Bioprinting: Prospects, considerations and challenges for application in South African clinical environments

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Bioprinting advances have revolutionised drug discovery and are set to disrupt biomedical research and medical application through the development of reproducible, fine-tuned functional 3D tissues and, eventually, whole organs. This intersectional bottom-up approach of additive manufacturing requires collaboration between tissue engineers, materials chemists, software and electrical engineers and medical practitioners for the software, hardware and wetware required by this disruptive technology. This review provides a current overview of the state of the art of bioprinting and the biomaterials/bioinks required, as well as the challenges and prospects for medical application in South Africa.

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Additive manufacturing (AM), a computer-controlled bottom-up building approach, has recently found its place within the medical sphere with an increasing portfolio of healthcare applications. There are four main foci of this technology in medicine: (*i*) anatomical models; (*ii*) surgical equipment; (*iii*) organic and non-organic implants; and (*iv*) prostheses.[1]

Three-dimensional (3D) printing is an interdisciplinary AM technology that has rapidly advancing applications in medicine.[2] Although prototyping, design and development remain the top uses of AM (~64%), applications in the medical field are seeing a rapid increase. In 2010, 3% of hospitals in the USA made use of centralised 3D printing facilities, whereas in 2016, this increased to 99% of US hospitals, where applications ranged from custom prosthetics to 3D printed organ models from CT scans for patient education and surgical guides.[3,4] Official statistics regarding 3D printer usage in South African hospitals are largely unknown.

As part of regenerative medicine, 3D printing applications focus largely on the engineering of functional human tissue termed bioprinting that makes use of specially designed bioinks (hydrogel-based biomaterials) in the fabrication of tissue constructs by patterning using modified 3D printing technology. It reduces the gap between *ex vivo* cell cultures and *in vivo* cellular tissue models, and there has been a significant increase in research and development for biomedical application.[5] 3D printing has been used in healthcare for manufacturing of hearing aids, prosthetics and dental apparatuses. In the medical field, AM has been confined to the production of static structures, such as patient-specific craniofacial implants, hip and mandibular prostheses and the manufacturing of scaffolds for tissue engineering. While it is far from being a commonplace clinical application, the field has seen rapid growth in bioink/biomaterial development, hardware and software advancement, and preclinical testing for eventual clinical translation.

Bioprinting meets the demand of highly personalised clinical treatments by using magnetic resonance imaging (MRI) or computed tomography (CT) scans and computer-aided design (CAD) software to design patient-specific constructs (both organic and non-organic) to address concerns of rejection currently faced in organ transplant therapies by using the patient's own cells. One of the most important and immediately applicable technologies is the ability to create *in vitro*, patient-specific disease models for tailored drug discovery in personalised medicine.[6] Aside from the ethical and regulatory issues faced with implementing bioprinted constructs towards clinical translation, consideration must be given to the difficulties in developing accurate and adequate biomaterials, manufacturing strategies and vascularisation.[7] Previously, the choice in biopolymers used for conventional 3D tissue engineering has typically been based on availability and previous experience with these materials; however, the focus has recently shifted towards more complex bioink formulations to more accurately resemble the responsiveness of native extracellular matrices.[8] Furthermore, these novel bioinks are not always compatible with the growing number of commercially available 3D bioprinters and typically lack the structural integrity needed for optimal bioprinting.[9] Engineering vascular networks within tissue constructs is perhaps the greatest challenge faced by researchers,[10] as tissues of the simplest structures will not survive without the necessary network for waste disposal and nutrient delivery.[7]

