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Review

Biosensor technology in aging research and age-related diseases

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ABSTRACT

Cell- and tissue-based biosensors comprise genetically engineered proteins that are incorporated into cells *ex vivo* or into cells of tissues *in vivo*. They enable the investigator to sense levels of hormones, drugs, or toxins, continuously and noninvasively, using biophotonics or other physical principles, and could potentially be used over the entire lifespan of an experimental animal. The present work reviews the state of the art of cell- and tissue-based biosensors and discusses how they could be of value in aging research. Examples of recently developed biosensors are given, including those that detect levels of a cytokine (TNFα) and drugs (activators of the mTOR pathway). Finally, we discuss the hurdles that would have to be overcome for biosensor technology to be used in humans in monitoring health status and disease treatment in late life.

1. Why consider biosensor technology for use in aging research?

Much of modern research into the regulation of longevity in mammals, particularly mice, comprises long-term experiments in which animals are subjected to an intervention of some kind, and subsequently the experimenter compares the resultant survival curves for the treated and control populations. Dietary restriction has been the most often employed paradigm for an intervention that extends lifespan (Nelson et al., 1995; Heydari et al., 1996; Masoro, 2009). Over the period of a lifespan study, typically 3–4 years in a single experiment, the animals are not usually subjected to sampling of tissues or fluids (such as blood), because even such minor disturbances may alter their physiological status and thereby change the nature of the experiment. Thus, over the period of the lifespan experiment, the investigators are often "blind" with respect to monitoring the intervention. For example, if a drug is administered in food, the investigators do not know whether blood levels of the drug are being maintained at desired levels; similarly, investigators typically do not monitor hormone levels over the lifespan when studying interventions such as dietary restriction that are known to alter levels of various hormones. For example, in a series of studies, mice are being fed drugs and other compounds that could affect lifespan, the concept being that such interventions might slow aging and delay death (Miller et al., 2007; Harrison et al., 2009; Miller et al., 2011). In that case, monitoring the circulating levels of the drug continuously and noninvasively over the lifespan would be very desirable. In dietary restriction and other interventions, hormones such as insulin show profound changes, both over the course of a day and also over the course of the lifespan of the animals (Nelson et al., 1995; Hauck et al., 2001; Wanagat et al., 1999; Martin et al., 2007). To be able to continuously monitor blood levels of insulin or other hormones would provide the investigator with valuable insights into the mode of action of the intervention. If levels of drugs, hormones or other factors could be monitored by less invasive or totally noninvasive methods, this would enable a continuous assessment of the progress of the experiment over long periods. This would allow, for example, adjustment of the experimental conditions to maintain target plasma drug levels or to achieve specific changes in hormone levels.

Currently, the noninvasive methods that would be useful in this type of research have not been adequately developed and are not in routine use. The aim of this review is to suggest how biosensor technology, in the form of cell-based and tissue-based biosensors, could begin to fill this role. Biosensors include proteins that provide real-time monitoring of intracellular events, such as autophagy and proteostasis (Bampton et al., 2005; Morimoto, 2008). Many other

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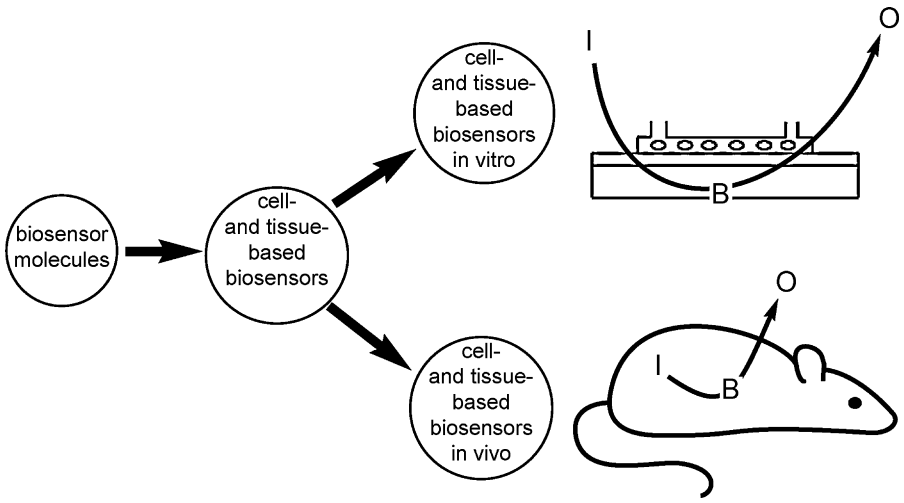

Fig. 1. Cell- and tissue-based biosensors are an extension of the concept of biosensor molecules (proteins) that can be incorporated into living cells. Biosensor cells (B) can be used in devices in vitro that transduce an input, I (the concentration of molecule to which the biosensor responds), to an output, O, comprising a physical signal that can be measured within the device or by some external method. Similarly, biosensor cells can be transplanted into a living animal, or alternatively biosensor molecules can be incorporated into the endogenous tissues of the animal. Biosensor cells/tissues in the living animal also transduce an input, the concentration of the sensed molecule, into a physical output. The output can be measured by a device external to the animal, or potentially by an embedded device.

biosensors have been developed for purely in vitro measurements (Serra, 2011). Here we focus on those cell- and tissue-based biosensors that can be used in living animals, and potentially in human patients, to monitor extracellular soluble molecules, thus providing noninvasive and continuous monitoring of levels of hormones, drugs, or other substances. We review the background to this field, which is generally not part of the gerontological literature, and assess the potential for the use of the technology by describing several examples that have been developed in the authors' lab. Work in the authors' lab developed from work on cell transplantation (Thomas et al., 1997; Thomas and Hornsby, 1999; Sun et al., 2004; Huang et al., 2007a). These studies led to the concept that the transplantation of genetically modified cells, expressing appropriate sets of proteins, that could be used as biosensors (Huang et al., 2007b; Acha et al., 2010). We hope that by providing insights into this field, investigators working in long-term aging experiments in mice or other mammals will be able to have the information necessary for them to evaluate whether this technology could be of future use to them in their research. A second aim is to consider the idea that similar technologies might potentially be used in human patients for long-term monitoring of health-related parameters and drug levels.

