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

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1 **Smart polymer materials that are non-living yet exhibit complex “life-**
2 **like” or biomimetic behaviours have been the focus of intensive re-**
3 **search over the past decades, in the quest to broaden our under-**
4 **standing of how living systems function under nonequilibrium con-**
5 **ditions. Discovery of how chemical and mechanical coupling can**
6 **generate resonance and entrainment with other cells or external en-**
7 **vironment is an important research question. We prepared Belousov-**
8 **Zhabotinsky (BZ) self-oscillating hydrogels which convert chemical**
9 **energy to mechanical oscillation. By cyclically applying external me-**
10 **chanical stimulation to the BZ hydrogels, we found that when the**
11 **oscillation of a gel sample entered into harmonic resonance with the**
12 **applied oscillation during stimulation, the system kept a “memory”**
13 **of the resonant oscillation period and maintained it post stimulation,**
14 **demonstrating an entrainment effect. More surprisingly, by system-**
15 **atically varying the cycle length of the external stimulation, we re-**
16 **vealed the discrete nature of the stimulation-induced resonance and**
17 **entrainment behaviours in chemical oscillations of BZ hydrogels, i.e.,**
18 **the hydrogels slow down their oscillation periods to the harmonics**
19 **of the cycle length of the external mechanical stimulation. Our the-**
20 **oretical model calculations suggest the important roles of the de-**
21 **layed mechanical response caused by reactant diffusion and solvent**
22 **migration in affecting the chemomechanical coupling in active hy-**
23 **drogels and consequently synchronising their chemical oscillations**
24 **with external mechanical oscillations.**

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

1 **S**ynchronisation of oscillations is abundant in nature from
2 physical, chemical to biological systems. Oscillations are
3 also found in various biological systems and can operate at the
4 molecular level (e.g. cardiac cell beating) and at the organism
5 level (e.g. sleep–wake cycles). When two oscillating systems
6 interact, their oscillations can be tuned to the same frequency
7 with a certain phase difference (1). This kind of entrainment
8 process serves a basis for synchronisation. From genes, body
9 and cell physiology, to our daily routines, activities are influ-
10 enced by the day-and-night cycle, e.g., transcription of genes,
11 protein synthesis and repair of tissues are fundamentally en-
12 trained to the rhythm of the sunlight cycle. At the cellular
13 level, synchronisation of electro-chemical oscillation can occur
14 through interactions between single cells and with their envi-
15 ronment. For example, it is well established that calcium wave
16 across the heart generates the mechanical **heartbeat** as a single
17 organ, i.e., individual heart cells synchronously contract in
18 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 inter-
action between different thermodynamic forces, i.e., chemical-
mechanical coupling, to produce stable synchronisation be-
tween 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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38 be induced by a variety of modalities, mechanical, chemical
39 and electrical coupling between two systems. It was originally
40 demonstrated using two pendulum clocks coupled through
41 a wooden structure (1). Synchronisation in this system was
42 achieved via mechanical vibrations through the wooden cou-
43 pling bar. In physical chemical systems, aqueous drops con-
44 taining Belousov-Zhabotinsky (BZ) solutions were shown to
45 have a variety of synchronous regimes of chemical reaction,
46 including in- and anti-phase oscillations, and stationary Turing
47 patterns (3).

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

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

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

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

98 To the best of our knowledge, this is a first study demon-

99 strating that internal chemical oscillations in physical chemical
100 systems can be truly "reprogrammed" by applying external
101 mechanical stimulation. Such reprogramming can be realised
102 not only during the stimulation via synchronisation, but more
103 promisingly also post stimulation via sustained 'entrainment',
104 leading to the relaxation process.

