

# Thinking Soils

A synthetic biology approach to material-based design computation

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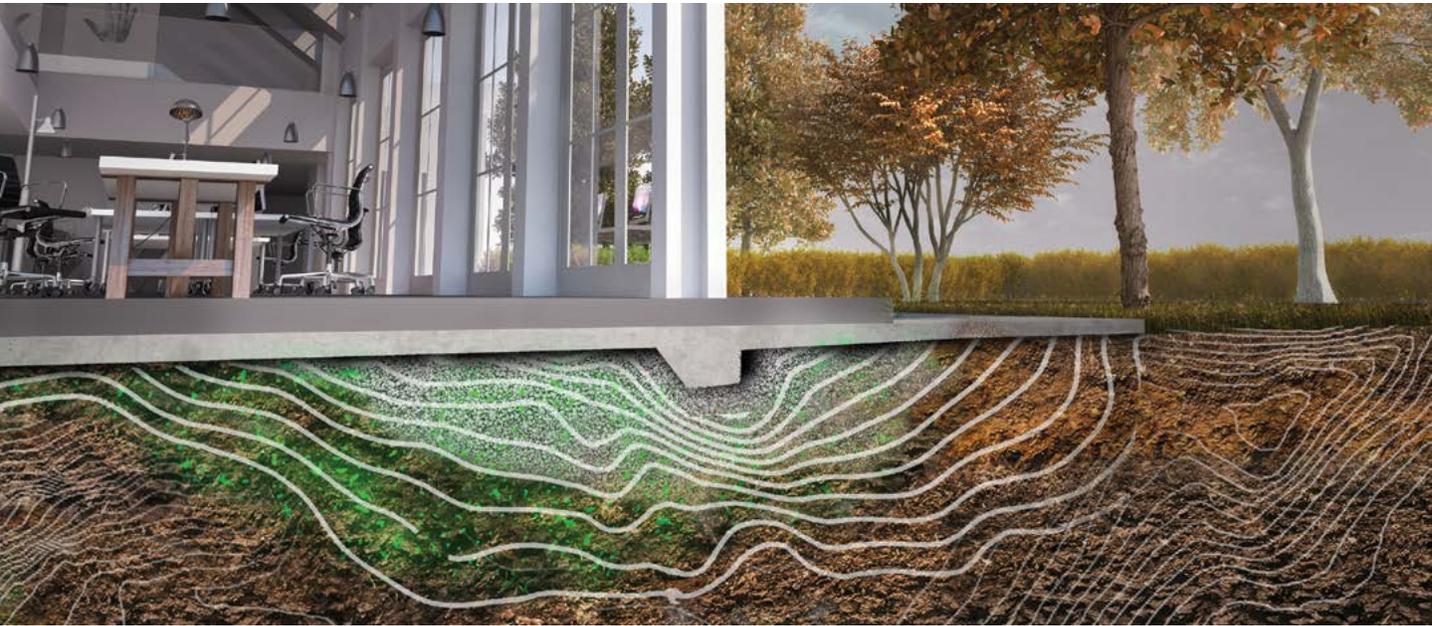
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## ABSTRACT

The paper details the computational modelling work to define a new type of responsive material system based on genetically engineered bacteria cells. We introduce the discipline of synthetic biology and show how it may be possible to program a cell to respond genetically to inputs from its environment. We propose a system of synthetic biocementing, where engineered cells, living within a soil matrix, respond to pore pressure changes in their environment when the soil is loaded by synthesising new material and strengthening the soil. We develop a prototype CAD system which maps genetic responses of individual bacteria cells to geotechnical models of stress and pore pressure. We show different gene promoter sensitivities may make substantial changes to patterns of consolidation. We conclude by indicating future research in this area which combines both *in vivo* and *in silico* work.

1 Artists impression of a bio-based self-constructing foundation.

## INTRODUCTION

In this paper we will introduce a computer model for a novel type of material-based design computation. Our model is based on bacteria which are engineered to sense pressure changes in their environment. Our project involves *in vivo* (within the living) experiments to identify genes which respond to pressure by regulating their expression, but our paper will focus on the *in silico* (in the computer) component of the project to map values of gene expression into a geophysical context. The project has the broader aim of developing a system of intelligent material synthesis, where bacteria, growing within soils or soil-like matrices, could respond to mechanical change by producing materials to improve soil resistance. Such a system could be implemented without the need for soil excavations since the bacteria could be seeded and would grow throughout the soil matrix. Advances in computational processes have enabled modelling and simulation to be conducted much earlier in the design process and computational models are used to *synthesize* design outcomes. For example, processes based on *material design computation* use models of material performance to find the optimal form for a structure based on the most efficient distribution of materials given a set of design requirements (Oxman and Rosenberg 2007). We propose a new design process based on systems in which both modelling and manufacture are combined into an engineered biological system. In this case, the designer does not define the material form, but rather designs a system where the material is synthesised in direct response to an environmental context.

