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Printed Electronic Devices and Systems for Interfacing with Single Cells up to Organoids

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The field of bioelectronics with the aim to contact cells, cell clusters, biological tissues and organoids has become a vast enterprise. Currently, it is mainly relying on classical micro- and nanofabrication methods to build devices and systems. Very recently the field is highly pushed by the development of novel printable organic, inorganic and biomaterials as well as advanced digital printing technologies such as laser and inkjet printing employed in this endeavor. Recent advantages in alternative additive manufacturing and 3D printing methods enable interesting new routes, in particular for applications requiring the incorporation of delicate biomaterials or creation of 3D scaffold structures that show a high potential for bioelectronics and building of hybrid bio-/inorganic devices. Here the current state of printed 2D and 3D electronic structures and related lithography techniques for the interfacing of electronic devices with biological systems are reviewed. The focus lies on in vitro applications for interfacing single cell, cell clusters, and organoids. Challenges and future prospects are discussed for all-printed hybrid bio/electronic systems targeting biomedical research, diagnostics, and health monitoring.

1. Introduction

Classical approaches for manufacturing electronic devices in bioelectronics have come a long way and feature an impressive track record in what kind of measurements are feasible nowadays on single cells up to tissues, organoids or even in vivo.[1] In particular, the development in microfabrication for electrodes and microelectrode arrays (MEAs),[2–5] and complementary metal-oxide semiconductors (CMOS) technology[6] have given rise to a myriad of applications in monitoring[7,8] and stimulating[9,10] single and groups of cells. Still, these approaches have inherent difficulties in respect to organic material integration, biocompatibility, and for applications that require flexible devices. Additionally, when biomedical applications are aimed at, disposability and manufacturing

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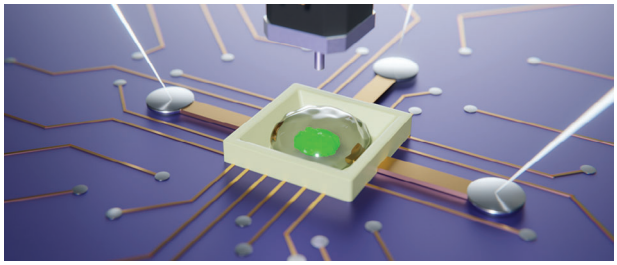

Figure 1. Depiction of a printed biolelectronic device in which a cell and electronics form a hybrid structure.

costs impose further constraints and challenges. Therefore, printed electronics,[11] hybrid approaches aiming at combining "the best of both worlds" from organic and inorganic materials, and connected processing technologies have attracted increasing attention in recent years.[12–15]

Printed electronics in 2 and 3D space comprises several additive manufacturing schemes including the most widespread digital printing technologies, such as (super)inkjet printing, laser printing, electrohydrodynamic printing[16] as well as capillary printing. Compared to classically manufactured silicon devices, printed electronic structures can be conductive, transparent and bendable or stretchable at the same time, photoresponsive, and biocompatible with living organisms. Moreover, their surfaces can be designed in such a way, that they form the best possible contact with the biological specimen. Surface design includes tuning the wettability,[17] controlling the surface roughness,[18] tuning the mechanical properties and deploying chemical patterning[19] which often requires an additional post-processing steps. Despite all these compelling advantages of printed electronic devices, a great challenge in this regard lies in the choice of the electronic read-out system and how it can be seamlessly integrated with the (biological) object under study.

As of the vast scope of potential papers of interest dealing with the electronic read-out and stimulation of cells and tissues, we set ourselves some principal selection criteria. First, the main focus of the review is on printed systems. Therefore, we aim at highlighting publications that feature fully printed devices, or comprise a crucial component that is printed (**Figure 1**). Furthermore, we will discuss printable materials with the potential for printed bioelectronics. In the applications, we focus on single cells, cell clusters and organoids as targets in ex vivo experiments, while in particular not exhaustively addressing the field of implantable/wearable and flexible devices aiming at measuring or stimulating tissues or organs in vivo. Some publications not matching these criteria are included when important as comparison, or to exemplify potentials for printed electronics. There, we aim in particular in showing untapped potential in 3D printing methods that could impact the field of bioelectronics when transferred to this field.

2. Materials for Cell-Interfaced Printed Bioelectronics

This section will give an overview over materials that can be used in printed electronics and are of interest especially for bioelectronics, either as connecting/interfacing material in electrodes or as active materials able to give stimuli to interfaced cells.

2.1. Electrode Materials

A wide range of electrode materials are used in bioelectronics to reach direct contact with cells or tissues, mainly based on the biocompatibility and processability of the materials **Table 1**. Also, many materials can be rendered biocompatible by appropriate coating.[20] Still, inherent biocompatibility is preferred to reduce processing steps and avoid risks from degradation of such coatings.

Among the classical metals used in electronics, gold (Au) and platinum (Pt), for example, have both good conductivity and biocompatibility, making them commonly used as interface electrodes.[10,21–27] Additionally, the powdered form of platinum, known as Pt black,[10,22,25,28] possesses a large surface area and good catalytic properties, which can be electroplated as a coating to reduce electrode impedance. However, highly conductive materials like silver (Ag), despite their good printability, cannot be used in direct contact with cells due to their high surface energy, which leads to oxidation and the release of toxic ions when exposed to aqueous media and air.[29]

Furthermore, the use of indium tin oxide (ITO) as a transparent electrode with good conductivity has been reported, aiding in the monitoring of cultured cells with optical microscopy.[30] Recently, the utilization of conductive polymers such as PEDOT:PSS has been increasing. The sheet resistance of PEDOT:PSS on silver and bare substrates is reported to be around 2.3 and 150 Ω/sq, respectively.[31,32] In addition to its good conductivity, PEDOT:PSS exhibits excellent ionic conductivity and biocompatibility, which are crucial for biosensors. Moreover, PEDOT:PSS's printability makes it a popular material for additive manufacturing. However, a significant weakness of PEDOT:PSS-based devices is their water stability. In various studies, the water stability of PEDOT:PSS has been improved by incorporating a crosslinker such as ethylene glycol (EG) or (3-glycidyloxypropyl)trimethoxysilane (GOPS).[31,33]

Metals with a low melting point, often called liquid metals (LMs) are also part of active research on electrode materials for biointerfacing.[34–37] In addition to having an extremely low elastic modulus (typically under 200 kPa), it also has a low flow resistance, minimizing mechanical mismatches with soft tissue by complying with rough surfaces.[38] A systematic investigation of the toxicology of LM-NP/L also revealed no obvious toxicity at the treatment dose, favoring its bioelectronics applications.[39–41] LM-based electrodes were used for noninvasive ECG recording[34] and for in vivo bullfrog sciatic nerve electrical stimulation, which also showed that the LM is nontoxic to cells.[36,37] Because of their inherent stretchability and flowability, LMs are also used as interconnection material between chips, overcoming the problems associated with rigidity of the chips in bioelectronics. This stretchable bioelectronics technology was used, for example, to acquire multichannel electrograms from the hearts of living animals.[42]

2.2. Materials for Active Cell Stimulation

To facilitate stimulation and manipulation of cells on printed bioelectronic devices, biologically "active" materials are often

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Table 1. Common electrode materials for bioelectronic applications.

