Growing Semi-Living Sculptures: The Tissue Culture & Art Project

Oron Catts and Ionat Zurr

ABSTRACT

Tissue engineering promises to replace and repair body organs but has largely been overlooked for artistic purposes. In the last 6 years, the authors have grown tissue sculptures, "semi-living objects," by culturing cells on artificial scaffolds. The goal of this work is to culture and sustain for long periods tissue constructs of varying geometrical complexity and size, and by that process to create a new artistic palette to focus attention on and challenge perceptions regarding the utilization of new biological knowledge.

The core of the Tissue Culture & Art (TC&A) Project is the artistic manipulation of living materials using the tools of modern biological research in order to sharpen questions arising from the utilization of these new sets of tools. Prevailing Western views of a nature-culture dualism can be challenged by putting into practice newly acquired knowledge in biology. Synthesizing biological processes and materials can help us understand that humans and their extended phenotype (the external manifestation of our genes expressed through our culture and technologies) are an integral part of what we call nature, and we therefore have to develop a new set of references in order to understand the implications of our deeds.

Many artists are directing their attention to the consequences of deciphering the genetic code. Our work deals with another level of the biological system—that of the cell and communities of cells: tissue. The interaction with nature that we offer is the manipulation and direction of the growth and three-dimensional formation of tissue on scaffoldings that we provide. Our work is conceptually closer to cybernetics, machine/nature hybrids and the effect of technologies on complex biological systems, than to molecular biology–based art—although we often use genetically modified cells and utilize other aspects of molecular biology. We are exploring the formation of a new class of objects/beings, which we refer to as "semi-living" objects.

The Tissue Culture & Art Project (initiated by Oron Catts in 1996) was set up to explore questions arising from the use of living tissues to create/grow semi-living objects/sculptures and to research the technologies involved in such a task.

WHAT IS TISSUE ENGINEERING?

Tissue engineering deals with constructing artificial support systems (with the use of bio-materials) to direct and control the growth of tissue in a desired shape in order to replace or support the function of defective or injured body parts. It is a multi-disciplinary field that involves biologists, chemists, engineers, medical practitioners and now, artists. "In essence, new and functional living tissue is fabricated using living cells, which are usually associated in one way or another with a matrix or scaffolding to guide tissue development" [1].

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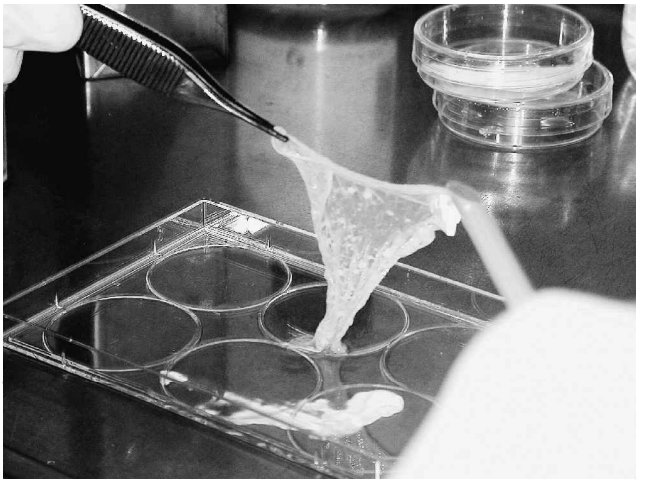
The use of embryonic and progenitor (adult) stem cells increases the potential for tissue engineering to fabricate complex organs outside of the body. In principle, stem cells can differentiate into any kind of specialized cells by entering discrete lineage pathways (which involves the action of specific growth factors and/or cytokines and other internal and external factors). This means that stem cells can be seeded on a 3D scaffold laced with different growth factors. Growth factors are proteins that bind to receptors on the cell surface, with the primary result of activating cellular proliferation and/or differentiation. Many growth factors are quite versatile, stimulating cellular division in numerous different cell types, while others are specific to a particular cell type [2] and can be used in specific areas in order to grow complex organs that consist of many cell types [3].

