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3-D Bioprinting, a Science of New Possibilities and New Cures

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Abstract—*Bioprinting is an emerging technology by way of which artificial organs, tissues or implants can be made for human welfare. This technology uses biocompatible polymers or biomaterials to recreate a lost function. Combined with tissue engineering techniques scientists have been able to mix biomaterials like hydrogels and stem cells to create new tissues and organs in several parts of the world. 3 D printed implants are also being tested as controlled delivery systems of drugs for treatment of epilepsy or wound healing. An artificial organ is no more a figment of imagination. Since the technology is still in its infancy therefore, the ethical issues pertaining to the same must also be established. As 3D bioprinting technology has flourished over time, this article investigates all the possible applications of this technique. This article also tries to compare the advancement made by Indian scientists in the field of 3 D bioprinting. Our country boasts of one of the best engineers and medical doctors of the world, therefore if the field of 3D printing has to be elaborated in our country then the experts of material science and experts of human science must act in conjunction.*

1. INTRODUCTION

The process of 3D Bioprinting begins with taking the MRI/CT scan of the organ that needs to be created. On the basis of this scan a 3D model is created on computer software, this model is called as 'Computer Aided Design'. This design guides the usage of a 'bioink' to print a 3D structure using a biomaterial impregnated with stem cells [1]. Several types of printing technologies can be utilized for 3D Bioprinting. Inkjet technology uses piezoelectric or thermal actuator method to deposit bioink onto hydrogel substrate or culture dish. Thermal actuator method of 3D printing uses an inflated bubble to force out ink from narrow nozzle onto the substrate [2]. The drawback of thermal actuator method is heat generation. In piezoelectric actuator method, piezoelectric ceramic actuator is present in each nozzle of the printer to eject bioink onto substrate. As this technique has a tendency to hurt the cell membrane and burst cells, thus thermal printing is thought to be more biocompatible. Laser Assisted Bioprinting or LAB utilizes laser as the source of energy for depositing biomaterials onto a substrate [3].

There are only four reports from India on 3 D Bioprinting. Out of these four, three are review articles and only one is an original research article [4, 5, 6, 7]. This original research article reports the usage of silk fibroin hydrogel that has prospects of allowing several stem cells so as to develop an organ. This work was carried out at Indian Institute of Technology in New Delhi, India. While we are still optimizing the hydrogels the rest of the world has already started creating organs and organoids. Prof. Anthony Atala of Wake Forest Institute already has created a 3 D printed kidney. Few of his creations have been implanted in human beings who have now been able to lead better lives [8].

2. BIOMATERIALS AND THEIR EVOLUTION

Biomaterials are identified as any material that can be introduced in a living body without causing any reaction in the body but still restoring a lost function. Long back it was established that a biological problem may have a non-biological solution leading to development of biomaterials. These included sutures and stitches made from animal intestine fiber dating back to the Neolithic times in Europe (9500-1000 B.C.), Gold used in dentistry by Aztec, Chinese and Roman civilisations (500 B.C.) [9], blue nacre shell to replace lost teeth by Mayans (600 A.D.) [10]. In 1829, Henry Levert tested the compatibility of metals in dogs to find that platinum was better tolerated than gold, silver or lead by living tissues[11]. In 1926, the invention of stainless steel offered a cost-effective metal for implants [12]. In 1930s, Polydimethylsiloxane (PDMS) synthesised was recognised to be useful for making silicone' implants[13]. In late 1940s, plastics became readily available with advantages of being easy to shape, light and inert. In 1949, poly (methyl methacrylate) (PMMA) plastic was used to create intraocular lenses to treat cataracts. In 1952, blood vessel replacement was achieved using parachute material. In 1960s a hip arthroplasty was carried out. In 1975 Society for Biomaterials was formed which marked the formal beginning of the era of Bionics and tissue engineering. In 1960s-1980s, a flurry of bioimplants like knee replacements, breast implants, stents, heart valve replacements were carried out by using teflon, hydrogels and bioglass[14]. Amongst metal implants, those made of titanium are most popular because of their low density, high strength and like iron or steel it does not corrode too quickly. Metal implants can be treated with hydroxyapatite, a natural component of human bones, to allow for the inward growth of bone cells. This helps in fixing of the implant within the bone, phenomenon called as 'osteoinduction'[15]. Metal implants include artificial knees, artificial hip implants as metals provide a cost-effective and strong options for load bearing. Biomaterials have evolved immensely since then. Currently, such stents have been developed which not only provide structural support to blood vessels but also act as depots of drug and later dissolve on their own without any need for a surgery to remove them. Invention of highly bio-functional materials and the advent of 3D printing tools have revolutionized the field of regenerative medicine. 3 D printing and regenerative medicine can give option to a person to get a replacement of his/ her osteoporotic bone with a freshly 3D printed' osteoporosis free bone implant. Such a technology will truly metamorphose the orthopaedic care.

