

Music for Cells? A Systematic Review of Studies Investigating the Effects of Audible Sound Played Through Speaker-Based Systems on 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 sounds that were used as stimuli, and relevant biological outcomes. The studies are categorized into simple and complex sounds depending on the type of sound employed. Some of the promising effects reported were 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 parameters as cellular stimuli.

Keywords

Mechano-acoustic stimuli, sound stimuli, music stimuli, cell cultures, mechanobiology

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Introduction

Is there such a thing as *music for cells*? The use of musical sound to induce a beneficial physiological and psychological effect on biological beings is not a novel idea (Babikian et al., 2013). In light of this, the efforts to attain a deeper understanding of the relationship between sound and the human body have become more specific, now at a minuscule level in the field of mechanobiology in the last few decades. Mechanobiology has developed substantially with more than 33,000 publications up until 2016 (Sokabe, 2016; Wall et al., 2017). More recently, there has been an indication of escalating interests and efforts to understand mechanotransduction through mechanical cues induced by acoustical perturbation on cellular responses (Exbrayat & Brun, 2019). Various cell types have been investigated including: plant cells (Lim & Bae,

2014), micro-organisms (Algieri et al., 2018), auditory cells (Exbrayat & Brun, 2019), and other mammal cells *in vitro* (Kumeta et al., 2018) or *in vivo* (Wei et al., 2016). The effects of sound on plant cells have been reported and reviewed numerous times previously (Hamant &

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Haswell, 2017; Hendrawan et al., 2020; Jung et al., 2018; Mishra et al., 2016). Hence, the aim of this review is to get an overview of studies that have used audible sound played through speaker-based systems to induce mechanical perturbation in bacteria, yeast, and mammalian cell cultures. We start with a brief background to mechanobiology and the basic physical properties of sound. Subsequently, the selected studies are discussed concerning the experimental setups, sound used (categorized into “simple” and “complex” sounds), and 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 (Mishra et al., 2017), and it has been suggested that cells are sensitive to physical forces (Kung, 2005). Mechanical signals, such as adhesiveness or extracellular matrix (ECM) stiffness, are also sensed by cells (Zhang et al., 2020). Different types of mechanical cues instigate specific responses in cells where certain genes can be activated through specific signaling pathways (Fedorchak et al., 2014). For instance, YAP and TAZ of the Hippo pathway, which has been identified as conserved mechanical transducers, change their locations to the nucleus in cells if the stiffness of the surface is rigid (Argentati et al., 2019). This nucleus localization is critical for driving stem cells' fate (e.g., targeting the cell type that stem cells change to through differentiation process; Zhang et al., 2020). At the same time, its altered activity is involved in aberrant cell mechanics transduction and several diseases (Pancier et al., 2017). Cells have different means to sense the mechanical changes in their immediate surroundings, which include mechanosensitive ion channels, membrane mechanosensors, and mechanosensitive proteins inside the cells (Yang et al., 2015). Interestingly, it has been reported that cells cannot only sense mechanical stimulations but also generate mechanical signals themselves. For example, bacterial cells can generate sound waves between 8 and 43 kHz (Matsuhashi et al., 1998), and mechanical vibrations of yeast cells (Pelling, 2004) and stem cells have also been observed (Ventura et al., 2017).

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 a mechanical stimulus and starts shaking and vibrating, sound energy is produced (Wood, 1946). This energy is propagated in the form of pressure waves from the sound source, usually as a longitudinal wave consisting of compressions and rarefactions of matter (Huber, 2010). Sound waves can travel through different mediums, including gas, liquid, and solids, with speeds dependent on the medium (Nandanwar et al.,

2017). Typically, sound travels faster in a liquid or solid medium (e.g., water or a steel bar) than in a gas medium (Huber, 2010; Khilyuk et al., 2000).

A medium's density impacts energy transmission when sound is traveling from one medium to another (Wood, 1946). This physical property of sound is a factor that needs attention in cellular experiments, in which sound energy transmits from the air into a liquid-based solution such as cell culture media. For a progressive plane wave, the physical property of a medium can be expressed as the acoustic impedance, defined in equation 1.

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

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

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

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

The medium's thickness also impacts the transmitted energy. If the thickness of the second medium is much smaller than the wavelength of the incident sound, for instance, the amount of transmitted energy will be minimized (Wood, 1946).

Manipulable Sound Parameters

When exploring the use of sound for the experimental set up of cells, there are several properties to consider. Some of the basic parameters of sound include its frequency content (which contributes to the perception of pitch and timbre), amplitude (which corresponds to perceptual loudness), and exposure time. Additionally, there are different types of waveforms, from the most basic (sine tones) to synthetic waveforms (sawtooth, square, triangle), and more complex and natural waveforms (noise, speech, music) (Farnell, 2010).

When it comes to basic descriptions of sounds, the sound frequency (Hz) is represented as the number of cycles or vibrations per second. It can be defined as:

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

In equation 3, T is the period (s) which is the time taken for a vibrating motion to complete one cycle, λ is the wavelength (m) which is the distance between two successive matching points in any section of the sound wave cycle.

When the period and wavelength increase, the frequency will decrease in a given material, but the speed of sound will remain the same unless the temperature changes (Wood, 1975). In the following, we will discuss the review method and selected studies.

Review Method

We searched for articles using 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 July 1, 2019 and June 20, 2020.

As illustrated in Figure 1, a total of 95 records were identified through database searching. Of these, seven records were removed as duplicates, 74 records were investigated but considered not relevant and hence removed. This included studies that were not related to cell studies *in vitro*, or not related to audible sound stimulation, or publications not in English. The full texts of two records were unobtainable and were not considered either. This left us with 12 studies that were included for further qualitative consideration (Table 1).

Results

When looking broadly at the selected studies, the sound used in the literature can be divided into two main sound types:

- simple sounds (e.g., sine tones): six studies
- complex sounds (e.g., music and speech): six studies

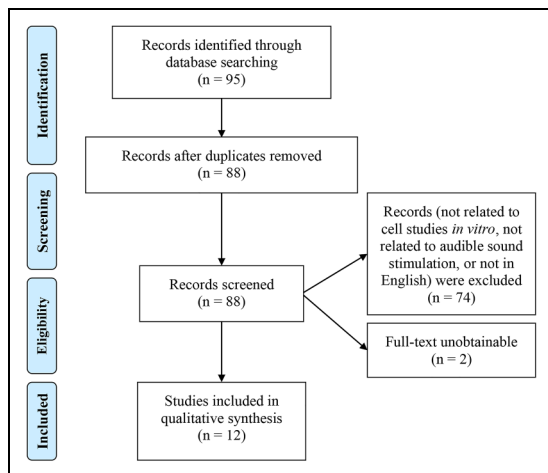


Figure 1. The PRISMA flow diagram for systematic review (search and filtering results).

