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**Abstract:** Advances in cell and tissue therapies are slow to be implemented in the clinic due to the limited standardization of safety and quality control techniques. Current approaches for monitoring cell and tissue manufacturing processes are time and labor intensive, costly, and lack commercial scalability. One method to improving in vitro manufacturing processes includes utilizing the coupled magnetic and mechanical properties of magnetoelastic (ME) materials as passive and wireless sensors and actuators. Specifically, ME materials can be used in quantifying cell adhesion, detecting contamination, measuring biomarkers, providing biomechanical stimulus, and enabling cell detachment in bioreactors. This review outlines critical design considerations for ME systems and summarizes recent developments in utilizing ME materials for sensing and actuation in cell and tissue engineering.

Keywords: magnetoelastic materials; cell therapies; biosensors; actuators; tissue engineering



Citation: Meyers, K.M.; Ong, K.G. Magnetoelastic Materials for Monitoring and Controlling Cells and Tissues. *Sustainability* **2021**, *13*, 13655. https://doi.org/10.3390/ su132413655

Academic Editor: Changhyun Roh

Received: 25 October 2021 Accepted: 8 December 2021 Published: 10 December 2021

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#### 1. Introduction

Tissue engineering, cell therapies, and cell products (i.e., collagens, albumin, etc.) are critical components in regenerative medicine for replacing or repairing diseased and damaged tissues. With an increasing number of patients needing organ transplantation, the importance of these biotechnologies has considerably risen to address shortages in organ donor availability and reduce risks associated with allografts [1,2]. Despite positive initial results with in vivo studies, implementation of novel cell therapies in regenerative medicine applications are slow and limited in scale due to a lack of consistent cell and tissue manufacturing processes [3]. For example, current cell preparation methods are time and labor intensive, which hinders scalability to meet clinical demands [4]. As a result, the cost of cell and tissue-based therapies are often not commercially viable for the general public. In fact, it was estimated that the implementation of hematopoietic stem cells in autologous and allogenic transplantations were \$378,000 and \$930,000, respectively [5]. Additionally, current cell manufacturing techniques lack reliable and cost-effective quality control processes, which significantly reduces yield and quality since cell and tissue manufacturing techniques need to account for inter-donor cell heterogeneity [6]. This presents a need for technologies that can provide effective optimization and normalization of cell and tissue preparation methods to ensure the safety and efficacy of cell-based therapies [7,8].

Many existing systems for tracking cell quantification and quality in vitro utilize fluorescent staining, flow cytometry, and polymerases chain reaction (PCR) systems, but a lot of these methods are time intensive, costly, or lack real-time monitoring capabilities. Recently, impedance-based sensors were engineered to detect cellular and biological parameters [9,10]. However, many of these techniques require internal power sources or connecting wires, limiting their applications for isolated and sterile in vitro systems and bioreactors. Optical techniques have also been explored for monitoring cell manufacturing, but their performance is highly dependent on the homogeneity and optical properties of the medium. An effective approach that is currently being explored to counter these traditional methods includes using magnetoelastic (ME) materials as sensors and actuators to remotely monitor and control cells and tissues in real-time during in vitro manufacturing processes.

Common examples of ME materials include amorphous ferromagnetic materials like iron, nickel, chromium, phosphorous, boron, molybdenum alloys (Metglas 2826MB), crystalline iron-gallium alloys (Galfenol), and iron-terbium-dysprosium alloys (Terenol-D). These materials have high magentostrition and magnetoelastic coupling. For example, Metglas 2826 has a typical magnetostriction of 12 ppm and a magnetoelastic coupling as high as 0.98. Galfenol and Terfenol-D, on the other hand, have very high magnetostrition that can be greater than 300 ppm and 1000 ppm, respectively. Due to strong magnetoelastic coupling, the magnetic permeability (e.g., permeability of Metglas 2826MB can reach 800,000 after annealing) of these materials change with applied force, enabling them to quantify pressure (on the order of kilopascals) and strain by directly measuring variations in their magnetic signatures [11,12]. Additionally, ME materials undergo mechanical deformation (i.e., elongation and shrinkage) via rotation of magnetic domains within the material in the presence of a magnetic field [13,14]. Thus, by exposing them to an alternating magnetic field, these materials can be set to vibrate and act as actuators. Vibrations in ME materials can also be used to detect changes in mass loading by monitoring differences in resonance frequency and/or resonance quality [14,15].

Due to their ability to provide wireless mechanical sensing and actuation, ME materials are ideal for aseptic in vitro cell and tissue manufacturing applications. ME sensors have the potential to streamline cell manufacturing processes and make advances in cell therapies more clinically translatable by reliably quantifying cell adhesion in real-time, monitoring cells for quality control, detecting contamination (i.e., bacteria), assessing cell adhesion strength, and measuring pH, glucose, and other biochemical metrics in bioreactors [16] (Figure 1). Additionally, ME actuators can provide mechanical stimulus via cyclic, longitudinal loading to supply cell microenvironment biomechanical cues that are especially vital in stem cell differentiation. Furthermore, mechanical stimulus can be used for aiding cell detachment during harvesting processes and allow researchers to study the effects of mechanical loading on cells [17] (Figure 1). The relatively low-cost of ME materials also allows them to be easily integrated in disposable consumables often associated with cell and tissue manufacturing processes. In this review, design considerations for ME systems utilized in vitro are summarized and recent advancements in ME system fabrication and implementation for cell sensing and actuation purposes are explored.



**Figure 1.** (**A**) ME system containing a ME material seeded with cells, an excitation coil to provide magnetic stimulus and a receiving coil to measure resonance frequency shifts associated with mass loading. (**B**) ME materials used in in vitro applications can remotely quantify cell adhesion, (**C**) monitor for contamination (i.e., bacteria), (**D**) detect biomarkers (i.e., glucose, growth factors, etc.), (**E**) provide mechanical stimulus to cells, and (**F**) mediate cell detachment.

