ACS SyntheticBiology

pubs.acs.org/synthbio

Research Article

Development of a Cell-Based Biosensor for Residual Detergent Detection in Decellularized Scaffolds

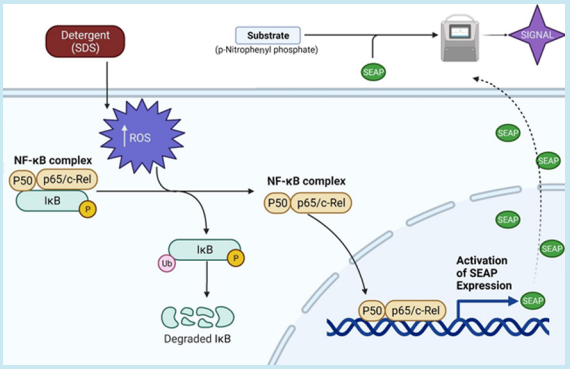
Fatemeh Ghorbani, Mohammadreza Abdihaji, Mehryar Habibi Roudkenar, and Ammar Ebrahimi*

Cite This: *ACS Synth. Biol.* 2021, 10, 2715–2724

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Ex vivo engineering of organs that uses decellularized whole organs as a scaffold with autologous stem cells is a potential alternative to traditional transplantation. However, one of the main challenges in this approach is preparing cytocompatible scaffolds. So far, high-precision and specific evaluation methods have not been developed for this purpose. Cell-based biosensors (CBBs) are promising tools to measure analytes with high sensitivity and specificity in a cost-effective and noninvasive manner. In this paper, using the NF-κB inducible promoter we developed a CBB for residual detergent detection. Proximal and core sections of the inducible promoter, containing NF-κB binding sequence, are designed and cloned upstream of the reporter gene (secreted alkaline phosphatase (SEAP)). After transfection into HEK293 cells, stable and reliable clones were selected. After confirmation of induction of this gene construct by sodium dodecyl sulfate (SDS), the stability and function of cells treated by qPCR and SEAP activity were measured. This biosensor was also used to evaluate the cytocompatibility of decellularized tissue. Results showed that the developed biosensor could detect very small amounts of SDS detergent (3.467 pM). It has the best performance 8 h after exposure to detergent, and its stability in high passage numbers was not significantly reduced. Applying this biosensor on decellularized tissues showed that SEAP activity higher than 4.36 (U/L) would lead to a viability reduction of transplanted cells below 70%. This paper presents a novel method to evaluate the cytocompatibility of decellularized tissues. The developed CBB can detect residual detergents (such as SDS) in tissues with high sensitivity and efficiency.



KEYWORDS: *cell-based biosensor, organ transplantation, decellularization, NF-κB*

INTRODUCTION

Organ transplantation is the only therapeutic option for many patients with end-stage diseases. Therefore, in addition to saving the lives of patients with the final phase of organ failure, organ transplantation can also be a treatment for people who have organ failure in the early stages and want to improve their quality of life.[1] Although organ transplantation is a proven and life-saving treatment approach for these patients, it faces many challenges. Currently, organ shortage is a major barrier to transplantation, and the gap between the number of patients on the waiting list and the limited number of available organ donors continues to widen. As a result, more than 6000 patients die each year while waiting for a transplant.[2] Meanwhile, for people with respiratory failure due to chronic obstructive pulmonary disease (COPD), emphysema, cystic fibrosis, etc., if not diagnosed early, the only treatment option left will be lung transplantation. In addition to organ shortage, there are other limitations such as transplant rejection and long-term posttransplant problems. Transplant recipients are always exposed to transplant rejection and problems related to immune suppression. Bioengineering is seen as an emerging solution aimed at producing the tissue and organs needed for transplantation, which may be a way to access a potentially endless source of transplantable organs that do not require posttransplant suppression of the immune system. Tissue engineering is a field related to regenerative medicine that focuses on the repair and replacement of poorly functioning organs or tissues. Therefore, one of the new strategies to overcome organ deficiency and reduce immune suppression is the use of natural scaffolds, including the use of decellularized organ scaffolds obtained from animal or human donors for tissue engineering.[3] These natural scaffolds are produced during the decellularization process by preserving the natural components of the extracellular matrix and proper structural and mechanical properties of the organs and removing DNA and cellular compounds. Due to the preservation of the main structural

Received: July 12, 2021
Published: September 22, 2021

https://doi.org/10.1021/acssynbio.1c00321
ACS Synth. Biol. 2021, 10, 2715–2724

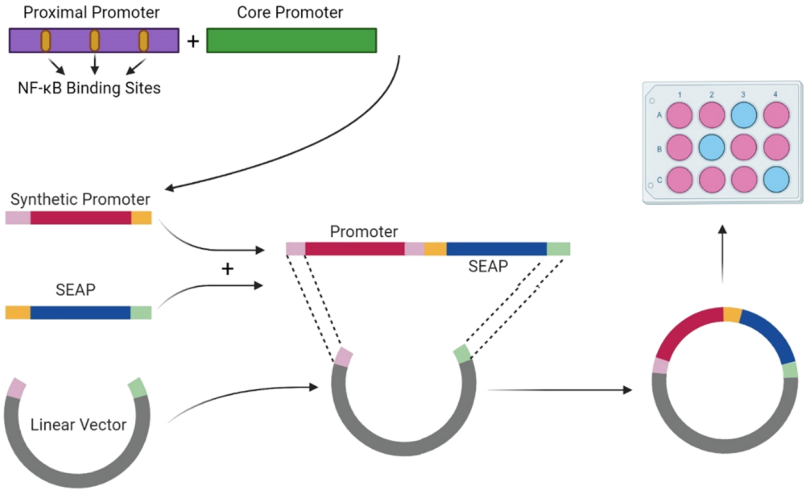


Figure 1. Generation of the recombinant vector. The NF-κB-inducible promoter is made by joining core and proximal promoters. The promoter was then cloned upstream of the reporter gene (SEAP). The recombinant vector was then transfected into the cells after confirmation.

architectures of the organ, vascular networks, adhesive molecules, and various markers for signaling, these scaffolds are known as biomimetic scaffolds, which have biocompatibility, biodegradability, and mechanical properties including elasticity, tensile strength, fracture resistance, and so on.

There are several techniques for decellularization of an organ, including physical, chemical, or enzymatic methods.[4] Physical methods include agitation, sonication, pressure, and freezing and thawing to destroy the cell membrane. Chemical methods that are the second category of treatments also include acids, bases, detergents, organic solvents and chelating agents, and hypertonic and hypotonic solutions that lead to the destruction of cell membranes and connections that are responsible for intracellular and extracellular communication. The third category of treatment, i.e., enzymatic treatment, also includes the use of protease and nuclease enzymes, which lead to the cleavage of peptide and nucleotide bonds, respectively. Detergents include nonanionic detergents, such as Triton X100, which have a hydrophilic polyethylene oxide group and a lipophilic or hydrophilic hydrocarbon group. Another type is sodium dodecyl sulfate (SDS) detergent, which is an anionic detergent used in sanitary and laundry products. Neutral detergents include CHAPS, which is used in laboratories to dissolve large biological molecules such as proteins. Among all kinds of available detergents, chemical detergents such as sodium deoxycholate, SDS, and Triton X100 are used for this purpose because they help in removal of the maximum cell content with the least damage to the extracellular matrix (ECM). Hence, the majority of protocols depend on a series of detergents used to lyse and remove cells while maintaining the structure and composition of the ECM. However, the scaffolds should be evaluated after decellularization for cytocompatibility as an essential criterion for all recellularization studies to determine their effect on the behavior and viability of new cells. Decellularized tissues should also be evaluated for cytotoxicity due to the toxic effects of the residual detergent and even lysed cell compounds on the scaffold. For successful scaffold recellularization, removal of lysed cell components and toxic residual detergents after the decellularization process is vital. This can be challenging, especially in large organs such as pig and human lungs, as large amounts of washing may be required to remove and wash away detergents.[5,6] Removing detergents, especially anionic detergents, from decellularized scaffolds is known to be very difficult.[5,7] There are currently no noninvasive tests to evaluate the removal of detergents from decellularized organs, so researchers mainly use cultures of one or two cell types on thin slices to test cytocompatibility before recellularizing larger sections or whole organs.[8−11] In addition to cell-based cytocompatibility tests, other studies have previously evaluated the presence of residual detergents in pig or human tissue-derived scaffolds by staining.[7,12] Another important consideration in evaluating decellularized scaffolds is predicting their ability to support cell survival and growth during the recellularization process.