105 Results and Discussion of BZ hydrogel experiments

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

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

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

157 We observed multiple modes of resonance in every C1–

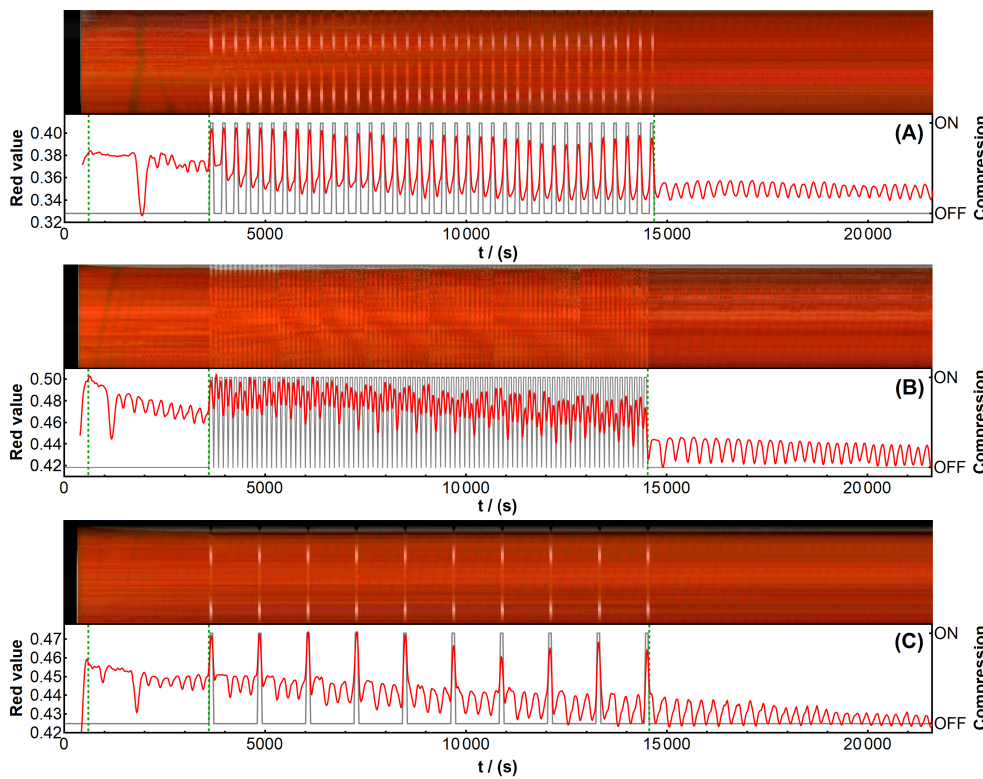


Fig. 1. Six-hour chemical oscillation time series of BZ hydrogel samples, illustrating the three possible types of resonance during mechanical stimulation. (A) Fundamental frequency resonance (1-to-1 synchronisation): catalyst-free BZ solution concentration: C2 (see SI); reference $T_{\text{nat}}=211 \pm 11$ s; $CL=5$ min. (B) $n \times CL$ harmonic resonance (1-to-3 synchronisation here): catalyst-free BZ solution concentration: C2 (see SI); reference $T_{\text{nat}}=211 \pm 11$ s; $CL=2$ min. (C) $(1/n) \times CL$ harmonic resonance (4-to-1 synchronisation here): catalyst-free BZ solution concentration: C3 (see SI); reference $T_{\text{nat}}=279 \pm 11$ 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.

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

163 In Fig. 1 (A), when $CL=5$ min (300 s) stimulation was
 164 applied to a gel with natural oscillation period $T_{\text{nat}}=211 \pm$
 165 11 s, the chemical oscillation was observed to slow down to
 166 $T_{\text{stimulation}} = 306 \pm 9$ s, thus achieving a $T_{\text{stimulation}}/CL \approx 1$
 167 ratio. The gel could enter into a resonant mode which was
 168 characterised by higher oscillation amplitudes. Since the syn-
 169 chronisation and resonance took place in a 1-to-1 manner with
 170 the stimulation CL , this type of interaction was termed "fundam-
 171 ental frequency" resonance. We observed that fundamental
 172 frequency resonance could only emerge when some particular
 173 conditions were met, especially that T_{nat} needs to be suitably
 174 smaller than CL so that the gel’s natural oscillation period
 175 could increase during stimulation and reach the required level
 176 for resonance. Crucially, it was observed in our entire study
 177 that cyclic mechanical compression always caused the hydrogel’s
 178 inherent oscillation to slow down, it could never prompt
 179 it to become faster. Furthermore, the amount of T increment
 180 was found to depend on how much compressing force was
 181 applied, with higher 35% volume compression giving higher
 182 $T_{\text{stimulation}}$ than 25% compression.

