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Biohybrid Soft Robots, E-Skin, and Bioimpedance Potential to Build Up Their Applications: A Review

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ABSTRACT Soft Robotics is a new approach towards better human-robot interaction and biomimicry in the robotics field. Its integration with biological materials (Biohybrid soft robotics) is one of the topics being focused on in the soft robotics research in the last fifteen years. The motive for this approach is to combine the best of biological and artificial systems. In this article, Biohybrid soft robots and Electronic Skin (E-skin), which is considered one of the advances of soft robotics, are reviewed. Their most significant milestones and the highlights of their most researched applications are listed. Bioimpedance, which is the impedance of biological tissue to an applied AC signal, has been used recently in many agriculture and medical applications for its non-destructive nature. It has been used in many bio-related applications for its ability to assess the state of living tissues. However, it has not been applied in biohybrid soft robots yet. Bioimpedance equivalent electrical models are surveyed showing both integer and fractional order based models. Finally, its potential to enhance biohybrid soft robotics and e-skin applications is discussed and validated.

INDEX TERMS Bioimpedance, fractional order calculus, soft robotics, biohybrid robots, e-skin.

I. INTRODUCTION

Robots are machines built to perform tasks in a fast, accurate and repetitive manner. Robotics is the science of developing these machines at the desired movement pushing the boundaries of its perception and intelligence. Since the continuous development of robots abilities is the goal of robotics science, building robots with soft matter in respect of soft texture, elastic actuators, advanced compliance and biological inspiration approach has been recognized as the prevailing challenge.

Biological inspiration has been always driving the direction towards new trends in the field of robotics [1]. However, since the early days of robots design and manufacturing, robots were made of hard and stiff links that didn't exploit compliance and flexibility present in living organisms. Robots classification based on the underlying materials and the degrees of freedom that can be exploited is shown in Fig. 1.

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The main differences between hard and soft robots are clear in degrees of freedom variance and the ability to control or sense. The soft robots have infinite degrees of freedom but on the other hand it is difficult to control its movement and capture its deflections and surface transformation [2]. Contrarily, hard robots are easy controlled and have constant kinematics. Its dynamics is well studied, movement is easily captured and surface has no irregularities. However, the trade off of hard robots advantages is limited degrees of freedom. Softness of robots made a shared environment between humans and robots possible and improved Human-Robot Interaction (HRI). This push the boundaries to more efficient exploitation of tasks.

The rise of soft robotics domain has led researchers to explore the best definition that describe this new aspect of robotics. A definition presented in [2] states that soft robots underline the shift from robots with rigid links to bio-inspired continuum robots that are inherintly compliant and exhibit large strains in normal operations. Another more identifying definition described soft robotics manipulators as continuum

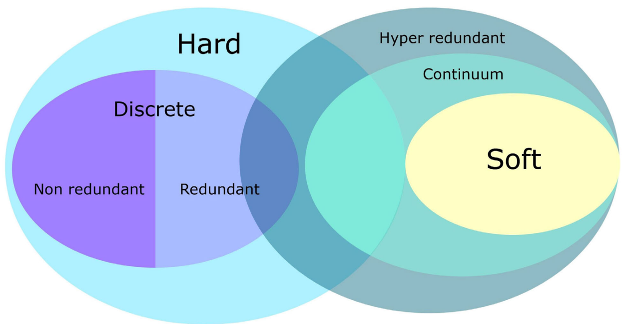

FIGURE 1. Robots classification.

robots made of soft materials that experience elastic deformation and produce motion through the generation of a smooth backbone curve [3].

The authors in [4] related between soft robots and animals. They reported that soft robotics is a growing field that is concerned with robots mechanical qualities and the integration of materials, structure and software. In addition, it aims to achieve enhanced mechanisms through mechanical intelligence of soft materials like the animal movements which are based on both neural and mechanical controls.

Biohybrid soft robots are considered to be one form of soft robots that conform the use of biological tissues within its structure [5]. The use of tissues adds some advantages present in biological systems to the artificial robotic system. Tissues are naturally known for its ability to self-heal, bio-compatibility and biodegradability that is crucial for some applications and finally its light weight [6]. This is an ambitious approach to combine both advantages of biological and artificial systems into one system. The success of this approach depends on the ability to merge different disciplines into one interdisciplinary paradigm [7].

The control of biohybrid soft robots is based on two main strategies [8]. The first strategy is directly stimulating the biological cells used within the biohybrid soft robot to achieve a desired motion or response. While, the other strategy is generating a force indirectly through an external stimuli that affects a synthetic element integrated with the biological one. The latter control strategy can be magnetic, optical, chemical, or electrical control. The advantages of this method are being remotely controllable and its non-invasive nature. Moreover, it has fast response time, usually below 1 second, except for chemical control methods where response time is dependent on chemical diffusion rate.

Modeling biohybrid soft robots remains a challenge in the biohybrid soft robots development. Soft robotics research related to biological cells and tissue culturing is based on trial-and-error approach, while not encompassing solid mathematical models as well. A few trials was reported to model biohybrid microswimmers. One of these instances is a trial to model the stochastic motion of bacteria propelled spherical microbeads [9]. A model to predict the behavior of bacteria swarm on a curved surface was built. The proposed model is intended to be used later on targeted drug delivery. Another work discussed the effect of near and far-wall effects on the three-dimensional motion of bacteria-driven microbeads [10]. Optical tracking method was used to detect the motion of $5\mu m$ polystyrene beads in three dimensions. Finally, the motion of a bead by a single bacteria is modelled and analyzed with respect to applied forces and torques.

Biocompatible and Biodegradable robots are different from Biohybrid soft robots in definition. Biocompatible soft robots are soft robots in which material decides the ability to be used within a biological system; however, biodegradable ones are robots which can degrade within this system. Yet, the three of them are always being used for the same applications. Thus, in this review, Biocompatible, Biodegradable and Biohybrid soft robots are treated as one category.

Skin for any organism is the channel of communication between it and the external environment. It also protects and includes the underlying organs. The skin layer itself conforms a complex network of mechanical, thermal and neural receptors. These receptors convert physical changes on the skin surface to electrical signals sent to the brain. This shows how the skin contributes in providing organisms with perception.

E-skin is one of the popular subjects researched in soft robotics boosted by advances in stretchable, soft electronics [11], [12]. It is a promising approach to reproduce biological skin for robots. It is one form of enhancing biomimicry which provides more clear understanding of biological design paradigms and enables real-time sensing-actuating. E-skin is also applied in biomedical and smart prosthetics field. Traditional prosthetics provide actuation for disabled people. However, they lack the embedded sensing interface.Thus, the integration between E-skin and prosthetics leverages the user experience and enhances its functionality.

Electric impedance spectroscopy (EIS) has been one of the promising non-destructive techniques for testing and evaluating biological tissues. This technique is based on relating stress or quality traits of tissue's to electric (impedance) parameters. This is owing to the fact that electric conductivity of tissue's exist as a result of ions presence and the current is related to ionic content and mobility [13]. Thus, cellular components and their structure rule the conductivity throughout all the frequencies. Changes in cellular structure or injuries to tissues can be correlated to electric parameters which are measurable in a non-destructive manner. This attribute enabled the implementation of EIS in various fields as discussed in Section IV.

This review article is organized as follows, Section II is a survey on Biohybrid soft robots milestones and its most important applications. Section III is focusing on e-skin advances and its two main applications, followed by Section IV where bioimpedance and its equivalent electrical models are presented and their fields of application are listed. Finally, the potential of integration between bioimpedance on one hand and biohybrid soft robots and e-skin on the another hand is explored in Section V.

