Music for cells? A systematic review of studies investigating the effects of audible sound played through speaker-based systems to cell cultures.

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Abstract

There have been several studies investigating whether musical sound can be used as cell stimuli in recent years. We systematically searched publications to get an overview of studies that have used audible sound played through speaker-based systems to induce mechanical perturbation in cell cultures. A total of 12 studies were identified. We focused on the experimental setups, the sound materials used as stimuli, and the outcomes. The stimuli were categorized into simple and complex sounds. The effects were reported as enhanced cell migration, proliferation, colony formation, and differentiation ability. However, there are significant differences in methodologies and cell type-specific outcomes, which made it difficult to find a systematic pattern in the results. We suggest that future experiments should consider using: 1) a more controlled acoustic environment), 2) standardized sound and noise measurement methods, and 3) a more comprehensive range of controlled sound stimuli.

Introduction

Music for cells. Is there such a thing? The use of (musical) sound to induce a beneficial physiological and psychological effect on biological beings is probably not a novel idea. Already some of the Greek philosophers, such as Pythagoras, recognized the fascinating relationship between musical sound and the human body (1,2). With more advanced technological development, the efforts to attain a deeper understanding of the relationship between sound and body have become more specific, now at a minuscule level in the field of mechanobiology. Mechanobiology has been developing substantially in the last few decades with more than 33,000 publications in the field up until 2016 (3,4). More recently, there have

been escalating interests and efforts to understand mechanotransduction through mechanical cues induced by acoustical perturbation on cellular responses (5). Various cell types were investigated including: plant cells (6), micro-organisms (7), auditory cells (5) and other mammal cells *in vitro* (8) or *in vivo* (9). However, the cell types, experimental setups, sound measurement methods, and outcomes are widely dispersed in the literature. In this review, the aim is to get an overview of studies that have used audible sound played through speaker-based systems to induce mechanical perturbation in cell cultures (particularly bacteria and mammalian cells). The paper starts with a background to the mechanobiology of cells and some fundamental physical properties of sound. A review of the literature focuses on the experimental setups, the sound material used (categorized into simple and complex sounds), and the experimental outcomes.

Background

The mechanobiology of cells

Cells *in vivo* are endlessly perturbed by different types of mechanical forces such as tension, compression, and shear stress (10), and it has been suggested that cells are sensitive to physical forces (11). Mechanical signals, such as adhesiveness or extracellular matrix (ECM) stiffness, are also sensed by cells (12). Different types of mechanical cues instigate specific responses in cells where they activate certain genes through distinct signaling pathways (13). For instance, the Hippo pathway has been identified as conserved mechanical transducers, which is critical for driving stem cells fate and regeneration, translocate to the nucleus in cells that are subjected to a stiff surface (12,14). At the same time, its altered activity is involved in aberrant cell mechanics transduction and several diseases (15). Cells sense not only the

mechanical signals but also generate signals themselves. It has been reported that bacterial cells can generate sound waves between 8 and 43 kHz (16), yeast cells can generate mechanical vibrations (17), and stem cells have shown unique vibrational signatures to sound stimulation (18).

Sound as a mechanobiological stimulus

Sound is essentially vibration. The word *vibration* originates from the Latin word *vibrationem*, which means shaking. When an object is disturbed by mechanical stimulus and starts shaking and vibrating, sound energy is produced (19). This energy is propagated in the form of pressure waves in all directions from the sound source, usually as a longitudinal wave consisting of compressions and rarefactions of matter. In some cases, sounds in solid mediums can travel as a transverse wave, but also as a longitudinal wave. Such a wave consists of up and down motion perpendicular to the direction of the vibration. Sound waves can travel through different mediums, including gas, liquid, and solids, but with different speeds dependent on the medium. Typically, sound travels faster in a liquid or solid medium, for example, water or a steel bar, than in gas medium (20–23).

A medium's density impacts energy transmission when sound is travelling from one medium to another. This physical property of sound is a factor that needs attention in cellular experiments, in which sound energy must be transmitted from the air into a liquid-based solution, or another possible cell medium. For a progressive plane wave the physical property of a medium can be expressed as the acoustic *impedance*, defined in [equation 1](#).

85

$$Z = \rho c \quad (1)$$

86

87 In [equation 1](#), ρ is the density (kg/m^3) of a medium and c is the speed of sound (m/s) in the
 88 medium. At the interface between two media of mismatched acoustic impedance, part of the
 89 incident energy will not be transmitted but will be reflected back to the initial medium. The
 90 amount of acoustic energy that will undergo reflection at the interface between a medium of
 91 impedance Z_1 and a medium of impedance Z_2 can be evaluated by the acoustic energy
 92 reflection coefficient defined in [equation 2](#).

93

$$R = \left(\frac{Z_2 - Z_1}{Z_2 + Z_1} \right)^2 \quad (2)$$

94

95 For example, sound pressure waves travelling from the air at 20 °C ($Z_1 \approx 413 \text{ Pa}\cdot\text{s/m}$) will be
 96 reflected almost entirely ($R \approx 0.9989$) at the surface of the water ($Z_2 \approx 1.5 \cdot 10^6 \text{ Pa}\cdot\text{s/m}$).

97

98 The medium's thickness also impacts the transmitted energy. If the thickness of the second
 99 medium is much smaller than the wavelength of the incident sound, for instance, the amount
 100 of transmitted energy will be minimized (19).