2. Implementation of cell- and tissue-based biosensors in living animals

Cells, tissues and whole organisms can be used as biosensors to detect or measure substances of importance. Apart from hormones and drugs, substances detected/measured could be of environmental/biodefense importance, such as hazardous bacteria, or could be of food safety importance, such as mycotoxins and pesticides. The molecular basis for most biosensors comprises proteins that interact with the substance that is being detected. Biosensor proteins can be incorporated into physical (non-living) sensors, or into living cells, tissues and organisms by genetic modification. These biosensor cells transduce the concentration of the substance being monitored to a physical signal (e.g. electrical, photonic, or magnetic) (Fig. 1). Biosensor proteins can be incorporated into tissues either via the transplantation of cells that have been modified to express them, or by the expression of the biosensor proteins in host tissues. The latter may be accomplished by viral or nonviral vectors that are introduced into the host animal organs. For example, an implant formed from an appropriate material could be coated with a vector encoding the biosensor proteins. When inserted into tissues, the vector-coated implant would accomplish the genetic modification of the surrounding cells. Transfection may be achieved by using commonly used implant materials, such as poly(D,L-lactic-co-glycolic acid) (PLGA), that have been processed to form a modified surface (Zheng et al., 2000; De Laporte and Shea, 2007; Kim et al., 2008). Although current technology employs plasmids or viruses, modified mRNA would likely be the best vector for biosensor protein expression (Warren et al., 2010), having the advantage that no permanent genetic modification of the host cells is involved. Such an implant could both achieve the necessary expression of biosensor proteins of adjacent cells and could also facilitate the physical detection of the output of the biosensor cells (electrical, photonic, or magnetic). Biosensor tissues formed by modification of the animal's own tissues would have the advantage that the body would not react to the biosensor tissue as foreign; the tissue would not be subject to rejection and would not encounter biocompatibility issues (although it should be noted that the biocompatibility of the implant itself must still be addressed). Such implants may be envisaged as a future development in this field. Such implants, or technologies based on similar principles, might eventually be used in human patients (see Section 4 below).

The technology described above involves the modification of differentiated tissue cells. A potentially more powerful technique comprises the genetic modification of stem cells, thereby creating a continuously renewing source of biosensor cells. For example, hematopoietic stem cells could be modified, resulting in genetically modified white blood cells. The output from such cells could be detected at many sites in the body, depending on the physical nature of the output. Such methods in essence would make the entire animal into a biosensor. Implants that can genetically modify cells that they contact, and biosensors based on genetic modification of stem cells, are examples of exciting future developments of cell- and tissue-based biosensor technology. Most of the current examples of this technology involve biosensor cells transplanted into experimental animals to form artificial tissues.

One example of a biosensor based on transplanted cells is provided by pioneering studies by Edelberg and colleagues. They showed that cardiomyocytes can be implanted into the external ear of the mouse, and that there they can survive and form beating