Material	Fabrication method	Function	Device	Application	Comments	References
Pt / Pt black	Photolithography (sputtering)	Electrode	MEA	Intercellular recording	+ Well established material for thin films / lithography	[21]
	Photolithography (ion mill)	Electrode	Microfluidic	Extracellular potentials recording	+ Good conductivity + Fair bio-compatibility	[22]
	Photolithography (e-beam evaporator)	Electrode	MEA	Extracellular & intracellular recordings	+ Surface area enhancement – Hard to print	[23]
	Molding	Electrode	Microelectrode	Electrophysiological activity and field potential	– Limited post-functionalization	[24]
	Electrodeposition	Electrode/Coating	MEA	Extracellular & intracellular-like recordings	– expensive	[10,25,28]
PEDOT:PSS	Aerosol-jet printing	µ-Needle Electrode	µ-Needle Electrode Array	Extracellular recording	+ Well established material for printing	[26]
	Inkjet printing	Ag electrode coating	Skin sensor	Bio-impedance measurement	+ Good biocompatibility	[31]
	Inkjet printing	Electrode	MEA	Extracellular recording	+ Easy to print	[43]
	Photolithography	Electrode	Electrochemical transistor arrays	Extracellular recording	+ Many postfunctionalization options	[44]
	drop-casting	Electrode coating	MEA, Skin sensor	Surface electromyography, electrocardiography measurements	+ Inexpensive	[45]
	Electro-polymerization	Electrode coating	MEA	Electrophysiological recording	– Lower conductivity	[3]
Au	Inkjet printing	Electrode	MEA	Extracellular recording – Action potentials	+ Well established material for thin films/lithography and printing	[27]
	Photolithography (e-beam evaporator)	Electrode	MEA	Impedance measurement of cancer cells	+ Excellent conductivity + Fair bio-compatibility	[46]
	Commercial	Fractal-like electrode	MEA	Intracellular recordings	o Fair to print o Limited postfunctionalization	[47]
	Photolithography	Hollow electrode	MEA	Extracellular recording	– Expensive	[48]
ITO	Photolithography (e-beam deposition)	Electrode	MEA	Extracellular recording	+ Well established material for thin films/lithography + Fair bio-compatibility + Many postfunctionalization options	[2,28]
	Photolithography (sputtering)	Electrode	MEA	Extracellular & intracellular recordings	+ Transparent o fair conductivity – Hard to print – Expensive	[4]
Ti	Photolithography (e-beam deposition)	Electrode	MEA	Extracellular recording	+ good bio-compatibility o Limited post-functionalization o Fair conductivity – Hard to print	[2]
TiN	Commercial	Porous electrode	MEA	Intracellular recordings	+ Good bio-compatibility + Good conductivity + Surface area enhancement o Limited post-functionalization – Hard to print	[47]

(*Continued*)

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Table 1. (Continued).

Material	Fabrication method	Function	Device	Application	Comments	References
Nafion	PDMS molding	Electrode coating	MEA	Electrophysiological recording	+ Easy to print + Good passivation + Easy to print – Nonconductive (ion conductivity)	[3,49]
Ag	Inkjet printing	3D electrode (Au and Pt electrodeposited coating)	MEA	Extracellular recording	+ Well established material for thin films/lithography and printing + Excellent conductivity o Fair bio-compatibility (toxic to bacteria) o Fair to print o Limited post-functionalization – Expensive	[50]
LM	Direct printing	3D electrode	Transistor arrays	Electrophysiological recording	+ Good conductivity + Fair bio-compatibility o Fair to print o Limited postfunctionalization – Mechanical stability	[35]

incorporated with the biologically "passive" materials to maintain device functionality without direct cell interfacing. Particularly materials that can regulate their interactions with cells without relying on a direct connection to an external voltage supplier are promising. These materials leverage wireless stimuli, such as applied electric fields or mechanical stress induced by sonic waves, to directly manipulate cells on the device. The printing of such materials opens up new possibilities for designing and controlling interactions, allowing the generation of electric fields or mechanical stress at specific locations within the cell without affecting the surrounding environment. These materials can be integrated into functional devices, providing an additional manipulating parameter, or they can constitute complete devices themselves, enabling precise stimulation of cell growth or localized temperature changes in a highly controllable manner. This section aims to provide a comprehensive overview of these materials, their manipulation techniques, and the utilization of wireless control in cell stimulation.[51]

2.2.1. Piezoelectric Materials

Piezoelectric materials belong to a class of materials capable of converting electric fields into mechanical stress, and vice versa. This unique characteristic arises from their noncentrosymmetric crystal structure, which lacks an inversion center within the unit cell. Consequently, upon deformation of the crystal, such as by exposure to sonic waves, a positive and negative charge imbalance occurs within the unit cell, resulting in the generation of an electric dipole moment. This dipole moment creates an electric field directly within the crystallite, extending its influence to nearby cells. By harnessing this feature, it is possible to apply an electric field, resulting in the generation of mechanical stress, or apply mechanical stress, leading to the creation of an electric field within the piezoelectric particle. Through the application of oscillating fields or oscillating mechanical stress, such as a sonic wave, the corresponding counterpart can be directly induced within the particle, and its characteristics can be modulated based on factors such as frequency, intensity, and other properties of the originating source. This feature has been made applicable in devices by printing piezoelectric nanoparticles, bringing them into contact with cells and subsequently applying ultrasonication to induce oscillating electric stimuli for cells.[52,53]

Various techniques have been utilized to print a wide range of piezoelectric materials, including inkjet printing, two-photon lithography, and digital light processing, fabricating different structures comprising piezoelectric particles for bioelectronic applications.[54] Barium titanate ($BaTiO_3$), a well-known piezoelectric ceramic material, has been a primary focus of investigation.[55] Although not all printed piezoelectric materials are subsequently intended for applying mechanical stress or electric fields, these techniques demonstrate the feasibility of printing such systems and enable the development of novel devices that combine piezoelectric properties with printing methodologies.

For biologically relevant purposes, $BaTiO_3$ has been printed using various techniques. In one study, a commercially available 3D printer was employed to fabricate scaffolds for bone tissue engineering. The ink used in this case consisted mainly of a mixture of $BaTiO_3$ and polyethylmethacrylate (PEMA), which was subsequently subjected to a sintering process.[56] Another study coated $BaTiO_3$ particles with different polymeric and organic species and then 3D-printed piezoelectric scaffolds with these using shape memory polymers, thereby adding an additional avenue for manipulation to the scaffold.[57]

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Another approach involves coating $BaTiO_3$ onto 3D printed scaffolds. This was achieved by applying hydrothermal synthesis to deposit $BaTiO_3$ onto 3D printed Ti_6Al_4V scaffolds, followed by polarization. The polarized $BaTiO_3/Ti_6Al_4V$ scaffold exhibited significant effects on macrophages and osteoblasts, effectively inhibiting an inflammatory cascade.[58] Inkjet printing has also been employed to structure $BaTiO_3$ particles,[59] while mixing commercial photoresist with $BaTiO_3$ produces inks that can be used for two-photon lithography (2PL). In this case, the printed scaffolds demonstrated enhanced differentiation of bone-like cells.[60]

A recent study utilized the inherent mechanic/electric conversion property of piezoelectric particles, specifically of polarized $BaTiO_3$ nanoparticles, to generate an electric field inside tumor tissue. Endothelial cells were exposed to low-intensity pulsed ultrasound in the presence of polarized $BaTiO_3$ nanoparticles. The study demonstrated that stimulated polarized $BaTiO_3$ nanoparticles, emitting an electric field due to the ultrasound, could counteract pathological angiogenesis in tumor tissue, promoting vascular normalization and enhancing the efficacy of chemotherapeutic drugs by 1.8 times.[61] Other promising studies also utilize wireless piezoelectric mechanic/electric conversion of $BaTiO_3$ for various applications, such as micromotors for neural stem-like cell stimulation,[62] neural behavior control,[63,64] antitumor treatment,[65] and regenerative therapy for neurodegenerative disorders.[63]

A study by Paci et al. combined printing with ultrasonication of $BaTiO_3$ particles. They used an alginate/Pluronic-based ink mixed with $BaTiO_3$ nanoparticles to print muscle cell-laden hydrogels. The application of ultrasound stimulation was found to enhance myogenesis during the early phases.[66]

In addition to $BaTiO_3$, other ceramic piezoelectric nanoparticles have attracted interest. For example, zinc oxide (ZnO) was combined with polyvinylidene fluoride (PVDF) to 3D print a scaffold for wound healing. During patient movement, the resulting friction between particles generates an electric signal that benefits wound healing.[67] Similarly, purely polymeric hydrogels have been used to create stable mechanical/electrical responsive materials under pressure.[68] Films composed of polymers and piezoelectric materials are also under investigation, particularly their response to ultrasound treatment.[69]

While this review primarily focuses in this chapter on the piezoelectric conversion of electric fields to mechanical forces and vice versa, it is important to note that other features of piezoelectric materials triggered by wireless polarization can lead to interesting properties in printed devices as well. For instance, piezoelectric catalyst materials that produce reactive oxide species for biological processes can be switched on and off upon application of ultrasonic treatment.[70,71]

Furthermore, particular cell types, especially cardiac cells, have contraction characteristics in addition to producing electrical signals. These contractile forces can be measured, e.g., by measuring the deflection of micropillars by the cells in imaging analysis[72] but also electronically by piezoelectric materials such as PVDF, as described, e.g., by Yang and Hsu[73] and Huang et al.[74] In these works, circular micro-grooved shaped PVDF membranes have been used to measured contraction force profiles of aligned cardiomyocytes.

