

4D Bioprinting, Newer Prospects in Regeneration

Utkalika Das¹, Gatha Mohanty², Rashmita Nayak³

¹Tutor, ²Senior Lecturer, ³Professor, Department of Periodontics and Oral Implantology, Institute of Dental Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India

Abstract

The new concept which has come up in recent times being integrated with 3D bioprinting is 4-dimensional bio-printing. This is now considered the most appropriate solution for “tissue engineering”. It has the potential to construct functional structure and also fabricate 3D patterns of biological architecture which can change shape under various stimuli by use of “stimulate responsive material”. 4Dbio-printing provides a great potential for tissue engineering of bone. The properties like shape memory of the printed structure is used for repair of personalized bone defect and the properties such as functional maturation helps in promoting osteogenic differentiation of stem cells. This review aims to discuss the concept of 4D bio-printing and its application in “bone tissue engineering”.

Keywords: 4Dbioprinting, Bone tissue engineering, Periodontal regeneration.

Introduction

In the recent time, the 3D bio-printing technology concept has integrated time as the 4th dimension to fabricate complex functional biological architectural models. The 4Dbio-printing structure can go through a transformation in shape or function overtime by the use of various stimuli-responsive biomaterials and the series of 4D bio-printing strategies. The application of bioprinting in tissue engineering guided tissue regeneration procedure remains unpredictable due to close proximity to the tooth surface.¹ To solve this dilemma 3D bio-printing was proposed by **Hull** et al in 1986.² 3D-bioprinting has been used for the fabrication of blood vessels, liver tissue, heart tissue and bone. However, there are certain limitations such as the initial condition of a printed object is only considered and assumed to be inanimate and static. Since the process of natural

tissue regeneration involves 3D microarchitectures and extracellular matrix formation along with the generation of a tissue with a unique function, according to **Y.C.Li** et al³ the complex conformational changes on tissue acquired by a mechanism that can respond to external or internal stimuli, 3Dbioprinting cannot mimic. **Skylar** et al in 2014⁴ at MIT for the first time demonstrated 4Dbioprinting technology where multi-material prints were introduced having the capacity to transform overtime or change its shape. Fabrication of various 3D design architectures that are capable of undergoing dynamic configuration transformation in response to various stimuli were done thereby overcoming the limitation of 3Dbioprinting. 4Dbioprinting was defined by **Gao** et al. This is considered as the generation of cell filled 3D printed structures that can respond to external stimuli or internal stimuli respectively. They also included the functionalization and maturation of tissue with time, change in the shape, and change in functionality of the printed material overtime as two main characteristics of “4D bioprinting”. The shape transformation feature has been used to carry out personalized bone regeneration in the irregular type of bone defects. Reassembly of stimuli-responsive material and program cross-linking could be modulated in the mechanical properties of 4D constructed architecture.⁵ Further, the functional maturation of 4D construct of bone could also help in establishing a micro environment that

Corresponding Author:

Dr. Gatha Mohanty

Senior Lecturer, Department of Periodontics and Oral Implantology, Institute of Dental Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India

e-mail: gathamohanty@soa.ac.in

enhances the differentiation of stem cells and influences cell behavior.⁶

Important characteristics feature of 4D-bioprinting

1. Functional transformation
2. Shape transformation

Stimuli-responsive material

(a) Physical stimuli

- Magnetic responsive
- Humidity responsive
- Temperature-responsive
- Electro responsive
- Photo responsive

