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Biological engineering as an approach to construct smart nanostructured systems

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Abstract

In this short review we explore the design of smart nanostructured systems that are assembled with inspiration from biology. Here we consider the design and assembly of smart nanostructured systems based on "biological engineering", which was a term introduced formally in 1970 with the intention to integrate engineering with biological systems to move beyond single disciplinary areas such as medicine, agriculture, or fermentation engineering. We further refine the discipline of "biological engineering" to be one that embodies the approach of assembling smart nanostructured systems by utilizing biological proteins, molecules and lipids in combination with synthetic materials. This approach is illustrated with two examples of smart nanostructured systems that utilize ion channels as control elements; the first by genetically modifying a liver cell to secrete insulin, and the second by assembling an artificial cell with purified ion channels incorporated in a lipid bilayer membrane. These examples of smart nanostructured systems are taken from my plenary presentation made at the European Biotechnology Congress that was held at the Harbiye Military Museum in Istanbul, Turkey, from 3rd to 5th October 2024.

Keywords: smart nanostructured system, biological molecules, biotechnology, biological engineering

Introduction

Many physical devices, which are capable of providing measurements and can be interfaced to other recording instruments, incorporate components that are nanostructured, whether by default or by design. However, those technical characteristics would not necessarily define such a system as being truly nanostructured. For biotechnology applications we can take inspiration from biology, where we can consider the cell as a smart nanostructured system that incorporates membrane proteins to respond to signals from the external milieu surrounding the cells. We can combine this biological inspiration with the best of the worlds of physics and engineering to assemble a biocompatible system that is capable of responding to biological signals and be designed to solve specific problems for medical applications. An appropriate illustration of this point is an implantable system that is required to exchange materials or energy with the body, which we can define as a smart nanostructured symbiotic system, or a "symbio-bot" [1].

To provide the capability for a symbio-bot to maintain such unimpeded duplex communication for long periods, the symbio-bot requires the body to recognize it as "self" to avoid the usual processes of opsonization and complement activation that lead to encapsulation [2-7]. The need for a symbio-bot to be recognized as "self" goes beyond the existing concepts of what has been defined as a biomaterial and biocompatibility; to extend those definitions to include a non-fouling interface that provides the intended long-term functional use of the implanted symbio-bot medical device. Returning to an inspiration from biology, we can assemble a symbio-bot with membrane proteins incorporated in a lipid bilayer interface to provide the non-fouling interface to maintain duplex communicating systems for ions or molecules. Here we illustrate this point with two examples of symbio-bots that utilize ion channels as control elements; first by genetically modifying a biological cell, and second by assembling an artificial cell with a lipid bilayer

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interface that incorporates engineered ion channels.

Role of biological engineering in the assembly symbio-bots

Defining the discipline of biological engineering applied to nanostructured systems is important for the conceptualization and realization for the type of human and material resources that are required to assemble smart nanostructured systems. As early as the 1930s, biological engineering was used to describe the incorporation of biological knowledge into traditional engineering. In 1970, the term biological engineering was introduced formally, and intended to provide an integrated approach to combine engineering with biological systems to move beyond single disciplinary areas such as medicine, agriculture, or fermentation engineering. During the 1980s and 1990s, biological engineering education, philosophy, and practice continued to grow and mature with engineers from agricultural, food, chemical and biomedical backgrounds increasing to recognize the underlying scientific basis of biology as the driving force for their engineering. Compared to the traditional applications-driven engineering disciplines, perhaps the most prominent aspect that defined this maturing discipline of biological engineering was to let the engineering design process be driven by the unique properties of a biological system, including non-linearity of system dynamics and time-variant behavior [8]. Those roots for biological engineering placed it as a science-based discipline in engineering that was not based on the science of physics, such as for mechanical engineering or electrical engineering. Moreover, neither was biological engineering an industrial applications-based discipline, such as for mining engineering, power engineering, or agricultural engineering [9].

For the development of symbio-bots we can further differentiate the discipline of biological engineering as one that includes the incorporation of biological elements as components in the design of these smart nanostructured systems. Biological components would include lipids, proteins, enzymes and other molecules that mimic their natural function of a biological system. Such biologically engineered smart nanostructured systems may include nanoparticles, but it is not the nanoparticles *per se* that define the assembly of a smart nanostructured system utilizing biological engineering. At this point we can consider that such a biologically engineered smart nanostructured system, a symbio-bot, does indeed provide a solution to the question posed by Richard Feynman in response to the suggestion of his colleague Albert R. Hibbs about the possibility for making relatively small machines that could be permanently incorporated in the body to assist some inadequately functioning organ. The question and challenge posed by Richard Feynman was *"How do we make such a tiny mechanism? I leave that to you"* [10].