We will now look more closely at each study in terms of the experimental setup, sound parameters, and biological outcomes.

Simple Sound Stimulation on Cell Cultures

The studies identified within the category simple sound stimulus all used a sine tone with a constant sound pressure level in the experimental settings. An overview of the findings can be found in Table 2.

In Shaobin et al. (2010), a speaker was placed above the petri dish inside a box-shaped temperature-controlled (37 °C) environment. The box was acoustically treated with sound absorption around the walls. Sine tones with frequencies of 1,000, 5,000, and 10,000 Hz were played separately at 90 dB SPL for 1 h at a time and repeated six times with 3 h breaks in between. *Escherichia coli* were spread on an agar plate and were therefore not in a liquid-based media. The petri 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 1,000, 5,000, and 10,000 Hz sine tones showed upregulation of the cell colony forming by 141.6, 130, and 131.1%, respectively.

In Aggio et al. (2012), 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 10,000 Hz were played using computer software at 92 and 89 dBA, respectively, for 14 h to *Saccharomyces cerevisiae* yeast culture. The results showed an effect on the exponential growth rate where it was increased by 8.7% ($p < .005$) when 100 Hz sine tone was played to the cell culture and by 11.5% ($p < .005$) with 10 kHz sine tone. Subsequently, the cells exposed to sine tones showed reduction in the biomass production which decreased by 14% ($p < .05$).

In Choi et al. (2016), an 8-inch active speaker (NA-818APR, NBN) was placed underneath (10 cm away) the cell culture dish inside a cell incubator. A 1000 Hz sine tone was generated by a function generator (FG-7002C, EZ digital) at 81 dB SPL. The sine tone was played for 7 days to Human Bone Marrow Mesenchymal Stem Cells (hBM-MSCs). The sound treatment induced neural differentiation, and MAP2 and NF-L protein levels significantly increased by more than 150% without altering the cell viability compared to the control group. Phosphorylation levels of Pyk2, ERK, Akt, and CREB were also tested at 10, 45, 90, and 180 min post-stimulation. Pyk2 level spiked (>200%) at 45 min and decreased after 90 min. The ERK level was increased (>150%) from 45 min and stayed until 180 min ($p < .05$). The CREB level was increased (>200%) from 90 min but slightly decreased (~150%) at 180 min ($p < .05$). The Akt level showed no difference. The Pyk2 level was rechecked at a different SPL (100 dB SPL), but did not show any effect, which suggests a possible SPL dependency.

Table 1. List of Included Studies (in order of Publication Year).

Publication year	First author	Title	Source
2010	Shaobin et al.	A pilot study of the effect of audible sound on the growth of <i>Escherichia coli</i>	<i>Colloids and Surfaces B: Biointerfaces</i> , 78(2), 367–371
2012	Aggio et al.	Sonic vibration affects the metabolism of yeast cells growing in liquid culture: a metabolomic study	<i>Metabolomics</i> , 8(4), 670–678
2016	Mohammed et al.	The effects of acoustic vibration on fibroblast cell migration	<i>Materials Science and Engineering: C</i> (69), 1256–1262
2016	Choi et al.	Sound waves induce neural differentiation of Human Bone Marrow-Derived Mesenchymal Stem Cells via ryanodine receptor-induced calcium release and Pyk2 activation	<i>Applied Biochemistry and Biotechnology</i> , 180(4), 682–694
2016	Lestard & Capella	Exposure to music alters cell viability and cell motility of human nonauditory cells in culture	<i>Evidence-Based Complementary and Alternative Medicine</i> , 2016: 6849473, 1–7
2016	Shah et al.	Sound stimulation can influence microbial growth and production of certain key metabolites	<i>Journal of Microbiology, Biotechnology and Food Sciences</i> , 9(5), 330–334
2017	Sarvaiya & Kothari	Audible sound in form of music can influence microbial growth, metabolism and antibiotic susceptibility	<i>Journal of Applied Biotechnology & Bioengineering</i> , 2(6), 212–219
2017	Ventura et al.	Cell melodies: when sound speaks to stem cells	<i>CellR4</i> , 5(2): e2331
2018	Mehrafsar et al.	Effect of exposure to Quran recitation on cell viability, cell migration, and BCL2L12 gene expression of human prostate Adenocarcinoma cell line in culture	<i>Health, Spirituality and Medical Ethics</i> , 5(4), 46–52
2018	Algieri et al.	Effects of music playing on biological molecules	<i>MATEC Web of Conferences</i> , 210: 05006
2018	Kumeta et al.	Cell type-specific suppression of mechanosensitive genes by audible sound stimulation	<i>PLOS ONE</i> , 13(1): e0188764
2018	Kothari et al.	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

In Mohammed et al. (2016), a thin mylar speaker ($\varnothing = 4.5$ cm, 0.2 W) was placed directly underneath the cell culture dish ($\varnothing = 3.5$ cm). A sine tone with different frequencies (100, 200, 400, 800, 1600 Hz) was generated using an Arduino microcontroller. This induced vibrations in the cell culture dish at a particular displacement amplitude (9, 5, 2, <1, <1 μm , respectively). The amplitude was measured by using a laser vibrometer (Polytech Ltd.). The dish was resting on the speaker. Different frequencies were applied for 5 min each time to two different cell types: human lung fibroblast cells (LL24) and mouse fibroblast cells (L929), placed in separate cell culture dishes. The lowest frequency (100 Hz) was found to increase the migration distance of both cell types by 6% ($p < .05$) over 4 h, without altering the cell viability. The migration distance decreased as the frequencies of the sine tone became higher.

In Kothari et al. (2018), a speaker (Minix soundbar) was placed 15 cm away next to the cell tubes inside a glass box-shaped chamber (25 (l) \times 25 (w) \times 15 (h) cm). A 300 Hz sine tone was played from a computer software NCH Tone Generator at five different SPLs (70, 76.5, 83, 87.5, 89.5 dB SPL) for 48 h each time. The stimulated cells were bacteria of type *Chromobacterium violaceum*. The noise level was below 40 dB SPL in the control chamber. The result showed an SPL dependency. It is important to note that the SPL difference of less than 13 dB SPL showed no significant alterations in the growth of cells.