2.2.2. Conductive Hydrogels

When it comes to the additive manufacturing, i.e., by 3D printing of bioelectronic devices the interaction with cells is very important and implies very distinct properties of the conductive materials in the devices. Biological tissues are volume conductors in electrical models, with moderate conductivity (e.g., electrical conductivity $\sigma = 0.1$ to 1 S m^{-1}) due to mobile ionic species dissolved in the media. In electrolytic tissue media, electrical communication between neural cells primarily depends on ionic fluxes since electrons cannot serve as charge carriers. Unlike biological tissues, conventional electronic systems rely on electronically conductive materials like metals, where free electrons act as mobile charge carriers. This difference creates a unique interface between tissue and electrode, exchanging ionic and electronic signals. Bioelectronic activities at tissue-electrode interfaces involve diverse ionic and electronic interactions across different length scales. Excitable cell membranes on the tissue side generate spikes of ionic currents or action potentials (APs), representing the fundamental element of bioelectronic activities at the nanometer length scale.[75]

Cells, as the smallest living entities in the human body, can transmit and receive electrical signals using ions and biomolecules. Electronically excitable cells have a cellular membrane described by the Hodgkin–Huxley model, with transmembrane ion-selective channels and pumps facilitating ion transfer.[76]

For bioelectronic devices, the goal is to transduce these chemical and ionic signals with high sensitivity, specificity, and spatiotemporal resolution, which can be achieved by 3D printing technologies even in presences of tissue and living material.

In recent years, the importance of soft materials for interfacing cells with materials has been highlighted, as most cell types are naturally in touch with relatively soft extracellular environments and are known to have mechanosensitive properties.[77] Therefore, also devices that are integrated or in direct connection with a living system need to be designed such that there does not occur a mechanical mismatch between material body tissue. Hydrogels are soft, hydrated polymers that have very well controllable mechanical properties and are therefore highly suitable to provide bioinspired environments for cells,[78] which is also important for keeping cells viable and in a naturally occurring phenotype in the context of bioelectronic systems. However, hydrogels are intrinsically nonconductive. They need to be made conductive by, e.g., adding conductive elements to the material. Organic conductive materials such as PEDOT:PSS stands out among different electrode materials for cellular stimulation and recording due to its excellent capability for high capacitive charge injection and furthermore its properties that are beneficial for a variety of 3D printing technologies. PEDOT:PSS exhibits a notable volumetric capacitor trait in cell recordings, allowing its charge storing capability to depend on both lateral dimensions and polymer layer thickness, yielding a capacitance per volume of $C^* = 39.3 \pm 1.3$ F cm^{-3}.[79] Serving as both a volumetric conductor and capacitor, PEDOT:PSS effectively facilitates signal transmission between cells and electronics in diverse device setups. Furthermore, it has been successful in reducing the impedance without compromising electrode density.[80] In addition PEDOT:PSS-based hydrogels

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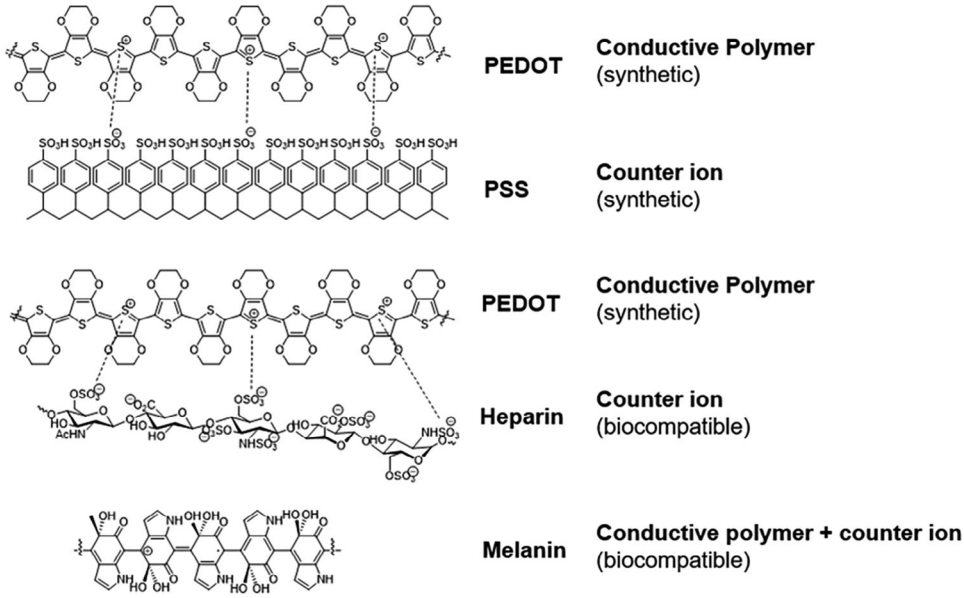

Scheme 1. PEDOT conjugated with different counterions as conductive hydrogels mimicking the extracellular matrix

have shown promise in this area due to their highly ion-sensitive properties resulting from correlated ionic and electronic conductivity of mixed ionomers. Hydrogels with a wide spectrum of stiffness based on the PEDOT:PSS type were highly investigated especially for the application of stimulating electrodes in very soft neuronal tissue. This is especially interesting if charge transport should be measured or the tissue should be stimulated by electric signals. However, there are many drawbacks. For example, printing brain tissue on microelectrodes directly limits the transfer of the electric signals as it has been shown that microelectrodes are inadequate for long-term implantation into brain tissue, with cellular encapsulation playing a significant role in limiting neural communication.[76,81] Fibrous tissue encapsulation is often the response to foreign materials. Generally, it consists of an inner layer of macrophages and a concentric fibrous tissue, and an outer vascularized tissue layer. This layer is insulating and passivating the electrodes. Presumably, the performance of electrodes in these devices could be improved by promoting better integration into the nervous tissue.[81] Despite reducing the nominal impedance through PEDOT:PSS coating, there are concerns about adverse biomechanical interactions between the electrode materials and target tissue, especially in implantable devices. These interactions could increase interfacial impedance and form an electrode-tissue cleft due to immunoreactions, potentially affecting bioelectronics' performance during stimulation and recordings.[75] Hydrogels can undergo volume changes in response to external electric stimuli. However, these electric stimuli are not rapidly transmitted through the hydrogel network because of the low electronic conductivity of the polymer matrix.

Conducting electroactive polymers (CPs) including polypyrrole, polyaniline, and polythiophene as well as poly-3,4-ethylene dioxythiophene (PEDOT) have been used to modify biosensors and bioelectronics.[82] The oxidation reaction required to convert conjugated organic monomers into polymers may be performed either with appropriate reagents such as Lewis acids (chemically) or with an externally applied current (electrochemically). The resulting polymers show charge conductivity when they are slightly oxidized. This is achieved by introducing negatively charged counter ions during synthesis. Typical counter anions that have been used include synthetic polymers (PSS), small molecules, or especially anionic biomolecules such as heparin or hyaluronic acid, which can be included as hydrogels from extracellular matrix.[83] CPs can be grown into hydrogels[84] creating material systems that are both electrically and ionically conductive, as well as mechanically robust.[85,86] It has recently been shown that by carefully controlling the rates of chemical polymerization and precipitation from solution of CPs during synthesis, swollen gels with a high degree of elasticity can be obtained by the addition of a hydrogel component. So far, the hydrogel-CP approach has not been implemented into the bioprinting of the living tissue itself by using the CP-monomers. Due to the varying behavior of the PEDOT:PSS novel approaches have been developed by exchanging the counterions by more physiological relevant entities such as heparin or hyaluronic acid,[87] heparin,[88] alginate, dextran, pectin, xanthan, deoxyribonucleic acid chondroitin sulfate, and polydopamine[89] and event to complete biocompatible polydopamine CPs such as derivatized melanin or the so called neuromelanin (**Scheme 1**). The molecular design of functionalized thiophenes includes an extended conjugated ring structure, with oxygen atoms located directly adjacent to the molecular backbone. This design leads to high chemical stability and charge transport properties. This is also true for polydopamine derivatives such as melanin. Natural melanin is also found in active brain tissue such as the substantia nigra, which is damaged in Parkinson's disease. Polydopamine or melanin shows negative resistance, a classic property of electronically active conductive polymers. Introduction of novel melanin-based derivatives into the cell-laden hydrogels during the printing process and in situ fabrication of the scaffold by controlled oxidation

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(i.e., tyrosinase addition) will eventually provide novel biobased, electronically active materials.

