Cell-bionics: tools for real-time sensor processing

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The accurate monitoring of the physiological status of cells, tissues and whole organisms demands a new generation of devices capable of providing accurate data in real time with minimal perturbation of the system being measured. To deliver on the promise of *cell-bionics* advances over the past decade in miniaturization, analogue signal processing, low-power electronics, materials science and protein engineering need to be brought together. In this paper we summarize recent advances in our research that is moving us in this direction. Two areas in particular are highlighted: the exploitation of the physical properties inherent in semiconductor devices to perform very low power on chip signal processing and the use of gene technology to tailor proteins for sensor applications. In the context of engineered tissues, cell-bionics could offer the ability to monitor the precise physiological state of the construct, both during ‘manufacture’ and post-implantation. Monitoring during manufacture, particularly by embedded devices, would offer quality assurance of the materials components and the fabrication process. Post-implantation monitoring would reveal changes in the underlying physiology as a result of the tissue construct adapting to its new environment.

Keywords: ISFET; analogue electronics; protein engineering; biosensors

1. BACKGROUND

Since the last quarter of the nineteenth century, there has been an enormous amount of work carried out on the physiology of the human body. In the 1940s, classical physiological studies began to be augmented by mathematical analysis. This work was complemented by the use of computers (from the 1960s onwards) for the mathematical modelling of metabolism, physiological function and biological signal processing. As the power of computers has increased over the last 30 years so has the ability of basic medical scientists to understand many areas of physiological function in detail. This knowledge, coupled with advance medical imaging techniques (e.g. magnetic resonance, computerized tomography, positron emission and ultrasound), has led to the current situation where clinicians are able to carry out detailed diagnosis of many diseases.

A key problem, which is still largely unresolved in many areas of medicine, is how to transform an accurate diagnosis into an effective treatment protocol. To date, electronics has been applied to the development of diagnostic devices, such as ECG monitors, ultrasound scanners (for the measurement of blood flow), blood pressure metres, etc. However, with one or two exceptions (e.g. cardiac pacemakers), medical electronic devices have been non-invasive and required high power consumption.

Medical technology on a chip encompasses some of the most challenging and technically demanding designs of implantable devices for therapeutic or long-term monitoring functions. These include implantable drug delivery systems, spinal cord bridges for neural stimulation, or the simulation of muscular or sensory

functions, multifunction catheters, systems for medical telemetry including sensors. In addition, there are other integrated medical devices such as cochlear and retinal implants under active development. A cardiac pacemaker is a classic example of a medical device that evolved from a discrete components-based design to its present form of an integrated system. This spectrum of activities strives for the development of new technologies for health care delivery modalities that include diagnosis as well as therapy, and it will continue to shape biomedical engineering in years to come.

There is a growing recognition that the effectiveness of disease management is improved by closer monitoring of critical biochemical indicators. The physiological state of both healthy and diseased tissues is typically indicated by the concentrations of small and large molecules, commonly referred to as *biomarkers*. Disease is indicated by the concentration of one or more biomarkers being outside the so-called reference range and the accurate measurement of these biomarkers can aid both diagnosis and management of the disease. Very rarely is a single biomarker of unambiguous use in diagnosis and hence a panel of markers is more commonly employed, often in combination with other physiological indicators, patient and family histories and such factors as the clinician may deem relevant. In assessing therapy or managing the disease, biomarker monitoring has been less extensively employed than in diagnosis, although the trend towards personalizing health care, the increasing importance of chronic rather than acute illness and the high cost associated with introducing novel pharmaceuticals suggests that monitoring of clinical biomarkers will play an increasingly important role in patient care.

One well-established area that has seen dramatic improvements in technology over the past 20 years has been diabetes where the use of colour strips, large,

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One contribution of 21 to a Theme Issue ‘Bioengineering the heart’.

finger stick, blood drops, visual assessment and cumbersome procedures have been replaced by fast (less than 10 s), low-volume electronic devices that have transformed the control of this disease. Even here, however, the episodic nature of measurement and the discomfort of taking regular blood samples demands further improvements in technology.