101

102 Manipulatable sound parameters

103 When exploring the use of sound for experimental set up of cells, there are several properties
 104 to consider. Some of the basic parameters of sound include its frequency (what is experienced
 105 as *pitch*), amplitude (which corresponds to perceptual *loudness*), and exposure time.
 106 Additionally, there are different types of waveforms, from the most basic (sine tones) to more
 107 complex, synthetic waveforms (sawtooth, square, triangle), and even more complex, natural
 108 waveforms (noise, speech, music). With modern digital signal processing tools, all of these
 109 can be created and/or modified systematically in different ways using various types of
 110 synthesis techniques (additive, subtractive, frequency modulation, amplitude modulation,
 111 etc.) and a broad range of effects and processing techniques in both the time and frequency
 112 domains (24).

113

114 When it comes to basic descriptions of sounds, the sound frequency (Hz) is represented as the
 115 number of cycles or vibrations per second, defined in [equation 3](#).

116

$$f = \frac{1}{T} \text{ or } f = \frac{c}{\lambda} \quad (3)$$

117

118 In [equation 3](#), T is the period (s) which is the time taken for a vibrating motion to complete
 119 one cycle, λ is the wavelength (m) which is the distance between two successive matching
 120 points in any section of the sound wave cycle. When the period and wavelength increase, the
 121 frequency will decrease in a given material, but the speed of sound will remain the same

depending on the temperature, in the air, for instance. There is more energy in sound frequencies with a higher amplitude than in sound frequencies with a lower amplitude. Amplitude is the difference between maximum and minimum values of the pressure with respect to the equilibrium pressure, and has a direct influence on the sound pressure level (SPL; dB SPL) (25).

In the following, we will differentiate between “simple” and “complex” sound stimuli. Simple waves, such as a sine tone, are constant and repetitive, and can be produced by “any vibrating body executing simple harmonic motion”. The Fourier theorem tells that any complex sound can be broken down to a given number of sinusoidal components (fundamental and its harmonics) when the signal is periodic. Similarly, any periodic sound can be constructed using a set of sine tones using the Fourier transform. There are also other types such as sawtooth and triangle waves that can be constructed using an infinite set of sine tones (26). Complex waveforms, such as music and speech, are usually not repetitive (21).

Review method

In this review study, an exhaustive literature search was performed using the University of Oslo Library’s Oria search engine, PubMed, and Google Scholar. The keywords included in the search were: “sound”, “music”, “acoustic stimulation”, and “cell culture”. The combinations of words used for the search were: “sound stimulation on cell culture”, “music stimulation on cell culture” and “acoustic stimulation on cell culture”. Publications were collected between 1 July 2019 and 20 June 2020.

144

145 As illustrated in [Fig 1](#), a total of 95 records were identified through the database searching.

146 Of these, 7 records were removed as duplicates, 74 records were investigated but considered

147 not relevant and hence removed. This included studies which were not related to cell studies

148 *in vitro*, or not related to audible sound stimulation, or publications not in English. The full

149 texts of 2 records were unobtainable and were not considered either. This left us with 12

150 studies that were included for further qualitative consideration ([Table 1](#)).

151

152 **Fig 1. PRISMA flow diagram.**

153

154 **Table 1. List of included studies.**

Publication year	First author	Title	Source
2010	Shaobin	A pilot study of the effect of audible sound on the growth of Escherichia coli	<i>Colloids and Surfaces B: Biointerfaces</i> , 78(2), 367–371
2012	Aggio	Sonic vibration affects the metabolism of yeast cells growing in liquid culture: a metabolomic study	<i>Metabolomics</i> , 8(4), 670–678
2016	Mohammed	The effects of acoustic vibration on fibroblast cell migration	<i>Materials Science and Engineering: C</i> (69),

1256–1262

		Sound waves induce neural differentiation of	
		Human Bone Marrow-Derived	<i>Applied Biochemistry</i>
2016	Choi	Mesenchymal Stem Cells via ryanodine	<i>and Biotechnology,</i>
		receptor-induced calcium release and Pyk2	<i>180(4), 682–694</i>
		activation	
		Exposure to music alters cell viability and	<i>Evidence-Based</i>
		cell motility of	<i>Complementary and</i>
2016	Lestard	human nonauditory cells in culture	<i>Alternative Medicine,</i>
			<i>2016: 6849473, 1–7</i>
		Sound stimulation can influence microbial	<i>Journal of</i>
		growth and production of certain key	<i>Microbiology,</i>
2016	Shah	metabolites	<i>Biotechnology and</i>
			<i>Food Sciences, 9(5),</i>
			<i>330–334</i>
		Audible sound in form of music can	<i>Journal of Applied</i>
		influence microbial growth, metabolism and	<i>Biotechnology &</i>
2017	Sarvaiya	antibiotic susceptibility	<i>Bioengineering, 2(6),</i>
			<i>212–219</i>
		Cell melodies: when sound speaks to stem	
2017	Ventura	cells	<i>CellR4, 5(2): e2331</i>
		Effect of exposure to Quran recitation on	<i>Health, Spirituality</i>
2018	Mehrafsar	cell viability, cell migration, and BCL2L12	<i>and Medical Ethics,</i>
		gene expression of human prostate	<i>5(4), 46–52</i>

Adenocarcinoma cell line in culture

2018	Algieri	Effects of music playing on biological molecules	<i>MATEC Web of Conferences</i> , 210: 05006
2018	Kumeta	Cell type-specific suppression of mechanosensitive genes by audible sound stimulation	<i>PLOS ONE</i> , 13(1): e0188764
2018	Kothari	Influence of a mono-frequency sound on bacteria can be a function of the sound-level	<i>Indian Journal of Science and Technology</i> , 11(4), 1–9

155

156 **Results**

157 When looking broadly at the selected studies, the sound material used in the literature can be
158 equally divided into the two main sound types identified above:

- 159 ▪ simple sounds (e.g., sine wave): 6 studies
- 160 ▪ complex sounds (e.g., music and speech): 6 studies

161

162 We will now look more closely at each of these two types of studies, focusing on describing
163 the experimental setup, the sound stimuli used, and the outcomes.