3. BIOMATERIALS IN REGENERATIVE MEDICINE

The power of regenerative medicine has multiplied with the advent of 3-D Bioprinting. In many cases like heel cancers where the solution was amputation of lower leg has now been changed. In these cases the cancer patient gets a new titanium heel. The orthopedic applications of 3D Bioprinting are immense. This is because the bones can be replaced by titanium implants which are inert and easily juxtapose with adjacent bones. To do so first the configuration of the bone to be printed is assessed by Magnetic Resonance Imaging/ MRI to find out the anchoring points, tissues and bones it articulates. The heel of the second leg is taken as a model to print the heel. Then minute details are noted like weight, surface polish, chemical nature of the biomaterial, whether the biomaterial allows the growth of soft tissue around the heel and grow into it or not. The idea is to develop an exact bioprinted replica of the bone that has to be replaced. The weight of the bioprinted devise can be reduced by making the structure hollow or by opening the pores of the structure. Here the objective could be simply to fulfil the mechanical function of the damaged bone like the use of stainless steel hip joints [16]. The aim could also be to develop a scaffold that could allow growth of living bone cells imprinted on the scaffold [17]. The Histological studies are being made exact to match the MRI scan of brain so as to help in brain surgeries [18]. Brain is a very challenging organ to recreate because it needs proper layering and much better 3D printers with finer resolutions.

The current era of 3D printing with metals is that of standardization as the field is relatively new therefore most of the effort goes in setting up the structure, playing with thermodynamics in order to reduce deformation during production. The CT/ MRI scans are first stored in software from which the scientists then focus on the structure of the bone/ area of interest that needs to be printed. Now the area to be printed is analyzed more closely for geometry, weight and strength parameters. Then a 3D model of the same is formed. This model is further analyzed by software by making digital slices of the same, followed by adding of a support structure and sent to the 3 D printer. Once the desirable structure is printed, the support structure is removed from it and the implant is then polished manually to remove any debris. First time after production the model may need further optimization of weight so the structure may need to be remodelled and undergo 3D printing again followed by processing to prepare the final implant. Implants are given all points/ structures for attachment to muscles or tendons. Once the implant is introduced into the body of a patient, the implant takes some time to settle into the body, this period is called as the healing period [16].

Selective Laser Melting or SLM is a process by which a controlled fabrication is done using a programmable laser. In this first a thin layer of metal powder which is melted only for a fraction of second so as to let them bind to the existing structure below [19]. This process is called as lasing. The other method called as sintering is a slower process which works on the principle of atomic diffusion. This technique is expensive involving huge infrastructure and space. Thus, it is improbable that this could have a wider usage [20].

Glaucoma is a disease which is caused due to pressure and fluid flow imbalances in the eye, requiring a surgery to fix [21]. But surgeries to fix glaucoma are imperfect, thus scientists have been exploring 3D implants for the same. But these implants are complex and difficult to make. The biggest challenge of these implants is to make them safe enough so as not to harm cornea. Other challenge is to devise these implants for delivering pharmaceutical agents into the eye. For such an implant high resolution products are needed thus one needs Inkjet Technology/ PolyJet technology which prints UV curable resins.

