Guidelines for Establishing a 3-D Printing Biofabrication Laboratory

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Abstract
Advanced manufacturing and 3D printing are transformative technologies currently undergoing rapid adoption in healthcare, a traditionally non-manufacturing sector. Recent development in this field, largely enabled by merging different disciplines, has led to important clinical applications from anatomical models to regenerative bioscaffolding and devices. Although much research to-date has focussed on materials, designs, processes, and products, little attention has been given to the design and requirements of facilities for enabling clinically relevant biofabrication solutions. These facilities are critical to overcoming the major hurdles to clinical translation, including solving important issues such as reproducibility, quality control, regulations, and commercialization. To improve process uniformity and ensure consistent development and production, large-scale manufacturing of engineered tissues and organs will require standardized facilities, equipment, qualification processes, automation, and information systems. This review presents current and forward-thinking guidelines to help design biofabrication laboratories engaged in engineering model and tissue constructs for therapeutic and non-therapeutic applications.

Keywords
Bioprinting, Biofabrication, Tissue Engineering, Cloud Manufacturing, Deep Learning

1. Introduction

The adoption of advanced manufacturing (AM) to create patient-specific devices and implants is resulting in improved life-changing outcomes. A one-size-fits-all approach is no longer desirable in today's personalised society, and this remains true within the hospital and healthcare sector with increasing demand for customised and personalised medicine. As we move towards the reality of customised implants containing the patient's own cells, or drug delivery systems personalised to the patient's genetic make-up, a major role exists within the AM sector to elevate these therapies for maximum clinical impact. The convergence of AM with medical scanning and 3D computer modelling enables improved personalisation, with medical implants designed to precisely fit defected tissue sites and improve identification of areas prone to re-fracture or injury. Furthermore, these digital technologies can allow computer modelling of tissue growth and mechanical load calculations to improve implant design, success and rehabilitation planning [1]. Beyond the implants themselves, personalised digital models can be 3D printed to assist clinicians in communicating procedural details with patients or for practicing the surgical procedure in advance [2]. These models can also be used to test the quality of fit of tools and implants prior to the operation, leading to improved economical and clinical outcomes [3].

With tissue engineering and bioprinting in its early stages, it is difficult to predict how a biofabrication industry will materialize. Many organizational models have formed and the growing interest of universities, pharmaceutical industry, medical technology companies, food industry, governments, private and public health systems could generate novel hybrid models. The healthcare industry is in a state of centralization along with many other industries. The economic conditions stemming from market and regulatory forces favor the

formation of certain types of business and organization models and determines the industry's expected innovation rate. Disruption can occur at any point in time making forecasts mostly conjectural. Thus far, biofabrication laboratories have arisen in the settings of university hospital partnerships (BioFab3D, Herston Biofabrication Institute, Wake Forest Institute of Regenerative Medicine) [4-6], biotechnology companies (Organovo, Inc., Aleph Farms), private health systems (Mayo Clinic) [7], and public-private partnerships (ARMI-BioFabUSA, CSIRO Manufacturing) [8-10]. This paper uses the perspective of 3D printing laboratories which have been developed in university hospital partnerships, public-private partnerships, and private health system models. However, the recommendations shared may be applied by all organization models in the 3D printing biofabrication community. Interested parties will be able to consult this paper for design considerations then tailor their laboratory spaces according to their budgets or their areas of specialization. This paper aims to provide a general set of laboratory design instructions within a single document in an attempt to increase the number of functional biofabrication laboratories which are successfully established. Developing effective tissue engineering technologies and successfully translating technologies into approved products is the greatest challenge ahead for biofabrication. However, the bottom-up approach to this challenge is put forward where everyday practices and interactions fostered by laboratory spaces become the prime movers in the growth of processes, technologies, and products. This comes in contrast to a top-down approach that starts with a successful product developed by large research universities, corporations, or government bureaucracies that prompt new firms to form. The bottom-up approach emphasizes spontaneity by trial and error rather than orderly top-down knowledge transmission. By increasing the number of laboratories and players we expect the following likely benefits to emerge from biofabrication development:

a.) Increase the number of innovators in the form of tinkerers, hobbyists, and entrepreneurs.
b.) Increase the number of hypotheses, experiments and trials that generate greater information and new knowledge.
c.) Increase the number of applications and their impact by bringing technology closer to the clinic.
d.) Provide greater training, diversification and employment of personnel for a biofabrication-ready work force.
e.) Diversify the scope of interests ranging from organ, tissue, bioink, and biomaterial development.
f.) Stimulate the organic formation of local supply chains for future industry growth.
g.) Increase competition for funding and commercialization to decrease the funding/survival of unsuccessful activities.
h.) Increase the number of biofabrication interest groups to advocate regulatory reform at local, regional, and national levels.
i.) Keeping new IP and commercialization opportunities onshore to improve local economy
j.) Enable clinicians to work closely with engineers to embrace innovation to create faster, cheaper and more automated healthcare solutions to improve patient quality of life.

Biofabrication is a field of advanced manufacturing where specialised 3D printers and biocompatible materials are used to produce personalised tissue constructs. This field has seen enormous growth over the years, and it is likely that hospitals around the world will eventually house facilities containing both advanced manufacturing and biological capabilities. These facilities will have the capability to fabricate tissue constructs matching the patient's anatomy and the technologies to process and culture the patient's own cells for tissue healing. Because of its significant impact on the healthcare sector, AM is projected to be a multi-billion-dollar market before 2024 [11]. Wohlers Report forecasts that the three-dimensional printing (3-DP) industry will have revenues exceeding $35 billion in 2024 [12], largely due to growth within the healthcare industry [11]. Recent advances in tissue engineering and printer technology has greatly increased the applications of 3-DP in hospital settings. As an example, *LimaCorporate* recently partnered with the Hospital for Special Surgery to create the first AM 3-DP facility for printing patient-specific orthopedic implants in a clinical setting [13]. The Mayo clinic, BioFab3D, Wake Forest IRM, and the Herston Biofabrication Institute are some of the earliest hospital-based biofabrication laboratories committed to developing personalized tissue-based therapeutics. The broader applications of advanced manufacturing in healthcare extend beyond tissue engineered constructs to the fabrication of personalised protective equipment and components (such as ventilator parts), the production of tissue-engineered structures for disease modelling and drug discovery, generation of anatomical models for medical training and surgical planning, and the engineering of implants and custom prosthetics [14]. As 3D printers utilize a variety of non-biological (e.g. polymers, metals, ceramics) and biological (e.g. decellularized matrices, cells) materials, their applications in the hospital setting also encompass rapid scalability during shortages, supply change adaptability, improved cost efficiency, and greater productivity [1-3,15]. With many healthcare centers seeking to establish 3D-P and biofabrication laboratories, we sought to establish a set of guidelines to inform the incorporation of a manufacturing center within a traditional healthcare infrastructure. Critical to the success of such a center is the requirement to facilitate collaboration between the transdisciplinary

workforce, with the need to enhance communication across the traditional domains of science, engineering, medicine and regulation. Silos cannot exist, and instead improved collaboration is required with a common goal of better patient outcomes. Beyond workforce collaboration, the facility needs to consider the different technological requirements of clinical scanning, computer modeling, 3D printing and tissue engineering and the need to design smoothly transitioning between the technology areas to maintain efficiency, biological sterility and good manufacturing practice (GMP) capabilities. In addition to the workforce and the space, the tools and equipment within the facility are also critical to enable the best possible technologies to be produced in a quality controlled and regulated manner.

As biofabrication services become more in demand at the point-of-care, many health delivery systems will experience numerous challenges associated with the conversion of facilities designed for a non-manufacturing sector or the construction of entirely new manufacturing facilities. Here, we review the specific building requirements, biofabrication equipment and supplies that are required to establish a functioning clinical 3-DP facility. In addition, we will discuss the basic quality management systems that are required to mitigate product variation and defects as well as improve operational efficiency. Finally, we will examine the systems that healthcare organizations will need to develop to manage teams of biofabrication experts, data management and storage, tissue and biologic repositories, regulations, quality control and commercial processes [16].

