator was switched off.

We observed multiple modes of resonance in every C1–

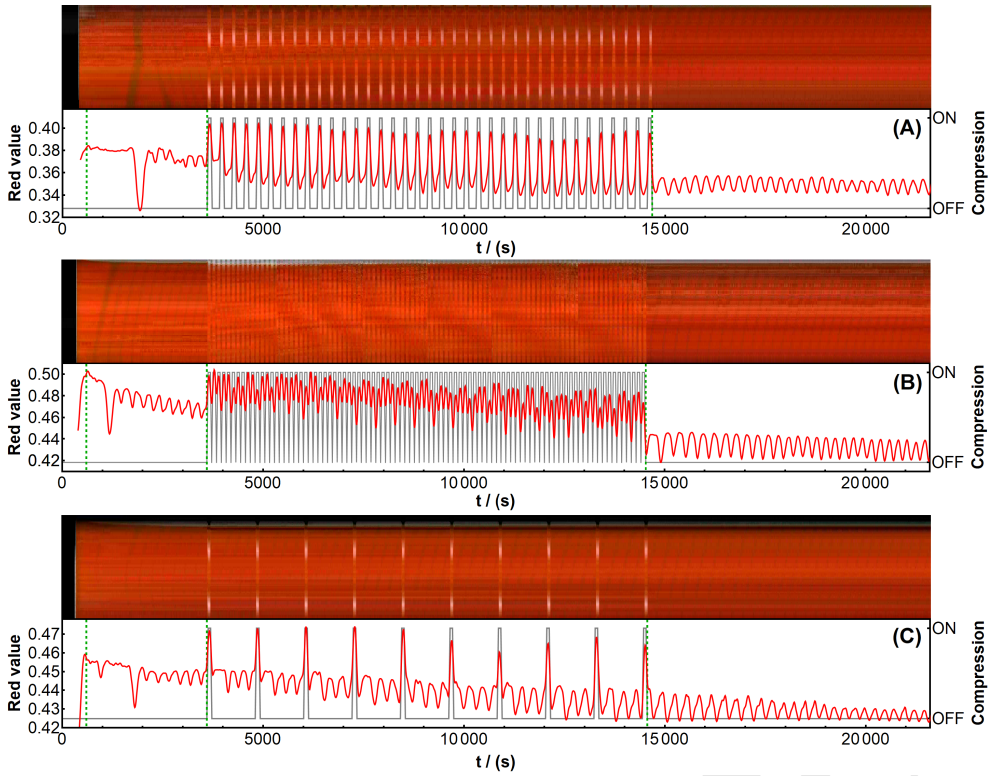

Fig. 1. Six-hour chemical oscillation time series of BZ hydrogel samples, illustrating the three possible types of resonance during mechanical stimulation. (A) Fundamental frequency resonance (1-to-1 synchronisation): catalyst-free BZ solution concentration: C2 (see SI); reference T_{nat}=211 ± 11 s; CL=5 min. (B) n×CL harmonic resonance (1-to-3 synchronisation here): catalyst-free BZ solution concentration: C2 (see SI); reference T_{nat}=211 ± 11 s; CL=2 min. (C) (1/n)×CL harmonic resonance (4-to-1 synchronisation here): catalyst-free BZ solution concentration: C3 (see SI); reference T_{nat}=279±11 s; CL=20 min. Top image for all (A–C): visualisation of wave propagation as explained in Fig. S5 in the SI. **Red curves – left axes:** changes in the BZ gel's red value over time - see Fig. S4 in the SI regarding how peaks and fluctuations can be correlated to redox changes and compression effects; **Grey curves – right axes:** ON-OFF times of the cyclic mechanical compression. Green dashed lines indicate an initial 600 s cut-off for data analysis and the 1 hour and 4 hour marks where cyclic stimulation begins and ends.

C4 BZ reactant composition, and the manifestation of the phenomenon depended on the T_{nat}/CL ratio. Fig. 1 contains three example time series of oscillating BZ gel samples that were obtained for 1-to-1 fundamental frequency, 1/n and n harmonic resonances during stimulation, respectively.

