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Electroconductive scaffolds for tissue engineering applications

Pawel Sikorski,*[a]

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Many material systems that can conduct electronic current have been in recent years studied in the context of tissue engineering. It is suggested that materials that can carry electronic current are necessary or beneficial in tissue engineering of cardiac, muscle, nerve and bone tissues. The mechanism by which such systems could influences cells is however unclear and the complexity of the interface between biological systems and electroconductive artificial systems is often underestimated. In this contribution, I review some of the recent literature in this field and highlight uncertainties, aiming to stimulate more theoretical and experimental work. Progress in the field of scaffold-based tissue engineering of electroactive tissues is tightly coupled to our understanding of biophysical processes that take place at scaffold-cell interface. Some authors consider electronic and ionic conductance as equivalent and develop novel materials based on this assumption. However, lack of good theoretical understanding hampers development of new materials and novel regenerative strategies.

1 Introduction

In recent years, large number of studies have combined bioelectronic processes with material science and engineering to development materials and systems for applications in tissue engineering, regenerative medicine, drug delivery, interfacing electronics with biological systems, electrotaxis and bio-sensing[1–15]. Biological electricity includes phenomena and cellular processes, where electric fields, charge separation or ionic currents are a key ingredient. Many artificial electroconductive material systems (materials that can conduct an electronic current) have been studied in the context of interconnection bioelectronic signals. This includes recently proposed conductive peptides[10,16–25], composites containing gold nanoparticles and nanowires[14,26,27], carbon based materials like graphene[28–30], carbon nanotubes[31,32], and synthetic conductive polymers originating from the field of organic electronics[33–35]. Applications of electroconductive biomaterial for cardiac tissue engineering have been recently reviewed by Solazzo et al[36].

Electroconductive biomaterials are not synonymous with *bioelectronic materials*. Research within bioelectronic materials focuses on interfacing electronic devices with biological systems for application among others in wearable or implantable devices and bionic neural interfaces[10,33].

The complexity of successfully interfacing biological systems with electroconductive materials is often underestimated. This is especially clear when electroconductive materials are researched for applications in tissue engineering (TE) and regenerative medicine[5–7,9,26,34,37–40]. It is suggested that electroconductive materials (materials that can carry electronic current such as metals or conductive polymers) can engage with bioelectronic signals. Furthermore, that those are necessary or beneficial in TE of cardiac, muscle, nerve and bone tissues. It is often assumed that bioelectronic signals can generate electrical signals in the scaffold, providing downstream effects. However mechanisms by which this could happen are unclear and often insufficiently discussed. Many authors consider electronic conductance in artificial systems and ionic conductance in bioelectronic as equivalent and compatible. It is assumed that a signal can travel freely between the two environments. Some authors talk about electrocinductive materials that should match physiological tissue in terms of conductivity, overlooking the inherent incompatibility of the ionic and electronic conductivities. This in my opinion, hampers progress in this important field of research and technology development.

In living systems and at physiological conditions:

1. ion concentration gradients across membranes are responsible for generating electric fields and conducting signals along biological membranes[41].
2. ion concentration gradients are used to store electrostatic and chemiosmotic energy[42]
3. electron conductance is very rare and typically observed in specialized systems where electron transport is a part of a redox reaction (in respiration or photosynthesis)[19,41–43]; electron conductance was proposed in conductive biofilms[25].

[a] *Department of Physics, Norwegian University of Science and Technology, NTNU. Trondheim, Norway; E-mail: pawel.sikorski@ntnu.no*

4. proton circuits instead of electron circuits are common[42]

For a tissue like the heart muscle, synchronous contraction of individual cells is achieved by a propagating electrical signal called the action potential (AP). Action potentials are however fundamentally different from signals found in electronic devices. Action potentials are local transient depolarization events that locally change the membrane potential and travel along the cell membrane. Local change in the membrane potential induces biological effects, such as local opening or closing of voltage sensitive ion channels[41]. In a muscle cell, AP changes intracellular calcium concentration, which in turn results in muscle contraction. Depolarization propagates to adjacent cells via gap junctions (electrical synapses) or chemical synapses[44]. Quoting Carmeliet who gives a detail historical perspective on signal propagation in cardiac tissue[44]: "From 1939 on, propagation of the axon action potential was considered a physical process originating in the membrane."

For a metal electrodes in physiological condition, current can flow between the ionic and the electronic environments only if redox reactions take place at the interface[45,46]. Signal transduction from the electronic to the ionic part is possible and is often used in delivery of external electrical stimulation[36]. This have successfully been applied to *in vitro* stimulation of cellular constructs in order to achieve myocardium maturation, direct stem cell differentiation or neurite growth[47,48]. It is also used to direct regenerative processes in vivo. When external stimulation is applied using the correct electrode geometry, electrical signal can be transduced into an ionic signal through redox reactions. The flow of ionic current between two electrodes result in a local electric field that can affect cells located away from the electrode surface[42].