2. Site Choice and Regulations

Ideally a hospital-based 3D-P biofabrication facility should be located as close as possible to the point of care, with the site being integrated within a hospital campus. This enables critical interaction and rapid communications between clinical staff, researchers and technical experts in all areas of scanning, imaging modelling and 3D-P. The spaces within the institute should be connected in such a fashion as to support the workflow. For example, the scanning area should be adjacent to an area that patients can enter and exit the building discreetly, 3D modelling should be connected to this space, and the 3D-P area should be quite separate and encompass post processing areas and workshops. Those designated “dirty areas” such as workshops should not be located too close to the cell biology and cell culture facilities. It’s also important to consider the implant journey and the need for quality control, regulation, and GMP including the installation of restricted access to these spaces ensuring only authorised personnel can enter. New laboratory facilities will need to be in compliance with one or more municipal, regional, and national jurisdictions. These governing units determine the appropriate building codes, construction methods, building use classification, connection of utilities, fire district regulations, permits for laboratory ventilation systems, etc. Approvals by local governing boards should be obtained before establishing a new laboratory and this is particularly important when these facilities are established on hospital campuses. Industrial insurance carriers should be involved in building plans to determine key design criteria. The International Building Code (IBC, 2019) classifies laboratory buildings engaged in clinical medicine, research, and education at Class A building construction and use Group B. Biosafety Level 1 (BSL-1) laboratory standards has been proposed for bioprinting, however we recommend BSL-2 laboratory for bioprinting [17]. Laboratories should adhere to the biosafety specifications designated in their respective countries (HHS, Council Directive 90/679/EEC, CBS, ABSANZ, etc.) [18-20].

3. Power Considerations

Albeit not unique to 3-DP labs, electrical requirements for printers do present unusual challenges. Prior to the installation of the 3-DP, it is necessary to allow for both a safe environment and future compatibility (i.e. “future proofing”) considering 3-DPs are often upgraded within 24-36 months. The National Electrical Code (NFPA 70, 2011) or International Electrotechnical Commission (IEC 60364) should be consulted to determine wire size and insulation type needed current loads expected for the printing facility. Requirements for electrical machinery and control processes can be found in the NFPA 79 for laboratories based in the United States, while European laboratories should review IEC 60204. Poor power quality can lead to device malfunction, premature failure, or inability to operate. Common power quality problems include blackouts, noise, and frequency or voltage variations (21). Unexpected power disturbances can cause damage to equipment, materials, automated testing devices, which ultimately causes productivity losses. It is strongly advised to install uninterrupted power supplies (UPS) to all equipment to negate any power interruptions, which will be detailed subsequently. Periodic inspection of electrical circuits and components is necessary, and annual preventative maintenance should be completed (NFPA 70B). Inspections should occur more frequently in clean rooms and manufacturing areas. In Europe, periodic inspections are provided by local regulators.

3.1 Voltage/Amperage Requirements

The first consideration for 3-DP installation is adequate allotment for the building’s electrical utility. The standard electrical utilities are often inadequate in their ability to provide appropriate power requirements for high current loads. Non-industrial outlets are limited to 20 amps, while most industrial (high current) loads

require at least 30 amps. A powerful heated build plate in 3-DP plays a critical role in printing large components. To set the plate temperature up to 150°C and avoid thermal contraction of large components, high current power supply is mandatory. For example, a 3-DP with 12" bed needs at least 1000-Watt power supply to properly manage the temperature of the plate. Therefore, the current limit should be considered in early design of a 3-DP lab to accommodate future expansion of the number of printers and decrease the cost of renovations. It is highly recommended to have a 30-amperage breaker with 10-gauge wire run to the outlets.

Laboratory designers should consult an electrical engineer to ensure that power is clean and the voltage does not drop considerably during the operation of 3-DPs. Quality design limits voltage drop across feeders to 2% and branch circuits to 3%. An electrical engineer should perform a power quality analysis to determine possible sources of "dirty" power by examining the building's current electrical bus. For example, a Fluke 123 Industrial ScopeMeter could be used to analyse and monitor any voltage fluctuations caused by other electric sources. By identifying these sources, the printers could be isolated from unwanted loads via an isolated and dedicated electrical bus for the lab. In addition, 3-DPs should be isolated from unwanted high magnetic fields sources such as MRI magnet. These considerations would prevent power loss to 3-DPs and negate irregularities and printing failures.

Another consideration is the importance of having both 220V and 110V outlets in the labs. Higher voltage/current may be required if powering large printers, multiple printers or devices involved in printing process [22]. A power consumption monitoring device (*Watts*Up power meter) can be connected with the 3-DP and installed into the outlet to measure consumption during printing processes. One study used *PronterFace* software to characterize and fine-tune the energy output for motor, heater and fan components [22]. Recording available power to 3-DPs can also provide liability protection should prints fail. A 25% reduction in energy utilization by 3-DPs occurred after using *PronterFace*. In addition to power supply, the location of the outlets is critical and areas should be designed to ensure that the power can be continually and optimally supplied (i.e. from the ceiling, beneath the floor or on the walls).The venting systems should be considered as they dictate that equipment must be located adjacent to external walls (see section 6).

3.2 Redundant Power Sources

Another critical feature for the lab is establishing an emergency power supply and uninterruptible power supply (UPS) rated for the printers, devices critical to the 3-DP's operation, and critical process systems—HVAC systems, cold rooms, refrigerators, and freezer equipment containing valuable materials. Emergency power sources can be storage batteries, diesel engine generators, and natural gas generators. Emergency power should be available within 10 seconds making diesel engine generators preferable to natural gas engine sets [23]. Installing UPSs provide an excellent solution since some labs are not part of the building's emergency power system and these redundant power sources can take a few seconds to restore power. A general rule when deploying a central UPS is to calculate the cumulative amperage requirements of the devices and then design a UPS to handle double this value. This will ensure that there is an adequate surplus for severe overload. In case the UPS experiences continuous overload conditions, its own circuit protection will command it to shutdown resulting in an abrupt loss of power. It is important to determine if the emergency power system will be used to power the 3-DPs for completion of prints already in progress which can be upwards of 20 hours. These few seconds would not only result in the loss of an ongoing print (which can already be 20+ hours in) but can additionally harm the sensitive electronics of the 3-DP. Therefore, it is recommended that these systems be placed on emergency power or UPS. Heated extrusion printers often rely on the onboard fans for adequate cooling. Improper fan function may cause damage to internal printer components which are not apparent to the user if exposed to a temperature greater than 230° C. This may give rise to defects, failed prints, and the second order effects of wasted material, labor, maintenance costs if the manufacturer used fluorinated hydrocarbon-based materials for thermal breaks. Bioprinting laboratories should consider the effect of power interruption on the completion of batch processes (e.g. cell cultures and bioinks) to avoid jeopardizing intermediate and final products. Finally, automated testing systems require clean electric power and continuous operation to ensure quality control measures. Table 1 provides a summary of the electrical codes and standards laboratories should adhere to for particular countries.

Codes and Standards	AU	EU	US
Installation	AS/NZS 3000	IEC 60364	NFPA 70 (NEC)
Industrial Machinery	AS/NZS 3000	IEC 60204	NFPA 79

Table 1: Electrical codes and standards for pharmaceutical plants from Australia (AU), European Union (EU), and United States (US).

4. Procedural Safety

Adequate "policy" of the lab's power outlets dedicated to the printers, is necessary as non-familiar staff may plug in non-critical components into the UPS's circuit. Therefore, battery depletion prior to effective back up time or circuit overload can occur since the UPSs often have a lower output rating than non-UPS circuits. Another common pitfall is not "tagging out" these outlets or making them physically inaccessible to avoid depletion by non-essential equipment. As such, we recommend adequate signage at the entry of the labs prohibiting external devices or unplugging existing devices without the consent of the lab supervisor. It is also recommended that individual plugs be labelled with the following details: the plug's sustained amperage, service panel location for the plug's respective breaker, if it is dedicated to a UPS or a 3-DP, and color coded to indicate whether it is UPS-backed outlet, line filtering only, or can be used as a standard plug.

