Four-dimensional bioprinting: Current developments and applications in organ replacement engineering

Abstract:

The field of regenerative medicine has witnessed remarkable progress with the advent of 4-D bioprinting, a cutting-edge technology that holds the promise of revolutionizing organ replacement therapies. Traditionally, artificial organs have struggled to replicate the intricate functions of natural organs. However, with a growing interest in stimuli-responsive materials and cell-laden bioinks, a new concept called four-dimensional (4D) bioprinting was introduced with “time” as the 4th dimension. This technique proceeds on the assumption that after three-dimensional (3D) bioprinting, tissues need to remodel and mature. This abstract provides an overview of the state-of-the-art developments in 4-D bioprinting for organ replacement and highlights its potential impact on healthcare.

Introduction:

Three-dimensional (3D) printing can be traced back to its initial patent by Charles Hull in 1986 [1]. Over the years, the realm of 3D printing has witnessed significant advancements, resulting in a diverse array of techniques [2]. Among these innovations, a pivotal breakthrough emerged with the integration of cells into 3D printed constructs, giving birth to the field of 3D bioprinting [3]. Notable 3D bioprinting technologies include inkjet printing, microextrusion, and laser-assisted printing approaches [4].

In the context of inkjet 3D bioprinting, various mechanisms such as thermal, piezoelectric, or electromagnetic tools are employed to deposit minute bioink droplets through nozzles. Microextrusion is widely known due to its capacity to

achieve higher cell densities and accommodate a broader range of polymer viscosities [3]. Microextrusion 3D bioprinting entails the extrusion of bio ink through nozzles, which can be facilitated using mechanical methods [5].

Another remarkable approach, laser-assisted 3D bioprinting, harnesses laser energy to vaporize a sacrificial layer, propelling a payload towards a receiving substrate, resulting in nozzle-free bioprinting [6]. The synergy between 3D bioprinting technologies and microfluidic platforms has paved the way for precise control of bioink flow rates and the attainment of multimaterial bioprinting with high spatial resolution [7,8]. Consequently, this fusion of technologies enables the creation of complex, heterogeneous, and biomimetic structures, with profound implications for tissue engineering applications [9–11].

Three-dimensional bioprinting technologies have revolutionized the creation of intricate tissue structures, offering precise control in an automated fashion [12]. However, it's worth noting that the currently fabricated structures often fall short in mimicking the dynamic nature of living tissues. Natural tissue regeneration and repair processes often entail conformational changes within the tissue structure [13]. Thus, the incorporation of a time-dependent element into 3D printed tissue constructs becomes imperative to accurately replicate these structural changes in tissues.

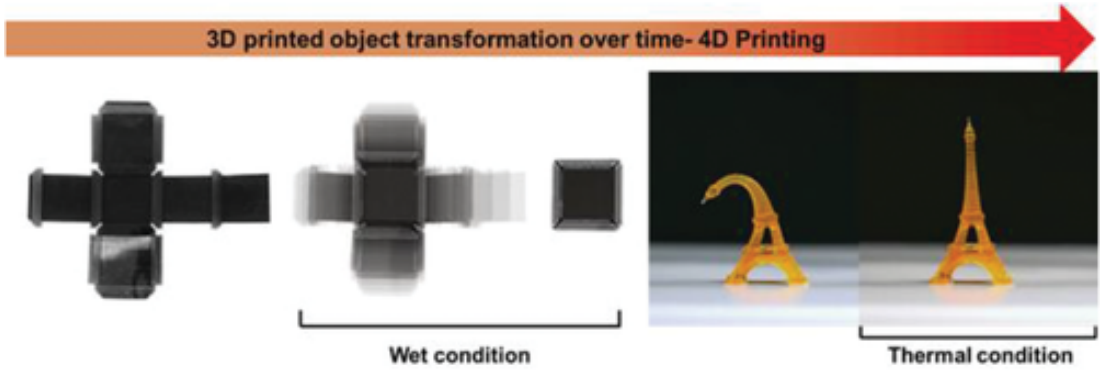

Figure 1

3D printed self-assembly objects change shape over time-4D printing. Reproduced with permission.[30] Copyright 2020, Advancetec.

