Open Source Biomaterials for Regenerative Medicine

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In 1997, a striking image depicting a mouse with a human ear growing on its back rapidly became a viral sensation circulating the media around the world (Figure 3-1).[5,6] It was through the media that the "earmouse" quickly became associated with fears about Dr. Frankenstein-created laboratory monstrosities and fears about the limits of human experimentation and genetic engineering. In spite of the fears, the earmouse kindled many hopes about future possibilities in medicine. Unfortunately, the image of the earmouse has often been circulated without any context, which rapidly generates many false assumptions about the origin and intent of this animal experiment. In fact, the original research group led by Dr. Charles Vacanti at the University of Massachusetts Medical School, is often left unmentioned. What is significant about the earmouse model is that although it sparked much debate about genetic modification, it was not actually a genetically modified organism. Rather, the procedure to create the earmouse consisted of several key steps.[3] First, an alginate cast of an ear from a three-year-old child was created. Then, using the cast as a mold, a synthetic biodegradable polymer (polyglycolic acid, or PGA) was shaped into an ear-like structure. At this point, cells naturally found in cartilage (chondrocytes) were isolated from slaughtered calfs and implanted into the polymer ear. Over time, the chondrocytes degraded the polymer scaffold while gradually replacing it with a new biological scaffold (cartilage) in the form of a human ear. This resulting sample of an ear-shaped cartilage was then implanted into mice in order to examine its durability and biocompatibility.

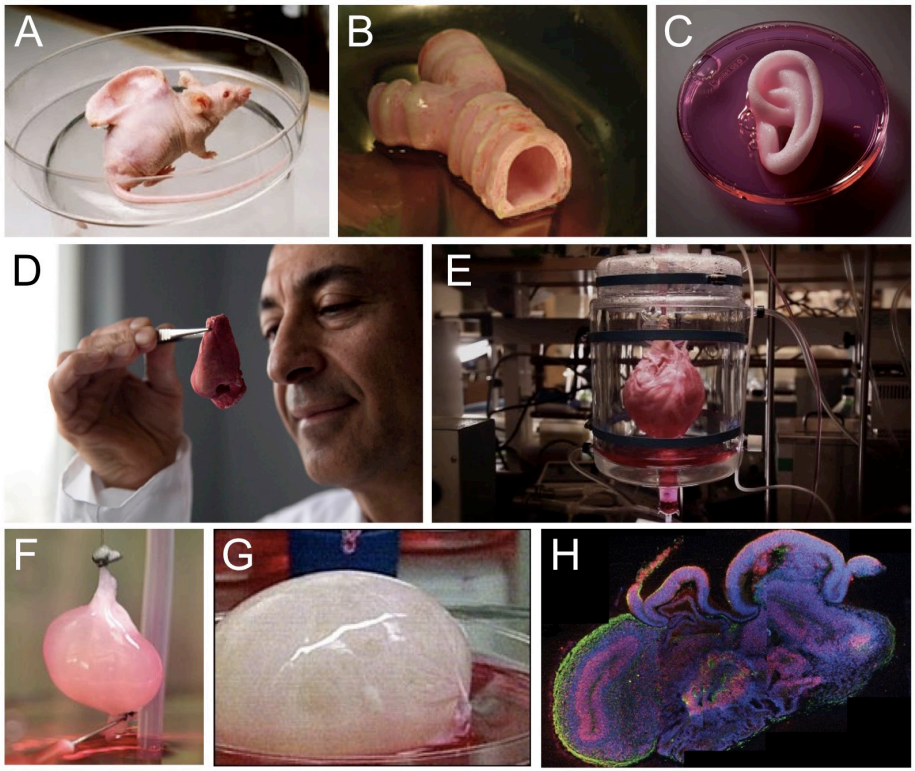

Figure 3-1. (A) The Vacanti earmouse.[1] Examples of other lab-grown organs and tissues using scaffolds of decellularized organs, including: (B) trachea[5] (C) ear[6] (D) nose,[7] (E) heart[8] (F) kidney[9] (G) bladder[10] and (H) model brain[11] Original image sources are provided in the relevant citations.[5–12]