Moreover, even with a short chain length, hydrogel based conducting polymers such as PEDOT:counterion exhibit significant absorption in the ultraviolet and visible wavelength range,[90,91] which poses a challenge for photoinitiation in light-based 3D printing techniques like stereolithography (SLA) and digital light processing (DLP)[92,93] based on their chromophoric structure. Therefore the most common technology is the inkjet-based 3D printing of conductive hydrogels based on PEDOT:counterions composites.

To realize contacts with living tissues and to meet with their dynamic mechanical and fluidic environment their further development into dynamic conductive hydrogels (DCHs) are very promising. DCHs are created using dynamic and reversible crosslinks, allowing the breaking and reforming of the hydrogel and CP polymer linkages. As a result, DCHs can provide dynamic environments for cellular functions while maintaining the integrity of the matrix. These dynamic materials can mimic some properties of native tissues, making them highly suitable for numerous biotechnological and medical applications.[94]

Considering the sensitivity of various cell types like epithelial cells, fibroblasts, and cardiomyocytes to material stiffness, adjusting the mechanical properties of DCHs significantly impacts cell behavior. Annabi et al. introduced a dynamic conductive hydrogel (DCH) using the cell friendly GelMA and PEDOT:PSS for constructing 3D C2C12 myoblast cell-laden structures through bioprinting.[95] The bioink preparation involved two steps: crosslinking PEDOT:PSS with bivalent calcium ions and a secondary photopolymerization step with visible light to crosslink GelMA after printing.

For instance, Engel et al. developed a DCH using collagen, alginate, and PEDOT:PSS[96] By identifying materials with desired stiffness and stress relaxation properties, they achieved ECM-mimetic fibrous structures and enhanced electrical coupling in engineered cardiac tissue, leading to notable improvements in beating characteristics. Moreover, enhanced cardiomyocyte maturation and beating properties were observed in human induced pluripotent stem cell-derived cardiomyocytes.[97]

In addition to PEDOT:counterion doped hydrogels particularly gold nanowires,[98] carbon nanotubes,[99] and graphene[100] have proven highly valuable in the context of hydrogel composites. They are frequently used for applications in cardiac tissue engineering, where the electrical stimulation of the cells is highly important. The microengineering of graphene tube-systems into hydrogels has proven to allow for a high conductivity of the materials, while keeping the conductive filler concentration at low levels.[101]

Conductive hydrogels (CHs) are becoming a highly promising and widely used platform for 3D printing of bioelectronic devices, offering the incorporation of electron signals as relevant physical cues. However, as described above conventional covalently crosslinked conductive hydrogels lack the necessary network dynamics (e.g., stress relaxation, shear shining, and self-healing) essential for complex cellular functions and various biomedical applications.

2.2.3. Magnetic Materials

Magnetic materials represent a distinct class of materials that exhibit responsiveness to external wireless influences, particularly electromagnetic fields. The interaction between magnetic particles, primarily Fe_3O_4, and electromagnetic fields gives rise to functional properties that enable cell manipulation that could be useful in bioelectronic devices. This manipulation can occur either with cells in close proximity to magnetic particles or with magnetic particles attached to cell surfaces.[102,103] While printing magnetic particles is a well-established field, the combination of printing techniques with wireless manipulation capabilities is currently less developed. Most studies that incorporate printing and magnetic particles focus on 3D-printed scaffolds for cancer therapy, incorporating magnetic nanoparticles for hyperthermia purposes. In this context, the magnetic particles are subjected to alternating magnetic fields, leading to heating and overheating of the surrounding environment, such as tumor cells.[104,105]

Hyperthermia studies involving printed structures commonly employ metal oxides as nanoparticles embedded within a polymer scaffold.[106] The printed scaffold plays a critical role since the preparation of magnetic nanoparticles in complex structures is often challenging. These scaffolds typically consist of hydrogels from polymers, biomaterials, and Fe_3O_4 as magnetic particles.[107–110] Utilizing 3D printing methods, these hydrogels can be shaped accordingly and employed in experiments involving various tumor cells. Studies report that these scaffolds, when exposed to alternating magnetic fields for 15 min, can achieve temperatures ranging from 50 °C to 80 °C, resulting in a cell death rate of approximately 75%.[111]

Some printed hydrogels also exhibit the capability to undergo shape changes, driven by the magnetothermal effect, thereby introducing an additional manipulative factor through controllable shape transformation.[112] However, despite the advancements in printing hydrogels, precise delivery to targeted cells remains a challenge. To address this, specific markers are often employed to label the printed hydrogels. Moreover, magnetic particles can be utilized to manipulate movement or accurately position cells. When incorporated into the hydrogel, an applied magnetic field can induce movement, as exemplified by a multifunctional hydrogel printed in the shape of an octopus.[113] This precise movement capability has been extended to non-magnetic printed structures or cells by attaching magnetic Fe_3O_4 nanoparticles, allowing for controlled movement under a magnetic field.[114–116] Studies have demonstrated the patterning of Fe_3O_4-linked cells using magnetic fields[117–119] as well as the ability to bring together different types of cells that would not typically interact, forming cell aggregates through this technique.[120] Printed magnetic structures were also used to capture magnetically marked cells, which could also be exploited in bioelectronic devices for targeted cell placement.

Beyond hyperthermia, other aspects of printed magnetic structures have also been explored. Examples include the development of printed scaffolds for drug delivery,[121,122] utilization as microrobots for theranostic cargo delivery,[123] and various sensoric[124,125] and therapeutic[126] applications, predominantly employing 3D printing approaches.

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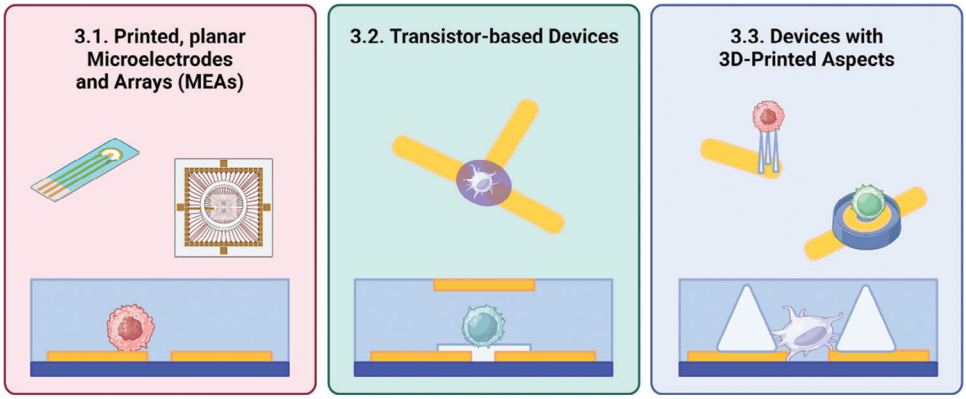

Scheme 2. The devices for single cell and cell cluster interfacing were classified into 3 groups that each are discussed in a dedicated subsection.

3. Devices for Single Cell and Cell Cluster Interfacing

In the following sections, we will discuss and exemplify different devices and systems for cell interfacing that are ideally fully printed, or have some vital printed component. For this, we classified the devices into three categories (**Scheme 2**), with raising device complexity.

3.1. Printed, Planar Microelectrodes, and Arrays (MEAs)

Planar electrodes are a basic component for many cell interfacing sensing and stimulation applications, including widespread analysis methods as cyclic voltammetry (CV),[127,128] electrochemical impedance spectroscopy (EIS)[129,130] and capacitive sensing,[131,132] as well as direct recording of field and action potentials in electrogenic cells.[133,134] Here, printed electronics can help to reduce complexity and cost of manufacturing (important in particular for biomedical applications) or open up routes to flexible devices that are better suited for interfacing with biological tissues. E.g., Bachman et al. introduced a fully-inkjet-printed gold MEA allowing the recording of extracellular action potentials in cardiomyocytes.[27] This approach was implemented on Kapton foil and overall reduced costs in comparison to standard manufacturing, making disposable printed MEAs feasible (**Figure 2**).