To address this challenge, four-dimensional (4D) bioprinting has emerged, capitalizing on stimuli-responsive biomaterials and cell traction forces to engineer structurally dynamic tissue constructs. In our context, 4D bioprinting is defined as the 3D printing of cell-laden materials with the ability to respond to external

stimuli or internal cell forces. This definition distinguishes it from Gao et al.'s interpretation [14], which associates 4D bioprinting not just with structural changes but also with cell maturation and functionality within 3D printed constructs over time (even if the geometry remains constant). The inception of 4D bioprinting can be traced back to the broader concept of 4D printing, as depicted in Figure 2, where multi-materials capable of undergoing transformations over time were introduced [15]. Since then, various studies have explored 4D printing materials and technologies [16–18]. Nonetheless, 4D bioprinting still demands further refinement to seamlessly adapt to post-printing changes in cell behavior and function, ensuring safety and predictability.

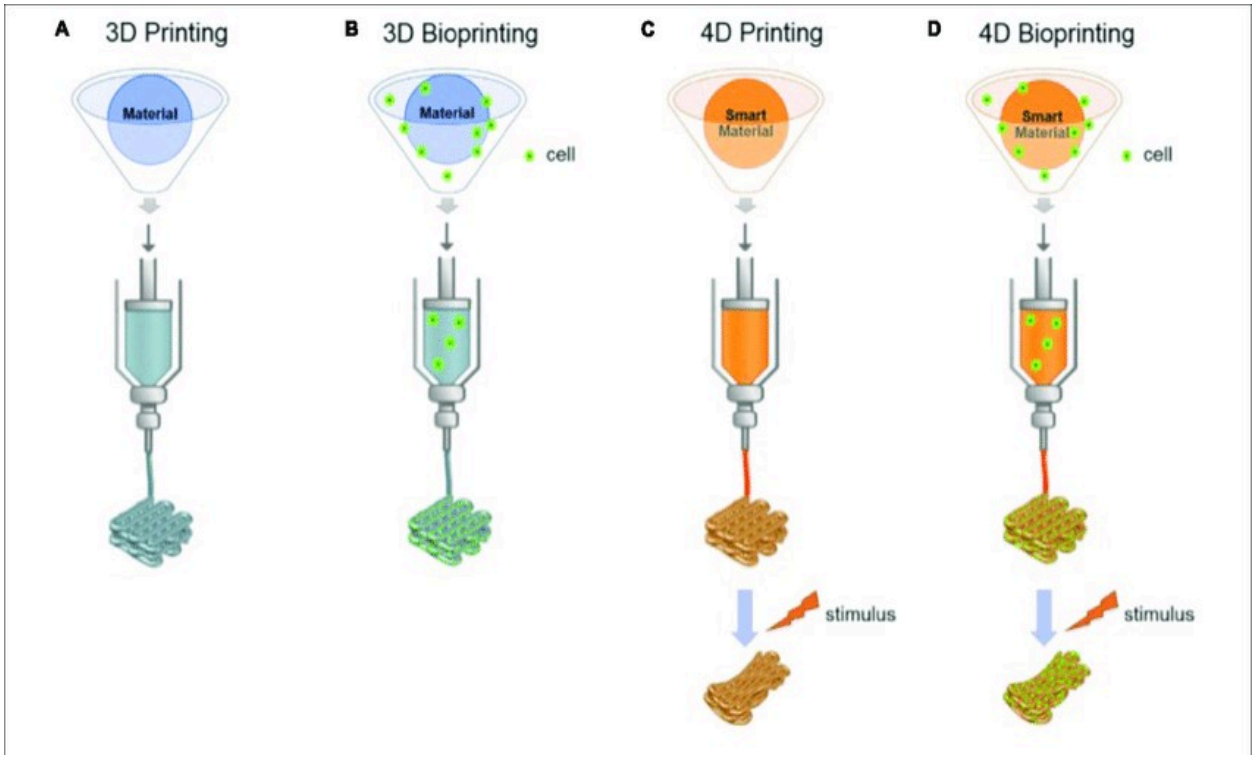

Figure 2

4D Bioprinting & Its Types:

4D bioprinting is analogous to 4D printing in that it is the printing of smart, environmentally responsive biological structures, tissues, and organs. 4D bioprinting begins with the printing of multiple cells or biological matrices resulting in structures that undergo subsequent, designed, and anticipated (not spontaneous) but self-originated development in response to an environment. Four distinct types of 4D bioprinting have been proposed.

There are four distinct categories within the realm of advanced bioprinting, each with unique characteristics and applications. The first category, referred to as "shape change," involves the use of 4D printed substrates constructed from intelligent biopolymers, and possibly containing cells. These substrates can undergo alterations in their 3D configuration or shape when stimulated.