Currently, the diabetes model for patients taking control of their own monitoring is the exception and a majority of such data are acquired through scheduled hospital or physician appointments. Not only is this disruptive to the patient's lifestyle, but it also may result in a delay in recognizing and treating adverse changes, with a potentially detrimental impact on the patient's condition. Thus, there is an urgent need for real-time data acquisition, processing and communication to health care personnel for remote critical health care monitoring and analysis. This paper reviews some of the authors' work at attempts to provide solutions based upon technologies that have evolved through the semiconductor and telecommunication industries as well as through the application of gene technology.

Tissue engineering also requires a highly integrated combination of specific changes in gene expression and environmental factors (chemical and physical). To date, there has been no means of monitoring or logically and simultaneously controlling these changes. Incorporation of such sensing devices in the bioreactors used to culture stem cells would lead to a greater understanding of the factors affecting stem cell proliferation and differentiation, as well as control of these factors via output to appropriate actuators. Tissue engineering is a bio-inspired technology aimed at harnessing the continual advances in the life sciences, so as to develop new treatments and therapies for the repair, replacement and regeneration of tissues and organs. From tissue engineering will come the next generation of medical implants. A critical issue is cell source. However, equally important is the incorporation of cells into structures that mimic native tissues and organs, both in architecture and function. Virtually all tissues are three-dimensional in nature; a vital lesson we have learnt in recent years is that cells in three-dimensional structures have characteristics far different from the same cells in monolayer. Although there have been initial successes in tissue engineering with skin substitutes, the full potential of this emerging technology will only be fulfilled by shifting its focus to more complicated tissues and organs. Opportunities abound in many areas, including orthopaedic tissue engineering, metabolic and secretory organs and cardiovascular substitutes.

Over the last decade, silicon chips have dramatically reduced both in their size and power consumption, while increasing greatly in information processing power. When combined with sensors and biotelemetry to communicate with external IT systems, such devices will open a vast range of possibilities in the analysis and treatment of disease, as well as in the monitoring of implants.

Device-tissue interfaces. One of the key technological challenges that will need to be overcome in moving from episodic to (semi)-continuous monitoring is the engineering of the interactions at the interface between

the sensor and the tissue. Sensors by their very nature measure local concentrations (or concentration gradients) and if these data are to be used to draw conclusions about the systemic significance of a change in biomarker concentration, then the sensor response should not be a consequence of local perturbations brought about by the very presence of the sensor itself. Given that the sensor is a foreign object, it will almost inevitably elicit a biological reaction; for *ex vivo* or short-term exposure, their effects are minimal. However, for long-term exposure of implanted devices, the tissues' responses are much more severe and present a significant challenge. The implantation of foreign objects is not of course limited to sensors. Prostheses have long had to confront the issues of biocompatibility and a number of successful strategies have been developed to ensure the long-term stability of implants. The issue with sensors is not purely to do with the body's toleration of the implant but rather the effect of local tissue response on the calibration of the sensor. This can be manifest as drift in calibration and/or sudden failure of the device.

2. TECHNOLOGY

The world is analogue and yet we have been exposed to a synthetic world that gives us discrete quantities to manipulate using Boolean logic. In the human space, however, we are entering a new wave of body-worn biosensor technology for medical diagnostics and therapy. This new trend is beginning to see the processing interface move back to continuous quantities that are more or less in line with the biological processes. We label this paradigm of processing as bio-inspired owing to the ability of silicon chip technology and the inherent physics of the device to now approach computational efficiencies of biology. From a conceptual viewpoint, this has led to a number of other morphologies, such as neuromorphic and retinomorphic processing (Mead 1989; Giles 2001). These processing paradigms have led scientist to model biological systems such as the cochlear and retina, with the key objective of using such models being to increase our understanding of biological and neurological processes.

We propose to apply a similar approach to 'chemically inspired' circuitry that would lead to portable, low-power micro-systems capable of fast chemical or biochemical discrimination and interrogation of data exactly integrated at the sensor end. Thus, in contrast to the digital approach, where a single operation is performed by a series of switched on or off devices, the physics of the elementary device itself either electrical, chemical or electrochemical can be exploited to perform the same operation in an analogue way; hence the energy per unit computation is lowered and power efficiency is increased.

The device under development is a wearable wireless personal sensor network, with wireless interfacing to a bespoke hospital- or community-wide database. The first generation device will comprise sub- and transcutaneous sensors for the measurement of critical biochemical parameters, e.g. pH, glucose, dissolved oxygen and carbon dioxide, as well as temperature and

Figure 1. A 1 μ W bio-inspired cochlear filter.