THE TC&A HYPOTHESIS

It is now feasible to use tissue-engineering techniques to create custom-made replacement organs. They can also be used for the design and construction of 3D living-tissue assemblies that can be sustained alive for long periods of time *in vitro*. If

Fig. 1. A layer of bone tissue differentiated from pig's mesenchymal cells (bone marrow stem cells) after 4 months of culture. (© Oron Catts and Ionat Zurr. Photo © Ionat Zurr.)



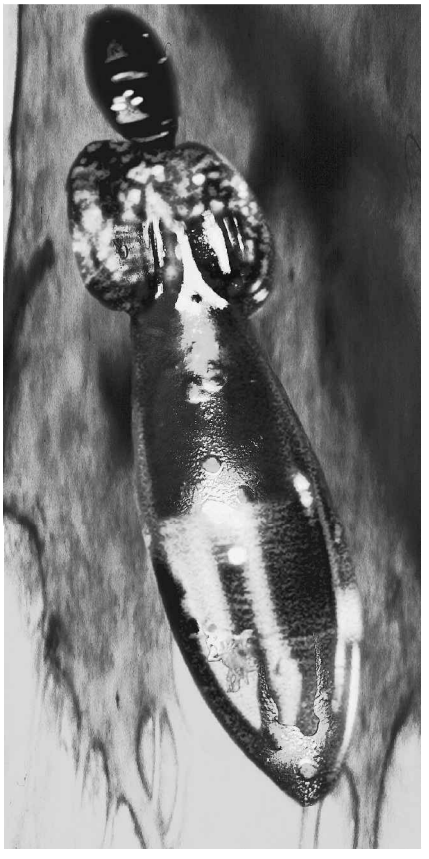


Fig. 2. *B(W)omb*, digital montage, 175 × 86 cm, 1998. (© Oron Catts and Ionat Zurr. Photo © Ionat Zurr.) The montage depicts epidermal and connective tissue grown over a glass figurine designed in a shape of a bomb.

we are able to grow something as complex as a fully functioning organ, why not change this design to suit other tasks? And if we can keep a complex organ *in vitro,* why not design semi-living objects that can be sustained alive outside of the body for the duration of their use? The TC&A Project also asks: If this is possible, should we go down this path?

Tissue engineering promises to replace and repair body organs, as well as change our relationship with the body. However, tissue engineering for artistic purposes, or any purpose other than medical, has largely been overlooked. In the last 6 years, our group has been applying tissue-engineering principles to artistic expression. We have grown tissue sculptures, semi-living objects, by culturing cells on artificial scaffolds in bioreactors. Ultimately, the goal of this work is to culture and sustain, for long periods, tissue constructs of varying geometrical complexity and size, and by that process to create a new artistic palette.

A unique set of issues and problems has arisen, because these living-cell tissue constructs will not be transplanted into the body. Some of the problems concern the practicalities of the procedure itself, while the acquisition and use of living cells for artistic purposes has focused attention on the ethical and social implications of creating semi-living objects. These entities (sculptures) blur the boundaries between what is born and what is manufactured, what is animate and what is inanimate and further challenge our perceptions and our relationships with our bodies and our constructed environment.

The ethical questions that have been raised by the project mainly concern our relationships with these semi-living objects: Are we going to care for them? Do these entities contribute to the objectification of living organisms? Their existence calls into question long-held belief systems and our perceptions of life and death. The realization that parts of the body (cells/tissues) can be sustained alive outside of the body and be made to grow into artificially designed shapes can lead either to a (false) sense of complete control over living materials (which seems to be the ideology governing the biotech industry) or to the understanding of the importance of communities and collaborative effort in the construction of complex systems (from the single cell to global society). Thus our goal is to create a vision of a future where some objects are partly artificially constructed and partly grown/born in order to generate a debate about the directions in which biotech can take us.