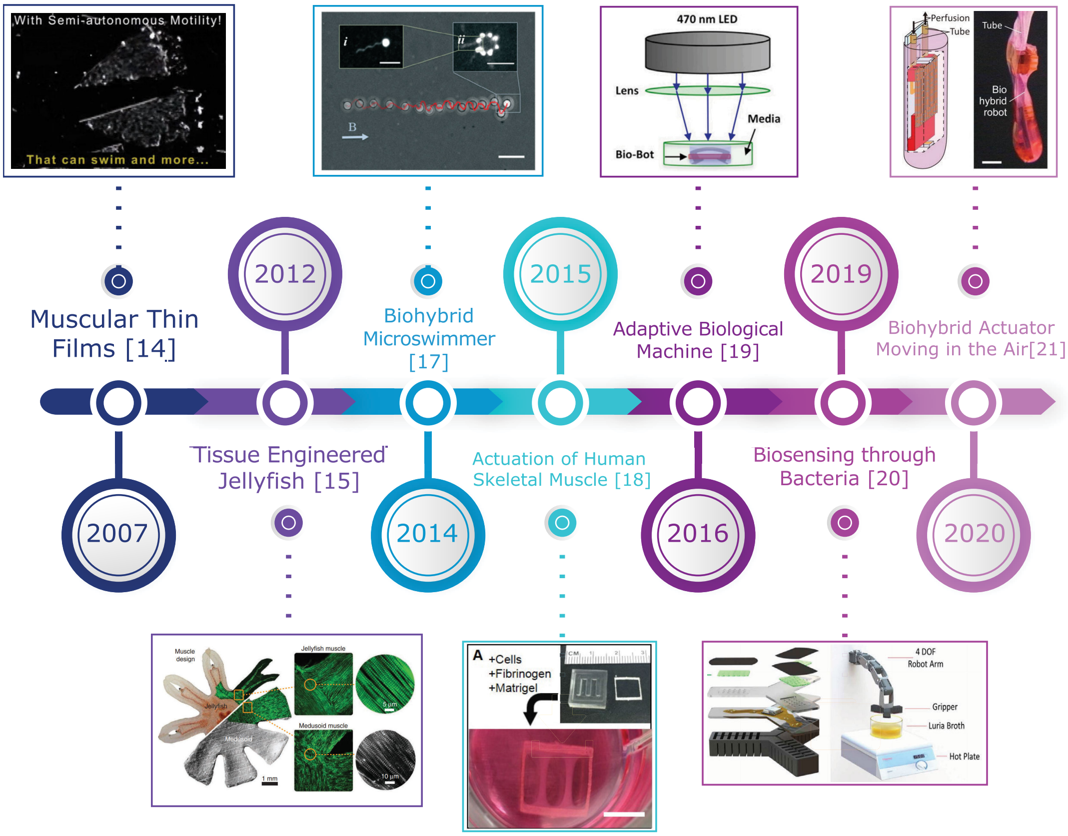

FIGURE 2. The milestones of developing biohybrid soft robots in literature.

II. BIOHYBRID SOFT ROBOTS

The use of living tissues within a soft robot has been first presented in [14] as shown in Fig.2. This article represents the integration between living tissues and synthetic polymer to develop what is now called ''living material''. The authors developed muscular thin films from neonatal rat ventricular cardiomyocytes cultured on polydimethylsiloxane (PDMS). The two dimensional myogensis was achieved by micro-patterning the PDMS films by extracellular matrix proteins. These centimeter-scaled muscular thin films were capable of performing gripping, walking and swimming. It also performed 4 millinewtons of force per millimeter square. This work is one form of direct stimulation of the biological element of the robot. Parallel platinum electrodes were used to connect the muscular thin film to a function generator. An electric signal, that amplitude was 10 V and pulse width of 10 ms, was generated at pace frequency ranged from 0.5 Hz to 5 Hz to operate the biological actuator.

Since then, studies have been deployed to investigate the potential of this new approach in building soft robots with respect to actuation, sensing, and control. The second milestone was reported in [15], where freely swimming jellyfish was constructed from rat tissues and silicone polymers (see Fig. 2). This article is a reverse engineering approach of mimicking jellyfish propulsion. Its output -engineered medusoids- is a representation of traditional soft robotics, biosynthetic compound materials, and computer-aided design approaches in molecular synthetic biology assimilation.

Microswimmers is a branch of robotics where micro scale robots are built in order to carry micro-cargoes to specific locations. Microswimmers are either bio-hybrid microswimmers, bacteria, or artificial microswimmers. Biohybrid microswimmers have advantage over artificial ones as they can sense their local environment [16]. The fact that biohybrid microswimmers are made of soft bodied bacteria enables it to be classified under biohybrid soft robots and accredit its advances to this emerging field [6]. One of the milestones presented in Fig. 2 is the development of biohybrid microswimmers for sensing applications [17]. In this article, *Bacillus subtilis* bacteria was used at different carrier to cargo ratios to realize what is called by the authors ''Two-Component Systems (TCS)'' that enabled the bacteria to sense and respond to different stimuli. The results of this work were very promising as this kind of bacteria is safe for human-related applications.

The biohybrid soft robots development was not limited to rat-muscles based systems as mentioned in [14]. However, researchers in [18] explored development and engineering of contracting human skeletal myobundles. The developed in-vivo model has reacted electrically and chemically, unlike previously developed models. The model, which was originally built to mimic clinical responses of this type of muscles to drugs, paves the way towards predictive drugs development and toxicology screening. The proposed methodology robustness was validated by building models from ten different donors. The models were tested at twitch and tetanus forces. Twitch force is providing electrical stimulation that contracts the muscle repeatedly before getting the chance to completely relax. While tetanus force is a prolonged continuous contraction free of any relaxation duration, the electrical stimulation to the models produced a force of 2.1 ± 0.9 mN/mm^2 and 7.0 ± 2.2 mN/mm^2 for twitch and tetanus forces, respectively.

The ability of biological systems to adapt towards different environment signals is one of its many advantages. Biological systems can sense, think and react in real time. This advantage motivated the development of an adaptive biological actuator named as ''biological adaptive machine'' in [19]. A ring-shaped actuator developed from skeletal muscle cells was connected to different 3D Printed skeletons and was controlled indirectly. This enabled the production of different contractile forces based on the bio-bot skeleton shape. The model produced different aspects of actuations including locomotion and steering. The actuation method here was non-invasive optic-based method, where an apparatus with a 470 nm light source stimulated the skeletal myotubes contraction. The ring-shaped actuator applied maximum 300 μN of contractile force, 1.3 $bodylength/min$ drive directional locomotion and $2^o/sec$ steering angle. This actuator is considered as a module which series or parallel combinations can form swimmers or pumps or other complext actuators.

The development of biohybrid robots is not limited to realization of bio-actuators, it extends to biological sensing and morphological control. Biosensing soft gripper used the chemical signals of engineered bacteria with flexible light-emitting diode circuit was introduced in [20]. This biosensing gripper converts chemical signals of biomaterial (bacteria) to an electrical signal that actuate soft pneunet gripper. This allows to use the flexibility of bacteria in detecting chemical signal and convert it to direct actuation for the gripper. Various membrane compositions; Polycarbonate Track-etched (PCTE), Polydimethylsiloxanxe (PDMS) and Polyethersulfone (PES) were investigated to choose the membrane with best permeability. PES provided the best results within the three materials.

The main issue of developing skeletal tissue-based actuators is its lack of durability. Skeletal tissues when being actuated in the air are always subject to dryness which leads to its damage. This gap led the researchers in [21] to propose a solution for this obstacle. They proposed a skeletal tissue based actuator which was covered by a collagen encapsulation. The collagen provided the tissue with hydration needed to operate and perform some tasks in the air without damage as shown in Fig. 2. The soft robot could perform object manipulation through contraction when pushed a bead over a surface when electrically stimulated for 1 second. Another important investigation was the possibility to actuate the robot in the presence of perfusable tubes which allows cell culturing. This approach prolonged the life time of the actuator from 15 minutes to an hour.

As per EngineeringVillage™, medical applications and biomimicry are the two most subjects conducted within biohybrid Soft Robots. Thus, the most significant work in these two areas are discussed in the following subsections.

A. MEDICAL APPLICATIONS OF BIOHYBRID SOFT ROBOTS

Soft robots have been deployed in multiple rehabilitation [22] and nursing-care applications [23]. Biohybrid soft robots are believed to be applicable in the medical field as well. One of the exciting throughputs of this approach was using the mud eel in a biomedical application [24]. A mud eel was inserted in a tube, and through microelectrodes on its surface, its locomotion has been controlled at high accuracy. The mud eel could reach deep parts of the intestine and this has very high potential in drug delivery applications. In [8], the actuation of a hydrogel was achieved through applying current in a medium through a pair of electrodes.