ECG. Low-power wireless transmitters will be integrated onto the sensor chips.

When weakly inverted, a Metal Oxide Semiconductor Field Effect Transistor (MOSFET) is characterized by diffusing carrier flow. Their weak inversion operating region is characterized by drain current levels ranging typically from 1 pA to 10 nA. Thus, the exploitation of weakly inverted MOSFETs powered by low (approx. 3 V) power supply voltages leads readily to the realization of analogue micropowered designs. Generally, weakly inverted MOSFET devices are governed by a pristine exponential $V-I$ characteristic (dictated at carrier level by the Fermi-Dirac distribution function)

$$I_{DS} = I_{n0} \left(\frac{W}{L} \right) \exp \left[\frac{V_{GS}}{nV_T} \right].$$

One key research opportunity proposed here is therefore to identify and exploit other niches where analogue signal processing can naturally have an edge over digital signal processing techniques, both in terms of power consumption and effectiveness. The current-mode approach (Toumazou *et al.* 1993) enables the design of high dynamic range systems since signals are represented by currents: junction leakage currents and noise constitute the lower signal limit, whereas degraded g_m/I values and distortion constitute the upper signal limit. Typically, four decades of current signal levels can be accommodated within the weak inversion region of a MOSFET device.

Figure 1 illustrates a practical, less than 1 μ W, fully tunable micropower log-domain filter designed for a cochlea implant (Georgiou & Toumazou 2005). The design employs MOSFETs operating in weak inversion exploiting the exponential characteristic to create filter functions. The design is characterized by 200 nW per pole power consumption. It is this technology which forms the core of much of the bionics activity reported to date.

3. BIOCHEMICAL SIGNAL PROCESSING

Most biological monitors whether implantable or wearable are based upon monitoring electrical activity and then performing the analysis off-chip using digital 'engines', such as microprocessors and computers. In this paper, we propose an alternative processing engine based upon monitoring chemical activity directly arising from biochemical reactions. We have labelled

this processing concept a 'biochemical signal processing engine' (figure 2).

This engine will perform on-chip temporal and spatial signal processing on inputs from an array of biochemical sensors. This processing will include sensor drift and temperature compensation, failure tolerance (redundancy), as well as mathematical manipulation, such as real-time calculation of rate constants and critical ratios and alarm/actuator triggering. There will also be logic elements for instantaneous thresholding.

4. SENSOR TECHNOLOGY

The ISFET is a chemical sensor, based on a standard transistor structure, but whose current-voltage characteristics are shifted linearly with changes in pH due to changes in charge distribution at the sensing surface. The use of chemically selective membranes or enzyme layers at the ISFET sensing surface enables the manufacture of devices known as ChemFETs and EnFETs, which are specific to certain chemical or biological species (figure 3; Bergveld 2003).

The attractiveness of ISFETs and their FET-based counterparts is that they are compatible with the standard CMOS fabrication process used to mass produce computer chips; therefore, they can be reliably and cost-effectively produced (Jakobson *et al.* 2002; Hammond *et al.* 2004). Furthermore, processing circuitry can be integrated onto the same chip as the ISFET device itself. The integration of intelligent circuitry with the sensing device itself is what is required for robustness to non-ideal sensing conditions and intelligent real-time interpretation of biochemical phenomena.

Our group are the first to have reported that it is possible to measure biochemical parameters such as pH using a novel ultra-low power method (Shepherd & Toumazou 2005b). This technique exploits the Boltzmann exponential characteristic between current and voltage that transistors exhibit when biased in the nanoamp 'weak inversion' range. Proof of principle has been demonstrated by a correlation between output current and the ionic concentration of standard solutions. Moreover, we have shown using a blood gas analyser that this correlation is also observed in clinical blood samples.

(a) The ISFET as more than a pH sensor

The ISFET is a chemical sensor, based on a standard transistor structure, but whose current-voltage characteristics are shifted linearly with changes in pH due to changes in charge distribution at the sensing surface. The use of chemically selective membranes or enzyme layers at the ISFET sensing surface enables the manufacture of devices known as ChemFETs and EnFETs, which are specific to certain chemical or biological species.