Researchers in the fields of tissue engineering and regenerative medicine (RegenMed) often cite the promise held by the example of the earmouse. There is strong hope that interdisciplinary scientists will be able to harness advances in stem cell biology, polymer chemistry, material science, and 3D bioprinting to grow replacement body parts in laboratories to produce them at an industrial scale. Regardless of the fears and criticisms of the earmouse, the desire to regenerate defective body parts is expected to be at the center of the next-generation of medical treatments and therapies. In addition to creating molded polymer scaffolds,researchers have shown that it is also possible to remove all the cells from a donated organ, leaving behind the naturally occurring scaffold matrix. This process is known as decellularization and is a commonly employed technique to

produce "ghost organs." The ghost organs lack any of the cells from the donor and can be subsequently cultured with healthy cells derived from the patient or another source. Such regenerated organs have been shown to be functional and have even already been utilized in surgeries to repair defective tissues.[4] In the past several years, many body parts have been created using polymer-scaffold and decellularization approaches, including the trachea, ear, nose, heart, kidney, bladder, and brain (Figure 3-1).[5–11]

Commercially Available Biomaterials

The field of RegenMed is defined by a large spectrum of technical possibilities that share the same goal: the regeneration or replacement of defective organs. Approaches include the use of surgery, implants, and biomaterial scaffolds that will replace or regenerate the defective tissues or organs. This type of work is often complemented with the introduction of stem cells into the surgical sites. However, the development of novel biomaterials for RegenMed, which is the principal focus of this article, is undergoing exponential growth. Many commercial companies and universities around the world are driving this type of research. Advances in the field are being encouraged by many successful RegenMed treatments that utilize synthetic biomaterials and the significant potential for financial gain through lucrative intellectual properties and licensing opportunities. Some estimate the biomaterials market to reach a value between $30 billion and $90 billion in the next few years.[13,14]

At the moment, there is an enormous array of biomaterial scaffolding products available commercially. These products vary considerably, from both biological and synthetic polymers, and are available in different forms such as powders, gels, membranes, and pastes (Table 3-1). Aside from creating replacement organs, such biomaterials are often employed to repair damage to various tissues and structures, such as skin, gum, cartilage, and bone. One of the defining characteristics of all these products is their high cost. The high prices are often due to several factors, such as the production procedures (which require specialized devices and sterile conditions), and the costs associated with research and development, licensing, and intellectual property.

Table 3-1. Commercial biomaterials and current pricing. Price ranges take into account any options of size format.

Product (company)	Material	Price[3] (USD/cm–USD/ml)
3D Insert™-PCL (3D Biotek)	Polycaprolactone	$30—$300
3D Insert™ (3D Biotek)	Poly(lactic-co-glycolic acid, or PLGA	$60
Optimaix 3D™ (Matricel)	Collagen type I/III	$40—$220
c-graft putty™ (Citagenix)	Demineralized bone matrix and carboxymethylcellulose (human and plant)	$150—$320
c-blast putty™ (Citagenix)	Demineralized bone matrix, cancellous bone, and carboxymethylcellulose (human and plant)	$150—$320
Neoderm (Citagenix)	Decellularized dermal grafts (human)	$260—$1,440
DynaMatrix™ (Citagenix)	Extracellular matrix (porcine)	$900—$1,980
DynaMatrix™Plus (Citagenix)	Extracellular matrix (porcine)	$623—$1,490
BioXclude™ (Citagenix)	Allograft amnion and chorion tissue (human)	$1,380—$3,170
Neomem® (Citagenix)	Resorbable collagen membrane (bovine)	$720—$1,660
DynaGraft D™ (Citagenix)	Demineralized bone matrix (human)	$200—$300
DynaBlast™ (Citagenix)	Demineralized bone matrix and cancellous bone (human)	$180—$365
Raptos® (Citagenix)	Cancellous particulate, cortical particulate, corticocancellous, and demineralized irradiated bone (human)	$565—$1,540
Raptos Flex® (Citagenix)	Demineralized cortical bone graft (human)	$1,370—$1,610
Bicon Resorbable Membrane (Bicon)	Resorbable collagen products	$555—$1,300
BioMend® (Zimmerdental)	Absorbable collagen membrane (bovine)	$810—$1,740
Puros® allograft (Zimmerdental)	Cancellous particulate, cortical particulate, and corticocancellous (human)	$145—$250