Origami Soft robots was no far from the medical field. Origami robots which are basically a 2D sheet, that is folded in a specific manner to achieve a 3D morphology. It has been used in many applications like; space robotics, medical devices, morphable wheels, and actuated solar panels [25]. Developing biodegradable and bio-compatible origami robot had been discussed in [26]. The built prototype was an origami robot encapsulated inside an ice cube to be ingested by humans for drug delivery purposes. The prototype was controllable and could perform underwater maneuvers through magnetic field. However, this origami robot was tested in a simulated environment not a real one.

One of the micro-scale advances in biomedical applications of bio-compatible soft robots is a micro device that reproduces the human lung feature of alveolar capillary interface [27]. The built prototype simulates how nano-particles navigate from epithelial and endothelial cell layer towards the underlying channels named ''microvascular channels''. This has a great impact as it provides another alternative to time consuming animal tests and clinical experiments. However, this goal require some improvements on the proposed model to mimic the lungs function at more accurate level. The fabrication of this device was based on PDMS, which is one of the most popular materials in micro scale bio-hybrid and bio-compatible robotics.

Drug delivery has been one of the popular subjects addressed in medical applications of biohybrid soft robots. It always requires interdisciplinary research to produce functional prototypes as presented in [28]. Where material science, magnetic field and Near Infrared (NIR) technology were combined to build a controllable untethered carrier that is able to release its cargo when it reaches the desired position. The cargo in this case is a biological agent, and the carrier is a soft microrobotic structure. This microrobot was designed based on 2D foldable robots paradigm. It was manufactured from bilayer hydrogel which protected and encapsulated the microparticles. Moreover, a NIR responsive layer was added, so the microrobot unfolded when NIR (785nm) beam was triggered. The microrobot was controlled through non-invasive magnetic field and performed manipulations at high accuracy. This prototype is one of the best representations of the indirect control strategy. The authors combined best features of the magnetic and NIR control methods in one application. The fast response of the magnetic field and the non-invasive nature of NIR technology are utilized together to achieve accurate locomotion and delivery.

3D printing manufacturing technology is nowhere far from biohybrid soft robots development. 3D printing is one form of additive manufacturing techniques where objects are built by adding material layer by layer to construct a desired design. This approach enabled the rapid manufacturing of complex designs and accelerated prototyping. The authors in [29] successfully developed a complete soft heart through lost-wax molding technique. An injection mold was 3D printed by a fused deposition molding (FDM) 3D printer using poly(acrylonitrile-co-butadiene-co-styrene) commercially known as ABS. The mold was injected by RTV silicone at room temperature and after solidification, the heart was ready to be extracted from the mold. The only rigid parts in the valve was the directional valves used as there is no soft alternative for it yet. However, this study presented a simple low cost flexible prototype rather than a functional heart for transplantation. The prototype mimicked the heart movement but the blood flow rate and pressure didn't meet the human needs yet.

B. BIOMIMICRY APPLICATIONS OF BIOHYBRID SOFT ROBOTS

Biological systems have always been a source of inspiration for engineers [1]. Its unique advantages of self-healing, real time sensing-processing and actuation, adaptability, and environmental reconciliation were some of the motives behind building biohybrid systems as biohybrid soft robots. Thus, mimicking biological organisms has been one of the significant subjects addressed in this field. Biomimicry is boosted by the engineers desire to deeply understand the underlying design principles that govern the biological systems behavior. In this section, advances in biomimicry and bioinspired designs are discussed.

The study of bimimetics always conforms a pre-study of biomechanics. The authors in [30] presented a pigeon-like robot controlled by wrist movement after a comprehensive study of how pigeon's fly in [31]. In their study on the biomechanics of rock pigeon *Columba livia*, the authors found that it has a special mechanism of keeping wing feathers tied together and of having multi-wing positions during flying. Small lobbed hair called lobate cilia lock with hooked rami (the conjunction of feather and wing) to prevent the formation of tiny feather gaps. These small hairs unlock automatically at relaxation, and they are considered to be the source of Velcro-noise (wing noise) as they are absent in silent fliers.

The authors proceeded their research to develop a flying prototype that realizes the new model of the pigeon. The constructed pigeon-like robot could achieve the three main flying movements; yaw, roll and pitch. The results of the pigeon-bot were satisfying as it reached 42 degrees of freedom and it -as most soft robots- exploit safer human machine interaction. This is due to the fact that the robot was equipped by real feathers where 20 feathers were attached elastically to each wing.

The octopus is one of the heavily studied animals in soft robotics biomemtics. Its nature of being completely soft and its ability to achieve difficult tasks increased the trials to mimic it [32]–[35]. However, the authors in [36] produced one of the most successful prototypes. It was an untethered, micro-fluidic based and completely soft robot, which was fabricated through 3D printing, molding and soft lithography. The robot had on board fuel reservoirs, which their reactions produced a gas that was responsible for inflating and contracting the octopus tentacles by filling and emptying specific micro channels. The elastomer used to fabricate the Octobot was PDMS.

Small scale organisms have also been an inspiration for robotics engineers to mimic their locomotion. Several work has discussed and reproduced caterpillars different locomotion forms like rolling, inching, and crawling [37]–[41]. However, the authors in [42] had developed a modular pneumatically driven omnidirectional soft robot. The modularity as an approach in developing robots has its advantages on both cost and time. Modular robots are also more adaptable to the working environment as different functions can be achieved by special arrangement and relocation of the modules. They successfully developed a prototype that was able of mimicking caterpillar's crawling motion at three degrees of freedom. It reached a speed of 18 m/hr, and omnidirectional locomotion was also achieved.

As for caterpillars, cockroaches' strength and firmness had been an inspiration for others. The authors in [43] successfully manufactured a cockroach-like soft robot which was relatively fast (up to 20 body length/sec) and could perform load carrying, sloap climbing and could withstand high loads. The cockroach robot could withstand 1 million times its weight as a load when a 60 kilogram man stood over it, it could continue locomotion afterwards. These soft robot's features are achieved thanks for its curved unimorph piezoelectric structure.

Many researches were conducted to realize and build underwater soft robots [44]–[46]. However, a few could develop untethered soft robot that successfully mimics fish movement at a reasonable speed as the work proposed in [47]. The robot -electronic fish- developed has a speed of 6.4 cm/sec and could actuate for 3 hours at each charge without having any motor. This is owing to the used hydrogel (ionically conductive) and the dielectric elastomer. Thus, the robot has on-board power source and is also controlled remotely.

III. ADVANCES ON E-SKIN

E-skin is one instance of biomimicry applications of soft robotics. It is a result of mimicking the soft morphology of human skin [48]. The development of e-skin systems is boosted by the desire to build highly complex bionic systems. Moreover, e-skin with tactile sensing capabilities

