In this review paper, we introduce some potentials of egg cell-coupled gate field-effect transistor (FET) in the field of drug screening and clinical diagnostics. Egg cells, such as oocyte, ovum, and embryo, were located on the gate surface of ion-sensitive FET (ISFET), resulting in the electrical monitoring of ionic behaviors based on cellular functions in a label-free, noninvasive, and real-time manner. In particular, this paper provides an outline of three topics regarding the egg cell-based ISFETs: a noninvasive monitoring of the transporter-drug interaction at the oocyte membrane; a real-time electrical detection of molecular charges at the ovum surface of sea urchin; a novel monitoring device of single mouse embryo activity after in vitro fertilization (IVF). The ISFET-based biosensor realizes to directly detect ionic behaviors at the cell/gate interface, which result from cellular functions. The electrical signal of ISFET biosensor should become an effective indication to evaluate objectively cellular activities as the morphology of living cells is observed subjectively by a microscopy. Thus, a platform based on the ISFET biosensor will contribute to promote drug screening and clinical diagnostics in the future.

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The changes in the surface potentials at the gate surface of the oocyte-based FETs were monitored after adding a substrate (Figs. 3a–3c). Here, two types of oocyte-based FETs were prepared; one was the FET with oocyte in which transporters were expressed, and the other was the FET with oocyte in which transporters were not expressed. With the use of these oocyte-based FETs, differential measurements were performed in order to eliminate the common background noises such as temperature change, change in ion concentration, and so on. The oocyte-based FET chip was treated as a single use tool for monitoring transporting function at cell membrane for the present. The reference FET without the oocyte itself was used in order to take the effect of oocyte placed at the gate surface into account. The estrone-3-sulfate (E3S) was used as a substrate in the uptake measurement for a human organic anion transporting peptide C (hOATP-C). When the E3S was introduced into two kinds of oocyte-based FETs and the reference FET, the surface potential of the oocyte FET with hOATP-C increased drastically during the uptake of E3S, while the oocyte FET without hOATP-C expression and the reference FET showed little surface potential changes. This result was similar to those obtained with RI measurement (Fig. 3d), in which [3H]-labeled E3S was used as a substrate. Monotonous increase of the E3S uptake during incubation time of 2 h was found. However, the time course of the uptake signal obtained with the oocyte-based FET was different from those of the conventional RI measurements. The surface potential of the oocyte-based FET reached steady-state in about 30 min after introduction of E3S, while the intensity of radioactivity increased over 2 h in the RI measurement. This means that the observed phenomena based on the oocyte-based FET would be disparate from the uptake amount of [3H]-labeled E3S. Although the molecular mechanism during the uptake of E3S through hOATP-C has not yet been elucidated, the uptakes of substrates mediated by some transporters are known to be associated with a substrate-dependent current under voltage-clamped conditions.25 In the case of OAT1, one of the family of organic anion transporters, entry of organic anion is reported to be accompanied by efflux of dicarboxylate by a 1:1 ratio.26 This exchange of singly charged organic anion with doubly charged dicarboxylates leads to a net loss of one negative charge per transporter cycle and change of the membrane potential in the positive direction. Since the positive change of the surface potential was obtained for the oocyte-based FET (Fig. 3), similar exchange of charged species is considered to take place during the uptake of E3S in the case of hOATP-C. The constant

Figure 1. Schematic illustration of ISFET. The Si$_3$N$_4$ or Ta$_2$O$_5$ gate surface with hydroxyl group in solutions is sensitive to pH based on the concentration of hydrogen ions. The charge density changes at the gate surface induce the electrostatic interaction with electrons at the channel in silicon crystal resulting in the change of drain current.