Product (company)	Material	Price[3] (USD/cm–USD/ml)
Puros® allograft (Zimmerdental)	Block allograft (human)	$290—$730
IngeniOs™ synthetic particles (Zimmerdental)	Hyaluronic acid (HA) bone (synthetic) and β-TCP bioactive bone (synthetic)	$60—$200
Puros® Dermis (Zimmerdental)	Dermis allograft tissue matrix (human)	$250—$1,420
Puros® pericardium (Zimmerdental)	Pericardium membrane allograft (human)	$500—$1,050
HydroMatrix™ (Sigma Aldrich)	Peptide nanofiber three-dimensional scaffold	$35—$60
HyStem® Cell Culture Scaffold Kit (Glycosan BioSystems)	HA-based matrix (synthetic)	$320
CellCeram™ insert (Scaffdex Oy)	Hydroxyapatite and ß-tricalciumphosphate	$240
BiostructureMatrix Scaffold Sheets (Synthecon Inc.)	PGA, PLLA, or PLGA 10:90	$35—$140
BiostructureMatrix Disc Scaffolds (Synthecon Inc.)	PGA, PLLA, or PLGA 10:90	$230
DirectGen™ Bone grafting-allograft (Implant Direct)	Cancellous particulate, cortical particulate, corticalcancellous blend, and demineralized cortical particulate (human)	$70—$120
DirectGen™ Putty (Implant Direct)	Demineralized bone (human)	$140—$220
BioResorb® Macro Pore (Implant Direct)	beta tricalcium phosphate (β-TCP; synthetic)	$80—$130
DirectGen™ Derm (Implant Direct)	Decellurized dermal allograft tissue (human)	$200—$800

Although some of the materials can be relatively inexpensive (~$30/cm), this can still represents a significant cost in some regions of the world.[3] According to the World Bank, about one-third of the world's population (~2.2 billion people) live on less than $2/day.[15] Therefore, for these people, a piece of the least expensive biomaterial in Table 3-1 (smaller than a sugar cube) would require two weeks of their salary. One must also keep in mind that this is only the cost of the raw

biomaterial and does not include shipping, processing by the hospital, overhead, consumables, pharmaceuticals, or other expenses. These expenses can, in some cases, lead to a significant increase in the cost associated with next generation RegenMed and healthcare. RegenMed biomaterials have the potential to effectively treat impaired tissues/organs due to birth defects or following catastrophic tissue damage. However, given the cost of these specialized biomaterials, it is unclear if they will be available to those in regions of the world where resources and accessibility are scarce. Will they be available in war-torn areas of the world? Will companies be willing to make such biomaterials available or affordable in regions of the world facing significant economic challenges? The prohibitive cost of these materials creates a situation that limits their widespread use and availability. In addition, these materials are closed source. Intellectual property, licensing agreements, and specialized production facilities make it impossible for local communities to produce these biomaterials directly on site; thus, scaffolds cannot be produced in the hospital in which they will be used).

Accessibility to these specialized biomaterials remains an important open question. Many of the biomaterials in Table 3-1 are of animal and human origin, raising concerns about the processes employed to obtain the source components, as well as human/animal welfare controls. In 2006, this issue became headline news in the US with respect to the origin of implants derived from human samples.[16] American body-harvesting labs illegally obtained tissue samples from cadavers from hospitals all over the country, without consent from donors or relatives. Although these crimes occurred in a wealthy country, it is not hard to imagine that economic pressures could drive a similar situation in the poorest parts of the world. The scandal in the United States has provoked many concerns about what oversight is applied to a billion-dollar industry that supplies essential biomaterials to hospitals worldwide so that they can perform millions of transplants and reconstructive surgeries per year.