Lessons to be learned from naturally smart nanostructured systems in biology

In applying a biological engineering approach, it is important to consider biological systems that are capable of providing bioinspiration for the assembly of smart nanostructured systems. For this, we can consider the cell as an individual component of a larger engineering system. Although the cell can function individually, it is the communication between cells that provides the capability for tissues and organs to function as a system in the body. The membrane of each cell provides the interface for each of these functional "cell" units within the overall system of cells in the tissue or organ. The membrane proteins have important roles to provide the output effectors for each cell to communicate with its neighboring cells. Membrane proteins also provide the neighboring cells with input channels and receptors that assist the communication between cells to support the coordinated overall function of the tissue.

Clearly, inside each cell there are complex interactions of genetic material and molecules with the cell nucleus and other organelles. These complex interactions are essential for the biological life of the cell. Also, essential for biological life is the immune system, comprising complement activation and the reticulo-endothelial system. The immune system protects the tissues of the body from infection, and is thus an essential aspect for maintaining the ongoing health of biological tissues. It is not possible to create life, but it is possible to engineer an individual functioning nanostructured "artificial cell" to mimic the membrane transport functions of a living biological cell [11]. That said, the assembly of an artificial cell with a lipid bilayer membrane also provides the capability to incorporate molecules to ensure the body recognizes such a symbio-bot as "self". If the defining feature of a biological system is the capability of the individual cells to communicate together, then a smart nanostructured system can thus be engineered to mimic the overall function of a biological system. In this context, the biological system responses are the important functional outputs to be mimicked by a smart nanostructured system.

In describing naturally occurring biological systems, we will utilize the terminology of a "network" in a similar sense to that of a "system". In a strict engineering usage of these terms, a "network" usually refers to a system that connects two or more computing devices for transmitting and sharing information. The computing devices are connected using either physical wires, including fibre optics, or wireless transmission of signals. A "system" is usually defined as a group of interacting or interrelated elements that act according to a set of rules to form a unified whole to produce a certain outcome. The interaction between biological cells does not occur via wires, but by various molecules that are secreted by cells and then sensed by receptors on the cell membrane. In excitable cells, such as nerves or muscles, there is also a capacitive coupling between cells that relies on nan-Faradaic interactions of electrical potential. Hence, the communication in a network of cells is due to combined electrochemical forms of transmission. Biological cells are capable of self-assembly into complex 3D structures. This implies that a network of biological cells is also a system, but one in which there may not be an overarching set of predefined rules for the output of the unified whole of cells

to produce a certain outcome.

Networks of second messengers inside cells that are important for cell functions

Each cell of the body comprises a complex network of intracellular second messenger molecules that are essential for specific functions of specific cells. A generalised function for an intracellular signaling network is illustrated in Figure 1. The second messenger network inside the cell is triggered by the binding of a first-message signal molecule from the extracellular fluid to a protein receptor incorporated in the cell membrane. The first-message molecule can be either naturally occurring (e.g., neurotransmitters, cytokines) or therapeutic molecules to treat diease. This ligand/receptor binding then triggers a cascade of intracellular biochemical events that contribute to an intracellular networked system. The advantage of such a second-messenger signaling network is that there is a biochemical amplification of the initial ligand/receptor first-message to ensure that there is an appropriate response from the cell, which can be sustained.

Despite being a smaller subset of the total number of proteins encoded by the human genome, membrane-bound proteins provide a major target for first-message signal molecules. Uhlén et al provide an analysis of the total human genome that indicates there are 20,344 putative protein-encoding genes [12]. Notwithstanding that there are differences in protein expression between different tissues and that around two-thirds of protein-encoding genes have splice variants for different cellular locations, there are about 5,500 genes that are predicted to encode membrane-associated proteins, with the proportion of specifically identifiable membrane-bound proteins being 14% (2,804 genes). Those membrane-bound proteins include ion channels or molecular transporters, enzymes, and anchors for other proteins [12]. Membrane-bound proteins are essential for several important cell functions that include the transmembrane transport of ions and small molecules, signal transduction, and enzymatic activity [13]. Indeed, such membrane-bound proteins provide the targets for more than