The initial idea for the TC&A Project came from Oron Catts's product-design studies research. Catts was looking at future interactions between biology and design. To illustrate this idea he imagined a theoretical product he called Custom Grown Organic Surface Coating (CGOSC). Ivy growing over a wall illustrates the basic principle behind CGOSC. Technology is needed to maintain it (a wall to support it, secateurs to prune it); ivy not only serves an aesthetic function, it acts as an insulator from the environment, produces oxygen and removes pollutants (such as heavy metals). However, Catts was looking at a more "sophisticated" living surface, using living tissues from complex organisms. The use of living tissue outside and independent of the organism raises many issues that go beyond strictly design principles. Catts's thesis also explored the way in which CGOSC would be perceived by a society drawing on the work of Stelarc [4] and Orlan [5], who deal (each in their own way) with the relationship between tissue (the flesh) and technology. Unlike these artists, TC&A is looking at parts of the body (tissue) that are sustained alive outside of the body and form autonomous entities.

Shortly thereafter, as part of a photomedia degree, Ionat Zurr wrote a thesis discussing tissue technologies as an art form. TC&A explores the dichotomy of nature/culture by using cells (nature) over constructed materials (culture) to create a version of a "constructed nature." Zurr was also examining the gap between the fast pace of development in science and technology and the slower pace of cultural understanding and adaptation. She saw TC&A as a form of art expression that deals with that gap by placing living and growing cells in a new context (out of the organism body and into artificial constructs).

Many people find this new context threatening to their cultural beliefs because of what seems to be an inability to categorize it. TC&A crosses borders between socially constructed dichotomies that are yet to be comprehended, let alone become part of our language. For that reason, we coined the term "semi-living objects/products/sculptures" to describe things that are both animate and inanimate, both part of an organism and outside of it.

THE PROCESS

The process of creating a tissue-engineered sculpture starts with obtaining the desired cells or tissue. There are two sources for tissue and cells: cell lines and primary tissue. Cell lines are cells that have been transformed by using viruses that ultimately cause the cells to grow indefinitely in culture. Cell lines can be ordered from cell and tissue banks around the world. Primary cells are explanted directly from a donor organism. They have a finite number of divisions in culture and given the right conditions can survive for some time. Obtaining primary tissue is usually referred to in the laboratory as harvesting. Cells and tissues are harvested from the animal either by means of biopsy from a living animal or by dissection of a freshly killed animal. Cells are then isolated by mechanical and chemical means. Once we obtain the cells or tissue, we either seed them directly onto 3D scaffolds or propagate them in tissue flasks until we have enough to use. All the primary tissues we obtain are left over from either meat production or scientific research. We consider ourselves scavengers.

We use different methods of seeding the cells over and/or into the scaffolds

depending on the kind of cells and the makeup of the scaffold. The seeding techniques can be either dynamic or static. Dynamic seeding usually involves flow or movement that assists the cells to get deep into the scaffold and attach to it. We are currently exploring (in collaboration with Adam Zaretsky) the use of vibrations produced by music and audible sound waves as a method for dynamic seeding. Static seeding entails combining the cells/tissue with the constructs in stationary conditions: we either drip the cell-media solution over the scaffold or inject the solution directly into it. When we deal with large bits of tissue we usually fix them to the scaffold in a mechanical way and let the cells migrate to the rest of the scaffold. All this work is done in sterile conditions inside a biological safety hood.

To date we have grown epithelial (skin) tissue from rabbits, rats and mice, connective tissue from mice, rats and pigs, muscle tissue from rats, sheep and goldfish, bone and cartilage tissues from pigs, rats and sheep, mesenchymal cells (bone-marrow stem cells) from pigs (see Fig. 1) and neurons from goldfish.