The most common cause of problems and outages is the result of improper power system and equipment grounding. These failures occur from poor design and installation of power systems, rather than failure of power systems themselves. New 3-DPs require proper electrical grounding to avoid safety incidents, which often stem from coupling equipment from different manufacturers [24]. Grounding of equipment not only mitigates the risk of electrical shock to lab staff in the event of an electrical fault but also reduces the risk of stray electrical charge such as static discharge e.g. "static shock" or electromagnetic interference (EMI) that damages sensitive electrical components. A ground bus bar should have the shortest distance to the grounded devices to minimize the length of the ground wire and therefore its resistance. The ground bus bar's location should be easily visible by lab staff to enable ease of periodic inspection of connections to guard against loose or damaged ground straps. Sensitive electrical equipment to EMI often has an attachment point for a grounding strap. Alternatively, the 3-DP manufacturer can recommend locations to install a proper chassis ground. Some 3-DP labs may be co-located in a medical building that has high voltage devices (e.g. medical imaging devices and defibrillators). Care should be taken that the building's earth ground is in good repair as these are often metallic rods installed during the building's initial construction and may degrade over time.

Another consideration is the proper allocation of demarcated physical space and electrical outlets for wet stations, which are becoming common place in 3-DP post-processing areas. Wet stations ideally should be in a dedicated portion of the lab demarcated for use of liquids. Physically spacing non-ingress protected (IP) rated devices from fluids minimizes corrosion from the evaporation of volatile chemicals as well as decreases the risk of unintentional splashes or spills. Flooring should not be carpeted but rather a non-slip, non-absorbent surface which is tolerant to chemicals used in post-print processing (e.g. acetone, ethanol alcohol). The electrical outlets should be equipped with ground fault circuit interrupter variant (GFCI) which mitigate the likelihood of electrical shock from liquids.

5. Vibration Isolation of the Printers

Reducing vibrational effects is often overlooked for fused deposition modelling (FDM) printer installations. Facilities should identify potential vibrational sources (traffic, trains, turbulent airflow, people walking by, etc.) and consider taking vibration suppression measures [25]. Laboratory layouts should avoid placing printers near elevators, mechanical rooms, and heavily used pathways to further reduce vibration. Printers are also prone to inducing vibrations within the cabinetry they rest upon. If the printers are left undamped, vibrations can cause artifact in the ongoing print as well as other printers sharing the workspace. These vibrations are often caused by the jerk from sudden effector plate movements and are exacerbated by increasing printer speeds. These vibrations can have deleterious effects during the printing process especially if it induces harmonic resonance of the printer's chassis. This results in significant amplification of otherwise imperceptible oscillations.

Vibrations can be mitigated by installing the printers on a "floating surface" or mounting device. This is often accomplished by placing a vulcanized rubber mat between two solid surfaces prior to machine installation. Further damping can be accomplished by applying acoustic damping materials (ADMs) such as *Dynamat* to the chassis. Care must be taken to ensure that ADMs placement which will not interfere with the printer's mechanical operation. ADMs should make contact with the printers manufacture or move the build plate as well as effector plate to all end limits to avoid mechanical interference. The material effectively converts vibration to thermal energy thereby providing further damping. Therefore, ADMs should be applied centrally to larger portions of the printer's metallic exterior chassis. Another consequence of the aforementioned processes is reduction in acoustic dB within the lab. This provides a quieter work environment for lab staff especially during multiple printers operating, simultaneously. Acoustic and structural engineers can be consulted for further recommendations on laboratory design and vibration control methods.

6. Ventilation Requirements/Considerations

Proper design for HVAC systems is essential for environmental control and active monitoring of AM clean room conditions (e.g. temperature, humidity, pressurization, and filtration). Isolating the 3-DP laboratory's heating, ventilation and/or air condition system (HVAC) from the rest of the building is highly preferred as FDM printers can be significantly affected by ambient room temperature, humidity as well as air currents over the build space. Having a dedicated HVAC system for the medical 3-DP laboratory would be ideal, and the location of the controls should be mounted in a location that is accessible to lab staff. A 3-DP laboratory should be designated as a classified space under the International Organization of Standardization (ISO) 14644-1. Classified spaces are designed to reduce airborne contaminants below a certain threshold. In addition, classified spaces are more tightly controlled for temperature and humidity than the ambient environment. HVAC systems should also have redundancies since failure of adequate cooling/ventilation would often require lab staff to suspend printing operations. Additionally, by having dedicated HVAC systems for the laboratory the system can be designed to decrease ingress of dust/foreign materials through the utilization of *high efficiency particular absorbing* (HEPA) rated filters. Filter classifications can be found in the HVAC Systems and Equipment Handbook from ASHRAE. Pre-filters should be installed to decrease the particulates reaching HEPA filters. Filters should be changed per manufacturer's recommendations. Most HVAC systems will not have HEPA rated filters, and therefore, will not adequately decrease aerosolized microparticulates that can cause premature failure of linear ball bearings. This may result in decreased tolerances and increased resistance. Increases in resistance can also negatively impact stepper motors, which subsequently increases current requirements and places greater strain on motor controllers. Decreased tolerance from mechanical wear permits excessive movement between the guide rods and linear ball bearings thereby decreasing print accuracy and increasing artifact. Inadequate filtration and airflow control can also cause contamination in bioprinting process. Sterile facilities should ensure unidirectional airflow and appropriate speed to move particles away from manufacturing or testing areas. Personnel flows should also be unidirectional to minimize the risk of contamination for bioprinting operations. Airlocks offer a physical solution to segregate areas, regulate airflow and control pressurization to further prevent cross contamination and ingress of contaminants in manufacturing areas. The U.S. FDA cGMP regulations are general for HVAC systems with regard to pharmaceutical products, however, we recommend ISO 5 (Grade A) standards for biosafety cabinets, ISO 6 (Grade B) for biomanufacturing clean rooms, and ISO 7 to 8 (Grade C/D) for support areas. Table 2 provides a summary of clean room environmental standards for different regulatory bodies.

The air exchange rate or flow of a laboratory's HVAC system is another consideration especially if working with materials that contain or release volatile solvents. In the case of metal printers, integral processes are in place which can decrease the amount of ambient oxygen. Air quality monitors should be considered/installed that measure harmful volatile organic compounds (VOCs) as well as the oxygen content in areas housing metal printers. Oxygen alarms at the entrance of the lab should be installed in these applications as many metallic printers operate in an inert atmosphere and this can lead to an oxygen deficient environment. To decrease VOCs, ventilation hoods should be appropriately placed and/or filtration systems such as IQ Air Chem filtration systems should also be considered. HVAC engineers should be consulted to design, optimize, and control laboratory conditions (temperature, humidity, air exchange, pressure) for new builds or modified spaces.

EU Grade	ISO (Standard)	US Federal Standard	Air Change per Hour
A	5	100	600
B	6	1,000	35
C	7	10,000	25
D	8	100,000	15

Table 2: Clean room environmental standards

7. Laboratory space and storage

Prior to the construction of the laboratory, careful deliberation should occur when selecting certain types of printers and their particular space requirements. Space requirements will be influenced by the size and number of 3-DP, post-print processes, workspace for lab technicians, storage of printing materials and tooling, and clean/sterile workspaces. At the entrance of the laboratory space, there should also be signage communicating required safety placards, a diagrammatic layout of the lab space, and areas requiring special garments or equipment prior to entry (these areas can also be demarcated by high visibility tape/paint on the floor if physical barriers are not a viable option). Lab spaces will be quite different in a service provision laboratory which is run by technical staff compared to a university research space for example which may have a higher turnover of users, some of whom may initially not be adequately trained on equipment or aware of safety considerations. Swipe access is advised for all spaces and appropriate training should be delivered to ensure safe activity within the spaces before secure access is granted.

Within the lab space there should also be an inventory list documenting printer location and their respective SN or local network addresses. This information will often be inaccessible once the 3-DP is installed and will make troubleshooting more straightforward when contacting the manufacturer (many printers have this information within their settings, but this can become inaccessible if the printer has a failure of its onboard power supply or display screen). This information might also extend to paperwork covering maintenance dates, contact details for technical assistance and the owner or "super user" of the equipment who should be the first point of contact within the lab. Another important placard would contain a diagrammatic representation of the laboratory's electrical layout, current power requirements of each of the 3-DP, and ancillary post-processing equipment. It should indicate the locations of the breaker enclosure and outlets, each outlet's supplied voltage and maximum amperage draw, and the 3-DP's location and its required voltage and amperage. At the breaker enclosure each breaker should list the 3-DP (if hardwired) and outlet that is supplied by the respective circuit and the circuit's amp rating.