In Fig. 1 (A), when CL= 5 min (300 s) stimulation was applied to a gel with natural oscillation period T_{nat}=211 ± 11 s, the chemical oscillation was observed to slow down to $T_{\mathrm{stimulation}} = 306 \pm 9$ s, thus achieving a $T_{\mathrm{stimulation}}/CL \approx 1$ ratio. The gel could enter into a resonant mode which was characterised by higher oscillation amplitudes. Since the synchronisation and resonance took place in a 1-to-1 manner with the stimulation CL, this type of interaction was termed "fundamental frequency" resonance. We observed that fundamental frequency resonance could only emerge when some particular conditions were met, especially that T_{nat} needs to be suitably smaller than CL so that the gel's natural oscillation period could increase during stimulation and reach the required level for resonance. Crucially, it was observed in our entire study that cyclic mechanical compression always caused the hydrogel's inherent oscillation to slow down, it could never prompt it to become faster. Furthermore, the amount of T increment was found to depend on how much compressing force was applied, with higher 35% volume compression giving higher $T_{\mathrm{stimulation}}$ than 25% compression.

In the following studies, we specifically chose CL values shorter than T_{nat} to see if and how resonant oscillation of BZ gels could happen in this parameter range (see Table S2 in the SI for combinations). Another type of resonance emerged in such systems when the hydrogel's inherent oscillation synchronised to a multiple of CL, i.e. to a harmonic of it, denoted as n×CL harmonic resonance, see Fig. 1 (B) for an example where the BZ hydrogel with $T_{\mathrm{nat}} = 211 \pm 11$ s was stimulated with CL=2 min (120 s). It shows that the gel inherent oscillation period increased so significantly that it could reach 3–4 times CL, eventually stabilising to produce 3×CL harmonic resonance with $T_{\mathrm{stimulation}} = 362 \pm 5$ s towards the end of the stimulation phase.

Finally, when the BZ hydrogel samples were compressed with much longer cycle lengths than the natural oscillation period, we also abundantly found the type of (1/n)×CL harmonic resonance, i.e. $T_{\mathrm{stimulation}}$ synchronised to an integer number ratio of CL, while still displaying the characteristic amplitude increase of resonance. Figure 1 (C) shows one example time series where (1/4)×CL resonance (n=4) was achieved. Owing to the fact that compression itself would always slow down the oscillation and increase the period, we found that the system was able to reach stable 1/n×CL resonance patterns where $n = \{2, 3, 4, 5, 6\}$ full oscillation periods were completed during one CL.

Phase diagram for resonant behaviour. The types of resonances mentioned so far – fundamental frequency, n×CL and (1/n)×CL types – were the three possible manifestations of resonance, and we referred to the second and third types collectively as harmonic resonance behaviours. In Fig. 2 all of the obtained oscillation periods during the stimulation phase (extracted from peak-to-peak analysis, as detailed in (16)) are plotted as coloured dots with error bars, with colours assigned according to the catalyst-free BZ solution concentrations in the C1–C4 range. Data points were arranged into coordinates according to their reference oscillation period T_{nat} along the x-axis and the stimulated $T_{\mathrm{stimulation}}$ along the y-axis, in both cases normalised by the corresponding, applied cycle length CL (a fixed constant parameter throughout one measurement) to enable comparison across all various experimental conditions. In order to aid the interpretation of the

results which appeared to all sit generally on a line, all possible fundamental and harmonic $T_{\text{stimulation}}/CL$ ratios were drawn with dashed horizontal lines, moreover, the $y = x$ curve, i.e., T_{nat}=$T_{\text{stimulation}}$ here, was also included for navigation.

First, we found in Fig. 2 that sample points only deviated from the T_{nat}=$T_{\text{stimulation}}$ line in the positive direction which indicated that cyclic compressions always caused the hydrogel to slow down its inherent oscillation (or occasionally if too long CL was applied, compressions had no notable effect on the hydrogel's oscillation period and it stayed around T_{nat}, as observable for some points in the bottom left corner of the diagram). For example, starting from an initial $T_{\text{nat}}/CL \approx 0.7$ ratio, the oscillation period increased so that it achieved a $T_{\text{stimulation}}/CL \approx 1$ ratio during stimulation, meaning fundamental frequency resonance. Further data points that were sitting above the T_{nat}=$T_{\text{stimulation}}$ line and above $T_{\text{stimulation}}/CL$=1 were those samples that showed n×CL type harmonic resonance, and similarly data points above the T_{nat}=$T_{\text{stimulation}}$ line, and under $T_{\text{stimulation}}/CL$=1 belonged to (1/n)×$CL$ type harmonic resonance.