The second category, termed "size change," is linked to in vivo bioprinting. In this approach, a 3D-printed device, often composed of hydrogel, is implanted within the body. Subsequent biological processes lead to the formation of tissue at the site where the initially implanted structure was, as it is absorbed.

The third approach, known as "pattern change," revolves around the precise deposition of micro-droplets of cells in a specific pattern or orientation. Following the initial printing, this pattern can be manipulated and transformed according to a pre-envisioned design by applying specific stimuli. Like 4D printing, these three types of bioprinting entail engineered and anticipated modifications in the post-printing physical shape, size, or pattern [19].

The fourth type of bioprinting introduces a novel dimension by focusing on dynamic biological development and responses. In contrast to traditional 4D printing, 4D bioprinting goes beyond merely altering physical structures. It encompasses functional and pre-designed changes that depend on or result in non-structural yet biologically significant activities. These engineered cellular and biological developments can directly influence the collective biological phenotype, functionality, and even the shape and structure of the printed product. In essence, 4D bioprinting is a domain where non-structural, highly predictable biological changes are integrated into the printed structures, yielding outcomes that have profound implications for the field of bioprinting [20].

Examples include component cells responding to such environmental actuators/mediators as immunological signals and input; adjacent cell or basement membranes; juxtacrine/paracrine / endocrines, etc; physicochemical “stress” or response inducers; differentiation signals and epigenetic modulators; nutritional / factor components and their gradients.

Such non-structural 4D bioprinting offers post-printing cellular “CD” development and display, cell receptor characteristic development, cellular type differentiation/adaptation, functionality development in organoid culture, cellular polarization influencing self-organization, dynamic, pluri-consequential or

reversible responses, mutations and chromosome silencing in transplantation chimera

Examples of anticipated, but purely biological responses include multipotent *in situ* cell differentiation, restricted chimerism with human naïve iPSCs, *in situ* tissue vascularization or organ development, admixtures determining the potency of cells in organ development.

Mathematical modeling:

Mathematical modeling plays a pivotal role in predicting the 4D bioprinting process and the eventual state of the printed materials when subjected to stimuli. Specifically, mathematical modeling provides valuable insights into the desired shape, properties, and functions of printed materials [22]. By doing so, it significantly streamlines the experimental process, reducing both time and costs in the quest to optimize 4D bioprinted structures. This modeling involves four core elements: the print path, the desired (final) shape, ink properties, and stimulus properties [23].

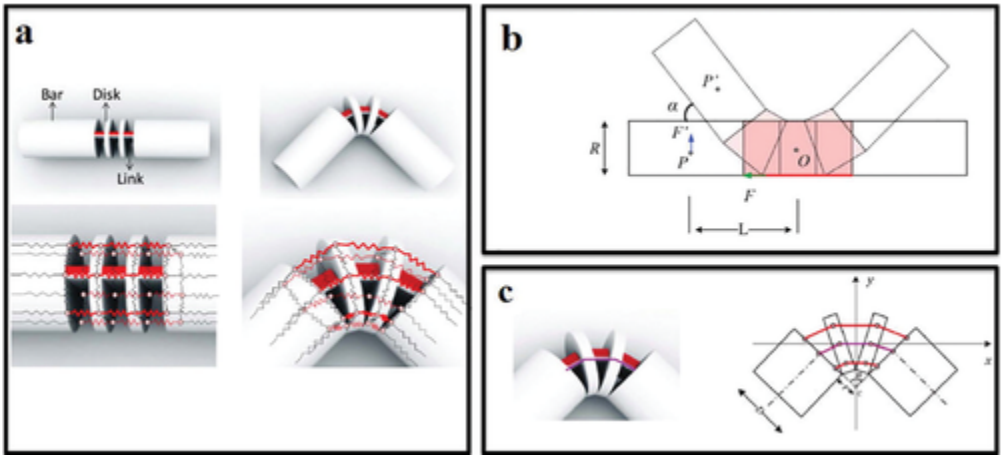

Figure 3

a) The upper row represents renderings of the initial joint and its folding and the lower row shows spring-mass systems corresponding to the renderings. The lateral black springs indicate the rigid bars and disks. The red springs represent the links causing the joint to fold. b) A schematic diagram consisting of variables that are used for the measurement of stiffness coefficients. c) Representation of computing the joint length, which is modeled utilizing two disks and length of each inner limb that is calculated based on its distance measured from the center of rotation. The link marked as red in the center remains constant over the time. Reproduced with permission.[27] Copyright 2014, Springer Nature.