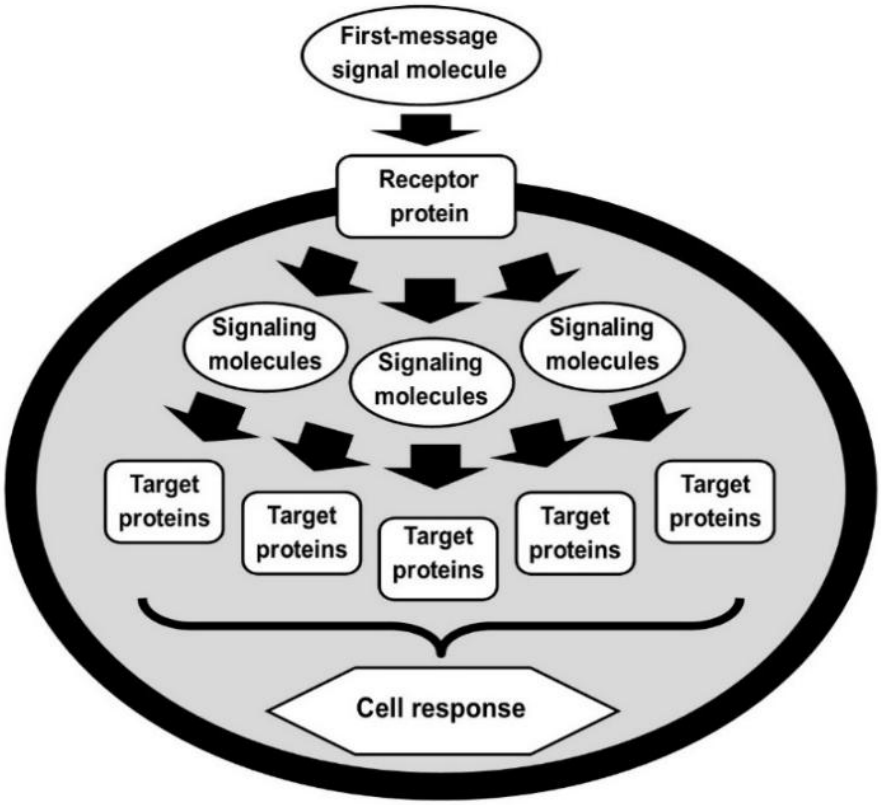

Figure 1. A generalised system of an intracellular second-messenger signaling network. The thick black line represents the cell membrane that encloses the interior of the cell (colored gray). The receptor protein incorporated in the cell membrane binds a ligand molecule from the extracellular fluid. This binding triggers the release of signaling molecules that are usually the or subunits of G-proteins associated with the receptor protein. The target proteins inside the cell can include protein kinases, enzymes or calcium-binding proteins. This type of intracellular signaling network can can lead to a multitude of cell responses, which include the activation of ion-transporting proteins, the phosphorylation of proteins, cell contraction, secretion of other molecules, changes in cell metabolism, immune responses, or regulation of gene expression.

60% of the drugs in clinical use [14].

Biological engineering of a liver cell to function as an "artificial β cell"

Glucose control could theoretically be improved by genetically engineering "an artificial β cell" that is capable of synthesizing, storing, and secreting insulin in response to metabolic signals. As the starting cell for such genetic engineering, hepatocytes (liver cells) were chosen since these cells are known to play a crucial role in intermediary metabolism and synthesis of proteins in the liver. Most importantly liver cells express the high-capacity glucose transporter GLUT2 [15] and the glucose phosphorylation enzyme glucokinase [16], which comprise the key elements of the "glucose sensing system," which regulates insulin secretion from pancreatic β cells in response to small external nutrient changes. The insertion of insulin cDNA into a human hepatoma cell line (HEPG2) that lacked native GLUT 2 expression to produce the cell-line HEPG2ins, resulted in both the synthesis, storage, and release of insulin to β cell secretagogues, but not to glucose [17]. A second insertion of the glucose transporter GLUT 2 resulted in near physiological release of insulin to glucose and other stimuli in the doubly transfected HEPG2ins/g cells [18].

As a smart nanostructured system, the novelty of the HEPG2ins/g cells is that dual expression of the glucose transporter GLUT 2, and the insulin gene has induced functional KATP channels in the resting cells. Transfection of the parent HEPG2 cell-line with both the GLUT2 transporter and insulin cDNA resulted in cells (HEPG2ins/g) that stored insulin in secretory granules and secreted insulin in response to glucose stimulation, at near physiological levels [18]. That genetic manipulation created a smart nanostructured system that expressed functional KATP channels in the resting HEPG2ins/g cells to provide a mechanism to trigger insulin secretion in response to stimulation of the cells by glucose [19, 20].

At this point it is important to highlight the steps in the genetic manipulations to show how this "an artificial β cell" was developed. Patch-clamp electrophysiology was used to measure that the doubly transfected HEPG2ins/g cells, in which cDNA for insulin and GLUT2 genes had been inserted, had a more negative membrane potential (–68.9 ± 7.1 mV) than either the untransfected HEPG2 cells (–18.2 ± 5.8 mV) or the HEPG2ins cells that had only the insulin gene inserted (–45.3 ± 4.4 mV). Furthermore, the increased membrane potential was due to the functional activity of KATP ion channels induced in the HEPG2ins/g cells (Figure 2). The activity of the KATP channels was inhibited cAMP, ATP, and glucose [19, 20].