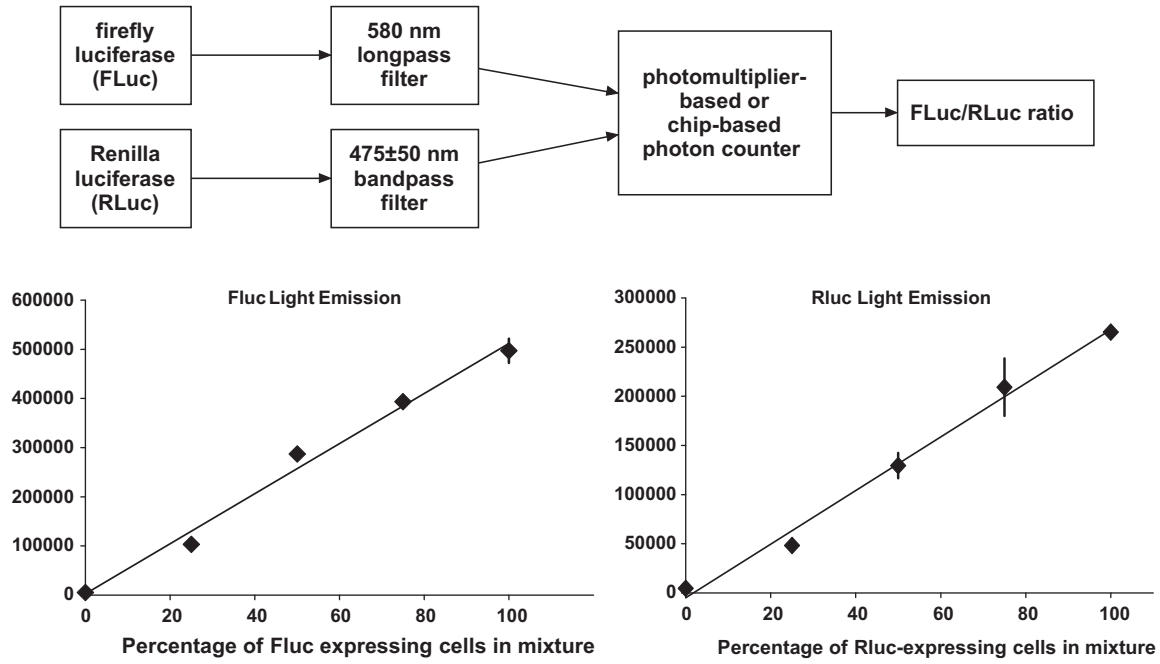

Fig. 2. Simultaneous measurement of light from two luciferases. Firefly (FLuc) and *Renilla* (RLuc) luciferases were separately introduced into target cells (MBA-MD-231) as follows. One population of cells was transduced with lentivirus FuhLucW (Morizono et al., 2005) to provide cells stably expressing FLuc under the control of a constitutive CMV promoter. A second population of cells was transduced with retrovirus pLEGFP-RLuc (generated from pLEGFP-N1, obtained from Clontech Laboratories Inc., by replacing the *neo* gene with RLuc) to provide cells stably expressing RLuc. FLuc-expressing cells and RLuc-expressing cells were mixed together in various ratios, as indicated in the figure, maintaining a total cell concentration of 106 cells/ml in 100 μl physiological buffer. Using a mixture of substrates (3 mg/ml D-luciferin and 24 μM coelenterazine), we measured light emission at two different wavelengths: >580 nm, suitable for detection of light from FLuc, and 475 ± 50 nm, suitable for detection of light from RLuc. The optical filters are located in a motorized wheel (Oriel model 74041, Newport Corp., Irvine, CA) connected to a photon counter (Hamamatsu H7467; Hamamatsu Photonics, Bridgewater, NJ). A fiber optic lightguide provides a path for light from a heated block, holding the cell suspension in a glass tube, to the filter wheel/photon counter. Both photon counter and filter wheel have RS-232 interfaces, allowing computerized measurement of light and control of the position of the wheel. Light emission at these different wavelengths (FLuc and RLuc; photons detected in a 20 min acquisition period) was plotted against the percentage of FLuc/RLuc-transduced cells in the mixture. The increase in light emission at >580 nm was linear with respect to the percentage of Fluc-expressing cells, with $r = 0.988$; and the increase at 475 ± 50 nm was linear with respect to the percentage of RLuc-expressing cells, with $r = 0.991$.

heart-like structures (Edelberg et al., 1998; Christini et al., 2001; Edelberg et al., 2002; Tang et al., 2003). By measuring the electrical activity of the transplanted cardiomyocytes these investigators were able to monitor circulating levels of adrenergic hormones and monitor the bioactivity of circulating drugs. For example, they engineered cardiomyocytes to monitor the local coagulation potential by overexpression of the human thrombin receptor, protease activated receptor-1 (PAR-1). PAR-1 engineered cells implanted *in vivo* detected local increases in thrombin by changes in chronotropic activity (Tang et al., 2003).

At the current state of development of the field there are a variety of questions regarding the optimal design and use of cell- and tissue-based biosensors. Published work has established the proof of principle that cell- and tissue-based biosensors can be used in living animals, but their potential has been largely unexplored to date. The following examples are given to show the progress that has been made in the field and the potential for future developments.

2.1. Light output: fluorescence and bioluminescence

Whereas biosensors based on electrical output have the advantage of simplicity of measurement of the output, biosensors based on principles of biophotonics are attractive because in theory they can be designed to detect almost any biological or chemical substance, and because light may be measured with a high degree of precision. Desirably, light output from the biosensor tissues would be monitored continuously and noninvasively, without direct contact, in freely moving conscious animals. Light could be detected from areas of the skin where the blood comes into close proximity to the surface, such as the external ear. There are no theoretical obstacles to achieving this aim, although there are many practical issues. Current methods do not yet approach these ideals. We consider here the general principles of light-based biosensors, both those based on fluorescence and those based on bioluminescence, their current capabilities and limitations, and potential future developments.

Photonics-based biosensors can be based on either fluorescence or bioluminescence: there are advantages and disadvantages to each technology. Fluorescence is more complex because it requires both an input and an output light path. Several fluorescence-based biosensors that function in living animals (mice) have been developed; some examples, among many others, are: (i) transgenic mice have been generated which express functional fluorescent calcium indicator proteins under control of a tetracycline-responsive promoter (Hasan et al., 2004; Palmer et al., 2011). (ii) Synaptically targeted pHluorins were expressed in the olfactory sensory neurons of transgenic mice. Using these mice, it was possible to investigate spatial patterns of glomerular activity after odorant stimulation within the olfactory bulb *in vivo* (Bozza et al., 2004; Newman et al., 2011). (iii) A calcium-sensing protein was expressed and analyzed in smooth muscle of transgenic mice (Ji et al., 2004; Manck and Griesbeck, 2008). Current methods of imaging of tissues in mice using fluorescence methods involve the imaging of exposed tissues under a microscope, and typically the animal must be anesthetized or immobilized. A severe challenge arises from poor transmission of light through tissues, including the skin, which absorb light strongly at wavelengths <600 nm (Weissleder and Ntziachristos, 2003). It is very likely that many of those issues will