The cell growth was upregulated the most by 15.02% ($p < .001$) at 70 dB SPL. Quorum Sensing regulated pigment production was affected the most which increased by 59.63% at 89.5 dB SPL ($p < .01$).

In Kumeta et al. (2018), a 4-inch active speaker (Fostex 6301NB) was placed above (25 cm away) the cell culture dish inside a cell incubator. A 440 Hz sine tone (triangle, square tones, and white noise were tested too) was generated at different SPLs (76, 82, 88, 94 dB SPL) using NCH Tone Generator on a computer. Each tone was played for 2 h. The background noise inside the incubator was measured to be around 65 dB SPL. The results showed a dependency on the type of waveform used on mice cells (ST2). The sine tones downregulated the gene (Ptgs2, CTGF, TNC) expression level between 60% and 80% ($p < .01$). The triangle tones downregulated only one of the genes (TNC), and the square tones and white noise did not show any significant effect compared to the sine tones. Different frequencies (55, 110, 4000 Hz) were also tested for the sine tone condition, but the effect was similar to that of 440 Hz. The sound level of 94 dB SPL showed a significant impact, while tones with other SPL showed little effect. Another important finding in this study was the temperature change in the cell culture media liquid. When sound was played at 94 dB SPL, it caused the temperature in the media to increase by 0.4 °C. A noteworthy assumption was that the sound energy

Table 2. Simple Sound Stimulus Studies (in order of Publication Year).

Author (Year)	Cell type	Sound type	Sound frequency (Hz)	Sound pressure level (dB SPL) /Amplitude (μm)	Duration	Key outcome
Shaobin et al. (2010)	Bacteria cell (<i>Escherichia coli</i>)	Sine tone	1,000	90 dB SPL	60 (stimulation) + 180 (break) \times 6 times	Cell colony forming upregulated to 141.6%
			5,000			Cell colony forming upregulated to 130%
			10,000			Cell colony forming upregulated to 131.1%
Aggio et al. (2012)	Yeast cell (<i>Saccharomyces cerevisiae</i>)	Sine tone	100	92 dB SPL	14 h	Growth rate 8.7% higher ($p < .005$) ^d , biomass production up to 14% lower ($p < .05$)
			10,000	89 dB SPL		Growth rate 11.5% higher ($p < .005$) ^d , biomass production up to 14% lower ($p < .05$)
Choi et al. (2016)	Human cell (Human BM-Mesenchymal Stem Cells (hBM-MSCs))	Sine tone	1,000	81 dB SPL ^c	168 h	Induced neural differentiation (increased Pyk2 and CREB level $p < .05$)
Mohammed et al. (2016)	Human cell (LL24; lung fibroblast)	Sine tone	100	9 μm^a	5 min	Increased cell migration by 6% ($p < .05$), diminishes after 2~3 h
			200	5 μm^a		Decreased cell migration compared to 100 Hz (no significant change)
			400	2 μm^a		Decreased cell migration compared to 400 Hz (no significant change)
			800	<1 μm^a		No change in cell migration
			1,600	<1 μm^a		Decrease in cell migration by 50% ($p < .05$)
Kothari et al. (2018)	Bacteria cell (<i>Chromobacterium violaceum</i>)	Sine tone	300	70, 89.5 dB SPL	14 h	70 dB SPL: Cell growth of <i>C. Violaceum</i> by 15.02% ($p < .001$); 89.5 dB SPL: QS-regulated pigment production increased by 59.63%
Kumeta et al. (2018)	Mouse cell (ST2; bone marrow)	Sine tone	55	76, 82, 88, 94 dB SPL	120 min	Similar effect to 440 Hz in the same study
			110			Similar effect to 440 Hz in the same study
			4,000			Similar effect to 440 Hz in the same study
			Sine, Triangle, Square tones ^b			440

^aThe amplitude was measured by a laser vibrometer.

^bWhite noise was also tested but the effect was insignificant.

^cThe sound was also played at 100 dB SPL, but did not show any effect

^dIt caused less biomass production up to 14% ($p < .05$).

would be reflected at the lid of the petri dish by more than 10 dB SPL. The transmitted sound pressure into the liquid-based media eventually was calculated to be approximately 10.4 mPa.

Complex Sound Stimulation on Cell Cultures

Here we will look at studies that have used different types of complex sound. This includes the use of music (different styles and genres) or speech (human voice) to stimulate the

cell cultures in various experimental settings. An overview of the findings can be found in Table 3.

In Aggio et al. (2012), the setup is described in the previous section. The description of the music is limited; they describe it as “broad-band music” sampled at a bit rate of 320 kbps. The growth rate showed an increase by 12.4% ($p < .005$) when the music was played to the cell culture, which caused a decrease in biomass production by 14% ($p < .05$). This observation was similar when the sine tone was played to the cell culture in the same study.

Table 3. Complex Sound Stimuli Studies (in order of Publication Year).

Author (Year)	Cell type	Sound type	Sound frequency range (Hz)	Sound pressure level (dB SPL)	Duration	Key outcome
Aggio et al. (2012)	Yeast cell (<i>Saccharomyces cerevisiae</i>)	Broad-band music	-	80–92 dBA	14 h	Growth rate 12.4% higher ($p < .005$) ^a
Lestard and Capella (2016)	Human cancer cell (MCF-7 & MDA-MB-231)	Mozart's Sonata for Two Pianos in D Major, KV. 488	-	70–100 dB SPL	30 min	Caused apoptosis in MDA-MD-231, not in MCF-7 ($p < .05$)
		Beethoven's 5h symphony, 1st movement	-			Decreased the number of living cells 48 h post-stimulation, inhibited the migration of MDA-MD-231 ($p < .05$)
		Ligeti's Atmospheres	-			Caused apoptosis in both cell lines ($p < .05$)
Shah et al. (2016)	Prokaryotic & eukaryotic microorganisms	Hindustani classical music (1. <i>Ahir Bhairav</i> & 2. <i>Piloo</i>)	1. 172–581 2. 86–839	1. 70–90 dB SPL 2. 85–110 dB SPL	-	Growth rate of the microorganisms was changed depending on the cell type (refer to discussion below)
Sarvaiya and Kothari (2017)	Prokaryotic & eukaryotic microorganisms	Indian classical piece (Raag Malhar)	41–645	95–110 dB SPL	-	Increased growth rate and antibiotic susceptibility except <i>Serratia marcescens</i> ($p < .05$)
Ventura et al. (2017)	Human cell (hASCs)	Percussive music based on African & Latin American traditional rhythms and sounds	70 (fundamental frequency)	~50 dB SPL	-	Different multispectral imaging patterns were generated by stem cells in response to different sound
		Human voice (soft / loud)	100 / 200 (fundamental frequency)	~40 / 30 dB SPL	-	
Algieri et al. (2018)	Biological molecules (tyrosinase)	Original music composition (Reminiscenza)	523.25, 587.33, 659.26	~75 dB SPL	-	Increased production by 30% ($p < .05$)
		Original music composition (Pioggia)	261.63, 293.66, 523.25, 622.25, 698.46, 830.61	~60.5, 75.5, 81 dB SPL	-	Decreased production by 50, 60, 80% at 60.5, 75.5, 81 dBA respectively ($p < .05$)
Mehrafsar and Mokhtari (2018)	Human cancer cell (PC-3)	Quran recitation (Surah Al-Fatina, voice by S.A. Basit)	-	90 dB SPL	120 min	91.8% ($p < .05$) of cells survived, migration rate decreased to 95.4% ($p < .05$), mRNA level of BCL2L12 decreased by a factor of 0.48 ($p < .001$)