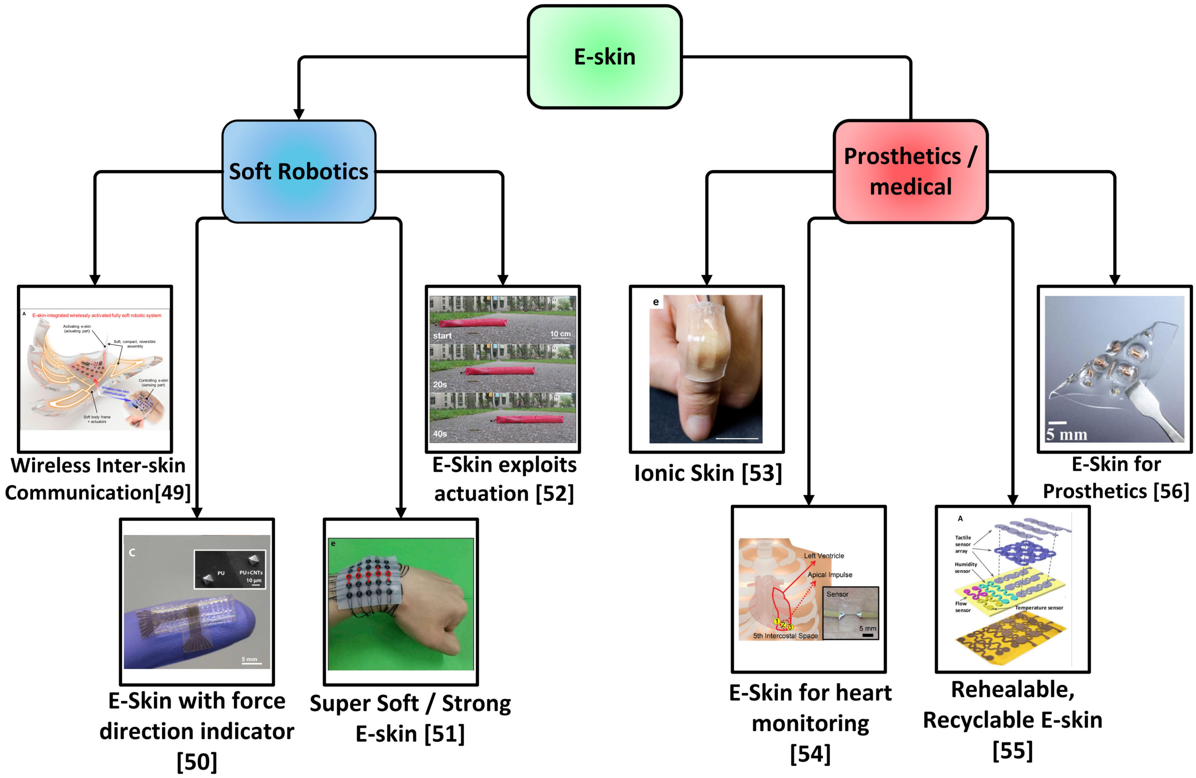

FIGURE 3. E- skin examples classified based on application.

enhances existing soft robots and augments real-time sensing-processing.

One of the drawbacks in existing soft robotic systems and models is that it inherits rigidity from its driving components and its solid electronics. Thus, the authors in [49] addressed this issue through developing a soft e-skin at exceptional thickness ($< 1\ mm$) and weighed about 0.8 grams. This was possible when using a new class of electronics which are soft and stretchable named "stretchable hybrid electronics (SHE)". These electronic components are placed over a soft conducting substrate. The e-skin is actually a pair of skins; one is for controlling and the other is for activating. This pair can be used in a scenario were a human controls a robot through the controlling e-skin. The channel of communication between the pair is constructed through RF transmitter at the controlling e-skin and RF receiver embedded in the other activating skin.

Handling fragile and sensitive objects is an advantage in soft robots over traditional rigid ones. In order to achieve best realization of this feature, it requires to embed tactile sensing feature in soft robots and e-skins as proposed in [50]. The authors developed a biologically inspired e-skin, that can detect forces and its direction. Detecting force direction adds another important feature which is slip detection. This was achieved by building clone of dermis-epidermis interlock interface in human skins by an array of capacitors. This e-skin was mounted on a gripper attached to a commercial Kuka^{TM} robot, and the tactile sensor provided a normal, shear force feedback for the robot at real time.

The soft nature of e-skin and soft robots doesn't mean it should be weak or of low durability. Human skin for its softness also has very good mechanical properties. This was source of inspiration to build a strong e-skin which is ultimately soft [51]. The developed prototype was built using carbon black/silicone composite. The use of this material provided high softness, stretchability and high durable skin. The e-skin had 5×5 tactile nodes which were capable of monitoring applied forces and its dynamic stress distribution.

Biological skin for some organisms provide more than just sensing abilities. Snakes' skin facilitates its movement and locomotion -crawling- through its kirigami-like structured skin. Different 3-Dimensional kirigami structures were discussed in [52] and how they can boost the crawling locomotion of a soft actuator. The authors extended their study and developed the snake like soft-robot out of the lab in an untethered design, where all the sensing, processing and actuating components were embedded in a 25 mm^3 volume space.

Another field of e-skin applications is biomedical wearables. E-skin has shown a great potential in the medical field in terms of rehabilitation, implanted sensors and smart prosthetics. One example of e-skin developed for wearables is presented in [53]. The e-skin named "Ionic skin" fabrication is based on hydrogels and an acrylic elastomer (VHB 4905 3M) to be used as dielectric. The proposed strain sensor is basically a capacitive sensor, where two layers of hydrogels are separated by a dielectric (VHB) and covered by the dielectric layers from up and down as enclosure. This elastomer was used as an enclosure for its stretchability, transparency and biological compatibility to human skin. The fabricated strain sensor could capture 1% to 500% strain, measure pressure starting from 1 *kpa*, and detect position of a touch when a number of sensors are distributed within a sheet.

The e-skin has gone beyond usage over skin to be used over the body organs. The fabrication of a heart-skin to be used as an Apexcardiogram (ACG) sensor was reported in [54]. The previously fabricated on-heart ACG sensors had produced inconvenient results, as for a proper ACG sensor it should be highly sensitive to strain at very small space. This could be achieved by a process called "2D percolation of metal nanocparticles". This process was implemented over the PDMS rubber substrate by a technique named "dry rubbing". The sensor reading was captured by a Field Programmable Gate Array (FPGA) and transferred wirelessly to a portable device. The sensor thickness was no more than $150\mu m$ and had fine contact to the skin. Its final results were validated using a conventional ECG device.

Biological skin has the ability to reheal when subjected to damage. The self-healing attribute has been one of the attractive advantages that pushed the biohybrid soft systems forward. This encouraged the development of a healing/recyclable e-skin that has multiple sensing capabilities embedded [55]. The fabricated e-skin is flexible and wearable thanks for its polyimine substrate. If any of its underlying channels is damaged, it can be rehealed by applying healing agent and heat pressing.

The most highly sensitive tactile sensor for prosthetics was reported in [56]. The tactile sensor is based on Giant Magneto-Impedance (GMI), where a GMI material is embedded with an air gap over an inductor within a pdms substrate. The mentioned inductor is a component in an LC oscillator circuit. The sensor operates when a tiny force is applied over it, the magnetic particles are displaced, changing the impedance of the inductor and finally changing the oscillator's output frequency. The sensor is reported to have a sensitivity of 120 $newton^{-1}$, minimum loading of 50 *micronewtons* which means lower threshold for sensing than biological skin. The sensor output form, which is oscillation frequency, is highly compatible to the human brain as its is similar to the pulse waveform of humans. This makes the prototype highly adaptive to human body and suitable for prosthetics integration.

Table 1 summarizes all the mentioned work in the area of biohybrid soft robots and e-skin. It is important to note that unlike Biohybrid soft robots, there is no recorded literature conformed the use of biological component in e-skin (highlighted column). In this review, we refer to bioimpedance as a cornerstone where biohybrid e-skin prototypes can be realized upon. This approach is discussed in Section V.

IV. BIOIMPEDANCE

Bioimpedance is the impedance of a biological tissue to an applied AC current. The bioimpedance depends on the tissue structure of a specific living organism. A typical tissue structure is composed of a number of cells, extracellular which is the fluid separating the cells from each other and intracellular which is all the cell contents [57]. The intracellular and extracellular are separated by the cell wall. The cell is composed of a lot of other organelles as shown in Fig. 4, but the most important one is the nucleus. The nucleus is the heart of the cell, it has its DNA which is the plants identity and decides all its physical properties. Nucleus and other organelles swim in a medium called cytoplasm. Cytoplasm is a semi-fluid substance that surrounds all the cell organelles within the cell membrane [57].

EIS is measuring the tissue impedance at a range of frequencies in a selected spectrum. The tissue impedance is different at low frequencies from that at high frequencies. This can be explained upon understanding the cell wall. Cell wall is a rigid complex structure that introduces very high impedance at low frequencies. This high impedance is enough to block the current from flowing inside the cell [58]. Thus, the variations in the impedance value at low frequencies describe the state of the extracellular and the state of the cell wall whether it is healthy or damaged. In Figure 5(a), we can see how current flow at low frequencies. On the other hand, at high frequencies the cell wall resistance isn't high enough to block electric current as shown in Fig. 5(b).