While we will highlight the *in vivo* factors in designing such a system, this paper focuses on the *in silico* part of our process through the development of a modelling and editing tool which bridges the design of individual DNA molecules and the large-scale physical modelling of volumes of soil. The project is conducted using knowledge from synthetic biology, which will be introduced here. By describing the demonstrator application and the results obtained from it, we will show how it may be possible to design material forms through genetic manipulation of living cells, thus extending the work of material-based design computation into a new sort of *in vivo* material computation. Our starting point is to consider a design scenario where a heavy load is placed on a weak soil. We imagine an engineered bacteria, living in the soil, that is capable of sensing pore pressure changes within the soil and responding by producing material to solidify the soil matrix. Such a system would create a synthetic foundation beneath the structure. However, designing such a system is not straightforward, and as we shall show here, the computational modelling illustrates some unforeseen emergent properties of such a system.

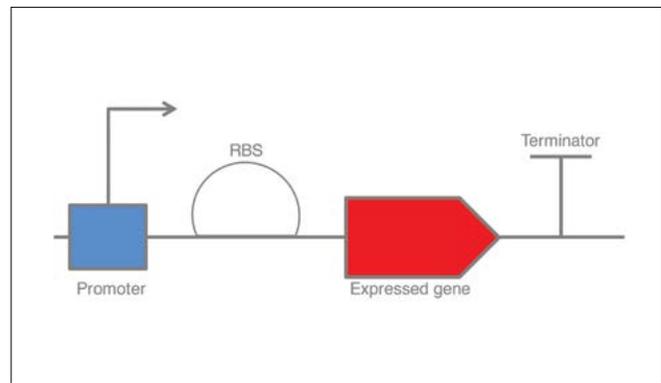
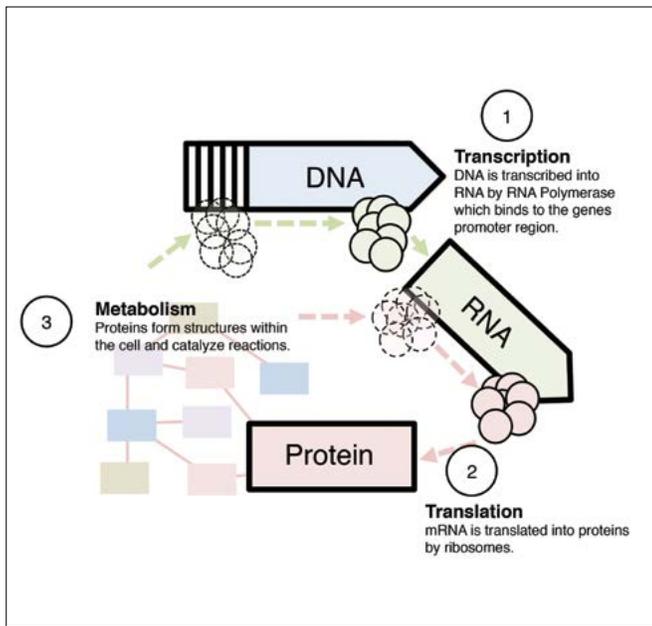
## BACKGROUND

### Synthetic biology

Before describing the application, it is worth briefly introducing the aims of synthetic biology and some basic biological understanding. The aim of synthetic biology, according to the Royal Society of Engineers is to “design and engineer biologically based parts, novel devices and systems as well as redesigning existing, natural biological systems” (Voigt 2012).

All living cells contain chromosomes which are long chains of deoxyribonucleic acid (DNA) molecules made of smaller molecules known as nucleotides of adenine, thymine, guanine and cytosine (abbreviated as A, T, G and C). Defined sequences of nucleotides can be grouped into genes. Genes are the molecular units of heredity and are transcribed by an enzyme called RNA polymerase into messenger ribonucleic acid (mRNA). The mRNA is then read by molecular machines known as ribosomes and translated into proteins (long chains of amino acids) according to the codes carried by the genes. Proteins, in turn, provide the structural parts of a living cell and drive the cell's metabolism (see Figure 2). Importantly for synthetic biology, the expression of genes is regulated. In many cases, genes are not simply being expressed constitutively, but are turned on or off depending on whether the cell needs their product at a given time. The regulation of gene expression, it is proposed, can be harnessed by building gene circuits. Regions of DNA can be broken into ‘parts,’ which not only encode for the proteins (genes), but also have promoter regions which, through their interaction with other molecules in the cell, can either inhibit or promote the transcription of a gene. Other parts include areas for the ribosome to bind when translation is initiated (ribosome binding sites) and terminators which indicate the transcription of a gene. These different parts can be assembled into ‘devices,’ as shown in Figure 3. Promoters can be used to control a single gene or a gene cluster and different promoters are sensitive to different chemical or physical conditions within the cell. By recombining promoters that are sensitive to a specific condition with genes that express a protein or proteins that we want to produce, we can create new genetic circuits.