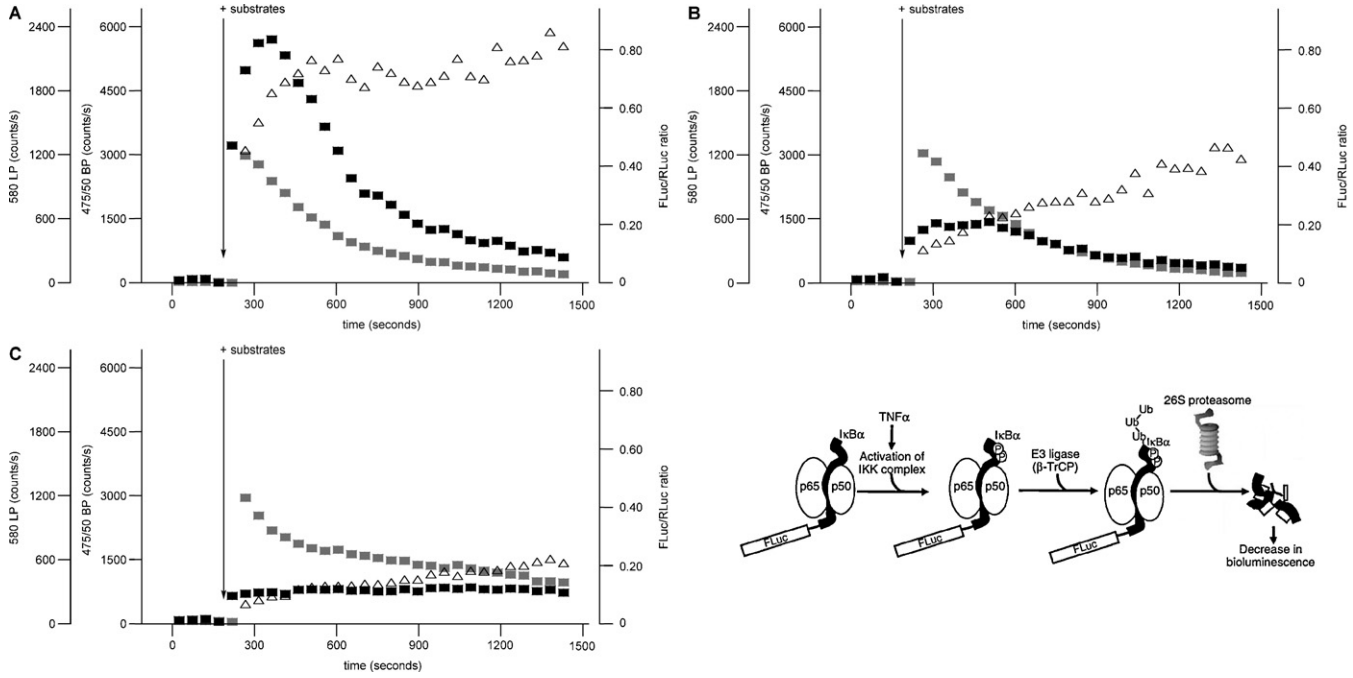

Fig. 3. A biosensor for TNFα. This biosensor is based on the design previously published (Gross and Piwnica-Worms, 2005). The principle is illustrated in the right lower portion of the figure. HeLa cells were first transfected with a plasmid vector encoding a fusion protein comprising IκBα at the N terminus and FLuc at the C terminus under the control of a constitutive promoter (CMV). The construct also encodes *neo*, enabling the selection of stably transfected clones by growth in 1 mg/ml G418. RLuc was then introduced into a clone expressing a high level of FLuc by retroviral transduction (see legend to Fig. 2). Clones of cells stably expressing IκBα-FLuc fusion protein and RLuc were used in these experiments. When TNFα is present and acts on the cell, the IKK complex is activated, resulting in the phosphorylation of the IκBα fusion protein. This stimulates its ubiquitination and destruction by the proteasome. The decreased luciferase level leads to decreased bioluminescence, the decrease being proportional to the concentration of TNFα. The operation of this biosensor is shown in the experiments illustrated in the top panels. Because TNFα present in serum could chronically affect the level of the IκBα-Fluc fusion protein, cells were grown in serum-free medium for 24 h before being assayed. Additionally, to avoid an effect of enzyme treatment on TNFα receptors, the biosensor cells were cultured on microcarrier beads (Cytodex 1) so that they could be directly transferred to the light measurement apparatus. The apparatus, system for light measurement, and other details of the experiments were the same as described in the legend to Fig. 2. Cells were incubated in buffer containing TNFα or in buffere alone for 10 min before the addition of D-luciferin and coelenterazine. In **A**, no TNFα was added to the cells; at the indicated time, coelenterazine and D-luciferin substrates were added to the cells and light emission was measured at the RLuc and FLuc wavelengths (gray squares and black squares, respectively). The FLuc/RLuc emission wavelength ratio is also shown (triangles). In **B**, 1 ng/ml TNFα was added to the cells 20 min before the substrates were added. In **C**, 10 ng/ml TNFα was added to the cells 20 min before the substrates were added.

be solved in future developments. For example, development of fluorescent proteins that have excitation and emission wavelengths that are in the far red and infrared ranges will enable the detection of biosensor proteins in cells and tissues relatively deep in the animal (Chudakov et al., 2010; Shcherbo et al., 2010). With sufficient sensitivity and specificity, biosensors based on genetic modification of animal tissues with fluorescent proteins have excitation and/or emission wavelengths in the far red and infrared range, combined with detection in a dark environment, could fulfil the ideals described above.

Currently, biosensors based on bioluminescence have some advantages over those that use fluorescence (Roda et al., 2009). Bioluminescence requires only an output path, because light production is endogenous. However, bioluminescence requires administration of a substrate, a factor that must be considered in the choice of technology. A key factor that favors bioluminescence is a superior signal to noise ratio, at least in comparison to fluorescence that uses standard visible light. In a detailed study of autofluorescence and autobioluminescence in living mouse tissues, detection of bioluminescence required far fewer cells than detection of fluorescence. When cells that express both fluorescent and bioluminescent proteins were injected subcutaneously, 3.8×10^5 cells were required to obtain a fluorescence signal above background, but only 400 cells were required to obtain a bioluminescence signal above background (Troy et al., 2004).

In addition to molecular processes that depend on changing the intracellular level of the bioluminescent protein as the result of action of the detected molecule, a particularly powerful form of biophotonics-based biosensor is based on protein–protein interactions. In one form of protein–protein interaction-based assay, the gene encoding a bioluminescent protein is split into two sections, each encoding a half-protein. Neither half-protein is bioluminescent, but when the two halves are brought together in the presence of a detected molecule bioluminescence is restored (Paulmurugan et al., 2002; Hida et al., 2009). In another form the protein–protein interaction is linked to light output via resonance energy transfer. The key feature of such interactions is that resonance energy transfer can occur between the two proteins when they are in close proximity (~10 nm). In one form of this phenomenon, each of the two interacting proteins is a fusion protein with a fluorescent protein. In fluorescence resonance energy transfer (FRET), light energy is transferred between two different fluorescent protein molecules without emission of a photon (Li et al., 2006). Bioluminescence resonance energy transfer (BRET), by contrast, involves one bioluminescent protein and one fluorescent protein (Massoud et al., 2007). The bioluminescent protein is capable of converting some form of chemical energy into light energy. The wavelength of the light emitted from the bioluminescent protein is suitable for excitation of the fluorescent protein, although (as in FRET) the energy is transferred between the two molecules without emission of a photon. Because of the versatility of BRET, like FRET, it can be adapted via fusion proteins to almost any protein–protein interaction and thereby to the detection of a huge variety of molecules.