Therefore, EIS has been widely deployed in multiple application like, fruit quality and medical applications. As for fruit quality, bioimpedance of different plants and fruits were investigated in order to relate its electric characteristics to its physical ones. For example, the researchers in [59] have found a direct relationship between bruises of apples and the change in its resistance. Bruises that resulted from 50 cm drop influenced the apples resistance more than a 10 cm drop. Moreover, EIS enabled fruit quality research that discussed the effect of heating and freezing injuries to plant tissues as in [60], [61]. In [61], four samples (Eggplant, tomato, zucchini, and carrots) were chosen to investigate the effect of heating and freezing injuries to their tissues. It was found that these injuries highly reduce the impedance of the inspected fruits at low frequencies. This indicates the damage of cells which matches other biological studies to these injuries.

Another agriculture related application is growth monitoring of plants and fruits. On the micro-scale, bioimpedance has shown to be a reliable parameter to monitor the cell growth throughout a week [62]. It also had been applicable in other growth monitoring experiments that included the use of a whole fruit. In [63], the strawberries ripening process has been monitored in conjunction with its equivalent model parameters changes. The authors used the Cole model as the strawberries equivalent electrical model. There was a clear relation between the degree of ripeness and the fractional order capacitor of the Cole model. Another work presented in [64] discussed the relation between apples aging and its bioimpedance. This experiment showed how the apples impedance was in constant increase throughout the aging phase. The authors concluded that this increase was due to the vaporization effect and relative humidity.

TABLE 1. Summary of the Biohybrid soft robots, and the E-skin research mentioned in this work.

Biohybrid Soft Robots					
Reference	Synthetic element	Biological element	Biomimicry	Actuation/Stimulation method	Application
[14]	PDMS	neonatal rat ventricular cardiomyocytes	–	Electric Stimulations	Gripping, walking, pumping and swimming
[15]	silicone polymer	rat tissues	jelly fish	Electric Stimulation	Propulsion actuator
[17]	silica particles	*Bacillus subtilis* bacteria	–	–	Microswimmer for sensing
[18]	–	Human skeletal myobundles	–	Electric Stimulation	Contracting actuator
[19]	3D printed polymer	Human skeletal myotubes	–	Optical Stimulation	Modular actuator
[20]	Polycarbonate Track-etched (PCTE), Polydimethylsiloxanxe (PDMS), and Polyethersulfone (PES)	Bacteria	–	Light Emmiting Diode	Embedded Sensing
[21]	PDMS	Skeletal tissue covered by collagen	–	Electric Stimulation	Biohybrid Actuator operates in the air
[24]	–	Mud eel	–	Electric Stimulation	Drug Delivery
[26]	Polyolefin	–	–	Magnetic field	Patching Stomach Wounds
[27]	PDMS	–	Lung alveoli	Electric power	Regenerating lung function on chip
[28]	Multi=layer polymers, Hydrogel bilayers	–	–	NIR	Drug Delivery
[29]	RTV Silicone	–	Human heart	Pump	Artificial heart
[30] [31]	3D printed polymer	feathers	Pigeon	Servomotors	Biomimicry
[36]	PDMS	–	Octopus	Chemical Reaction	Biomimicry
[42]	Silicone	–	Caterpillar	Pneumatic	Reconfigurable Omnidirectional Robot
[43]	Polyvinylidene difluoride (PVDF) polyethylene terephthalate (PET)	–	Cockroach	Electric Stimulation	High Durable Soft Robot
[47]	Dielectric elastomer, hydrogel	–	Fish	on-board battery	Biomimicry
E-Skin					
Reference	Synthetic element	Biological element	Application	Description	
[49]	PDMS	–	Robotics	Controlling a robot Through pair of E-skin	
[50]	PU Elastomer	–	Robotics	Tactile Sensing, Detects direction of force	
[51]	CB silicone composite	–	Robotics	High durable, tactile sensing skin	
[52]	Polyster plastic	–	Robotics	Facilitating actuation kirigami skin	
[53]	stretchable dielectric, and stretchable ionic conductor	–	Prosthetic	Skin works as pressure and strain sensor	
[54]	Metal nanocparticles	–	medical	Apexcardiogram (ACG) sensor	
[55]	Polyimine substrate, and polymer solution	–	Prosthetic	E-skin that has multiple sensing capabilities and can be recycled and rehealed	
[56]	PDMS	–	Prosthetic	E-skin for tactile sensing at extremely high sensitivity	

Bioimpedance is also presented in medical application through two major techniques. The first technique is EIS and it has been used in dental applications, cancer related applications, body composition [65] and hydration process modeling [66]. In the dental field, it was used in monitoring the osseointegration process of a dental implant [67]. It also proved its ability to classify chemoresistant tumor cells from chemosensitive tumor cells [68]. The second technique is Electric Impedance Tomography (EIT). It is a cheap and safe technique to perform tissue imaging based on impedance measurements [69]. It has been used in monitoring the regional intratidal gas distribution in acute lung injury beside the acute respiratory distress syndrome [70]. It has also been used in breast cancer detection through detection. Tumor cells are deformed cells which have different conductivity that make tumors appear as foreign body in impedance images [71].

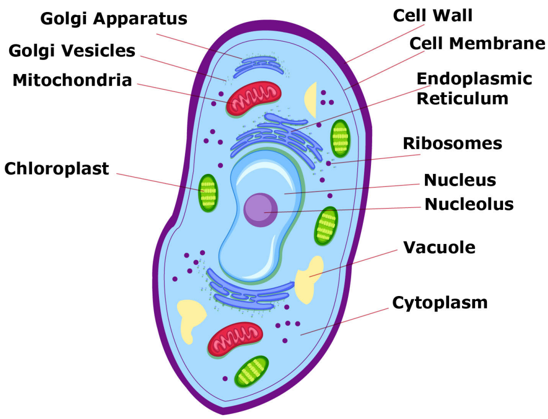

FIGURE 4. Cell structure.

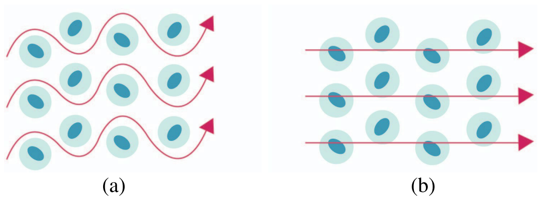

FIGURE 5. Representation of current flow (red arrow) in a tissue with respect to cells (greenish blue circles). In (a) low frequency current flow in the extracellular medium without penetrating cell walls, while in (b) current is at high frequency and can penetrate the cell wall.

A. ELECTRIC MODELS OF BIOLOGICAL TISSUES

Electrical models have been proposed to model the structure of the biological tissue of plants [72]. Those models ranged between traditional integer based models as presented [73]–[75], and there are fractional order [76] based models which are a new prospective in calculus. Fractional order calculus is a branch of mathematics that was discovered when the two scientists L'Hopital and Leibnitz were discussing the meaning to replace the n in the latter's notation by $\alpha = 0.5$. This important question then was not answered. However, it was considered as a paradox which its solution could led to many advances in science. This turned to be true and fractional calculus applications are widely spread now [77]. Fractional order calculus has shown great potential in modeling as presented in [78]. In order to comprehend the fractional order calculus meaning, Caputo presented one of the most used definitions as [79]:

$$ {}^{C}_{\alpha}D^{\alpha}_{t}f(t) = \frac{1}{\Gamma(m-\alpha)} \int_{a}^{t} (t-\tau)^{m-\alpha-1} f^{(m)}(\tau)d\tau \quad (1)$$

whereas $m - 1 \leq \alpha \leq m$ and $m \in \mathbb{N}$ and its laplace representation is:

$$\mathcal{L}\{{}^{C}_{\alpha}D^{\alpha}_{t}f(t)\} = s^{\alpha}F(s) - \Sigma_{k=0}^{m-1} s^{\alpha-k-1}D^{k}f(0) \quad (2)$$

Interpretation and analysis of tissues' impedance behavior have been facilitated since the use of fractional order calculus [72]. Figure 6 shows the different models used to model the bioimpedance of plant tissues. These electric models and their applications are discussed briefly in the following subsections.