An important factor in this project is that a gene can rarely be said to have been turned on or off, but rather their expression is up- or downregulated. Expression profiles can be mapped for specific genes showing the genetic response (in terms of the amount of a gene product produced) against a given input. In our case, we are interested in potentially pressure-sensitive genes; i.e. the expression of the genes that will be regulated by changing pressures in their environment.



- Schematic illustration of the 'central dogma' in biology, the processes of translation and transcription from DNA to proteins.
- Diagram of a genetic 'device' consisting of a promoter which regulates the genes transcription into mRNA, a ribosome binding site (RBS) which recruits the ribosome which initiates the process of translation and a gene which encodes for a protein. The diagram is produced using SBOL (Synthetic Biology Open Language) visual.

### Design basis in biology

In our project, we hypothesised the existence of a pressure-sensitive gene or genes in the bacteria *Escherichia coli* (*E. coli*). Pressure sensing systems have been discovered in bacteria, including *E.coli* (Welch et al. 1993). We performed transcriptome shotgun sequencing (RNA-seq) to annotate and quantify all mRNAs presented in *E. coli* cells under higher pressure and compare them with the control, that is, *E. coli* cells under normal atmospheric pressure. Using this technique, we have identified 122 genes which are upregulated at pressures of 10atm, and 16 which are downregulated (defined by genes which have a more than 3 fold change in their expression when compared to normal atmospheric pressure).

In our system, we want to locate one or more pressure-sensitive gene promoters. Using this pressure-sensitive promoter, we can then build a gene circuit which controls the synthesis of extra cellular materials. In this way, soils would be cemented where pressures were highest.

### Dynamic behaviour of saturated soils

The proposed system requires an understanding of the behaviour of granular materials (like soils) under load and the relationship between this behaviour and the environment of the bacteria cell itself. In this paper, we are interested in saturated soils which would carry bacteria and the nutrients that enable them to survive.

In saturated soils, the pores between the grains are filled with water. In unloaded sediments, the pressures in the pore water are hydrostatic over the depth of the soil layer, but as saturated

sediments are loaded, pore pressures can locally increase, which exerts a counteracting force in the material. Because water is incompressible, a load will cause a localised increase in pore pressure before the water is allowed to flow away from the pores and pressure is equalized throughout the system. This implies that fluid flow through the material must be slow or stagnant, thus allowing pockets of saturated sediment to build different pressures (Bredehoeft and Hanshaw 1968).

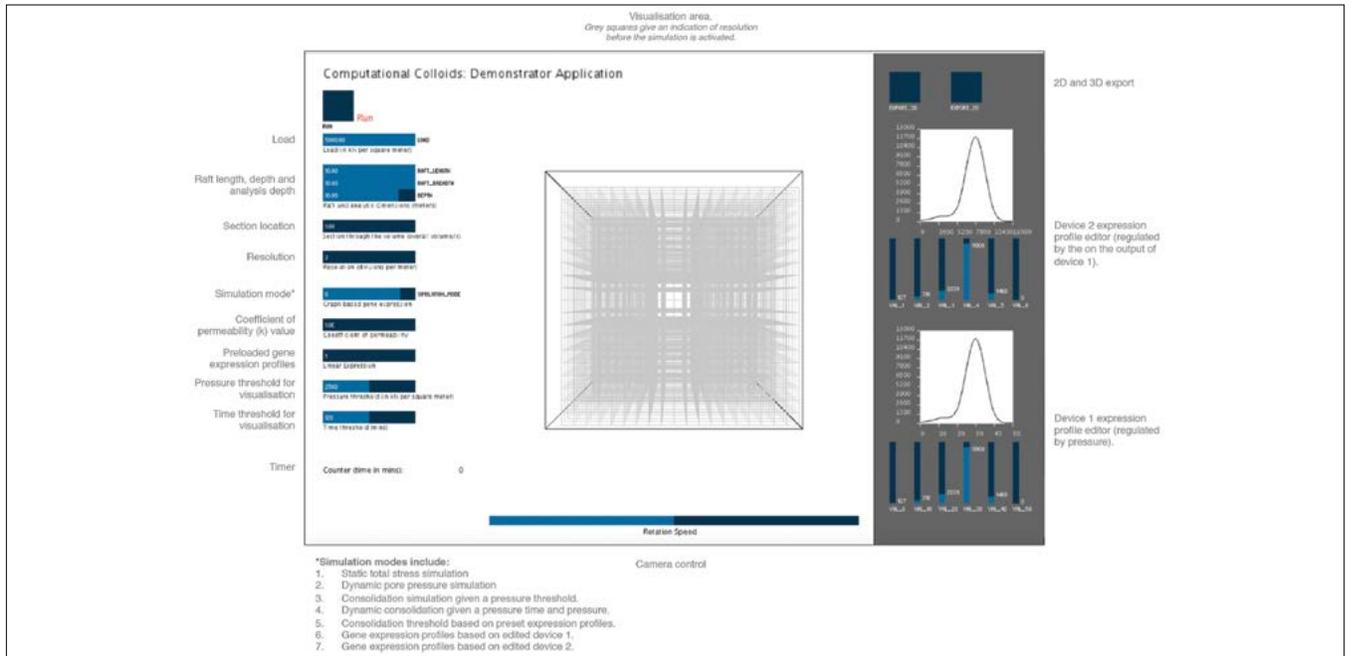
Pore pressure is defined as “the pressure of the fluid in the voids (pores) between the individual grains comprising a soil’s matrix” (Strout and Tjelta 2005). Pore pressure is important because it is a factor in the fundamental geotechnical concept of effective stress, where actual stress is calculated as total stress minus pore pressure. Pore pressure is therefore a function of:

- The permeability of the soil.
- The amount of excess pressure
- The length of the drainage path through the soil.