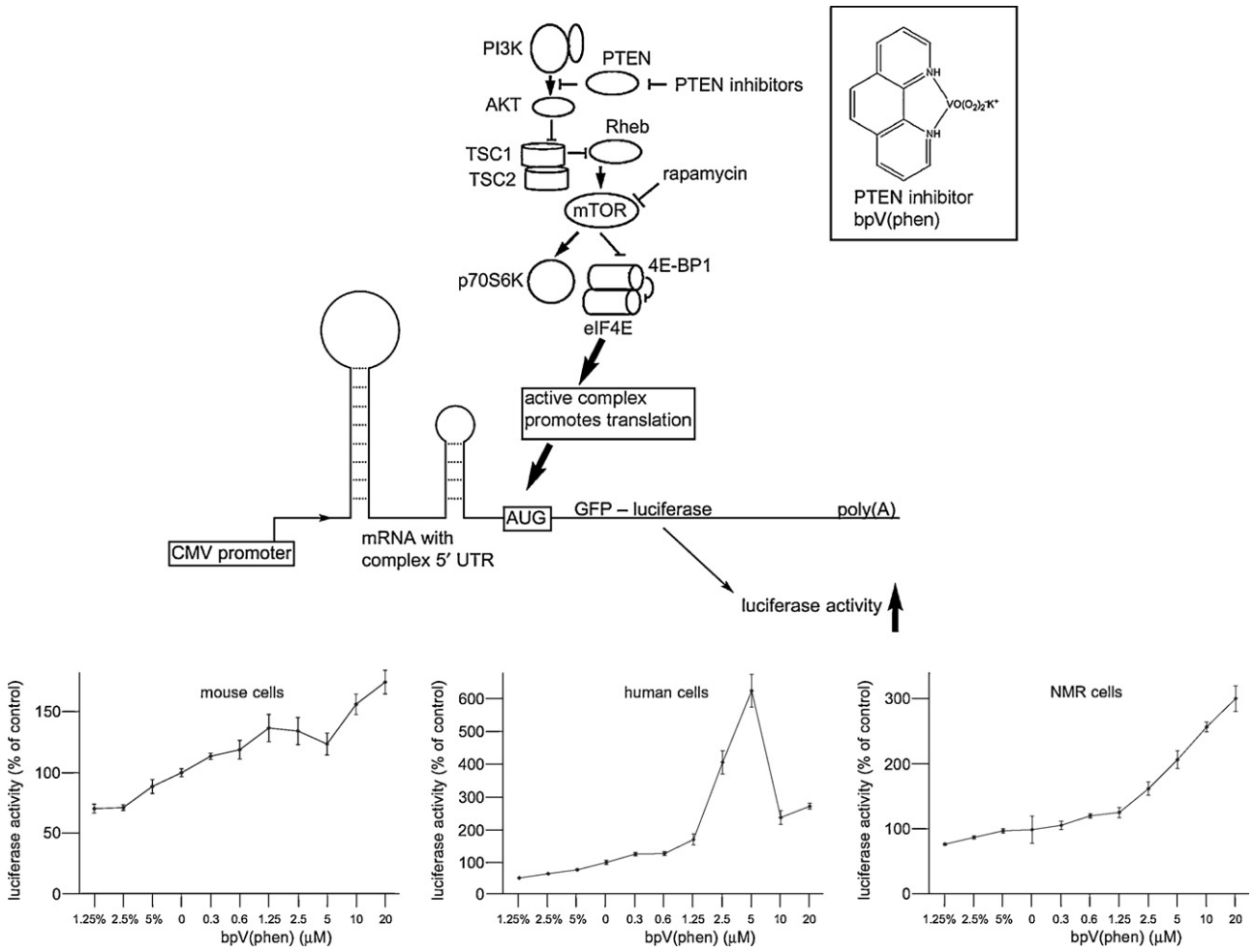

Fig. 4. A biosensor for compounds that stimulate mTOR activity. The top central diagram shows a simplified version of the mTOR pathway. The biosensor is based on the principle that mRNAs with complex 5′ untranslated regions (hairpins or other secondary structures) are highly dependent for translation on a high level of eIF4E complex, which is activated by mTOR via phosphorylation of 4E-BP1 (Larsson et al., 2006; Mamane et al., 2007; Silvera et al., 2010). The figure illustrates the pathway by which activation of mTOR leads to phosphorylation of the inhibitory protein 4E-BP1. This results in the release of active eIF4E, a key component of the translation initiation complex (termed eIF4F). Binding of the complex to the mRNA m^7GpppN cap increases association of the mRNA with ribosomes, forming protein-synthesizing polysomes. The presence of complex secondary structures in the 5′ UTR makes the successful initiation of translation of the mRNA highly dependent on the eIF4F complex, which includes eIF4E, eIF4G and eIF4A (an RNA helicase that unwinds secondary structures in the 5′ UTR). The biosensor is based on an mTOR-dependent mRNA. The coding region is a fusion protein of GFP and FLuc; this is flanked on the 5′ side by a 55 nucleotide UTR with two hairpins, as illustrated. This UTR is a consensus sequence based on the 5′ UTRs of a large number of mTOR-responsive mRNAs (Larsson et al., 2006). To ensure that only translational control by mTOR affects FLuc protein levels and therefore luciferase activity, the construct is placed under the action of a constitutively active promoter (CMV). A particular example of a class of drugs that increases mTOR activity is provided by inhibitors of PTEN (Posner et al., 1994). PTEN is a phosphatase that acts to dephosphorylate Akt, thereby decreasing its activity (Li et al., 1997). When PTEN is inhibited, Akt activity is greatly increased (Posner et al., 1994). Via TSC1/2 and Rheb, Akt increases mTOR activity. Increased mTOR activity increases the translation of the biosensor mRNA, thus increasing levels of GFP and FLuc in the cells. The construct illustrated was incorporated into a lentiviral vector (pGreenFire1; System Biosciences, Inc.), enabling its testing in primary skin fibroblasts. We tested the biosensor in three species: mouse, human and naked mole-rat (NMR). Following transduction of the skin fibroblasts with the mTOR biosensor lentivirus, cells were exposed to various concentrations of the PTEN inhibitor bpV(phen) (Posner et al., 1994), or alternatively the cells were incubated with lowered concentrations of fetal bovine serum (5, 2.5, and 1.25%, instead of the standard 10%). After 48 h, FLuc activity was measured in the cells. Other details of the FLuc measurements are the same as those provided in the legend to Fig. 2. The results of triplicate determinations ± S.E. are shown. A control construct in which the GFP-FLuc cDNA lacked a complex 5′ UTR did not show any responsiveness to PTEN inhibitors.