The biocompatible substrates that we have used to produce 3D scaffolds/constructs are: glass (see Fig. 2), hydrogels (P(HEMA) [see Fig. 3], collagen), biodegradable/bio-absorbable polymers (poly-glycolic acid [PGA], PLGA, P4HB) and surgical sutures. We are attempting to grow tissue over corals and cuttlefish endoskeletons. We have used both cell lines and primary tissue. We have experimented with different techniques to isolate the primary tissue and cells; we have used an array of nutrient media (according to the cell type) and experimented with different concentrations of serum, growth factors and antibiotics. The 3D constructs have been handcrafted, blown, cast and output from CAD files using different methods of 3D printing (CAD/CAM rapid prototyping, computer-operated milling machines, a 3D printer and stereo-lithography). The forms we have worked with range from representations of technological artifacts such as cogwheels, surgical instruments and pre-historic stone tools (see Fig. 3) to cultural artifacts (Guatemalan worry dolls [see Color Plate A No. 1 and Fig. 4] and found glass objects) and mythological animal body parts (e.g. pigs' wings).

The semi-living sculptures that have resulted from combining cells and tissue with 3D scaffolds/constructs have been grown and sustained alive in bioreactors—devices used for growing and sustaining living cells and tissues outside of their natural environment. This task is achieved by emulating the conditions in the bodies from which the cells and tissue have been derived. The most basic requirements for a bioreactor are the supply of nutrients and other biological agents, the removal of waste and the constant maintenance of homeostasis (including temperature, pH levels, dissolved gas levels), while keeping the content of the bioreactor sterile (free of microbial contamination). In their application to tissue engineering, bioreactors should also be designed to enhance the attachment of cells to the scaffolds/substrate, to support 3D formation of tissue (e.g. in micro-gravity), to control the release of biological agents (such as growth factors and inhibitors), to apply controllable stress on specific tissue types (e.g. pulsatile flow for the formation of blood vessels [6], directional stress for the alignment of muscle fibers) and to enable the operator to change settings [7].

It is of great importance for us to communicate our ideas to as broad an audience as possible. Due to the nature of the project, we are not always able to present semi-living sculptures. Therefore we use and develop biological-imaging techniques involving different types of microscopes, scientific imaging softwares, computer graphics and our interactive web site. We are also developing bioreactors for long-term installations that will enable us to present our semi-living sculptures in varied situations and places.

Fig. 3. *Spear 1*, digital montage, 70 × 130 cm, 1999. (© Oron Catts, Ionat Zurr and Guy Ben-Ary. Photo © Ionat Zurr.) Muscle tissue from mice was grown over a hydrogel replica of a neolithic stone tool c. 10,000 years BP. The image was acquired using an inverted microscope and an X/Y motorized stage. Each square represents a frame from a microscope.