## 2. ME System Fabrication and Design Considerations for In Vitro Applications

When designing a ME system for in vitro applications, many mechanical and biochemical parameters can be tailored to optimize the platform for specific cell and tissue types. Some of the design aspects for ME resonance sensor systems are highlighted in detail below.

### 2.1. ME Substrate Materials

Since in vitro environments are humid and warm, metal ME sensors are subject to accelerated rates of corrosion and degradation. Specifically, corrosion and degradation of ME materials can affect their performance as resonance frequencies are partially dependent on the material mass [18]. ME materials are often coated with layers of highly unreactive metals including gold or chromium via sputtering or thermal evaporation to aid in corrosion prevention [19,20]. Compared to chromium, gold is slightly more active on the metal reactivity series, but generally cheaper in cost. However, hybrid coatings containing both chromium and gold layers have been successfully utilized in ME materials [21]. Metal coatings are typically applied on ME materials at a thickness on the order of hundreds of nanometers, however thickness can be easily optimized to balance the effects of coating on improving corrosion resistance and decreasing sensitivity due to a contribution in greater initial ME material mass [22].

While some ME materials are biocompatible, many are inherently toxic to cells and tissues as they are composted of cytotoxic elements such as nickel and molybdenum [18,23]. Therefore, bioinert polymer coating methods are typically utilized with these toxic ME materials to prevent adverse effects on cell viability [24]. Common coatings for ME materials include parylene and its derivatives, polyurethanes, and silanes. In metallic biomedical

implants, parylene coatings are most often used to provide electrical isolation and improve corrosion resistance due to their low water permeability, ease of application (i.e., conformation to complex geometries and simple control over coating thickness), and wear resistance properties [25]. Despite many advantages, parylene is difficult to functionalize with agents requiring aqueous solvents due to its hydrophobic character [25]. Polyurethanes are widely used in the medical field due to their highly biocompatible and abrasion resistant properties [26]. Specifically, Bayhydrol 110 (Covestro AG: Leverkusen, Germany), an aliphatic polyester urethane resin, is a common polymer coating used with ME materials to enable bacterial cell adhesion [27,28]. Silane-based coatings can also be used to prevent corrosion of ME materials and provide biofunctionalized surfaces [19]. The thickness of polymer coatings on ME materials typically ranges from one to tens of micrometers. Therefore, initial polymer mass loading effects on ME system sensitivity should also be considered. In fact, in one study parylene coating mass dramatically decreased resonance amplitude and quality in a linear fashion [29]. Both polymeric and metallic coatings can be combined on ME materials to prevent corrosion and improve cell adherence and viability. For example, Metglas 2826MB coated with platinum, gold, and Bayhydrol 110 had significantly increased cell viability (98%) when compared to samples only coated with platinum and gold (55%) after 48 h [21].

Polymer and metal coatings provide leaching and corrosion protection but are generally not conducive to cell attachment and growth. Therefore, physical, chemical, and biological surface functionalization of ME materials are employed to improve bioactivity and cell adherence. One common surface treatment method used with ME materials coated with parylene for cell applications includes oxygen plasma etching. With this approach, the physical and chemical properties of the polymer surface are altered with the generation of nanoporosity and hydrophilic oxygen-rich regions [30]. Therefore, plasma etching is typically utilized on polymer coated surfaces to improve cell adhesion [30,31]. However, the influence of plasma etching time on initial polymer mass loading should be accounted for, as longer etching periods leads to greater mass loss which subsequently influences resonance characteristics of the ME system [29]. Biochemical surface modification techniques provide more selectivity over physical approaches like plasma etching. For instance, the conjugation of antibodies and other bioactive elements can create ME systems that are conducive to adhesion for distinct cell types. One example of a highly specific biochemically functionalization ME sensor used bacteriophages to detect Salmonella typhimurium cells [32]. Biofunctionalized ME sensors are also beneficial for monitoring biological agents (i.e., nutrient, growth factor, and enzymatic levels) associated with cell culture environments or conditions. For example, ME sensors have been incorporated with biotin-functionalized polyethylene glycol (PEG) to detect avidin and horseradish peroxidase conjugation to quantify glucose levels [32–34]. These types of ME sensing platforms could be particularly useful in bioreactors for remote quality control in cell and tissue manufacturing.

To prevent non-specific protein adsorption from potentially affecting ME material performance, researchers have used passive blocking agents including natural proteins (i.e., bovine serum albumin (BSA) and chicken serum albumin (CSA)) and synthetic polymers (i.e., self-assembled monolayers (SAMs)) [35–37]. Coating ME surfaces with small-molecule natural proteins provides a physical barrier to avert non-specific protein adsorption, but desorption of the protein layer over time is a major limitation of this approach [37]. Implementing chemical surface modification techniques provides another means of restricting non-specific protein adsorption. A common antifouling method used in the medical field and with ME materials includes creating various types of hydrophilic SAMs and PEG to deter non-specific protein attachment [34,38]. Compared to protein blocking agents, synthetic polymer coatings are typically more robust, however they are still susceptible to oxidative damage over time [37]. As with other surface modification techniques, the effects of blocking agent coating mass should be considered and optimized when designing ME sensing and actuating systems. An overview of ME materials utilized

in vitro for sensing and actuating various cell types as well as material functionalization methods applied are listed here (Table 1).