One of the promising tools for this purpose is the use of biosensors. Cell-based bioassay means using living cells as sensor elements to detect the effects of extracellular compounds that can respond in a shorter time than other assessment methods.[13] Studies have suggested that the NF-κB signaling pathway is induced by an increased amount of ROS production in cells.[14,15] Therefore, it can be concluded that oxidative stress caused by detergents (such as the residual detergent in decellularized tissues) may increase the ROS production levels and subsequently activate NF-κB signaling. It seems that using NF-κB binding sequences in the proximal promoter upstream of a reporter gene can first indicate the activation of NF-κB after exposure of the cells to detergent-induced stress; moreover, by using complementary tests, the biosensor can estimate the amount of residual detergent and therefore cytocompatibility in the decellularized organ or tissues.

In this paper, we explore the possibility of using a novel cell-based biosensor (CBB) for scaffold cytocompatibility. We developed a biosensor containing the secreted alkaline phosphatase (SEAP) reporter gene, inducible by NF-κB pathway activators. Thus, the toxic effects of the residual SDS detergent on cells can be investigated by measuring the activity of SEAP in the culture medium.

METHODS

Cell Culture. Human embryonic kidney 293 cells (HEK293), human dermal fibroblasts (HDF), human lung epithelial cells (HBE), and a bone marrow-derived stromal progenitor cell (hMSC) were obtained from the Royan Institute cell bank and maintained in DMEM containing 10% fetal bovine serum (FBS; Gibco, USA), 100 μg/mL amphotericin, 50 μg/mL

https://doi.org/10.1021/acssynbio.1c00321
ACS Synth. Biol. 2021, 10, 2715−2724

streptomycin, and 100 U/mL penicillin. The cell cultures were kept at 37 °C in an incubator with 5% CO_2.

Generation of Stable Reporter Cell Lines. The gene construct containing the NF-κB-inducible promoter and the SEAP reporter gene, which is a secreted alkaline phosphatase, was designed by Vector NTI software (Life Technologies, USA).

To build the promoter, the core promoter sequence of the E-selectin gene was combined with a proximal promoter containing three NF-κB binding sites (with the GGGGACTTTCC sequence) and located upstream of the SEAP coding sequence. After synthesis, it was cloned into the pcDNA3.1/Zeo vector. Clones obtained after transformation into DH5a were cultured and their plasmids extracted and confirmed. (Figure 1).

HEK293 and HDF cells were seeded at a density of 4×10^4 cells per well in 0.5 mL of complete growth media on 24-well cell culture plates 24 h before transfection; 2 μL of the Lipofectamine 2000 reagent (Life Technologies) and 500 ng of pcDNA3.1/Zeo-NFkB plasmid were used for transfection. The complete cell culture medium was replaced with a selected medium containing zeocin as a selective antibiotic, 24 h after transfection. The kill curve assay was used to determine the optimal zeocin dose, and 100 and 450 μg/mL of antibiotic as lethal concentrations for HEK293 and HDF cells, respectively, were chosen after 1 week of exposure for further experiments.[16,17]

To perform single clone selection, transfected and reproduced cells were subcultured and highly diluted (50 cells per well on a 12-well plate). Using trypsin-soaked paper disks (Whatman filter paper; sterilized by autoclaving), clones were isolated and reproduced. After reproduction of single colonies, eight of them (with the highest vitality and growth rate) were induced with LPS and screened to find the most sensitive one. One of them was selected with the linearity, lowest background, and smallest standard deviation.

First, we needed to confirm that the designed gene construct can induce SEAP gene expression when the NF-κB pathway is activated. To this end, we used LPS as one of the most important activators of the NF-κB pathway. To determine the optimum LPS concentration for biosensor performance, the following was done: first, 500,000 HEK293 and HDF cells were cultured in a 6-well plate, and after reaching 50% confluency, 0.001, 0.01, 0.1, 15, and 50 μg/mL LPS solution was added and incubated for 8 h. Then, the SEAP expression from the cells was investigated. It was also necessary to confirm that SDS exposure could activate the NF-κB pathway in cells. To this end, manipulated HEK293 and HDF cells and control cells were treated with 0.5% SDS solution. Induction of the NF-κB pathway was assessed by measuring the expression of the IκBα gene by real-time PCR. We also investigated the expression of the IκBα gene in cells treated with concentrations of 1, 0.1, and 0.01% of SDS solution at different time points after treatment (2, 4, 6, and 8 h).

SEAP Activity Assay. Transfected and stable cells were stimulated with SDS (0.5%) or LPS (25 μg/mL) solutions, $t = 6$ h. The cell culture supernatants were then placed in new Eppendorf tubes and centrifuged for 10 min at 10,000 rpm to obtain clarified solutions. A total of 20 μL of cell culture supernatants were transferred to a new 96-well plate. The first step in inactivating endogenous alkaline phosphatase was to heat samples at 65 °C for 10 min (SEAP is heat stable). Then, the samples were mixed with (50 μL) assay buffer (20 mM L-homoarginine (Merck), Tris base (Merck), and 1 mM $MgCl_2$ (Sigma) pH 9.0) and incubated in a cell culture incubator for 15 min. In the final step, 35 μL of 100 mM *p*-nitrophenyl phosphate was added and samples were incubated for 1 h at 37 °C. SEAP catalyzes the hydrolysis of *p*-nitrophenyl phosphate, producing a yellow product that can be measured on a plate reader at 410 nm.

ROS Assay. After treating the cells with SDS detergent at different time points (0, 2, 4, 6, and 8 h), cellular ROS was measured by the dichlorodihydrofluorescein diacetate method as reported by Kang et al. (2005).[18] Briefly, the cells growing in the 96-well plates were given 2,7-dichlorofluorescein diacetate (DCFDA), a fluorogenic dye that measures ROS activity within the cells. DCFDA was oxidized by ROS after diffusion into the cells to a highly fluorescent molecule, DCF. A fluorescent microplate reader was used to detect the fluorescence of DCF, which has maximal excitation and emission spectra of 495 and 529 nm, respectively.

Assessment of Efficiency and Sensitivity of the Designed Biosensors. This was performed by studying the toxic effects of different concentrations of SDS detergent on manipulated cells by measuring the amount of SEAP activity expressed from the gene construct. To this end, in a 96-well plate, 20,000 cells were seeded, and after 24 h of incubation, SDS detergent concentrations of $1-10^{-10}$ were added, and after 2, 4, 6, 8, 12, 24, and 48 h, the levels of SEAP activity were examined by a spectrophotometer at 410 nm.