To provide a pharmacological means to control the KATP channels during experiments, we also demonstrated that the KATP channels were activated by diazoxide and inhibited by glibenclamide (Figure 3).

The activation by diazoxide would be expected to inhibit the glucose-stimulated cell depolarisation, and thus provide an important control for the experiments. Furthermore, we confirmed by Western blotting that the KATP channels were induced in the HEPG2ins/g cells. This confirmation was achieved with the use of an antibody against the conducting pathway of the KATP channels, Kir6.2 (Figure 4).

The HEPG2ins/g cell ("artificial β cells") also responsed to stimulation by glucose with an increase in the intracellular Ca^{2+} concentration (Figure 5).

Ca^{2+} is an important intracellular messenger that is needed to trigger the secretion of insulin. We confirmed that the HEPG2ins/g cells were capable of secreting inclusion in response to glucose, which was controlled by the activity of the induced KATP channels (Figure 6). Note that activating KATP channels leads to hyperpolarisation of the cell, which then does not lead to an increase in intracellualar Ca^{2+} concentration and hence there is an inhibition of insulin secretion.

Thus, this nanostructured system of the HEPG2ins/g cells follows the classical triggering pathway whereby glucose enters the cell and there is a rise in the ATP-to-ADP ratio leading to closure of KATP channels, which causes membrane depolarization and subsequent activation of insulin secretion by exocytosis of secretory granules [21]. The combined transfection of the GLUT2 and insulin genes induces the activation of functional KATP channels that do not require pharmacological stimulation to generate currents in this "artificial β cell".

The overall function of this genetically engineered "artificial β cell" is illustrated in Figure 7. The expression of functional KATP channels in the resting HEP G2ins/g cells provides a mechanism for the rapid triggering of insulin secretion. Glucose in the extracellular environment is sensed and transported across the membrane by the facilitative glucose transporter GLUT2 and converted to glucose-6-phosphate by glucokinase; there is a rise in the ATP:ADP ratio leading to the closure of KATP channels that leads to cell depolarization, influx of extracellular Ca^{2+} to increase the intracellualr concentration, increased release of Ca^{2+} from intracellular stores, exocytosis, and secretion of insulin. As for the situation of a normal pancreatic β-cell it seems likely this increase in Ca^{2+} would also have a wide range of effects including the activation of glycerol phosphate dehydrogenase and other mitochondrial enzymes and activation of protein kinases and phospholipase C. The subunits that comprise the KATP channel in natural pancreatic β-cells are SUR1 and KIR 6.2. The subunits comprising the KATP channel in the parent HEP G2 cells were reported to be SUR2 and Kir 6.2, whose mRNA expression was unaltered by glibenclamide. In this "artificial β cell", following overexpression of the insulin and GLUT 2 cDNA, the HEPG2ins/g cells express KATP channels that are blocked by both tolbutamide and glibenclamide and activated by diazoxide. This pharmacology suggests that the expression of the insulin and GLUT 2 genes in these cells possibly modified the SUR2 subunit of the existing channels to enable the KATP channels to be functional. This "artificial β cell" supports the possibility to engineer liver cells to mimic the physiology of pancreatic β cells and the eventual use for treatment of type I diabetes.

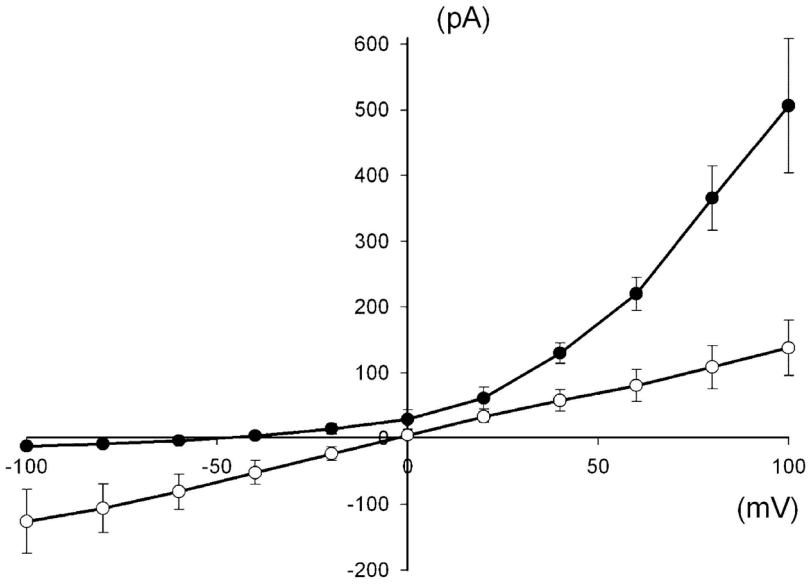

Figure 2. Summary of whole cell I V curves for the samples of HEPG2ins/g (n=3) and HEPG2 cells (n=5). Vertical bars indicate SEM. The position of the reversal potential ($E_R \approx 50$ mV) for the HEPG2ins/g cells indicates a potassium selective current, whereas the current for the HEPG2 cells is non selective ($E_R \approx 0$ mV). Figure reproduced from [20].