Printed electrodes can also open a route to new material options by allowing broader material processing capabilities. Adly et al. have created silk fibroin MEAs capable of enabling both, electrophysiological recording and drug delivery, with high quality and non-invasive electrical signals. These arrays are fabricated using inkjet printing and enable the recording of electrical activity in various cell and tissue types. The utilization of silk fibroin films as a material for bioelectronic interfaces offers benefits such as softness and flexibility.[135]

The use of liquid metals (LMs) in electrode/cell interfaces is also widespread due to their low toxicity and high conformity. Chow et al. developed a liquid metal-based microcapillary pipette that enables high-frequency electrical stimulation and measurement, allowing for 4D single-cell manipulation. This simplified fabrication method eliminates the need for complex microfabrication steps, making it cost-effective and accessible for biomedical applications with significant potential impact.[136] Cheeseman et al. reported that adhesion between living cells and gallium-based liquid metal droplets is driven by chemical interactions rather than physical forces, as evidenced by surface wrinkling and oxide layer rupture.[137]

Guo and Liu developed liquid metal nerve electrode arrays via spraying technology, which exhibited excellent compliance, conductivity, stability, and biocompatibility. Also, bullfrog sciatic nerve electrical stimulation showed that the liquid metal is non-toxic to cells, making LM promising for future implantable prostheses.[36]

3.2. Transistor-Based Devices

In addition to electrode arrays, also active devices such as transistors can be used to interface with biological and bioinspired systems. In recent years, there has been a growing interest in utilizing transistors for single cell applications, where the ability to interface with individual cells opens new possibilities in healthcare, biotechnology, and scientific research. There are several transistor types that have found wide interest in bioelectronics for interfacing with cells and biological systems as artificial cell membranes in form of phospholipid bilayers. Here, in particular, printable electrolyte-gated field-effect transistors (EGFETs) working as solid state devices[138] or in liquid[139] as well as liquid metal based fully printed transistors[140] are among the promising candidates for interfacing with biological systems, as of their inherent compatibility and non-toxicity (**Figure 3**).

The earliest attempt to use transistor-based devices for bioelectronic interfacing was reported in the 70s by Bergveld et al. who demonstrated that ion selective FETs could be used to measure extracellular ion concentration outside the cells.[141] Later, Sprössler et al. successfully measured the electrical properties of rat cardiomyocytes in cell culture.[142] As the direct contact of membrane proteins to solid based devices causes the denaturing of proteins, ultrathin polymer supports (thickness < 30 nm) have been used as the soft "cushions" interfacing membranes and semiconductors.[143]

One promising approach for single cell transistors is the use of nanowires.[144] These nanostructures exhibit excellent electrical properties and can be fabricated to have dimensions comparable to the size of cells. By carefully engineering the surface

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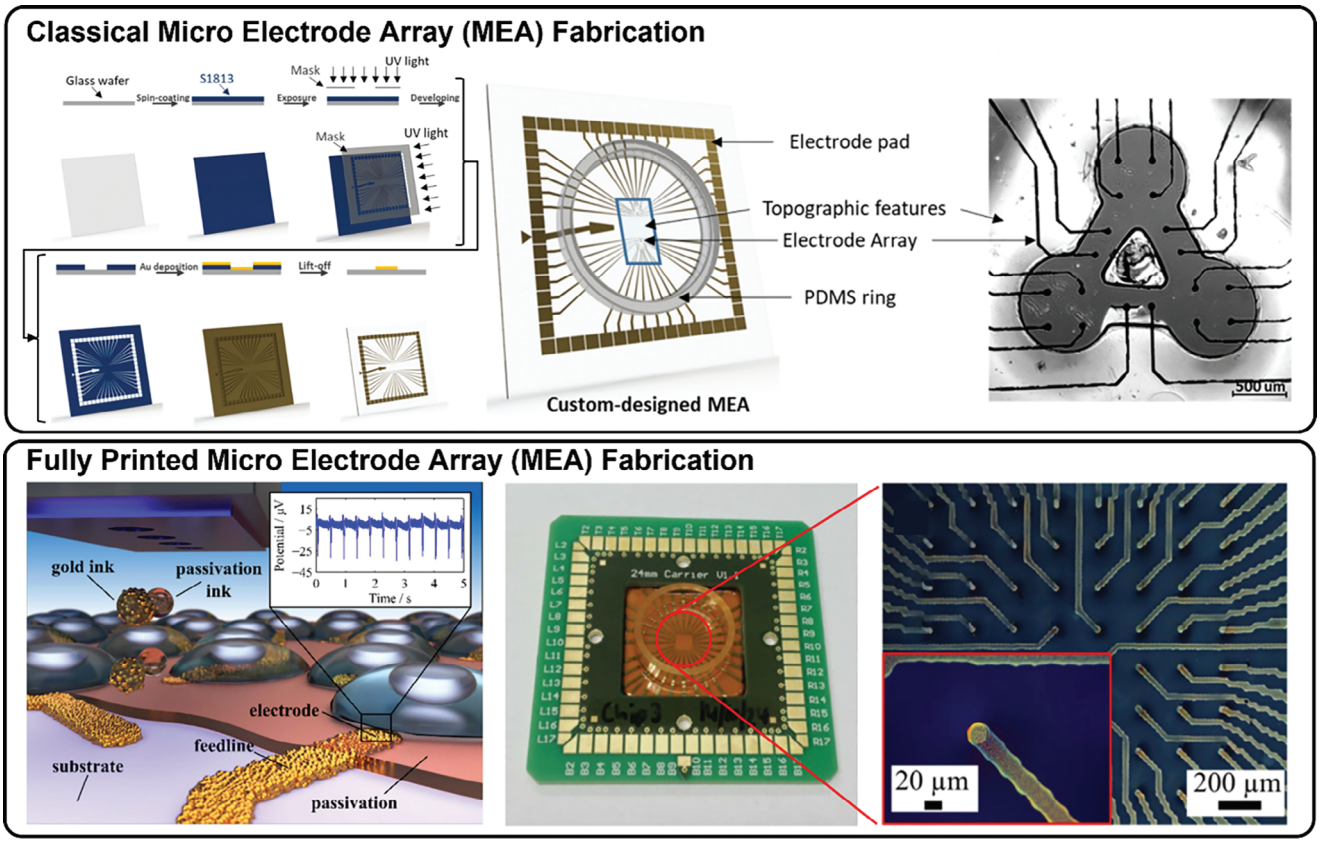

Figure 2. Comparison of classical photolithography-based approach for fabrication of a MEA (top) to a fully printed fabrication route (bottom) and resulting electrode arrays. Top is adapted under the terms of the CC BY 4.0 license.[5] Copyright 2021, by the authors of.[5] Bottom is adapted with permission.[27] Copyright 2017, IOP Publishing.

properties of these nanomaterials, it becomes possible to interface with cells at the molecular level, enabling precise control and measurement of cellular processes. Merz and Fromherz demonstrated snail neurons can be stimulated and electrically measured with silicon-based transistors.[145]

Recently, electrolyte gated transistors have found great interest in the field of bioelectronics due to the use of aqueous based saline solutions as gate. EGFETs have also been used as sensors with phospholipid bilayers.[147] Zhang et al. reported the use of lipid bilayers in PEDOT:PSS films on transistors to sense ion pores.[148] Kawan et al. used EGFETs to monitor lipid bilayers and understand electrochemical properties.[149] Mehlhose et al. employed GaN-based EG-FETs to monitor the electron transfer along the helical peptides and the specific binding of proteins on the membrane surface.[150,151] These studies show the potential of such devices to study the mechanisms in the outer cell membrane by model membranes or to exploit such model membranes to induce artificial cell–cell communication. While fully printed EGFETs have been demonstrated,[138] the application of biomaterial elements in them, as the phospholipid bilayers in above examples, show the potential of multi-method processes for such devices. Scanning probe lithography (SPL) techniques as dip-pen nanolithography (DPN)[152] with phospholipids (L-DPN)[153] can print such functional components precisely for better and more targeted integration, enabling multiplexed devices.