183 In the following studies, we specifically chose CL values
 184 shorter than T_{nat} to see if and how resonant oscillation of BZ
 185 gels could happen in this parameter range (see Table S2 in the
 186 SI for combinations). Another type of resonance emerged in
 187 such systems when the hydrogel’s inherent oscillation synchron-
 188 ised to a multiple of CL , i.e. to a harmonic of it, denoted
 189 as $n \times CL$ harmonic resonance, see Fig. 1 (B) for an example
 190 where the BZ hydrogel with $T_{\text{nat}} = 211 \pm 11$ s was stimulated

with $CL=2$ min (120 s). It shows that the gel inherent oscillation
 191 period increased so significantly that it could reach 3–4
 192 times CL , eventually stabilising to produce $3 \times CL$ harmonic
 193 resonance with $T_{\text{stimulation}} = 362 \pm 5$ s towards the end of the
 194 stimulation phase.

195 Finally, when the BZ hydrogel samples were compressed
 196 with much longer cycle lengths than the natural oscillation
 197 period, we also abundantly found the type of $(1/n) \times CL$ har-
 198 monic resonance, i.e. $T_{\text{stimulation}}$ synchronised to an integer
 199 number ratio of CL , while still displaying the characteristic
 200 amplitude increase of resonance. Figure 1 (C) shows one exam-
 201 ple time series where $(1/4) \times CL$ resonance ($n=4$) was achieved.
 202 Owing to the fact that compression itself would always slow
 203 down the oscillation and increase the period, we found that the
 204 system was able to reach stable $1/n \times CL$ resonance patterns
 205 where $n = \{2, 3, 4, 5, 6\}$ full oscillation periods were completed
 206 during one CL .
 207

Phase diagram for resonant behaviour. The types of resonances
 208 mentioned so far – fundamental frequency, $n \times CL$ and
 209 $(1/n) \times CL$ types – were the three possible manifestations of
 210 resonance, and we referred to the second and third types col-
 211 lectively as harmonic resonance behaviours. In Fig. 2 all of
 212 the obtained oscillation periods during the stimulation phase
 213 (extracted from peak-to-peak analysis, as detailed in (16))
 214 are plotted as coloured dots with error bars, with colours
 215 assigned according to the catalyst-free BZ solution concentra-
 216 tions in the C1–C4 range. Data points were arranged into
 217 coordinates according to their reference oscillation period T_{nat}
 218 along the x-axis and the stimulated $T_{\text{stimulation}}$ along the y-
 219 axis, in both cases normalised by the corresponding, applied
 220 cycle length CL (a fixed constant parameter throughout one
 221 measurement) to enable comparison across all various experi-
 222 mental conditions. In order to aid the interpretation of the
 223

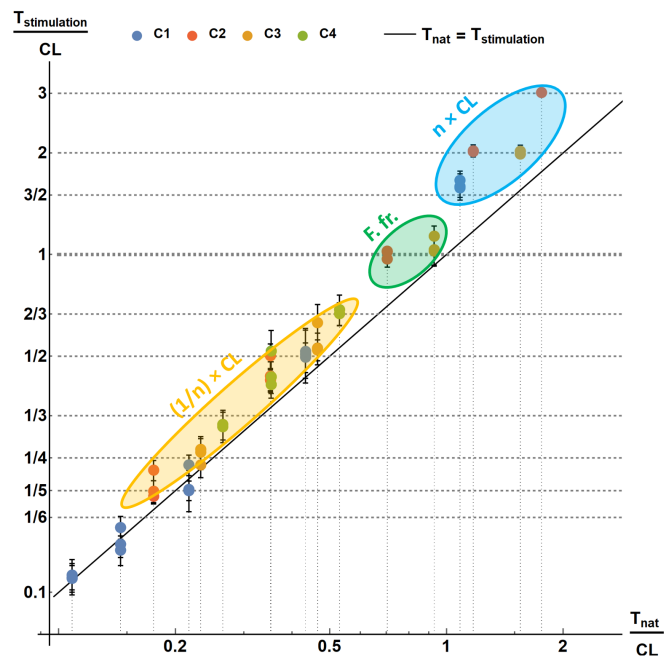
224 results which appeared to all sit generally on a line, all possible
 225 fundamental and harmonic $T_{\text{stimulation}}/CL$ ratios were drawn
 226 with dashed horizontal lines, moreover, the $y = x$ curve, i.e.,
 227 $T_{\text{nat}}=T_{\text{stimulation}}$ here, was also included for navigation.