Along with the development of more efficient bioprinting processes, there are simultaneously, rapidly growing alternative technologies/research areas that aim to target organ shortages and patient-specific therapies within the field of regenerative medicine. Organ decellularisation and recellularisation is of particular interest as a newly emerging tissue engineering strategy.[11] Organ decellularisation is a top-down approach to tissue engineering and focuses on the use of chemically and enzymatically decellularised extracellular matrices (ECMs) to allow for cell seeding.[12] This strategy has been studied with regards to volumetric muscle loss,[12] intervertebral disk degeneration,[13] vascular grafts,[14,15] constructing miniature humanised hearts for improved drug discovery systems and understanding cardiac biology,[16,17] as well as diabetes, through pancreatic organ engineering.[18,19] Issues faced with transplanting of bioengineered organs, such as vascular perfusion, recellularisation, engraftment and, importantly, animal survival, have recently been tackled with great success by Nichols *et al.*[20] in a recellularised lung transplantation in a preclinical porcine model. Organ complexity with respect to level of organisation and cellular heterogeneity is a

major stumbling block in this technology, and the same can be said about it being a major hurdle for the future of bioprinted organs.

This review aims to outline AM for medical application, the state of the art of 3D bioprinting with respect to available and developing hardware and related techniques, biomaterial research and development and application in personalised drug discovery and regenerative medicine. A proposal of the future of this technology in clinical application within South Africa (SA) is also discussed.

Non-organic additive manufacturing in medicine

The rapid prototyping nature of 3D printing speaks to the needs of the demanding clinical environment. To find its place in biomedical applications, the technology must meet the demands of high complexity, improved accessibility to the technology (for both patients and clinicians), ease of customisation, patient-specific necessities, small production quantities and, most importantly, easy public access.[21] The small production quantity speaks to the bespoke manufacturing of highly complex objects nature of 3D printing. As of 2018, the main types of AM techniques used in the biomedical field include: powder-bed fusion; inkjet printing and contour crafting; stereolithography; and direct energy deposition (Table 1).[21] A hallmark of AM is that it allows engineers to rapidly manufacture and iterate designs, which ultimately reduces time to market. This is important in the medical field as the success of any newly manufactured medical device relies heavily on the feedback from physicians and patients, and AM can then allow for a more rapid speed at which the design improvements may be implemented. This fast feedback loop accelerates design development and will encourage rapid diagnostics and treatment. The biomedical market is one of the greatest drivers for AM advancement – it represents 11% of the total AM market share to date.[21] AM is proving to be vital in many patient-specific therapies that rely heavily on the ability to customise treatments to individual patients. Hearing aids,[22] drug delivery methods,[23,24] dental,[25] orthopaedic[26] and paediatric implants [27] are a few of the therapies that have been revolutionised by the development of AM technologies. Furthermore, in the hope of reducing complication rates, researchers have found 3D printing to be invaluable in providing medical students with more realistic models for better preparation before performing complicated surgeries on real patients.[28]

These applications further highlight 3D printing as an attractive endeavour within the clinical field in terms of its highly personalised patient-specific designs, on-demand fabrication of complex structures and high precision. The National Institutes of Health (NIH) 3D Print Exchange (https:// https://3dprint.nih.gov) is an online repository of biomedical 3D printing files, modelling tutorials and educational material.[29] With AM being medicine's next frontier, making the

Table 1. Additive manufacturing technologies used in medicine*

Application	Technology	Percentage (%)
Anatomical models	Powder-bed fusion	24[59]
	Material extrusion	32[60]
	Material jetting	12[61]
	Vat photopolymerisation	15[62]
	Binder jetting	8[63]
	Bioprinters	1[64]
Prosthetics and/or orthotics	Powder-bed fusion	23[65]
	Material extrusion	38[66]
	Material jetting	15[67]
	Vat photopolymerisation	8[68]
	Binder jetting	9[67]
Dentistry	Powder-bed fusion	16[69]
	Material extrusion	16[70]
	Material jetting	19[69]
	Vat photopolymerisation	26[71]
	Binder jetting	3[72]
Non-resorbable patient-matched implants	Powder-bed fusion	45[73]
	Material extrusion	10[74]
	Material jetting	5[67]
	Vat photopolymerisation	10[75]
	Binder jetting	5[76]
	Direct energy deposition	10[77]
Resorbable patient-matched implants (including scaffolds)	Powder-bed fusion	23[3]
	Material extrusion	15[78]
	Material jetting	8[79]
	Vat photopolymerisation	15[80]
	Binder jetting	15[81]