1) INTEGER MODELS

a: HAYDEN MODEL

The Hayden model shown in Fig.6 was first introduced in [73]. Small pulses of AC current have been applied to potato tissues (Solatium tuberosum) and alfalfa (Medicago sativa), and their introduced impedance was represented by:

$$Z(j\omega) = \frac{R_1(R_2+R_3)(R_1+R_2+R_3)+B}{(R_1+R_2+R_3)^2+\omega^2\, C^2R_3^2(R_1+R_2)^2} - j\frac{\omega C R_1^2\, R_3^2}{(R_1+R_2+R_3)^2+\omega^2 C^2 R_3^2(R_1+R_2)^2}$$

where

$$B = \omega^2 C^2 R_3^2 R_1 R_2 (R_1+R_2) \quad (3)$$

where R_1 is donated for the extracellular resistance, R_2 for the the intracellular resistance, R_3 for the cell membrane resistance, and C for its capacitance. The model parameter calculations method was presented in [80] on 10 different fruits. The fruits were inspected at frequency range between 20Hz to 300kHz. The calculation method found that R_2 was not constant and was inversely proportional to frequency applied at low range and had nearly constant value at high frequencies. Also the capacitance component showed a non-constant behavior unlike the proposed assumptions in [73].

b: THE DOUBLE-SHELL MODEL

The double shell model [74] was deployed to characterize plant tissues in several measurements. It consists of one more branch than the hayden model to model the vacuole through R_4 and an extra C component as shown in Fig.6. The double shell model was used to characterize impedance of carrot roots and potato tubers in [74], where the protoplasmic resistance R_2 in the Hayden model is split into R_2 which stands for the cytoplasm resistance and R_4 which expresses the vacuole resistance in the double shell model. Also, the cell membrane capacitance C in the hayden model is replaced by the tonoplast and plasma membrane resistance donated by C_5 and C_3 respectively. Thus the model impedance is donated by:

$$Re(Z) = \frac{N_1(\omega)}{D(\omega)}, \quad Im(Z) = \frac{N2(\omega)}{D(\omega)} \quad (4)$$

$$\begin{aligned} N_1(\omega) = {} & R_1(1-\omega^2 C_3C_5R_2R_4) \\ & (1-\omega^2C_3C_5(R_2R_4+R_1(R_2+R_4))) \\ & +\omega^2R_1(C_3R_1+C_5(R_2+R_4)) \\ & (C_3(R_1+R_2)+C_5(R_2+R_4)) \end{aligned} \quad (5)$$

$$\begin{aligned} N_2(\omega) = {} & -\omega R_1((1-\omega^2C_3C_5R_2R_4) \\ & (C_3(R_1+R_2)+C_5(R_2+R_4))) \\ & -(1-\omega^2C_3C_5(R_2R_4+R_1(R_2+R_4))) \\ & (C_3R_2+C_5(R_2+R_4)) \end{aligned} \quad (6)$$

$$\begin{aligned} D(\omega) = {} & (1-\omega^2C_3C_5(R_2R_4+R_1(R_2+R_4)))^2 \\ & +\omega^2(C_3(R_1+R_2)+C_5(R_2+R_4))^2 \end{aligned} \quad (7)$$

In [81] The double shell model was used to examine the freezing injury to the tissues of potato tubers, It was also

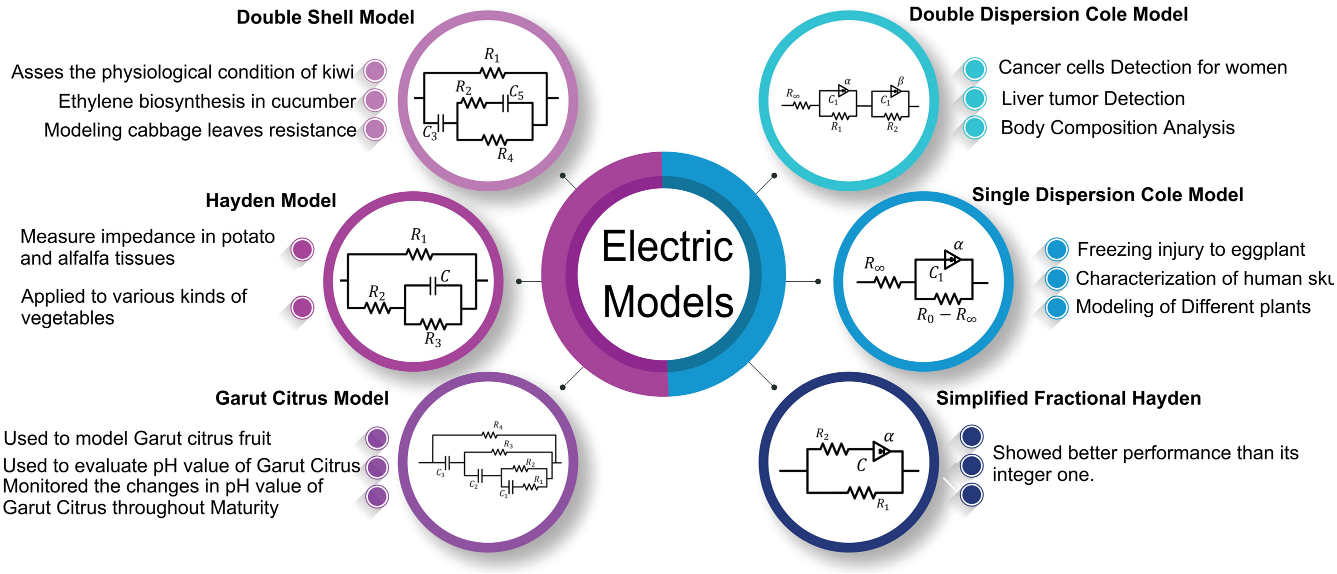

FIGURE 6. Taxonomy of plant models based on Integer/Fractional order based classification.

deployed to model cucumbers and eggplants in [82] and [60] respectively. In [60] the freezing- thawing injury and its effect on the impedance parameters are discussed similar to the work presented in [61]. However, the double-shell based experiment examined an Eggplant pulp unlike a complete whole non-destructed different kinds of plants in [61].

c: GARUT CITRUS FRUITS MODEL

This models shown in Fig.6 was originally designed to model the Garut Citrus fruits impedance [75]. It was compared to other integer models like Hayden model and Double-shell model, and it showed to fit more than both of them. Different elements of the Garut Citrus fruit; outer shell, flavedo, albedo, seeds, segments, and segment walls are represented by resistors and capacitors as follows:

- R_1: Seed resistance
- R_2: Segment resistance
- R_3: Segmeent wall resistance
- R_4: Outer shell resistance
- C_1: Segment capacitance
- C_2: Albedo capacitance
- C_3: Flavedo capacitance

The authors who first presented the Garut Citrus model applied it in applications related to fruit maturity. A trial to relate the model electric parameters to physiochemical properties of the fruit like the pH value is discussed in [83]. The results of this article showed that the resistance, reactance, and inductance per unit weight decreased with respect to higher pH value fruits. On the other hand, the capacitance of albedo and flavedo per unit weight increased on higher pH values of the inspected samples.

2) FRACTIONAL MODELS

a: SINGLE DISPERSION MODEL (COLE IMPEDANCE MODEL)

The single dispersion model [76] shown in 6 is simple for its ability to model plant tissues through only four parameters. However it is not the best model to describe a specific cellular structure, It was found that it is more robust when describing the state of tissues with different structures. Consisting of high frequency resistance R_∞, low frequency resistance R_0 and a constant phase element (CPE), the impedance is expressed as:

$$Z(s) = R_\infty + \frac{R_0 - R_\infty}{1 + s^\alpha (R_0 - R_\infty) C_1} \tag{8}$$

where α is the dispersion constant and holds a constant value. The single dispersion model is used in [60] and the fitting results showed to be more accurate than Hayden and Double-shell models. It was used on many other plants like banana, potato, apples, garlic, kiwi and lemon in [84] and [85].

The single Dispersion Cole model have been extended to medical applications as well. It has been used in human skull modeling as presented in [86]. Also, the storage effect on the red blood cells and its effect on the electrical attributes of the blood based on the Cole model is discussed in [87]. One of its widely deployed applications is human body composition [88].

b: DOUBLE DISPERSION MODEL (COLE IMPEDANCE MODEL)

he single dispersion model is extended to the double dispersion Cole model [76] which has shown to be the most robust and its fitting results had the least errors compared to other models [72]. The double dispersion model is constructed from three resistor and two fractional capacitors [76] as shown in Fig.6. The model has two dispersion coefficients α and β and two time constants $\tau_1 = (R_1 C_1)^{1/\alpha}$ and $\tau_2 = (R_2 C_2)^{1/\beta}$.