The restructuring process of a saturated soil under loading is known as consolidation.

### DEVELOPING THE *IN SILICO* MODEL

An *in silico* model was developed to examine the vertical stress and pore pressure encountered by soils under load and map soil mechanics over time. Specifically, the model looks at the initial vertical stress developed in a soil under load and the transition from pore pressure to stress within the soil skeleton. This is then used to predict how bacteria in different locations within the soil



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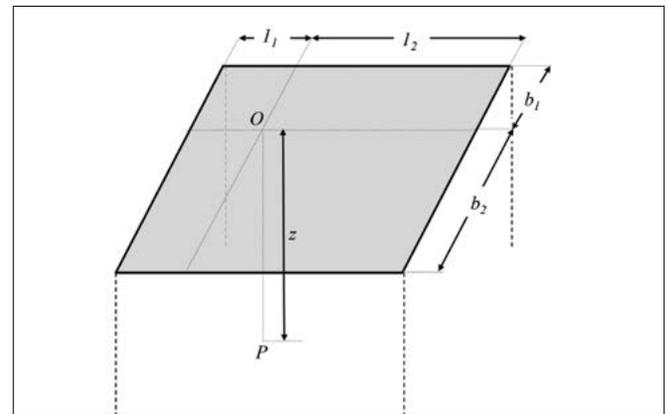
might respond in terms of the regulation of a pressure-sensitive gene promoter. A graphical user interface was built to allow the model to be explored interactively and for the results to be visualized dynamically (see Figure 4).

### Modeling vertical stress

The vertical stress model is based on the Boussinesq equation (Boussinesq 1871) (initially adapted for point loads and based on the simulation of wave propagation), which assumes a saturated, homogeneous, isotropic, elastic material. Whilst this is not strictly true for soils, it has proven to be sufficiently accurate for most geotechnical contexts, although its limitation will be discussed later. The Boussinesq equation is then coupled with an equation which calculates the influence of a load distributed over a rectangular foundation. While influence of a load on a rectangular foundation will also occur outside the limits of the foundation plate itself, this mode only simulates the distribution of stress directly underneath a foundation (where the most significant stress would be expected). The process, as defined by (Tomlinson 2001), is in two stages. For each point of interest beneath the foundation, two values are required:

- $m = b/z$
- $n = l/z$

where  $b$  is the breadth of the foundation,  $l$  is the length of the foundation and  $z$  is the depth at the point of measurement. In the case of a rectangular foundation, for each point of interest the foundation is split into four rectangles by lines intersecting at point  $O$  as seen in Figure 5.

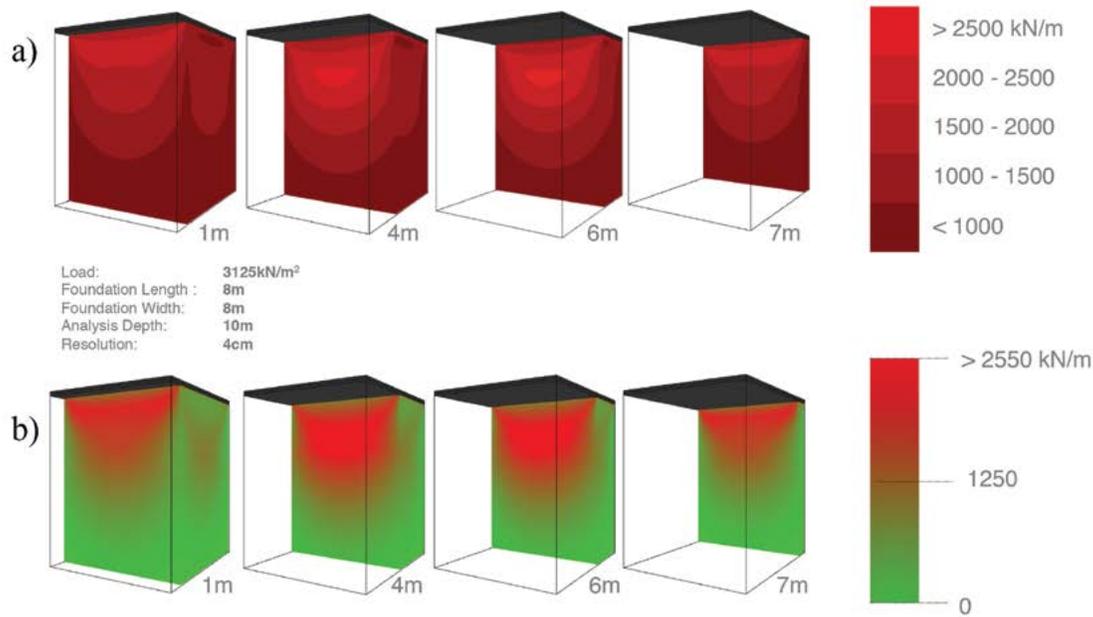


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- 4 Annotated screenshot of simulation software developed for the geotechnical simulations.
- 5 Diagram to illustrate the method of calculation of stress underneath a rectangular load.