As a main discovery of the resonance of self-oscillating gels based on harmonics, we found that in Fig. 2 sample points of resonant oscillation periods systematically sat very close to the navigatory dotted lines along the y-axis, i.e. specific integer $T_{\text{stimulation}}/CL$ ratios, confirming the discrete nature of the resonance when the self-oscillating gels were compressed by rhythmic mechanical oscillation. The type of resonance that could arise was primarily determined by the simple ratio of T_{nat} and CL, and concerning all three resonance types, the relationship between the resonant oscillation periods and the external mechanical frequencies can be fully summarised quantitatively. Fundamental frequency resonance could only emerge if T_{nat}/CL<1, specifically we found that samples possessing ratios approximately in the 0.7<T_{nat}/CL<0.9 interval were successful. When the system started from a T_{nat}/CL>1 ratio, given that the hydrogel's oscillation period could only increase due to compression, the hydrogel had the possibility to achieve even as high as $T_{\text{stimulation}}/CL$=2 or 3 ratios during stimulation and find n×CL harmonic resonance. However, we point it out here that the initial T_{nat}/CL>1 ratio could not be arbitrarily higher than 1 because above a certain ratio self-oscillation could be completely suppressed and paused by too frequent compressions, therefore the eventual relationship was determined as approximately 1<T_{nat}/CL<~2 for n×CL resonance. Finally, we found that (1/n)×CL harmonic resonance could emerge in the ~0.12<T_{nat}/CL<~0.6 region.

Post-stimulation behaviour and relaxation. Looking back at the study of Nitsan et al. (2), they applied mechanical stimulation to living cardiac cells in the form of cyclic oscillation and found complex resonance behaviours (fundamental frequency and bursting harmonics) in the cells' beating rhythm. Even more importantly, they also found conditions where the beating frequency of the heart cell became entrained to the stimulation frequency in a sustained manner, meaning that the cell kept beating at the modified frequency for a while even after the mechanical stimulation was stopped. However, the function of reprogramming, relaxation process to its natural oscillation after being entrained is not explored.

So, some biological mechanism would be switched to change the calcium oscillation affecting the RvR-ATP cycle in sarcoplasmic reticulum. We specifically designed our cyclic mechanical compression experiments for BZ hydrogels to include the aforementioned post-stimulation phase, where data acquisition was still continued for further two hours after compressions were removed, in order to find out if any similar sustained effects could emerge in BZ hydrogels.