164

165 Simple sound stimulation on cell cultures

166 In all six studies identified within the category simple sound stimulus, a sine wave with a
167 constant SPL was typically used in various experimental settings. An overview of the
168 findings can be found in [Table 2](#).

169

170 **Table 2. Simple sound stimulus studies (in order of frequency, low to high).**

Author (Year)	Cell type	Sound type	Sound frequency (Hz)	Loudness (dB SPL) / Amplitude (μm)	Duration (min)	Outcome
Kumeta et al. (2018)	Mouse cell (ST2; bone marrow)	Sine wave	55	76, 82, 88, 94 dB SPL	120	Similar effect to 440 Hz in the same study
Mohammed et al. (2016)	Human cell (LL24; lung fibroblast)	Sine wave	100	9 μm^a	5	Increased cell migration by 6% ($p <$ 0.05), diminishes

						after 2~3 hours
						Growth rate
						12.4 %
	Yeast cell					higher (p <
Aggio et al.	(<i>saccharomy</i>	Pure tone	100	92 dB SPL	14 hours	0.005),
(2012)	<i>ces</i>					biomass
	<i>cerevisiae</i>)					production
						14 % lower
						(p < 0.05)
						Similar
Kumeta et	Mouse cell					effect to 440
al. (2018)	(ST2; bone	Sine wave	110	76, 82, 88,	120	Hz in the
	marrow)			94 dB SPL		same study
						Decreased
						cell
	Human cell					migration
Mohammed	(LL24; lung	Sine wave	200	5 μm^a	5	compared to
et al. (2016)	fibroblast)					100 Hz (no
						significant
						change)
	Bacteria cell					70 dB SPL:
Kothari et	(<i>Chromobac</i>	Sine wave	300	70, 89.5 dB	14 hours	Cell growth
al. (2018)	<i>terium</i>			SPL		of C.

	violaceum)						Violaceum by 15.02% (p < 0.001); 89.5 dB SPL: QS- regulated pigment production increased by 59.63%
Mohammed et al. (2016)	Human cell (LL24; lung fibroblast)	Sine wave	400	2 μm^a	5		Decreased cell migration compared to 400 Hz (no significant change)
Kumeta et al. (2018)	Mouse cell (ST2; bone marrow)	Sine, Triangle, Square waves ^b	440	76, 82, 88, 94 dB SPL	120		Sine: Ptgs2, CTGF, TNC expression downregulat ed to 60~80 % (p < 0.01),

						Triangle: downregulat ed only TNC, Square: no significant effect
Mohammed et al. (2016)	Human cell (LL24; lung fibroblast)	Sine wave	800	< 1 μm^a	5	No change in cell migration
Shaobin et al. (2010)	Bacteria cell (Escherichia coli)	Sine wave	1000	90 dB SPL	60 (stimulation) + 180 (break) x 6 times	Cell colony forming upregulated to 141.6% (p < 0.05)
Choi et al. (2016)	Human cell (Human BM- Mesenchym al Stem Cells (hBM- MSCs))	Sine wave	1000	81 dB SPL ^c	168 hours	Induced neural differentiation (increased Pyk2 and CREB level p < 0.05)
Mohammed et al. (2016)	Human cell (LL24; lung	Sine wave	1600	< 1 μm^a	5	Decrease in cell

	fibroblast)						migration by
							50% (p < 0.05)
							Similar
Kumeta et al. (2018)	Mouse cell (ST2; bone marrow)	Sine wave	4000	76, 82, 88, 94 dB SPL	120		effect to 440 Hz in the same study
						60	Cell colony forming
Shaobin et al. (2010)	Bacteria cell (Escherichia coli)	Sine wave	5000	90 dB SPL	+ 180 (break) x 6 times		upregulated to 130% (p > 0.05)
						60	Cell colony forming
Shaobin et al. (2010)	Bacteria cell (Escherichia coli)	Sine wave	10000	90 dB SPL	+ 180 (break) x 6 times		upregulated to 131.1% (p > 0.05)
							Growth rate
							12.4 %
	Yeast cell						higher (p < 0.005),
Aggio et al. (2012)	(<i>saccharomyces cerevisiae</i>)	Pure tone	10000	89 dB SPL	14 hours		biomass production
							14 % lower

(p < 0.05)

^aAmplitude is measured by a laser vibrometer. ^bWhite noise was also tested but with insignificant effect. ^c100 dB SPL did not show any effect

In Shaobin et al. (27), a speaker was placed above the plate inside a box-shaped temperature-controlled (37 °C) environment. The box was acoustically treated with sound absorption installed around the walls. Sine tones with frequencies of 1000, 5000, and 10000 Hz were played separately at 90 dB SPL for 1 hour at a time and repeated 6 times with 3-hour breaks in between. The bacteria cells (*Escherichia coli*) were spread on an agar plate and were therefore not in a liquid-based medium. The cell dish holder was made to rotate using a motor installed outside the chamber. It is not stated whether the control group was placed on the holder while the holder was rotating. Cell colony-forming efficiency was tested, and it was shown that 1000, 5000, and 10000 Hz sine tones upregulated the cell colony-forming by 141.6, 130, and 131.1 % respectively (p < 0.05).