Zhang et al. used an organic co-polymer poly(N-alkyldiketopyrrolo-pyrrole-dithienylthieno[3,2-b] thiophene) DPP-DTT in between the source and drain electrodes to increase the mobility and sensitivity of the electrolyte thereby, aiding in better electrochemical measurements of mesenchymal stem cell (MSC) layers on the surface. The study also tested the cytotoxicity of various chemicals to cells, showing the validity of such devices for applications in cell monitoring.[154]

Scuratti et al. reported the real time monitoring of homo sapiens duodenum adenocarcinoma (HUTU-80) and human embryonic kidney (HEK-293) cells using polymer wrapped carbon nanotubes which were printed in between source and drain. This approach improved cell/electrode interaction and thus allowed to study attachment and detachment of cells and time-based absorbance.[155] In general, graphene-based interfaces also offer good cell-contacts and biocompatibility, thus printable graphene inks should also have a good potential to construct fully printed devices. In Hess et al. HEK-293 cells were cultured on graphene-based transistors and the patch clamp method was used to study ion concentration and sensitivity of cells. The results from the electrical studies were consistent with simulation models, showing that also complex systems as cell/electronic hybrid devices can be modelled with appropriate effort.[156] Graphene-based transistors have also been used to monitor the action potentials of cardiomyocytes[157,158] and kidney cells[159] in real-time.

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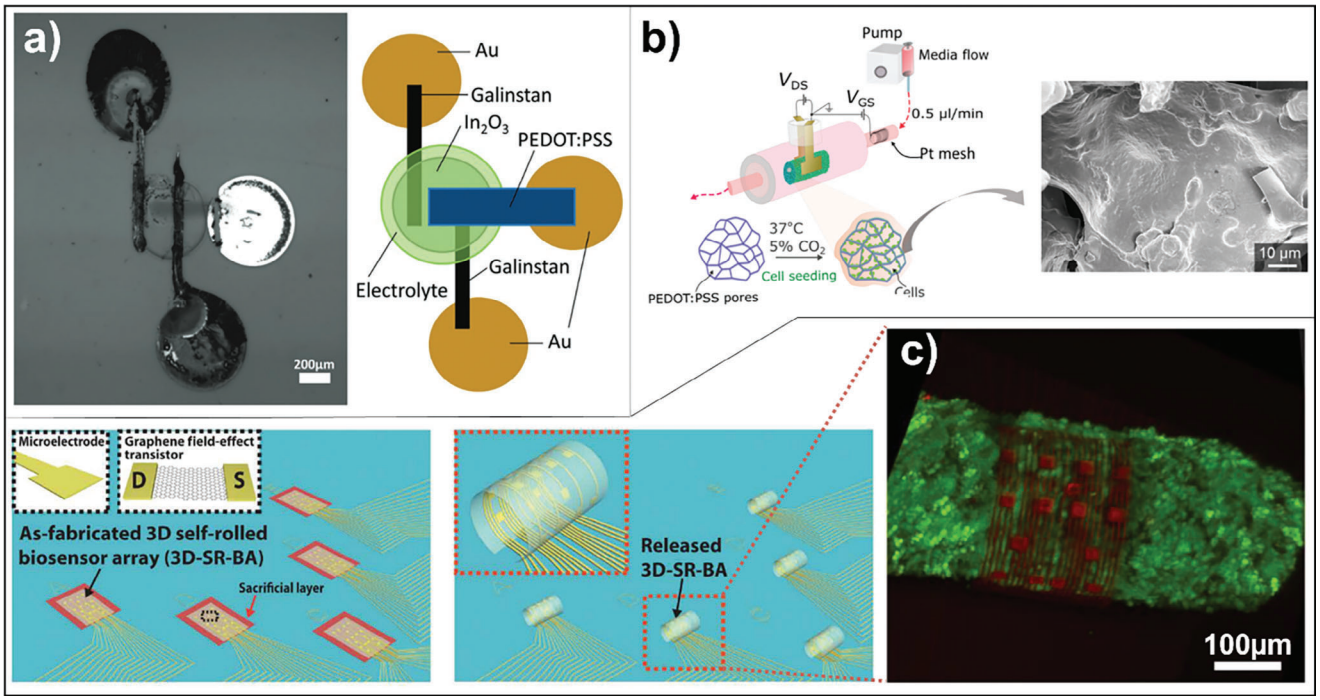

Figure 3. a) Fully printed EGFET with LM source/drain electrodes. Reproduced under the terms of the CC BY 4.0 license.[140] Copyright 2021, by the authors of.[140] b) Scheme of a "transistor in a tube" allowing to measure signals from mm sized cell cultures in PEDOT:PSS-based porous scaffolds. Reproduced under the terms of the CC BY-NC 4.0 license.[33] Copyright 2018, by the authors of.[33] c) Self-rolling transistor/microelectrode arrays on flexible substrate for conformal contact of spheroids. Adapted under the terms of the CC BY-NC 4.0 license.[146] Copyright 2019, by the authors of.[146]

Generally, EGFETs used in electrophysiology can achieve a high signal to noise ratio,[160,161] thus enabling sensitive recordings of cell activity.

As exemplified above, printable electronic devices offer versatility in terms of material selection, footprint, and conformity for many bioelectronics systems. It allows the use of various functional inks, including conductive, semiconductive, and insulating materials, to even create complex electronic circuits that can in turn be used for readout. In conclusion, transistors designed specifically for single cell applications hold great promise for advancing our understanding of cellular behavior and revolutionizing various fields, including healthcare and biotechnology. As researchers continue to explore new materials, fabrication techniques, and integration strategies, we can expect even more sophisticated and powerful transistor-based devices that will enable transformative discoveries in the realm of single cell analysis and manipulation.

3.3. Devices with 3D Printed Aspects

Development in micro- and nanofabrication enable the creation of more and more complex 3D micro-/nanostructures with many implications in bioelectronics. These structures can act as passive components in electronic devices (e.g., as structural elements, or as scaffolds and "cages" for cells), or also as active parts (e.g., as electrode, or for cell manipulation and stimulation). One important use of 3D-structured elements is the functionalization of electrodes with needle-like microstructures that allow for more conformal contact between cells, or can be used for intrusion into an organoid or even a single cell for intracellular measurement. Conventionally, these structures are implemented by photolithography and etching approaches.[162] In particular with direct laser writing (DLW), also the combination of classical manufactured electronics in combination with printed structures as protruding electrodes is used.E.g., Brown et al. used DLW written microneedles, later metallized by evaporation, to record neural signals from zebra finches and mice.[163] Kim et al. reported a multimodal sensor that is composed of an active-matrix pressure-sensitive transistor array with integrated soft LM electrodes inserted inside an organoid to form the intraorganoid interface for simultaneous electrophysiological recording and stimulation.[35] However, Zips et al. demonstrated that such approaches can also be achieved fully printed by fabricating a MEA that used passivated silver electrodes with protruding PEDOT:PSS microneedles to record extracellular signals from cardiomyocytes (**Figure 4**).[26] Overall, conductive polymers seem especially promising for the direct interfacing with cells, as of their favorably mechanical properties and options for biofunctionalization.[164]

For single cell applications, 3D printed structures can be used for trapping or aligning cells in respect to other parts of a device. Simple flow control microfluidic structures can be used to align cells appropriately. Here, e.g., Clark et al. demonstrated fabrication of a millimeter scale PDMS microwell array using a silicon master mold and razor blade stencil to form cross sectional polymer walls to trap and uniformly orient isolated adult cardiomyocytes in a flow of fluid.[166] However, 3D DLW