^aIt caused less biomass production up to 14% ($p < .05$).

In Lestard and Capella (2016), a 60 W coaxial speaker was installed at the top of the acoustically treated (with cork and foam) incubator. The first movement from Mozart's Sonata for Two Pianos in D Major, KV. 488, the first movement from Beethoven's 5th symphony, and Ligeti's *Atmospheres* were played to the cells. These excerpts were played at around 70–100 dB SPL for 30 min each to human breast cancer cells (MCF-7 and MDA-MB-231). Mozart's piece did not alter the viability of MCF-7 but caused cell death (apoptosis) in

MDA-MD-231 ($p < .05$). The Beethoven and Ligeti pieces decreased the number of living cells 48 h post-stimulation and inhibited the migration of MDA-MD-231 ($p < .05$). The Ligeti piece caused significant cell apoptosis in MCF-7 ($p < .05$).

In Shah et al. (2016), a speaker was placed next to (15 cm away) the cell tubes inside a glass chamber (22.5 (l) × 22.5 (w) × 12.5 (h) cm). The SPL was measured with an SPL meter (ACD Machine Control Company) that was positioned 15 cm away from the speaker. Two pieces of

music (*Ahir Bhairav* and *Piloo*) were played at 70–90 dB SPL and 85–110 dB SPL, respectively, to six different microorganisms (prokaryotic and eukaryotic). The recordings were from a CD album named *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%, $p < .01$), *Chromobacterium violaceum* (4%, $p < .05$), *Xanthomonas campestris* (6.66%, $p < .05$), *Saccharomyces cerevisiae* (8.94%, $p < .01$), *Lactobacillus plantarum* (6.14%, $p < .01$), and *Bacillus parabrevis* (18.75%, $p < .01$) were changed compared to the control. After playing *Piloo*, the growth rate of *Serratia marcescens* (−7.46%, $p < .01$), *Chromobacterium violaceum* (−24.19%, $p < .01$), *Xanthomonas campestris* (9.32%, $p < .05$), *Saccharomyces cerevisiae* (4.68%, $p < .01$), *Lactobacillus plantarum* (3.41%, $p < .01$), and *Bacillus parabrevis* (18%, $p < .05$) were changed compared to the control.

In Sarvaiya and Kothari (2017), the experimental setup was the same as in Shah et al. (2016). A piece of music (*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* ($p < .05$). Another key finding was the enhanced alcohol tolerance of *Saccharomyces cerevisiae* was significantly enhanced by 33.69% ($p < .001$) after

24 h of incubation when the concentration of ethanol was 5%v/v, 132.94% ($p < .001$) after 24 h of incubation when the concentration of ethanol was 10%v/v, 27.92% ($p < .01$) after 48 h of incubation when the concentration of ethanol was 12.5%v/v, and 1200% ($p < .001$) after 120 h of incubation when the concentration of ethanol was 15%v/v.

In Ventura et al. (2017), the cell culture was placed 2 m in front of a live performance. Percussive music based on traditional rhythms and sounds from Africa and Latin America and an actor's voice were played for 45 and 30 min, respectively, to Human Adipose Stem Cells (hASCs). The tempo of the percussive music was taken from the performer's own heartbeat, and the actor's voice was performed with two different intensities (soft and loud). The most dominant frequency for the percussive music was around 70 Hz at ~50 dB SPL, and for the actor's voice was around 100 Hz at ~30 dB SPL (soft voice) and 200 Hz at ~30 dB SPL (loud voice). The sound analysis was done through Praat, a software package developed for speech analysis. The cell culture was observed in real time under the Multi-Spectral Imaging (MSI) system (Nikon Eclipse TS 100). The MSI analysis showed spectral changes of cell imaging with specific vibrational signatures in relation to the performances.

In Algieri et al. (2018), a set of 3 W internal speakers (LENOVO E93z) was placed underneath (10 cm away) the reactor vessel on a magnetic plate. The SPL measurement was done with a FUSION −1 dB meter and analyzed using dB Trait. Two distinctively composed pieces of music, *Reminiscenza* (constant rhythm and melody) and *Pioggia* (irregular rhythm with more variable devices such as irregular tempo changes, and melody) were played to biological molecules (tyrosinase). These were placed in a reactor vessel with a stirring mechanism and a constant temperature of 30 °C. In *Reminiscenza*, only C5 (523.25 Hz), D5 (587.33 Hz), and E5 (659.26 Hz) musical notes were used and played at ~75 dBA. In *Pioggia*, C4 (261.63 Hz), D4 (293.66 Hz), C5 (523.25 Hz), D#5/Eb5 (622.25 Hz), F5 (698.46 Hz), and G#5/Ab5 (830.61 Hz) were used, and played at different SPL (60.5, 75.5, and 81 dBA). As one of the key findings, the 1-DOPA production rate was reported. Playing *Reminiscenza* resulted in a significant increase in the 1-DOPA production, by 30% more compared to the control group. Playing *Pioggia* resulted in decreased the 1-DOPA production by 50% at 60.5 dBA, 60% at 75.5 dBA, and 80% at 81 dBA ($p < .05$).

