4D Printing: The Dawn of “Smart” Drug Delivery Systems and Biomedical Applications

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Abstract

With the approval of first 3D printed drug “spritam” by USFDA, 3D printing is gaining acceptance in healthcare, engineering and other aspects of life. Taking 3D printing towards the next step gives birth to what is referred to as “4D printing”. The full credit behind the unveiling of 4D printing technology in front of the world goes to Massachusetts Institute of Technology (MIT), who revealed “time” in this technology as the fourth dimension. 4D printing is a renovation of 3D printing wherein special materials (referred to as smart materials) are incorporated which change their morphology post printing in response to a stimulus. Depending upon the applicability of this technology, there may be a variety of stimuli, most common among them being pH, water, heat, wind and other forms of energy. The upper hand of 4D printing over 3D printing is that 3D printed structures are generally immobile, rigid and inanimate whereas 4D printed structures are flexible, mobile and able to interact with the surrounding environment based on the stimulus. This capability of 4D printing to transform 3D structures into smart structures in response to various stimuli promises a great potential for biomedical and bioengineering applications. The potential of 4D printing in creating smart drug delivery, smart pharmacology, targeted drug delivery and better patient compliance. The paper highlights the recent advancements of 4D printing in healthcare sector and ways by which 4D printing is doing wonders in creating smart drug delivery and tailored medicine. The major constraints in the approach have also been highlighted.

Keywords: 4D printing, smart, drug delivery system, patient compliance, biomaterials, tailored medicine

1. INTRODUCTION:

Innovation is never-ending. With the beginning of the 3D printing era in medicine and drug delivery, 4D printing has become a reality. Based on the foundation of 3D printing, 4D printing includes a fourth dimension; that is, time. Products designed through 4D printing can often change their configuration over time in response to external or internal stimuli (such as heat, pH, temperature or even water). Conversion into this ‘fourth dimension’ can be a result of the smart materials (feedstock) and the predetermined design in which the products are fabricated (known as smart design).1-5

2. BACKGROUND AND EMERGENCE OF 4D PRINTING:

In 1994, 3D printing technology was introduced by Charles (Chuck) Hull of 3D Systems Corporation in a process known as stereolithography (SLA) which employs materials, designs, and 3D printers to create 3D structures by successive layering of the materials based on a predetermined design6. The official term “3D printing” (formerly referring to a powder bed adhesive jetting technology) was introduced later at MIT, which developed at MIT. In 2012, 4D printing was revealed to the world by the Massachusetts Institute of Technology (MIT) at the TED Conference where Skylar Tibbits demonstrated how a static printed object transformed over time7. This marked the start...
of the 4D printing concept, where the fourth dimension is time, highlighting that the printed structures are no longer static; rather, they are programmable and can transform independently, alter their morphology when exposed to various stimuli like light, temperature, water, and pH. 4D printing is predictable and targets shape, property, and functionality evolution which allows for self-assembly, multi-functionality, and self-repair. Since then, 4D printing has become an advanced branch of 3D printing that is gaining substantial attention from scientists and engineers of different disciplines.

3. HOW 4D PRINTING WORKS?

4D printing requires the integration of a time component into the 3D printed structures. This makes the designing process more important. Furthermore, 4D structures need to be programmed prior printing to make the time-dependent transformation mechanism of smart materials possible. Fig. 1 depicts the working of a 4D printer.

![Figure 1: Working of a 4D printer.](image)

4. SMART MATERIALS USED IN 4D PRINTING:

The smart materials used in 4D printing for drug delivery and medical device applications are appended in Table 1.

