Three dimensional printing has been around for some time. The first commercially available 3D printer appeared in 1986. Unlike fabrication techniques typical throughout the industrial age, 3D printing used an additive process, building objects layer by layer. As the layers bind to each other, the cumulative thin layers transform into a three-dimensional object or shape. The term "3D printing" has come to include many additive manufacturing techniques which use ink jet printing technology to apply layers of material to binding agents. But unlike print-on-paper two dimensional images, the 3D printers spray a microscopically thin layer of material (frequently titanium, stainless steel, resin or plastic) on to a bed that drops imperceptibly to accommodate each ensuing layer of material. Guided by a virtual 3-D image, complex shapes or objects can be "grown" in a single piece.

Three dimensional printing or additive manufacturing has found an eager audience among businesses, companies, individuals and industries anxious to test the notion that the process will save time and money. For example, Ford relied significantly on 3-D printing to design and test its engineer's ideas in a prototype hybrid automobile in 2013. The vehicle's transmission was printed on a 3-D printer and two days later a working prototype was available. The ability to create prototypes in this was reduces expenses dramatically. Ford estimates the entire process of developing a working prototype cost only $300,000.00. Designers had a working model in hand within a few days compared with a previous wait of two to three months. Instead of waiting for tools and parts to come back from a machine shop or injection-molding house, product developers on a tight schedule get more hands-on time with their models. The flexibility of 3-D printing allows innovators to test and update more versions of their product in rapid succession, greatly refining the ultimate product and reducing the risk of having to retool once production is underway.

Observers of the 3-D printing phenomenon are effusive in their predictions. Chris Anderson, author of the book, *Makers* (the process, not the whiskey) predicts that 3-D printing, the "maker revolution" as he describes it, will rival both the industrial revolution and the PC revolution in its impact on society. "The introduction of a digital manufacturing model to the general public means...the democratization of technology", says Anderson. Once individuals are able to produce small numbers of products comparable to those made by large corporations, he envisions a future with everything from custom body parts to "instant vaccines made by a DNA printer".

The future may be now if current events are any indication. In August 2013 at the Hangzhou University in China a bio material 3-D printer was used to print a small working kidney that lived for 4 months. Earlier in 2013, a two year old child in Philadelphia successfully received a wind pipe built with her own stem cells. The technology is being used around the world to create various human tissues. In fact the director of regenerative medicine at Wake Forrest has used regenerative medical techniques to replace bladders in dozen of children over the past several years. 3-D printing involving human tissue is also known as bio printing. It has evolved simultaneously with the previously described 3-D printing or additive manufacturing process used in industrial applications. Naturally, 3-D printing that involves human tissue is extremely complex and comes with its own set of problems. But as is the case with many emerging technologies 3-d bio printing has rather humble roots.

In 2000, bioengineer Thomas Boling the self described "grandfather" of bioprinting, spied an abandoned Lexmark printer in his laboratory at Clemson University. He and his colleagues modified the ink jet printer to print fragments of DNA in order to study gene expression. Boling decided that if an ink jet could print genes perhaps the same hardware could print other bio materials. After all, the smallest human cells are 10 micrometers, roughly the dimension of a standard ink drop. Boling emptied the Lexmark ink cartridge and filled it with collagen. He then glued a thin black silicon sheet onto blank paper and fed it into the printer. He opened a word document on his pc, typed his initials, and hit print. The papers spooled out with "tb" clearly delineated on off white proteins.

By the end of 2000 Boling and his teammates had configured a different printer to produce ecoli bacterial impressions. They soon left that behind for larger mammalian cells formed from Chinese hamsters and lab rats. After printing, 90 percent of the cells remained alive which meant the product was useful and not simply a novelty. In 2003 Boling patented the process.

Cell printing consists of three principal components: the bio-printer, the bio-ink and the bio-paper.

The bio-printer employed most frequently in cell printing uses the same technology as the ink jet printers used in homes and offices around the world. Those printers contain ink reservoirs with very small nozzles that spray fine droplets of ink onto paper in precise patterns dictated by computer-fed data. Inkjet printing technology is well-developed and cheap. It can produce features that are close to the size of individual cells—perfectly sized building blocks to construct a tissue drop-by-drop.

But getting a living cell to survive the process of being shot through a bioprinter nozzle is tricky. Special ink is required and getting the ink right is a major challenge in cell printing. The simplest form of ink is like salt water, but because the cells sink to the bottom of the solution, nozzles become clogged and the cells tend to clump and cease being effective. The sinking problem has been solved by introducing microgel particles that interact with the cells, allowing them to remain perfectly suspended while at rest. No sinking here. A second problem involved the surface tension of the water molecules present in solution. The water molecules tend to stick to one another. This problem is usually solved by use of a surfactant that isn't toxic to the living cells. So to succeed, the bio-ink must be developed in such a way that it maintains its liquidity, suspends the cells so they don't clump and clog and doesn't create a toxic environment for the cells. Simple, huh? The problems have been solved to date sufficiently well to enable engineers to use simple and inexpensive ink jet type printers to print muscle and nerve tissue that not only survived but thrived. But much like high quality photography, the finished product depends on the paper as well as the ink.