The fluorescent protein emits light at a longer wavelength than the bioluminescent protein. Thus the amount of light emitted at the longer wavelength, as a fraction of the total light emitted, gives a measure of the fraction of the molecules with close apposition of the two proteins. This measures the extent of the protein–protein interaction thereby measures the concentration of the agent that caused the conformational change, e.g., a ligand such as a hormone or toxin. BRET has been used in the study of a large number of protein–protein reactions, particularly with plasma membrane receptors (Pfleger and Eidne, 2005).

Many of the challenges involved in optimizing the performance of tissue-based biosensors that use bioluminescence are the same as those that use fluorescence. Several approaches are possible to overcome this limitation, including the development of bioluminescent proteins that emit light at longer wavelengths and the use of more sensitive detection devices. In this lab, we used skin/body wall windows that permit light to reach the detector without impedance by intervening tissues. In our published work on use of tissue-based biosensors employing bioluminescence, we used a skin window over the kidney (Huang et al., 2007b). We subsequently readily adapted the skin window to skeletal muscle. Bioluminescence can be measured from other organs by appropriate modifications of this “window” technique.

Another set of challenges arises from the need for a substrate to be supplied to the bioluminescence-emitting protein. In order to produce light, luciferases need oxygen and a specific substrate, and in some cases also ATP. Two commonly used luciferases are firefly luciferase (FLuc) and *Renilla* luciferase (RLuc). For FLuc, the