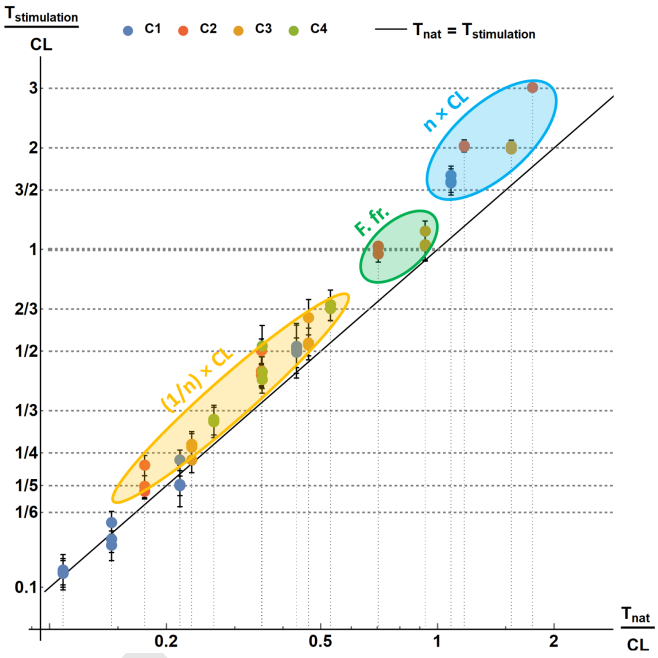

Fig. 2. Quantitative phase diagram summarising all resonance results obtained in BZ hydrogels during the stimulation phase. Both the natural oscillation periods T_{nat} and the stimulated oscillation periods $T_{\text{stimulation}}$ of the hydrogels are normalised by the corresponding CL cycle lengths, for better comparison across various experimental conditions. Dots with error bars correspond to extracted T values, coloured acccording to the C1–C4 reagent concentrations (see SI), with vertical dotted lines marking their x-axis positions for easier interpretation of the governing T_{nat}/CL ratios. Horizontal dashed grey lines have been drawn to indicate the fundamental 1 ratio and all the possible harmonics achieved in our experiments. The black line indicated the y=x line, in this case the T_{nat}=$T_{\text{stimulation}}$ line for navigation. Coloured ovals indicate the approximate regions where certain resonance behaviours emerged: F.fr = Fundamental frequency resonance; n×CL and (1/n)×CL harmonic resonances.

Figure 3 plots example chemical oscillation period (T, black) and amplitude (A, blue) values that were directly extracted from the time series presented earlier in Fig. 1 for (1/4)×CL harmonic resonance: as shown in Fig. 3 part (A), both parameters increased significantly and then stabilised during stimulation, as a direct consequence of cyclic compressions. Following this, in the first 30 minutes of post-stimulation they both remained at their entrained high levels, then went through a relaxation process, i.e., decreasing to approximately their natural unstimulated levels. Such entrainment and the following relaxation process were further confirmed by plotting the return map of the post-stimulation T and A values in Fig. 3 (B): in case of T, values still lingered around the entrained $T_{\text{stimulation}}/CL$=1/4 level for a while, before decreasing to T_{nat}/CL; concurrently, A showed the same, parallel relaxation process, signalling a strong connection between the two parameters due to mechanical stimulation effects.

Theoretical model simulations. In a previous publication (16), we proposed a theoretical model based on the original work of Yashin *et al.* (17, 18) for describing chemomechanical oscil-

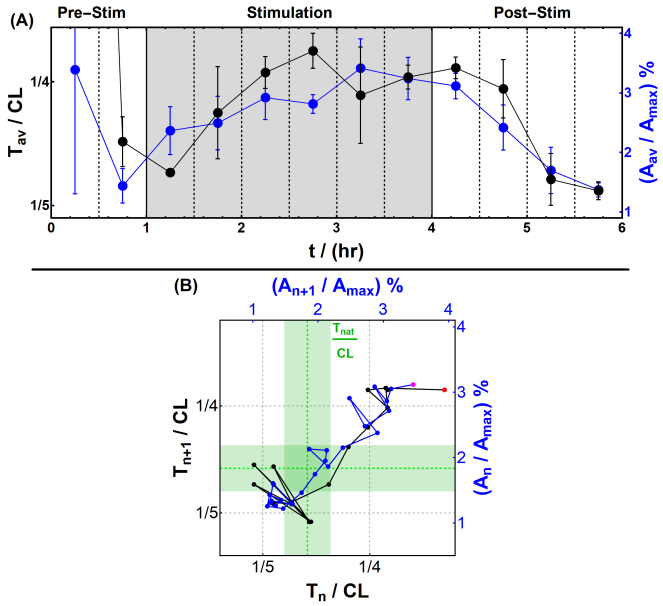

Fig. 3. An example of change in the amplitude and oscillation period of the BZ hydrogel's chemical oscillation throughout the entire 6-hour length of a mechanical stimulation experiment, comprised of 1-hour pre-stimulation, 3-hour stimulation and 2-hour post-stimulation phases, corresponding directly to the (1/4)×CL harmonic resonance time series presented earlier in Fig. 1 (C). (A) Normalised chemical oscillation period $T_{\rm av}/CL$ (black) and normalised chemical amplitude percentages $A_{\rm av}/A_{\rm max}$ (blue), averaged and plotted for each 30 min interval of the experiment to reveal trends over time. (B) Return map of the post-stimulation phase, showing the normalised oscillation period values (T/CL, black) and the normalised chemical amplitude percentages ($A/A_{\rm max}$, blue), to illustrate simultaneous relaxation in both parameters (red and pink dots denote first period and amplitude values, respectively, in the post-stimulation phase right after mechanical stimulation stops). Additional dashed lines mark the possible resonant T/CL ratios to which period synchronisation is possible. The reference $T_{\rm nat}/CL$ value with its uncertainty is also drawn as a green dashed lines and darker green regions.