For each rectangle, an influence value is calculated using values for length and breadth ( $l_1$  or  $l_2$  and  $b_1$  or  $b_2$ ) and the depth of the point of interest ( $z$ ). Using values obtained for  $m$  and  $n$ , in each case the following equations are used to calculate the influence ( $I$ ) of each rectangle using equations taken from (Newmark 1935):

$$I = \frac{1}{4\pi} \left( \frac{2mn\sqrt{m^2+n^2+1}}{m^2+n^2+m^2n^2+1} \cdot \frac{m^2+n^2+2}{m^2+n^2+1} + \tan^{-1} \frac{2mn\sqrt{m^2+n^2+1}}{m^2+n^2+1-m^2n^2} \right) \quad (1)$$



6 Simulations used to show the magnitude of vertical stresses under an 8m x8m raft foundation with a total stress of 200,000 kN. The images show two different visualisations based on (a) contours and (b) a continuous gradient.

When  $m^2 + n^2 + 1$  is larger than  $m^2n^2$  the value for  $I$  ends up as a negative so this equation is used instead:

$$I = \frac{1}{4\pi} \left[ \frac{2mn\sqrt{m^2+n^2+1}}{m^2+n^2+m^2n^2+1} \cdot \frac{m^2+n^2+2}{m^2+n^2+1} + \tan^{-1} \left( \pi - \frac{2mn\sqrt{m^2+n^2+1}}{m^2+n^2+1-m^2n^2} \right) \right] \quad (2)$$

The influence is calculated for each of the four rectangles and the stress for point P ( $\sigma$ ) can then be calculated using the equation:

$$\sigma_z = q(I_1 + I_2 + I_3 + I_4) \quad (3)$$

where  $q$  is the load per square meter over the foundation.

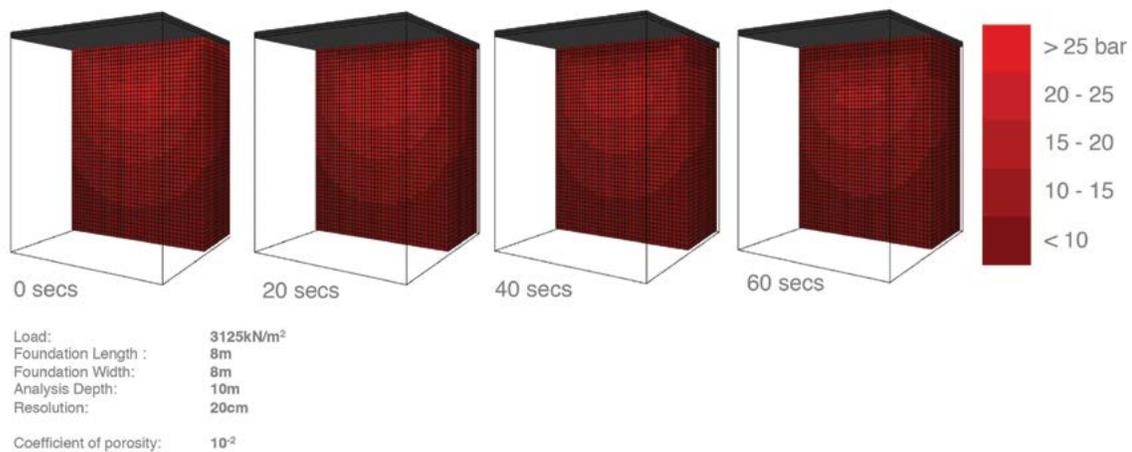
To build up a profile of stresses underneath the foundation, these equations were implemented in software code to systematically iterate over a matrix of points beneath the foundation and return a stress value for each point. The code was implemented in *Processing* (v. 2.2.1), a programming environment developed at MIT and used predominantly for visualisation. The *Processing* language is based on Java and uses the same syntax and structure, but has the advantages of built-in libraries for graphics processing and presentation and a simplified programming

environment. This makes it a useful language for generating quick software 'sketches' and quickly developing complex visualisations which may require many more lines of code or the extensive use of external libraries in other programming languages. The code implements a type of finite element analysis, where the area underneath the loaded foundation is split into voxels. The values for each point in the voxel matrix was calculated using the equations described above for each position in the grid. The output of the Influence equation was validated using lookup table from Tomlinson (2001).