Two primary strategies have been employed in mathematical modeling for 4D printing: the forward problem and the inverse problem approaches [21]. In the forward problem approach, the final shape of the material is unknown, focusing on the fundamental concepts and mechanisms of 4D printing technology. In contrast, the inverse problem approach is more application-oriented, where the print path is unknown.

Figure 4

Mathematical modeling showing a) the connection between print paths, which is quantified by the angle θ between first and second layer and b) final desired shape quantified based on curvature tensor (κ), mean curvature (H), and Gaussian curvature (K). Reproduced with permission.[29] Copyright 2017, Elsevier.

As shown in Figure 3a, this study demonstrates the print path of the first and second layer which aligns along the x and y direction respectively and can be presented through the equation cos(θ)*x* + sin(θ)*y*, where θ represents the angle between the two successive layers. The relationship between angle θ and final desired shape can be established using the following Equations (1) and (2)[28]

$$H = c_1 \frac{(\alpha_{\parallel} - \alpha_{\perp})}{h} \frac{\mathrm{Sin}^2\theta}{c_2 - c_3\cos(2\theta) + m^4\cos(4\theta)}$$

$$K = -C_4 \frac{(\alpha_{\parallel} - \alpha_{\perp})^2}{h^2} \frac{\mathrm{Sin}^2\theta}{c_5 - c_6\cos(2\theta) + m^4\cos(4\theta)}$$

Here κ is the curvature tensor that quantifies final desired shapes using mean curvature,

$$H = \frac{1}{2}\mathrm{Tr}\,(\kappa) = \frac{1}{2}(\kappa_{xx} + \kappa_{yy})$$

and Gaussian curvature,

$$K = \det(\kappa) = \kappa_{xx}\,\kappa_{yy} - \kappa_{xy}^2$$

Here $\alpha_{\perp}$ and $\alpha_{//}$ represents the longitudinal and transverse swelling strain respectively (Figure 3b).[28] In addition, m is the ratio of layer thicknesses (ratio of a_{bottom} and a_{top}), h is the sum of a_{top} and a_{bottom}, which defines the total thickness of the bilayer. c_i denotes functions of Poisson's ratio(v), m, longitudinal ($E_{\perp}$) and transverse ($E_{//}$)Young's modulus, respectively. Hence, the print paths can be calculated based on the final desired shape.

The application of mathematical modeling in 4D printing is well-documented. For instance, Raviv et al. utilized a spring-mass mathematical model in a forward problem approach to predict the final structure of printed shape-memory materials [24]. They assumed an elastic behavior in their simulation, a concept applicable to biomaterials in 4D bioprinting. Yu et al. introduced a quantitative analysis to assess thermo-responsive and shape-memory polymers, examining energy storage and release during the shape-memory cycle [26]. While their model was relatively simple, it laid the foundation for more complex polymer structures. Mathematical modeling has also been instrumental in quantifying hydrogel properties in 4D printing. Gladman et al. employed an inverse problem approach to determine the print path of hydrogels, taking into account hydrogel swelling-induced deformations [21]. Additionally, Kwok et al. employed a design optimization technique for the 4D printing of origami and kirigami structures [25].

Mathematical modeling can be extended to capture intricate tissue structures in the design of printed constructs. Identifying mathematical relationships between external or internal stimuli and tissue structure and function holds immense potential for engineering dynamic and precise tissue constructs. On a broader scale, such information can contribute to our understanding of structural biology, cell biology, tissue morphogenesis, and disease modeling. Furthermore, there is room for further development of modeling approaches to encompass cells and their interactions with biomaterials in both the printing and post-printing phases. This would be a significant advancement in the field of 4D bioprinting [22].

Advantages of 4D Printing:

The advantages of 4-D bioprinting include precise control over tissue architecture, improved cell viability, and the potential for real-time adaptation to changing conditions within the body. This technology offers new avenues for the creation of patient-specific organs, reducing the risk of rejection and addressing the chronic shortage of donor organs. Furthermore, 4-D bioprinting holds promise

for replacing traditional autografts, reducing donor site morbidity and invasive surgeries. The technology's applications extend to creation of artificial organs that can mimic natural functions of specific organs like the heart and lungs.