| Material | Description | Stimulus | Examples | Ref |
|----------|-------------|----------|----------|-----|
| Shape Memory Alloys (SMAs) | SMAs are usually subjected to a programming process between two transformation phases of metal alloys. These phases depend on differences in temperature or magnetic field. | Thermo-responsive | TiNi (Titanium Nickle) alloy | 11 |
| Shape Memory Ceramics (SMCs) | SMCs exhibit either superelasticity with the ability to deform to large strains and recover, or exhibit the shape memory effect where they can transform between states that have been predefined with the aid of an external stimulus. These crack at low strains and after only few cyclic strains are applied. | Thermo-responsive | Superelastic zirconia ceramics | 12,13 |
| Shape Memory Polymers (SMPs) | SMPs withstand large amounts of deformation strain over a range of temperatures, for radical and impressive transformation versatile processing and possess abilities required for a range of AM technologies. | Thermo-responsive | Inks: Methacrylate-based monomers, photoinitiator Phenylbis (2,4,6-trimethyl benzoyl) phosphine oxide (BAPO), Sudan I (0.05 wt%) and Rhodamine B (1 wt%) | 11 |
| Stimuli-responsive hydrogels | Hydrogels are used to fabricate structures in which the diffusion of water into the polymer network manipulates the product morphology by causing the structure to swell up. | Water | | 7 |
5. 4D PRINTING AND SMART DRUG DELIVERY SYSTEMS:

Owing to its novelty, the use of 4D printing in developing drug delivery systems is yet to be explored fully. As of now, there are two major mechanisms by which 4D printing can be used in fabricating smart drug delivery systems viz bio-adhesion and encapsulation.5

5.1. Bio-Adhesion:

5.1.1. Smart Microneedles with backward facing bars:

A major constraint in controlled long-term drug delivery or biosensing using microneedles is its low tissue adhesion. Inspired by natural microscopic structures with high tissue adhesion found in living creatures like microhooks of parasites, barbed stingers of honeybees and quills of porcupines, Daehoon et al. fabricated microneedles with bioinspired backward-facing curved bars for enhanced tissue adhesion using 4D printing. The microneedles with horizontal bars were fabricated using a 3D printer. Post-printing, upon exposure to UV light as stimulus, these horizontal bars containing resin undergo bending and curvature due to desolvation-induced deformation utilizing cross-linking density gradient in a photocurable polymer and change into backward facing bars that result in enhanced tissue adhesion. These backward facing barbed micro needles have demonstrated 18 times more stronger tissue adhesion than the barbless microneedles.

5.1.2. Tri-layered Mucoadhesive Drug Delivery System:

Using 4D printed materials, a smart sustained drug delivery system has been fabricated by He et al. The device is provided with an outer layer of a pH-sensitive hydrogel. As it reaches the intestinal pH of 6.5, it changes its shape in such a way that it gets anchored to the intestinal wall. This has been successfully demonstrated in the porcine small intestine (using a pH 6.5 buffer), providing a longer residence time in comparison with a standard poly(ε-caprolactone) patch (103 and 72 min respectively), minimizing the drug exposure to intestinal fluid. Using this self-folding mechanism, this tri-layered mucoadhesive drug delivery system device increases the duration of adhesion of the drug-loaded mucoadhesive layer with the intestinal wall, thus resulting in more amount of drug getting traversed across the mucosal epithelium.

5.1.3. Theragrippers:

These are temperature-sensitive multi-fingered drug delivering devices developed by Malachowski et al. After entry into the body at room temperature, this device spontaneously grips onto the tissue when exposed to temperatures beyond 32°C. The drug loading property is attributed to the porosity of the structure that can release...
the drug for about 7 days, following the release kinetics of first order. A successful demonstration by in-vitro studies has shown enhanced sustained release of doxorubicin in comparison with a control. Some researchers have further incorporated iron oxide nanoparticles into the porous hydrogel layer of these theragrippers for magnetically guiding the drug delivery system to the target site, providing targeted drug delivery. In some cases, the devices were able to terminate cells from a fibroblast cell clump, showing their potential use in validating some form of surgery themselves.