Figure 6 shows the typical output from a rendering that maps vertical stresses through a volume of soil 8x8x10 loaded with 200,000 kN. In this case, points at 3 cm intervals are analysed through the soil volume. The resulting visualisations show two types of rendering, where each voxel is given a colour value where either red indicates higher stress and green indicates low stress or isobars depict blocks of red shades indicating different stress ranges. The visualisations are also shown with sections taken at different points through the soil volume.

#### Modeling pore pressure dissipation with time

The vertical stress through a material given a constant load does not change. However, in the context of soils, stress will be transferred from pore pressure to the soil skeleton over time. In saturated soils, it is assumed that the initial load will be taken entirely by pore pressure, but that as water flows out of the pores, the water pressure in the material will dissipate and



7 Example simulation of pore pressure dissipation over time.

the load will eventually be taken entirely by the soil skeleton. The speed and spatial distribution of this is important for this study, as the proposed system depends on the soil maintaining high pore pressures long enough for the bacteria to detect and respond to the higher pressure levels.

In civil engineering, it is rarely necessary to do detailed calculations of pore pressure changes underneath a foundation. The process of consolidation leads to the soil underneath a load being compressed, and civil engineers are required to understand the maximum bearing load for a given soil and the time and magnitude of settlement. To this end, civil engineers tend to use a one-dimensional model of consolidation which assumes that pressure is even across the underneath of a foundation and that water flows upwards to the lowest areas of pressure on top of the soil. There are two and three-dimensional models of pore pressure, but these are complex and computationally expensive to implement. In this case, a one-dimensional method based on Darcy equations of flow (Atkinson 1993) has been chosen. The equation—which assumes that the flow of water through the soil is upwards as pore pressure rises—calculates the velocity of flow ( $V$ ) based on the known coefficients of permeability for different soils ( $k$ ), the unit weight of water ( $\gamma_w$ ), the excess pore pressure ( $\delta\bar{u}$ ) in the location and the depth of point of interest in the soil ( $\delta z$ ).

$$V = \frac{k}{\gamma_w} \cdot \frac{\delta\bar{u}}{\delta z} \quad (4)$$

The velocity is used to calculate the rate at which the pore pressure will subside. The effect shown by running simulations over time is that the areas closest to the top of the soil surface (the underside of the foundation raft) tend to dissipate quickly, while deeper points tend to show slower flow velocities.

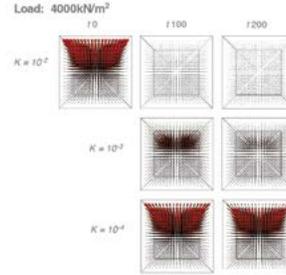
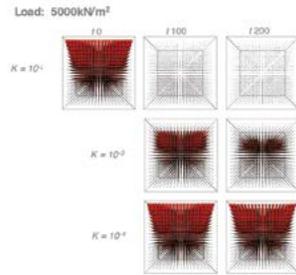
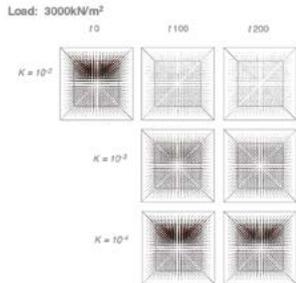
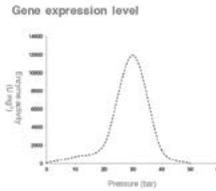
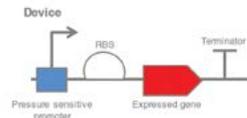
#### Modeling integration with gene data

The different models were implemented in the same code and a graphical user interface was developed that allowed the different simulation modes to be accessed and run and for the variables to be edited using sliders. The interface allowed for different simulations to be run quickly and without accessing the main code and for the results to be output as images or as 3D models for further analysis. In addition, the dynamic pressure values were referenced against hypothetical gene promoter profiles that simulate gene expression as measured in terms of enzyme activity (measured as U mg<sup>-1</sup>). The units in this instance, however, are less important than the relative expression, which are indicated as promoter profiles on editable graphs on the left hand side of the interface. These promoter profiles could be adjusted and the test rerun. Two scenarios were modelled in this context. In the first scenario, a pressure-sensing promoter is placed on the same gene as a hypothetical material synthesis gene. In other words, both the sensing and material synthesis are done as part of the same genetic device in the same organisms (see Figure 6). In the second scenario, two devices are modelled—imagining two different organisms with two different genetic circuits. In cell type 1 genes, the pressure-sensing promoter is connected to a

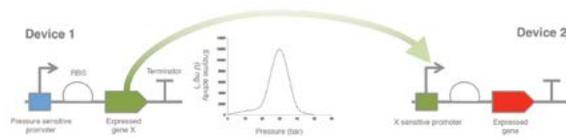
8 Annotated screenshot of simulation software developed for the geotechnical simulations.