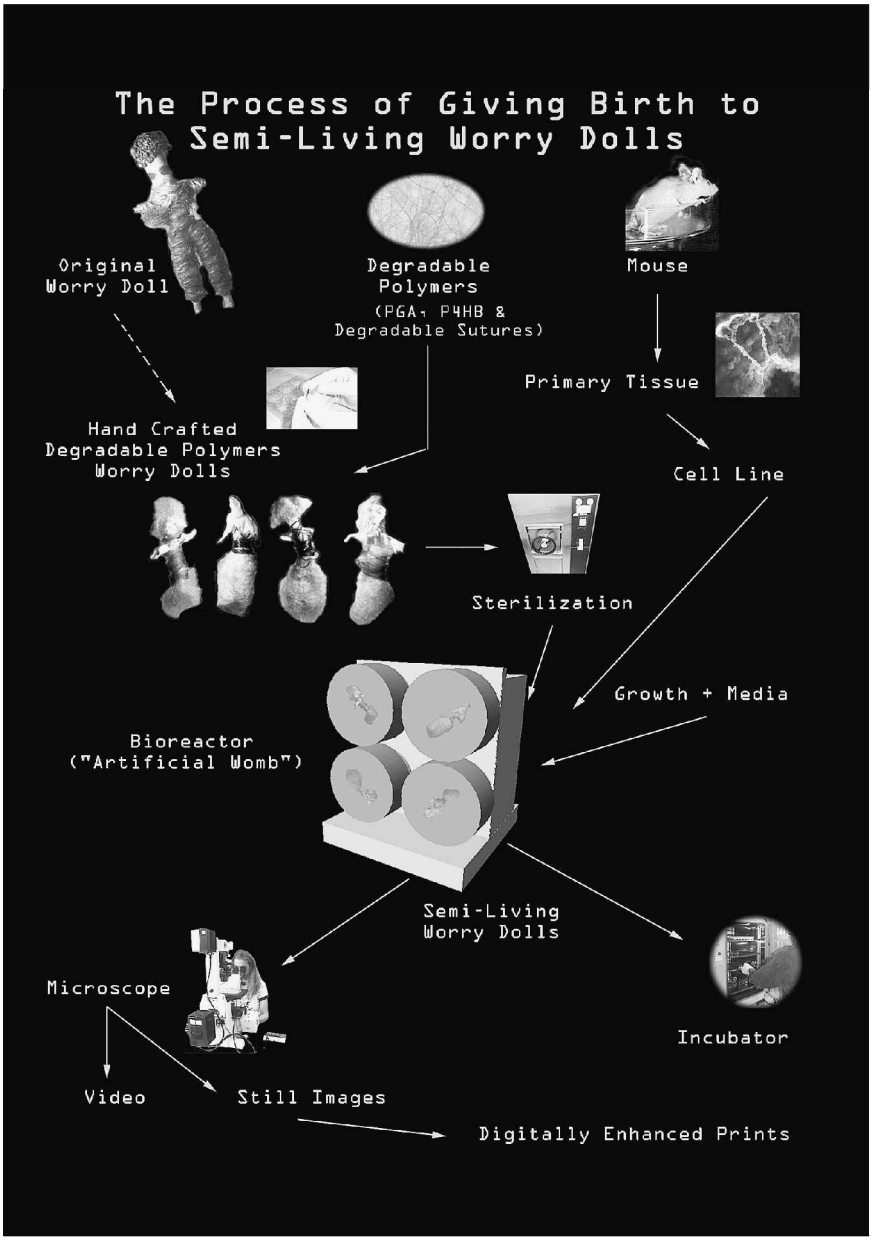


Fig. 4. ***The Process of Giving Birth to a Semi-Living Worry Doll.*** **Digital print, 58 × 84 cm, 2000. (© Oron Catts, Ionat Zurr and Guy Ben-Ary. Photo © Ionat Zurr.) A diagram illustrating the methods and materials used in the creation, growth and imaging of a semi-living worry doll.**

A Case Study

Tissue Culture and Art(ificial) Womb 2000, also known as *The Process of Giving Birth to Semi-Living Worry Dolls,* featured the first living-tissue engineered structures to be presented as art in a gallery context. The dolls were first shown at the Ars Electronica Festival 2000 in Linz, Austria.

Conceptual Background

We chose to grow modern versions of the legendary Guatemalan worry dolls in the artificial womb. A note attached to a package of worry dolls purchased from a comic shop in Boston, U.S.A., said:

> The Guatemalan Indians teach their children an old story. When you have worries you tell them to your dolls. At bedtime children are told to take one doll from the box for each worry & share their worry with that doll. Overnight, the doll will solve their worries. Remember, since there are only six dolls per box, you are only allowed six worries per day.

We decided to give birth to seven dolls, as we are not kids anymore; they might not be allowed to have more than six worries, but we surely do. The genderless doll figures represent the current stage of cultural limbo, characterized by child-like innocence and a mixture of wonder and fear of technology. We gave the dolls alphabetical names that represented our worries and anxieties. Readers are welcome to find new worries and new names and post them on our web site so that we can whisper your worries to these dolls and hope they will take those worries away.

Doll A: stands for the worry about Absolute Truths and people who think they hold them.

Doll B: represents the worry of Biotechnology and the forces that drive it (see Doll C).

Doll C: stands for Capitalism, Corporations.

Doll D: stands for Demagogy and possible Destruction.

Doll E: stands for Eugenics and the people who think that they are superior enough to practice it.

Doll F: the fear of Fear itself.

G: not a discrete doll, as Genes are present in all semi-living dolls.