5.2. 4D Smart Encapsulation Devices:

These are self-folding structures that shapeshift to create an enclosure in which the API or even cells can be encapsulated. Fibroblasts and β- cells of the pancreas have successfully been encapsulated and displayed viability for more than a week. Yeast cells have also been encapsulated in such devices using self-folding capsules that released their contents in response to the surrounding temperature. This technology can be utilized in the concept of enteric drug delivery, wherein, the contents are released in response to change in pH.17-19

5.2.1. 4D Microrobot device containing hydrogen bilayer:

A ‘micro-robotic’ device containing a bilayer of hydrogel has been designed by Li et al using conventional lithography. The first layer consists of a pH-responsive gel that can change the morphology of the structure to trap as well as release the contents. The second layer contains iron oxide particles, able to magnetically guide the device to the target site (similar to the aforementioned theragrippers).20 The pH of tumor tissue is approximately between 4.5 to 6.0 owing to the high rate of metabolic activity of the tumor microenvironment as well as the low partial pressure of oxygen21-23. This approach is utilized by the micro-robot that is designed in such a way that it releases its contents only after encountering acidic tumor tissue. Microbeads loaded with the anti-cancer drugs Paclitaxel and Docetaxel have been encapsulated within these devices and guided magnetically to the target cells. This structure has demonstrated successful release of the drug at the intended site with 70% reduction in cell viability. However, further reduction in size is needed for the device to pass throughout the circulatory system, and become viable clinically. This shows that utilizing this technology can open new doors in targeted drug delivery with minimal side effects, especially in cardio-oncology. By focussing drug release at the tumor site, the therapeutic effects of the drug is maximized and at the same time, while the amount of drug entering the systemic circulation is limited. This lowers the chances of serious side-effects that often limit the use of many anti-cancer medicines.5 Fig. 4 depicts the encapsulating mechanism of fibroblast cells in a polymeric container.17

![Figure 4](image)

**Figure 4:** (a) Images showing the self-folding process of a polymeric container at 60°C (b) bright-field and (c) fluorescence z-plane stack image of a non-porous polymeric container containing stained fibroblast cells.

5.3. 4D Bioprinting:

Bioprinting is presently being utilized extensively in 3D printing. With the help of 3D bioprinting, the fabrication of living structures is already old school. However, by transporting this concept into 4D printing, it is possible to fabricate living structures that will have an added feature of being able to respond to their external environment. As of now, Hydrogels as smart materials have been extensively employed in 4D bioprinting mainly because of their osmotic properties and high porosity.24 As a matter of fact, these hydrogels are being utilized in scaffold-free 4D bioprinting of cells that have the ability to self-assemble, resembling normal tissue growth. This has been demonstrated in one study wherein chondrocytes were encapsulated inside a cylindrical hydrogel, enabling the tissue strands inside the hydrogel lattice to merge over time, producing viable tissues.25 This proliferation of cells inside hydrogel lattices is referred to as “Bio-ink”.26 All of this signifies that 4D printing is stepping forward to a level wherein selected bio-inks could be used to print complex organs composed of many tissues that will respond to external as well as internal stimuli, similar to that of real physiological organs. Some researchers have used these smart hydrogels to create muscular tissue.25 The tissue displayed contraction in response to an influx of calcium ions, much like normal muscular physiology. This demonstrates the potential of 4D printing in improving 3D bioprinting, creating complex structures responsive to their environment. Although a lot of work still needs to be done to achieve this aim, research done so far demonstrates the unique ability of this technology to create stimuli-responsive structures in which the cells can mature.5

5.3.1. 4D Smart Airway Splints for tracheobronchomalacia:

Children with tracheobronchomalacia, a disease that causes the windpipe to collapse during breathing, have benefited from 4-D printing technology at CS Mott Children's Hospital, part of the University of Michigan. Before the invention of these 4D printed splints, babies with severe tracheobronchomalacia had little chance of survival. The first clinical assessment came into sight in the journal “Science Translational Medicine”. This device referred to as
“airway splint”, is a porous, hollow tube that can be sewn around the affected trachea and bronchi to keep them open. Initially, the size of some of the baby bronchi is no longer than a pencil lead. The expandable shape of the airway splint allows the airways to grow with time—typically doubling in size—until they are strong enough to support themselves. A child normally comes out of this condition by the age of three. The splint is made up of biodegradable material (polycaprolactone) which then dissolves harmlessly in the body. Fig. 5 shows the pictures of three 4D printed tracheal airway splints (that grow with time) created using CT scans and 3D imaging software.