Noninvasive monitoring of the uptake kinetics of substrates mediated by membrane-bound transporters.—The schematic illustration of the oocyte-based FET is shown in Fig. 2. A *Xenopus laevis* oocyte is placed on the surface of the gate insulator of the FET. The n-channel depletion type FET was used and the width (W) and length (L) of the gate channel were 340 μm and 10 μm, respectively. The oocyte-based FET is immersed in a measurement solution together with an Ag/AgCl reference electrode with a saturated KCl solution. The potential of a measurement solution is controlled and fixed by the gate voltage ($V_g$) through the reference electrode. The extracellular potential changes are induced at the interface between the gate insulator and cell membrane by the uptake of substrates. The change in the electrical characteristics caused by interaction between transporters and substrates at the cell membrane is measured and monitored using the oocyte-based FET system. The electrical characteristics of FET such as the $V_{DG}$-$I_D$ characteristic and the surface potential at the gate surface are measured in a solution using a semiconductor parameter analyzer (B1500A, Agilent). As the basic electrical characteristic, $\Delta V_T$ is defined as the difference of $V_{DG}$-$I_D$ characteristics at a constant $I_D$. The time course of the surface potential at the gate surface is monitored using a source follower circuit24 with which the potential change at the interface between an aqueous solution and the gate insulator can be read out directly at a constant $I_D$. The FET chip was mounted on a polyimide printed circuit board and encapsulated with a polymer cover with holes in which cells are cultured. The details of the FET device and the fabrication process have been reported previously.24

The changes in the surface potentials at the gate surface of the oocyte-based FETs were monitored after adding a substrate without any leakage. It would be preferable to monitor the interaction between membrane proteins/transporters and ligands at the cell membrane noninvasively while the cells are cultured on the materials.
Discrimination of transporting ability among genotypes of the transporters using the oocyte-based FET.—hOATP-C is a liver-specific transporter involved in the hepatocellular uptake of a variety of endogenous chemicals, such as taurocholate, estrone sulfate, estradiol 17β-D-glucuronide, leukotriene C₄, prostaglandin E₂, and thyroid hormone. Genetic polymorphisms in hOATP-C have been investigated because of pharmacologic, toxicologic, and pathologic significances. We attempted to detect the difference of the transporting ability between the wild-type and mutant-type transporters using the oocyte-based FETs. The estradiol 17β-D-glucuronide (E217βG) was used as a substrate mediated by the wild-type hOATP-C*1a (the same transporter as hOATP-C) and the mutant-type hOATP-C*15. The uptake of the RI-labeled substrate was first measured for both the wild type and the mutant type transporters as a control experiment. The [3H]-labeled E217βG was detected in both hOATP-C*1a and hOATP-C*15-expressed oocytes (Figs. 4a and 4b). The uptake amount of [3H]-labeled E217βG for the wild-type transporter was 2 times larger than that of the mutant-type transporter. The difference of the uptake amount among the genotypes in the transporter was 2 times larger than that of the mutant-type transporter.

The oocyte with the wild type hOATP-C*1a or the mutant type hOATP-C*15 transporter was placed on the gate surface of the oocyte-based FET. Figure 4c shows the potential behaviors of the oocyte-based FETs during the uptake of E217βG without the RI label. The surface potentials of both oocyte-based FETs increased after the introduction of E217βG while the control oocyte FET showed a little change in the surface potential. The amount of the surface potential change of the oocyte-based FET with the wild-type transporter was approximately twice as large as that of the oocyte-based FET with the mutant-type transporter. The difference of the surface potential change between the wild type and the mutant type transporters was in good agreement with that obtained in the RI measurement (Figs. 4a and 4b). Thus, the transporting kinetics of the substrate mediated by the wild-type and the mutant-type transporters were distinguished by use of the oocyte-based FETs.

It is possible to integrate multiple oocyte-based FETs and signal processing circuits in a single chip using advanced semiconductor technology. Simultaneous analyses of different transporters and membrane proteins can therefore be realized based on the integrated oocyte-based FETs. Because of the output of the oocyte-based FET is an electrical signal, it would be easy to quantify the transporting ability of various transporters in the future. The platform based on the oocyte-based FETs is suitable for a simple, accurate, and inexpensive system for high-throughput screening in pharmaceutical lead discovery.

Real-Time Electrical Detection of Molecular Charges at Ovum Membrane

Understanding molecular behavior at cell membrane in the integrated field of biology and electronics is very important for life science. Glycoproteins or glycolipids exist in the plasma membrane and have important roles in interactions of the cell with its surroundings. Some of them have charges, exposing their sialic acid residues or sulfated saccharides at the cell surface. Information on molecular charges at the surface of cell membrane is therefore useful not only for understanding the cell functions but also for practical application.