To determine cytotoxic thresholds from the detergent, we followed ISO 10993:5 (tests for in vitro cytotoxicity: Standard I. Biological Evaluation of Medical Devices) and set the cytotoxic threshold at 70% viability compared to the cell cultivation medium (negative, nontoxic control). Biosensor cells, hMSCs and HBE, were seeded at 10,000 cells in 96-well plates and left to adhere overnight. Media was then changed to media containing different amounts of detergents over a range of concentrations and incubated for 24 h. Then, cytotoxicity was determined using an MTT assay (Takara, Japan) according to the manufacturer's instructions. The results were normalized to the medium (negative control) and presented as a percentage of the control values.

Stability of the Biosensors. The stability of the reporter cell lines was tested at various passage numbers for each cell type. Freshly thawed cell clones (time passage 1) were compared with cells that had undergone a weekly splitting for 4 or 8 months (passage 15 and 30, respectively). Cells corresponding to different passage numbers (1, 15, and 30) were seeded in 96-well plates and cultured overnight. HEK293 or HDF clones were stimulated with 0.1% SDS solution. Finally, cells were analyzed at 48 h after stimulation by alkaline phosphatase assay.

To see if genetic changes affected the properties of the cells, the established stable sensor cell lines were compared to untransfected cells. To compare cell viability, DMSO, as a known inducer of cell death, was used. After 24 h of incubation with 1−10% (v/v) DMSO, EC50 of both nonmodified and modified cell lines was calculated and plotted.

Validation of the Biosensor Performance. Cells were seeded in 96-well plates with a seeding density of 2.5×10^4 cells/well and cultured overnight. LPS (25μg/mL) as the proinflammatory stimulus was added simultaneously with dexamethasone (2 μM). Cells were further incubated for 24 h and subsequently analyzed by SEAP assay, as described previously. As controls, cells without treatment and cells that had only been treated with the stimulus (LPS) were included.

https://doi.org/10.1021/acssynbio.1c00321
ACS Synth. Biol. 2021, 10, 2715−2724

Tissue Preparation and Decellularization. In order to evaluate the condition of decellularized tissue for the presence of residual SDS detergent, in the first step, a male rat weighing 220 g underwent surgery after intraperitoneal injection of ketamine and xylazine, and the lung was immediately removed and washed with PBS + ampicillin 10% solution. It was cut into fresh slides with 80 μm thickness by a cryosectioning device (Leica Biosystems). The obtained slides were placed in 24-well plates and treated with 0.1, 0.5, and 1% SDS concentrations. The plate was covered with parafilm and placed at 4 °C for 24 h. After this time, the solution was removed from the wells and the tissues were washed with PBS−ampicillin solution, and SDS was added again. These steps were repeated for the second and third 24 h. Finally, all tissues were treated with DNAse for 24 h and then washed again with PBS−ampicilin and then exposed to UV light for 20 min to continue the sterilization process. The decellularization process was confirmed by hematoxylin and eosin (H&E) and Masson's trichrome staining.[19,20] The tissues were kept at 4 °C until use.

At this stage, the tissues that had been decellularized with SDS solutions were exposed to HEK293 and HDF biosensors to evaluate the toxicity of this detergent by measuring the activity of SEAP secreted in the culture medium. Thus, 20,000 HEK293 or HDF biosensors were seeded in the 96-well plate, and 150 μL of DMEM + 10% FBS was added and incubated for 2 h. Wells without tissue were considered as control. After 2 h of incubation, the medium was collected to evaluate the secretion of SEAP, and this step was repeated at 4, 6, 8, 12, 24, and 48 h time points. Then, to evaluate the detergent-based toxicity and viability, after 48 h, these cells were used for MTT assay.

Cell viabilities were estimated using MTT assay. Briefly, HEK293 or HDF cells were placed into 96-well plates with tissue slices at the bottom for 48 h. MTT (Takara, Japan) was added to each well and incubated at 37 °C for another 4 h. The nonmetabolized MTT was then aspirated after centrifugation, and to solubilize the formazan, 150 μL of DMSO was added. An ELISA plate reader was then used to measure the absorption at a wavelength of 495 nm.

Statistical Analysis. GraphPad Prism software (version 8) was used to analyze the experimental data. All data from three independent experiments were expressed as mean ± SD. The difference was evaluated by a one- or two-way ANOVA and *t*-test. The nonlinear regression fit was also used for curve fitting. Statistical significance was defined as values of $p < 0.05$. Significant differences are denoted by a single asterisk (*), which indicates $p < 0.05$. Additionally, a double asterisk (**) indicates $p < 0.01$ and a triple asterisk (***) indicates $p < 0.005$.

RESULTS

In aerobic species, ROS is engaged in signal transduction and maintaining cellular redox equilibrium as a major signaling molecule.

We first confirmed that cell exposure with detergent works by increasing ROS production in the cells. Figure 2 shows the results of the ROS production experiment. With respect to each control, SDS treatment caused an overall increase in ROS production in all analyzed cells in a time-dependent way. The maximum ROS level In HEK293 cells was found after 8 h of treatment with 0.5% SDS solution (7125 RF), significantly higher than control cells (413 RF) ($p < 0.01$). Similarly, the levels of ROS in HDF cells increased dramatically from 422 RF in control to 6142 RF in cells treated with 0.5% SDS solution ($p < 0.01$).

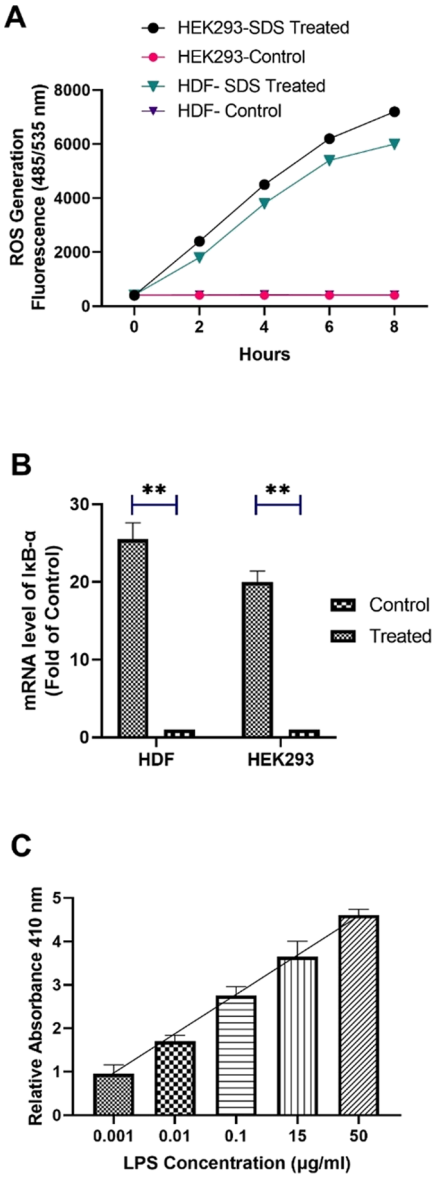


Figure 2. (A) Treatment of HEK293 and HDF cells with SDS solution significantly elevated ROS generation in these cells ($p < 0.01$). (B) Examination of NF-κB pathway activation at the mRNA level in HEK293 and HDF cells treated with SDS solution showed that the NF-κB pathway was activated significantly, compared to the control groups. (C) Response of the selected clone (clone number 8) after stimulating with LPS solutions with linear fitting.