If the lab contains hazardous materials, their quantity and location should be detailed at the entrance of the lab and in compliance with local safety/fire codes. These local safety/fire codes should be reviewed prior to the acquisition of the materials and often local fire departments have fire marshals/liaisons that can provide further guidance regarding local ordinance/registration requirements. In addition to the building's previously existing fire suppression system, bespoke halon systems can be considered as their activation/use will not damage sensitive electronic equipment. Lab staff should also have scheduled/recurrent safety briefings on the laboratory's safety equipment and its proper use.

To ensure minimal unscheduled lab downtime, replacement parts and necessary tools for the 3-DP's repair maintenance should be kept on site. There also should be procedures written for proper storage as well as which staff are allowed to perform the 3-DP repair/maintenance that is in compliance with the 3-DP manufacturer. These procedures help protect the device's functionality and avoid violating any warranties/service contracts.

8. Bioprinting aspects for a medical 3-DP laboratory

8.1 Facility requirements

Apart from the aforementioned requirements for an AM laboratory, there are many more to be satisfied for a 3D-P laboratory dedicated to bioprinting. Bioprinting is defined as "the use of computer-aided transfer processes for patterning and assembling living and non-living materials with a prescribed 2-D or 3-D organization in order to produce bio-engineered structures serving in regenerative medicine, pharmacokinetic and basic cell biology studies" [26] and is considered an upcoming technology in the AM field. In bioprinting, living materials i.e. cells and other materials with a biological origin are employed.

The facility requirements for bioprinting overlaps with modern biotechnology facilities in the pharmaceutical industry. Both should have distinctive areas for manufacturing and manufacturing support. Standard biomanufacturing areas include designated rooms or operational areas for media preparation, buffer preparation, cell separation, harvesting, and purification. Support areas in biotechnology facilities typically include a cell bank, quality control laboratory, weigh and dispense room, freezer room, and cold rooms. Facilities should have surface finishes that are durable, cleanable, functional, sustainable, maintainable, and cost-effective [27]. Design teams should create layouts using a process segregation approach to organize areas, their adjacencies, and the flow of personnel, material, equipment, and waste. This strategy mitigates contamination risk along with selecting clean space classifications for each operational area. Airlocks should be used to maintain area classification by allowing transition of people, equipment and materials without altering room pressurization. Laboratory managers should employ spaghetti diagrams, which visually represent the flow of materials and people to further eliminate process flows for redundancies and contamination risks. This section will focus on features unique to bioprinting laboratories compared to generic biotechnology facilities and clinical laboratories.

It is crucial that any laboratory dedicated to bioprinting has a variety of facilities/equipment besides the bioprinters (manufacturing), including cell culture facilities (production) and microscopy equipment (monitoring). The facility should be designed so each of these activities has a dedicated space, and their cleanliness matches the safety risk outlined in current Good Tissue Practices (cGTP) Code of Federal Regulations (CFR) Title 21, Part 1271 and CFR 21, Parts 210 & 211. In addition, the FDA's Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing-cGMP and EU's Annex 1 can provide greater detail on appropriate laboratory control [28,29]. Furthermore, operation of the equipment and the facility as a whole should be carried out by highly qualified staff with backgrounds in cell biology, materials science and bioengineering to cover all the unique sections in a bioprinting facility, which are discussed in detail below.

8.2 Quality Management Systems

Advanced therapy medicinal products for human use (ATMPs) have different regulatory frameworks in the European Union and United States. In the EU, tissue-engineered products are governed by Directive 2009/120/EC, whereas, the US is governed by Title 21 CFR 600-680. Table 3 summarizes the review by Oberweise et al., which provides a worldwide overview of regulatory frameworks for tissue-based products [30]. Biofabrication laboratories should familiarize themselves with their national standards before pursuing products intended for humans [24]. The purpose for current Good Manufacturing Practices (cGMP) and current Good Tissue Practices (cGTP) is to verify the purity, identity, viability, and stability of manufactured products throughout production. If there is an intention to implant anything biofabricated then establishments will need to implement a quality system to ensure compliance with cGMP and cGTP requirements as well as seeking FDA, CE or TGA approval for the final product. Quality Management Systems (QMS) assure product specification, ensure quality is maintained throughout its process and mitigates potential risk. QMS for product manufacturing is based upon a set of standards outlined in ISO 9001:2008. The International Conference on Harmonisation (ICH) adapted QMS standards specifically for the pharmaceutical industry [32]. QMS for cell therapies generally includes validations, quality control programs, quality assurance programs, and standard operating procedures (SOPs) that describe the activities in each category. Quality control (QC) monitors and reviews QMS of the starting materials, process, and product. Additionally, QC ensures testing and validations are executed and evaluates the associated documentation. For large operations, quality assurance (QA) units routinely audit records independent of the manufacturer to ensure all SOPs are adhered to and QC meet their criteria. Smaller facilities may manage QC and QA responsibilities by a single individual. A support laboratory should be devoted to QC/QA, and furnished with the necessary tests, processing equipment, and environmental controls. QC and QA provide surveillance for deviations (e.g. process changes, non-conforming specifications, or GMP non-compliance) and reports them for quality improvement and risk management. Diagnostics identifies the source of faults, and prognostics (continuous monitoring) is performed to detect early signs of structure, systems, and component decline. Before the final product can be used as a treatment, it must be formally cleared by the QA manager. Formal clearance is completed after reviewing batch records, deviation reports, QC testing, and monitoring records.

Establishing documented evidence during the manufacturing process is a critical component according to the FDA's Guideline on General Principles of Process Validation [33]. Documentation ensures a process will consistently yield a product meeting predetermined specifications and qualifications [33]. Validation entails planning specific tests and acceptance requirements in advance, which should be summarized into a protocol. Once a manufacturing process is validated, the process can be monitored continuously using statistical controls to achieve specific quality standards [34]. Bioprinter validation should be performed by the supplier or specialist to certify it is fit for purpose with periodic calibration described by the manufacturer [35]. Equipment should be stored in locations that do not interfere with airflow. Cleaning and maintenance of bioprinters should be performed using well-defined procedures and schedules [36]. Validation engineers specialize in documenting and executing protocols based on approved procedures and standards (ISO, IEC and FDA) and should be consulted for commercial development. Table 4 provides an overview of QMS and its core components (QC/QA and Validation).

Tissue-Based Product Regulations						
Country	**Australia**	**Canada**	**EU**	**Japan**	**South Korea**	**USA**
Regulatory Body	The Therapeutic Goods Administration	Health Canada	European Medicines Agency	Ministry of Health, Labour and Wealth	Ministry of Food and Drug Safety	The Food and Drug Administration
Regulation	ARTG & TGA 1989*	Food and Drugs Regulations	EC No. 1394/2007#	Pharmaceuticals and Medical Devices Act	Pharmaceutical Affairs Act	PHSA section 351 & 21 CFR 1271^

Table 3: National regulatory bodies and regulations governing tissue-based products.
*Australian Register of Therapeutic Goods and The Australian Government Therapeutics Goods Act 1989
#European Commission Number 1394/2007
^The Public Health Services Act section 351 and 21 Code of Federal Regulations 1271

Quality Management System		
Quality Control	**Quality Assurance**	**Validation**

Facility monitoring	Surveillance, Diagnostics,	Equipment
Facility maintenance	Prognostics	Facilities
Equipment monitoring	Document control	Reagents
Materials management	Audits	Processes
Tissue processing	Batch release	
Packaging, labelling, delivery	Clinical trials management	

Table 4: Quality Management System

8.3 Cell Production facilities

Laboratories should maintain and update information data sheets listing specifications for all required materials to ensure bioprocess and product consistency. Batch records should document the development of patient material in each stage of manufacturing e.g. biopsy, shipping, and disposal in compliance with (21 CFR 211.188) along with all equipment, reagents, and supplies used during their manufacturing [36].