Ovum of sea urchin is surrounded by gelatinous layer called the jelly coat that is made up of a mixture of sulfated fucose-rich polysaccharides and sialic acid-rich glycoproteins. Sulfated fucose-rich polysaccharide is believed to be a major component for acrosome reaction at the jelly coat of sea urchin ovum surface (Fig. 5), although this reason is not still clarified as to whether the pure sulfated polysaccharide activates the acrosome reaction or not. Thus the ovum surface of sea urchin have negative charges of molecules such as sialic acids and sulfated saccharides at glycolipids and glycoproteins in an aqueous solution so that intrinsic molecular charges at the ovum surface related to the interaction between ovum and sperm can be detected in principle by measuring the change in the electrical characteristics of FET devices. Since sialic acids at cell membrane are related to various recognition processes for cells, quantitative analysis of molecular charges of sialic acids at cell membrane would be a good measure to analyze cell functions. In particular, molecular charges at the ovum surface of sea urchin might be significant for fertilization, because sulfated oligosaccharide units at the ovum surface of sea urchin function as receptor for sperm. In this Real-time electrical detection of molecular charges at ovum membrane section, we show the real-time electrical detection of intrinsic molecular charges of sialic acids and sulfated saccharides, which play an important role on fertilization, at the ovum surface of sea urchin by use of the FET devices.

Real-time electrical detection of intrinsic molecular charges at ovum membrane.—The same FET devices as those shown in the above sections were used in this Real-time electrical detection of molecular charges at ovum membrane section. Sea urchin ova were seeded on the surface of the gate insulator of the ISFET. The ISFET...
Figure 3. Noninvasive monitoring of uptake of a substrate mediated by human organic anion transporting peptide C (OATP-C). (a) Surface potential change of the oocyte FET with the transporter based on the uptake of estrone 3-sulfate (E3S). (b) Surface potential change of the control oocyte FET based on the uptake of E3S. (c) Surface potential change of the reference FET based on the introduction of E3S. In (a), (b) and (c), 100 mM E3S was introduced at 10 minutes at room temperature. (d) Time-dependent uptake of [3H]-labeled E3S. Data were expressed as mean ± S.E. (n = 8). Reproduced with permission.9 Copyright 2008, American Chemical Society.

was immersed in a measurement solution together with an Ag/AgCl reference electrode with a saturated KCl solution. The molecular charges at the ovum surface on the gate interact electrostatically with electrons in silicon crystal through the thin gate insulator and induce electrical signals by the field effect, and are monitored as the change in the surface potential on the gate at a constant ID using the FET measurement system.

The change in the surface potential at the gate surface of the ISFET was monitored after adding sea urchin ova (Fig. 6). Here, two types of ISFETs were utilized; one was the ISFET on which sea urchin ova were seeded, and the other was the ISFET on which nothing is introduced. Using these ISFETs, differential measurements were performed in order to eliminate the common background noises such as temperature change and so on. In case of the reference ISFET, the surface potential have never shifted because no ova was introduced (Fig. 6a). When the sea urchin ova were introduced into the ISFET, the surface potential decreased during the adhesion of sea urchin ova on the gate surface (Fig. 6b). As shown in Fig. 7, two or three sea urchin ova were observed on the gate surface of ISFET. The change in surface potential at the gate of FET device depends on the change in charge per unit area of the gate. Since the change of surface potential of ISFET was about 40–50 mV after introducing the sea urchin eggs on the gate surface as shown in Fig. 6, the surface potential shift of about 10–20 mV would be obtained for one egg by use of the ISFET. The negative shift of surface potential by use of the FET system indicates the increase of negative charges on the gate surface. The negative charges are based on mainly sialic acids and sulfated saccharides at the ovum surface of sea urchin. In the other work, sulfated polysialic acid chains at carbohydrate-rich vitelline envelope (under the jelly coat shown in Fig. 1), which is believed to be important for fertilization and has been found as a component of the receptor protein for sperm, inhibited fertilization, while the nonsulfated form of this polysialic acid chains has little inhibitory activity.35 Therefore, the ovum with low ability for acrosome reaction and subsequent fertilization may be distinguished from the normal ovum by comparing the surface potentials based on the charge amount at the ovum surface using the FET devices.