In Aggio et al. (28), a pair of mid-high frequency speakers and a subwoofer was placed around shaker-flasks in a cell incubator. The distance between speakers and flasks was 20 cm. Sine tones of 100 and 10000 Hz were played using computer software at 92 and 89 dBA respectively for 14 hours to a yeast cell culture (*Saccharomyces cerevisiae*). The results showed an effect on the exponential growth rate where it was increased up to 12.4 % (p < 0.005), but after around 10 hours the biomass production was decreased by 14 % (p < 0.05) compared to the control (silence).

192

193 In Choi et al. (29), an 8-inch active speaker (NA-818APR, NBN) was placed underneath (10
194 cm away) the cell culture dish inside a cell incubator. A 1000 Hz sine tone generated by a
195 function generator (FG-7002C, EZ digital) at 81 dB SPL. It was played for 7 days
196 continuously to Human Bone Marrow Mesenchymal Stem Cells (hBM-MSCs). The result
197 showed that the sound treatment induced neural differentiation, and MAP2 and NF-L protein
198 levels significantly increased without altering the cell viability compared to the control group.
199 Phosphorylation levels of Pyk2, ERK, Akt, and CREB were also tested at 10, 45, 90, and 180
200 minutes post-stimulation. Pyk2 level spiked at 45 minutes and decreased afterwards. The
201 ERK level was increased from 45 minutes and stayed until 180 minutes ($p < 0.05$). The
202 CREB level was increased from 90 minutes but slightly decreased at 180 minutes ($p < 0.05$).
203 The Akt level showed no difference. The Pyk2 level was rechecked at a different SPL (100
204 dB SPL), but did not show any effect, which suggests a possible SPL dependency.

205

206 In Mohammed et al. (30), a thin mylar speaker ($\varnothing=45$ mm, 0.2 W) was placed directly
207 underneath the cell culture dish ($\varnothing=35$ mm). A sine tone with different frequencies (100, 200,
208 400, 800, 1600 Hz) was generated using an Arduino microcontroller. This induced vibrations
209 in the cell culture dish at a particular displacement amplitude (9, 5, 2, < 1 , < 1 μ m
210 respectively). The amplitude was measured by using a laser vibrometer (Polytech Ltd.). The
211 dish was resting on the speaker. Different frequencies were applied for 5 minutes each time to
212 two different cell types: human lung fibroblast cells (LL24) and mouse fibroblast cells
213 (L929), placed in separate cell culture dishes. The lowest frequency (100 Hz) was found to
214 increase the migration distance of both cell types by 6 % ($p < 0.05$), without altering the cell
215 viability. The migration distance decreased at higher frequencies.

216

217 In Kothari et al. (31), a speaker (Minix soundbar) was placed 15 cm away next to the cell
 218 tubes inside a glass box-shaped chamber (L: 250 x W: 250 x H: 150 mm). The stimuli
 219 consisted of sine tones played from the computer software NCH Tone Generator
 220 (<https://www.nch.com.au/index.html>) at 300 Hz and at five different SPLs (70, 76.5, 83, 87.5,
 221 89.5 dB SPL) each for 48 hours. The stimulated cells were bacteria of type *Chromobacterium*
 222 *violaceum*. The noise level was below 40 dB SPL in the control chamber. The result showed
 223 an SPL dependency, and the SPL difference of less than 13 dB SPL showed no significant
 224 alterations in the growth of cells. The cell growth was upregulated the most at 70 dB SPL, by
 225 15.02 % ($p < 0.001$). Quorum Sensing regulated pigment production was affected the most, at
 226 89.5 dB SPL by 59.63 % ($p < 0.01$).

227

228 In Kumeta et al. (8), a 4-inch active speaker (Fostex 6301NB) was placed above (250 mm
 229 away) the cell culture dish inside a cell incubator. Different types of sounds (440 Hz sine,
 230 triangle and square waves, as well as white noise) were generated at different SPLs (76, 82,
 231 88, 94 dB SPL) using NCH Tone Generator. Each sound was played for 2 hours. The cells in
 232 question were of mice (ST2). The background noise inside the incubator was measured to be
 233 around 65 dB SPL. The results showed a dependency on the type of waveform used. The sine
 234 tones downregulated the gene (Ptgs2, CTGF, TNC) expression level to 60~80 % ($p < 0.01$).
 235 The triangle tones downregulated only one of the genes (TNC), and the square tones and
 236 white noise did not show any significant effect compared to the sine tones. Different
 237 frequencies (55, 110, 4000 Hz) were also tested for the sine tone condition, but the effect was
 238 similar to that of 440 Hz. The SPL of 94 dB SPL showed a significant impact, while tones
 239 with other SPL showed little effect. Another important finding in this study was that the

temperature changed in the cell culture medium. When sound was played at 94 dB SPL, it caused the temperature in the cell culture medium to increase by 0.4 °C. In this experimental setup, the sound energy was calculated to be reflected by 10.1 dB SPL at the lid of the cell culture dish. Subsequently, the transmitted sound pressure into the liquid-based medium was calculated at approximately 10.4 mPa.