In Mehrafsar and Mokhtari (2018), four speakers were placed around the cell culture plate. A voice recording (*Surah Al-Fatima*, voice by *Sheikh Abdul Basit*) was played at 90 dB SPL for 2 h to the human cancer cell line (PC-3; Human Prostate Adenocarcinoma Cells). The cell viability, migration, and BCL2L12 gene expression level were reported. After the recorded audio was played to the cells, it was reported that 91.8% ($p < .05$) of PC-3 cells

Table 4. The List of Proposed Cellular Sensing Mechanisms or Type of Forces Induced by Sound Waves.

First author/date	Proposed cellular sensing mechanisms/ type of forces induced by sound waves at the cellular level
Shaobin et al. (2010)	<ul style="list-style-type: none"> Induced oscillation of cells Induced motion of internal fluid (inside the cells) Deformation of plasma membrane Increased membrane fluidity
Choi et al. (2016)	<ul style="list-style-type: none"> Mechanical stress on the membrane of the cells
Lestard and Capella (2016)	<ul style="list-style-type: none"> Mechanical stress on the cells
Shah et al. (2016)	<ul style="list-style-type: none"> Induced vibration within the cell medium (liquid) Different pressure distribution in membranes ("pressure profile of membrane") of different cell types
Sarvaiya and Kothari (2017)	<ul style="list-style-type: none"> Induced vibration within the cell media (liquid)
Ventura et al. (2017)	<ul style="list-style-type: none"> Induced oscillation of the cells
Mehrafsar and Mokhtari, (2018)	<ul style="list-style-type: none"> Mechanical stress on the cells
Algieri et al. (2018)	<ul style="list-style-type: none"> Induced oscillation of the cells
Kumeta et al. (2018)	<ul style="list-style-type: none"> Induced oscillation of the cells: leading to molecular and cytoskeleton sensing

survived, the migration level decreased to 95.4% ($p < .05$), and mRNA level of BCL2L12 decreased by a factor of 0.48 ($p < .001$).

Possible Cell Sensing Mechanisms of Sound

Many of the reviewed studies have proposed possible ways that cells sense mechanical cues due to sound stimulation. Some of the speculations reported are compared in Table 4.

In the subsequent sections, we will discuss some of the central issues from the selected studies.

Discussion

Many different types of sound (simple and complex), with an extensive range of audio frequency and SPL have been tested. The results reported for both simple and complex sound studies are promisingly beneficial. Some of the important observations include enhanced cell migration, proliferation, colony formation, and upregulation of certain genes and proteins or decrease in cancer cell migration and inducing apoptosis of cancer cells. However, it is both challenging to find a systematic pattern across studies and to compare the results to test for publication bias because the studies used different statistical analysis and effect sizes, sounds, experimental setups, sound measurement methods, and observation strategies. Below, we synthesize and discuss the results while considering the practical aspects of the studies (e.g., experimental setups and measurement methods) and the physics of sound.

Experimental Setup

Many of the reviewed studies were based on a speaker position above (Figure 2; scenarios 1 and 2, position a.), underneath (Figure 2; scenarios 1 and 2, position b.) or outside an incubator or specially constructed chamber for the cell cultures (Figure 2; scenario 1, position c.). The distances between the speaker and petri dish varied, ranging from

~0 (directly underneath the dish) to 200 cm. These speaker placements claimed to have resulted in cellular responses regardless of whether the speaker was placed outside or inside a specially constructed chamber or cell incubator. Whether the observed biological alterations are a direct effect of sound pressure waves is doubtful in some cases such as speaker position c in scenario 1 in Figure 2. As discussed earlier, there are several physical factors that contribute to the transmission loss of sound energy. This was discussed by one of the studies where the polystyrene cover of petri dishes and significant impedance mismatch between water and air were taken into consideration to estimate the transmission loss (Kumeta et al., 2018). Theoretically, the amount of acoustic energy that reaches the cells would be insignificant compared to the incident energy as elaborated above.

It would be of value to perform a systematic experiment of how different positioning of the sound source (the speaker) results in various types of mechanical force in the cell culture media. For example, whether a speaker is coupled (in direct contact) with the petri dish. Several of the reviewed studies have not clearly stated how the experimental environment was acoustically treated. Based on its physical properties, sonic vibrations may interact with the testing environment. Ideally, sound experiments should be performed in an acoustically controlled environment. For example, an anechoic chamber is designed to imitate the free field conditions (i.e., no reflected sound waves), and to allow no or minimum external sound to enter the chamber. Using such an environment can greatly improve the accuracy of acoustic measurements (González et al., 2018). Additionally, for practical reasons, speakers should ideally be placed inside or facing directly into an acoustically treated chamber. Another reason would be to prevent hearing damage of the researchers. For example, a continuous sine tone at a loudness level of 70 dB SPL or higher can cause temporary or even permanent hearing damage (Huber, 2010). Although, a full anechoic

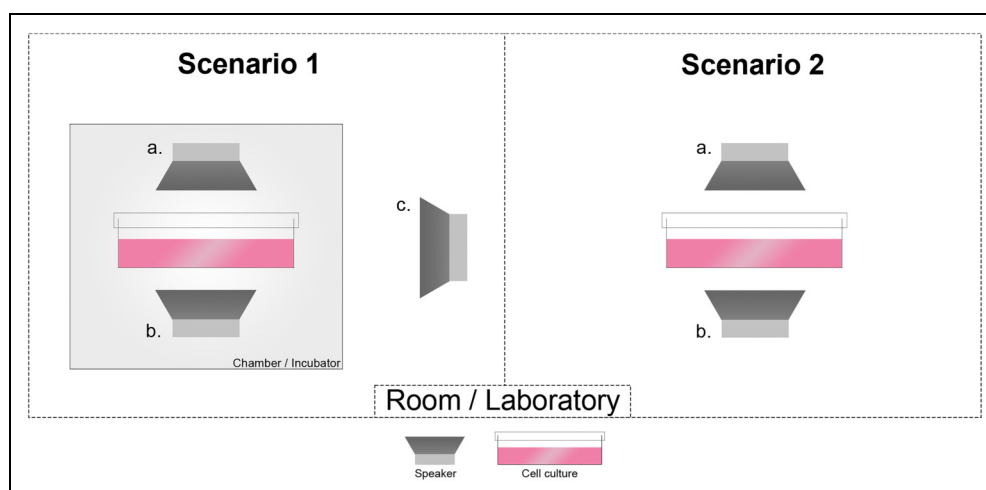


Figure 2. Different positions (scenarios) of the speaker used in the experiments.

chamber would not be feasible considering the cost and size, it would still be useful to try to reduce the acoustic impact of the environmental “noise” by using an acoustic damping device. In that way, it becomes more possible to measure and characterize how the sound behaves in an environment. This could also be used to address potential obstacles during experiments (e.g., amplitude distortion at specific frequencies, standing waves, and phase cancellation) and increase the chance to infer how different acoustic conditions are related to observed cellular responses.