Bioengineering is a term given to the science of generating new tissues from patients own tissue to grow a new tissue so as to replace the faulty one. 3 D printing of these tissues and organs have been explored by scientists. This technique is fairly complex and includes 3 D printing of stem cells in a scaffold material. For this, scientists have utilized 2 D printers 'bio ink' for 3 D printers which contain biofunctional polymers along with stem cells [22]. This bio ink is utilized in a 3 D printer to create complex organs like urinary bladder and liver wherein multiple cell forms are put together to mimic the natural environment or anatomy for the stem cells to grow in. Here an organ is 3 D printed with appropriate stem cells, the organ is then treated appropriately for stem cells to develop into expected lineages [23]. This process leads to the development of a functional organ outside the body of a human being and later on after functional tests such a 3 D printed organ is implanted into the body of a patient [24]. The printers using bioinks cannot heat up as heat destroys stem cells, thus these printers use piezoelectric crystals in cartridge [25]. The cartridges are loaded with specific bioinks to print a specific tissue. Several nozzles are utilized for inkjet printing wherein each nozzle is controlled independently by the software to form 2 D and then 3 D structures. These structures are later conditioned for specific tissue growth.

Challenge to 3 D print with cells is that the cells are extremely delicate and loose viability very quickly. So there are a number of variables that need to be controlled to obtain a completely viable tissue. Therefore the biomaterial used for printing must provide sufficient support or protection to cells while printing and also after printing. During the process of printing enough nutrition needs to be supplied in order to maintain cells in viable state. Maintenance of viability is not enough because some cells lose their true nature and subsequently function if not maintained in proper conditions. Thus coaxial extrusion technique is utilized wherein two materials are utilized [26]. In this process one nozzle puts the material containing the living cells into the core of a shell made of another material which provides support to the cells. In this structure, the living cells are accessible thus the nutrients can be supplied to them. The same system provides feasibility of supplying biologically active molecules to the living cells and also a temporal control of this accessibility. A control on accessibility to the biologically active components like pharmacoactive agents is important for medical implants. In the beginning the biomaterials or biopolymer acts as a cement but later they self-degrade once the cells within them mature and secrete connective tissue i.e. the natural cement of the cells that hold them together in a tissue. These support biopolymers include hydrogels mixed with gelatin as they allow cells to grow within them [5, 27].

A biopen is a handheld printing tool that can be used by surgeons at the time of surgery to deposit a layer of implant at the time of surgery that may later develop into the tissue of choice. This pen has two nozzles, from one comes out the biopolymer and from second comes out the stem cells. The Biopen allows the surgeon to make a tailor-made implant for the patient during operation. Biopen works on the principle of coaxial extrusion [28].

Cartilage regeneration is another application of 3 D bioprinting wherein ‘biopen’ is utilized to create the cartilage [29]. This application can be utilized to create articular cartilage that allow for articulation between adjacent bones at joints. This cartilage is usually degraded in arthritis or by trauma or in cancer. Mode of action is to first surgically removed the defective or damaged portion and then introduce a cartilage patch between the bones. A cartilage patch is a 3D printed structure formed by using a biopolymer that contains osteochondral cells and chondrocytes which secrete collagen and rebuild cartilage naturally. The surface of this patch which stays in contact with the adjacent bones contains osteoblasts that develop into bone that was lost due to accident or disease. As the cells used in this patch are derived from the patient himself therefore chances of rejection by patient’s immune system are bleak. Nano-electrodes are also incorporated within this patch which can be stimulated to induce healing and growth post implantation. The biopolymers making the cartilage implants are [30] UV curable biopolymers which are strong enough to prevent crushing of implant post operation. These implants are in early stages of clinical trials and they may be one of the first 3 D printed tissues that would soon become popular implants. This is also because cartilage lacks vascularity as a result creating it is simple, while creating a highly vascular organ is an extremely difficult task [31].