Single Embryo-Coupled Gate Field Effect Transistor for Elective Single Embryo Transfer13,14

Recently, assisted reproductive technology (ART) has been expected to be one of therapeutic methods of sterility. Engineers other than obstetricians have been required for assured success of ART programs. For in vitro fertilization (IVF) of one of ART programs, how to evaluate embryo quality and select an embryo in good condition are significant. Morphological evaluation has been widely used to rank embryo quality because microscopic analysis is noninvasive and useful in predicting pregnancy rates.36,37 However, the standard of classification for embryo quality seems to be ambiguous among...
operators because it is a subjective method. Moreover, elective single embryo transfer (eSET) will be recommended in the future in order to prevent a multiple pregnancy. Therefore, a novel principle to evaluate the quality of a single embryo quantitatively and noninvasively in a real-time manner is required for practical use in ART. The detection principle of ISFET is based on the potentiometric detection of charge density changes at the gate insulator and is applied for various biosensing. Since the gate insulator usually consists of Si$_3$N$_4$ or Ta$_2$O$_5$ with hydroxyl group at the surface in solutions, furthermore, the ISFET is sensitive to the concentration of hydrogen ion with positive charge and should be utilized as the pH sensor. Therefore, pH variation based on respiration activity of the embryo will be monitored quantitatively and noninvasively in a real-time manner using a single embryo-coupled gate FET for eSET (eSET-FET), because pH at the interface between the embryo and gate membrane of FET will change sensitively according to dissolution of carbon dioxide into medium generated by metabolism and respiration activity in an embryo. Thus, the platform based on the eSET-FET sensor will be valuable for the development of an evaluation system to select a single embryo with good quality for eSET in the future.

On the other hand, oxygen consumption has been considered to be the parameter that provides the best indication of overall metabolic activity of a single embryo, although embryo metabolism has previously been assessed by measurement of nutrient consumption, such as glucose, pyruvate, and amino acids. As one of the detection methods for the evaluation of embryo quality, the electrochemical system is being developed. Shiku et al. reported previously the detection concept of oxygen consumption based on the respiration activity of an embryo. In this method, the oxygen reduction current was detected near the surface of a single embryo using the cyclic voltammetry technique. However, this method is unsuitable for real-time measurement for a long-term such as cleavage of mammalian embryo.

Therefore, we propose a semiconductor-based embryo sensing device for eSET (eSET-FET) to monitor not only sea urchin embryo but also single mouse embryo activities based on cellular respiration in a real-time, quantitative, and noninvasive manner. Additionally, we report to have developed the simultaneous analysis system composed of microscopic observation and electrical measurement of eSET-FET under the adequate embryo culture condition in an incubator. In this section, sea urchin embryos and a single mouse embryo were treated for evaluations.

**Sea urchin embryo**—Figure 8 shows the conceptual structure of the bio-ISFET intended to detect embryo activity after fertilization. Sea urchin ova were seeded on the surface of the gate insulator of the ISFET. Then, sperms were added to ova, resulting in the formation of fertilization membrane. The gate surface of the ISFET was immersed in a measurement solution together with an Ag/AgCl reference electrode.
trode with a saturated KCl solution. The charge density changes based on hydrogen ions could be detected as the shift of surface potential of the bio-ISFET, as mentioned in the above sections. Basically, the ion or molecular charges at the gate interact electrostatically with electrons in silicon crystal through the thin gate insulator and induce electrical signals by the field effect, and are monitored using the bio-ISFET system. Furthermore, two types of bio-ISFETs were prepared for differential measurements in this study; one was the bio-ISFET on which sea urchin ova were kept and fertilization was accomplished by induction of sperms, and the other was the control bio-ISFET on which there were ova, but sperms were not introduced. Using these bio-ISFETs, differential measurements were performed in order to eliminate the common background noise such as temperature change, non-specific adsorption and change in ion concentration.