Open Source Biomaterials

This situation begs the question, "Is it possible to create low-cost, DIY, open source biomaterials?" Biology and evolution have produced a nearly infinite number of natural structures and architectures in the plant world. Therefore, we hypothesize that it should be possible to find a naturally occurring scaffold candidate that possesses the qualities of a commercial RegenMed biomaterial scaffold. Indeed, we recently published work in the open-access journal, *PLOS ONE*, in which we describe the simple preparation of apple tissue to create a functional biomaterial (Figure 3-2).[17] In this study, we decellularized apple tissue, which created porous 3D scaffolds. This work proved that plant cellulose (specifically

apples) could act as a scaffold for the 3D growth of human and mouse cells *in vitro*. It is already known that cellulose is a good biomaterial candidate, as it has already been used for different purposes, such as permeable dialysis membranes and as diffusion-limiting membranes within biosensors.[18] In our study, we were able to demonstrate that mammalian cells were able to proliferate inside the apple cellulose scaffold *in vitro* and reach a very high density. While these results are promising, cellulose is not perfect. Mammalian cells do not naturally grow on cellulose structures and do appear to have a decreased proliferation rate compared to commercial scaffolds. Yet, in spite of a slower growth rate, we did observe that mouse and human cells still fully invaded and infiltrated the scaffold. Importantly, our intent here is not to extol the virtues of apple-derived cellulose scaffolds. Rather, our work is intended to provoke a discussion about the use of natural, renewable, and organic resources to create scaffolds, as opposed to proprietary (bio)chemicals and processes.

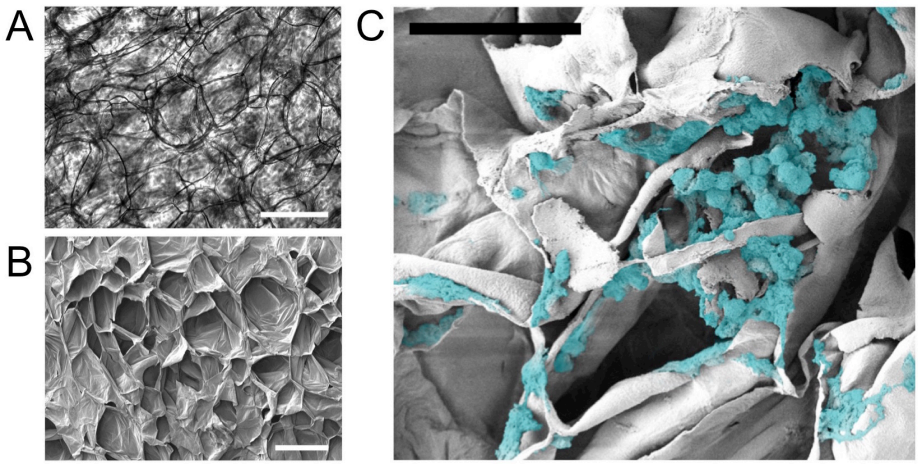

Figure 3-2. (A) Phase contrast microscopy and (B) scanning electron microscopy (SEM) of apple-derived decellularized cellulose scaffolds (scale bars = 200 µm). (C) SEM of mouse muscle cells attaching and growing on the cellulose scaffolds (scale bar = 50 µm). Figure is adapted from the Modulevsky et al. study[17].

Compared to commercial products, apples typically cost <$0.01/cm^3, representing a >10,000 fold decrease in cost compared to the average price listed in Table 3-1. Moreover, to prepare apple tissue as a biomaterial scaffold, one only requires boiled water and liquid dish soap. For those interested, we present a DIY protocol for preparing apple-derived cellulose biomaterial scaffolds in Chapter 6.

Although one can enhance the production of these materials with the use of antibiotics, biological buffers, and specialized surfactants, they are not necessary. Therefore, DIY open source biomaterials can be easily prepared with readily available plants and supplies from the grocery store. Moreover, there is no need for specialized equipment, and the biomaterials can be kept sterile with boiling water and proper training. An interesting implication of the apple-based biomaterials is that the apple tissue can be easily shaped (e.g., through CNC milling, laser cutting, or simply with wood carving tools) into almost any desired structure. Moreover, plants naturally possess many intriguing structures (e.g., leaf vasculature) that might be exploited in some way in order to elicit additional functionality.