Table 1. Example	s of ME	Materials	Used In	Vitro.
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Intended Use	Cell Type	ME Material	Functionalization Method	Detection Range	Reference
Sensor	L929 fibroblasts	Metglas 2826MB	Parylene and plasma etching	$\begin{array}{c} 10\times10^375\times10^3\\ \text{cells/sensor} \end{array}$	[39]
	Human breast cancer cells (MCF-7)	Metglas 2826MB	Bayhydrol 110	$\begin{array}{c} 50\times10^31\times10^6\\ \text{cells/mL} \end{array}$	[40]
	Salmonella typhimurium	Metglas 2826MB	Chromium, gold, and rabbit polyclonal antibody to S. typhimurium	10 <sup>5</sup> –10 <sup>9</sup> colony forming units/mL	[41,42]
	Escherichia coli	Metglas 2826MB	Gold and anti-E. coli antibodies	$10^2$ – $10^6$ cells/mL	[43]
	Pseudomonas aeruginosa	Metglas 2826MB	Bayhydrol 110	$10^{3}$ – $10^{6}$ cells/mL	[44]
Actuator	MC3T3 preosteoblasts	Cobalt ferrite	Polyvinylidene fluoride scaffold		[45]
	Human adipose derived stem cells	Magnetite	Kappa-carrageenan hydrogel		[46]
	L929 fibroblasts	Metglas 2826MB	Parylene and plasma etching		[29,47]
	Escherichia coli, Staphylococcus aureus, and Staphylococcus epidermidis	Metglas 2826MB	Parylene and plasma etching		[48]

#### 2.2. *ME Substrate Geometry*

ME materials used for cell and tissue engineering can have the form of flat sheets or particles depending on their intended application. Rectangular ME sheets are most commonly utilized individually or in arrays for sensing purposes while ME particles provide a simple method of forming magnetoactive composites. The physical geometry of ME substrates influences detection parameters for ME sensor applications. For instance, the resonance frequency of a ME material is inversely proportional to the dimension that is parallel to its vibration direction (typically along the length of rectangular ME substrates) [14], thus larger sensors may be preferred if there is an upper limit in operating frequency. Furthermore, due to the reduction in ME material volume, smaller sensors have lower signal amplitude and hence a shorter detection range, limiting their use in applications where the detection coil cannot be directly adjacent to the sensor. However, smaller and thinner ME sensors have been shown to exhibit lower detection limits and larger resonance frequency shifts for equal mass loading [41]. Therefore, smaller sensors may be advantageous for low-limit detection applications.

Investigations have also shown that the sensitivity to stress of a vibrating ME material varies with the location on the substrate's surface. In general, at the fundamental resonance frequency (the same frequency as the magnetic field that the sensor vibrates with), the middle of a ME material has the least vibration and sensitivity compared to the edges. However, the least-vibrating position of the ME material shifts from the middle to other geometric positions at different resonance modes (the substrate also vibrates at a frequency different from the applied magnetic AC field). The difference in the sensitivity across the surface of the ME substrate for different resonance modes can be utilized advantageously to detect numerous biological parameters [49,50]. For example, using different functionalization agents on specific areas of ME surfaces associated with different resonance modes could create a single platform for sensing multiple biomarkers, aiding in quality control during cell and tissue manufacturing processes. Altering the geometrical shape and aspect ratio of a ME material can also improve overall sensitivity. For instance, using the tips of triangular shaped ME sensors can increase sensitivity when compared to the ends of rectangular ME sensors with equal mass loading [50].

#### 2.3. ME Excitation and Receiving System

Utilizing electromagnetic coils to transmit and receive magnetic signals provides ME materials with their unique ability to act as passive and wireless sensors and actuators. Typically, a ME substrate excitation/detection system is composed of an excitation and detection coil. A frequency-varying AC current is usually produced by a signal generator and passed through the excitation coil to create the excitation field, while the detection coil is ether connected to a spectrum analyzer, oscilloscope, or a lock-in amplifier to measure the generated magnetic field from the substrate at each frequency point (Figure 2). Optimizing the number of turns and wire diameter for in vitro ME excitation and detection systems should be performed and error produced by potential variations in alignment and distance between coils and ME material should be considered [51]. While the ME substrate can be detected using separate excitation/detection coils, some systems combine these two coils into a single coil and use an electronic relay/switch to separate the excitation/detection phase into their respective circuits. Although the single-coil design has a smaller footprint and generally requires lower-power, it does not allow for optimization of coil dimensions for excitation and detection, thus limiting detection ranges with larger substrates [14].



**Figure 2.** Overview of ME excitation/detection system design. (**A**) A driving AC electric signal of varying frequency is produced by a signal generator. (**B**) ME excitation and detection coils (either combined or separate) send and receive the electromagnetic signals to and from the ME sensor containing adhered cells or other biologics. (**C**) The ME sensor response can be measured by either a spectrum analyzer, oscilloscope, or lock-in amplifier.

### 2.4. Additional In Vitro Design Considerations

For cell and tissue manufacturing applications, ME materials will typically be kept at a relatively constant temperature (37 °C) so the possibility of variations in temperature affecting sensor performance is less critical. However, if needed, altering biasing fields can compensate for changes in temperature as they shift the base resonance frequency of the sensors [15,52,53]. In a bioreactor, ME sensors and actuators utilized with cells and tissues The presence and consumption of these elements can lead to changes in solution viscosity which can alter ME material performance since resonance frequency is decreased as the viscosity of fluid increases [14]. To compensate for changes in viscosity, an unfunctionalized ME surface can be utilized to compare shifts in resonance behavior with biofunctionalized ME sensors containing the target of interest. Sterilization techniques utilizing ethylene oxide or heat have been previously used on ME materials designed to be implemented for aseptic in vitro environments. Selecting an appropriate sterilization procedure for a ME sensor or actuator depends on how the material was surface treated. For example, a biologically functionalized ME sensor should not be exposed to excessive heat during sterilization to prevent protein degradation.

## 3. ME Materials for Cell Sensing

Detecting cell adherence and tracking cell counts in vitro can be used as means of measuring cell proliferation in cell manufacturing technologies and drug screening applications. The mechanisms involved in ME sensor detection and some examples of using ME sensors to monitor mammalian and bacterial cells are described in the following sections.