9 Visualisations to show gene expression levels in a three dimensional volume of soil given the interaction of two gene circuits where the product of pressure regulated Device 1 regulates the expression of Device 2. The simulations are based on different response profiles for Device 2. The size of the cubes in each matrix indicates the relative level of expression and the colors indicate levels of stress in the soil.

Foundation Length : 10m  
 Foundation Width: 10m  
 Analysis Depth: 10m  
 Resolution: 25cm

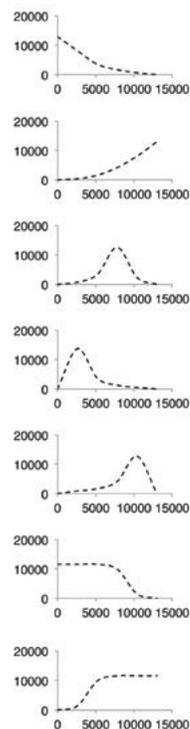
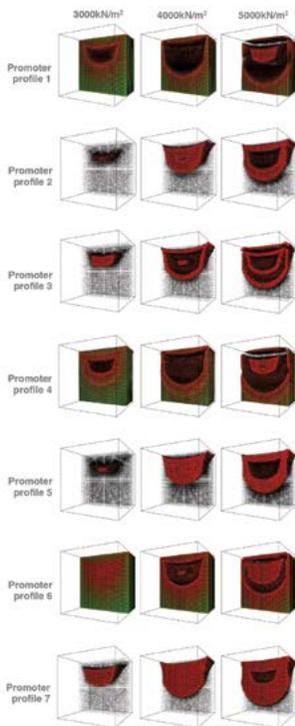


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3D Expression profiles different loads and promoter profiles from Device 2

Gene Expression Profile for variations of Device 2 (x axis = input value, y axis = output value)



gene which codes for a signalling molecule. A signalling molecule is a substance that can be exported outside the cell and detected by other cells. The second cell contains a device with a promoter that is sensitive to the signalling molecule expressed by Device 1. The promoter belonging to Device 2 is used to control the material synthesis gene. Both scenarios were run with different loads, different soil permeability and scenario two was run for different promoter profiles for Device 2. In this case, the cube size at each point in the matrix is proportional to the level of gene expression from the product of Device 2. A section is also cut through the soil volume to show the internal profile of the soil matrix (see Figure 8). The computational model was run for various loading scenarios on a 10 m x 10 m raft foundation with an analysis depth of 10 meters.

## RESULTS

### Spatial distribution of high pore pressure

The stress models shown in Figure 6 illustrate the uneven nature of stress transfer through the soil with comparatively low pressure directly underneath the foundation and a zone of high pressure some distance below. However, these results need to be treated with caution. The method for calculating vertical stress assumes that the material being analysed is an elastic solid. Whilst this model (based on the wave theory of Brosses) is often used in soil mechanics for modelling basic consolidation, more sophisticated models exist. The stress characteristics are likely to vary for different soil types. It is anticipated that further research will show that it is unlikely that stress distributions in many types of soil would appear as they do in Figure 6, and that the highest

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stress should be felt directly below the foundation plate (see for example (Powrie 2014)).

While this vertical stress model needs more development, the model of pore pressure changes with time, which shows that an area of high pressure persisting deep into the soil volume (see figure 7) is more reasonable as the water deeper into the volume of soil has further to travel. This is, however, a theoretical case and should also be investigated in more realistic geotechnical contexts where, for example, there is the presence of bedrock or soils with variable porosities. Any boundary between a porous material and a non- or less-porous material would usually be considered as a means of water escape and many standard geotechnical models assume water escaping vertically and horizontally. To cope with these more complex situations, the model would have to be developed to a full model for 3 dimensional consolidation.

### Gene expression in the soil volume

In the absence of detailed data on pressure sensitivity at the magnitudes of pore pressure highlighted in this study, this aspect of the research is largely hypothetical. However, the application of hypothetical data has proven to be valuable and has highlighted some design challenges and potentials of our proposed system. Using hypothetical data has also enabled us to build a framework on which real data can be interpreted.

The model has been developed while assuming a pressure-sensitive promoter with a similar profile but which is an order of magnitude more sensitive, thus showing activity between 1MPa and 5MPa. Running the simulation with different loads shows that, for loads that exceed the peak promoter sensitivity, zones of low gene activity exist within the highest pressure areas of the soil volume. The situation becomes more complicated if the model simulates a device containing a pressure-sensitive promoter that signals to another device with its own sensitivity to the signalling product of Device 1 as shown in Figure 9. The simulations show gene expression levels 20 minutes after loading for a range of different profiles for Device 2, including profiles with peaks of activity and situations where the promoter is positively and negatively regulated by the product of the first genetic device. The results show a wide range of expression patterns with some of the most interesting results demonstrating an interference-like effect resulting in two bands of high or low expression.

These results have potential implications for design. In a synthetic consolidation system, the appearance of low areas of gene expression within high-pressure areas of the soil matrix could cause problems of instability and their effects may need to be mitigated. For example, using soils with comparatively higher

porosity would mean that areas of highest pressure would diminish quickly, thus bringing the pressurized volume of soil within the range of the pressure-sensitive promoter. More than one type of promoter sensitive to different pressure ranges and sensitivities to the signal of Device 1 might also be tried.