In bioprinting, cells are often the most important/delicate component of any bioink. Therefore, cell culture facilities should be at the core of any bioprinting facility. Cell culture facilities should be tailored to the specific cell types used in the inks. For example, facilities that employ the use of cells isolated from primary tissue should have dedicated equipment for processing the obtained donor tissue and may involve a separate quarantine incubator and tissue culture hood to maintain isolated primary cells for screening of potential infection prior to cell expansion. The instrumentation of these facilities can vary as established isolation protocols can differ between different tissue types. Independent of the tissues processed, the facility requires a fridge to store cell culture media as well as a *-20°C or -80°C freezer where growth factors and other media supplements can be stored over an extended period of time. Liquid nitrogen storage is essential for aliquots of expanded cells which may require freezing down to store for a later date. Furthermore*, all of the facilities which are involved in the production of cells will need to comply with 'good manufacturing practice' (GMP) standards which is required for cell-based therapy approaches [37]. GMP is a quality system certifying that products are manufactured safely and consistently and to specified standards.

With regard to biosafety, it is generally recommended that human and other primate cells are handled within biosafety level 2 (BSL2) facilities [38]. *This requires the use of class II biosafety cabinets (BSC) throughout the facility. These BSC contain HEPA filters which filter the exhaust air from the cabinets. This air can be safely recirculated into the laboratory or directly exhausted to the outside. Such BSC are required to be tested and certified at least on an annual basis. Any biologically contaminated waste produced from a BSL2 facility needs to be decontaminated and the facility therefore requires instrumentation to do so. Autoclaving, chemical disinfection or incineration are common methods for decontamination but any validated method for decontamination can theoretically be employed [38].*

While GMP and biosafety requirements are similar in many aspects such as the need for restricted access or mandatory personal protective equipment (PPE), they differ significantly with respect to ventilation requirements. While GMP facilities keep any contaminants out of the facility to protect the product (positive pressure against the surrounding environment), biosafety facilities require that the contaminants are contained within the facility to prevent the escape of materials to the outside (negative pressure versus the environment). To solve this problem, biosafety areas contained within a GMP facility can be put under positive pressure compared to the outside environment but at a negative pressure compared to the rest of the facility i.e. the GMP facility is at the highest pressure, the biosafety area at the second highest pressure and the outside is at the same or lower pressure as the BSL2 area [39]. The inward directional airflow needs to be maintained to ensure containment of the BSL2 facility within a GMP environment, hence redundant ventilation aggregates are required for backup should the first one fail.

Depending on the tissue targeted in the bioprinting process, the size of the construct as well as cell density within the bioprinted construct (#cells/ml) can vary. However, generally a large number of cells are required for the creation of a bioprinted construct and large-scale culture systems therefore become important. Single-use (disposable) bioreactors are usually employed for cGMP production approximating 2,000 L scale in clinical manufacturing, which simplifies the maintenance of an axenic environment for staff. Bioreactors require additional gases (e.g. N2, CO2, O2) to control conditions within the bioreactor. For anchorage dependent cells, a variety of methods exists, however, the stacked plate system as well as cell carriers in a spinner flask are considered most viable [40]. The number of cells produced within a certain time period needs to be considered when choosing the right incubators for the production facilities.

8.4 Cell Preparation

Cell characterization assessments should be performed on pre-production bulk cells to verify their identity, purity, viability, and safety [34,35]. In-process testing should be performed on samples during each critical step

of biomanufacturing. Viability tests are necessary to assess whether growth conditions are resulting in cell death [43] these tests include: Tryptan blue exclusion method, MTT assay, and live/dead assays. In addition, sterility assessments are required to prevent culture contamination by bacteria or fungus. Current GMP guidelines should be followed for all cell therapies. All cell cultures should occur within a clean room protected by a HEPA filter to eliminate airborne contaminants. Confirmatory sterility tests can be performed periodically on small amounts of cell batches during expansion [44, 45]. Commercial purposes require additional sterility tests from regulatory bodies i.e. Food and Drug Administration (FDA) or European Pharmacopoeia (EP) [46]. Inoculator or filtration methods are two common practices to confirm sterility [47]. Viral contaminants can be detected using Enzyme-linked immunosorbent assay (ELISA). These processes ensure no impurities are transmitted to patients. Medium fill simulation should be used to certify aseptic status at each stage of the manufacturing process [48-50].

8.5 Cell Monitoring Systems – environment and microscopy

Commencement of a process operation requires verification from the QA team that a process room has passed documented sterility testing, which includes sampling room surfaces for microbial organisms and particles. GMP recommends using both contact and settle plates (e.g. tryptone soya or Sabouraud agar) for monitoring bacterial and fungal contamination. Settle plates test for the number of microorganisms deposited by air within cleanrooms, while contact plates test for the number of microorganisms on any surface within the cleanroom [32]. Requirements for contamination control are summarized in Table 5. Air circulation should also be monitored using an air sampler at high pressure inlets. Building automation systems (BAS) and their sensors play an important role in environmental control and should be validated, periodically. BAS should be supplemented by manual monitoring to detect any faults in the automated system [32].

To monitor the quality of the cultured cells and the bioprinted constructs, a 3-DP laboratory would require specialized facilities which can be used to examine and characterize the manufactured cells and tissues. Whilst these processes may be done manually, there are advantages to using automated systems that can replace the cell culture media. Some advanced systems may also contain plate readers for biological assays and have the capability to take brightfield or even fluorescent images. Both methods can be used to track the progress of the tissue maturation and determine the appropriate time to release the manufactured tissue to be implanted into the patient. Automated cell culture systems have a relatively large footprint, but their advantage lies in minimizing the required manual manipulations which, apart from imparting increased reproducibility, also reduces health and safety risks associated with tissue culture. Several classes of image analysis software have been developed to keep pace with automated microscopy, specifically, companion packages (e.g. MetaMorph—Molecular Devices, Elements—Nikon), commercial programs (e.g. Imaris—Bitplane), and open-source packages (e.g. CellProfiler, Icy, KNIME, ImageJ/Fiji) [51]. Laboratories should consider the advantages and drawbacks when selecting image processing tools. As one of the goals of bioprinted tissues is to make the tissues patient-specific, standard protocols might not be suitable with respect to timelines and constant monitoring of the tissue maturation would require dedicated staff to do so. Furthermore, most automated systems are optimized for 2-D cell culture and the evaluation of the 3-D bioprinted constructs might still require dedicated and highly skilled technicians to perform the monitoring.

Confirming cellular phenotype is an important step to avoid cellular dedifferentiation during cell expansion. Immunophenotyping and immunohistochemical analysis are two preferred techniques to identify phenotypic properties and determine if the cells are healthy or abnormal [52]. If the chosen cell type can be clearly identified through surface markers, fluorescence assisted cell sorting (FACS) is an excellent method to assess the identity of all the cells within a population. Genomic assessments can also be performed using quantitative PCR, karyotyping, fluorescent in-situ hybridization (FISH), telomere length assay, or beta-galactosidase quantification. PCR arrays systematically screen numerous genes to ensure cells retained their desired phenotypes [53]. Karyotyping can detect chromosomal instability or fragmentation that may accrue in cell cultures [54, 55]. Telomere length assays and beta-galactosidase identify cell senescence that may limit their proliferative capacity in bioprinted structures [56, 57].