Complex sound stimulation on cell cultures

Here we will look at studies that have used different types of complex sound stimuli. This includes the use of music (different styles and genres) or speech (human voice) to stimulate the cell cultures in various experimental settings. There were six studies identified in this category. An overview of the findings can be found in [Table 3](#).

Table 3. Complex sound stimuli studies (in order of published year).

Author (Year)	Cell type	Sound type	Sound frequency range (Hz)	Loudness (dB SPL)	Duration (min)	Outcome
Shah et al. (2016)	Prokaryotic	Hindustani	1. 172-581	1. 70-90 dB	-	Growth rate
	&	classical		SPL		of the
	eukaryotic	music (1.	2. 86-839	2. 85-110		microorgani
	microorgani	<i>Ahir</i>				sms was
	sms	<i>Bhairav &</i>		dB SPL		changed

2. Pilo)

depending
on the cell
type (refer
to section
4.2)

Lestard & Capella (2016)	Human cancer cell (MCF-7 & MDA-MB-231)	Mozart's Sonata for Two Pianos in D Major, KV. 488	0-10000	70-100 dB SPL	30	Caused apoptosis in MDA-MD-231, not in MCF-7 ($p < 0.05$)
Lestard & Capella (2016)	Human cancer cell (MCF-7 & MDA-MB-231)	Beethoven's 5h symphony, 1st movement	0-15000	70-100 dB SPL	30	Decreased the number of living cells 48 hours post-stimulation, inhibited the migration of MDA-MD-231 ($p < 0.05$)
Lestard & Capella	Human cancer cell	Ligeti's Atmosphere	0-15000	70-100 dB SPL	30	Caused apoptosis in

Algieri et al.	Biological	Original	523.25,	~75 dB SPL	-	Increased
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(2018)	molecule	music	587.33,			production
	(tyrosinase)	composition	659.26			by 30 % (p
		(Reminiscen				< 0.05)
		za)				
			261.63,			Decreased
						production
		Original	293.66,			by 50, 60,
Algieri et al.	Biological	music	523.25,	~60.5, 75.5,		80 % at
(2018)	molecule	composition	622.25,	81 dB SPL	-	60.5, 75.5,
	(tyrosinase)	(Pioggia)	698.46,			81 dBA
			830.61			respectively
						(p < 0.05)
						91.8 % (p <
						0.05) of
						cells
		Quran				survived,
		recitation				migration
Mehrafsar et	Human	(Surah Al-				rate
al. (2018)	cancer cell	Fatina, voice	-	90 dB SPL	120	decreased to
	(PC-3)	by S.A.				95.4% (p <
		Basit)				0.05),
						mRNA level
						of BCL2L12
						decreased

by a factor
of 0.48 ($p <$
0.001)

253

254 In Lestard and Capella (32), a 60 W coaxial speaker was installed at the top of the
255 acoustically treated (cork and foam) incubator. The stimuli included the first movement from
256 Mozart's Sonata for Two Pianos in D Major, KV. 488, the first movement from Beethoven's
257 5th symphony, and the first movement from Ligeti's *Atmospheres*. These excerpts were
258 played at around 70~100 dB SPL for 30 minutes each to human breast cancer cells (MCF-7
259 and MDA-MB-231). Although it was not reported in the paper, we have found that the
260 frequency range in Mozart's piece is, approximately, between 0 and 10000 Hz, and 0 and
261 15000 Hz in both the Beethoven and Ligeti pieces. Mozart's piece did not alter the viability
262 of MCF-7 but caused cell death (apoptosis) in MDA-MD-231 ($p < 0.05$). The Beethoven and
263 Ligeti pieces decreased the number of living cells 48 hours post-stimulation and inhibited the
264 migration of MDA-MD-231 ($p < 0.05$). The Ligeti piece caused significant cell apoptosis in
265 MCF-7 ($p < 0.05$).

266

267 In Shah et al. (33), a speaker was placed next to (15 cm away) the cell tubes inside a glass
268 chamber (225 x 225 x 125 cm). An SPL meter (ACD Machine Control Company) was
269 positioned 15 cm away from the speaker. Two Hindustani classical pieces *Ahir Bhairav* and
270 *Piloo* were played at 70~90 dB SPL and 85~110 dB SPL, respectively, to six different
271 microorganisms (prokaryotic and eukaryotic). The recordings were from a CD album named
272 *Call of the Valley* (Saregama Indian Ltd.). The fundamental frequency ranges of the two

pieces were 172~581 Hz (*Ahir Bhairav*) and 86~839 Hz (*Piloo*). After playing *Ahir Bhairav*, the growth rate of *Serratia marcescens* (-4.32 %, $p < 0.01$), *Chromobacterium violaceum* (4 %, $p < 0.05$), *Xanthomonas campestris* (6.66 %, $p < 0.05$), *Saccharomyces cerevisiae* (8.94 %, $p < 0.01$), *Lactobacillus plantarum* (6.14 %, $p < 0.01$), and *Bacillus parabrevis* (18.75 %, $p < 0.01$) were changed compared to the control. After playing *Piloo*, the growth rate of *Serratia marcescens* (-7.46 %, $p < 0.01$), *Chromobacterium violaceum* (-24.19 %, $p < 0.01$), *Xanthomonas campestris* (9.32 %, $p < 0.05$), *Saccharomyces cerevisiae* (4.68 %, $p < 0.01$), *Lactobacillus plantarum* (3.41 %, $p < 0.01$), and *Bacillus parabrevis* (18 %, $p < 0.05$) were changed compared to the control.