It is however not as straightforward to transduce bioelectric signals to electrical signals. Electric field that exist in a living system is always a consequence of ion separation across a membrane. Therefore the membrane potential can only be measured across the cell mambrane[49]. Other much weaker signals can also be detected extracellularly and these are, for example, exploited in multielectrode arrays technology[50,51]. Signals recorded extracellulary are very week, as the detection method is based on detecting charge imbalance and extracellular ionic currents created by an action potential in the space between an electrically active cell and the electrode[52–55]. One area in which electroconductive materials could make an impact, is in real-time monitoring of the local electrical activity during the regeneration processes[13]. Combine with delivery of external stimulation, this could be a valid approach to TE.

Cell-cell coupling by an electroconductive scaffold achieved without external stimulation is far more challenging and remains an area of significant research effort. Numerous studies have demonstrated the effectiveness of electroconductive scafolds in TE, but fail to show conclusively that the effect is correlated with electrical conductivity and the propagation of an electrical signal through the scaffold[7,27,37,56,57]. Response of cells to an artificial scaffold is a consequence of a complex interplay between material chemistry (which for example will influence protein corona formation), topography, mechanical properties, surface charge density and perhaps electrical conductivity. Designing experiments that can separate between these effect is challenging, but one can not simply assume that electrical conductivity is a key property responsible for the observed function. Below, I describe several studies selected from a large number of similar investigations, that propose to use materials that conduct electrons in TE applications. I focus on the motivation for the development of such materials and how these are envisaged to be used in TE application.

2 Electroconductive Biomaterials

Methods for 3D printing and structuring of conductive GelMA/PEDOT:PSS hydrogels were developed by Spencer *et al* with the aim to obtain materials that can improve bioelectric function of the tissues during regeneration[37]. It was suggested that that conductivity* is an important parameter for scaffold assisted regeneration excitable tissues such as cardiac, skeletal and smooth muscle, as well as neural tissues. The aim of this study was to engineer complex, conductive and cell-laden structures. Developed materials show tunable mechanical properties, tunable conductivity and good biocompatibility. Electrical properties were characterized with electrochemical impedance spectroscop. The gels were washed with DiW to remove excess salt ions and measurements were recorded between 0.1 and 1000 Hz with an AC amplitude of 10 mV. Only very moderate changes (approximately factor of two) in the impedance between doped and undoped gels were observed. It is unclear if these changes can be attributed to elecronic conductance of the scaffold, or if it is a result of other processes that take place at the hydrogel-electrode interface. More importantly, if such electroconductive scaffold should induce coupling of electrically disconnected cells for example to improve cardiac function, mechanism by which electric signals could be induced in a conductive scaffold *in vivo* needs to be understood. Proofs of concept that verify that scaffold concept can indeed direct cellular processes are difficult to obtain, due to the complexity of the system under investigation. The authors only shown that used materials have high printing fidelity and allowed attachment, spreading and support high viability of the encapsulated immortalized mouse myoblast (C2C12 cell line).

In a similar study, You *et al* investigated macropores hydrogel scaffold containing gold nanoparticles. The study was motivated by a hypothesis that scaffolds made from electroconductive material could produce a unique cellular microenvironment. In addition it was suggested that tissue function, exemplified in this study by cardiomyocytes, could be improved by the use of a scaffold which conduct electrical current. The authors state that, "Therapeutic opportunities may exist in increasing the conductivity of damaged cardiac tissue by amplifying cell-cell communication"[7]. However the mechanisms by which cell-cell communication could be enhanced is unclear. It was observed that neonatal rat cardiomyocytes exhibited increased expression of Connexin 43, a protein that is involved in gap junction channels and elec-

* By saying conductive many authors mean electronic conductance, that is material that can conduct electric current

trical synapses between cardiomyocyte[7,58]. Increased expression was observed for experiments which were conducted with and without external electrical stimulation. The authors suggested that conductive nature of the scaffold was response for this effect. Hypotheses other than electronic conductivity of the scaffold were not explored and the mechanism by which scaffold properties induce this increased expressions was not explained.