Doll H: symbolizes our fear of Hope (see Color Plate A No. 1).

The process in which the natural (tissue) takes over the constructed (polymers) is not a precise one. New shapes and forms are created in each instance, depending on many variants such as the type of cells, the rhythm of polymer degradation and the environment inside the artificial womb (the bioreactor). This means that each doll transformation cannot be fully predicted and is unique to itself. Our practice is in the realm of a dialogue with nature rather than control over it.

Methods and Materials

Our worry dolls were handcrafted from biodegradable polymers, PGA mesh, P4HB, PLGA and various surgical sutures (see Fig. 4). The dolls are approximately 10 mm tall by 7 mm wide by 5 mm deep. The polymer constructs were sterilized using ethylene oxide (ETO) at 55°C for two hours; we seeded the dolls with McCoy Cell Line (derived from human, now classified as mouse endothelial cells, and used in virology studies). We statically cultured the dolls for 14 and 21 days in a 37°C/5%CO_2 incubator. We then moved them to the Synthecon RCCS ID4 (a rotating bioreactor that provides conditions of micro gravity) for the duration of the exhibition. The tissues were cultured until proliferated cells largely covered the polymer surface, growing into the porosity of the polymer scaffold.

The living worry dolls were photographed throughout the stages of growth using inverted and dissecting microscopes. In addition, tissue growth was documented using time-lapsed movies. During a gallery presentation, the semi-living worry dolls were displayed and

viewed via microscope and as they were in the Synthecon RCCS.

The Imaging System

One of the worry dolls was seeded with cells and put into a Focht Chamber System 2 (FCS2), which is a closed system, live-cell micro-observation chamber that combines constant flow of nutrient media with precise temperature control. The surface of the upper slide of the chamber (or microaqueduct) is heated, and thus the content of the chamber is kept at 37°C. The surface of the chamber contains "T" shaped grooves, which allow for laminar flow perfusion. Through these grooves and chamber ports we let nutrient media solution flow to the culture at a slow and steady rate using a peristaltic pump [8].

The chamber was kept closed (and thus sterile) and was mounted on an inverted microscope and a video camera for 5 days. The video camera was connected to a frame-grabber imaging board controlled by a time-lapse application developed in Image Pro Plus scripting language [9]. An image of the doll was grabbed every five minutes and stored in different formats (TIFF and JPEG) and sizes on a networked computer's hard drive. This computer functioned as a web server. An Active Server Pages (ASP) script scanned the web server's hard drive for new images every 20 minutes and uploaded the new images to a Web page. The images were then added to a column of images monitoring the development of the semi-living sculpture (the doll) for 5 days. The viewer could scroll the column of images, enlarge them for higher resolution and follow the growth of the semi-living sculpture.

Results

A preliminary form of tissue-engineered art has been successfully produced. Under laboratory conditions, a close-to-confluent layer of McCoy cells was achieved on the worry dolls in approximately 3 weeks. After the tissue was placed in the RCCS chamber, its 3D growth exceeded our expectations, to the degree that clumps of tissue can seen with the naked eye. All but one of the worry dolls maintained their structural integrity during exhibition.

Our semi-living sculptures have met with varied reactions. The general reaction has been one of immense curiosity surrounding the objects themselves, the production process and its implications for the future. Our art challenges many people to examine their perception of the boundary between the living and the inanimate. We have been able to engage the public with our art by providing informative and contextual explanations and by the use of humor.

Future Projections

The main barrier to achieving a large-scale tissue-engineered sculpture is the lack of an internal plumbing system (large blood vessels and capillaries) to deliver nutrients and other agents and to remove harmful waste. Diffusion alone cannot sustain thick formations of tissue. We share this problem with tissue engineers who are trying to produce complex organs for eventual transplantation. The Tissue Engineering and Organ Fabrication Laboratory at Massachusetts General Hospital, Harvard Medical School, in Boston, U.S.A. (where we were research fellows in 2000–2001) is exploring ways to overcome this problem using techniques borrowed from silicon-chip manufacturers and exploring the use of high-resolution 3D printing to create a scaffold or a mold as a template for a bio-artificial capillary system. An artificial capillary system would enable us to grow sculptures of a size that would allow the viewer more direct interaction. The development of a capillary system would also facilitate the creation of a living barrier—a skin—to protect the sculptures from harmful agents in the environment. This would enable us to take our sculptures out of containment and provide an element of tactile interaction.