#### 3.1. ME Material Sensing Mechanisms

Changes in resonance frequency associated with alterations in mass loading of ME sensors can be correlated to cell adherence number to provide a platform for quantifying cell proliferation. Specifically, frequency sweeps performed on ME devices can detect resonance frequency shifts that linearly decrease with increased mass loading and changes in resonance amplitude which decreases with increased mass loading [14]. Monitoring alterations in ME sensor phase of impedance at resonance frequency provides another method of quantifying cell number. ME sensors can also be designed to detect particular cell types by conjugating their surfaces with cell-specific antibodies [54].

### 3.2. Sensing Mammalian Cells

One example of using ME sensors to detect cell adherence in vitro includes monitoring the attachment of L929 fibroblast cells [39]. In this study, annealed Metglas was parylene coated and plasma etched to produce a cell counting platform. This device rapidly quantified cell number based on the phase of impedance at the resonance frequency of the sensor. The ME detection system was able to provide a linear sensitivity range from 10,000 to 75,000 cells per sensor when tested with varying cell densities of L929 cells. Changes in cell adherence were also monitored in real-time with the sensor using various cell seeding densities over 24 h. By generating cell-specific calibration curves, the versatile ME device could be utilized with other cell types needed in tissue manufacturing technologies.

In addition to tracking cell adherence, ME sensors can also be used in vitro to detect cell detachment in drug screening applications. For example, one study utilized ME devices to determine breast cancer cell proliferation in response to various chemotherapy drug types and concentrations [40]. Metglas coated with Bayhydrol provided a system for detecting human breast cancer cell (MCF-7) attachment and proliferation based on changes in ME sensor resonance amplitude (Figure 3A). Results indicated that with increasing cell adhesion, the greater the change in resonance amplitude of the sensor in a linear fashion over a range of  $5 \times 104$  to  $1 \times 106$  cells/mL (Figure 3B). When evaluating the effects of anti-cancer drugs fluorouracil and cisplatin, the ME sensor successfully monitored dose-dependent cell detachment (Figure 3C,D). Furthermore, data gathered from the ME device was able to show the fluorouracil concentration at which the maximum inhibitory efficiency was achieved. Therefore, this ME system could be combined with patient-specific cells to create a platform to determine effective dosing parameters for cancer drug screening and lab-on-a-chip applications. In cell and tissue manufacturing, monitoring cell detachment



could also indicate how well or how long cell removal techniques (i.e., trypsinization) are performed.

**Figure 3.** (**A**) ME detection system for monitoring changes in breast cancer cell proliferation in response to chemotherapy drugs. (**B**) A linear relationship between cell seeding density and changes in sensor resonance amplitude was used to generate a calibration curve. (**C**,**D**) Changes in ME sensor resonance amplitude indicating the dose-dependent drop in cancer cell adherence with fluorouracil and cisplatin anti-cancer drugs [40]. Reprinted from Biosensors and Bioelectronics, 24(2), Xilin Xiao et al., In-situ monitoring of breast cancer cell (MCF-7) growth and quantification of the cytotoxicity of anticancer drugs fluorouracil and cisplatin, 247–252, 2008, with permission from Elsevier.

## 3.3. Sensing Bacterial Cells

Identifying cell contamination is vital in quality control during cell and tissue manufacturing processes. Multiple ME platforms have been developed to monitor for bacteria in the food quality industry [41,42]. One example of an ME sensor that could be used to assess for bacterial contamination related to cell manufacturing processes includes gold-coated Metglas conjugated with anti-*Escherichia coli* (*E. coli*) antibodies [43]. The platform created a sandwich enzyme-linked immunosorbent assay with adhered *E. coli* cells and utilized subsequent biocatalytical precipitates to amplify changes in mass to detect cell adherence. Results indicated that shifts in resonance frequency linearly decreased with logarithmic values of *E. coli* cell number over 102 to 106 CFU/mL *E. coli*. This approach to detecting contamination could potentially be modified to monitor for multiple types of bacterial cells or cell quality control during cell and tissue manufacturing processes.

Another type of deadly antibiotic-resistant bacteria that is common in clinical environments associated with moisture (i.e., ventilator breathing tubes or catheters) includes *Pseudomonas aeruginosa* (*P. aeru*). A ME sensor designed to detect *P. aeru* based upon its growth behavior in culture has been developed [44]. This platform utilized Metglas coated with Bayhydrol to detect shifts in resonance frequency associated with bacterial cell attachment. A linear relationship between the logarithmic value of *P. aeru* concentration and changes in resonance frequency was identified over a range of 103 to 108 cells/mL. Bacterial consumption of nutrients in culture media also provided another means of tracking *P. aeru* proliferation with the ME sensor since decreasing the viscosity increased the apparent resonance frequency of the device. Studying shifts in the real-time growth curves of cells

in culture with ME sensors could supply another method for determining if bacterial contamination is present in cell and tissue manufacturing applications.

## 4. ME Materials for Cell Mechanical Stimulation

Many cells in bone, cartilage, and cardiac tissues undergo cyclic mechanical loading during normal physiological processes. Since these biomechanical cues guide cell growth and differentiation, it is vital that cell manufacturing methods consider both passive (i.e., substrate stiffness) and active (i.e., shear, compressive or tensile loading) elements to ensure cells exhibit standard phenotypical behavior [55]. Controlling mechanical stimulus with ME material vibrations can facilitate normal cell growth in addition to aiding in cell detachment in vitro. Examples of utilizing cell mechanical stimulation to control biomechanical cues and cell adherence are outlined below.

#### 4.1. ME Material Vibration Mechanisms

Appling an alternating magnetic field to an ME material causes sub-micrometer to micrometer-sized vibrations which can be transmitted to cells directly adhered to the ME substrate or indirectly through cell adherent coatings or scaffolds. Varying the magnitude, frequency, or duration of ME actuation could provide controlled biomechanical stimulus or aid in cell or tissue detachment.