lation behaviours of BZ hydrogels in the absence of external force. Instead of assuming an instantaneous equilibration of the osmotic pressure in the gel system (17, 18), we phenomenologically took into account the effects of reactant diffusion and solvent migration in and out of the gel region which can cause a phase difference between the mechanical and chemical oscillations. Our modified model was able to qualitatively describe the delayed mechanical response of BZ gels to chemical kinetics.(16) Here we extend this model to simulate the chemo-mechanical behaviours of BZ gels under external stimulation. The model details and parameter values can be found in SI. We note that the BZ gels studied in the theoretical model need to be sufficiently small, e.g. with side lengths comparable to smaller than the cross-section dimension (around $1mm$) of our experimental samples, for them to undergo uniform swelling-deswelling. Despite lack of chemical wave propagation, the model simulation results can help understanding the stimulation-induced synchornisation behaviours observed in our experiments (e.g., Fig.1).

Figure 4(a) presents the model simulation results on the time series of the dimensionless concentrations of reagent in solution, $u(t)$, and oxidised catalyst grafted to the polymer backbones, $v(t)$, and also the gel cross-section area measured by the squared transverse deformation factor $\lambda_\perp(t)^2$ for a model BZ hydrogel with natural oscillation period $T_{v,nat} \approx 695.4s$. Similar to experiments, the external mechanical stimulation was applied cyclically with one-minute constant compression and $CL-1$ minutes of release. Here CL was taken to be 12 min (720s) which is slightly higher than $T_{v,nat}$. The compression led to a 35% reduction in the average gel thickness, corresponding to a deformation factor of $\lambda_\parallel = 0.65$ in the compression direction. The chemical oscillation periods, T_v and T_u, were measured as the time intervals between the adjacent peaks in the $v(t)$ and $u(t)$ curves, as shown in Fig. 4(b). Figures 4(c,d) provide two more sets of T_v and T_u results obtained from simulations using stimulation cycle lengths CL= 4 min (c) and 35 min (d), respectively. In all three cases, the chemical oscillations in the hydrogel show synchronisation with external mechanical oscillation. More specifically, the average chemical oscillation periods during stimulation, $T_{\rm v(u),stimulation}$, demonstrate the fundamental ($\sim CL$), $3\times CL$ (1-to-3) and $(1/3)\times CL$ (3-to-1) harmonic synchronisation, which are qualitatively consistent with our experimental observations. Further simulation results can be found in Fig.S7 of SI for the $T_{\rm v,stimulation}/CL \approx 2$, 1/2 and 1/4 harmonic synchronisation. On the other hand, we have run extra simulations by assuming instantaneous equilibration of osmotic pressure in the system (17, 18), the so-obtained $T_{v(u)}$ results did not clearly manifest the harmonic synchronisation behaviour, see Fig.S8 of SI.

Our theoretical model calculations thus reveal the important role of the reactant diffusion and solvent migration processes in affecting the chemomechanical coupling in the BZ hydrogels and consequently leading to their resonance or synchronisation behaviours under external stimulation. This finding is consistent with the discovery of Yashin *et al.* in their gel lattice spring model simulations of BZ gel patches separated by neutral polymer network immersed in solutions that allowing diffusion of reaction activators (u) from the gel patches into outer solution can produce the experimentally observed oscillation synchronisation among these patches (19).

We note that in the $(1/n)\times CL$ synchronisation cases, only simulation results obtained at $n=2$ show relatively constant oscillation periods during stimulation, see Fig.S7(c,d). When $n \geq 3$ (e.g., in Figs. 4(d) and S7(f)), $T_{v(u)}$ within each stimulation cycle started with high values following the sudden compression and then gradually decreased towards $T_{v,nat}$ until the onset of next compression. It reflects that the impact of the external compression is decaying with time in the relatively long releasing interval ($(CL-1)min \gg T_{v,nat}$) and the system gradually recovered its unperturbed state. This memory losing effect is stronger in the simulation systems than that observed in experiments where the $(1/5)\times CL$ synchronisation or resonance can still be obtained. One possible reason is the simplified theoretical treatment of the reactant diffusion and solvent migration effect by using a single characteristic relaxation time (see Eq.(9) in SI). Other factors may also include some internal structural (e.g., physical cross-linking) and volumeric changes in the experimental samples which are not incorporated in the theoretical model we used. The same reasons can also explain the absence of the sustained post-stimulation entrainment behaviours in the simulation results. In addition, the experimentally observed increase in the oscillation amplitude during stimulation (resonant mode) is not so significant in the time series obtained in simulations, which may imply that the compression-induced gel volume change