Sound Features

We will look at four basic sound features here: frequency (Hz), sound pressure level (dB SPL), waveform (simple & complex), and rhythm. The overview of the studies is illustrated in Figure 3.

Frequencies. In the studies where single-frequency sound was used, a single frequency selected in the range of 55–10000 Hz were explored on bacteria cells (*Escherichia coli*, *Chromobacterium violaceum*, *Serratia marcescens*), mouse cells (ST2), and human cells (hBM-MSCs, LL24). In the studies where the more complex sounds were used, musical pieces or human voices over a wide frequency range were explored on a biological molecule (tyrosinase),

microorganisms (various prokaryotic & eukaryotic), bacteria cells (*Escherichia coli*), human cancer cells (MCF-7, MDA-MD-231), and human cells (hASCs).

To summarize, bacteria and yeast cells showed an effect of sound across a wide range of frequencies: cell colony-forming and growth rate increase using 100, 300, 1,000, 5,000, and 10,000 Hz (Aggio et al., 2012; Choi et al., 2016; Lestard & Capella, 2016). On the other hand, mammal cells showed a more selective and frequency-dependent effect. For example, a lower frequency (100 Hz) induced cell migration increase in human cells, but higher frequencies (above 1600 Hz) induced the opposite effect on cell migration (Mohammed et al., 2016). Also, a sine tone of 440 Hz induced gene (Ptgs2, CTGF, TNC) expression increase in mouse cells (ST2), while 55, 110, and 4,000 Hz showed a less or similar effect (Kumeta et al., 2018).

In the complex sound studies, the styles of music and human voice employed varied widely (Table 5). 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, varied 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 cellular responses. Regardless, a notable

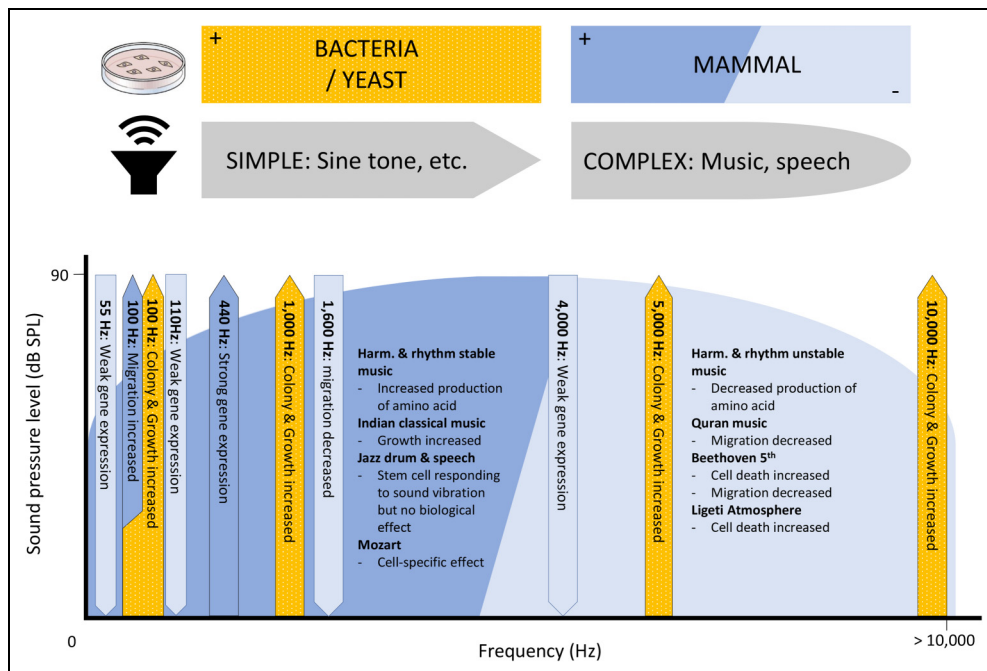


Figure 3. A simplified schematic diagram of the outcome of the studies. A wide range of sound frequency was tested (approximately between 20 and 10,000 Hz) with the sound level being around 90 dB SPL. The studies are divided into bacteria/yeast and mammal cells studies. The studies are further categorized into biological outcome as positive (upregulation) and negative (downregulation), and types of sound as simple (sine tone) and complex (music and speech). Since each experiment had a specific cell line and a setup, it was not possible to draw a full conclusion. However, the diagram provides an overview and some recognizable patterns in the result. Concerning these specific sets of outcomes, mammal cells showed more frequency dependency than bacteria cells. A group of studies of bacteria/yeast cells reported a general increase of colony-forming and growth rate over a wide range of frequencies, whereas, in mammal cell studies, the effects of the sound depended more on which frequency was used.

outcome was 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*. A musical composition with regular characteristics, *Reminiscenza*, resulted in 1-DOPA production increase. This piece of music is described, by Algieri et al. (2018), as having stability in terms of harmony and rhythm. The entire piece is based on a thetic rhythm and centered around 523.25 Hz (musical note: C5), 587.33 Hz (D5), and 659.26 Hz (E5). Another piece of music that had a contrasting characteristic, *Pioggia*, resulted in 1-DOPA production decrease. Unlike the first piece, *Pioggia* is described as more unstable and based on agogic accents

and rubato, and frequencies varied more 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) (Algieri et al., 2018). This indicates a possible role of the degree of pitch (frequency) and rhythm in the produced outcome.

Sound Pressure Level. In the investigated studies, although the sound pressure level range used varied extensively, from 40 to 110 dB SPL, the majority of the studies used the range between 75 and 95 dB SPL. The range centered around 90 dB SPL, which is about 632 mPa. This pressure level is small compared to the periodic blood flow pressure, for example, which can typically be around 120/80 mmHg (approximately 10–16 kPa) (Zhou et al., 2017). The transmitted sound energy in the cell media (liquid) from the air is estimated to be even smaller: approximately 29.67 mPa (when the temperature is 20 °C, $R \approx 0.9988$, transmitted sound energy from the air to water is $1 - R \approx 0.0022$ when there is no absorption) (Kumeta et al., 2018). This is a insignificant mechanical stress, but the cells have mechanisms that can sense such small forces and changes involving cytoplasmic molecules α -catenin, for example (Maki et al., 2016).