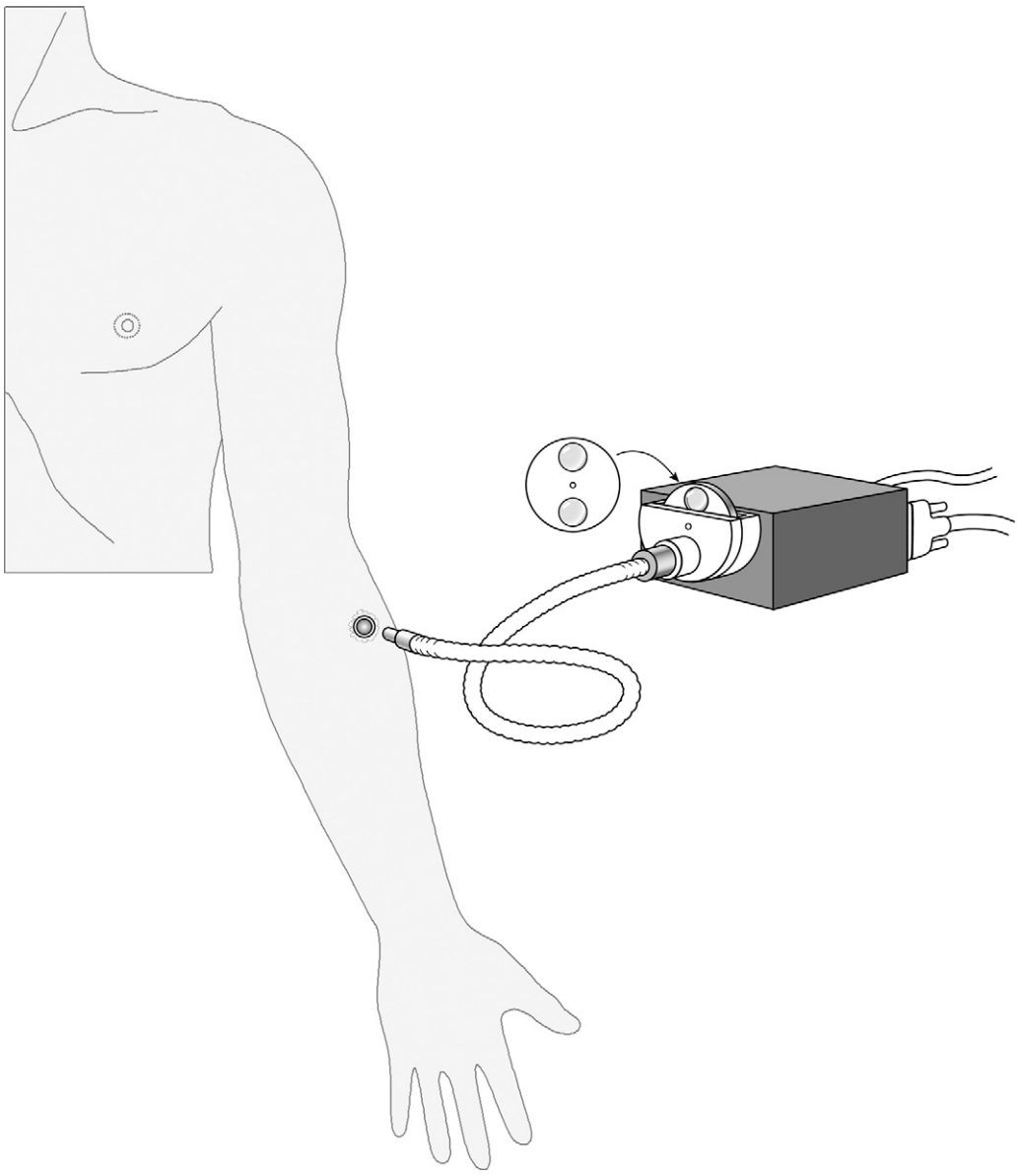

Fig. 5. Concept of cell- and tissue-based biosensor technology applied to human medicine. A device is implanted into the patient to enable the continuous monitoring of a hormone, drug, etc. The biosensor transduces the level of the monitored substance into a signal that can be measured externally. In the diagram, one possible mode of detection is shown. In this example, light output is measured via a light guide connected to a photon counter with changeable filters. While this is one example, there are many possibilities for the biosensor technology and for the mode of measurement of the output of the biosensor. For biosensors that involve the expression of biosensor proteins in the patient's tissues, the main issues that would need to be solved are: (i) the safe introduction of biosensor proteins; (ii) a simple and noninvasive means of measuring the output from the biosensor cells; and (iii) a useful lifespan for the biosensor, but without permanent effects on the host tissues.

substrate is D-luciferin; for RLuc, the substrate is coelenterazine. These molecules are cell permeable and are usually delivered by intraperitoneal or intravenous injection for observation of bioluminescence in experimental animals (Bhaumik and Gambhir, 2002). Ideally, substrates would be directly and continuously supplied to biosensor cells in the living animal under the investigator's control. A pioneering example of *in vivo* bioluminescence monitoring via continuous supply of luciferase substrate in conscious freely moving animals is a study in which FLuc was expressed in the brain under the control of a circadian rhythm-responsive promoter. Bioluminescence was measured continuously using an indwelling fiber optic probe (Yamaguchi et al., 2001). Continuous delivery of D-luciferin by micro-osmotic pumps was also shown to enable real-time bioluminescence imaging of FLuc activity *in vivo* (Gross et al., 2007). Thus long-term administration of luciferase substrate *in vivo* can be performed without encountering apparent toxicity.

3. Examples of cell- and tissue-based biosensors based on bioluminescence

3.1. Biosensors based on light output from two luciferases

Key issues for any biosensor, and for biosensors based on bioluminescence in particular, are to make the assay independent of cell number and independent of the distance of the light-monitoring apparatus from the light source (cells). In this way the assay is made completely robust and enables its use in living animals. The key solution to this problem is to measure a ratio, the relative light emission at two wavelengths, rather than an absolute number. One wavelength serves as the signal and the other wavelength as the reference (internal control). In tissue-based biosensors used in this lab we either used BRET (which measures a ratio) or we used the ratio of bioluminescence output from two different luciferases (Huang

et al., 2007b; Acha et al., 2010). For example, if the biosensor protein is a form of modified FLuc, then RLuc may be expressed in wildtype form at a constant (constitutive) level. In this way the ratio [biosensor protein light output/control protein light output] becomes the measurement of interest. FLuc and RLuc emit light at distinct wavelengths, and have separate substrates for light production, thereby enabling light output from the two luciferases to be measured simultaneously. The internal control protein should be expressed from a constitutive promoter so that its levels are invariant.

In order to demonstrate the feasibility of independent measurement of FLuc and RLuc in a mixture, we investigated the separate detection of the two light wavelengths using mixtures of FLuc-expressing and RLuc-expressing cells. If detection of FLuc and RLuc is specific, then light output at each of the two wavelengths will vary in proportion to the fraction of FLuc or RLuc cells in the mixture. The results of this test (Fig. 2) confirmed that this is the case, and that therefore dual-wavelength assays can measure FLuc and RLuc accurately in a tissue or cell population in which both are expressed. This principle forms the basis for many varieties of biosensor, in which the FLuc is under regulation by the detected substance and RLuc is a constitutive. Two examples of these are presented below: for TNFα, and for agents that activate mTOR.

3.2. A biosensor for TNFα

Various molecular mechanisms can be employed to link the molecule being detected to the output of light in the form of bioluminescence. For example, the gene encoding the bioluminescent protein may be placed under the control of a promoter that is regulated by the molecule being detected. This would be appropriate for detection of a molecule that acts via a transcriptional response (e.g. hormones like thyroid hormone or estrogen; Freitas et al., 2011; Weatherman, 2008). Alternatively, the protein level may be under translational regulation, or under post-translational regulation, e.g. by proteolysis. The latter can be used to link the level of a biosensor protein to an intracellular pathway that is regulated by protein breakdown.

A proteolysis-based biosensor can be used to detect TNFα. The basis for the biosensor is a fusion protein of IκBα and FLuc that is degraded by the proteasome under the control of an intracellular pathway stimulated by TNFα (Gross and Piwnica-Worms, 2005; Zhou et al., 2010). Thus, a cell expressing this fusion protein can serve as a biosensor for TNFα and other factors that activate the same intracellular pathway. We used HeLa cells stably expressing this gene under the control of a constitutive CMV promoter, together with a constitutively expressed Rluc gene as an internal control. As shown in Fig. 3, prior incubation of the cells in increasing concentrations of TNFα resulted in decreased light emission at the wavelength characteristic of FLuc, whereas light emission at the RLuc wavelength was unaffected. Note that it was necessary to add TNFα first, before the luciferase substrates, because the decrease in FLuc occurs is maximal at about 20 min following TNFα. Luciferase substrates produce a burst of light emission that lasts ~10–15 min. We performed control experiments in which cells were transfected with a construct that encodes FLuc only, rather than the IκBα-FLuc fusion protein. In these control cells, addition of TNFα had no effect on FLuc light levels even at the highest concentrations. These results show that (1) cells expressing an IκBα-FLuc fusion protein can be used as a biosensor for extracellular TNFα, and (2) constitutive RLuc can be used as an internal control for FLuc, when cells are exposed to a mixture of substrates for the two luciferases.