The change in the surface potential at the gate surface of the bio-ISFET was monitored after adding sea urchin ova and sperms in turn (Fig. 9a). First of all, the surface potential showed a negative shift after introducing ova at t₁ on the gate because of negative charges of the ovum membrane in contact with the gate surface. The negative charges of the ovum membrane derived from mainly sialic acids and sulfated saccharides of its jelly coat, as shown in the Real-time electrical detection of molecular charges at ovum membrane section. After ova were kept on the gate area, sperms were added there at t₂ but little surface potential change of bio-ISFET could be detected, although some noise based on temperature and ion concentration changes could be found. The size of a sperm is smaller than that of an ovum (about 100 μm) and the diameter of the head is about 5 μm. They were running around above or on the gate surface. Since most of small sperms attached and detached on the gate repeatedly, a continuing change of surface potential would not be detected after the introduction of sperms. Moreover, the fertilization occurred on the gate surface a few minutes after introducing the sperms, which was confirmed by the formation of a fertilization membrane. Cell division occurred on the gate area to 2-cell at about 1 h, 4-cell at about 2.5 h, 8-cell at about 3.5 h, 16-cell at about 5.5 h and morula at about 8 h after fertilization. The surface potential of the bio-ISFET increased gradually to the 8-cell stage, then decreased after that. The increase of surface potential at the beginning of cell division indicates an increase of hydrogen ions induced by dissolved carbon dioxide created by respiration. Since little surface potential change was measured before fertilization, when virginal ova were kept on the gate surface, the shift of surface potential based on the increase of hydrogen ion might reveal the change of function of the respiration system due to fertilization. On the other hand, the control bio-ISFET showed little the electrical response due to non-fertilization (Fig. 9b).

Mitochondria play an important role to respiration inside cells. Pyruvate molecules produced by glycolysis are transported into the mitochondrion matrix through the inner membrane, where they are oxidized and combined with coenzyme A to form carbon dioxide, acetyl-CoA, and NADH. The fertilized ova would become activated, accompanied by cell division, resulting in changes of shape and amount of mitochondria, which produce ATP and are closely related to the metabolism of the embryo. Cell division progressed to the 8-cell phase in a relatively short time, so the surface potential shifted increasingly due to the increase of hydrogen ions. On the other hand, the surface potential decreased gradually after reaching the 8-cell phase. The cell division of each blastomere occurred at random, and each step of cell division took longer than the previous step. For example, the transition from 8-cell to 16-cell took longer than that from 2-cell to 4-cell, resulting in the degeneration of respiration for one fertilized ovum in a constant period. This explains why the concentration of hydrogen ions would decrease based on the diffusion from the interface between the embryo and the gate surface to the bulk solution after the 8-cell phase. This means the effect of diffusion of hydrogen ions on the electrical signal was stronger than that of soluble carbon dioxide due to respiration. Thus, the embryo activity at the beginning of development can be easily detected in a real-time and noninvasive manner by use of the bio-ISFET.

**Single mouse embryo**—A single mouse embryo was put on one gate sensing area of the eSET-FET (Fig. 10a). The embryologist put a single mouse embryo on a gate area by use of a micropipet. The mammalian embryo is very sensitive to external environments such as ion strength of culture medium, temperature, and so on. This is why the total system for evaluation of embryo based on ISFET should be easily detected in a real-time and noninvasive manner by use of the bio-ISFET.
measurement using the eSET-FET sensor in the incubator. Using the simultaneous analysis system, the subjective and objective evaluation of embryo under the adequate condition of embryo culture can be performed in a real-time and noninvasive manner. Moreover, the sensing area was covered by a 20 μL droplet culture medium, where a single mouse embryo was kept on the gate of semiconductor, as shown in Figure 10c. The mineral oil of 1 mL was introduced onto a dropped culture medium of 20 μL to prevent drying of the medium. The droplet culture is useful to control the position of the single embryo on the gate and detect ion concentration change due to low volume.