4. ROLE OF 3 D PRINTED STRUCTURES IN CONTROLLED DRUG DELIVERY

Controlled drug delivery could be another application of 3 D printed materials and they could be utilized for treating epilepsy. Epilepsy is another disease that affects millions of people across the world. Currently the 3 D printed materials are being explored as tools to allow for controlled delivery of immunosuppressants, anti-inflammatory agents etc. Here we are looking at an implant that may be stimulated by a seizure to release a drug in the brain so as to immediately control the situation [32]. Skin implants are also being experimented upon by 3 D printing. As skin is a highly compartmentalized organ therefore inkjet or polyjet printing is being explored to create skin [33]. Cells of the patient receiving the implants can be utilized to create the implant so that chance of rejection of skin implant is minimal. Such implants may be useful in skin cancers or in accidental skin damage to restore lost skin. These implants are also being explored as tools to release bioactive molecules in controlled fashion to promote wound healing.

Developing a brain by 3 D printing may be in its infancy but doctors at Boston Children’s Hospital have been saving lives of babies with the help of 3 D printing. At Boston Children’s Hospital, the surgeons used a simulation tool to rehearse the surgery on the exact replica of the baby’s brain made by a 3 D printer. This way the surgeon had the chance to weigh its outcome before the surgery itself thus the chances of the surgery to be successful was higher (http://simpeds.org/news/a-3d-printed-brain-saved-this-toddlers-life/). The life of one toddler has been saved by using this technique. Thus 3 D Bioprinting has the potential to make human lives better.

5. ETHICAL CHALLENGES

As clinicians are well versed with the hip joint replacement and liver transplantations therefore the 3-D printed material do not offer any new ethical challenges [34]. But as the newly printed material may offer new safety issues thus trials have to be done so as to ascertain the safety and stability of the bioprinted devise. For this reason it is essential to compare the treatment offered by 3D printed materials or the currently available treatment. The 3 D printed materials are example of personalized medicine therefore it is very difficult to assess the average efficacy of each type of printed material for common use. But before human use, parameters like propensity of the material to cause inflammation or induce abnormal cell development, or half-life of printed material within the body can be assessed. This can be achieved by traditional methods of testing *in vitro* by tissue culture techniques followed by testing on animals [34]. The 3 D imprinted organs will have advantage that they will be made up of patients own cells thus problems of biocompatibility will be minimal, the patient would need to wait for donated organs for which there is usually long bookings and organs of appropriate sizes can be generated for children. With advancement in 3 D imprinted organ development the possibility of creating myriads of organs like pancreas, liver, bladder, internal ear etc. may rise. At the same time the ethical questions like playing with nature, availability to common man and safety of implants would always exist. As the 3 D printing for regenerative medicine is still in its formative years therefore currently it is a good time to contemplate and make proper ethical regulations for future use [35].

6. CONCLUSION

This review article has explored all the possible cures and remedies promised by 3D printing. But currently India stands a long way off from the exciting field of 3D bioprinting. If at all, this branch has to be developed then collaboration between medical doctors and engineers of our country is a prerequisite. The medical doctors and engineers of our country are world renowned; therefore, the need of the hour is to develop this branch by providing maximum funding for inter-disciplinary research on 3D bioprinting.

REFERENCES

[1] Reid JA, Mollica PA, Johnson GD, Ogle RC, Bruno RD, Sachs PC. “Accessible bioprinting: adaptation of a low-cost 3D-printer for precise cell placement and stem cell differentiation”, Biofabrication. 2016;8(2):025017.

[2] Juuti JA, Jantunen H, Moilanen VP, Leppävuori S. “Manufacturing of prestressed piezoelectric unimorphs using a postfired biasing layer”, IEEE Trans Ultrason Ferroelectr Freq Control. 2006 May;53(5):838-46.

[3] Devillard R, Pagès E, Correa MM, Kériquel V, Rémy M, Kalisky J, Ali M, Guillotin B, Guillemot F. “Cell patterning by laser-assisted bioprinting”, Methods Cell Biol. 2014;119:159-74.