Deep learning methods performing cellular image analysis using low-resolution images can now be implemented via open-source convolutional neural networks (ConvNets), such as CellProfiler and Cell Cognition Profiler [58,59]. ConvNets have shown proficiency in identifying cells within mixed populations along with individual phenotypes. Microscopic images with poor resolution or low signal-to-noise ratio can be restored in real time using a combination of deep learning and content-aware image restoration networks [51]. Thus, downstream analysis is improved and allows microscopes to operate at higher frame rates, lower light intensities, and shorter exposures [60]. Some deep learning software packages can adapt to new cell types or imaging modalities more readily than others, so laboratories should consider these capabilities if their

laboratory intends to specialize or generalize their biomanufactured outputs [61]. Implementing automated process monitoring systems can improve phenotypic assessment accuracy and reduce time devoted by staff towards visual inspection. To effectuate computer vision for phenotypic profiling, laboratories must develop algorithms or use commercial off-the-shelf software tools for cell segmentation, feature extraction, feature selection, dimensionality reduction, and cluster or classify resultant profiles [62,63]. Segmentation algorithms execute edge detection, region growing, thresholding Markov random fields or machine learning to distinguish cells from their environment [64]. Large-scale datasets examined in biofabrication laboratory settings should use high-performance algorithms since they take less time to run [65]. Feature extraction algorithms derive morphological and textural attributes from the microscopic images and the previously mentioned image analysis packages can perform this function [66]. Next, the feature selection algorithms sort useful features from uninformative features. After selecting useful features, laboratories can implement supervised learning (e.g. classification) or unsupervised learning (e.g. clustering) methods to categorize phenotypic profiles. Intelligent systems are now being used to automate large-scale phenotypic screening procedures by combining reflection-based autofocusing microscopes with machine learning platforms (Micropilot, Cellprofiler Analyst) [51,64,67]. A crucial step in computer vision is image pre-processing for improving image quality such as image denoising, deblurring and image normalization [63]. The normalization matches the fundamental visual features (e.g. resolution, color distribution, denoising, range of intensity values, and de-blurring) for each image to improve the cell profiling [68-72]. Image registration enables visual analysis from heterogenous image sources or different acquisitions of the same image modality. Images also undergo data augmentation which transforms images via cropping, rotations, mirroring, and flipping to increase the quantity and diversity of training data for machine learning algorithms. Other reviews are available that concentrate on deep learning and computer vision techniques for cell image analysis [72]. Finally, cell sorting is an important but time-consuming task during cell culturing and prior to selecting cells for inclusion in bioinks. Intelligent image-activated cell sorting (iIACS is a machine-intelligence technology allowing real-time automated operation for sorting of specified cells [73]. A guide is available detailing how to design, build, and use an iIACS machine, which requires a microfluidic chip, a cell focuser, a microscope, a speed meter, specialized optics, an image processor, neural network and a cell sorter [73]. Constructing a cell sorting system will require expertise in optical system design, digital system design, image processing, microfluidic chip design, sensor-actuator system construction, and flow cytometry experimentation.

Data processing time is a computational bottleneck for implementing automated cell image analysis. Groups are aiming to address this problem with one group developing a deep learning program (e.g DenseDeconNet) that achieves a 50- to 160-fold increase in image deconvolution for optical microscopes [74]. Implementing deep learning methods for cytology analysis can lead to improvements in quality assurance for biospecimen selection, enhance reproducibility, and improve specimen quantitation [75]. Laboratories should be aware of these process improvements when designing their image analysis workflows. As previously mentioned, computational constraints may be encountered with deep learning image analysis such as insufficient dynamic random access memory (DRAM) [76]. This may require laboratories to use multiple GPUs for processing large batch sizes or reducing batch sizes while training the algorithms. Advances in GPUs and/or introducing cloud computing can alleviate this bottleneck. In summary, cellular and tissue biomanufacturing will require extensive online process monitoring via microscopic and sensor monitoring to achieve consistent and predictable quality standards [77].

Grade	Settle Plates, cfu/4 hours	Contact Plates, cfu/plate	Air Sample, cfu/m^3
A	<1	<1	<1
B	5	5	10
C	25	25	100
D	50	50	200

Table 5: Limits for Microbial Contamination (EU cGMP Annex 1)—colony-forming units (cfu)

8.6 Manufacturing facilities – the bioprinters

Depending on the type and capabilities of the bioprinter employed for the tissue manufacturing, different safety precautions need to be set in place with regard to the PPE and warning signs. Personnel entering ISO 5/6 (Grade A/B) areas should remove outdoor shoes and clothes put on sterilized gloves, hood, coveralls, shoe covers, face mask and safety glasses. Clothing requirements for clean rooms can be found in IEST Recommended Practice (RP-CC-003.2) and EU Guidelines. Common safety hazards around a bioprinter include moving parts, high pressure (extrusion bioprinting), lasers (e.g. stereolithography printers and laser induced forward transfer bioprinters [78] and ultraviolet (UV) radiation. Most of these hazards can be controlled by placing the printers in a biosafety cabinet which needs to be closed for the printer to operate. Some bioprinters are already designed and integrated within biosafety cabinets to maintain sterility, many of these bioprinters have been reviewed

extensively in terms of their capabilities [79-82]. Video cameras independent of the bioprinter cameras should be installed to monitor the printing process. Independent cameras are akin to flight data recorders, which provide a performance record should the prints fail, abruptly.

Bioink development and optimization of printing parameters [83] is an iterative process and does not need to adhere to the same GMP standards as the final tissue printing process. An ideal bioink should possess scalable features relating to hydrogel design, printability, and biological outcomes. Hydrogel design should exhibit a cell friendly gelation behavior, cytocompatibility and a homogenous distribution of components. Printability generally includes rheological requirements and shape fidelity but is further constrained by the specific printer platform. For example, extrusion bioprinting assesses bioinks for extrudability and filament formation where lithographic printing would assess for photo curing and light penetration depths. Finally, a bioink needs to ensure proper cell viability, proliferation, and differentiation. Rheological requirements (e.g. viscosity, shear thinning, yield stress, elastic recovery) describe deformation and flow behaviors of materials under applied forces [84]. These physicochemical parameters have the largest influence on hydrogel printability. While, shape fidelity refers to shape retention ranging from single filaments to geometric properties in planar and multilayered constructs.

As it has been established throughout literature [36][77], there is no such thing as a universal bioink. This makes bioink requirements dependent on printer technology, application and tissue, which forces ongoing development of novel bioinks. The bioprinting process complicates bioink development, since these activities often have different (and opposite) material requirements during the printing process compared to the final printed construct. Embedding cells into bioinks further complicates bioink properties by disrupting cross-linking efficiency and changing viscoelasticity [85]. Apart from the difficult bioink development process, the translation of bioprinted products is currently also limited by poor reproducibility of printing processes as well as limited bioink availability. Many laboratories are introducing novel quantitative tests, qualitative tests, and predictive models for mainly extrusion-and lithography-based bioprinting [86]. These issues have been extensively covered by Schwab et al. and laboratories would benefit from measuring the parameters listed in their review to evaluate bioink printability and create more consistent protocols. Laboratories should implement standardized testing protocols to characterize rheological and morphological properties in bioinks both with and without cell inclusion [87]. Laboratories can also review ASTM/ISO guidelines for tensile measurements for bioinks, which can help yield more consistent results.

Laboratories can install an open source platform that automates the manufacturing of bioinks thus improving their reproducibility and throughput [88]. The open source workstation enables automated pipetting of materials with validation and verification by absorbance measurements. This platform is modular and can easily be customized to adapt to laboratory needs or changing research requirements. Laboratories should consider installing this platform to convert their operation to high-throughput production. It is also highly recommended to have a second bioprinter installed with the exact same configuration as the printer intended for tissue manufacturing. The purpose of the secondary printer is to optimize printing parameters via benchmark models and test novel bioinks for their printability. Although many ink properties related to printability and shape fidelity can be determined using rheological analysis [45][89], others, such as the extent of die swell or time dependent changes during the printing process, are best assessed by directly performing the printing procedure. By using two bioprinters, it is assured that all the optimization and development happens on one printer while the second printer in the GMP environment is limited to the use of optimized ink and processes. The use of the second printer is therefore minimized, and with it, the risk of contamination or potential equipment failure. Machine intelligence has been used to find relationships between rheological data and predict printability outcomes for extrusion-based printers [90]. Developers used these tools to improve bioink design and these techniques could be advanced to minimize trial and error testing for bioink development.