The activation of NF-κB signaling after SDS exposure of HEK293 and HDF cells was studied in order to select a stress response pathway for the later development of sensor cells; 0.5% SDS solution was used to stimulate cells for 6 h. As expected, examination of NF-κB pathway activation in HEK293 and HDF cells at the mRNA level showed that the NF-κB pathway was activated approximately 20- and 24-fold, respectively, compared to control groups. The results of this experiment showed that SDS can activate the NF-κB pathway in cells and therefore by measuring the activation of NF-κB we can detect the presence of SDS in the vicinity of the cells. Also, as shown in Figure 2B, evaluation of the effect of different concentrations of SDS on the activation of the NF-κB pathway in HEK293 cells showed that with increasing the concentration of SDS solution, the expression level of the IκBα gene also increases. Examination

https://doi.org/10.1021/acssynbio.1c00321
ACS Synth. Biol. 2021, 10, 2715−2724

of the expression pattern of this gene at different time points also showed that the highest amount of expression occurs after 2 h of treatment with SDS solutions. According to these results, the NF-κB-inducible promoter was designed to investigate the presence of SDS detergent.

Generation of NF-κB Sensor Cells. After confirming that the NF-κB pathway is activated in HEK293 and HDF cells in response to SDS exposure, the next step is to develop a sensor cell line that would allow rapid determination of SDS effects based on reporter gene activation. To this end, an inducible promoter was created by combining an E-selectin promoter with three NF-κB binding sites (5′-GGGGACTTTCC-3′) and was placed upstream of the SEAP reporter gene (as shown in Figure 1). In the absence of NF-κB pathway activation, the reporter displays low activity. Induction of NF-κB activates the promoter resulting in expression of the reporter gene (SEAP) and enables monitoring the toxic effect of SDS. After synthesis of the gene construct, the plasmid transfected into the HEK293 and HDF cells. According to the kill curve results to determine the minimum lethal dose of the zeocin antibiotic, cell density of HEK293 and HDF cells reached zero with 100 and 450 μg/mL, respectively, after 1 week of treatment.

Induction of SEAP Gene Expression in Cells. Single colony selection was carried out after stable transfection. The goal of single clone selection was to achieve 100% clonal purity. Stimulation with LPS was used as a proof-of-concept setup to screen clones and establish, optimize, and validate the sensor cell-based assay. LPS is a well-known NF-κB pathway activator. After single colony reproduction, eight of them (with the best viability and growth rate) were screened to find colnes with a linear output.

First, the standard curve of alkaline phosphatase activity with 25 μg/mL LPS concentration was plotted for clones, and the results showed linear induction of SEAP gene expression in selected LPS-treated cells (Figure 2C).

Evaluation of Efficiency and Sensitivity of the Designed Biosensor. The study of SEAP activity secreted from cells after 2 and 4 h of SDS treatment revealed that not only alkaline phosphatase activity was very low, but it also had a nonlinear trend. However, the results after 6 and 8 h of treatment were linear, with a significant increase in SEAP secretion and activity. It was no longer in a linear or incremental mode after 12 and 48 h. Therefore, it seems that the best time to check the SEAP activity is 6−8 h. On the other hand, the results of MTT assay and viability of the biosensors also showed that after 12 h of cell treatment with SDS solution, cell viability gradually begins to decrease, leading to a decrease in SEAP activity, possibly due to cell death at 12 and 48 h after treatment. As expected, the lowest activity and lowest viability occur at an SDS concentration of 1%.

Determination of Cytotoxic Detergent Thresholds. We assessed the in vitro cytotoxicity of the commonly used detergent across a wide range of potential detergent concentrations in two representative human cells, a bone marrow-derived stromal progenitor cell (hMSC) and human lung epithelial cells (HBE), since a variety of cell types will be investigated for use in recellularization of decellularized lung scaffolds. After being exposed to SDS solutions, we discovered that cells had slightly different cytotoxic thresholds. HEK293 cells were the most sensitive to SDS, and hMSC cells were more viable than HBE cells at similar SDS concentrations (Table 1).

Stability of the Biosensors. It is crucial to test the biosensor's performance with different cell passages to ensure that the assay is being used correctly. Many subculturing procedures can cause alterations in cells.[21] This could lead to phenotypic heterogeneity, with cells unable to react to stimulation correctly over time. In order to determine the long-term stability of the reporter cell lines, cells with different passage numbers were seeded representing freshly thawed cells (passage 1), cells subcultivated for 4 or 8 months (passage 15 and 30, respectively), and cultured overnight. Then, with 0.1% SDS solution, cells were stimulated for 8 h and NF-κB activation was measured by determining of SEAP activity.

Table 1. SDS Detergent Cytotoxic Thresholds Estimated for the Biosensors and Each Cell Type Commonly Used in Whole Lung Recellularization

cell type	SDS %
HEK293	0.0008
HDF	0.0011
HBE	0.0023
hMSC	0.0016

The transfected HEK293 cells produced stable reporter signals even after 8 months of storage and repeated reactivation, although in the case of HDF cells, over time, clones lost their stability and ability to respond to stimuli.

Furthermore, the genetic modification had no effect on cell sensitivity to DMSO, a well-known inducer of cell death. Both transfected and control cells were equally sensitive after 24 h of incubation in the presence of 1−10% DMSO (Figure 3). The findings clearly show that genetic alterations to cells have no effect on the cell growth rate or viability.

Validation of the Biosensor Performance. Dexamethasone (DEX), is a synthetic glucocorticoid receptor agonist and a potent anti-inflammatory drug that works in different cell contexts by inhibiting DNA binding, IKK activity, and transactivation. Therefore, we selected this synthetic inhibitor and cells without treatment and cells treated only with LPS were included as controls. DEX treatment significantly reduced NF-κB activation (as measured by SEAP activity) in HEK293 and HDF clones, as shown in Figure 3B.

The NF-κB transactivation potential was investigated using quantitative RT-PCR (RT-qPCR) of IκB-α expression to validate the connection between mRNA levels of activated NF-κB elements and levels of reporter protein activity to further characterize the NF-κB-mediated stress response to SDS exposure. The synthesis of the IκB-α inhibitory subunit, which is NF-κB-dependent, is required to stop NF-κB transcription. As a result, the expression of IκB-α mRNA was evaluated in order to assess NF-κB activation by qPCR (Figure 3E). The obtained results are consistent with the expression of SEAP protein. The maximum expression of IκB-α mRNA was observed after 2 h of stimulation with 1% SDS, whereas the maximum activity of reporter protein was observed after 6 h of stimulation. Naturally, cells require more time for translation and posttranslational modification than transcription, which may explain the observed time shift.

Evaluation of Residual SDS Toxicity in Decellularized Tissues. First, H&E and Masson trichrome staining were used to confirm the decellularization of lung tissues prepared by SDS detergent. As expected, no cell nucleus was observed.