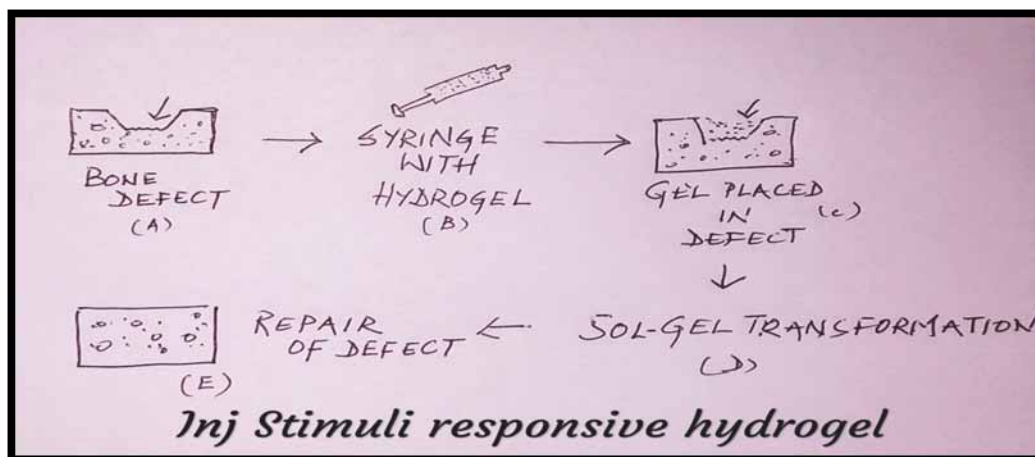
(b) Chemical stimuli

(c) Biological stimuli

3. CTF

Application: 4Dbioprinting is used for personalized bone defects especially the minor bone defects. The microvascular system and network of nerves within the bone tissue can be also constructed for a large bone graft substitute.

(a) Use of injectable stimuli-responsive hydrogel: In simple terms the thermostatic hydrogel is injected to bone defect and overtime gets transformed to a gel-like state at a temperature that is conducive to the human body, thereby repairing the defect.



The hydrogels are thermo-responsive and are made up of polysaccharides. They also carry cells, HAP and CaPO₄ cements, growth factors, inorganic composites like bioactive glass.⁷ PNIPAM is one of the classical thermoresponsive material. This material along with chitosan and hyaluronic acid forms a suitable hydrogel that can be injected into the defect.⁸ Another type of thermosensitive material available is a copolymer of polylactic acid, polyethylene glycol, and polyglycolic acid which can convert to gel under physiological condition.⁹ These injectable hydrogels have mechanical strength that can be improved by the use of nanohydroxyapatite tricalcium phosphate and bioactive glass.¹⁰ These hydrogels carry osteoblastic cells. The cells further have “alkaline phosphatase” activity and fill up irregular cells defect thereby providing of “mini-

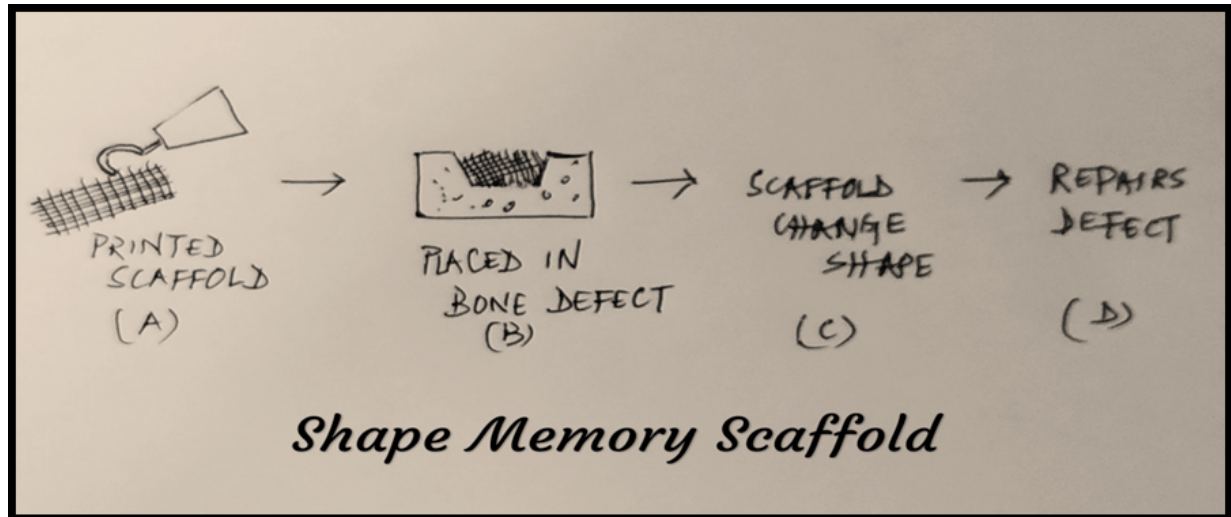
invasive repair” of bone defects. Mesenchymal stem cell differentiation has been enhanced with bioactive cues such as angiogenetic and osteogenic growth factors and are used by orthopedics in recent times.