assumed in the model calculations was smaller than the actual change in the experimental samples. Further understanding of the microscopic mechanisms underlying the experimental observations is thus still needed for developing more quantitative theoretical and computational models for describing the dynamic behaviours of BZ gel systems of various sizes and geometric shapes, including those studied in our experiments where the larger BZ gel samples undergo chemomechanical wave propagation. This will benefit from multiscale computer simulations using a bottom-up approach.

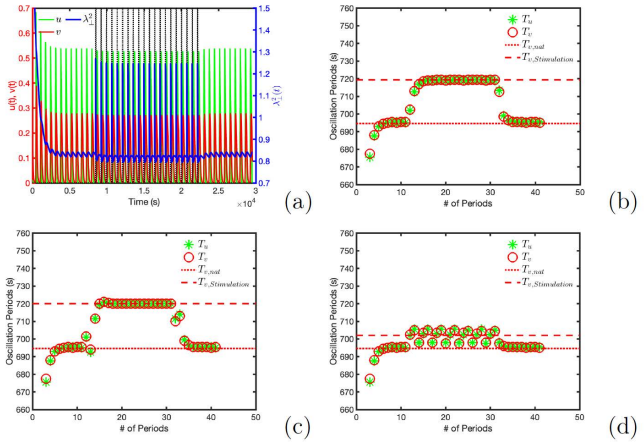

Fig. 4. (a) Theoretical model simulation results on the time series of dimensionless concentrations of reagent in solution, $u(t)$, and oxidised metal-ion catalyst grafted to polymer backbones , $v(t)$, and the gel cross-section area measured by the squared transverse deformation factor $\lambda_{\perp}(t)^2$ of a model BZ hydrogel with natural oscillation period $T_{v,nat} \approx 695.4s$. The applied stimulation cycle length is CL=12 min (720s); (b-d) Chemical oscillation periods T_v and T_u of the same BZ gel as studied in (a). The stimulation cycle lengths are CL=12 min (720s) (b), 4 min (240s) (c) and 35 min (2100s) (d), respectively. In all cases, the stimulation cycle consists of one-minute constant compression (marked by vertical dashed lines) and $CL - 1$ minutes of release. The compression leads to a reduction of 35% of the average thickness of the gel sample, corresponding to the deformation factor $\lambda_{\parallel} = 0.65$.

Discussions. We have shown that the inherent oscillation of the hydrogel can be regulated via multiple modes of stimulation application, including fundamental and harmonic modes even after the removal of the external mechanical oscillation. The simultaneous analysis of the stimulation and post-stimulation behaviours clearly revealed that any significant impact of the mechanical compressions – e.g. oscillation period and amplitude increase/decrease – could only manifest gradually in the hydrogel, and never abruptly. Our results of entrainment, harmonics and memory subject to the relaxation process strongly indicate a complex underlying mechanism behind those functions that went above mere time-based interference between the frequencies of the hydrogel and the mechanical stimulation. Note that the combination of resonance during stimulation and the corresponding relaxation process post-stimulation was termed 'entrainment' in our study.

In a prior study Shiota et al. observed fundamental-type synchronisation in isotropic BZ gel samples (20). Since in their experments the compression always appeared to coincide with the hydrogel being in reduced state and then oxidation peaks seemed to emerge immediately after realease, they speculated that the compression significantly reduced the gel volume and excluded crucial BZ reactants, e.g., BrO_3^-, into the outer solution. The lower concentration of reactants decreased the reaction rates and maintained the gel in reduced state. Their focus is on the regime of synchronisation under the cyclic compression, in our study, we opened up the paradigm of self-oscillating gels towards the memory functions after the removal of external stimulation, thus, some new mechanisms need to be explored to discuss the universal mechanism in cell biology.