The results showed that the effects of residual SDS toxicity were significant in decellularized tissues with a concentration of 1% SDS compared to the other two groups with concentrations of 0.5 and 0.1%. According to the MTT assay results, cells

https://doi.org/10.1021/acssynbio.1c00321
ACS Synth. Biol. 2021, 10, 2715−2724

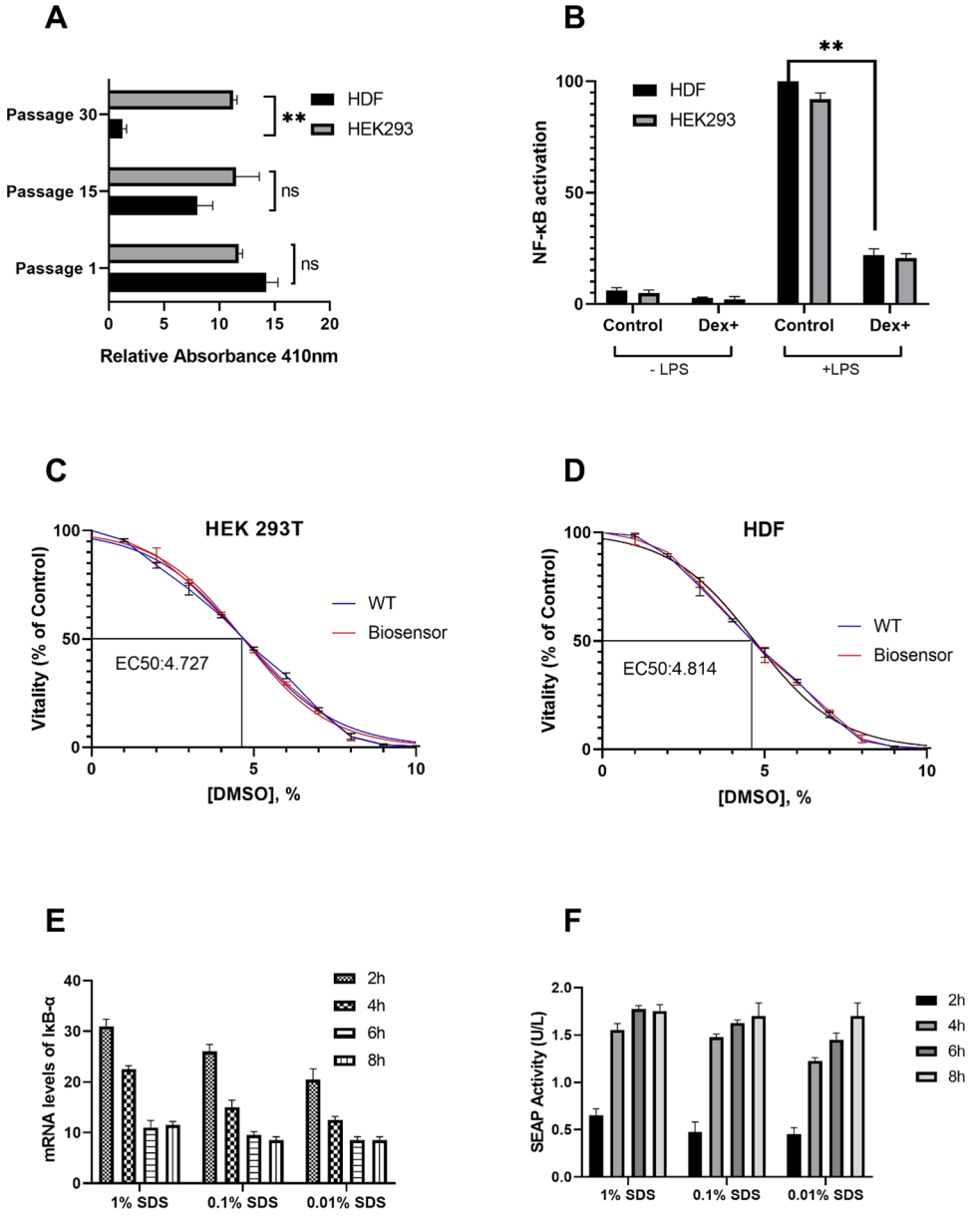


Figure 3. (A) Investigation of the biosensor stability after 1, 15, and 30 subcultivations. In high passages, immortal HEK293 cells maintained good viability, whereas normal HDF cells gradually lost their viability. (B) Modulation of LPS-induced NF-κB signaling in HEK293 and HDF cells. Cells were treated with the NF-κB inhibitory compound, DEX. NF-κB activation and cell viability were evaluated by SEAP assay. Data were normalized considering the LPS control as 100% activation. (C,D) Dose response sensitivity comparison of modified and nonmodified cell lines. T = 24 h, stimulation with DMSO (1−10% v/v). (E,F) Evaluation of the induction of IκBα gene expression at mRNA and protein levels after stimulation by different concentrations of SDS solutions. In all groups, the highest gene expression occurred 2 h after stimulation and the maximum yield at the protein level was observed after 8 h (controls = 1).

showed a decrease in viability. As expected, the results also showed that increasing the number of washes of decellularized tissues from one to four times increases the survival rate of cells (Figure 4A−D).

The results of decellularized tissue examination with biosensors showed that the biosensor output signal was correlated to the cell viability in the following days. Considering viability of 70% as an acceptable threshold, evaluation of the output signal of biosensors after 6 h of coculture with decellularized tissues showed that enzyme activity greater than 4.36 (U/L) in HEK293 cells would lead to a viability reduction below 70%. In other words, if 48 h after biosensor coculture with decellularized tissue, the culture medium is collected and the activity of SEAP enzyme is examined, the activity is greater than 4.36 (U/L), indicating that the tissue is not ready for cell transplantation and detergent should be removed by repeated washing (Figure 5).

DISCUSSION

Challenges of organ transplantation include acute or chronic rejection of a transplant, side effects of immunosuppressive drugs, and organ transplant deficiency itself. In addition to existing supportive and pharmacological treatments, new strategies for treating these patients rely on genetic engineering technologies, gene therapy, cell therapy, and regenerative medicine. For example, ex vivo engineering of lung tissue that