In Sarvaiya and Kothari (34), the experimental setup was the same as in Shah et al. (2016). A Hindustani classical piece (Raag Malhar) was played at around 95~110 dB SPL to various microorganisms (prokaryotic & eukaryotic). The fundamental frequency range of the music was between 41 and 645 Hz. All tested cells showed an increase in growth rate, and in antibiotic susceptibility, except *Serratia marcescens* ($p < 0.05$). The alcohol tolerance of *Saccharomyces cerevisiae* was significantly enhanced by 33.69 % ($p < 0.001$) after 24 hours of incubation when the concentration of ethanol was 5 %v/v, 132.94 % ($p < 0.001$) after 24 hours of incubation when the concentration of ethanol was 10 %v/v, 27.92 % ($p < 0.01$) after 48 hours of incubation when the concentration of ethanol was 12.5 %v/v, and 1200 % ($p < 0.001$) after 120 hours of incubation when the concentration of ethanol was 15 %v/v.

In Ventura et al. (18), the cell culture was placed two meters in front of a live performance. Percussive music based on traditional rhythms and sounds from Africa and Latin America

296 and an actor's voice were played for 45 minutes and 30 minutes, respectively to Human
 297 Adipose Stem Cells (hASCs). The tempo of the percussive music was taken from the
 298 performer's own heartbeat, and the actor's voice was performed with two different intensities
 299 (soft and loud). The fundamental frequency for the percussive music was around 70 Hz at
 300 ~50 dB SPL, and for the actor's voice was around 100 Hz at ~40 dB SPL (soft voice) and 200
 301 Hz at ~30 dB SPL (loud voice). The sound analysis was done through Praat, a software
 302 package (www.fon.hum.uva.nl/praat/) developed for speech analysis. The cell culture was
 303 observed in real-time under the Multi Spectral Imaging (MSI) system (Nikon Eclipse TS
 304 100). The MSI analysis showed spectral changes of cell imaging with specific vibrational
 305 signatures in relation to the stimuli.

306

307 In Algieri et al. (7), a set of 3 W internal speakers (LENOVO E93z) was placed underneath
 308 (10 cm away) the reactor vessel on a magnetic plate. The SPL measurement was done with a
 309 FUSION -1 dB meter and analyzed using dB Trait. Two distinctively composed pieces of
 310 music, *Reminiscenza* (constant rhythm and melody) and *Pioggia* (irregular rhythm with more
 311 variable devices such as irregular tempo changes, and melody) were played to biological
 312 molecules (tyrosinase). These were placed in a reactor vessel with a stirring mechanism and a
 313 constant temperature of 30 °C. In *Reminiscenza*, only C5 (523.25 Hz), D5 (587.33 Hz), and
 314 E5 (659.26 Hz) musical notes were used and played at ~75 dBA. In *Pioggia*, C4 (261.63 Hz),
 315 D4 (293.66 Hz), C5 (523.25 Hz), D#5/Eb5 (622.25 Hz), F5 (698.46 Hz), and G#5/Ab5
 316 (830.61 Hz) were used, and played at different SPL (60.5, 75.5, and 81 dBA). The 1-DOPA
 317 production rate was tested. Playing *Reminiscenza* resulted in a significant increase in
 318 production, by 30 % more compared to the control group. Playing *Pioggia* resulted in

decreased production by 50 % at 60.5 dBA, 60 % at 75.5 dBA, and 80 % at 81 dBA ($p < 0.05$).

In Mehrafsar et al. (35), four speakers were placed around the cell culture plate. A recording of Quran recitation (*Surah Al-Fatina*, voice by *Sheikh Abdul Basit*) was played at 90 dB SPL for 2 hours to the human cancer cell line (PC-3; Human Prostate Adenocarcinoma Cells). The cell viability, migration, and BCL2L12 gene expression level were tested. After the sound stimulation, 91.8 % ($p < 0.05$) of PC-3 cells survived, the migration level decreased to 95.4 % ($p < 0.05$), and mRNA level of BCL2L12 decreased by a factor of 0.48 ($p < 0.001$).

Discussion

As we have seen from the various studies above, many different types of both simple and complex sound types, with an extensive range of frequencies and SPLs have been tested. The results reported for both simple and complex sound stimulation studies are promising, either they can enhance cell migration, proliferation, colony formation, and differentiation ability or decrease cell migration and cause apoptosis of cancer cells. However, it is challenging to find a systematic pattern and to compare the results because the studies use widely different sound materials, experimental setups, sound measurement methods, and observation strategies. Below, we synthesize and discuss the results while considering the practical matters (e.g., experimental setups and measurement methods) and also the physics of sound.