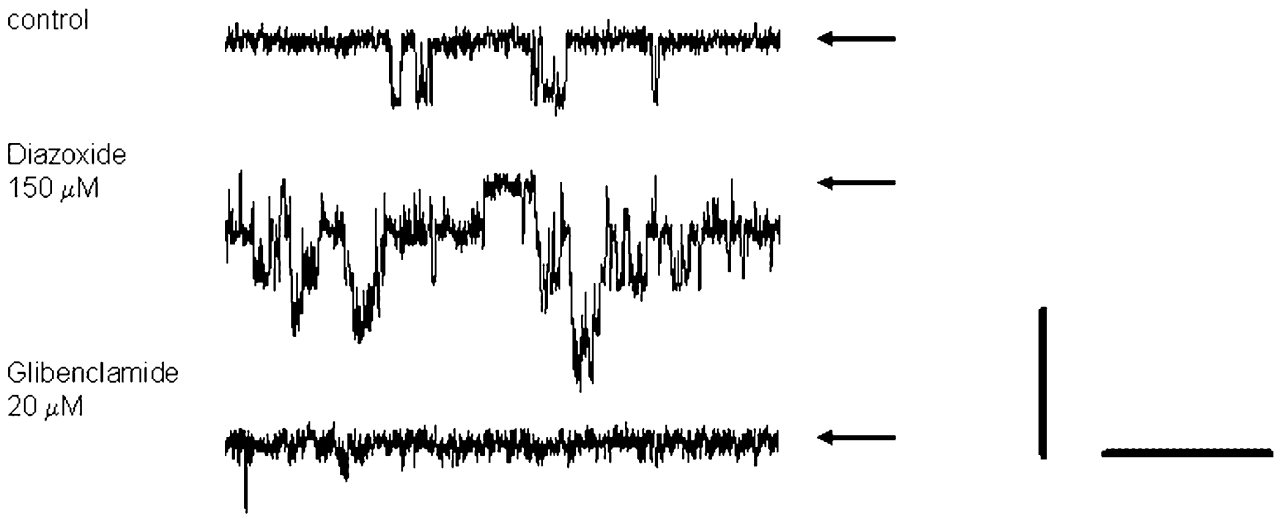

Figure 3. Single channel activity of K ATP in an outside out patch was increased by the channel opener diazoxide (150 μM) and inhibited by the channel blocker glibenclamide (20 μM). Closed channel level is indicated by the horizontal arrows, and open levels are downward deflections. Transmembrane potential = 40mV. The vertical scale bar represents 5 pA, and the horizontal scale bar represents 100 ms. Figure reproduced from [20].

Biological engineering of an artificial cell as a smart nanostructured systeml

We have seen how a liver cell can be used as the "chassis" for genetically engineering a smart nanostructured system to achieve a different purpose compared to the original function of the liver cell. However, for a more generalized biological engineering approach we need to consider how to assemble a nanostructured system that does not required the modification of an existing living cell. In other words, we need to utilize the principles we have learned from the genetic engineering approach, but apply these to the assembly of a synthetic nanostructured systems. We will illustrate such a biological engineering approach using the example of an artificial cell that was assembled using synthetic and biological components [11]. The synthetic component for that artificial cell was a hollow PSS/PAH polyelectolyte polymer capsule of 6 μm in diameter (Figure 8). The nanostructured biological components comprised a lipid bilayer membrane to enclose the hollow

Figure 4. Qualitative Western blot analysis for Kir6.2 in HEPG2ins/g (lane 1), MIN 6 (lane 2), HEPG2ins (lane 3), and HEPG2 cells (lane 4). The molecular weight of Kir6.2 is 35 kDa. The MIN 6 cells (positive control) had the strongest staining for Kir6.2. There was a strong presence of Kir6.2 in the HEPG2ins/g cells. There was weaker staining for Kir6.2 in the HEPG2ins cells and almost no staining for Kir6.2 in the HEPG2 cells. This pattern of Kir6.2 expression follows the electrophysiology data, which indicated th at the functional presence of the K ATP channels was greatest in the HEPG2ins/g cells and absent in the HEPG2 cells. Figure reproduced from [20].