*Adapted from Awad *et al.*,[82] and Medical Additive Manufacturing/3D Printing Annual report.[4]

technologies publicly available is an essential step toward successful application. Despite its noteworthy progress in the medical field, applying AM technologies to existing therapies has limitations. Although non-organic implants have favourable mechanical strength and friction-resistance, the high strength and elastic modulus do not match that of native bone tissue and therefore can cause prosthetic loosening due to a stress-shielding effect.[30] Furthermore, the preferred materials used for non-organic implants include gold, titanium and stainless steel, which are incompatible with magnetic resonance imaging (MRI). This limits the possibility of postoperative MRI examinations.[30] Hypersensitivity reactions can also be triggered with long-term presence of non-organic materials within the human body.[30] While non-organic AM technologies are leading the charge for AM to the clinic, there remains a growing demand for a more regenerative approach. Bioprinting, through the use of stem cells, biomaterials and controlled patterning, provides a viable option within tissue engineering.

Bioprinting: A highly specialised manufacturing approach

Biofabrication has shown to be of great importance and potential in regenerative medicine by allowing the generation of constructs that closely resemble the complexity of tissues and organs.[31] Biofabrication can be defined as the exploitation of self-arrangement and self-assembly of biological systems to form biologically functional and structurally organised constructs;[31] positional control through the bioprinting process further facilitates this. Bioprinting and bioassembly fall under and constitute a pedestal of biofabrication.[31]

A rapidly advancing technology such as bioprinting has many different approaches. However, all follow the same pre-processing (imaging, design approach, material selection, cell selection), processing (bioprinting inkjet, microextrusion, laser-assisted, direct light processing) and post-processing (application, maturation, implantation, *in vitro* testing) workflow.[7,32,33] The three pillars of successful bioprinting include hardware, software and wetware considerations, as outlined in Fig. 1. Liu *et al*.[34] provide an in-depth review of the different types of bioprinter. There are five main principles for bioprinting: stereolithography, extrusion-based, laser-assisted, inkjet-based and nano-printing.[35] Extrusion-based bioprinting has been largely focused on for the manufacture of 3D tissue constructs, owing to its many advantages compared with other methods, including but not limited to the following: high cell viability;[7] flexible geometric shapes;[36] ability to incorporate multiple biomaterials and cell types;[37] homogenous and heterogeneous structures can be created;[33,36] easily updated software and hardware; and the ability to include multiple bioinks/biomaterials in order to account for the high level of complexity within tissues and organs. Disadvantages such as the need for low viscosity of bioinks, lack of precision of droplets, distortion of cell structure, long printing times, high cost and use of intense UV light make inkjet, microextrusion, laser-assisted and direct light processing bioprinting less favourable than extrusion based bioprinting for regenerative cell therapies.[7,55,56] Although extrusion-based bioprinting has been the most widely accepted method for tissue engineering, developments are still being made such as the multi-head deposition system which allows for the manufacturing of increasingly complex tissues.[38] *In situ* bioprinting has also been cited as a promising approach to regenerative medicine in terms of facilitating graft or implant customisation and also, providing the building blocks to drive translational research.[38] Bioprinting has the potential of revolutionising personalised medicine and drug discovery.[24] It has been predicted to cause a substantial paradigm shift in drug