228 First, we found in Fig. 2 that sample points only deviated
 229 from the $T_{\text{nat}}=T_{\text{stimulation}}$ line in the positive direction
 230 which indicated that cyclic compressions always caused the
 231 hydrogel to slow down its inherent oscillation (or occasion-
 232 ally if too long CL was applied, compressions had no no-
 233 table effect on the hydrogel's oscillation period and it stayed
 234 around T_{nat} , as observable for some points in the bottom
 235 left corner of the diagram). For example, starting from an
 236 initial $T_{\text{nat}}/CL \approx 0.7$ ratio, the oscillation period increased
 237 so that it achieved a $T_{\text{stimulation}}/CL \approx 1$ ratio during stimu-
 238 lation, meaning fundamental frequency resonance. Further
 239 data points that were sitting above the $T_{\text{nat}}=T_{\text{stimulation}}$
 240 line and above $T_{\text{stimulation}}/CL=1$ were those samples that showed
 241 $n \times CL$ type harmonic resonance, and similarly data points
 242 above the $T_{\text{nat}}=T_{\text{stimulation}}$ line, and under $T_{\text{stimulation}}/CL=1$
 243 belonged to $(1/n) \times CL$ type harmonic resonance.

244 As a main discovery of the resonance of self-oscillating gels
 245 based on harmonics, we found that in Fig. 2 sample points
 246 of resonant oscillation periods systematically sat very close
 247 to the navigatory dotted lines along the y-axis, i.e. specific
 248 integer $T_{\text{stimulation}}/CL$ ratios, confirming the discrete nature
 249 of the resonance when the self-oscillating gels were compressed
 250 by rhythmic mechanical oscillation. The type of resonance
 251 that could arise was primarily determined by the simple ratio
 252 of T_{nat} and CL , and concerning all three resonance types,
 253 the relationship between the resonant oscillation periods and
 254 the external mechanical frequencies can be fully summarised
 255 quantitatively. Fundamental frequency resonance could only
 256 emerge if $T_{\text{nat}}/CL < 1$, specifically we found that samples
 257 possessing ratios approximately in the $0.7 < T_{\text{nat}}/CL < 0.9$
 258 interval were successful. When the system started from a
 259 $T_{\text{nat}}/CL > 1$ ratio, given that the hydrogel's oscillation
 260 period could only increase due to compression, the hydrogel
 261 had the possibility to achieve even as high as $T_{\text{stimulation}}/CL=2$
 262 or 3 ratios during stimulation and find $n \times CL$ harmonic
 263 resonance. However, we point it out here that the initial
 264 $T_{\text{nat}}/CL > 1$ ratio could not be arbitrarily higher than 1
 265 because above a certain ratio self-oscillation could be
 266 completely suppressed and paused by too frequent
 267 compressions, therefore the eventual relationship was
 268 determined as approximately $1 < T_{\text{nat}}/CL < \sim 2$ for $n \times CL$
 269 resonance. Finally, we found that $(1/n) \times CL$ harmonic
 resonance could emerge in the $\sim 0.12 < T_{\text{nat}}/CL < \sim 0.6$ region.

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

282 So, some biological mechanism would be suitable to change
 283 the calcium oscillation affecting the RvR-ATP cycle in sar-
 284 coplasmic reticulum. We specifically designed our cyclic me-



285 **Fig. 2.** Quantitative phase diagram summarising all resonance results obtained in BZ
 286 hydrogels during the stimulation phase. Both the natural oscillation periods T_{nat} and
 287 the stimulated oscillation periods $T_{\text{stimulation}}$ of the hydrogels are normalised by the
 288 corresponding CL cycle lengths, for better comparison across various experimental
 289 conditions. Dots with error bars correspond to extracted T values, coloured according
 290 to the C1–C4 reagent concentrations (see SI), with vertical dotted lines marking their
 291 x-axis positions for easier interpretation of the governing T_{nat}/CL ratios. Horizontal
 292 dashed grey lines have been drawn to indicate the fundamental 1 ratio and all
 293 the possible harmonics achieved in our experiments. The black line indicated the
 294 $y=x$ line, in this case the $T_{\text{nat}}=T_{\text{stimulation}}$ line for navigation. Coloured ovals
 295 indicate the approximate regions where certain resonance behaviours emerged: F.fr.
 296 = Fundamental frequency resonance; $n \times CL$ and $(1/n) \times CL$ harmonic resonances.