polyelectrolyte capsule, and ion channels incorporated into the lipid bilayer membrane. This complete system provides a structurally stable microcapsular system and addresses the concern expressed previously about liposomes that incorporated a porin to facilitate membrane permeability [22]. The ~20nm thick wall of the hollow polyelectrolyte capsule allows the transfer of transition metal complexes with overall large positive or negative charges as well as neutral species while providing enough structural strength to maintain a spherical shape when coated with a lipid bilayer. The lipid coating provides a seal, containing both large and small solute species within the microcapsule. The incorporation of the engineered ion channel, gramicidin (bis-gA), provides a platform that can be used to effectively control transport of molecules from the microcapsule. This engineered micro/nanosystem has potential applications in physiologically relevant delivery or sensing applications. Indeed, we have reported an algorithm and method that would enable such a system to be used efficiently in a biosensing application [23]. The opening of bis-gA suggest that the bis-gA ion channels incorporated rapidly into the lipid bilayer around the microcapsules and provided functional and gateable pathways to control ion transport across the microcapsules.

In this type of "artificial cell" as a smart nanostructured

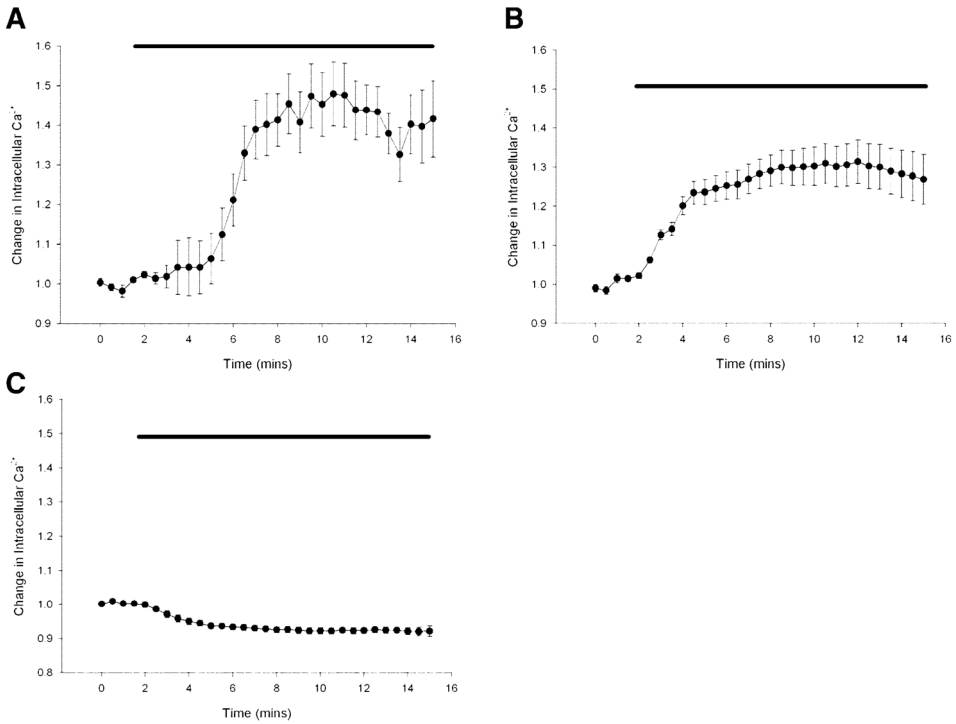

Figure 5. Intracellular free Ca^{2+} changes in HEPG2 and HEPG2ins/g cells induced by glucose and the K ATP channel antagonist glibenclamide. The vertical bars on each data point indicate SE where this is larger than the symbol. (Glucose 20 mM stimulated an increase in the intracellular free Ca^{2+} level in HEPG2ins/g cells (n=12). (Glibenclamide 100 μM also stimulated an increase in the intracellular free Ca^{2+} level in HEPG2ins/g cells (n=9). (Glucose 20 mM failed to stimulat e an increase in intracellular Ca^{2+} level in the HEPG2 cells (n=10). The horizontal bars indicate the time of application of glucose or glibenclamide. Figure reproduced from [20].