The "paper" plays an important role in the survival of the living cells. As already mentioned earlier, successfully shooting a living thing through a nozzle is a dicey proposition. Recall the old joke about jumping off a building: it's not the fall that kills you it's the sudden stop at the end. The same principal holds true for the bioprinted cells. The printer actually fires the cellular ink onto pads of collagen gel. Collagen is part of the normal matrix for living cells. The gel keeps the cells hydrated and provides biological attachment points for the cells.

The process described thus far is two dimensional and is useful for studying how two systems like nerves and muscles interact. But to achieve the goal of printing functional tissues and organs, the third dimension must be added.

By printing cells and gels in a layer by layer configuration it is possible to build three-dimensional structures. The gel material is provided by a third print head. The accuracy of the equipment enables engineers to fabricate structures containing multiple cell types, biomaterials and other important biomolecules positioned in specific locations to mimic a real tissue.

Boling was not alone in the field. Other scientists, doctors and engineers were working on applications of 3-D printing for the medical community. Doctors printed bone grafts from ceramic substances, dental crowns from porcelain, hearing aids from acrylic and prosthetic limbs from polymers. These products were much simpler than the ones Boling was trying to create because those doctors and scientists were dealing with printing in three dimensions from common materials and not human tissue. Boling was able, however, to learn an important lesson from these medical applications because he determined that 3-D printing of human tissue was in fact possible.

Boling and his fellow pioneers modified their printers and added elevator like platforms that raised and lowered the printing surface. By printing one layer of cells at a time bioengineers went from drawing life on a flat canvas to building living sculptures. But even the remarkable progress shown to date leaves questions unanswered. For example doctors at Cornell can create a meniscus from cartilage identical in appearance to a human knee meniscus. Unfortunately the finished product may look like the knee cartilage but it is extremely weak. Doctors are still studying how to make the created tissue behave like real tissue.

As far as organ creation is concerned doctors are close to major breakthroughs. One leader in the field of regenerative medicine, Organova, claims that it will produce a functioning biofical liver by the end of 2014. The development of biofical replacement organs appears to be the Holy Grail of regenerative medicine.

Remember the bladders transplanted into child patients mentioned earlier? The doctor responsible for those surgeries, Dr. Anthony Atala of Wake Forrest implanted these lab grown bladders into seven patients between 1999 and 2001. As director of regenerative medicine at Wake Forrest, he developed several techniques for creating artificial scaffolds on which cells were seeded by 3-D printers. The scaffolds eventually melted away once the cells had grown together to create the bladders.

As these scaffolds have progressed over the years scientist have been able to produce more complex organs. For example bladders can be made with just two types of cells. Kidneys however require 30 different types of cells. "When you try to engineer more complex tissue there is no way you can manually place different cell types into different locations that can replicate the native tissue structures says Dr. Raymond Yoo." Doctors initially place the cells on the scaffolds by hand but this proved ineffective. So at Wake Forrest Drs. Yoo and Atala came up with a bio-printer that could not only work faster than its predecessors but it could also simultaneously spray many types of cells onto

the scaffolds. The printers were designed to create both the synthetic scaffold and tissue. This equipment is now able to produce intricate ears, noses and bones.

But as important as the scaffolding process has proven to be, some scientist believe that the scaffolds are not necessary at all. Dr. Gabor Forgacs of Organovo believes that a more effective solution is allowing the cells through the process of morphogenesis to fuse together and form structures independent of scaffolding. Dr. Forgacs and his colleagues have learned that placing cells where scientist think they should be in a finished organ is not the proper technique. Forgacs teaches that the cells should be arranged so they can interact and to start forming an organ just like cells would do in an embryo. "The cells know what to do because they have been doing it for millions of years. They learned the rules of the game during evolution." Organovo's technique involves cellular aggregates which ultimately combine to create the various structures needed to create complex systems like a human organ. Once researches can scale the size and complexity of a vascular system to provide blood and nourishment to the bio-synthesized organ doctors will be well on their way to creating viable replacement body parts.

Some of the most dramatic work involving 3-D printing of biofical organs is being done in Louisville at the Cardiovascular Innovation Institute. The efforts there are nothing short of revolutionary. Doctors and engineers expect to produce a fully functional biofical heart within ten years. They have already succeeded in making functional biofical blood vessels, a crucial step in the developmental process. The ultimate goal according to Dr. Stuart Williams is the ability to extract a patients fat cells via liposuction, isolate appropriate cells, mix them with glue and "print" a heart. All within an hour. A heart not subject to rejection because it is made from the patient's own cells.

For now though, goals and applications are not quite so ambitious. A liver by the end of 2014 is not really possible, but production of viable liver tissue in sufficient quantity for laboratory testing of drugs is. Availability of such tissue would save billions of research and development dollars for the pharmaceutical industry. Use of 3-D bioprinting to create models is another important medical application available now. Doctors can 3-D print an organ targeted for surgery and use the model to plan surgical strategy with no risk to the patient.

3-D printing of human organs has the potential to revolutionize medicine. One researcher predicts a day when every hospital will be able to provide a patient with a biofical organ from the hospital's own on-site laboratory, perfectly resolving the patient's needs without risk of rejection or waiting for a donor.

But the coming revolution will not be without controversy. Ethical issues will arise, as will concerns about stem cells. And what about patient infringement? And funding?. These topics, while compelling, exceed the scope of this paper. Stay tuned.

Respectfully Submitted this March 6, 2014.

John Atkins