Yang *et al*[56] equate electronic and ionic conductivity, stating that myocardium is an conductive tissue capable of transferring electrical signals. They hypothesise that nonconductive materials which do not allow electrical signal propagation, if used *in vivo*, prevent effective cell–cell communication. This argument is used as a motivation for developing electrically conductive double-network hydrogels[56]. Also for this scaffold, increased expression of Connexin 43, this time by brown adipose-derived stem cells was observed. Increase in expression was especially strong for experiments where electrical stimulation was applied to the cells in culture. The effect was attributed to intracellular generation of reactive oxygen species (ROS), that activates signalling cascades involved in growth and differentiation of stem cells[59]. It was suggested that the conductive properties of used hydrogel enhance this effect. This is however quite surprising, as the stimulation was done by sending electrical pulses with an amplitude of 1 V and a duration of 2 ms through an ITO conductive support coated with the conductive scaffold material. Cells were seeded on top of the scaffold. In this geometry it is unclear how much current flows through the highly conductive ITO support and how much current flows through the scaffold itself.

In one of the earlier studies that investigated the effect of conductive components in a hydrogel matrix, Dvir *et al* studied the effect of alginate-gold nanowire composites on neonatal rat ventricular myocytes. It was observed that nanowires embedded in the scaffold had a significant influence on beat synchronization between cells in different parts of the scaffold[27]. The exact mechanism by which this improvement was achieved was however unclear, and the authors speculated that this could be due to conductive bridges formed across the hydrogel connecting adjacent pores and cell bundles. The authors have however not excluded the fact, that improved synchronization could be caused by nanowire-induced change in mechanical properties of the scaffold, nanowire-induced change in ECM produced by cells or nanowire-induced changes in gen expression. In many articles citing this work†, synchronization between cells is attributed to presence of conductive nanowires. Interestingly, Navaei *et al* have investigated the role of electronic conductivity in a similar system and found that nonconductive nanomaterials could also influence maturation and excitability of cardiac tissues[60]. They concluded that nanomaterials increased expression of cardiac-specific markers through modification of the mechanical properties of the scaffold[60].

Song *et al* motivate the development of injectable, conductive 3D elastic network materials based on a hyprothesis that electrical conductivity is important to maintain the physiological function of the heart[12]. They showed that elastic, conductive spring-like coils supported cardiomyocytes attachment and the cells on the scaffolds formed highly oriented sarcomeres. Developed materials were tested *in vivo* in a rat infarct model and had an ability to improve cardiac function and helped to reduce the size of infarct area. However, the mechanism by which they function was unclear. In the rat model, increased revascularization in the infarct area was observed, but it is difficult to conclude that it was directly connected to the conductive nature of the scaffold.

Several authors develop and study electroconductive scaffolds that are used with electrical stimulation without careful consideration of the field direction or current flow paths and how these can influence cells cultured on or within an scaffolds[3,6,12,30,61,62]. It is for example unclear how current passed through a highly conductive substrate could effect cells that are cultures on the top of such substrate[6,62]. Others suggest that piezoelectric effect could be used to influence cell behaviour[9,34] without explaining how an electric field that is present inside a piezoelectric material, could influence cells that are located at the interface.

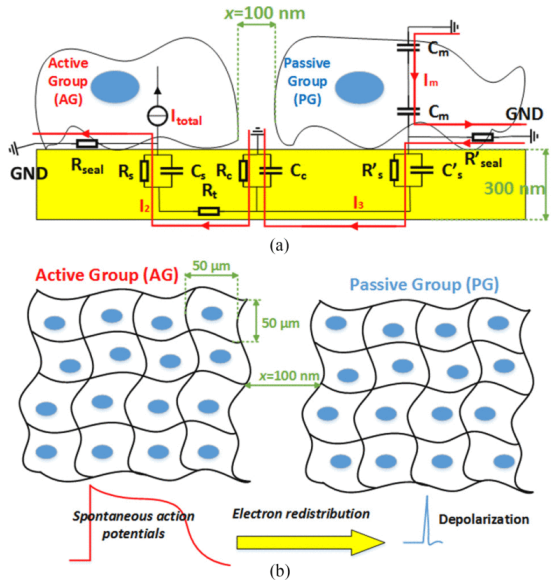

Fig. 1 Model of an interface between electrically active cells and a conductive scaffold proposed by Wu et al in which two groups of cardiomyocytes adhering to a conductive substrate. The active group (AG) is firing action potentials and the signal is transduced to the passive group through the conductive substrate[63]. © 2018 IEEE. Reprinted, with permission, from Wu, Y., & Guo, L. (2018). Enhancement of Intercellular Electrical Synchronization by Conductive Materials in Cardiac Tissue Engineering. IEEE Transactions on Biomedical Engineering, 65(2), 264–272.

Studies describe above open several interesting questions, that to my knowledge are not answered in the current TE literature. In depth understanding of the interactions between ionic and electronic conductance would without a doubt contribute to new strategies for TE. For example, what conditions are necessary for an electrically active cell to induce an electronic current in the scaffold or a substrate? Is such current at all possible? Could this signal induce downstream effects such as propagation of an action potential in cells that are contact with the scaffold?