297 chanical compression experiments for BZ hydrogels to include
 298 the aforementioned post-stimulation phase, where data acqui-
 299 sition was still continued for further two hours after compressions
 300 were removed, in order to find out if any similar sustained
 301 effects could emerge in BZ hydrogels.

302 **Figure 3** plots example chemical oscillation period (T , black)
 303 and amplitude (A , blue) values that were directly extracted
 304 from the time series presented earlier in Fig. 1 for $(1/4) \times CL$
 305 harmonic resonance: as shown in Fig. 3 part (A), both pa-
 306 rameters 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 approxi-
 mately 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 con-
 nection between the two parameters due to mechanical stimu-
 lation effects.

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

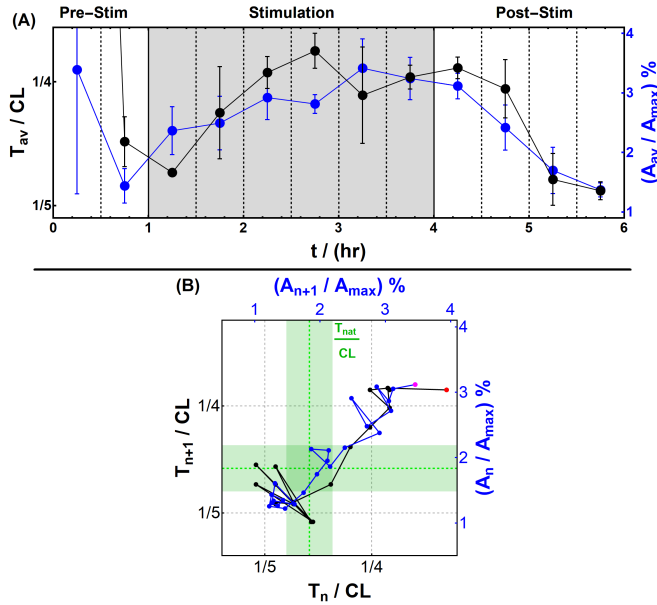


Fig. 3. An example of change in the amplitude and oscillation period of the BZ hydrogel's chemical oscillation throughout the entire 6-hour length of a mechanical stimulation experiment, comprised of 1-hour pre-stimulation, 3-hour stimulation and 2-hour post-stimulation phases, corresponding directly to the $(1/4) \times CL$ harmonic resonance time series presented earlier in Fig. 1 (C). (A) Normalised chemical oscillation period T_{av}/CL (black) and normalised chemical amplitude percentages A_{av}/A_{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_{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_{nat}/CL value with its uncertainty is also drawn as a green dashed lines and darker green regions.

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 $\lambda_{||} = 0.65$ 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, $T_{v(u),stimulation}$, 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 $T_{v,stimulation}/CL \approx 2, 1/2$ 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 volumetric 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

310 lation behaviours of BZ hydrogels in the absence of external
 311 force. Instead of assuming an instantaneous equilibration of
 312 the osmotic pressure in the gel system (17, 18), we phenomeno-
 313 logically took into account the effects of reactant diffusion and
 314 solvent migration in and out of the gel region which can
 315 cause a phase difference between the mechanical and chemical
 316 oscillations. Our modified model was able to qualitatively de-
 317 scribe the delayed mechanical response of BZ gels to chemical
 318 kinetics.(16) Here we extend this model to simulate the chemo-
 319 mechanical behaviours of BZ gels under external stimulation.
 320 The model details and parameter values can be found in SI.
 321 We note that the BZ gels studied in the theoretical model
 322 need to be sufficiently small, e.g. with side lengths comparable
 323 to smaller than the cross-section dimension (around 1mm)
 324 of our experimental samples, for them to undergo uniform
 325 swelling-deswelling. Despite lack of chemical wave propaga-
 326 tion, the model simulation results can help understanding the
 327 stimulation-induced synchronisation behaviours observed in
 328 our experiments (e.g., Fig.1).