340 **Experimental Setup**

341 Many of the reviewed studies were based on a speaker position above, underneath or next to
 342 the cell cultures. The distances varied, ranging from ~0 (directly underneath the dish) to 200
 343 cm. All three placements resulted in cellular responses without any apparent difference. It
 344 would be of value to perform a systematic experiment of how different positioning of the
 345 sound source (the speaker) results in various types of mechanical force in the cell medium,
 346 for example whether or not a speaker is coupled (in direct contact) with the cell dish or plate.
 347 In terms of the acoustic treatment of the experimental environment, this is sometimes not
 348 clearly stated. Based on its physical properties, a sound stimulus may interact with the testing
 349 environment. Ideally, sound-related experiments should be performed in an acoustically
 350 controlled environment. The ideal solution might be to use an anechoic chamber, which is
 351 designed to imitate the free field conditions (i.e., no reflected sound waves), and which would
 352 allow no or minimum external sound to enter the chamber (36). This would improve the
 353 measurements of the dependent variable and ultimately help reach conclusions about how
 354 different variables are correlated (37). Also, for practical reasons, speakers should ideally be
 355 placed inside an acoustically treated chamber in order not to damage the researcher's hearing
 356 in the lab. For example, a continuous sine tone at a loudness level of 70 dB SPL or higher can
 357 cause temporary or even permanent hearing damage (21). In most cases, an anechoic
 358 chamber would not be feasible considering the cost and effort. However, it would still be
 359 useful to try to reduce the acoustic impact of the environment by using some kind of damping
 360 element. It would also be essential to measure how the sound stimulus behaves in the
 361 environment. This could be used to address potential obstacles during experiments, for
 362 example, amplitude distortion at specific frequencies, standing waves, and phase cancellation.

363

364 **Sound features**

365 We will look at four basic sound features here: frequency (Hz), sound pressure level (dB
366 SPL), and waveform (simple & complex).

367

368 **Frequencies**

369 In the studies where single-frequency sound stimuli were used, single frequencies selected in
370 the range of 55~10000 Hz were explored on bacteria cells (*Escherichia coli*,
371 *Chromobacterium violaceum*, *Serratia marcescens*), mouse cells (ST2), and human cells
372 (hBM-MSCs, LL24). In the studies where the more complex sound stimuli were used,
373 musical pieces or human voice in the frequency range between 0 and 15000 Hz were
374 explored on biological molecule (tyrosinase), microorganisms (various prokaryotic &
375 eukaryotic), bacteria cells (*Escherichia coli*), human cancer cells (MCF-7, MDA-MD-231),
376 and human cells (hASCs). To summarize, bacteria and yeast cell studies showed an effect of
377 sound across a wide range of frequencies: cell colony-forming and growth rate increase using
378 100, 300, 1000, 5000, and 10000 Hz (28,29,32). Mammal cells studies showed a little more
379 selective and frequency-dependent effect. For example, a lower frequency (100 Hz) induced
380 cell migration increase in human cells, but higher frequencies (above 1600 Hz) induced the
381 opposite effect on cell migration (30). Also, stimuli of 440 Hz induced gene (Ptgs2, CTGF,
382 TNC) expression increase in mouse cells (ST2), while 55, 110, and 4000 Hz showed a
383 smaller but similar effect (8).

384

For the complex sound stimulation studies, there are too little reports in the literature, but the results are intriguing. The tested music and human voice varied widely: Western classical music such as Mozart, Beethoven, and Ligeti (32), Hindustani classical music such as *Ahir Bhairav*, *Piloo*, and *Raag Malhar* (33,34), an African and Latin American percussive music and a voice of an actor (18), a contemporary composition such as *Reminiscenza* and *Pioggia* (7), and Quran recitation such as *Surah Al-Fatina* (35). The most present frequencies of the music and voices tested stayed between 40 and 840 Hz. However, it is most likely that other frequency content, especially overtones, varies between different types of music or speech style due to distinctive *timbre* differences between instruments and human voices. Since complex sounds typically consist of many frequencies, it is difficult to know if a particular frequency had an impact on the outcome. However, a notable reported outcome is where two musical compositions of contrasting characteristics in terms of melody and rhythm (regular/constant vs irregular/spontaneous) showed contrasting results in 1-DOPA production rate in *tyrosinase*. Music with regular characteristics (constant harmonic and rhythmic structure) using 523.25 Hz (musical note: C5), 587.33 Hz (D5), and 659.26 Hz (E5) resulted in 1-DOPA production increase. Music with more irregular characteristics (disharmonic and irregular rhythm and tempo structure), on the other hand, using 261.63 Hz (C4), 293.66 Hz (D4), 523.25 Hz (C5), 622.25 Hz (D#5/Eb5), 698.46 Hz (F5), and 830.61 Hz (G#5/Ab5), resulted in 1-DOPA production decrease (7). This indicates a possible role of the degree of pitch (frequency), also possibly of harmonic versus disharmonic frequency content, and temporal regularity in the produced outcome.

407 Sound pressure level

408 In the investigated studies, the sound pressure level range used varied extensively, from 40 to
 409 110 dB SPL. The majority of the studies used the range between 75 and 95 dB SPL, which is
 410 as loud as a vacuum cleaner (3 meters away) and a subway train (6 meters away),
 411 respectively. The range centered around 90 dB SPL, which is about 632 mPa. This is above
 412 the loudness level where sustained exposure can cause hearing damage. However, the
 413 pressure is small compared to the periodic blood flow pressure, for example, which can
 414 typically be around 120/80 mmHg (approximately 10~16 kPa) (38). The transmitted sound
 415 energy in the cell medium from air is estimated to be even smaller: approximately 29.67 mPa
 416 (when the temperature is 20 °C, $R \approx 0.9988$, transmitted sound energy from the air to water is
 417 $1 - R \approx 0.0022$ when there is no absorption). This is a small mechanical stress, but the cells
 418 have mechanisms that can sense such small forces and changes, for example, α -catenin
 419 (8,39).