Another area that we are researching is the use of muscle tissue to provide movement to the sculptures. Satellite skeletal muscle cells (myoblasts), sometimes referred to as progenitor muscle cells, are isolated, cultured and proliferated until a sufficient amount of cells can be attached to the scaffold. Then, modifications in the growth media will transform the cells into multi-nuclei myotubes (muscle fibers), which will start to twitch randomly. We have reached this stage and are now looking at using electrical pulses to harmonize these twitching muscle fibers. Again, in order to achieve visible movement, a capillary system would have to be in place to meet the high demand for energy through nutrients and oxygen.

Until we can use a capillary system, which still seems to be years away, we are interested in developing a bioreactor for long-term installations. In the context of our project, the bioreactor should be treated as an art object and not a mere tool. Conceptually a bioreactor (in conjunction with the semi-living sculptures growing inside it) represents an artificial "life-giving" and maintaining force. The development and production of a bioreactor for artistic purposes represents a different set of problems and solutions than those offered by science and industry. These considerations are mainly (but not entirely) to do with the nature and objectives of the constructs we are planning to grow in the bioreactor and the settings in which the bioreactor will operate.

Our constructs are designed to confront the viewer with a unique class of object/being that is partly grown and partly artificially constructed. The design of the bioreactor should aim to enhance this point. We envisage a bioreactor that would provide constant, undisturbed visual contact with the sculptures, both aided (via a monitoring system) and to the naked eye. We would also like to offer some degree of interactivity in which the viewer (either physically present or online) can change some of the bioreactor's settings and experience the results of her/his actions.

The aim of the installations of which the bioreactor is part is to explore artistic outcomes, not scientific or commercial ones. Therefore, some aspects of current bioreactors (such as biocompatibility with patients, the need for accurate biological sampling, etc.) are irrelevant, while others (such as robustness, automation, ease of use, transportability and imaging/monitoring features) are essential.

Conclusion

The concept of semi-living objects is at the heart of the TC&A Project. We are interested in exposing gaps between our cultural perceptions of life and scientific knowledge and its implementation. A growing number of entities challenge our long-held notions of life. Objects that consist of parts of animals, sustained alive outside the body by artificial support, are just one example. According to Sherry Turkle [10], children are starting to perceive e-toys as alive (not in the same sense as dogs, but still alive). The creation of semi-living sculptures that lack intelligence but are perceived as living is on the other side of this continuum. Following Wilson's conception of "biophilia," i.e. our need of natural things and natural processes for our well-being [11], we are looking at a high-tech version of the natural environment.

Interaction with semi-living entities will further blur the concept of the body as one entity that stands separate from its environment. As defined by Lynn Margulis [12], a body is a community of cells and, furthermore, the biosphere is one interdependent entity. Semi-living objects are a tangible example of such a concept; we are able to view parts of our body growing as part of our environment.

We believe that the work we have done in the last 5 years demonstrates that tissue technologies can produce valid artistic expression, by enabling us to create living art—and by presenting contentious objects that represent the flux in our understanding exposed by the introduction of new biological technologies. When presenting our work we do not attempt to give a utopian vision, nor are we overly pessimistic. This ambiguity is designed to make the viewer aware of our lack of cultural understanding in dealing with new knowledge and control over nature.

Our project is about life, a dialogue with life's different levels, and the notion that we are all made out of communities of cells. It is an important part of our practice that we need to care for our semi-living sculptures.

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9. The microscope was a Nikon Eclipse TS100; the video camera was a Nikon Coolpix 950; the imaging board, a PIXCI SV4. The scripting language was developed by Media Cybernetics.

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