Resonance itself by an external oscillation is not surprising: e.g. when applying mechanical oscillation to a mass-damper-spring setup, there will be resonance based on the natural, inherent frequency of the system. However, this is a passive system, needless to say, it does not produce any mechanical forces spontaneously. For active systems, living or non-living, if the system continues to oscillate at the resonant frequency after the removal of the external oscillation, this opens up a new venue as an open non-equilibrium system which has a memory function to remember the environmental information.

In the beating heart, million cardiomyocytes contract in synchronisation, generating contractile wave fronts that propagate through a whole organ. Coordinating this wave front requires fast and robust signalling mechanisms between cells. The primary signalling mechanism has long been identified as chemical communication between cells: gap junctions conduct calcium ions, triggering membrane depolarisation, intracellular calcium release, and actomyosin contraction. Generally, this has been understood as one directional chemical-to-mechanical interactions. Recently, it was found that external mechanical oscillation can modify the calcium oscillation within the cell (2). Chemo-mechanical coupling as a form of cell-to-cell communication can thus be a key candidate to explain the robust heartbeat against perturbations.

Our results showed that it is possible to "reprogram" the inherent chemical oscillation by an external mechanical oscillation in a fundamentally simple physical-chemical system, providing fascinating parallels with those results obtained by Nitsan et al. for cardiac cells stimulated by and synchronising to a mechanical probe in (2), where also a wide range of cell-to-probe frequency ratios could produce interactions in the heart cell's behaviour and reliably regulate its beating rate. However, the memory function of entrainment was not exhibited after the removal of the external fields, which is necessary to reprogram the chemical oscillations repeatedly. Using the artificial active matter, we showed the relaxation process into the natural and original frequency for the first time, indicating that the self-oscillating gels can be reprogrammed repeatedly to different oscillation frequencies.

To explain the synchronisation and entrainment in chemo-mechanical coupling found in the cardiomyocytes (2), Cohen and Safran theoretically, based on nonlinear oscillator subject to external oscillations, found that transitions from spontaneous beating to dynamical entrainment of cardiomyocytes induced by the mechanical oscillation (21). The possible scenario is that mechanical force is coupled to acto-myosin, which is sequentially coupled to calcium concentration. Te mechanical pacing releases calcium normally bound to actin back into the cytosol, efectively changing the calcium concentration. In summary, their scenario involves cell contractility as a necessary mediator in entraining calcium oscillations.

Furthermore, they proposed that, in the early embryonic heart tube, the signaling mechanism coordinating beats is me-

chanical rather than electrical, presenting a simple biophysical model in which CMs are mechanically excitable inclusions embedded within the extracellular matrix (ECM), modeled as an elastic-fluid biphasic material (22). However, their theoretical models did not show the entrainment after the mechanical oscillation was removed. Thus, how the entrained calcium oscillation can 'remember' the entrained oscillation, the mechanisms of relaxation process has not been studied.

Broadly speaking, there is a mounting body of evidence that physical forces induce biochemical changes. The early embryonic heart provides illustration of the importance of mechanics in living matter; embryonic hearts use mechanical signaling through the heart. Chiou et. al. modelled the embryonic heart as mechanically excitable tissue, with cardiac myocytes that are triggered to contract under strain (23).

In the field of cell biology and soft active matter, all of the experimental results and theoretical models did not exhibit the memory function of entrainment after the removal of the external fields. Thus, our experimental and theoretical results using the simple artificial system, self-oscillating hydrogel in which a set of chemical species and polymer networks are coupled through chemical reaction and osmotic pressure can be a milestone to understand the universal mechanisms of entrainment and memory necessary constituents for 'reprogramming', bridging soft active matter with biology.