https://doi.org/10.1021/acssynbio.1c00321
ACS Synth. Biol. 2021, 10, 2715−2724

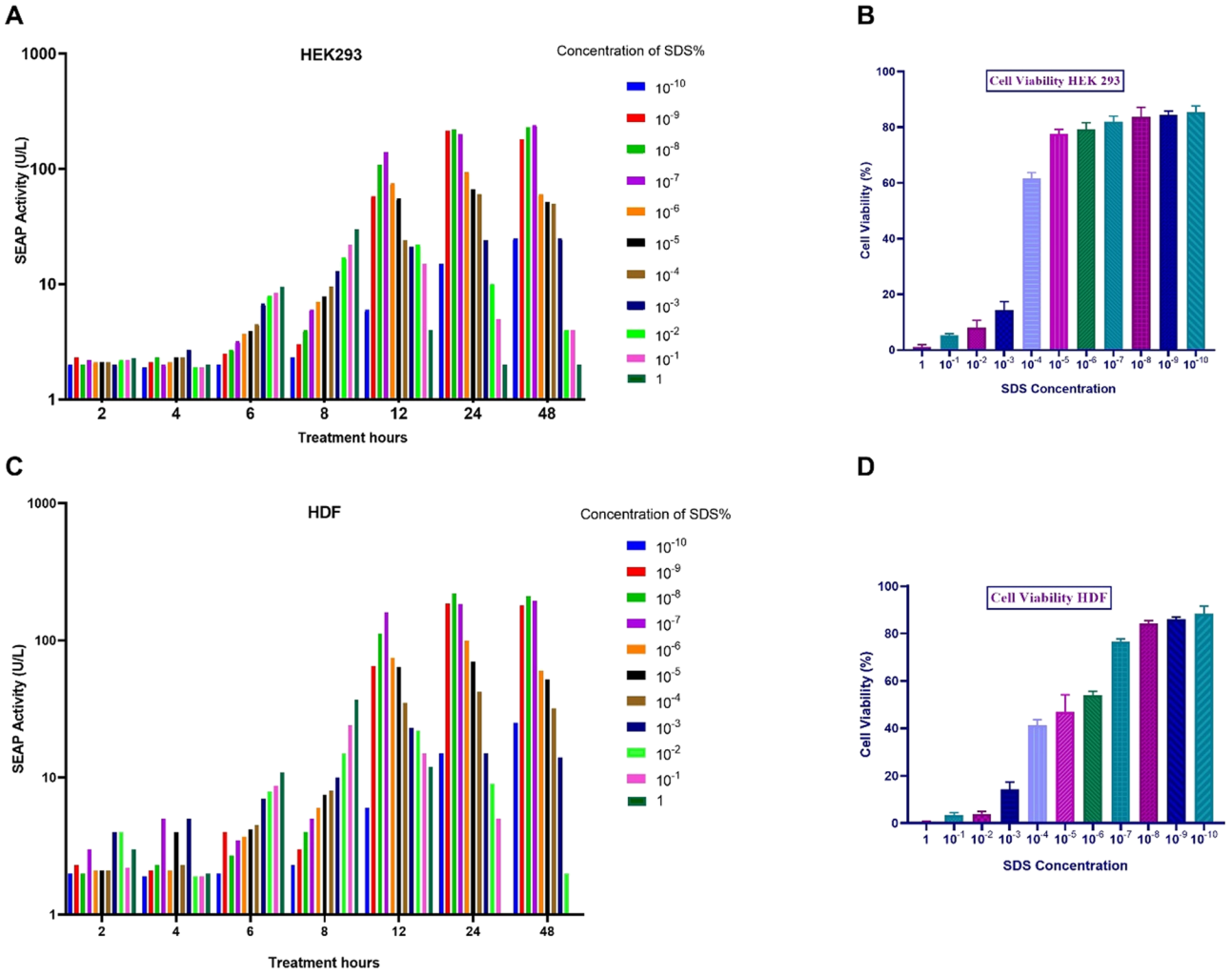


Figure 4. (A, C) Cells were stimulated with different concentrations of SDS at various time points to evaluate the function of biosensors. Although the highest SEAP activity was observed 24 and 48 h after stimulation, due to the nonlinearity of the results, reliable results were obtained 6 and 8 h after stimulation. (B,D) Effects of SDS solutions on cell viability of HEK293 and HDF biosensors. HEK293 and HDF biosensors treated with indicated concentrations of SDS solutions and their viability measured after 48 h.

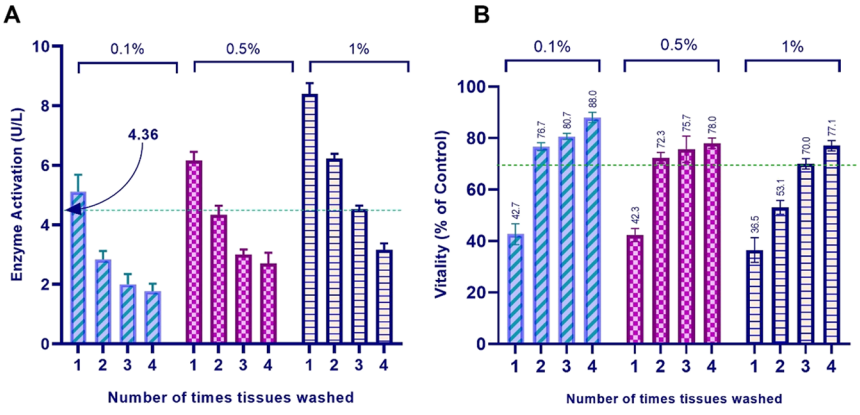


Figure 5. (A) Lung tissue samples decellularized with SDS solution concentrations of 0.1, 0.5, and 1% were washed one to four times, and the SEAP activity of the cultured biosensors was examined after 8 h. The dashed green line indicates a threshold, higher activity of this threshold (4.36 U/L) will lead to a reduction in vitality below 70%. (B) Illustrates the vitality results of seeded cells on decellularized tissues. The dashed line indicates the range with a vitality of 70%. These results were obtained by MTT assay, 48 h post treatment and normalized to control cells (vitality is considered 100%).

uses decellularized whole lungs or lobes as a scaffold with autologous stem cells obtained from a transplant recipient as a potential alternative to transplantation is one of these novel strategies.[22,23]

Engineered tissue traditionally requires a scaffold, or a three-dimensional (3D) matrix, which can be biologically derived or synthetically produced. To minimize immunological concerns and the use of immunosuppressive medications, scaffolds can be

https://doi.org/10.1021/acssynbio.1c00321
ACS Synth. Biol. 2021, 10, 2715−2724

seeded with an autologous stem, progenitor, or other cells acquired from the ultimate transplant recipient.[24]

Detergents are often used to decellularize and prepare these natural scaffolds.[25,26] However, since the presence of residual cytotoxic detergent used in the decellularization process can lead to oxidative stresses and negative effects on newly seeded cells, it is necessary to examine the presence of residual detergent in order to prevent these negative effects.[27]

Detergents, particularly anionic detergents, are generally difficult to remove from proteins and large acellular scaffolds.[26] There are currently no commonly used noninvasive techniques to detect the removal of anionic detergents from decellularized lungs.

Using the Stains-All solution, Gilpin et al. performed colorimetric analysis to detect residual SDS in tissue collected from decellularized human and porcine lungs.[7] While the general concentration of SDS employed throughout lung decellularization techniques is 0.1−1%, the Stains-All solution has previously been demonstrated to be specific for SDS, among other potential contaminating ions and detergents, in a linear way between 0−0.2% SDS. Excision of decellularized lung tissue, lyophilization, and enzymatic digestion of the tissue was used in this method of detergent analysis to detect SDS. It is unclear whether Stains-All can be used to detect other anionic detergents or effluents. Sudan III staining was employed by Price et al. to detect residual detergents in removed tissue slices from swine lungs. While this technique may be effective for visualizing detergent localization in the matrix, it requires tissue removal.[7,12] Sudan staining is not just for detergents; it can also stain other cellular components like lipids, making interpretation more difficult.[28]

Other than lung, detecting and removing residual detergents has been a significant endpoint in the development of acellular scaffolds.[8] The presence of anionic detergents in acellular human saphenous vein and acellular porcine liver and kidney tissue was determined using a methylene blue (MB)-based assay.[29]

While others have had success in detecting residual detergents in homogenates made from these acellular organs, the specificity of some of these approaches in detecting specific anionic detergents is unclear.[8] Furthermore, all of these procedures used destructive methods, making them unsuitable for clinical translation schemes or nondestructive cytocompatibility screening prior to costly and laborious recellularization procedures. Therefore, in this study, we present an innovative method to deal with this problem. A genetically engineered whole CBBs was used as an accurate, fast, and noninvasive method to determine the cytotoxic state of the residual detergent and also to evaluate the tissue readiness for accepting new cells.

When compared to complex experiments on tissue slices or animals, the benefits of utilizing cell-based sensors include quick growth and ease of culture cells. Cellular metabolism, bioavailability, and physiological responses relevant to humans and animals can all be reported by mammalian cells. This kind of data is critical for the development of functional biosensing. Mammalian cells' ability to mimic how an organism interacts with toxicants has become a critical tool for screening, monitoring, and evaluating environmental, food, and clinical hazards.

CBBs are usually made using immortal cells.[30] Immortal cells may originate from cancer cell lines or cells that have been immortalized by genetic modifications. In this study, we used HEK293 cells to develop the main biosensor. These immortalized cells are easy to transfect and have a longer life span than primary cells, making them useful in cell-based biosensing. Nevertheless, realizing that HEK293 cells differ from wildtype cells, which can be found in native tissues, we needed to confirm that genetic instability or changes in this cell line do not interfere with signal reception, transmission, and interpretation. Therefore, we also used normal HDF cell lines that do not have any genetic alterations.