3.3. A biosensor for compounds that activate mTOR

The mammalian target of rapamycin (mTOR) pathway has been implicated in the control of life span in mammals, and the homologous pathway has been shown to be critical in life span determination in *Drosophila*, *Caenorhabditis elegans*, and yeast (Bishop and Guarente, 2007). Feeding of rapamycin to mice extends maximal life span by 9% in males and 14% in females (Harrison et al., 2009; Miller et al., 2011). These intriguing observations raise the possibility that the mTOR pathway acts to integrate multiple inputs that can affect life span; however, the mechanism by which life span is regulated by mTOR in mammals is yet unknown. The observations raise important questions regarding the tissue/organ targets for the action of rapamycin and the portion of the life span over which mTOR inhibition is critical. This raises the issue of the feasibility of continuous noninvasive monitoring of compounds that alter mTOR activity in living cells and living animals.

A major action of mTOR is the stimulation of the translation of sets of mRNAs that encode proteins involved in growth and/or cell proliferation. While mTOR activation upregulates the translational machinery generally (e.g. ribosome activation/biogenesis), mTOR also regulates the translation of various classes of mRNAs. One class comprises mRNAs that have structured 5′ untranslated regions comprising stem-loops or more complex secondary structures, e.g., VEGF, survivin, MCL-1, BCL-2 (Larsson et al., 2006; Konicek et al., 2008).

The specific enhancement of translation of mRNAs with complex 5′ UTRs provides the basis for the design and implementation of a biosensor for compounds that active mTOR activity (Fig. 4). We linked such a complex 5′ UTR to a reporter coding region (GFP-FLuc fusion protein) in a lentiviral vector. When various primary cells expressed this construct, FLuc activity responded to the addition of compounds that increased mTOR activity and responded with decreased activity to deprivation of factors that stimulate or maintain mTOR. Currently, we have used this biosensor in model cell systems in culture, but it should be feasible to employ it to monitor the levels of mTOR-activating drugs in living animals, potentially over long periods in lifespan studies.

4. Application of biosensor technology to monitoring in chronic diseases of aging

Biosensors that are based on purely physical principles are under intensive development for use in human medicine and even, to some extent, already in use. The synergy of nanotechnology and implantable sensors creates an exciting future for the development of this field (Vaddiraju et al., 2010). However, the biocompatibility of implanted physical sensors is a major concern (Frost and Meyerhoff, 2006). Using the patient's own cells, by creating a cell/tissue-based biosensor, would likely offer the advantage of superior biocompatibility over purely physical biosensors. Of course, this would have to be accomplished with minimal inconvenience and with no risk to the patient. While many hurdles would have to be overcome to make this feasible, it is possible to speculate on how this might be achieved. In brief, the main issues would be (i) the safe introduction of biosensor proteins; (ii) a simple and noninvasive means of measuring the output from the biosensor cells; and (iii) a useful lifespan for the biosensor, but without permanent effects on the host tissues.

Genetic modification of the patient's cells could be accomplished using a vector-coated implant, as described earlier. Permanent genetic modification would be highly undesirable, in view of the real potential for adverse effects caused by integration of the vector into the genome of the patient's cells (Hacein-Bey-Abina et al., 2003). However, with the advent of successful expression of ectopic proteins via mRNA transfection (Warren et al., 2010), it becomes possible to envision the use of biosensor proteins that involves no long-term genetic modification of the cells. Potentially,

the modifying mRNA could be administered via an implant small enough to be surgically implanted with little inconvenience. Such an implant could both accomplish the introduction of the biosensor proteins and also facilitate the measurement of the output from the cells, either entirely within the body or partly external to it. Cell- and tissue-based biosensors would allow noninvasive monitoring of hormones, drugs or toxins, as required.

The process of monitoring the output from the cells depends on what kind of output is employed. For outputs based on the principles of biophotonics, such as biosensors that produce fluorescence or bioluminescence, the patient's tissues and skin provide a barrier that must be overcome, as we discussed earlier in the context of animal experiments. In animals, the use of skin/body wall windows provides a way to measure light very precisely and at multiple wavelengths (Huang et al., 2007b). In a human patient, placing the biosensor cells as close to the skin surface as possible would be desirable (Fig. 5). In animals, a fiber optic lightguide can be used to conduct the emitted light to a photon counter or other light sensitive device. In this lab we have tested the Hamamatsu Multi-Pixel Photon Counter, a miniature device based on photodiodes, and shown it to be equivalent in sensitivity to a traditional photomultiplier-based photon counter. Despite the versatility of biosensors based on biophotonics principles, such biosensors have the disadvantage that they need a completely dark environment for measurements to be made. Moreover, they need either a source of substrate, for those based on bioluminescence, or a light source, for those based on fluorescence. Purely physical problems associated with light measurement will almost certainly be solved by continued advances in electronics. Electronic devices will continue to shrink, while increasing in power, at a rate that exceeds the rate of advances in biomedical science. Issues related to the need for light to be detected with great sensitivity via the skin will likely be solved by the further development of fluorescent and bioluminescent proteins that emit in the far red and infrared wavelengths, as we discussed earlier for biosensors in living animals.

Finally, the useful lifespan of the biosensor should be long enough to accomplish the needs of the study. In some cases, short-term monitoring might be all that is required. In other cases the design of the implant should permit the vector encoding the biosensor proteins to be re-administered from time to time as needed. These and other practical considerations may limit the use of this form of biotechnology in human patients. Nevertheless, the exploration of these issues in experimental animal models may pave the way toward the implementation of nanotechnology-based biosensors that have the same molecular basis as the cell- and tissue-based biosensors considered here.

5. Conclusions

Overall, the use of tissues as biosensors, derived by transplantation of biosensor cells or by genetic modification of tissues *in situ*, has great promise for biomedical science. Tissue-based biosensors could provide *in vivo* readouts for hormones, toxins, drugs and other molecules. While the current state of development of the field limits use of these biosensors to experimental animals, the future application of this technology to human subjects must await the resolution of several practical and safety issues.

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