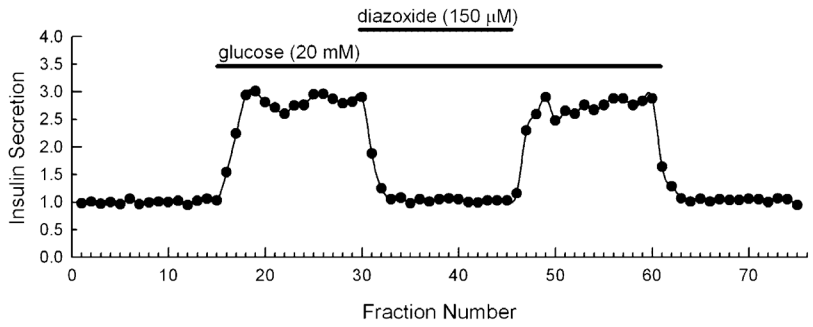

Figure 6. Static stimulation of insulin secretion in cultures of HEPG2ins/g cells. Data have been normalized to the average insulin secretion measured in samples between 0 and 15 min (1.00 ± 0.001 pmoles insulin/10^8 cells), during which cells were perfused with basal medium without any stimuli. The y axis shows the increase in insulin secretion as a multiple of the normalized basal secretion (= 1.0). Data are representative of three independent experiments. Figure reproduced from [20].

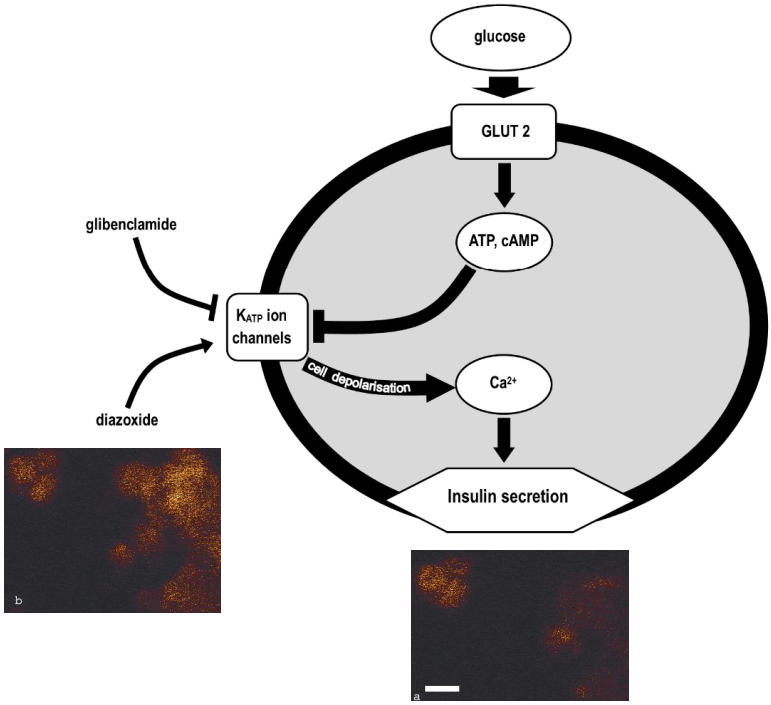

Figure 7. Schematic diagram of the biochemical pathway of glucose stimulated insulin secretion from HEP G2ins/g cells. A rise in extracellular glucose initiates the inhibition of K ATP channels, which leads to cell depolarization, exocytosis, and secretion of insu lin. In normal pancreatic β cells depolarization is due to influx of Ca^{2+} ions. The secretion of insulin is illustrated by the confocal microscopy images (a) and (b) of Zinquin E fluorescent labeling of secretory granules in HEPG2ins/g cells [53]. Image (a shows Zinquin staining of secretory granules in HEP Gins/g cells superfused with 5 mM glucose in DMEM medium (control). Image (shows the same HEPGins/g cells as in (a), after changing the superfusing solution to one containing 150 μM diazoxide in DMEM medium followed by the addition of 20 mM glucose in DMEM medium 15 min later. The image was taken after 45 mins' exposure to 20 mM glucose medium. The greater punctate staining in (a) indicates that the K ATP channel activator (diazoxide) has inhibited the glucose stimulated secretion of insulin containing granules in the HEP G2ins/g cells despite prolonged exposure to 20 mM glucose.

system, the intriguing aspects of variations in the kinetics of the gating of incorporated membrane-spanning bis-gA ion channels provids the possibility to use this "artificial cell" to gain further fundamental insights into the mechanisms underlying instability in the lipid coating of polyelectrolyte supports. Measuring the kinetics of channel open/close activity could be a valuable addition to techniques such as colloidal force spectroscopy [24] in enhancing an understanding of the mechanisms of interaction between lipid bilayers and polyelectrolyte supports. Notwithstanding, the "artificial cell" system provides a platform for the development of a sealed microcontainer for drug delivery or smart sensing that can navigate in the physiological environment and be controlled with physiologically relevant stimuli. Our evidence and