At high frequencies flows through both extracellular, cell walls and the intracellular. The impedance introduced R_e is expressed as

$$R_e = R_\infty + R_1 + R_2 \tag{9}$$

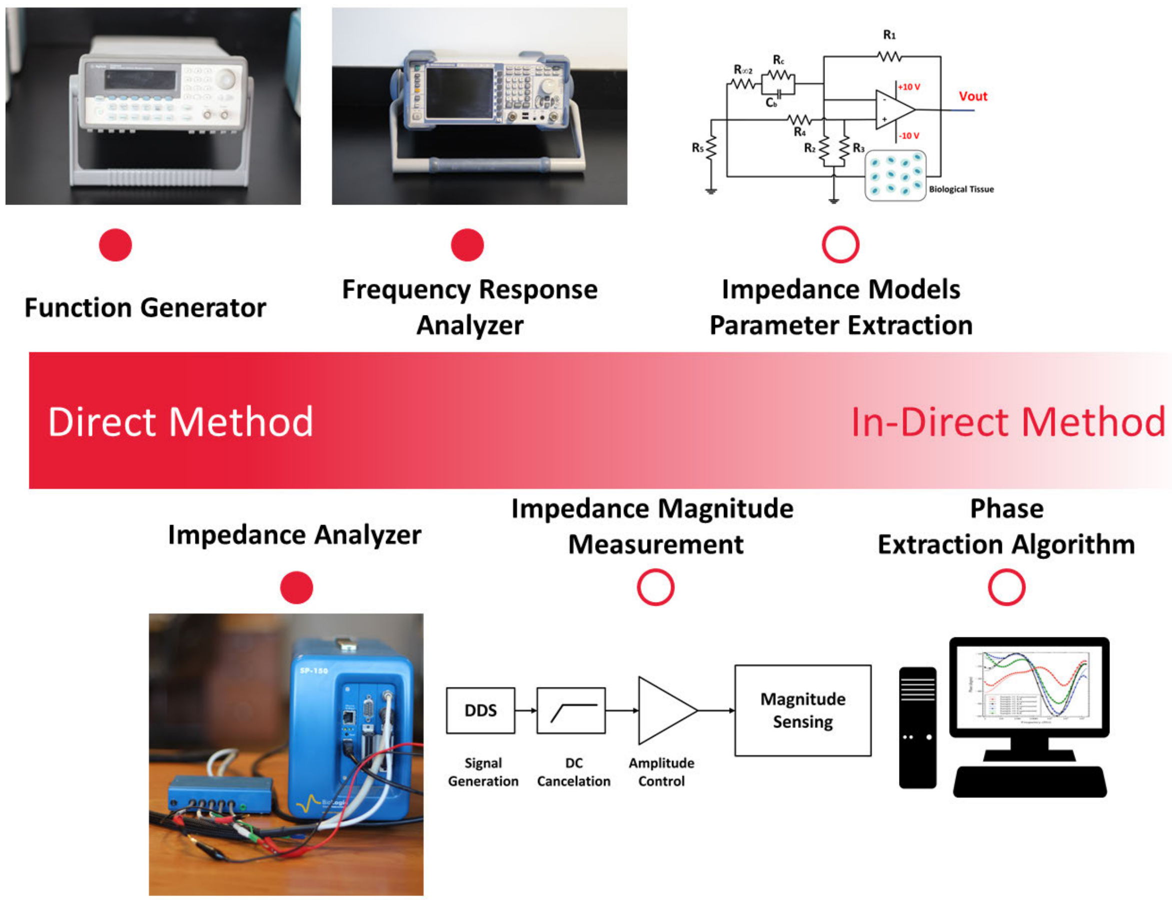

FIGURE 7. Bioimpedance direct and indirect measurement methods.

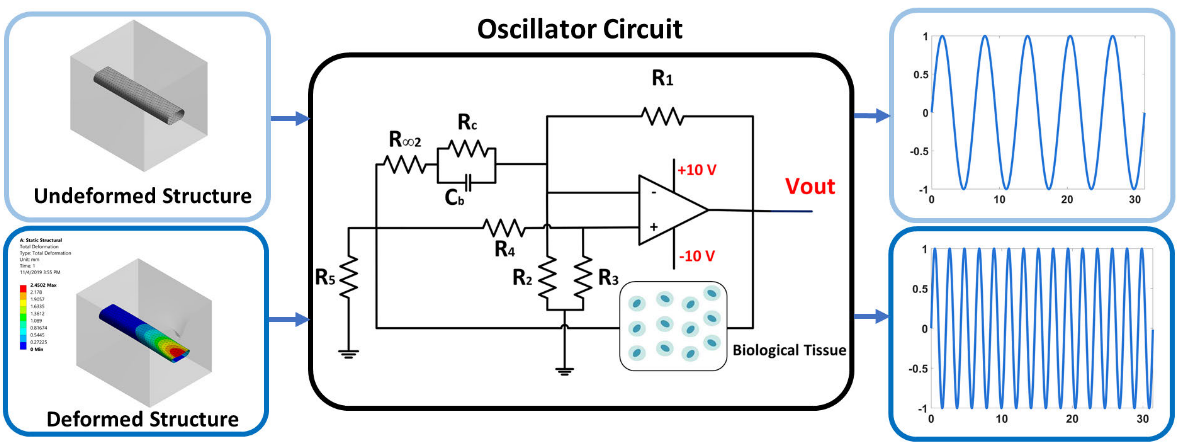

FIGURE 8. Bioimpedance integrated skin validation.

While at low frequencies as current flows in the extracellular only, the impedance R_i is expressed as:

$$R_i = R_\infty(1 + \frac{R_\infty}{(R_1 + R_2)}) \tag{10}$$

Double Dispersion Cole model had been used in many medical studies as well. It has been used in detecting cancer for women [89]. The study is based on the impedance spectroscopy device manufactured by the australian firm TruScreen^{TM}. Liver tissues classification between cancerous and benign has been achieved using modeling based on the mentioned cole model [90].

c: SIMPLIFIED FRACTIONAL HAYDEN

The simplified fractional Hayden model has been presented recently in [72] by replacing the capacitor of the integer model by a constant phase element. It was used to explore its ability to model plants' tissues in comparison to its integer model. The fractional model showed better performance than the integer one owing to the extra degree of freedom