5.3.2. 4D Bioprinting makes the smallest synthetic blood vessels till date:

At this point of the technology’s development, typical 3D bioprinters have the capacity to make tubes at a diameter scale of tens to hundreds of microns. If we compare, the diameter of the smallest blood vessels in the human body is about 5 microns. To achieve the fabrication of such small structures is a significant challenge to the technology, and the ability to create complex tissues for therapeutic treatments.

4D bioprinted vessels created in the Ionov Lab essentially doubles the resolution of a typical tube by allowing the diameter to be created in the act of self-folding. This method is capable of creating vessels with an average internal diameter of 20 microns which is not yet achievable by other existing bioprinting/biofabrication approaches. Additionally, the material used (made from alginate and hyaluronic acid) is not having any negative effect on the viability of the printed cells. This has been proved by supporting the survival of mouse bone marrow for up to 7 days.

Fig. 6 depicts the self-folding progress of the 4D bioprinted vessels (image via Advanced Materials)

![Figure 5: Images of the three 4D printed trachea implants created using CT scans and 3D imaging software.](image1)

![Figure 6: Self-folding progress of the 4D bioprinted vessels (image via Advanced Materials)](image2)
Figure 7 shows the screenshot taken from the Video clip demonstrating living, beating heart cells inside a self-rolled polymer tube. (*Video via leonid lonov*)

6. SUMMARY OF 4D PRINTING IN SMART DRUG DELIVERY AND BIOMEDICAL DEVICES:

The summary of 4D printing in the fabrications of smart drug delivery systems and medical devices is appended in Table 2.

Table 2: Summary of 4D Printing in Smart drug delivery and Medical devices

| Mechanism | Stimulus | Delivery System/ Device | Ref |
|-----------|----------|-------------------------|-----|
| Bioadhesion | UV Light | Smart Microneedles with backward facing barbs | Daehoon et al |
|           | pH       | Tri-layered Mucoadhesive Drug Delivery System | He et al |
|           | Temperature | Theragrippers | Malachowski et al |
|           | Temperature | Iron Oxide Nanoparticles incorporated theragrippers | Breger et al |
| Encapsulation | pH | Microrobot device containing hydrogen bilayer | Li et al |
|           | pH or Temperature | Self-Folding Capsule | Stoychev et al |
| Bioprinting |          | Smart Airway Splints for tracheobronchomalacia | Morrison et al |
|            |          | 4D bioprinted vessels | Alina et al |
|            |          | Bio Ink | Mironov et al |
|            |          | Smart Hydrogels | Yu et al |

7. MAJOR CONSTRAINTS OF 4D PRINTING: 30

- 4D printing technology may replace ample number of complex manufacturing processes which otherwise require large manpower. Hence, there will be no need of such manpower which would result in unemployment and financial crisis.
- At present, this technology may be approved for use only in a few specialized areas such as outer space stations, military, healthcare and war zones. Bringing 4D technology into routine practice may take decades.
- 4D printing may have gone a step further in the field of bioprinting. But, there is lack of research for identifying unknown complications associated with 4D bioprinting of organs and tissues.
- Another major constraint in 4D printing is the high initial cost partially due to there being only a few companies currently developing techniques that support 4D. After commercialization, there may be a reduction in the cost of 4D printing.

8. CONCLUSION:

The applications of 4D printing in the healthcare system and drug designing are going to be enormous. The medical industry accepted to make use of 4D printing, as shape changing 4D prints are expected to be more compatible with the body than rigid metal supports. The “smart” drug delivery systems fabricated via 4D printing will have better patient compliance, low toxicity, low manufacturing cost as well as targeted delivery. To conclude, 4D printing is the future that is going to revolutionize the healthcare industry. Although there is much more to explore and research is going on all across the globe to find new ways by which 4D printing can be used as a valuable tool in healthcare sector as well as in the development of drug delivery systems.
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10. COMPETING INTERESTS:
Authors have declared that no competing interests exist.

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