It should be noted that some of the SPLs specified in the literature are incomparable. This is because some of the reports were unclear about how the actual sound output was measured. Additionally, some of the commercially available SPL measurement devices typically come with filters that imitate the human hearing characteristics (less sensitive at very low and high frequencies within the audible frequency range), such as an A-weighting filter. There are other weightings, as illustrated in Figure 4

Table 5. The List of Music and Voice Styles From the Selected Studies.

First author	Type/style	Additional information
Lestard and Capella (2016)	Western classical music	Mozart, Beethoven, Ligeti
Sarvaiya and Kothari (2017); Shah et al. (2016)	Hindustani classical music	Ahir Bhairav, Piloo, Raag Malhar
Ventura et al. (2017)	African and Latin American Acting voice	Percussive music: Milford Graves Whisper and clang
Algieri et al. (2018)	Contemporary original music	<i>Reminiscenza</i> and <i>Pioggia</i>
Mehrafsar and Mokhtari (2018)	Quran recitation	<i>Surah Al-Fatina</i>

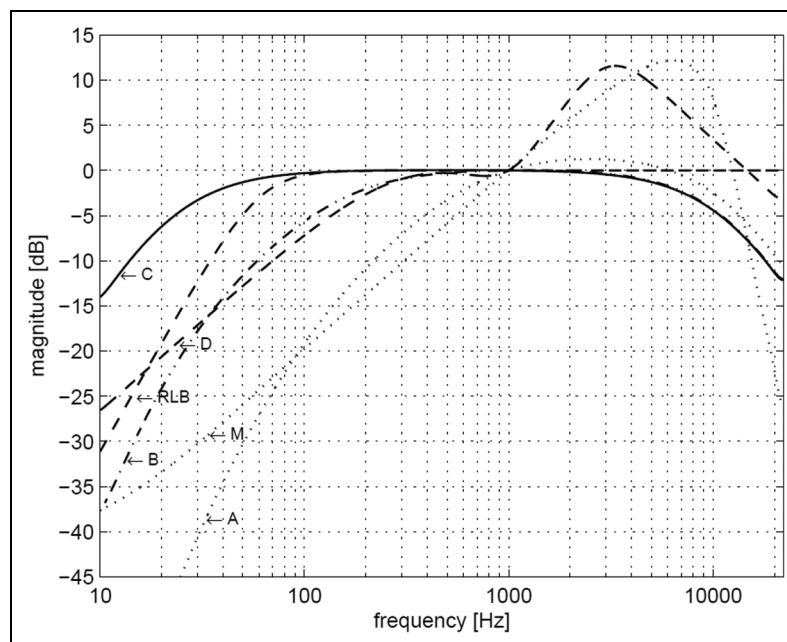


Figure 4. Frequency response of the A, B, C, D, M, and RLB frequency weighting filters. All the curves are aligned in level so that their magnitude response at 1 kHz is 0 dB. The figure is reproduced with permission (Skovenborg & Nielsen, 2004).

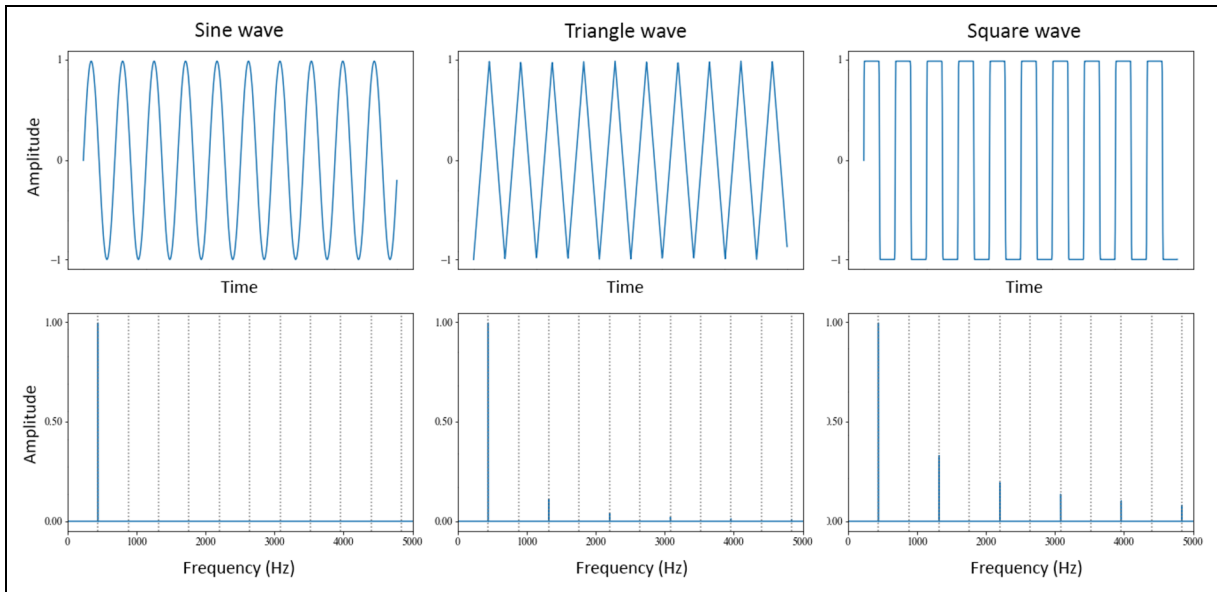


Figure 5. Comparisons between 440 Hz sine, triangle, and square waveforms (top) and Fourier Transforms (below).

(Skovenborg & Nielsen, 2004), designed for different applications. This is a small piece of information that may not be considered necessary, but it should be stated in the report, as a specific weighting filter readout can undermine a particular frequency range as shown.

Waveform. As pointed out earlier, the majority of the simple sound studies employed sine tones. There was only one study that compared different waveforms (triangle and square) and white noise. Interestingly, the different waveforms yielded contrasting outcomes in cellular responses (Kumeta et al., 2018). However, the underlying mechanism is not well understood. Sine waves do not have any harmonics (overtones), only the fundamental frequency. Triangle and square waves, on the other hand, contain odd harmonics of the fundamental frequency. The higher harmonics of the triangle wave get weaker faster than those of a square wave, as shown in Figure 5. When the amplitude and the frequency are the same, there is more sound energy in square waves and stronger harmonics than in sine and triangle waveforms. Although there is a need for further investigation, according to the differences we explore, a possible explanation of different outcomes between sine, triangle, square, and white noise could be the strength of harmonics present in the sound material.