Advancements in nanomaterials and nanotechnology offer the possibility of creating intelligent nanomaterials that can interact with cells and tissues at the cellular and molecular levels. These materials can release biomolecules, mimic the extracellular matrix, and modulate inflammatory responses.

Moreover, integrating 4D bioprinted constructs with wireless communication and data storage devices opens new horizons for multifunctional, smart cellular-based systems in tissue regeneration and controlled drug delivery. Precise control of 4D bioprinted construct function in vivo, enabled by electronics and sensor technology, holds the potential for personalized care of implanted devices and tissues.

As the technology continues to develop, we can expect to see 4D printed objects used in a wide range of applications, including implantable medical devices, such as self-fitting stents and biodegradable tissue scaffolds, robots that can morph and adapt to their environment, self-assembling products and to create molds and tooling that can adapt to changes in design.

Current Challenges and Outlook:

Four-dimensional bioprinting offers significant promise for crafting dynamic cellular structures in biomedical applications. This innovative approach harnesses cell traction forces and stimuli-responsive biomaterials to create adaptive 4D constructs. Yet, it faces several key challenges that necessitate exploration.

The printing process's influence on cell-laden biomaterials' responsiveness, the reciprocal effect of cells on stimuli-responsive biomaterials, and the impact of material dynamics on cell viability are fundamental questions that must be addressed before the widespread adoption of 4D bioprinting.

Leveraging stimuli-responsive materials within 4D bioprinted structures to interact seamlessly with host tissues and integrate with the native microenvironment is a compelling prospect. However, the often harsh local tissue environment,

particularly in inflamed pathological conditions, poses a challenge. Comprehensive studies are essential to understand and control interactions between printed tissues and the body's immune system.

While many stimuli-responsive materials can respond to a single stimulus type, the human body's intricate cellular activities are regulated by multiple physiological signals. Therefore, there's a growing demand for 4D printed constructs capable of responding to various signals, paving the way for their use in in vivo biomedical applications.

To bring 4D bioprinting to the forefront, considerations must be given to scalability, manufacturing, affordability, and user-friendliness. Exploring the use of portable bioprinters for in-situ fabrication, especially for constructs reliant on cell traction forces, is a promising avenue. Balancing complexity with manufacturability and addressing validation and cryopreservation challenges in large-scale production are pivotal steps in realizing the potential of 4D bioprinting.

Conclusion:

Recent advancements in four-dimensional bioprinting have introduced the capability to induce controlled conformational changes in printed structures using stimuli-responsive biomaterials and/or cells. This breakthrough allows for the creation of dynamic tissue structures capable of undergoing predetermined morphological transformations. Beyond its application in tissue engineering, four-dimensional bioprinting holds significant potential in various biomedical domains, including bioactuation, biorobotics, and biosensing. The utilization of stimuli-responsive biomaterials and cell traction forces as bioinks further enhances the versatility of 4D bioprinting. Employing mathematical modeling proves to be a valuable tool in predicting the transition and final state of 4D printed constructs. In summary, four-dimensional bioprinting stands poised for a promising future, offering a powerful technology to emulate the dynamic and hierarchical organization observed in native cellular structures.

References:

[1] Hull CW, 1986, US Patent, 4575330A.

[2] Tack P, Victor J, Gemmel P, Annemans L, *BioMedical Engineering OnLine* 2016, 15(1), 115. [PMC free article] [PubMed] [Google Scholar]

[3] Murphy SV, Atala A, *Nat. Biotechnol* 2014, 32, 773. [PubMed] [Google Scholar]

[4] Zhang YS, Oklu R, Dokmeci MR, Khademhosseini A, *Cold Spring Harb Perspect Med* 2017, 18, pii: a025718. [Google Scholar]

[5] Ozbolat IT, Hospodiuk M, *Biomaterials*. 2016, 76, 321. [PubMed] [Google Scholar]

[6] Dababneh AB, Ozbolat IT, *J. Manuf. Sci. Eng* 2014, 136, 61016. [Google Scholar]

[7] Liu W, Zhong Z, Hu N, Zhou Y, Maggio L, Miri AK, Fragasso A, Jin X, Khademhosseini A, Zhang YS, *Biofabrication*. 2018, 10, 5090/aa9d44. [PMC free article] [PubMed] [Google Scholar]

[8] Miri AK, Nieto D, Iglesias L, Goodarzi Hosseinabadi H, Maharjan S, Ruiz-Esparza GU, Khoshakhlagh P, Manbachi A, Dokmeci MR, Chen S, Shin SR, Zhang YS, Khademhosseini A, *Adv Mater*. 2018, e1800242. [PMC free article] [PubMed] [Google Scholar]