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Figure 2. The biochemical signal processing engine.

device itself. The integration of intelligent circuitry with the sensing device itself is what is required for the development of so-called 'smart sensors'. These are required for robustness to non-ideal sensing conditions, as well as for the use of electronics to discriminate between chemicals 'on-chip'—for use in early warning systems for example, or in locations where full computer analysis tools and trained operators are not available.

The *weak inversion* mode of operation involves maintaining the gate voltage lower than the threshold voltage such that the channel is depleted and only a thin inversion layer exists. In weak inversion, the mobile charge in the thin inversion layer is too low to contribute significantly to any drift current across the horizontal electric field. Drain current in weak inversion is due to the diffusion of electrons across a concentration gradient between the source and the drain.

- (i) Current proportional to concentration gradient
- (ii) Conc. gradient = conc. source – conc. drain (dependent on barrier potentials; dependent on applied voltages V_s and V_d) and on surface potential (dependent on V_g)
- (iii) (barrier potential = built in potential – q (surface potential – V_s or d))
- (iv) surface potential = $\phi_0 + kV_g$ (ϕ_0 related to V_{th})
- (v) Drain barrier potential higher than source barrier potential if $V_d > V_s$.

For the ISFET, the reference electrode acts as a remote gate and the chemically sensitive membrane deposited on top of the SiO_2 insulator is exposed directly to the sample solution. The extent of inversion in the channel beneath the insulator is dependent not only on the voltage applied to the reference electrode, but also on the accumulation of charge from ions in solution on the sensing membrane. The build-up of ions at the surface of the membrane is related to the concentration of the ionic species in the sample by the site binding and Gouy–Chapman double-layer models.

Figure 3. The ISFET.

Since any positive charge build-up on the membrane surface must be mirrored by a negative charge build-up in the channel, changes in ionic concentration of the sample will be reflected directly in the ISFET's weak inversion drain current.

A knowledge of the relation between membrane surface charge and species concentration, together with the fact that weak inversion ISFET current is proportional to membrane surface charge, means that electronic circuits performing simple mathematical manipulation can be used to obtain a direct relation between species concentration and current even in ChemFETs and EnFETs. Furthermore, the sensitivity of the weak inversion ISFET current to ion concentration is independent of temperature, since the temperature-scaled Boltzmann distributions of both the electrons in the channel and the ions in solution cancel each other out. This is a significant advantage over the use of the ISFET as a potentiometric device whose sensitivity increases linearly with temperature.

(b) *Mathematical manipulation for smart sensors*

The motivation for using ISFETs in weak inversion for the transduction stage of a chemical sensor has been explained. The use of MOSFETs in weak inversion is also advantageous for on-chip processing circuitry since the exponential relation between drain current and terminal voltages can be exploited to implement

mathematical manipulation using very simple, low-power circuits.

Low-power chemical signal processing circuits such as these are required for the development of true smart sensor systems taking the ISFET and its transistor-based counterparts beyond chemical-to-electrical transduction. Smart ISFET systems will have increased accuracy through the use of feedback and multiple sensors, and adaptivity to drift and changes in baseline conditions. Intercommunication between several sensors in an array will enable real-time chemical discrimination.

The exponential behaviour of weak inversion circuits has also been used extensively in the implementation of neural networks (Mead 1989), which could be integrated into ISFET systems in order to add intelligence features, such as learning and predictability (Shepherd & Toumazou 2005b).

(c) Translinear circuits

Circuits which exploit the exponential relationship between current and voltage in bipolar and subthreshold MOS transistors are known as translinear circuits (Andreou & Boahen 1996). These circuits exploit the linear relation between transconductance and drain current and can be used to perform multiplication, division and power-law functions on current signals. Gilbert's translinear principle for bipolar transistors (Toumazou *et al.* 1993) is a reformulation of Kirchhoff's voltage law and states that 'in a closed loop containing an even number of forward-biased junctions arranged so that there are an equal number of clockwise- and anticlockwise-facing polarities, the product of the current densities in the clockwise direction is equal to the product of the current densities in the anticlockwise direction',

$$\prod_{\text{clockwise}} \text{current density} = \prod_{\text{anticlockwise}} \text{current density}.$$

Andreou formulated the principle for application to the exponential region of MOSFETs (Andreou & Boahen 1996), which had already had much success in the field of biologically and neurally inspired engineering (Giles 2001), and work reported in this paper proposes that the principle is equally valid and useful for weakly inverted ISFETs.