Rhythms. Rhythm is one of the fundamental properties of music and is also an essential feature of biological life. The “synchronization of multiple rhythms” is vital in living organisms (Muehsam & Ventura, 2014), and rhythmicity as a parameter is underexplored. The human body is full of different rhythms, such as heart rate and breathing rhythms. If certain rhythms in the human body become irregular, it can be an indication of more severe health

problems, for example, abnormal respiration rhythms (Whited & Graham, 2020).

In the investigated simple sound studies, there were no rhythmic features in the sound. On the other hand, all the complex sound studies had an aspect of rhythm. The styles of rhythms varied between 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* (Figure 6a and b). 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 (Algieri et al., 2018). As discussed previously, 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, although other factors in the pieces of music could have contributed to the difference.

Other possible premises can be reflected upon when it comes to the rhythmic stimulation of *in vitro* cells. For instance, the regulation of intake of Ca^{2+} is a crucial factor for insulin secretion β -cells (Yang & Berggren, 2006), which is regulated by Ca^{2+} channels located in the cell membrane (Larsson-Nyrén et al., 2007). The Ca^{2+} intake of β -cells is known to be rhythmic (Idevall-Hagren & Tengholm, 2020), and the mechanical force can regulate the Ca^{2+} intake (Matthews et al., 2010; Yang et al., 2015). Therefore, it would be interesting to explore the question of whether a rhythmic mechanical stimulation can “rescue” irregular or un-synchronicity Ca^{2+} oscillation (Li et al., 2014; Yang et al., 2015) in unhealthy β -cells. Taken together, rhythmicity is a possible new avenue of sound stimulation of cells to be investigated further.

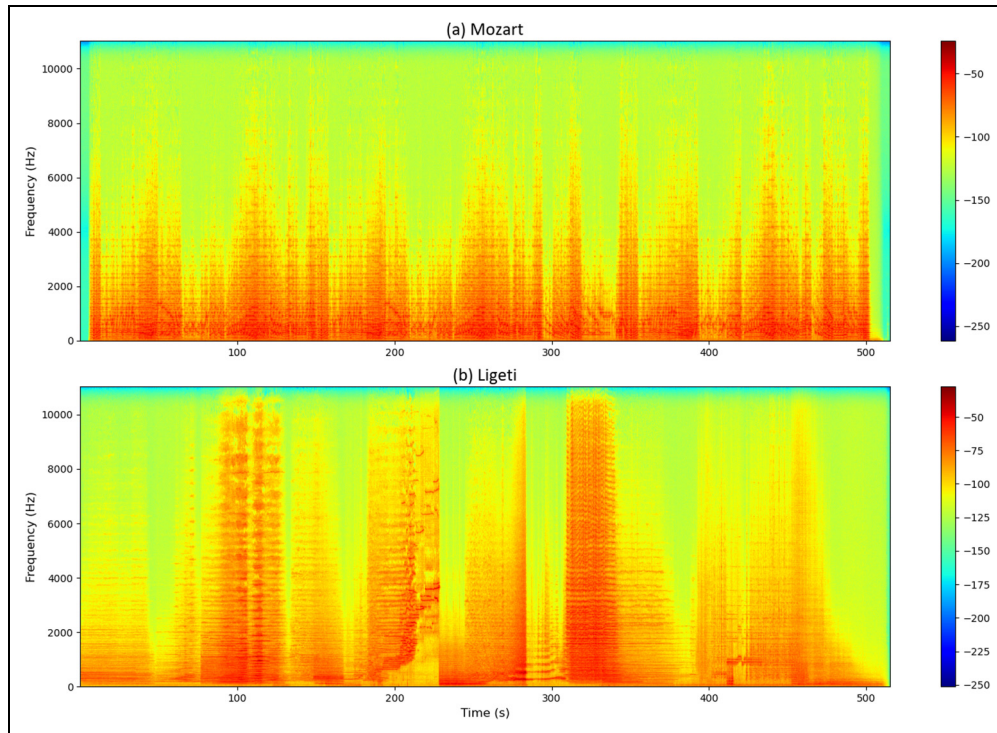


Figure 6. Spectrograms of (a) the first movement of Mozart's Sonata for Two Pianos in D Major, KV. 488 and (b) Ligeti's Atmospheres.

Cell Sensing Mechanisms of Sound

There have been some reports on cellular changes after a certain period of exposure to sound waves. Although several possible mechanisms have been suggested (Table 4), the studies in the current review unanimously acknowledged the lack of understanding of the mechanism by which cells are affected by sound waves. One of the aspects that needs more attention is a possible shear force generated by the flow of the fluid (cell culture media) within the petri dish when sound is played to the cell culture. Shah et al. (2016) and Sarvaiya et al. (2017) partly referred to a possible effect of shear force by suggesting that sound induces vibration within the cell culture media. Nevertheless, it is not clear whether fluid flow has any effect on the reported outcomes. Such mechanical oscillations could potentially have an impact on the cellular responses in addition to the sound waves (Kanie et al., 2019).

Conclusion

There appears to be promising effects of using audible sound on microorganisms and mammalian cells. However, 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 their acoustic environment. This makes it difficult to understand how the stimulation happened. In future research, therefore, we see the need for:

- **Controlled sonic environments:** An acoustically treated environment would make it easier to control the sound. This would reduce the level of external noise and avoid unwanted or uncontrolled reinforcement or cancelation of frequencies. What is being applied to the cells should ideally be the sound emitted directly from the sound source. The use of an anechoic chamber or appropriate acoustic treatment of the experimental environment is recommended with minimum acoustic barriers (e.g., incubator walls) between the sound source and the cell cultures.
- **Consistent measurement methods:** Due to the possible (sonic) influence of the environment, 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 appropriate SPL measurement method with a weighting filter should be used to estimate what the cells are stimulated with.
- **Broad and systematic synthesis of sound:** A more comprehensive range of controlled parameters of sound, or possibly music, should be tested. All the simple sounds used in the literature so far have been of a continuous nature. Considering the complexity of the ECM (environment) that the cells live in, learning the influence of pulsating or rhythmic stimulation on the cell cultures could also be valuable.

Many other possible non-sound related factors can alter the functionality of cells under examination (Goetzke et al.,

2018). Therefore, as to recommendations for future research in this multidimensional and uprising field, it is crucial to have the sound waves well under control and elucidate the practical issues. High priority should be given to investigating the optimal acoustic environment for cell stimulation that takes cell viability (e.g., temperature) into consideration. The 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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