One of the important aspects to consider when installing a bioprinter in a manufacturing line is that the bioprinter is installed within a BSC so that the printing of the tissue constructs can be performed under sterile conditions. Ideally, the room containing the bioprinter is within the BSL2 containment but in another location as to separate cell production from tissue bioprinting. Bioprinter parts which are in contact with the cells and the bioink need to be sterilizable or come as sterilized one-time use products. Different suppliers of bioprinters pursue different approaches to the sterilization problem. Some printers are comprised predominantly from 316L medical grade stainless steel which can be sterilized in an autoclave. Other parts, usually tips or cartridges for extrusion bioprinting, are inexpensive and commonly intended for single use. If sterilization of the printer is performed via UV-sterilization within a BSC cabinet, attention needs to be paid to which of the surfaces are actually exposed to the UV radiation and which ones are not. If a pressure dispensing system is utilized for the printing, medical grade sterile air filters need to be put in place to ensure that the pressurized air is sterile.

Many commercially available printers come with an optional camera. These cameras can be utilized for quality control purposes to monitor ongoing print processes and document the final product. Such setups can also be used to ensure reproducibility over extended periods of time. This is specifically relevant for bioprinted constructs that are not patient-specific, such as devices utilized for *in vitro* testing. Quality assessment for patient specific bioprinted constructs on the other hand is more difficult to perform as each printed construct is unique. This would require constant monitoring of the print using a camera to assess the fidelity of each printed layer and how it compares to its CAD equivalent. Currently there are no commercial printers offering such setups, although multiple bioprinting companies are seeking to integrate these features with future printers [91, 92]. In addition to bioprinters whose end goal is to print sterile constructs containing living cells, most 3D-P laboratories would benefit from the inclusion of other variations of 3D-P which may be used for creating anatomical models, non-implantable scaffolds for research purposes and drug screening, and have the ability to print lab consumables and spare parts for laboratory equipment. These technologies have been extensively reviewed [93] and are summarised in Figure 1, typically bioprinting would fall under the classification of an extrusion-based 3D-P approach.

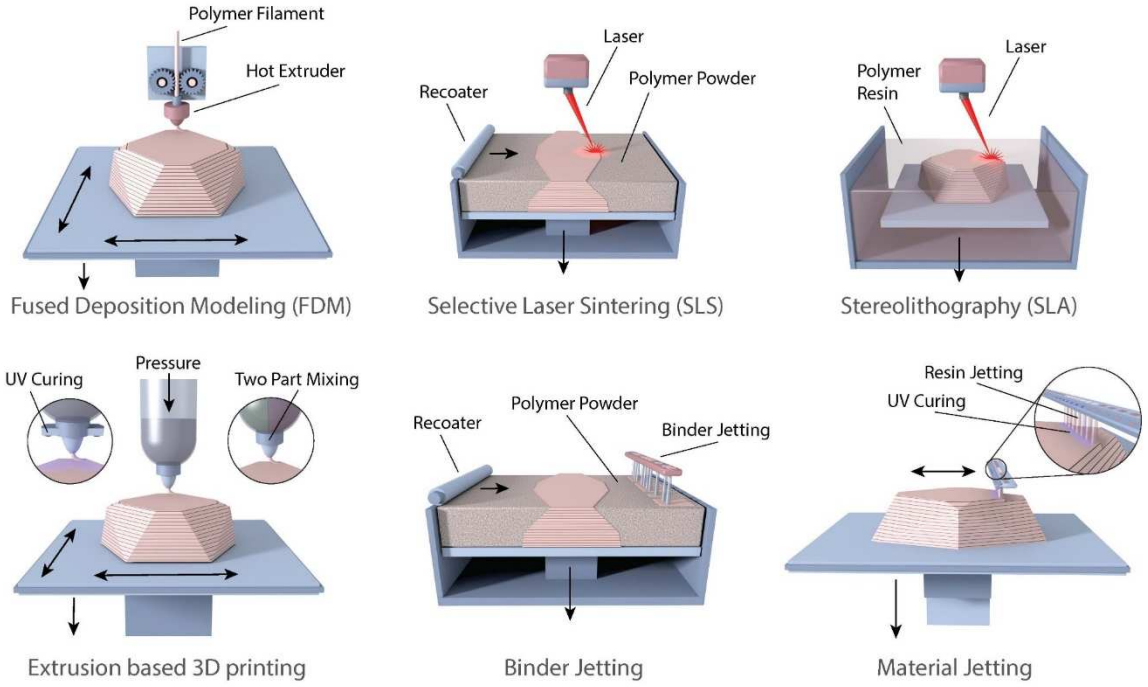

Figure 1. Illustrations of some common polymer 3D-P techniques which fabricate an object in a layer-by-layer manner. Top left: Fused deposition modeling (FDM); A molten polymer is extruded through a nozzle and onto a print-bed in order in a controllable manner to produce a series of stacked 2D patterns which make up the final 3D object. Top center: Selective laser sintering (SLS); A fine layer of polymer powder is coated onto the print area and a laser is used to sinter or melt the powder onto the layers below. After sintering/melting each layer, another layer is coated on top and the process repeated to build up a 3D object. Direct metal laser melting is a similar process that fuses layers of metal powder instead of polymers to produce 3D printed metallic objects. Top right: Stereolithography (SLA); A laser or projector selectively polymerizes a layer of liquid resin into the desired pattern. The laser/projector is either located above a vat of liquid resin in which case the polymerized layer is lowered into the vat or located below the vat where the completed layer is raised, resulting in the polymerizing of multiple layers to build up an object. Lower left: Extrusion 3D-P; Similar to FDM, a 3D object is produced layer-by-layer by selectively extruding the material from a nozzle using either pneumatic or mechanical means. The extruded material can be polymerized in various ways such as two-part polymerization or photopolymerization. Lower center: Binder Jetting; Similar to SLS, binder jetting binds layers of powdered polymer through selective extrusion of a liquid binder material (adhesive). After each layer is bound, another fine layer of powder is coated on top and the process repeats. Color additives can be included into the binder liquid to enable color full-color 3D-P. Lower right: Material jetting; Liquid resin is selectively extruded onto the top layer of the print area and then photopolymerized. The polymerized object is lowered and the process repeated layer-by-layer. Advanced implementations of this method involve multi-nozzle extruders capable of selectively depositing several different polymers prior to photopolymerization to enable differential control of the final material properties throughout the 3D printed object. "Reproduced with permission" statement need to go here after permission has been obtained for use of this figure from the publisher – Advanced Materials via the Copyright Clearance Centre) [94].

8.7 Bioprinting Monitoring Systems

Translating bioprinted products to the clinical setting requires careful control of printing parameters and cell viability. A major focus in bioprinting is establishing the printability of a bioink and the final mechanical properties of the printed constructs [95]. However, emphasis has shifted towards upscaling, repeatability and print fidelity, as printed constructs will need to be verified if they are to reach the clinic and be implanted into the patient. This requires adequate validation in the form of production batches to prove consistency of manufacturing process for the final product. Multiple batches will also be required to ensure product stability during specific storage and shipping conditions [96]. Additionally, printing processes will need to be recorded and optimized, and the results from the prints will need to be monitored. While verification and validation processes are common in the medical device industry and 3D-P, the growth of 3D bioprinting is making these processes more challenging as each single printed construct is unique to the patient. Online monitoring and recording during the printing process of each bioprinted construct would offer a potential solution to this issue. For example, Kang et al. used quantitative image analysis to determine print accuracy for selected geometries in 3D printed constructs [97]. Apart from the geometrical fidelity between the CAD design and the final printed construct, monitoring systems also need to be put in place to evaluate the conservation of the cells' phenotype and their viability after the printing process to ensure the safety of the printed construct. It has been shown in the past that these cell parameters can be affected during the printing process [98-101] and long-term monitoring might be required during the cell and tissue maturation process in the bioreactor to ensure the cells in the printed construct fulfill their intended role. This could include the analysis of soluble factors within the culture media produced by the maturing tissues or microscopical and spectroscopical analysis with label free methods such as second harmonic generation or FT-IR. Non-destructive machine learning approaches have been developed to automate cell profiling within 3-D scaffold architectures [102]. Moreover, emerging spectroscopy using artificial intelligence is an acceptable method for automated quality monitoring of stem cells and engineered tissue products [103,104]. Another non-destructive characterization method is the use of quantitative ultrasound to monitor cell growth and tissue formation [105]. Implementing these monitoring systems can reduce processing times and ensure product standardization.