[4] Melke J, Midha S, Ghosh S, Ito K, Hofmann S., “Silk fibroin as biomaterial for bone tissue engineering,” Acta Biomater. 2016 Feb;31:1-16.

[5] Das S, Pati F, Choi YJ, Rijal G, Shim JH, Kim SW, Ray AR, Cho DW, Ghosh S., “Bioprintable, cell-laden silk fibroin-gelatin hydrogel supporting multilineage differentiation of stem cells for fabrication of three-dimensional tissue constructs”, Acta Biomater. 2015 Jan;11:233-46.

[6] Kasoju N, Bora U, “Silk fibroin in tissue engineering”, Adv Healthc Mater. 2012 Jul;1(4):393-412.

[7] Datta P, Ayan B, Ozbolat IT, “Bioprinting for vascular and vascularized tissue biofabrication”, Acta Biomater. 2017 Jan 11. pii: S1742-7061(17)30035-1.

[8] Moon KH, Ko IK, Yoo JJ, Atala A, “Kidney diseases and tissue engineering” Methods. 2016 Apr 15;99: 112-9.

[9] Meston Nc, “Casting gold inlays in artificial teeth”, Dent Lab Rev. 1946 Dec;21(12):27.

[10] Valashani SM, Barthelat F, “A laser-engraved glass duplicating the structure, mechanics and performance of natural nacre”, Bioinspir Biomim. 2015 Mar 30;10(2):026005.

[11] Ehmke O. “Platinum stapedial prosthesis in middle ear surgery”, Arch otolaryngol. 1964 jan;79:54-66.

[12] O'connor Jt Jr., “The use of stainless steel wire as a suturing and ligating material in veterinary surgery”, J Am Vet Med Assoc. 1947 Nov;111(848):371-5.

[13] Swigert Vw, Fishback Hr, et al. “Silicone implantations in the rat.”,Fed Proc. 1948 Mar;7(1 Pt 1):122.

[14] Allder MA, Guilbeau EJ, Brandon TA, Walker AS, Koeneman JB, Fisk RL., “A hydrogel pericardial patch”, ASAIO Trans. 1990 Jul-Sep;36(3):M572-4.

[15] Brett E, Flacco J, Blackshear C, Longaker MT, Wan DC, “Biomimetics of Bone Implants: The Regenerative Road,”Biores Open Access. 2017 Jan 1;6(1):1-6.

[16] Barnes JE, “Manufacturing a human heel in titanium via 3D printing.”, Med J Aust. 2015 Feb 16;202(3):118.

[17] Yang M, Wang J, Zhu Y, Mao C., “Bio-Templated Growth of Bone Minerals from Modified Simulated Body Fluid on Nanofibrous Decellularized Natural Tissues.”, J Biomed Nanotechnol. 2016 Apr;12(4):753-61.

[18] Luciano NJ, Sati P, Nair G, Guy JR, Ha SK, Absinta M, Chiang WY, Leibovitch EC, Jacobson S, Silva AC, Reich DS. “Utilizing 3D Printing Technology to Merge MRI with Histology: A Protocol for Brain Sectioning.” J Vis Exp. 2016 Dec 6;(118). doi: 10.3791/54780.

[19] Pattanayak DK, Fukuda A, Matsushita T, Takemoto M, Fujibayashi S, Sasaki K, Nishida N, Nakamura T, Kokubo T, “Bioactive Ti metal analogous to human cancellous bone: Fabrication by selective laser melting and chemical treatments.”, Acta Biomater. 2011 Mar;7(3):1398-406.

[20] Shao H, Yang X, He Y, Fu J, Liu L, Ma L, Zhang L, Yang G, Gao C, Gou Z., “Bioactive glass-reinforced bioceramic ink writing scaffolds: sintering, microstructure and mechanical behavior.,” Biofabrication. 2015 Sep 10;7(3):035010. doi: 10.1088/1758-5090/7/3/035010.