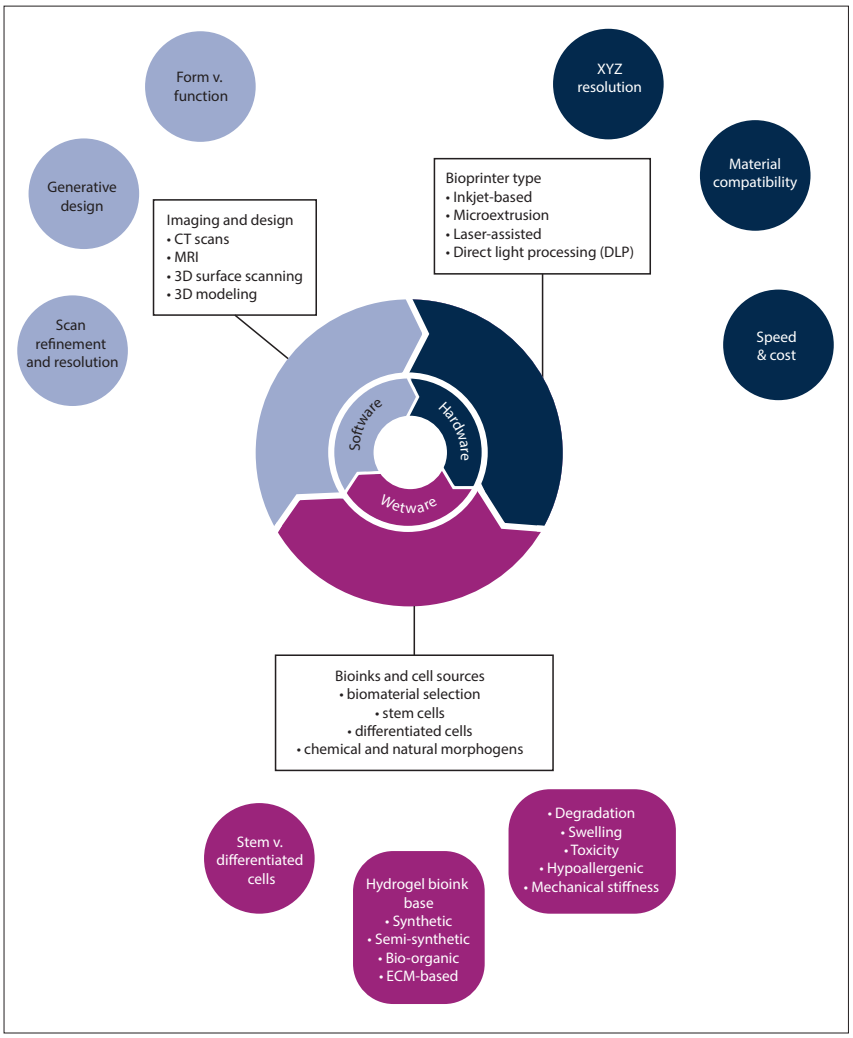

Fig. 1. Considerations for rapid prototyping in bioprinting. The interlinked process model describes the three pillars required for successful bioprinting, i.e. software, hardware and wetware development. Here, wetware is used as an all-encompassing term to describe bioprinted materials and tissues. Factors that influence imaging and design (software), types of bioprinter (hardware) and bioinks and cell sources (wetware) are shown in smaller circles outside the interlinked circles. (CT = computed tomography; MRI = magnetic resonance imaging; ECM = extracellular matrix.)

design, formulation and production by providing flexibility and autonomy to existing treatment processes.

Control of the printing process is highly reliant on positioning of the 3D printhead, which is itself dependent on the input data. The Radiological Society of North America (RSNA) 3D printing Special Interest Group (SIG) recently proposed guidelines with respect to the use of imaging data for application in medical 3D printing.[39] These include guiding principles on image resolution and processing, through to the eventual maintenance of image fidelity in 3D printing and post-processing of the printed construct. While these guidelines are highly applicable to the process of bioprinting, there should be consideration for the inclusion of generative design. Generative design is a computer-controlled human-guided design process that utilises a biomimicry approach to building stable, efficient structures that could assist greatly in bioprinting, considering the micron scales of biological detail.[40]

The levels of scale and organisation in biological tissues and organs cannot be ignored and is an important factor in bioprinter development and selection, as the XYZ resolution in current printing processes is controlled by both the printer as well as the bioink.