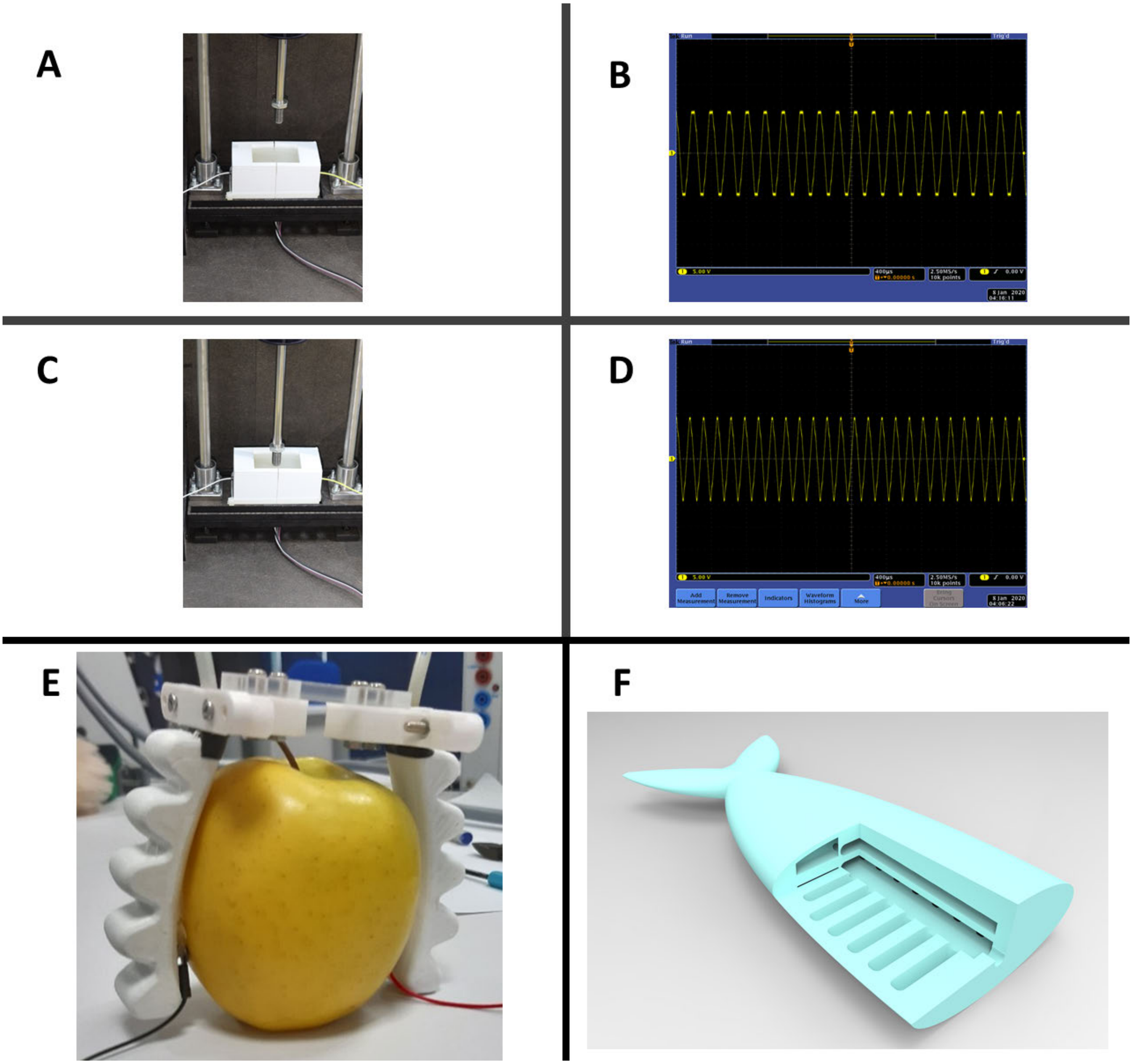

FIGURE 9. **View of the experimental setup and the experiment results. In (A) the stroke is stretching yet did not reach the surface of the soft silicone rubber structure. The oscillation in (B) shows to be around 4 kHz before the contact moment. (C) shows after loading force on the soft structure leading to a rise in the frequency recorded as clear in (D). (E) shows a successful trial to implement EIS on a ripe apple [97], while (F) shows a pilot design of a fish tail to fabricate a soft underwater robot.**

of the fractional element. Its impedance is represented by:

$$Z(s) = \frac{R_1(1 + s^{\alpha} R_2 C)}{1 + s^{\alpha}(R_1 + R_2)C} \qquad (11)$$

B. BIOIMPEDANCE MEASUREMENT

Bioimpedance measurement can be achieved through multiple direct and indirect methods. Direct methods include the use of stationary, and accurate laboratory instruments like function generators, frequency response analyzers, and impedance analyzers as shown in Fig.7. Function generators are capable of producing waveforms at different frequencies (frequency sweep). This enables the impedance calculation through recording the waveform across the inspected sample. However, impedance analyzers are more advanced devices which measure the impedance of samples under test across the selected spectrum without the need for any further calculations. Impedance analyzers also exceed the performance of frequency response analyzers which is originally used for transfer function characterization of filters or amplifiers. Impedance analyzers can measure impedance at lower frequencies which in our case are of higher value.

Direct methods conform the use of other portable solutions. The AD5993 chip is one of the heavily used solutions in literature [64], [91]–[94]. However, this chip has its limitations and drawbacks as well. It can not measure impedance beyond 100 *kHz* which is not sufficient in many biological applications. It also needs continuous calibration and requires a dedicated frontend. Recently, indirect methods have been proposed to provide portable and low cost solution for impedance measurements. For instance, a method to extract the phase parameters from magnitude-only measurements was presented in [95]. In this work, Kramers-Kronig transform was modified and used to extract phase information, and final results were compared to the results of an industrial impedance analyzer.

Another approach in indirect methods is concerned with equivalent electrical model parameters extraction. Measuring impedance spectrum is usually followed by fitting these results to the selected models' equations to calculate the circuit elements values.Thus, the authors in [96] could extract the models' parameters using different oscillators topologies. This work is of a great value to the biohybrid soft robotics and e-skin field, as these oscillators can be dealt with as sensors which convert the physical changes of biological tissues into electrical signals as discussed and validated in Section V.

V. BIOIMPEDANCE POTENTIAL IN BIOHYBRID SOFT ROBOTS AND E-SKIN APPLICATIONS

Bioimpedance has a wide range of applications as shown in Section IV. It showed high efficiency in detecting injuries for biological tissues, decaying and dying cells, and growth monitoring of tissues. It has also been used in classification and detection of abnormality in cells. These advances show that bioimpedance monitoring reflects a lot about the state of the inspected tissues. This shows that bioimpedance monitoring has a very promising potential in Biohybrid Soft Robots applications.

As mentioned earlier in Section II, biological tissues, rat tissues and human skeletal tissues, had been used to fabricate actuators without sensorial element. In these cases, bioimpedance change related to different mechanical deformation can be modeled and thus closed-loop control can be performed. This approach is enabled by advances in low cost impedance analyzers [98], and extraction of models' parameters by means of optimization [96]. Moreover, bioimpedance monitoring paves the way towards the use of biological components in e-skin applications which is absent as shown in Table 1.

Small forces applied to biological tissues result in deformation which consequently changes its impedance. This concept can be adopted to reproduce e-skin using biological elements in order to add the advantages of bio-materials to these prototypes. This approach is validated through the experiment shown in Fig. 8, where Aloe Vera pulp had been used to fill channels of a silicone rubber skin. When force is applied to the skin surface, it deforms the channel geometry changing its bioimpedance. The two sides of the Aloe Vera channels are connected to an oscillator circuit which changes its output frequency proportional to the change in bioimpedance. The live experiment is shown in Fig. 9.

This experiment shows the ability of biological tissues to be used in sensing applications which is not widely implemented in biohybrid robots. Its sensorial output (oscillation frequency) is very similar to the signals sent to human brain from biological skin. This reflects the biohybrid systems throughput in enhancing the biomimicry, which is the motive for developing e-skin. This would be valuable for applications like wearable devices, smart prosthetics and biomedical systems.

From another point of view, integrating bioimpedance measurements with soft robotics enables soft robots to perform bioimpedance analysis. As for agriculture applications, bioimpedance integrated grippers can perform EIS for different fruits which can assist in the picking decision as shown in Fig. 9 E [97]. This approach paves the way for gathering impedance data for underwater life through Soft Under-Water Robots (SUWR). SUWR have caught a lot of attention recently owing to its high biomimicry [99]. This ability,enhanced biomimicry, enable SUWR to blend in the marine life. The integration between bioimpedance and SUWR may facilitate gathering impedance data for underwater plants and reefs crucial for better understanding of marine life. Figure 9 F shows a pilot design of a SUWR which is one of the future work to reproduce the EIS experiment for underwater plants and reefs.

VI. CONCLUSION

In this review, Biohybrid soft robotics throughput in the last two decades has been listed and discussed. E-skin applications and advances had been reviewed and classified on application basis. Bioimpedance and fractional order calculus had been briefly presented. Moreover, their applications in agriculture and medical field were listed. This review is an approach to highlight the potential role of bioimpedance in the uprising field of biohybrid robots and e-skin. The ability of bioimpedance to upgrade existing biohybrid systems and to create new biological e-skin prototypes had been discussed. In addition, an experiment validating this methodology had been conducted and discussed. Bioimpedance applications were based on correlating physical properties of tissues to its electrical properties. Hence, mechanical deformation of biohybrid actuators can be modelled electrically by the same manner. Biohybrid systems, as an approach, are motivated and driven by interdisciplinary research where material science, mechanics, pneumatics, and electronics are combined. Finally, this article proposes the integration of bioimpedance with the other involved technologies and sciences.

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