[9] Yang Q, Lian Q, Xu F, *Biomicrofluidics*. 2017, 11. [PMC free article] [PubMed] [Google Scholar]

[10] Ma Y, Ji Y, Huang G, Ling K, Zhang X, Xu F, *Biofabrication*. 2015, 7(4), 044105. [PubMed] [Google Scholar]

[11] Ma Y, Ji Y, Zhong T, Wan W, Yang Q, Li A, Zhang X, Lin M, *ACS Biomaterials Science & Engineering*. 2017, 3, 3534. [PubMed] [Google Scholar]

[12] Bajaj P, Schweller RM, Khademhosseini A, West JL, Bashir R, *Annu. Rev. Biomed. Eng*. 2014, 16, 247. [PMC free article] [PubMed] [Google Scholar]

[13] Xiao Y, Ahadian S, Radisic M, *Tissue Eng. B* 2017, 23, 9. [PMC free article] [PubMed] [Google Scholar]

[14] Gao B, Yang Q, Zhao X, Jin G, Ma Y, Xu F, *Trends Biotechnol*. 2016, 34, 746. [PubMed] [Google Scholar]

[15] Tibbits S, *Architect. Design* 2014, 84, 116. [Google Scholar]

[16] Raviv D, Zhao W, McKnelly C, Papadopoulou A, Kadambi A, Shi B, Hirsch S, Dikovsky D, Zyracki M, Olguin C, Raskar R, Tibbits S, *Sci. Rep* 2014, 4, 7422. [PMC free article] [PubMed] [Google Scholar]

[17] Ge Q, Sakhaei AH, Lee H, Dunn CK, Fang NX, Dunn ML, *Sci. Rep* 2016, 6, 31110. [PMC free article] [PubMed] [Google Scholar]

[18] Su M, Huang Z, Li Y, Qian X, Li Z, Hu X, Pan Q, Li F, Li L, Song Y, *Adv. Mater* 2018, 30(3), 1703963. [PubMed] [Google Scholar]

[19] An J, Chua K and Mironov V, 2016, A Perspective on 4D Bioprinting. International Journal of Bioprinting, vol.2(1): x–x. http://dx.doi.org/10.18063/IJB.2016.01.003.

[20] Whitford W, Development Strategies for Bioprinting Media and Buffers, International Bioprinting Congress, 9-10 July 2015, Singapore.

[21] Gladman AS, Matsumoto EA, Nuzzo RG, Mahadevan L, Lewis J, *Nat. Mater* 2016, 15, 413. [PubMed] [Google Scholar]

[22] Suntornnond R, Tan EYS, An J, Chua CK, *Materials (Basel)*. 2016, 9, 10.3390/ma9090756. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[23] Momeni F, Mehdi SM Hassani N, Liu X, Ni J, *Mater. Design* 2017, 122, 42. [Google Scholar]

[24] Raviv D, Zhao W, McKnelly C, Papadopoulou A, Kadambi A, Shi B, Hirsch S, Dikovsky D, Zyracki M, Olguin C, Raskar R, Tibbits S, *Sci. Rep* 2014, 4, 7422. [PMC free article] [PubMed] [Google Scholar]

[25] Kwok T, Wang CCL, Deng D, Zhang Y, Chen Y, *J. Mechanical. Design* 2015, 137, 10. [Google Scholar]

[26] Yu K, Xie T, Leng J, Ding Y, Qi HJ, *Soft Matter* 2012, 8, 5687. [Google Scholar]

[27] D. Raviv, W. Zhao, C. McKnelly, A. Papadopoulou, A. Kadambi, B. Shi, S. Hirsch, D. Dikovsky, M. Zyracki, C. Olguin, R. Raskar, S. Tibbits, Sci. Rep. 2014, 4, 7422.

[28] Patronace's GRDXKN is a New Textile Printing Technology, Des. Archit. Des. Mag. 2018. [Google Scholar]

[29] F. Momeni, S. M. Mehdi, N. Hassani, X. Liu, J. Ni, Mater. Des. 2017, 122, 42.

[30] A Look At 4D Printing: The Next Big Thing?, https://advancetec.co.uk/tech-today/a-look-at-4d-printing-the-next-big-thing/ (accessed: November 2020). [Google Scholar]