#### 4.2. Controlling Biomechanical Cues

Biomechanical cues and cell loading has been shown to guide the differentiation of mechanosensitive mesenchymal stem cells into bone, cartilage, tendon, adipose, or muscle tissues [55–57]. ME actuators can supply submicron vibrations that mimic cyclic loading found in many connective tissues. In one study, a ME composite scaffold consisting of poly(vinylidene fluoride) (PVDF) incorporating cobalt ferrite nanoparticles (CFO) was utilized as a magnetoactive scaffold for bone tissue engineering [45]. This system was able to mimic trabecular bone pore microstructure by solvent casting the PVDF component and provide biomechanical stimuli to preosteoblasts (MC3T3s) seeded on the scaffold with the inclusion of magnetoelastic CFO particles (Figure 4A). Additionally, the composite could provide electrical stimulus through the electromechanical coupling of the magnetoelastic CFO particles and the piezoelectric properties of the  $\beta$ -phase of PVDF. In biocompatibility studies, scaffolds containing only PVDF and PVDF with CFO particles were compared to confirm that potential cytotoxic CFO nanoparticle leaching did not lead to cell death over relatively short (24 h) and long (7 days) timepoints (Figure 4B) [58,59]. With the application of a dynamic magnetic field, MC3T3 proliferation significantly increased on scaffolds with various pore size (60, 80, and 120  $\mu$ m) indicating that magnetoelastic and piezoelectric effects of the scaffold facilitated bone cell growth (Figure 4C).

In addition to bone tissue engineering, ME composites have been utilized to grow cartilage tissues from human stem cells [46]. Specifically, magnetite nanoparticles (MNPs) were incorporated in a carrageenan-based hydrogel where an external magnetic field could elicit actuation. Human adipose stem cells (hASCs) blended into the hydrogel structure showed an order of magnitude greater expression of chondrogenic markers (Collagen I, Collagen II, and Sox9) over 2 weeks with the application of magnetic actuation compared to control hydrogels without MNPs. These results confirm that mechanical stimulus provided by ME materials can assist in stem cell differentiation in vitro.



**Figure 4.** (A) Schematic showing ME composite scaffold containing a porous PVDF matrix and CFO nanoparticles that can produce local magneto-mechanical and magneto-electrical effects in the presence of a dynamic magnetic field. (B) No differences in cell viability among scaffolds with and without CFO particles was observed over 24-h and 7-day time periods. (C) Cell proliferation on scaffolds of all pore size (60, 80, and 120 µm) was significantly increased under dynamic magnetic field conditions when compared to static controls (\* p < 0.01) (\*\* p < 0.05) [45]. Reprinted with permission from Fernandes, M.M., et al., Bioinspired Three-Dimensional Magnetoactive Scaffolds for Bone Tissue Engineering. ACS Appl Mater Interfaces, 2019. 11(48): p. 45265–45275. Copyright 2019 American Chemical Society.

## 4.3. Controlling Cell Detachment

Using ME actuators in vitro could be useful in generating on-demand cell detachment and removing bacterial contamination in cell manufacturing processes. Modulating L929 fibroblast detachment with ME vibrations was achieved in a study utilizing Metglas coated with parylene [29,47]. Vibrations from the ME actuator caused a significant decrease in cell attachment in a vibration amplitude-dependent manner (Figure 5A–D). The effect of ME actuation on decreasing cell attachment was also able to be replicated after relatively short (6 h) and long (48 h) time periods from cell seeding indicating that ME vibrations could promote detachment at various stages of cell adhesion (Figure 5E). It was also determined that cell proliferation could be significantly reduced with the application of mechanical stimulus from the ME actuator, providing a potential method of controlling cell growth rate in vitro for manufacturing processes.

ME vibrations can also be utilized to remove and inhibit bacterial cells from colonizing on various surfaces. One study investigated the effects of magnetically controlled vibrations on parylene and poly-L-lactide (PLLA) coated ME materials that were contaminated with various bacterial species [48]. Initial results indicated that in the absence of magneto-mechanical stimulus, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Staphylococcus epidermis* (*S. epidermis*) adhered in greater quantity to the PLLA coatings when compared to parylene coatings. With the application of sub-micron vibrations, parylene coated ME materials could significantly reduce the adhesion of *S. aureus* and *S. epidermis* by 50% whereas PLLA coated ME materials could significantly reduce all bacterial species tested by about 90%. Therefore, ME actuators could be utilized in cell and



tissue manufacturing technologies to prevent unwanted cell adherence in certain regions of bioreactors.

**Figure 5.** Live/dead images of L929 fibroblasts seeded on a ME actuator with (**A**) no vibrations and vibrations with amplitudes of (**B**) 0.10 and (**C**) 0.15 $\mu$ m. (**D**) Cell adhesion was dependent on vibration amplitude in a dose-dependent manner (\* *p* < 0.05 vs. all other groups) († *p* < 0.05 vs. all other groups). (**E**) Cell adhesion could be significantly reduced with ME vibrations after relatively short (6 h after seeding) and long (48 h after seeding) time periods (\* *p* < 0.05 vs. non-vibrated controls) [47]. Reprinted from Journal of Biomechanics, 71, Hal Holmes et al., Control of cellular adhesion and myofibroblastic character with sub-micrometer magnetoelastic vibrations. 199–207., 2018, with permission from Elsevier.