329 Figure 4(a) presents the model simulation results on the
 330 time series of the dimensionless concentrations of reagent in
 331 solution, $u(t)$, and oxidised catalyst grafted to the polymer
 332 backbones, $v(t)$, and also the gel cross-section area measured by
 333 the squared transverse deformation factor $\lambda_{\perp}(t)^2$ for a model
 334 BZ hydrogel with natural oscillation period $T_{v,nat} \approx 695.4s$.

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

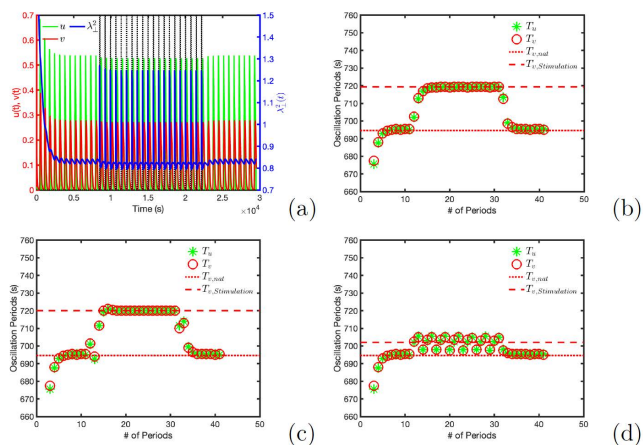


Fig. 4. (a) Theoretical model simulation results on the time series of dimensionless concentrations of reagent in solution, $u(t)$, and oxidised metal-ion catalyst grafted to polymer backbones, $v(t)$, and the gel cross-section area measured by the squared transverse deformation factor $\lambda_{\perp}(t)^2$ 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 experiments the compression always appeared to coincide with the hydrogel being in reduced state and then oxidation peaks seemed to emerge immediately after release, 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. The mechanical pacing releases calcium normally bound to actin back into the cytosol, effectively 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-

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

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

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

515 Regarding the mechanisms underlying resonant chemome-
516 chanical oscillation in BZ hydrogels, our theoretical model cal-
517 culations demonstrate that the diffusion of reactants, and also
518 proelastic effect caused by migration of solvent in larger BZ
519 gels, leading to the delayed mechanical response, are playing
520 an important role in synchronising the chemical and mechan-
521 ical oscillations. The abrupt application and release of the
522 external compression cause fast gel volume and polymer den-
523 sity changes, inducing flux of solvents and contained reactant
524 in and out of the gel phase. It is followed by a much slower
525 diffusion process of reactants and ions for recovering their
526 equilibrium distributions. The redistribution of the reactants
527 will affect the chemical kinetics, which can be modelled by
528 diffusion-reaction equations, and consequently the mechanical
529 response via chemomechanical coupling described by the Oreg-
530 onator model. These coupled processes construct a feedback
531 loop to synchronise the oscillation frequencies of the BZ gels
532 with the external stimulation. The reason behind the long-
533 lasting post-stimulation memory or entrainment phenomenon
534 still needs further exploration. It may be related to some slow
535 relaxing volumetric or internal structural changes, such as slow
536 releasing of physical cross-linking formed under compression.

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

550 On a larger scale, when the Ancient Egyptians recognised

the regular periodicity of the flooding of the river Nile and suc-
ceeded 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 antici-
pation 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 stimula-
tion 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 dur-
ing 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 calcu-
lations with consideration of the reactant diffusion and solvent
migration effect to chemomechanical coupling in BZ hydro-
gels are able to produce the stimulation-induced fundamental
and harmonic synchronisation behaviours. **Our findings help
bridge the functions of biological systems with nonequilib-
rium 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% rela-
tive catalyst concentration, following the newer two-step procedure
developed by Masuda *et al.* (25), later optimised for our experi-
ments 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 chemome-
chanical waves (whereas < 1 mm gel pieces display homogeneous,
isotropic oscillation only).

Experiments were performed at $20 \pm 0.2^\circ\text{C}$ 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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