Biomaterials and bioinks

There are two main types of cells that can be used for bioprinting – stem cells and differentiated cells. Stem cells are the favoured type of cell choice as they have properties of self-renewal and potency, allowing for an unlimited cell source for bioprinting. In addition to this, stem cells have low immunogenicity properties, favouring their use in bioprinting for regenerative medicine. Differentiated cells lack potency and have a finite life span as well as increased immunogenicity effects, which disadvantages them for use in bioprinting for regenerative medicine.[41,42]

Biomimicry, in which function follows form, is the most widely used approach to bioprinting; this is a strong consideration when approaching the development of wetware (Fig. 1).[7] In order to achieve a construct that closely mimics that of native tissue, specifically designed and formulated bioinks are used. There are two main types of bioink materials currently used in 3D bioprinting.[43] The first is a cell scaffold-based method in which the bioinks consist of biomaterials combined with living cells, used to print 3D tissue constructs. The second method makes use of cells printed directly in a manner in which it resembles normal embryonic growth and patterning.[43] These neotissues form functional tissue structures over time.[43] Biomaterials incorporated in the bioink formulations are included to promote *in vivo* revascularisation in the host tissue, safely degrade at a similar rate of tissue formation and prevent inflammatory responses which could cause rejection of the new tissue.[44,45]

In cell scaffold-based bioprinting, scaffolds are used in order to minimise and control the complexities found within native tissues.[8,46] An optimally designed scaffold will closely mimic the native structural and mechanical properties of a target tissue.[7] A poorly designed scaffold, on the other hand, will result in ineffective cell seeding and reduced cell viability, which will in turn result in poorly engineered tissue models.[47]

The term 'scaffold' refers to an artificially designed 3D structure which allows for the growth and proliferation of seeded cells such that the combination of cells and scaffold produce a viable tissue model.[2] Scaffolds are essentially artificially engineered extracellular matrices (ECMs). The design and optimisation of artificial scaffolds for tissue engineering is an attractive avenue in the advancement of bioprinting as the success of the printed tissue model relies heavily on the microenvironment in which the cells are grown.[8] Furthermore, the architectural design of scaffolds greatly influences mechanical properties and cell behaviour.[48] Scaffolds and other printed constructs are engineered from commonly used medical images using CAD, making bioprinting a highly custom- and patient-specific technology.[2,7] Scaffolds need to provide appropriate attachment factors, whether biological or structural, in order to support cells during deposition, which is a process characterised by substantial mechanical and thermal changes.[7]

The modification of scaffolds in terms of their structural design, surface properties, addition of various bioactive molecules or nanoparticles for the enhancement of attachment, proliferation and differentiation of specific target stem cells is referred to as 'smart scaffold' design. Designing appropriate scaffolds and bioinks remains the core challenge in the advancement of 3D tissue engineering and has frequently been cited as the most common inhibitor of bioprinting technology growth.[32]

Hydrogels are water-swollen polymers used as scaffold materials in 3D tissue engineering, as they are tunable, affordable, biocompatible, biodegradable, safe and have desirable mechanical properties similar to natural ECMs.[46] Hydrogels have either synthetic (polyethylene oxide (POE), poly(vinyl alcohol), poly(acrylic acid)) or natural (alginate, collagen, hyaluronic acid) polymer chains.[49] To address the issues related to lack of vascularisation in 3D-printed tissue constructs, there has been an increasing focus on the development of more complex and responsive scaffold materials. Earlier studies were focused on simpler alginate, collagen and agarose-based bioinks, while more recent studies have developed application-specific bioink formulations.[50] Supramolecular polymers have been investigated as potential alternatives to hydrogels as the primary scaffold material used in tissue engineering and AM.[51] Four-dimensional (4D) printed scaffolds have also been a focus of recent studies, tying into the 'smart scaffold' concept, where 3D shape memory polymers are exploited to deliver time-controlled stimulus on encapsulated cells.[9] It may be argued that bioprinting in itself may be classed as 4D printing (the fourth dimension being time), as cells and the printed construct as a whole change over time depending on the environment. This may be key to driving neovascularisation of printed constructs through the defined patterning of seed cells (endothelial) and homing beacons (e.g. vascular endothelial growth factor) to stimulate the vascularisation process.

Importantly, the success of a novel bioink as scaffold material in an engineered tissue construct relies heavily on the ability to 3D bioprint the newly designed bioink. The advancement of 3D printer technology is as imperative as the development of novel scaffolds in successful tissue engineering. Older 3D printers are not designed to print complex bioinks and have provided the basis for further 3D printing development.