Regarding the mechanisms underlying resonant chemomechanical oscillation in BZ hydrogels, our theoretical model calculations demonstrate that the diffusion of reactants, and also poroelastic effect caused by migration of solvent in larger BZ gels, leading to the delayed mechanical response, are playing an important role in synchronising the chemical and mechanical oscillations. The abrupt application and release of the external compression cause fast gel volume and polymer density changes, inducing flux of solvents and contained reactant in and out of the gel phase. It is followed by a much slower diffusion process of reactants and ions for recovering their equilibrium distributions. The redistribution of the reactants will affect the chemical kinetics, which can be modelled by diffusion-reaction equations, and consequently the mechanical response via chemomechanical coupling described by the Oregonator model. These coupled processes construct a feedback loop to synchronise the oscillation frequencies of the BZ gels with the external stimulation. The reason behind the long-lasting post-stimulation memory or entrainment phenomenon still needs further exploration. It may be related to some slow relaxing volumetric or internal structural changes, such as slow releasing of physical cross-linking formed under compression.

Our study of entrainment can be also associated with 'anticipation' as a form of intelligence in primitive organisms (24). Slime mold as a model species changed its metabolic cycle against an environmental dry-wet cycle: via applying a new frequency of dry-wet cycles, the motion of the slime mold became resonant with it, and still continued at its modified frequency even after the removal of the external cycle. Thus this simple organism exhibited the phenomenon of the entrainment, by spontaneously changing its motion in anticipation of the environmental stimulus even when it wasn't applied again. Sigusa proposed that the organism was able to remember periodic changes that it had not experienced before, indicating that the organism had a generalised capacity for learning.

On a larger scale, when the Ancient Egyptians recognised the regular periodicity of the flooding of the river Nile and succeeded in anticipating the next flood, this led to the invention of the calendar as a symbol of the dawn of civilisation. Thus, entrainment to an external cycle can be considered as anticipation where an intelligent agent predicts the next step of the environment, and hence prepare for it. It is thus remarkable that such a simple, non-living system as BZ hydrogels could show "anticipation".

Conclusions. We investigated the effect of cyclic mechanical stimulation by compression on BZ hydrogels that developed propagating chemomechanical waves. Determined by the ratio of the hydrogel's inherent oscillation period and the stimulation cycle length, it was possible to find resonance, either with the stimulation's fundamental frequency or an n$\times$ or (1/n)$\times$ harmonic of it. Moreover, we consistently found that when the inherent oscillation of the gel had entered into resonance during stimulation, the system kept a "memory" of the resonant oscillation period and maintained it at least for a short while post-stimulation, before relaxing back to its natural state, thus achieving full entrainment. Our theoretical model calculations with consideration of the reactant diffusion and solvent migration effect to chemomechanical coupling in BZ hydrogels are able to produce the stimulation-induced fundamental and harmonic synchronisation behaviours. Our findings help bridge the functions of biological systems with nonequilibrium chemical physics and pave the pathway to study the complicated biological problems using simpler bio-mimicking chemophysical systems.

Materials and Methods

Self-oscillating hydrogel samples were prepared with 10% relative catalyst concentration, following the newer two-step procedure developed by Masuda *et al.*(25), later optimised for our experiments in (16) (see the SI for methods and materials in detail). Hydrogels were first synthesised as a bisacrylamide cross-linked poly(NIPAAm-co-NAPMAm) gel (N–isopropylacrylamide and N–3–(aminopropyl)methacrylamide monomers, respectively); then to the NAPMAm groups a tris bipyridine ruthenium complex was conjugated covalently, fully saturating the polymer mesh. For each experiment, three pieces of approx. 1 mm$\times$1 mm$\times$10 mm size quasi-1D BZ gels were cut, and immersed in the catalyst-free BZ solution containing sodium bromate, malonic acid and nitric acid, which were used in four different concentrations C1–C4 (see Table S1 in the SI for exact values). Gels of the described size were already large enough in one spatial dimension to develop propagating chemomechanical waves (whereas <1 mm gel pieces display homogeneous, isotropic oscillation only).

Experiments were performed at 20$\pm$0.2°C constant temperature, following the same data collection procedure as detailed in (16). Self-oscillation was recorded using a USB microscope microscope in the form of time lapse image sequences, which enabled the following of chemical oscillation via colour changes in the hydrogel, and mechanical oscillation via size changes of the sample. These changes could be extracted from image parameters for each recorded time point, then averaged and plotted to reconstruct time series such as one ones in Fig. 1. See Fig. S1 and S4 in the supplementary information for more details about the image analysis method, as well as the process for determining crucial oscillation period and amplitude values from time series.

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