The results showed that in most of the experiments, the performance of the biosensors is consistent. The only major difference in these cells was their stability, which, as expected, HDF cells were not able to tolerate high passage numbers. Furthermore, the integrated extracellular reporter system (SEAP) is beneficial in 3D cell culture evaluations since it eliminates the need for cell lysates and hence further extraction and analyses. Notably, the expression kinetics of the reporter gene can be easily evaluated by collecting media from the same culture multiple times; the analyzed cells are not disturbed during SEAP activity measurement and remain intact for subsequent research. The endogenous alkaline phosphatase background can also be eliminated, and the assay can be easily automated using multiwell plates.

Transfected cells may display different levels of expression of proteins coded in plasmids depending on where the genomic DNA vector was inserted and in how many copies. This remark could explain the different sensitivity responses of the clones investigated after stimulation with LPS and SDS. The relationship between cell exposure to SDS detergent and ROS production was poorly investigated before. The results obtained by this study are in line with other results shown by Messina et al., where they demonstrated the close relation between SDS exposure and ROS production, in the mussel's hepatopancreas.[31]

Stability monitoring of the biosensors (Figure 3) indicates that transfected cells are characterized by a stable signal (in the meantime cells were frozen and thawed). Nonetheless, accomplished examination of HDF showed that immortal cells are needed for long-term use of these biosensors and primary cells cannot have that reliable performance in a long time.

The biosensor developed responds optimally to bioactive analytes and is distinguished by its quick response time, label-free research, and straightforward technique. As a result, the biosensor developed is a useful tool for studying the physiological impacts of detergents.

Biosensor cells detect the toxic effect of SDS, as shown in Figure 3, even after 2 h of exposure, NF-κB signaling belongs to fast-acting elements; thus, the NF-κB pathway appears to be a perfect screening signal for cytotoxicity at early stages. This is explained by the presence of inactive NF-κB transcription factors in the cytoplasm, which do not require new protein synthesis in order to be activated. This enables NF-κB to respond quickly to potentially harmful cellular stimuli. The expression of IκB-α mRNA was examined to quantify NF-κB activation by real-time PCR. Maximum expression of IκB-α mRNA (Figure 3) was observed after 2 h of SDS stimulation, whereas the maximum abundance of reporter protein was observed after 8 h of SDS and LPS stimulation. In comparison to transcription, cells require more time for translation and posttranslational modification, which could explain the observed time shift. Furthermore, after 4 h of SDS stimulation, mRNA levels decreased and plateaued, which can be attributed to the relatively short half-life of the transcript and the switch from proinflammatory to proapoptotic pathways, resulting in the

https://doi.org/10.1021/acssynbio.1c00321
ACS Synth. Biol. 2021, 10, 2715−2724

inhibition of reporter gene expression. Instead of expressing the reporter protein, highly stressed cells now direct their metabolism to death.

To our knowledge, this is the first study to examine the cytocompatibility of the scaffolds with a CBB. This is a novel solution for assessing the toxicity of residual detergents in decellularized tissues with high sensitivity and the developed biosensor can detect very low concentrations of SDS in solutions or tissues with a specific and robust signal.

AUTHOR INFORMATION

Corresponding Author

Ammar Ebrahimi − *Department of Medical Biotechnology, School of Paramedicine, Guilan University of Medical Sciences, 4256 Rasht, Iran; Department of Biomedical Sciences, University of Lausanne, Lausanne 1005, Switzerland;* orcid.org/0000-0002-4586-5468; Phone: +41 21 692 55 01; Email: Ammar.Ebrahimi@Unil.ch

Authors

Fatemeh Ghorbani − *Department of Medical Biotechnology, School of Paramedicine, Guilan University of Medical Sciences, 4256 Rasht, Iran*

Mohammadreza Abdihaji − *Center for Genomics and Bioinformatics, Indiana University, Bloomington, Indiana 47405, United States*

Mehryar Habibi Roudkenar − *Department of Medical Biotechnology, School of Paramedicine, Guilan University of Medical Sciences, 4256 Rasht, Iran*

Complete contact information is available at: https://pubs.acs.org/10.1021/acssynbio.1c00321

Author Contributions

F.G. performed the experiments, M.A. analyzed the data and co-wrote the paper, and M.H.R. designed and performed the experiments. A.E. designed and performed the experiments, performed bioinformatic analyses, analyzed the data, and wrote the paper.

Funding

This work was partially supported by Guilan University of Medical Sciences fund (97060705).

Notes

The authors declare no competing financial interest.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

(1) Santivasi, W. L.; Strand, J. J.; Mueller, P. S.; Beckman, T. J. In *The organ transplant imperative*, Mayo Clinic Proceedings, Elsevier: 2017; pp. 940−946.

(2) Kobashigawa, J.; Dadhania, D.; Bhorade, S.; Adey, D.; Berger, J.; Bhat, G.; Budev, M.; Duarte-Rojo, A.; Dunn, M.; Hall, S., *Report from the American Society of Transplantation on frailty in solid organ transplantation*. Wiley Online Library, 2019.

(3) Fu, R.-H.; Wang, Y.-C.; Liu, S.-P.; Shih, T.-R.; Lin, H.-L.; Chen, Y.-M.; Sung, J.-H.; Lu, C.-H.; Wei, J.-R.; Wang, Z.-W. Decellularization and recellularization technologies in tissue engineering. *Cell Transplant.* **2014**, *23*, 621−630.

(4) Gilbert, T. W.; Sellaro, T. L.; Badylak, S. F. Decellularization of tissues and organs. *Biomaterials* **2006**, *27*, 3675−3683.

(5) Nichols, J. E.; Niles, J.; Riddle, M.; Vargas, G.; Schilagard, T.; Ma, L.; Edward, K.; La Francesca, S.; Sakamoto, J.; Vega, S.; Ogadegbe, M.; Mlcak, R.; Deyo, D.; Woodson, L.; McQuitty, C.; Lick, S.; Beckles, D.; Melo, E.; Cortiella, J. Production and Assessment of Decellularized Pig and Human Lung Scaffolds. *Tissue Eng., Part A* **2013**, *19*, 2045−2062.

(6) Chen, X.; Sun, J.; Li, X.; Mao, L.; Cui, L.; Bai, W. Transplantation of oral mucosal epithelial cells seeded on decellularized and lyophilized amniotic membrane for the regeneration of injured endometrium. *Stem Cell Res. Ther.* **2019**, *10*, 1−13.

(7) Gilpin, S. E.; Guyette, J. P.; Gonzalez, G.; Ren, X.; Asara, J. M.; Mathisen, D. J.; Vacanti, J. P.; Ott, H. C. Perfusion decellularization of human and porcine lungs: bringing the matrix to clinical scale. *J. Heart Lung Transplant* **2014**, *33*, 298−308.

(8) Wang, Y.; Bao, J.; Wu, Q.; Zhou, Y.; Li, Y.; Wu, X.; Shi, Y.; Li, L.; Bu, H. Method for perfusion decellularization of porcine whole liver and kidney for use as a scaffold for clinical-scale bioengineering engrafts. *Xenotransplantation* **2015**, *22*, 48−61.