† https://scholar.google.com/scholar?cites=18319436645342824132

Could this signals induce changes in cellular processes on the long timescale, for example receptor clustering, changes in gen expression or cell differentiation?

3 Interface Models

Models of the interface between electrically active cells and a conductive substrates have been recently proposed by Wu *et al*[63] and Burnstine-Townley *et al*[64]. Based on electrical circuit simulation, Wu *et al* concluded that depolarization event in one cell group that is in contact with a conductive substrates (referred to as Active Group, see Figure 1), could induce some degree of membrane depolarization in adjacent cells (referred to as Passive Group, see Figure 1) that are coupled to the cells in the active group through the electroconductive substrate. The used model was constructed based on an approach typically used to model cell-electrode interface for recording of neural activity or for electrostimulation of cells in contact with an electrode[51,54]. The interface is described in terms of electrical equivalent circuit (Figure 1). Wu *et al* apply the approach based on the work of Franks *et al*[45], who uses Randles model[65] to describe processes that take place at the electrode. In this model, the electrode-electrolyte interface is described by interface capacitance C_s and reaction resistance R_s[54,63,64] that are connected in parallel. Complete, cell-conductive scaffold circuit is more complex and in addition includes seal resistance R_{seal}, membrane capacitance C_m and scaffold resistance R_t.

However, when applied to cell-elecroconductive scaffold interface, this model might only be partially correct. The current that flows through the reaction resistance R_s at the interface (see Figure 1) is due to the transfer of charge between the electrode and the electrolyte. This transfer can only happen through a redox reaction[45,51]. If no redox reactions are taking place at the electrode interface, the net DC current through the reaction resistance should be zero. Whenever such redox reactions can be coupled to cell activity and to the action potential is unclear. Based on the assumption that the action potential results in a transient decrease in the concentration of sodium ions at the cell-electrode interface, a coupling between action potential and redox reactions in unlikely. This aspect of the electronic-ionic interface is important for studies where electroconductive materials are used in TE.

Wu *et al* treats cells in the active group as a ideal source of an ionic current. This approach also has some limitations that are not discussed by the authors. On careful examination of the transfer function proposed by Wu *et al*, one see that the PG depolarization (U_{peak} and the amplitude of the transfer function) increases with increasing seal resistance R_{seal}. This is not unexpected. However, the maximum amplitude of the depolarization signal in the PG should not exceed the membrane potential of cells in the AG. In microelectrode arrays that are used to perform extracellular recordings of neuron activities, recorded potentials are typically below 1 mV, and are dependent on the electrode impedance. Therefore, a conclusion that large seal resistance can improve signal transduction through the scaffold might need further analysis.

Using a similar approach, Joye *et al* conclude that the signal at the electrode interface is at the order of 1-10% of the change in the membrane voltage[51] (30 dB attenuation of the amplitude and signal with maximum amplitude of 1 mV to 10 mV). Even if this signal could be transmitted without losses to the PG, this would most likely only result in a sub-threshold depolarisation that would not be sufficient to initiate action potential in the passive group[51]. Burnstine-Townley *et al* also investigated electrical properties of the interface[64]. In their model, a small depolarization amplitude was predicted, and it was suggested that this amplitude was sufficient to activate cells in the passive group. As the typical change in membrane potential needed for activation of an action potential is in the range of 20 mV to 50 mV basis for these conclusions are unclear[66].

Conclusion

Whether the models describe above are proven correct or need to be revised, the outcome should be used in rational design of scaffold materials that aim at interacting with ionic currents and electric fields found in living tissues and cell cultures. The goal of this review was to highlight some uncertainties when it comes to the use of materials that conduct electronic current in tissue engineering applications. Standardizing methods used to study material properties in relevant conditions are also needed. Today, various techniques are applied to show electronic conduction in a dry state and sometimes electronic (or ionic) conduction in physiological conditions. Often it is not clear what is measured and what is the significance of measured parameters. Researchers working in this field are encouraged to formulate hypotheses that could explain process that take place at the interface between ionic and electronic conductance and test these with material design approaches. In particular, (i) what processes can be induced in a electroconductive scaffold by electric fields originating from ion concentration gradients? (ii) what is the field strength and field direction and how can electric field mediated by the scaffold influence biological components such as voltage gated ion channels? (iii) what is the mechanism for charge transfer through the interface? (iv) what alternative mechanisms could explain observed biological effects? Addressing these questions would allow scientists and engineers to take full advantages of possibilities in this exciting area of research and technology development.

Conflicts of interest

There are no conflicts to declare.

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