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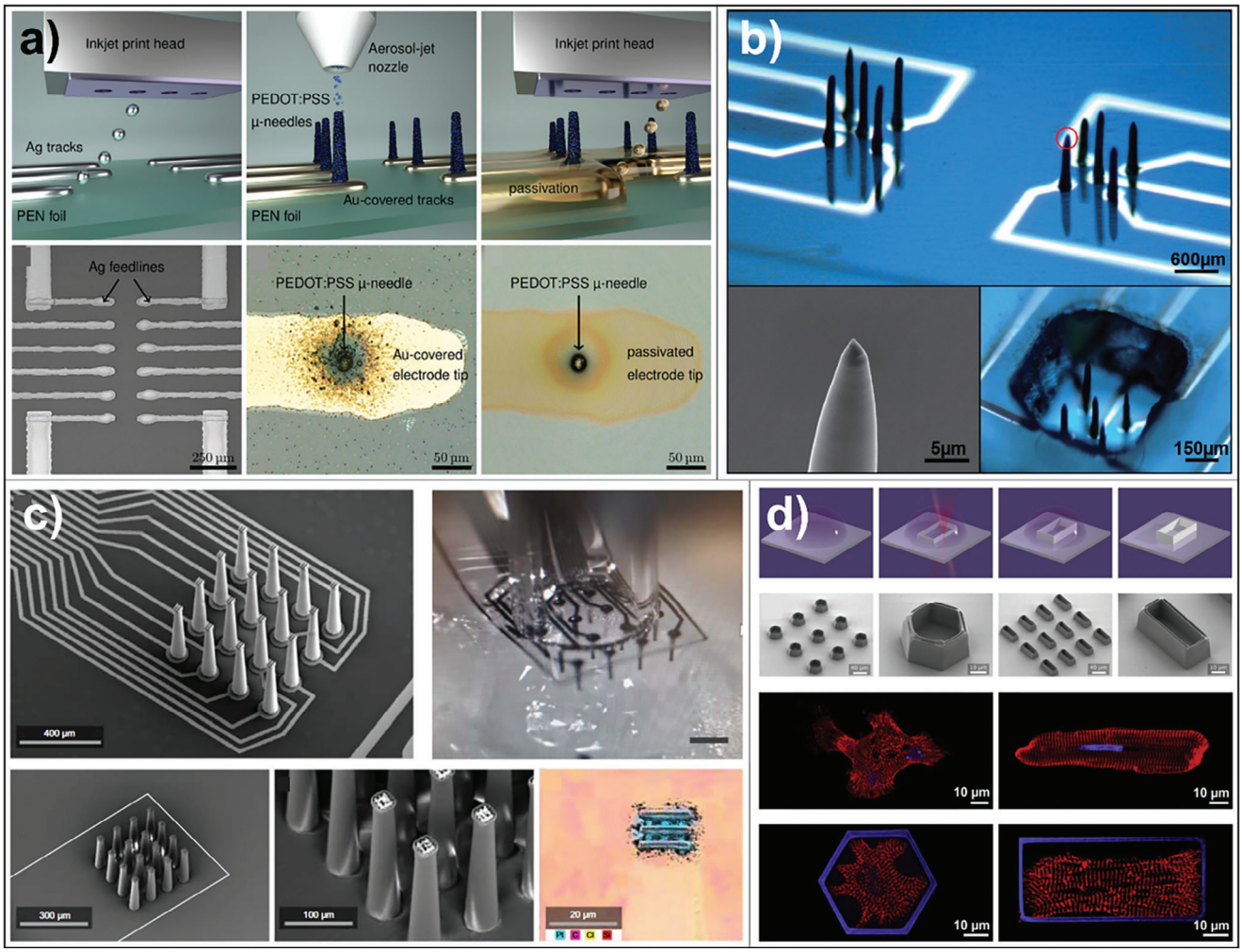

Figure 4. a) Fully printed PEDOT:PSS microneedle electrodes for recording signals from cardiomyocyte-like HL-1 cell layers. Reproduced with permission.[26] Copyright 2019, American Chemical Society. b) LM microneedle electrodes on classical metal connectors used for contacting cardiac organoids. Reproduced under the terms of the CC BY-NC-ND 4.0 license.[35] Copyright 2022, by the authors of.[35] c) Microneedle electrodes fabricated by DLW on a flexible substrate. Adapted under the terms of the CC BY 4.0 license.[163] Copyright 2023, by the authors of.[163] d) DLW written microcompartments for single cell alignment and shape restraint. Reproduced with permission.[165] Copyright 2020, Elsevier.

opens up enormous flexibility in creating tailored microstructures for aligning, manipulating and scaffolding cells in complex microstructures not obtainable in molding approaches.[167] Silbernagel et al. conducted a study to explore the effects of a structured 3D environment on the differentiation of single cardiomyocytes derived from induced pluripotent stem cells (iPSCs). They used 3D laser nanoprinting to fabricate the structures. The study revealed that iPSC cardiomyocytes grown in cuboid 3D-micro-scaffolds resembling the size and geometry of mature cardiomyocytes show an improved structural maturation. This was exemplified by a strong sarcolemmal remodeling process, myofilament reorientation and enhanced clustered expression of L-type Ca^{2+} channels and consequently faster Ca^{2+} transient kinetics. This improved structural maturation is accompanied by an improved functional maturation leading to robust Ca^{2+} handling during spontaneous and triggered activity. These findings demonstrate the potential of 3D-micro-scaffolds in enhancing iPSC-cardiomyocyte maturation and their potential applications in regenerative medicine.[165,168] DWL allows also for the generation of microreservoirs with integrated electrodes, as shown by Marques et al., making use of the reservoir for a solid electrolyte transistor.[169] These architecture of fully printed devices hold also promise for future single cell applications.

Recently, it has been demonstrated that printed 3D microscaffolds provide a versatile tool to study single cell mechanics. For example, 3D-printed soft metamaterials with different subcellular geometries offer a large degree of freedom to adjust their effective elastic properties. These bio-metamaterials can be used to mechanically regulate the morphology and active traction forces generated by mesenchymal stem cells.[170] Moreover, the integration of stimulus-responsive polymers[171,172] into the printed 3D micro-scaffolds offer the possibility to stimulate single cells mechanically. Whereas 3D microstructures printed by a photoresist doped with poly(N-isopropylacrylamide) (pNIPAM) exhibit a substantial response to changes in temperature,[173] stimulus-responsive, supramolecular hydrogels[174] enable the

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reversible actuation of cells under physiological conditions by non-cytotoxic chemical stimuli. For example, Hippler and co-workers introduced a stimuli-responsive host-guest system fabricated with direct laser writing to measure cellular forces during cell stretching.[175] More recently, this system has been applied to study the distinct roles of non-muscle myosin II isoforms for establishing cellular tension and during cellular homeostasis.[176]

The vascularization of intricate cell cultures, such as organoids, has historically posed challenges. Nevertheless, Grebenyuk and co-workers have made notable progress in this area by introducing a synthetic 3D soft microfluidic system, fabricated through two-photon hydrogel polymerization. This innovative system enables the generation of networks of small-scale vessels capable of perfusing large tissues exceeding >15 mm^3 in size. Through their research, the authors demonstrated the system's efficacy in sustaining engineered tissues for extended durations, effectively mitigating issues related to hypoxia and necrosis.[177]

Several studies have shown that electrical stimulation can induce the formation of vascular networks in vivo or in vitro in 2D monocultures.[61,178,179] The effect of electrical stimulation in in vitro 3D multicellular structures, including organoids and bioprinted 3D models, to induce directional migration, orientation, and elongation of endothelial cells has not yet been completely explored. In particular, organoids have a limited lifespan as a direct consequence of their growth to several millimeters in size, which deprives the cells from nutrients and oxygen resulting in a necrotic core.[180] In order to facilitate the accessibility to nutrients and oxygen in the core of organoids as well as in bioprinted specimens, it would be beneficial to facilitate the formation of vascular tubes by endothelial cells in these 3D systems. Overall, 3D printed electronics is a highly evolving field[134,181] that will enable more and more complex integration of 3D devices with living matter.

4. Engineering the Spatial Contact of Cells with Bioelectronic Systems

A highly important issue in bioelectronic systems is to position cells at a desired position, where an electrical stimulation or an electrical readout should ideally take place. In principle, there are two approaches for this, (I) to functionalize the desired positions with binding spots, allowing cells to settle only at defined places or patterns, and (II) direct positioning by physical means to place cells at or move to desired positions or in specific arrangements.