2. 4Dbioprinting based on shape memory scaffolds:

The shape memory scaffold is developed by 4Dbioprinting and implanted into the bone defect site. This scaffold changes its size to occupy the void space and repairs the defect. According to F.S. Senatov et al fused filament fabrication technique is used to obtain polylactide and hydroxyapatite porous scaffold.¹¹ The shape memory effect of the scaffold was stimulated by direct heating. These are used in self-fitting implants that repair small bone defects.

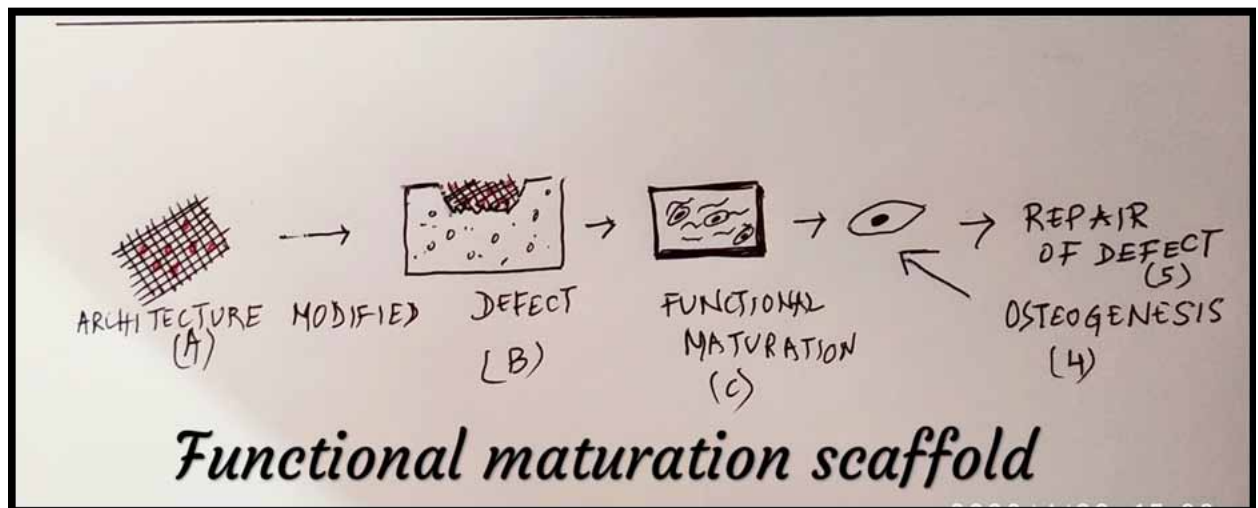
S. Miao et al synthesized smart polymer that is PCL and cross-linker with castor oil-producing renewable bio-scaffold with shape memory and shape recovery effect at physiological temperature.¹² These

scaffolds are biocompatible and also biodegradable. More recently S. Miao et al fabricated a temperature-responsive scaffold that contained epoxidized acrylate materials.¹³



3. **4Dbioprintingbasedonfunctionaltransformation mechanism:** During the post bioprinting stage, the biological activity of 3D-printed scaffolds and osteogenesis of stem cells can be improved by the biomimetic bony microenvironment. The establishment of this biomimetic microenvironment inspires the “functional maturation” of 3D-printed constructs, which is being reformed as polymeric “3D-printed scaffolds” by decorating them with

a “cell-laden mineralized extracellular matrix” to imitate bony microenvironment. As time passes, a rotary flask bioreactor culturing, the printed bone structures became mature. Further it can be seen that the “extracellular-matrix-adorned” scaffolds showed evidence of better osteoinductive and osteoconductive properties than those of “bare 3D-printed scaffolds.”



However, the bone tissues consist of complex hierarchical structures which further consists of both anisotropic mechanical as well as electromechanical properties. Barium titanate which is a smart biomaterial having piezoelectric effect, has the capacity of stimulating the physiological electrical microenvironment. This smart biomaterial will act in response to functional stress as well as promote the growth of osteoblasts by showing bone inducing abilities and favourable biocompatibility. Piezoelectric materials have shown promising development in post printing stages.