(9) O'Neill, J. D.; Anfang, R.; Anandappa, A.; Costa, J.; Javidfar, J.; Wobma, H. M.; Singh, G.; Freytes, D. O.; Bacchetta, M. D.; Sonett, J. R. Decellularization of human and porcine lung tissues for pulmonary tissue engineering. *Ann. Thorac. Surg.* **2013**, *96*, 1046−1056.

(10) Chen, C.; Dong, J.; Chen, H.; Wang, X.; Mei, J.; Wang, L.; Xian, C. J. Preparation of adriamycin gelatin microsphere-loaded decellularized periosteum that is cytotoxic to human osteosarcoma cells. *J. Cell. Physiol.* **2019**, *234*, 10771−10781.

(11) Ferdowsi Khosroshahi, A.; Soleimani Rad, J.; Kheirjou, R.; Roshangar, B.; Rashtbar, M.; Salehi, R.; Ranjkesh, M. R.; Roshangar, L. Adipose tissue-derived stem cells upon decellularized ovine small intestine submucosa for tissue regeneration: An optimization and comparison method. *J. Cell. Physiol.* **2020**, *235*, 1556−1567.

(12) Price, A. P.; Godin, L. M.; Domek, A.; Cotter, T.; D'Cunha, J.; Taylor, D. A.; Panoskaltsis-Mortari, A. Automated decellularization of intact, human-sized lungs for tissue engineering. *Tissue Eng., Part C* **2015**, *21*, 94−103.

(13) Gui, Q.; Lawson, T.; Shan, S.; Yan, L.; Liu, Y. The Application of Whole Cell-Based Biosensors for Use in Environmental Analysis and in Medical Diagnostics. *Sensors* **2017**, *17*, 1623.

(14) Bubici, C.; Papa, S.; Pham, C.; Zazzeroni, F.; Franzoso, G. The NF-kB-mediated control of ROS and JNK signaling. *Histol. Histopathol.* **2006**, *21*, 69−80.

(15) Chen, A. C.; Arany, P. R.; Huang, Y.-Y.; Tomkinson, E. M.; Sharma, S. K.; Kharkwal, G. B.; Saleem, T.; Mooney, D.; Yull, F. E.; Blackwell, T. S. Low-level laser therapy activates NF-kB via generation of reactive oxygen species in mouse embryonic fibroblasts. *PLoS One* **2011**, *6*, No. e22453.

(16) Delrue, I.; Pan, Q.; Baczmanska, A. K.; Callens, B. W.; Verdoodt, L. L. M. Determination of the Selection Capacity of Antibiotics for Gene Selection. *Biotechnol. J.* **2018**, *13*, No. 1700747.

(17) Rahim, F.; Abbasi Pashaki, P.; Jafarisani, M.; Ghorbani, F.; Ebrahimi, A. Runx2 silencing promotes adipogenesis via down-regulation of DLK1 in chondrogenic differentiating MSCs. *J. Gene Med.* **2020**, *22*, No. e3244.

(18) Kang, K. A.; Lee, K. H.; Chae, S.; Zhang, R.; Jung, M. S.; Kim, S. Y.; Kim, H. S.; Kim, D. H.; Hyun, J. W. Cytoprotective effect of tectorigenin, a metabolite formed by transformation of tectoridin by intestinal microflora, on oxidative stress induced by hydrogen peroxide. *Eur. J. Pharmacol.* **2005**, *519*, 16−23.

(19) Crapo, P. M.; Gilbert, T. W.; Badylak, S. F. An overview of tissue and whole organ decellularization processes. *Biomaterials* **2011**, *32*, 3233−3243.

(20) Abbasi Pashaki, P.; Rahim, F.; Habibi Roudkenar, M.; Razavi-Toosi, S. M. T.; Ebrahimi, A. MicroRNA Tough Decoy Knockdowns miR-195 and Represses Hypertrophy in Chondrocytes. *Appl. Biochem. Biotechnol.* **2020**, *191*, 1056−1071.

(21) Masters, J. R.; Stacey, G. N. Changing medium and passaging cell lines. *Nat. Protoc.* **2007**, *2* (9), 2276−2284.

(22) Tebyanian, H.; Karami, A.; Motavallian, E.; Aslani, J.; Samadikuchaksaraei, A.; Arjmand, B.; Nourani, M. R. A Comparative Study of Rat Lung Decellularization by Chemical Detergents for Lung Tissue Engineering. *Open Access Maced. J. Med. Sci.* **2017**, *5*, 859−865.

(23) Ebrahimi, A.; Kardar, G. A.; Toolabi, L.; Ghanbari, H.; Sadroddiny, E. Inducible expression of indoleamine 2, 3-dioxygenase

attenuates acute rejection of tissue-engineered lung allografts in rats. *Gene* **2016**, *576*, 412−420.
(24) Totonelli, G.; Maghsoudlou, P.; Garriboli, M.; Riegler, J.; Orlando, G.; Burns, A. J.; Sebire, N. J.; Smith, V. V.; Fishman, J. M.; Ghionzoli, M. A rat decellularized small bowel scaffold that preserves villus-crypt architecture for intestinal regeneration. *Biomaterials* **2012**, *33*, 3401−3410.
(25) Wagner, D. E.; Bonvillain, R. W.; Jensen, T.; Girard, E. D.; Bunnell, B. A.; Finck, C. M.; Hoffman, A. M.; Weiss, D. J. Can stem cells be used to generate new lungs? Ex vivo lung bioengineering with decellularized whole lung scaffolds. *Respirology* **2013**, *18*, 895−911.
(26) Xiong, X.; Yang, X.; Dai, H.; Feng, G.; Zhang, Y.; Zhou, J.; Zhou, W. Extracellular matrix derived from human urine-derived stem cells enhances the expansion, adhesion, spreading, and differentiation of human periodontal ligament stem cells. *Stem Cell Res. Ther.* **2019**, *10*, 396.
(27) White, L. J.; Taylor, A. J.; Faulk, D. M.; Keane, T. J.; Saldin, L. T.; Reing, J. E.; Swinehart, I. T.; Turner, N. J.; Ratner, B. D.; Badylak, S. F. The impact of detergents on the tissue decellularization process: A ToF-SIMS study. *Acta Biomater.* **2017**, *50*, 207−219.
(28) Chen, T. H. Tissue regeneration: From synthetic scaffolds to self-organizing morphogenesis. *Curr. Stem Cell Res. Ther.* **2014**, *9*, 432−443.
(29) Caralt, M.; Uzarski, J. S.; Iacob, S.; Obergfell, K. P.; Berg, N.; Bijonowski, B. M.; Kiefer, K. M.; Ward, H. H.; Wandinger-Ness, A.; Miller, W. M. Optimization and critical evaluation of decellularization strategies to develop renal extracellular matrix scaffolds as biological templates for organ engineering and transplantation. *Am. J. Transplant.* **2015**, *15*, 64−75.
(30) Liu, Q.; Huang, H.; Cai, H.; Xu, Y.; Li, Y.; Li, R.; Wang, P. Embryonic stem cells as a novel cell source of cell-based biosensors. *Biosens. Bioelectron.* **2007**, *22*, 810−815.
(31) Messina, C. M.; Faggio, C.; Laudicella, V. A.; Sanfilippo, M.; Trischitta, F.; Santulli, A. Effect of sodium dodecyl sulfate (SDS) on stress response in the Mediterranean mussel (*Mytilus Galloprovincialis*): Regulatory volume decrease (Rvd) and modulation of biochemical markers related to oxidative stress. *Aquat. Toxicol.* **2014**, *157*, 94−100.

https://doi.org/10.1021/acssynbio.1c00321
ACS Synth. Biol. 2021, 10, 2715−2724