420

421 It should be noted that some of the SPLs specified in the literature are incomparable. This is
 422 because some of the reports were unclear about how the actual sound output was measured.
 423 Additionally, some of the commercially available SPL measurement devices typically come
 424 with filters that imitate the human hearing characteristics (less sensitive at very low and high
 425 frequencies within the audible frequency range), such as an A-weighting filter. There are
 426 other weightings, as illustrated in [Fig 2](#) (40), designed for different applications. This is a
 427 small piece of information that may not be considered necessary, but it should be stated in the
 428 report, as a specific weighting filter readout can undermine a particular frequency range as
 429 shown.

430

431 **Fig 2. The frequency responses of A, B, C, D, M and RLB weighting filters (40).**

432

433 **Waveform**

434 As pointed out earlier, the majority of the simple sound studies employed sine tones. There
 435 was only one study that compared different types of simple waveforms (sine, triangle, square)
 436 and white noise. Interestingly, the different waveforms yielded contrasting outcomes in
 437 cellular responses (8). The underlying mechanism is not well understood, but an analysis of
 438 the waveforms can be made: sine waves are continuous, whereas triangle and square waves
 439 are discontinuous. Also, sine waves do not have any harmonic frequencies (overtones), only
 440 its fundamental frequency. Triangle and square waves, on the other hand, contain odd
 441 harmonics above its fundamental frequency. The higher harmonics of the triangle wave get
 442 weaker faster than those of a square wave, as shown in [Fig 3](#). Due to its wave shape, the
 443 amount of time that the square wave stays in its maximum amplitude is longer than other
 444 waveforms. This is a reason why square waves are perceived louder than sine waves. When
 445 the amplitude and the frequency are the same, there is more sound energy in square waves
 446 and stronger harmonics than in sine and triangle waveforms. Although there is a need for
 447 further investigation, according to the differences we explore, a possible explanation of
 448 different outcomes between sine, triangle, square, and white noise could be the strength of
 449 harmonics present in the sound material.

450

Fig 3. Comparison of sine, triangle, and square waves with a fundamental frequency of 440 Hz

Rhythms

Rhythm is one of the fundamental properties of music and is also an essential feature of biological life. In the investigated simple sound studies, there were no rhythmic features in the sound stimuli. On the other hand, all the complex sound studies had an aspect of rhythm. The rhythms varied widely: Western classical music, Hindustani classical music, and African and Latin-American traditional music. For illustration, Mozart's Sonata for Two Pianos in D Major, KV. 488 shows more regular and steady rhythmic patterns than Ligeti's *Atmospheres* (Fig 4a & Fig 4b). There was only one study in which the aspect of rhythm was deliberately tested and reported, and where regular/constant and irregular/inconstant rhythms were compared in terms of cellular responses (7). The results showed that the musical piece with regular rhythm produced an increase in the production of 1-DOPA compared to the control group, whereas the piece with irregular rhythms resulted in a decrease.

Fig 4a. Spectrogram of the first movement of Mozart's Sonata for Two Pianos in D Major, KV.488

Fig 4b. Spectrogram of Ligeti's Atmospheres

The “synchronization of multiple rhythms” is vital in living organisms (41), and rhythmicity is one parameter that is underexplored in the literature. The human body is full of different rhythms, such as heartbeats, which are paced by the so-called sinus node and respiratory rhythms that are controlled by the bilateral dorsal respiratory groups. If certain rhythms in the human body become irregular, it can be an indication of more severe health problems, for example, arrhythmia (42) or abnormal respirations (43). Together, rhythmicity is a possible new avenue of sound stimulation of cells and is of value to be investigated further.

Conclusion

As we have seen from this literature review, there appear to be promising effects of using audible sound on microorganisms and mammalian cells. The correlations are complex, and the exact cause of cell responses to audible sound stimulation is still largely underexplored. We have also found that there are considerable differences in experiment setups and sound features when it comes to the setups used for cellular stimulation. In many cases, it is also unclear exactly how the stimulation happened due to an uncontrolled (from a sonic point of view) environment. In future research, we, therefore, see the need for:

- **Controlled sonic environment:** An acoustically treated environment would make it easier to control the sound stimuli. This would reduce the level of external noise and avoid reinforcement or cancellation of particular frequencies. What is being applied to the cells should only be the sound emitted directly from a speaker. The ideal would be

to use an anechoic chamber, but even installing suitable dampening acoustic materials would improve the experimental environment.

- **Measurement method:** Due to the possible (sonic) influence of the environment as mentioned above, it is necessary to measure and analyze the environment with appropriate equipment (e.g., a flat frequency response calibration microphone). This would serve as a control, and an SPL measurement method should be used to know what the cells are stimulated with.
- **Sound stimuli:** A more comprehensive range of sound stimuli should be tested. All of the simple sound stimuli used in the literature so far have been of a continuous nature. Considering the complexity of the extracellular matrix (environment) that the cells live in, learning the influence of pulsating or rhythmic stimuli on cells could also be interesting. The control of the sound factors/parameters that are varied should be improved when using more complex sound stimuli.

Many other possible non-sound related factors can alter the functionality of cells under examination (44). Therefore, as to recommendations for future research in this multidimensional and uprising field, it is crucial to have the sound stimuli well under control and elucidate the practical issues. High priority should be given to investigate the optimal acoustic environment for cell stimulation that takes cell viability (e.g., temperature) into consideration. Focus should also be put on standardizing methods to measure and analyze the characteristics of the experimental sound output and the background noise. Finally, we suggest replication studies of the findings from the literature under the above-mentioned controlled conditions.

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627

Fig 1

Fig 2

Fig 3

Fig 4a

Fig 4b