4. 4D bioprinting with blood vessels and network of nerves: The major challenge in “bone-graft-substitute engineering is regeneration of microvasculature along with the nervous networks. A combination of MSC’s of mouse along with methacrylate alginate and hyaluronic acid hybrid hydrogels, a hollow self-folding tubes were fabricated. They had diameters closest to the smallest blood vessels. By entrapping enzymes in 4D hydrogel, pre-programmed calcification and fibrin biofilm formation could be triggered. “Bio-inspired 3D constructs” consists of bone-like similar structures that surround the constructed blood-vessels making a replica of vascularized alveolar bone constructs. 4D bioprinting of the “electro-responsive biomaterials” has shown scope for “nerve tissue regeneration”.

Conclusion

4D bioprinting has emerged as a newer field for constructing a multilayered, constructive and functional bone tissue having complex structures. Fabrication of “shape-programmed”, as well as functional structures, is possible in a more controlled manner. The advanced technology of this “4D bioprinting” will fulfill medical requirements and also it will help to explore its extensive purpose in the biomedical field. However, further researches are required in this field to work out the existing challenges so that 4D bioprinting can serve as a controlling means to ascertain dynamic along with hierarchical structures of natural tissues or organs.

Conflict of Interest: Nil

Ethical Permission: Approved

Funding: None

References

1. Das U, Mohanty G, Mohanty R, Nayak R. 3D Printing, Innovative Approach in Dentistry. Indian Journal of Public Health Research & Development. 2019; 10 (11):1124-1126.
2. Hull CW, Apparatus for production of three-dimensional objects by stereolithograph, US Patent US4575330 A (1986).
3. Li YC, Zhang YS, Akpek A, Shin SR, Khademhosseini A. 4D bioprinting: the next-generation technology for biofabrication enabled by stimuli-responsive materials. *Biofabrication*. 2016;9(1):012001
4. Qin Z, Compton BG, Lewis JA, Buehler MJ. Structural optimization of 3D-printed synthetic spider webs for high strength. *Nat Commun*. 2015;6:7038.
5. Suo H, Zhang D, Yin J, Qian J, Wu ZL, Fu J. Interpenetrating polymer network hydrogels composed of chitosan and photocrosslinkable gelatin with enhanced mechanical properties for tissue engineering. *Mater Sci Eng C Mater Biol Appl*. 2018;92:612-620.
6. Kim HD, Amirthalingam S, Kim SL, Lee SS, Rangasamy J, Hwang NS. Biomimetic Materials and Fabrication Approaches for Bone Tissue Engineering. *Adv Healthc Mater*. 2017;6(23):10.
7. Graham S, Marina PF, Blencowe A. Thermoresponsive polysaccharides and their thermoreversible physical hydrogel networks. *Carbohydr Polym*. 2019;207:143-159.
8. Yang J, van Lith R, Baler K, Hoshi RA, Ameer GA. A thermoresponsive biodegradable polymer with intrinsic antioxidant properties. *Biomacromolecules*. 2014;15(11):3942-3952.
9. Vojtova L, Michlovska L, Valova K, et al. The Effect of the Thermosensitive Biodegradable PLGA-PEG-PLGA Copolymer on the Rheological, Structural and Mechanical Properties of Thixotropic Self-Hardening Tricalcium Phosphate Cement. *Int J Mol Sci*. 2019;20(2):391.
10. Azevedo AS, Sá MJ, Fook MV, et al. Use of chitosan and β -tricalcium phosphate, alone and in combination, for bone healing in rabbits. *J Mater Sci Mater Med*. 2014;25(2):481-486

11. Senatov FS, Niaza KV, Zadorozhnyy MY, Maksimkin AV, Kaloshkin SD, Estrin YZ. Mechanical properties and shape memory effect of 3D-printed PLA-based porous scaffolds. *J Mech Behav Biomed Mater.* 2016;57:139-148.
12. Miao S, Wang P, Su Z, Zhang S. Vegetable-oil-based polymers as future polymeric biomaterials. *Acta Biomater.* 2014;10(4):1692-1704
13. Miao S, Zhu W, Castro NJ, et al. 4D printing smart biomedical scaffolds with novel soybean oil epoxidized acrylate. *Sci Rep.* 2016;6:27226.