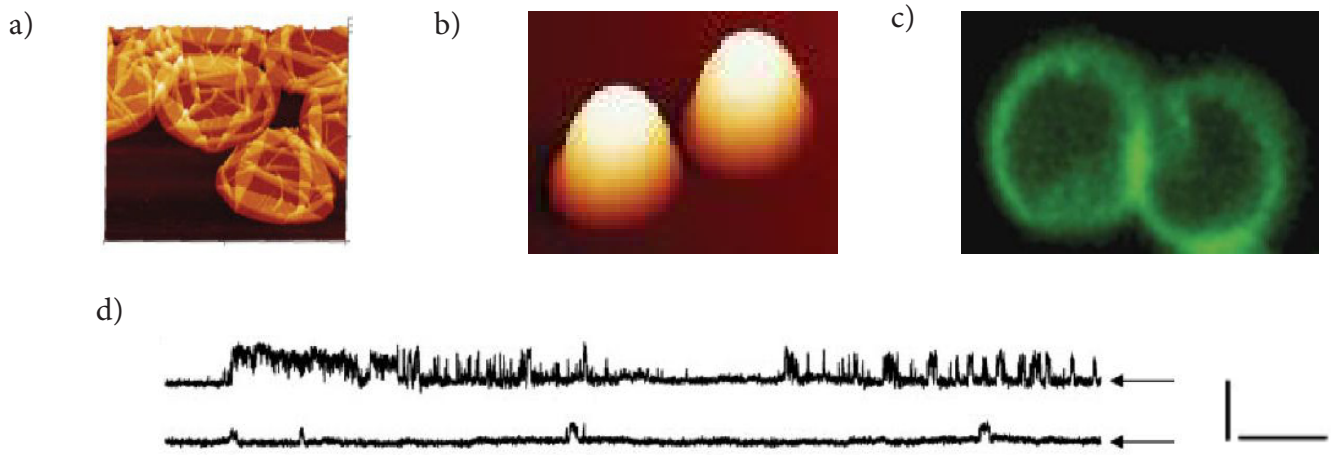

Figure 8. Structures to form an artificial cell. (3D rendered AFM images of hollow PAH/PSS polyelectrolyte microcapsules showing a folded and layered structure in an aqueous environment. (The microcapsules with a lipid coating do not collapse and maintain a spherical structure in an aqueous environment. (Confocal microscopy images of hollow lipid coated microcapsules loaded with dextran FITC by adding 10 µL of 10 kDa dextran FITC to 1mL of a 1mg/mL. After washing, the lipid coated microcapsules retained the fluorescence, which showed the lipid membrane formed an effective insulating barrier to seal the microcapsules. (Continuous 10 second patch clamp recordings of the gating of the bis gA channels incorporated in the lipid coating of the capsules by MBC (10 µM). MBC is an acronym for the compound *N,N0 dimethyl N (2 --[2 --(methyl 4 --[1,1,3,3 tetramethylbutyl]phenoxy)ethoxy]ethyl)benzylammonium chloride*. The upper trace is the control condition, where the upward deflections indicate the ion current permeating across the wall of the capsules to the opening of the bis gA channels incorporated in the lipid coating. The lower trace is recorded 2 min after addition of the drug to the solution bathing the capsules. The MBC blocked the opening of bis gA and hence th e ion permeation across the capsule wall, as shown by the reduction in the upward deflections. The horizontal arrow indicates the nonconducting condition of the bis gA channels where there is zero current recorded. The horizontal scale bar represents 1 s and the vertical scale bar represents 5 pA. The four panels were compiled from figues in [11].

formulation of a Hidden Markov Chain Model for automatic detection of changes in the ion channel gating reinforce the potential physiological applications of this "artificial cell" system that incorporates ion channels [23]. The controlled transport in this "artificial cell" system could be tuned for selective release biomimetically by controling the gating of incorporated ion channels. This system provides a platform for the creation of “smart” biomimetic delivery vessels for the effective and selective therapeutic delivery and targeting of drugs.

Concluding Remarks

For an implanted application the highly significant advantage of utilizing biological components is the ease of providing a more natural interface to living tissues. This is because a smart nanostructured system based on biological engineering utilises biological components, including lipids and proteins, which assist the system to be recognized as “self”. Here we have explored a biological engineering approach in the design and assembly of symbio-bot nanostructured systems with inspiration from biology. The term biological engineering is used to describe a discipline that embodies this approach. Indeed, in 1970, this term was introduced formally with the intention to integrate engineering with biological systems to move beyond single disciplinary areas such as medicine, agriculture, or fermentation engineering. We have further refined the discipline of biological engineering to be one that utilizes biological proteins, molecules and lipids in combination with synthetic materials to assemble smart nanostructural systems. We have illustrated this discipline with the assembly of two nanostructured systems.

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