These results might, however, also offer design potential. By combining promoters with different sensitivities through a signalling system, the designer might have tight controls over the morphology of the consolidated soils. While the immediate applications for such a process are not clear, it might be possible to imagine a process whereby underground structures are created using consolidated soils, where the more friable material is excavated, thereby leaving caverns and holes in the ground. When combined with different patterns of loading, complex structures could be created in this way.

To explore this further, a better understanding of how synthetic consolidation would occur would need to be developed with reference to threshold values for meaningful levels of consolidation—i.e. when the resulting material can be considered substantially more load-bearing than the material around it. The current model also doesn't take into account the fact that the process of consolidation itself will change the dynamics of pore pressure and prevent the flow of water from leaving the pores. This may mean that high pressure areas will persist in the pores permanently, and will in turn change the dynamics of consolidation.

## CONCLUSION

### Limitations of this study

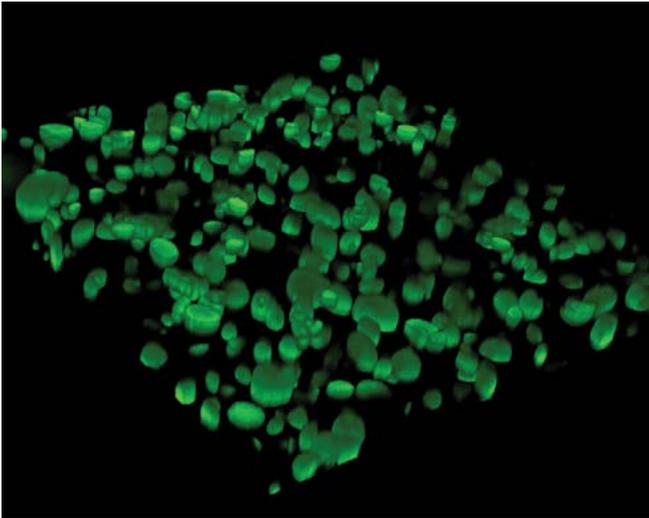
There are limitations to this analysis. Currently these broad values for pore pressure are obtained from standard formulas based on soils which are saturated with water. A bacteria mix is likely to be much thicker than water and the liquid flow through the soils will be slower, thus maintaining higher pressures in the soil for each condition longer.

The narrow range of loading values should also be expanded. The current values of loading are orders of magnitude higher than the bearing capacity of most soils because much of the research conducted so far on pressure sensing in bacteria is concerned with responses of high pressures. Based on our *in vivo* work, we believe that there are promoters which are sensitive to much smaller pressure changes and the model needs to be developed to reflect this.

The model also does not take into account many of the microbiological parameters which would need to be considered. It is likely, for example, that the bacteria would not distribute themselves



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10 Compression test being performed on a column of agarose gel.

11 Confocal (3D) microscope images of bacteria illuminated with GFP.

evenly through the soil; rather, they would migrate to the surface where oxygen levels are highest. The models would need to take these factors into consideration.

### Implications and future work

While there are limitations in the model described here, the editing interface hints at a new type of CAD application which connects the behavior of cells, defined at the molecular level (at scales of nanometers) with consolidation patterns measured

in meters. The sensitivity of the promoter parts, shown here as editable graphs, is hypothetical. But as our in vivo work develops, we should be able to plot promoter sensitivity for a selection of our known pressure sensitive genes, and in the future, we may be able to edit promoters to give us the desired sensitivity profile—sculpting material responses to force by altering sequences of DNA and through the interaction of many different genetic devices and engineered organisms. This work also hints at the possibility of extending our application domain. In addition to formally characterizing the profiles of pressure-sensing promoters, therefore, we have begun to extend the experimental work to growing the bacteria in hydrogels. Hydrogels act for us as a surrogate soil with some similarities with weak clay-type soils. The bacteria can grow through the 3D matrix of the hydrogel material and we can visualize their growth and activity in three dimensions. Our aim will be to build an engineered bacterium using our newly discovered pressure-sensing promoter. The pressure-sensing promoter will be connected to a signaling molecule (such as green fluorescent protein [GFP]) so that we can visualize the promoter activity in a volume of hydrogel. Figures 10 and 11 show us beginning to test the mechanical properties of hydrogels and visualizing bacteria which glow as they express GFP production. Such a demonstrator would indicate the first steps towards a new type of responsive material which might have much broader architectural applications.

### ACKNOWLEDGEMENTS

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## IMAGE CREDITS

Figure 1: Carolina Ramirez-Figueroa, Luis Hernan, Martyn Dade-Robertson, 2016

Figures 2-9: Martyn Dade-Robertson, 2016

Figure 10: Javier Rodriguez Corral, 2016

Figure 11: Javier Rodriguez Corral, Meng Zhang, 2016).

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