#### 5. Future Outlook

Initial results for in vitro ME systems appear promising for applications in cell and tissue manufacturing, however there are still some critical impediments to progress before these technologies can be fully executed in the medical field and incorporated in the standardization of tissue engineering techniques. Many of the ME systems reviewed are designed to be implemented in 2D culture settings. Therefore, developing innovative methods to interface ME materials with 3D culture techniques and bioreactors presents an area for improvement. More work in modifying ME sensors to specifically monitor cell quality control, which is needed to ensure cell and tissue cultures are producing consistent results during manufacturing, also needs to be performed. For example, quality control can be achieved with ME materials designed to identify contaminants such as bacteria or detect biomarkers associated with cell aging or mutation. Additionally, tissue engineering can encompass heterogenous cell populations so creating ME sensors that can identify and collect information from different cell types should be explored. One approach to monitoring various cell populations with ME materials includes developing arrays of ME sensors to detect multiple parameters (i.e., temperature, pH, growth factors, antibodies, ion concentrations) and cell types in bioreactors [53]. Another method to ensuring quality control during cell and tissue manufacturing includes the development of novel magnetoelastic microcarriers to monitor bioreactor conditions through the detection of cells and chemical biomarkers. These microsensors can be fabricated at different lengths to output distinct resonance frequencies and chemically functionalized to provide sensing information on a wide variety of biomarkers. This technology platform has the potential to provide simultaneous monitoring of multiple indicators of cell quality control while not disturbing manufacturing processes. Examining the evolution of sensors in the food safety

and quality industry may also help guide the advancement of ME materials for cell and tissue manufacturing processes.

# 6. Conclusions

ME sensor and actuator technologies can help address the rising demand for more cost-effective tissue engineering and cell products by improving the commercially scalability of cell manufacturing processes. Some advantages of using ME systems for in vitro applications include their wireless, passive nature as well as their relatively low cost compared to current methods used to monitor quality control in cell and tissue manufacturing. Specifically, ME sensors can detect many metrics to quantify cell proliferation, while ME actuators can provide mechanical stimulation to aid in cell growth, differentiation, and detachment. Proper selection and optimization of ME material functionalization methods and ME excitation and detection systems are vital to ensuring the proper function of sensors and actuators. Future developments in applying ME materials with 3D tissue culture techniques and generating multifunctional ME sensor arrays to identify different cell types and biomarkers simultaneously are anticipated.

**Author Contributions:** Writing—original draft preparation, K.M.M.; writing—review and editing, K.M.M. and K.G.O.; supervision, K.G.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

## References

- Bastani, B. The present and future of transplant organ shortage: Some potential remedies. *J. Nephrol.* 2020, 33, 277–288. [CrossRef]
  Goradel, N.H.; Hour, F.G.; Negahdari, B.; Malekshahi, Z.V.; Hashemzehi, M.; Masoudifar, A.; Mirzaei, H. Stem Cell Therapy:
- A New Therapeutic Option for Cardiovascular Diseases. J. Cell. Biochem. 2018, 119, 95–104. [CrossRef]
- 3. Rafiq, S.; Hackett, C.S.; Brentjens, R.J. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat. Rev. Clin. Oncol.* 2020, *17*, 147–167. [CrossRef] [PubMed]
- 4. Robb, K.P.; Fitzgerald, J.C.; Barry, F.; Viswanathan, S. Mesenchymal stromal cell therapy: Progress in manufacturing and assessments of potency. *Cytotherapy* **2019**, *21*, 289–306. [CrossRef] [PubMed]
- 5. Driscoll, D.; Farnia, S.; Kefalas, P.; Maziarz, R.T. Concise Review: The High Cost of High Tech Medicine: Planning Ahead for Market Access. *Stem Cells Transl. Med.* **2017**, *6*, 1723–1729. [CrossRef] [PubMed]
- Phinney, D.G.; Galipeau, J.; MSC Committee of the International Society of Cell and Gene Therapy. Manufacturing mesenchymal stromal cells for clinical applications: A survey of Good Manufacturing Practices at U.S. academic centers. *Cytotherapy* 2019, 21, 782–792. [CrossRef]
- 7. Lukomska, B.; Stanaszek, L.; Zuba-Surma, E.; Legosz, P.; Sarzynska, S.; Drela, K. Challenges and Controversies in Human Mesenchymal Stem Cell Therapy. *Stem Cells Int.* **2019**, *2019*, 9628536. [CrossRef]
- 8. Pigeau, G.M.; Csaszar, E.; Dulgar-Tulloch, A. Commercial Scale Manufacturing of Allogeneic Cell Therapy. *Front. Med.* **2018**, *5*, 233. [CrossRef]
- 9. Velasco-Garcia, M.N. Optical biosensors for probing at the cellular level: A review of recent progress and future prospects. *Semin. Cell Dev. Biol.* 2009, 20, 27–33. [CrossRef]
- 10. Couniot, N. Capacitive biosensing of bacterial cells: Analytical model and numerical simulations. *Sens. Actuators B Chem.* **2015**, 211, 428–438. [CrossRef]
- 11. Tan, E.L.; DeRouin, A.J.; Ong, K.G. Magnetoelastic-Harmonic Stress Sensors With Tunable Sensitivity. *IEEE Sens. J.* 2012, 12, 1878–1883. [CrossRef]
- 12. Tan, E.L.; DeRouin, A.J.; Pereles, B.D.; Ong, K.G. Design, fabrication, and implementation of a wireless, passive implantable pressure sensor based on magnetic higher-order harmonic fields. *Biosensors* **2011**, *1*, 134–152. [CrossRef]
- 13. Bras, Y.L. Magneto-Elastic Resonance: Principles, Modeling and Applications; IntechOpen: London, UK, 2017.
- 14. Grimes, C. Wireless Magnetoelastic Resonance Sensors: A Critical Review. Sensors 2002, 2, 294–313. [CrossRef]
- 15. Ren, L.; Yu, K.; Tan, Y. Applications and Advances of Magnetoelastic Sensors in Biomedical Engineering: A Review. *Materials* **2019**, *12*, 1135. [CrossRef]