[21] Harake RS, Ding Y, Brown JD, Pan T., “Design, Fabrication, and In Vitro Testing of an Anti-biofouling Glaucoma Micro-shunt.”, Ann Biomed Eng. 2015 Oct;43(10):2394-405.

[22] Rutz AL, Hyland KE, Jakus AE, Burghardt WR, Shah RN. “A multimaterial bioink method for 3D printing tunable, cell-compatible hydrogels.” Adv Mater. 2015 Mar 4;27(9):1607-14.

[23] Whitford WG, Hoying JB. “A bioink by any other name: terms, concepts and constructions related to 3D bioprinting.” Future Sci OA. 2016 Jul 22;2(3):FSO133. doi: 10.4155/fsoa-2016-0044.

[24] Shafiee A, Atala A. “Tissue Engineering: Toward a New Era of Medicine.” Annu Rev Med. 2017 Jan 14;68:29-40. doi: 10.1146/annurev-med-102715-092331.

[25] Hossain SM, Luckham RE, Smith AM, Lebert JM, Davies LM, Pelton RH, Filipe CD, Brennan JD. “Development of a bioactive paper sensor for detection of neurotoxins using piezoelectric inkjet printing of sol-gel-derived bioinks.” Anal Chem. 2009 Jul 1;81(13):5474-83.

[26] Gao Q, He Y, Fu JZ, Liu A, Ma L, "Coaxial nozzle-assisted 3D bioprinting with built-in microchannels for nutrients delivery." Biomaterials. 2015 Aug;61:203-15.

[27] Guan X, Avci-Adali M, Alarçin E, Cheng H, Kashaf SS, Li Y, Chawla A, Jang HL, Khademhosseini A. "Development of hydrogels for regenerative engineering." Biotechnol J. 2017 Feb 21. doi: 10.1002/biot.201600394.

[28] Han YL, Hu J, Genin GM, Lu TJ, Xu F., "BioPen: direct writing of functional materials at the point of care". Sci Rep. 2014 May 6;4:4872. doi: 10.1038/srep04872.

[29] O'Connell CD, Di Bella C, Thompson F, Augustine C, Beirne S, Cornock R, Richards CJ, Chung J, Gambhir S, Yue Z, Bourke J, Zhang B, Taylor A, Quigley A, Kapsa R, Choong P, Wallace GG. "Development of the Biopen: a handheld device for surgical printing of adipose stem cells at a chondral wound site." Biofabrication. 2016 Mar 23;8(1):015019

[30] Mouser VH, Melchels FP, Visser J, Dhert WJ, Gawlitta D, Malda J, "Yield stress determines bioprintability of hydrogels based on gelatin-methacryloyl and gellan gum for cartilage bioprinting." ,Biofabrication. 2016 Jul 19;8(3):035003.

[31] Datta P, Ayan B, Ozbolat IT., "Bioprinting for vascular and vascularized tissue biofabrication. Acta Biomater." , 2017 Jan 11. pii: S1742-7061(17)30035-1.

[32] Jost J, Preux PM, Druet-Cabanac M, Ratsimbazafy V, "How to reduce the treatment gap for people with epilepsy in resource-limited settings by innovative galenic formulations: A review of the current situation, overview of potential techniques, interests and limits", Epilepsy Res. 2016 Jan;119:49-61

[33] Cubo N[1], Garcia M, Del Cañizo JF, Velasco D, Jorcano JL. , "3D bioprinting of functional human skin: production and in vivo analysis." Biofabrication. 2016 Dec 5;9(1):015006.

[34] Yoo SS. "3D-printed biological organs: medical potential and patenting opportunity." Expert Opin Ther Pat. 2015 May;25(5):507-11.\

[35] Bauer HK, Heller M, Fink M, Maresch D, Gartner J, Gassner UM, Al-Nawas B., Social and legal frame conditions for 3D (and) bioprinting in medicine. Int J Comput Dent. 2016;19(4):293-299.