The change of surface potential at the gate surface of eSET-FET sensor was monitored after IVF (Fig. 11). The surface potential of the eSET-FET sensor with a single embryo to blastocyst increased gradually as shown in Fig. 11a. The increase of surface potential at the beginning of cleavage indicates the increase of hydrogen ions with positive charges based on dissolution of carbon dioxide generated by cellular respiration. Since little surface potential change was measured before fertilization, when virginal ova were kept on the gate surface, the shift of surface potential based on the increase of hydrogen ion would reveal the change of metabolism due to fertilization, as discussed in the above Sea urchin embryo section. On the other hand, the surface potential change of the eSET-FET sensor with a single embryo accompanied by a 2-cell block decreased gradually, which was similar with that of a control sensor without embryo, although it increased drastically only for several hours before a 2-cell stage. This electrical signal indicates an abnormal behavior of an embryo before a 2-cell block and that hydrogen ions with positive charges based on respiration activity increased drastically at the interface between embryo and sensor surface. This may be because metabolism in a single embryo was activated accompanied by autophagy before cell death, because proteins would be degraded to amino acids in autolysosome, which would be utilized for cellular respiration. Lastly, the control sensor without embryo showed actually the pH variation of culture medium for 90 h under the embryo culture condition. The surface potential change of the control sensor reached about ~20 mV at around 40 h, which could be calculated as pH 7.4 to 7.7 because the semiconductor sensor had the detection ability of about 60 mV/pH. Moreover, the differential electrical signals were calculated as shown in Fig. 11b. Basically, the electrical signal of sample sensor against control sensor should be evaluated considering unexpected signals such as temperature change, pH variation of culture medium, and so on. The electrical signal of the eSET-FET sensor with normal embryo against the control FET (normal eSET-FET) was clearly different from that of the eSET-FET sensor with abnormal embryo against the control FET (abnormal eSET-FET) after around the 2-cell stage. Interestingly, the surface potential for normal eSET-FET increased gradually after IVF, but its inclination changed significantly after around the 2-cell stage of 10−15 h. This might show the change of metabolism during cleavage at around a 2-cell stage. Figure 11c shows the differential signal at 40 h after IVF in Fig. 11b, which corresponds to around an 8-cell stage for normal eSET-FET particularly. All the signals were

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**Figure 8.** Schematic illustration for measurement of surface potential of bio-ISFET. A shift of the threshold voltage $V_T$ can be determined from the gate voltage ($V_G$)-drain current ($I_D$) characteristics in an artificial sea water (pH 8.1). The $V_G$ was controlled through the Ag/AgCl reference electrode with the saturated KCl solution. Sea urchin ova or sperms were introduced to the gate sensing area during the measurement of electrical signal. Reproduced with permission.13 Copyright 2011, Springer International Publishing AG.

**Figure 9.** Noninvasive and real-time monitoring of embryo activity of sea urchin using bio-ISFET. $t_1$ and $t_2$ shows the introduction time of ova and sperms, respectively. The fertilization occurred at a few minutes later after the induction of sperms to ova on the gate surface. They have been already added on the gate at 0 h (b). Reproduced with permission.13 Copyright 2011, Springer International Publishing AG.
The volume of culture medium might actually affect the signals of pH variation based on embryos. In this case, however, the volume of 20 μl used in this study was large enough for the size of the embryo (about 100 μm), and actually, we could not find electrical signals when the embryo did not have contact with the gate area and was placed far from the gate in the same volume. Therefore, we need to directly put the embryo on the gate surface for measurement. Thus, the electrical signal of eSET-FET sensor for single mouse embryo activity should become an effective indication to evaluate objectively embryo activity as its morphology is observed subjectively after IVF. The platform based on the eSET-FET sensor will contribute to promote eSET in human ART.

**Conclusions**

In this review, some egg cells were targeted for the electrical monitoring with the semiconductor devices. Generally, cells can be directly and noninvasively observed by microscopy, which enables the imaging of fluorescent-labeled biomolecules as well as the observation of cellular morphologies. A quality check of an embryo obtained by IVF is performed before transplantation according to the criterion of the embryo grade, as introduced in this paper, and the differential behaviors of stem cells such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are also investigated using green fluorescent protein (GFP)-induced gene transfection. Microscopic observation in vitro provides considerable information on cellular functions to researchers and doctors. In particular, observation by inverted microscopy enables the high-resolution imaging of cells on a conventional cell culture dish owing to its transparency. However, the quantitative analysis of invisible cellular activities such as ion behaviors through cell membrane proteins is difficult to per-
form at the same time as microscopic observation. It is very important to simultaneously analyze a number of functions of the same cell as rapidly as possible under the same conditions, because the evaluation of living cells for transplantation into a human body should be performed concurrently for identical cells in a noninvasive manner. However, silicon-based semiconductor devices focused in this paper are not suitable for microscopic observation because of their non-transparency. By developing transparent devices for cell sensing,54 therefore, a simultaneous measurement system that allows visible and invisible information to be obtained simultaneously will be realized in the medical fields of clinical diagnosis and tissue engineering in the future.

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