- 16. Zeng, K. Wireless Magnetoelastic Physical, Chemical, and Biological Sensors. IEEE Trans. Magn. 2007, 43, 2358–2363. [CrossRef]
- 17. D'Angelo, F.; Tiribuzi, R.; Armentano, I.; Kenny, J.M.; Martino, S.; Orlacchio, A. Mechanotransduction: Tuning stem cells fate. *J. Funct. Biomater.* **2011**, *2*, 67–87. [CrossRef]
- 18. Holmes, H. Biodegradation and biocompatibility of mechanically active magnetoelastic materials. *Smart Mater. Struct.* **2014**, 23, 095036. [CrossRef]
- 19. Liu, R. High sensitivity detection of human serum albumin using a novel magnetoelastic immunosensor. *J. Mater. Sci.* 2019, 54, 9679–9688. [CrossRef]
- Beltrami, L.V.; Kunst, S.R.; Birriel, E.J.; Malfatti, C.D. Magnetoelastic biosensors: Corrosion protection of an FeNiMoB alloy from alkoxide precursors. *Thin Solid Film.* 2017, 624, 83–94. [CrossRef]
- Vlaisavljevich, E.; Janka, L.P.; Ong, K.G.; Rajachar, R.M. Magnetoelastic Materials as Novel Bioactive Coatings for the Control of Cell Adhesion. *IEEE Trans. Bio-Med. Eng.* 2011, 58, 698–704. [CrossRef] [PubMed]
- Huang, S.; Hu, J.; Wan, J.; Johnson, M.L.; Shu, H.; Chin, B.A. The effect of annealing and gold deposition on the performance of magnetoelastic biosensors. *Mater. Sci. Eng. C* 2008, 28, 380–386. [CrossRef]
- DeRouin, A.; Guillory, R.; He, W.L.; Frost, M.; Goldman, J.; Ong, K.G. Magnetoelastic galfenol as a stent material for wirelessly controlled degradation rates. J. Biomed. Mater. Res. B 2019, 107, 232–241. [CrossRef] [PubMed]
- 24. Menti, C.; Beltrami, M.; Possan, A.L.; Martins, S.T.; Henriques, J.A.P.; Santos, A.D.; Missell, F.P.; Roesch-Ely, M. Biocompatibility and degradation of gold-covered magneto-elastic biosensors exposed to cell culture. *Colloids Surf. B Biointerfaces* **2016**, *143*, 111–117. [CrossRef] [PubMed]
- 25. Golda-Cepa, M.; Engvall, K.; Hakkarainen, M.; Kotarba, A. Recent progress on parylene C polymer for biomedical applications: A review. *Prog. Org. Coat.* **2020**, *140*, 105493. [CrossRef]
- 26. Das, A.M.; Mahanwar, P. A brief discussion on advances in polyurethane applications. *Adv. Ind. Eng. Polym. Res.* 2020, *3*, 93–101. [CrossRef]
- 27. Huang, S.J.; Wang, Y.J.; Ge, S.T.; Cai, Q.Y.; Grimes, C.A. Quantification of Staphylococcus epidermidis using a wireless, massresponsive sensor. *Sens. Actuators B Chem.* **2010**, *150*, 412–416. [CrossRef]
- Pang, P.; Cai, Q.; Yao, S.; Grimes, C.A. The detection of Mycobacterium tuberculosis in sputum sample based on a wireless magnetoelastic-sensing device. *Talanta* 2008, 76, 360–364. [CrossRef]
- Holmes, H.R.; Tan, E.L.; Ong, K.G.; Rajachar, R.M. Fabrication of biocompatible, vibrational magnetoelastic materials for controlling cellular adhesion. *Biosensors* 2012, 2, 57–69. [CrossRef]
- Golda, M.; Brzychczy-Wloch, M.; Faryna, M.; Engvall, K.; Kotarba, A. Oxygen plasma functionalization of parylene C coating for implants surface: Nanotopography and active sites for drug anchoring. *Mater. Sci. Eng. C* 2013, 33, 4221–4227. [CrossRef]
- Golda-Cepa, M.; Kulig, W.; Cwiklik, L.; Kotarba, A. Molecular Dynamics Insights into Water-Parylene C Interface: Relevance of Oxygen Plasma Treatment for Biocompatibility. ACS Appl. Mater. Interfaces 2017, 9, 16685–16693. [CrossRef]
- 32. Lakshmanan, R.S.; Guntupalli, R.; Hu, J.; Petrenko, V.A.; Barbaree, J.M.; Chin, B.A. Detection of Salmonella typhimurium in fat free milk using a phage immobilized magnetoelastic sensor. *Sens. Actuators B Chem.* **2007**, *126*, 544–550. [CrossRef]
- Pang, P. Determination of glucose using bienzyme layered assembly magnetoelastic sensing device. Sens. Actuators B Chem. 2009, 136, 310–314. [CrossRef]
- 34. Grimes, C.A.; Roy, S.C.; Rani, S.; Cai, Q. Theory, instrumentation and applications of magnetoelastic resonance sensors: A review. *Sensors* **2011**, *11*, 2809–2844. [CrossRef]
- Shen, W.; Li, S.Q.; Park, M.K.; Zhang, Z.W.; Cheng, Z.Y.; Petrenko, V.A.; Chin, B.A. Blocking Agent Optimization for Nonspecific Binding on Phage Based Magnetoelastic Biosensors. J. Electrochem. Soc. 2012, 159, B818–B823. [CrossRef]
- 36. Riquelme, M.V. Optimizing blocking of nonspecific bacterial attachment to impedimetric biosensors. *Sens. Bio-Sens. Res.* **2016**, *8*, 47–54. [CrossRef]
- 37. Lichtenberg, J.Y.; Ling, Y.; Kim, S. Non-Specific Adsorption Reduction Methods in Biosensing. Sensors 2019, 19, 2488. [CrossRef]
- 38. Park, M.K.; Chin, B.A. Novel Approach of a Phage-Based Magnetoelastic Biosensor for the Detection of Salmonella enterica serovar Typhimurium in Soil. *J. Microbiol. Biotechnol.* **2016**, *26*, 2051–2059. [CrossRef] [PubMed]
- Shekhar, S.; Karipott, S.S.; Guldberg, R.E.; Ong, K.G. Magnetoelastic sensors for real-time tracking of cell growth. Biotechnol. Bioeng. 2021, 118, 2380–2385. [CrossRef] [PubMed]
- Xiao, X.; Guo, M.; Li, Q.; Cai, Q.; Yao, S.; Grimes, C.A. In-situ monitoring of breast cancer cell (MCF-7) growth and quantification of the cytotoxicity of anticancer drugs fluorouracil and cisplatin. *Biosens. Bioelectron.* 2008, 24, 247–252. [CrossRef]
- Guntupalli, R.; Hu, J.; Lakshmanan, R.S.; Huang, T.S.; Barbaree, J.M.; Chin, B.A. A magnetoelastic resonance biosensor immobilized with polyclonal antibody for the detection of Salmonella typhimurium. *Biosens. Bioelectron.* 2007, 22, 1474–1479. [CrossRef]
- Guntupalli, R.; Lakshmanan, R.S.; Hu, J.; Huang, T.S.; Barbaree, J.M.; Vodyanoy, V.; Chin, B.A. Rapid and sensitive magnetoelastic biosensors for the detection of Salmonella typhimurium in a mixed microbial population. *J. Microbiol. Methods* 2007, 70, 112–118. [CrossRef]
- Ruan, C.; Zeng, K.; Varghese, O.K.; Grimes, C.A. Magnetoelastic immunosensors: Amplified mass immunosorbent assay for detection of Escherichia coli O157:H7. Anal. Chem. 2003, 75, 6494–6498. [CrossRef]
- Pang, P.; Huang, S.; Cai, Q.; Yao, S.; Zeng, K.; Grimes, C.A. Detection of Pseudomonas aeruginosa using a wireless magnetoelastic sensing device. *Biosens. Bioelectron.* 2007, 23, 295–299. [CrossRef]