Open source biomaterials also present an opportunity to explore physical biohacking. In contrast to the more well-known practice of biohacking through the manipulation of DNA, physical biohacking involves the re-purposing of the components of living matter (in this case, cellulose and animal cells). In essence, the objects prepared in our lab are a hybrid between plant and animal (in the spirit of the movie *Little Shop of Horrors*). The samples were created without the use of genetic engineering; instead, researchers relied on standard cell culture techniques. This work highlights the ability to create living, functional, biological composites that do not naturally exist in nature, without resorting to the manipulation of DNA. Recently, we presented this work in two bioart installations, *Re-Purposed* (TOXICITY, Plug In Gallery, Winnipeg, Ontario, Canada, 2014) and *Re-Purposed 46* (BioArt | Collabroating with Life, Karsh-Masson Gallery, Ottawa, Canada, 2015; see Figure 3-3). These works provoke a reflection on the hopes, fears, and possibilities associated with engineering biological objects that blur the lines between science fiction and reality.

Open Source Biomaterials: Too Good to Be True?

As is true for all good things, there may be potential limitations to the widespread usage of open source biomaterials. Although the academic and DIYbio communities may be able to develop these novel materials, bringing them into the clinic in a meaningful and global way represents a significant, but not insurmountable, challenge. As with all medical technologies and therapeutics, open source biomaterials will need to be tested in animals, followed by human clinical trials. Preclinical studies will require both *in vitro* and *in vivo* testing with different cell cultures and animal models. If the results from such studies are promising, human clinical trials will then need to be initiated and carried out. Such studies are remarkably expensive and time consuming and will require close collaboration and knowledge sharing between the DIYbio, academic, and clinical communities. Although open source biomaterials should be relatively cheap and easy to pro-

duce, significant financial support will be required to ensure that they are safe for human use. Companies, or national granting agencies, often support clinical biomedical research when they foresee a potential economic return on their investment. In the case of open source biomaterials that lack intellectual property protections, such clinical studies may only be possible through philanthropic investments.

Figure 3-3. Re-Purposed 46 by D. Modulevsky and A.E. Pelling. Apples, Human HeLa Cells, Cell Culture Plastic, Acrylic. Shown at BioArt | Collabroating with Life, Karsh-Masson Gallery, Ottawa, 2015. Forty-six slices of decellularized apples were impregnated with human cells derived from Henrietta Lacks and preserved (HeLa cells). Photo by Luc Lalande (@LucLalande).

Another challenge to the widespread use of open source biomaterials will be to develop standardized production protocols to ensure quality control and reproducibility. It remains unclear how one would ensure quality control over a material that could be easily and cheaply produced anywhere in the world. Moreover, there are potential dangers due to contaminants in local water sources and environments. These challenges are just some of the issues that will need to be

addressed in the future in order to pursue real-world clinical applications of open source and DIY biomaterials.

Conclusion

In this article, we have highlighted the potential benefits and challenges of using different materials, from artificial to natural origins, in the development of therapeutic strategies for RegenMed. Although there are challenges to bringing open source biomaterials into the clinical setting, they are certainly not impossible to solve. Open source biomaterials have the potential to immediately impact the costs of RegenMed healthcare, not just in terms of production but also in costs associated with licensing or buying the intellectual property. We want to challenge others to begin thinking beyond developing yet another closed source, proprietary biomaterial and begin developing simple, cheap, and open biomaterials. Our lab is entirely funded through public tax dollars, and it is a priority for us to ensure that the science being done is not only open, but will ultimately be accessible to anybody, regardless of state, geographic, or economic resources. By releasing the general protocols and intellectual property into the public domain, we hope that a larger body of researchers in the DIYbio, academic, and industrial spaces will be able to propel such work forward. We are certain that others can improve and continue to develop the protocol published alongside this article and look forward to seeing the type of work it disrupts, provokes, and inspires.

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