The South African bioprinting context

The South African Department of Science and Technology (DST) officially launched its Additive Manufacturing Strategy in 2015 outlining a road map for adoption of AM as the cornerstone of SA's contribution to the Fourth Industrial Revolution.[52] Approximately ZAR358 million has been invested into the advancement of 3D printing technologies since 2014. AM provides a unique opportunity to offer more localised and distributed research systems, a more sustainable and open-source economic model, and allows for an increase in investments and decrease in maintenance. While the strategy is largely targeted at the traditional manufacturing sector, the adoption of the technology in SA and the trickle-down effect

Table 2. Bioprinting and non-organic AM technology for drug discovery and clinical application in South Africa

Institution	AM technology	Application
North-West University	CellInk BioX	Drug discovery*
University of the Witwatersrand	EnvisionTec GmbH 3D Bioplotter (4th generation)	Drug discovery* and drug delivery
Rhodes University	Proprietary system under development (2nd generation prototype)	Drug discovery*
Central University of Technology	Multiple non-organic commercial systems	Clinical implants (titanium)

*Development of 3D tissue models.

on the medical and healthcare sector cannot be underestimated. The contribution to biomedical research and clinical applications in SA is yet to be determined. Table 2 presents current efforts at various institutions in SA with respect to biomedical AM and bioprinting. While the Central University of Technology Centre for Rapid Prototyping and Manufacturing has made a significant contribution to the development of medical AM applications through the development of titanium implants,[53-56] most of the current efforts around organic bioprinting with bioinks are centred on the development of reproducible preclinical human drug discovery models (see Sithole *et al.*[48]). The clinical importance of these systems cannot be ignored with respect to the establishment of a personalised medicine therapeutic pipeline. Stellenbosch University has recently launched a 3D printing laboratory in partnership with the Division of Orthopaedics at Tygerberg Hospital, which is focused on the delivery of patient-specific models for surgical planning and represents a further leap in establishing the promise of this technology within the SA clinical environment. These projects feed into the South African Bioeconomy Strategy, with the health sector being identified as one of three key economic sectors most in need of implementation of the aforementioned strategy. A strategic focus placed on bioprinting and medical 3D printing will therefore certainly aid SA to achieve the strategic goals within the health sector. Furthermore, projects focused on bioprinting aim to provide key research into the engineering of tissue, which will address the disease burden prevalent in SA and will therefore contribute substantially to the advancement of the biopharmaceutical industry in SA.[57] Research into the advancement of bioprinting and AM can in turn aid in transforming the SA economy into a knowledge-based economy, which is the second initiative of the Bioeconomy Strategy.

The African Tissue Engineering and Regenerative Medicine International Society (ATERMIS) was officially launched in 2017 at the 2nd International Conference on Tissue Engineering and Regenerative Medicine as the African chapter of the Tissue Engineering and Regenerative Medicine International Society (TERMIS) to build and strengthen tissue engineering research and translation thereof within Africa.[58] This Society provides a viable platform to propose bioprinting workshops and workgroups/networks of all researchers and practitioners currently working in or with an interest in the field. Workshops would be of benefit to bring together clinicians and researchers to build translational application of laboratory investigations. Arguably the largest grouping of scientists working within this field in Africa is in SA, owing to excellent research facilities and n environment conducive to to foster further development. Within the context of the DST BioEconomy and the AM strategies, available relevant expertise needs to be leveraged.

Conclusions

AM has the potential to produce viable and functional structures for the advancement of personalised medical care.[3] Three-dimensional printing meets various demands of individualised medical treatments and has numerous advantages. However, it is a relatively new technology in the clinical environment, and current challenges include: designing appropriate bioinks; manufacturing more efficient bioprinters; addressing the lack of vasculature present in printed constructs; and maintaining long-term survival of the printed tissue constructs.[5-8] To date this type of AM has not been extensively applied in the clinical environment but still shows great potential in providing alternative and reliable personalised therapies.[3] While whole-organ printing is not yet a reality, further dedicated research and time will help current AM technologies evolve into revolutionary treatment alternatives.

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