- 45. Fernandes, M.M.; Correia, D.M.; Ribeiro, C.; Castro, N.; Correia, V.; Lanceros-Mendez, S. Bioinspired Three-Dimensional Magnetoactive Scaffolds for Bone Tissue Engineering. *ACS Appl. Mater. Interfaces* **2019**, *11*, 45265–45275. [CrossRef]
- 46. Popa, E.G.; Santo, V.E.; Rodrigues, M.T.; Gomes, M.E. Magnetically-Responsive Hydrogels for Modulation of Chondrogenic Commitment of Human Adipose-Derived Stem Cells. *Polymers* **2016**, *8*, 28. [CrossRef]
- Holmes, H.R.; Vlaisavljevich, E.; Tan, E.L.; Snyder, K.L.; Ong, K.G.; Rajachar, R.M. Control of cellular adhesion and myofibroblastic character with sub-micrometer magnetoelastic vibrations. J. Biomech. 2018, 71, 199–207. [CrossRef]
- 48. Paces, W.R.; Holmes, H.R.; Vlaisavljevich, E.; Snyder, K.L.; Tan, E.L.; Rajachar, R.M.; Ong, K.G. Application of sub-micrometer vibrations to mitigate bacterial adhesion. *J. Funct. Biomater.* **2014**, *5*, 15–26. [CrossRef]
- 49. DeRouin, A. Multi-parameter sensing with a single magnetoelastic sensor by applying loads on the null locations of multiple resonant modes. *Smart Mater. Struct.* **2016**, *25*, 035044. [CrossRef]
- 50. Pacella, N. Geometrical modification of magnetoelastic sensors to enhance sensitivity. *Smart Mater. Struct.* **2015**, *24*, 025018. [CrossRef]
- 51. Karipott, S. An Embedded Wireless Temperature Sensor for Orthopedic Implants. IEEE Sens. J. 2018, 18, 1265–1272. [CrossRef]
- 52. Ong, K.G. Control of a Magnetoelastic Sensor Temperature Response by Magnetic Field Tuning. *IEEE Trans. Magn.* 2003, *39*, 3319–3321. [CrossRef]
- Ong, K.G. Quantification of Multiple Bioagents With Wireless, Remote-Query Magnetoelastic Microsensors. *IEEE Sens. J.* 2006, 6, 514–523. [CrossRef]
- Horikawa, S.; Bedi, D.; Li, S.; Shen, W.; Huang, S.; Chen, I.H.; Chai, Y.; Auad, M.L.; Bozack, M.J.; Barbaree, J.M.; et al. Effects of surface functionalization on the surface phage coverage and the subsequent performance of phage-immobilized magnetoelastic biosensors. *Biosens. Bioelectron.* 2011, 26, 2361–2367. [CrossRef]
- Wang, C.; Shan, S.; Wang, C.; Wang, J.; Li, J.; Hu, G.; Dai, K.; Li, Q.; Zhang, X. Mechanical stimulation promote the osteogenic differentiation of bone marrow stromal cells through epigenetic regulation of Sonic Hedgehog. *Exp. Cell Res.* 2017, 352, 346–356. [CrossRef] [PubMed]
- 56. Delaine-Smith, R.M.; Reilly, G.C. Mesenchymal stem cell responses to mechanical stimuli. *Muscles Ligaments Tendons J.* **2012**, *2*, 169–180.
- 57. Chen, J.C.; Jacobs, C.R. Mechanically induced osteogenic lineage commitment of stem cells. *Stem Cell Res. Ther.* **2013**, *4*, 107. [CrossRef]
- 58. Brito-Pereira, R. Silk fibroin-magnetic hybrid composite electrospun fibers for tissue engineering applications. *Compos. Part B* **2018**, 141, 70–75. [CrossRef]
- 59. De Stefano, D.; Carnuccio, R.; Maiuri, M.C. Nanomaterials toxicity and cell death modalities. *J. Drug Deliv.* **2012**, 2012, 167896. [CrossRef]