The DC characteristics of commercially produced discrete Al₂O₃ ISFETs from Sentron Europe B.V. were analysed with a semiconductor parameter analyser in buffer solutions of different pH.

The results are shown in figure 4 and clearly indicate the existence of a weak inversion region in which drain current is exponentially related to gate voltage. As can be seen, current levels are of the order of several nanoamperes.

(d) The HCell circuit: chemical-inspired processing

The 'HCell' circuit in figure 5 is presented as an example of application of the weak inversion ISFET translinear principle to manipulate the equation relating the drain current to the gate voltage to obtain an input stage whose output is a current directly proportional to the concentration of H⁺ ions (protons) in the bulk of the electrolyte. It is illustrative of how apt it is to exploit

the exponential behaviour of field effect transistors in weak inversion to obtain real chemical parameters directly. The ISFET used in the HCell has its ion-sensitive membrane chemically tailored such that its pH sensitivity gives $\alpha/n=0.5$ so that a squaring operation will render a linear relationship between [H⁺] and I_D. (Shepherd & Toumazou 2004, 2005a,b)

5. PROTEIN DESIGN AND ENGINEERING

(a) Biorecognition

Much of the impetus in integrating solid-state devices with biomolecules is to take advantage of the latter's key attributes of specificity, affinity and in the case of enzymes catalytic activity. The underlying mathematics of biorecognition is the same, irrespective of whether it is purely a binding interaction or an enzyme-catalysed reaction and this forms the basis of the sensing mechanism. Initially, a complex is formed between the two partners (*R*, reagent; *A*, analyte)

The equilibrium constant, *K*, usually expressed as a dissociation constant, defines the concentration range over which the equilibrium occurs.

In an enzyme-catalysed reaction, the formation of *RA* is followed by the conversion of *A* to some product. In both cases, the functional form of the relationship between what is measured as a signal from the sensor, *S*, the analyte concentration, [*A*], and *K* is given by

$$S = \frac{S_{\max}[A]}{K + [A]},$$

where *S*_{max} is the maximum value of *S*, i.e. the value when [*A*] ≫ *K*. The above equation has the form of a rectangular hyperbola yielding a linear response at low analyte levels and becoming independent of analyte at high concentrations.

(b) Mass transfer

Sensors are heterogeneous devices, the analyte being in solution while the reagent is typically immobilized on the sensor surface. This results in mass transfer having an important role to play in sensing devices and where the overall response is mass transfer limited, this will have important consequences for calibration. In the case of enzyme sensors, mass transport limitations can alleviate the effects due to changes in enzyme activity affecting calibration, and the relative contributions of mass transfer and catalytic activity can be manipulated through sensor design. Design factors include enzyme loading, sensor size and geometry as well as the use of mass transport barriers, such as membranes.

(c) Protein engineering

The use of proteins as selective coatings on sensor surfaces brings the power of biorecognition and catalysis to the devices. It should be realized, however, that the proteins we use in biosensors have properties honed through evolution that fit them to their physiological role rather than to the sensing one. Until purely synthetic coatings achieve the same degree of performance, the alternative to using the 'wild-type' (natural) form of the protein is to redesign some of its properties to make

Figure 4. ISFET transconductance curves showing weak inversion characteristics: (a) linear scale; (b) logarithmic scale.

them better suited to the sensor requirements. This protein engineering takes advantage of our ability to make precise changes in the sequence of the DNA coding for the protein that are expressed as an altered amino acid sequence and hence function (figure 6).

The advantage of such an approach is that we can treat the protein as comprising three functional modules:

- (i) an immobilization module;
- (ii) an analyte recognition module;
- (iii) a signal generation module.

Through the power of recombinant DNA methods, it is then possible to introduce and/or change these modules in a directed fashion.

The advantage of adopting a protein engineering approach is that a design cycle can be employed to optimize protein performance where there is an interplay between computation and experiment.