Following approach (I), a standard method to position cells in a desired pattern is to microstructure extracellular matrix (ECM) proteins by photopatterning methods,[182] but also microcontact printing is employed,[183] as well as self-assembly methods like block-copolymer micelle nanolithography (BCML).[184] Further complexity and more precise control can be achieved when different adhesion proteins can be incorporated in the same microstructures as, e.g., demonstrated by Richter et al. in DLW generated microstructures for cell-alignment[185] (**Figure 5**) or by Brinkmann et al. with polymer pen lithography (PPL) printed micropatterns for directing neural outgrowth.[186] Instead of using ECM proteins, also other capture or binding tags can trap cells in place. E.g., Striebel et al. demonstrated the use of specific antibodies immobilized on a general cell repellent polymer brush via microchannel cantilever spotting (μCS) to selectively trap highly adherent macrophages of different polarization into defined arrays.[187] Another interesting binding strategy is the use of DNA-directed immobilization (DDI), as demonstrated by Lemma et al. for selective deposition of two different cell types on 2PL written scaffold structures.[188]

The direct positioning of cells, approach (II), has been addressed with noncontact printing methods like extrusion/drop-on-demand (DoD) printing of cells and spheroids,[190,191] laser-based cell printing,[192] and inkjet based cell printing.[189] In addition, there are also physical methods to position cells. For example, optical tweezers can be used to position cells, even within collective assemblies[193] and more recently, also acoustic methods to arrange cells have been demonstrated[194] and could evolve as a highly interesting method to establish distinct cell assemblies also in 3D. In spite of these highly sophisticated methods, a very robust method to position cells for research purposes is to use a cantilever system with integrated microfluidics, which allows for picking up cells and releasing them at desired positions.[195] Also extrusion printing methods develop into systems to build more complex tissues and more precise deposition of different cell types. This can be achieved, e.g., in suspension as demonstrated by Daly et al. by printing and positioning spheroids into hydrogels that then join into the desired tissue shape,[196] or by the use of microreactor structures as shown by Cho et al. for a joint system of blood and lymph vessels for modelling more realistic setups in targeted cancer therapy.[197]

The big advantage of methods that in situ intervene with cellular systems is, that they also allow a dynamic control of cell position, whereas pre-defined materials or printing of proteins and/or cells does normally not allow further manipulation of the cell position.

However, recent advances regarding photocleavable and photoswitchable materials could here also allow for additional dynamic interventions.[198,199] In the future, also recently developed methods to manipulate cells with in situ 2PL[200] can evolve highly beneficial for achieving a dynamic manipulation and isolation of cells and also 3D systems of cells in bioelectronic devices.

5. Conclusion and Outlook

Printed electronics has shown the capability to interface with cells and organoids giving the advantage of a reduction in cost and complexity in fabrication and a wider range of accessible materials in particular biomaterials. While functionalization of 3D printed structures is still challenging, active elements, e.g., by thermo- or light-responsive materials can be created.[173,175,201] This can lead to responsive systems, as, e.g., 3D roll electrodes that are used in conventional fabricated bioelectronics for cell clusters and organoids,[146] and were shown in a printed version for interfacing small peripheral nerves,[202] but also even more complex mechanical control of cells and cell-interface contacts on devices in the future.

Also (bio)chemical functionalization of 3D printed microstructures (where in particular site-selective and multiplexed functionalization is still an obstacle) sees progress, e.g. by offering process

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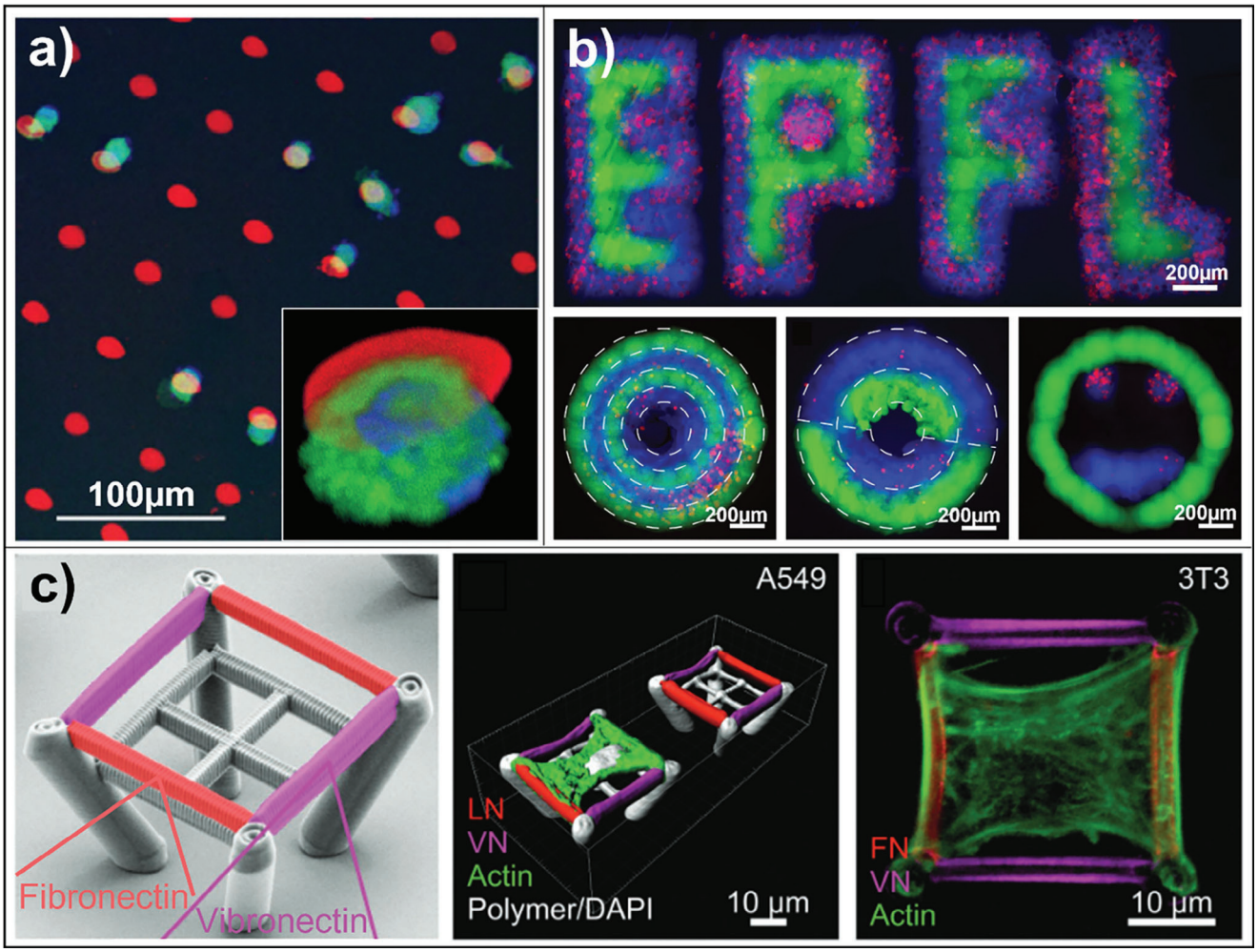

Figure 5. Examples of different cell positioning approaches. a) Macrophages, selectively captured on an antibody array. Inset shows a single macrophage (green/blue) on a capture dot (red). Adapted under the terms of the CC BY 4.0 license.[187] Copyright 2021, by the authors of.[187] b) Inkjet printed cell-laden hydrogels. Adapted under the terms of the CC BY 4.0 license.[189] Copyright 2018, by the authors of.[189] c) DLW fabricated structures with two distinct protein functionalization for selective cell attachment and alignment of single cells. Adapted with permission.[185] Copyright 2017, Wiley.

stable binding tags allowing the more sensitive proteins or even decorated cells to self-assemble onto specific parts of a printed structure.[185,188] Also alternative 3D micro-printing approaches via SPL methods can solve some problems in this regard for the creation of 3D micro- and nanostructures[203,204] and multiplexed inherent[205] or post-functionalization.[206,207]

These methods can either be used in combination with other printing approaches that construct the electronic part of a device, or as means of functionalization of printed electronic devices. In particular, for laser writing approaches, ongoing developments in processable resins allow now for laser printed electronics,[208,209] that, by combining different resin types, have promises for fully printed bio-hybrid devices in a monolithic process.

3D printed electronics have shown already their utility in cell- and organoid interfacing as described in our review. We believe that there is a bright future for these approaches, in particular when their full potential for complex and hybrid devices is leveraged in the future by combining the different approaches and leaving behind the exclusive interfacing or cell positioning for responsive devices that not only measure or stimulate cells electrically, but allow for additional controlled mechanical and chemical manipulation.

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Conflict of Interest

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