(d) *Immobilization*

The attachment of proteins to sensor surfaces is often a critical step and becomes more so as the dimensions of the sensor are reduced from millimetres to micrometres. This is partly a consequence of the direct effect of the protein loading on signal intensity and partly a change in the mass transfer characteristics. The attachment of fusion 'tags' is well established in the field of protein purification, and matching the tag chemistry to that of the surface allows a generic approach to immobilization

Figure 5. HCell with I_{out} linearly proportional to $[H^+]$.

Figure 6. DNA to protein. Changes in the DNA sequence lead to changes in amino acid sequence and hence function of the protein.

Table 1. Some examples of tag–surface complementarity used in biosensors.

tag chemistry	surface chemistry
(histidine) ₆	metal chelate, gold
cysteine	gold, polyaniline
streptag	streptavidin
avitag	streptavidin and avidin
E12	hydrophobic (e.g. polystyrene)

that in principle leads to controlled orientation on the surface and high retention of activity. Some examples of tag–surface complementarity are given in table 1 and while retention of activity is often observed, the tags are not always innocent (see Halliwell *et al.* 2001 for an example). Using a flexible spacer such as (Gly.Ser)_n to bridge the protein and tag can be beneficial in preserving activity both in solution and following immobilization (Shao *et al.* 2000; Wada *et al.* 2003).

Analyte recognition implies not just the more or less specific reaction of the analyte by the protein but also the matching of the responsive concentration range to that which is clinically relevant. Where the ‘natural’ affinity of the protein does not match the analytical range engineering, the binding site can be used to shift the dose–response curve by several orders of magnitude (figure 7).

Figure 7. Shifting the dose–response curves of the phosphate-binding protein through engineering the binding site. The native curve corresponds to the protein without binding site mutations. The numbers 1–3 are different binding site mutants.

It is also quite possible to change the analyte specificity as well as the dynamic range through directed mutations in the binding site.

Signal generation lies at the heart of biosensor technology and here too protein engineering has played a significant role in the introduction of efficient coupling between molecular recognition or catalysis and signal generation. In some cases, the engineering of specific label attachment sites can couple a ligand-dependent conformational change to fluorescence or redox changes. In other cases, the enhancement of enzyme activity can improve the signal to background ratio where the signal is not mass transport limited.

(e) Applications

Smart sensing concepts based on weak inversion operation of the transistor applications beyond chemical discrimination using the selectivity of the membranes are as follows.

- (i) Real-time reaction monitoring and analytical data processing.
- (ii) DNA sequencing.
- (iii) Fast acid–base titrations using kinetic models to extract the analytical information sought, well before reaching the equivalence point.
- (iv) Implementation of ‘chemical decision trees’ using ISFETs and other sensors directly as logic (true/false) devices with adjustable thresholds.
- (v) Medical diagnostics using real-time monitoring of blood, interstitial fluid and urine metabolite ratios.
- (vi) Neural bridges.
- (vii) Impurity detection.

This can then be combined with the redesign of proteins to provide customized sensor coatings with enhanced activity and stability.

6. SUB-CELLULAR BIONICS?

While the current generation of devices is large relative to the size of a cell, the natural extrapolation would be to

devices small enough to report from inside the cell. Intracellular molecular sensing has already been demonstrated using optical readouts, for example with molecular beacons to monitor gene expression (Santangelo *et al.* 2006) and PEBBLEs to monitor metabolites (Buck *et al.* 2004). In the former case, the reporter is purely molecular, while the PEBBLEs use polymer encapsulation to protect the reporter molecule from the cellular constituents and the cell from potential cytotoxicity of the reporter. We can imagine a logical extension of this approach to encompass ultra-low power, wireless, solid-state devices as nanoprobe reporting on the sub-cellular environment. Recent descriptions of the wireless, swallowed 'lab on a pill' (Johannessen *et al.* 2006a,b) point the direction in this respect, although orders of magnitude reduction in size and novel approaches to powering such devices will be needed.

7. CONCLUSION

The main technical challenges lie in the development of sensors, which are easy to calibrate, accurate in and well matched to the physiological range and resistant to biofouling. Another important consideration will be the monolithic integration of the sensors with their processing circuitry, requiring a well-controlled encapsulation process to ensure that the electrical components are insulated while ensuring the sensing elements remain exposed. If these challenges can be met, then there will be an explosion in the application of cell-bionics to tissue engineering, both during manufacture of the construct and post-implantation.

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