Machine intelligence has been applied to bioprinters in several studies to optimize process parameters including gas pressure as it relates to droplet number, size and position [106,107]; nozzle distance, voltage, and stage moving speed associated with cone modes [108,109]; fine-tune drop on demand printing [110]; and spheroid-based bioprinting [111]. Machine intelligence has yielded enhanced printing resolution by optimizing printing parameters based on shape fidelity features (e.g. layer adhesion, layer fusion, and pore infill stemming from construct collapse) [112]. These platforms require high-speed cameras, an LED light source, and computer processing. Biomanufacturing laboratories should automate process control systems as they become commercially available. Newer bioprinter models will begin incorporating real-time process monitoring and parameter adjustment into an all-in-one system for convenience [91,92]. Image analysis should be performed using images generated from microscopic evaluation. Commercial (e.g. Amira, Imaris, and Volocity) and open-source (ImageJ, Cell Profiler, Icy, V3D) software are commonly utilized for microscopic image processing and analysis to quantify cellular proliferation or profile cells [113]. Bioprinter accuracy can be calculated from light microscope images by measuring the dimensions (in pixels) of structures using ImageJ. In the future, these processes can be automated in real-time using high-resolution optical and laser technologies to capture features linked to discontinuities or defects [114-117]. Optical coherence tomography (OCT) is a readily available technology that can accommodate quantitative testing for morphological features such as filament size, surface area, pore size, porosity, and pore volume. OCT is one of the earliest proposed techniques for real-time monitoring [118,119]. Algorithms are already in development that adjust the printing process with OCT analysis [110]. Deep learning OCT image processing is developing in the field of ophthalmology that laboratories can model their algorithms for bioprinting applications [120,121]. Laboratories should be prepared to update their hardware and integrate their bioprinters with intelligent systems to minimize operator error, accelerate print times, and ensure accurate prints [111].

8.8 Post processing

Post processing is an important step for biofabrication and varies with fabrication methods and tissues laboratories develop. Laboratories should be aware of these requirements and procedures. For instance, lithography-based bioprinting may require more light exposure for curing. The post-bioprinting stage also includes conditioning constructs with nutrients and metabolites to promote tissue maturation. Failure to do this is a major reason for construct failure. Many laboratories use bioreactors (spinner flask, hydrostatic, flow perfusion, strain, compression) to create the proper conditions to support tissue constructs. Bioreactors are tissue and application specific, however, all bioreactors serve the purpose of providing the immature tissue with the right mechanical and biochemical cues to develop into the final tissue engineering product. The size and the complexity of such bioreactors can vary from small benchtop devices to larger instruments used to mature multiple tissue engineering constructs at the same time. Therefore, the special requirements of the bioreactor

will highly depend on the type of tissue the bioprinting facility is focused on. Another important factor that all bioreactors share is that they need to be easily assembled and sterilizable to be considered suitable for a 3D bioprinting facility. An automated system, which is able to exchange the cell culture media, would limit the manual manipulations required during tissue maturation and may contribute to a higher reproducibility. Therefore, such systems should be taken into consideration independent of the type of bioreactor and might be advantageous during cell production as well. Construct shrinking or swelling can occur in postprinting processing and laboratories should account for this when designing their bioinks and when performing postprocessing procedures. Laboratories can consult other articles focusing more on this topic [122].

9. Multitechnology Bioprinting

Multi technology bioprinting is able to blend complementary fabrication technologies into a single platform for delivering high-throughput functional tissue constructs as described by Castilho et al. who have detailed the latest examples of multitechnology biofabrication [123]. Future bioprinters will combine multiple printing techniques described in Figure 1 to fabricate constructs with enhanced cell distribution, more accurate biomimetic microstructures, and improved biomechanical functionality without compromising cell viability [124-126]. Next generation bioprinter systems will also co-opt complementary fabrication technologies (e.g. computational modelling, machine intelligence, and smart manufacturing) for real-time process monitoring and manufacturing tissue structures with enhanced functionality. To actualize previously mentioned semi-autonomous monitoring systems and tissue construct complexity, laboratories should consider the following section into their laboratory plans.

9.1 Mathematical Modelling in Tissue Engineering and Biomanufacturing

While monitoring is a requirement to verify parameters such as printing fidelity, and printing parameter optimization, achieving this fidelity is still performed in a reductionist iterative manner. To minimize operational costs, material waste and experimentation, advanced manufacturing facilities are moving towards design of multiscale, multiphysics modelling. Computational modelling has been used to predict component interactions, optimal filament dimensions, hydrogel properties, cell viability, along with printing parameters for AM processes [127-129]. For example, the power law and Herschel-Bulkley model are reliable tools for predicting bioink printability for initial screenings [89,130]. Such models could eliminate wasteful and costly trial and error activities that plague bioink development. Digital design is a process that generates 3D models using computer software and simulates their biomechanical performance. It encompasses numerous parameters, including multiscale architecture, hydrogel composition, and biomaterial interactions. At the molecular level, computational modelling plays an important role in understanding positive feedback-based switches determining cell fate. As an example, engineers have used toggle switch models to control transcription factor expression in mammalian cells [131]. At the cellular level, cellular automaton models have also been valuable in the computational analysis of stem cell variations in differentiation for different subpopulations in cell cultures [131]. Tissue-based models (e.g. reaction-diffusion, proliferative, and activator-inhibitor models) have also been useful in predicting tissue growth rates, cell numbers, and complex pattern formation [133-135]. Many simulation programs exist (COMSOL, MATLAB, Simul8, ANSYS, Abaqus, Mathematica etc.) to optimize these parameters, and each facility should select the program based on their preferences, printing setup, and the intended property to simulate [136]. Facility managers should ensure their computer system requirements correspond with their intended simulation software. Researchers have used this approach to isolate parameters impacting printability [137,138]. For example, one study generated viscoelastic rheology and surface tension models using IPS UBOFlow to simulate bioink deposition and material shape [138]. Another study utilized Abaqus for mechanical simulations and ANSYS for permeability simulations to optimize scaffold topology [137]. Eventually, biomanufacturing tissue engineered products will require complex process simulation modelling key inputs (materials, operating parameters, equipment, labor resources) with expected outputs (material properties, energy balances, cycle times, process scheduling, throughput analysis etc.) to standardize production. This will allow manufacturing plants to promote lean solutions focused on computational efficiency, streamlining production, eliminating waste, cycle time optimization, production scheduling, and reducing potential bottlenecks [139-141].

Another class of mathematical modelling that is trending now is predictive modelling, which forms the basis of machine learning and deep learning programs. Predictive modelling is the process of forecasting outcomes by uncovering relationships between data using powerful computers and model building software tools and platforms such as (JMP, WEKA, R, CRAN, Keras, Scikit-learn, Apache Spark, Google AI (Artificial Intelligence), IBM Watson, AWS, etc.). This forms the basis for the automated cell monitoring and bioprinting monitoring systems in previous sections. To effectively execute predictive modelling, data must be collected then undergo quality assessment (curation) to avoid detrimental data manipulation in later stages. Data quality assessments demand formalized systems of annotation (feature categorization), deduplication (similarity detection), data imputation methods, and outlier detection [142-145]. Data discretization methods can further reduce recording errors [146]. Laboratories can select from a range of supervised (regression analysis, Bayesian models, decision trees, neural networks, SVMs) [147-151] and unsupervised algorithms (e.g. K-means, hierarchical clustering, spectral clustering, and etc.) [152-154]. After constructing predictive models, they must undergo training and then performance evaluation to validate their outputs. A variety of cross-validation methods exist including leave-one-out [155], leave-P-out [156], k-fold [157], stratified k-fold [158], and repeated k-fold [159]. Predictive modelling has been instrumental in many areas affecting tissue engineering including biomaterial design, gene-editing, predicting cellular responses to biomaterial surfaces, designing scaffold properties, optimizing process parameters, automating phenotypic screening, and

predicting bioprinted construct performance [160,161]. Laboratories should look to integrate computational and predictive modelling to streamline the tissue fabrications bioprocesses.

9.2 Data Ecosystem and Cloud Manufacturing

Advanced manufacturing is beginning to develop big data ecosystems to create new applications related to product development, production, and business activities [162]. Specifically, the emergence of cloud computing, data science, and artificial intelligence has made it possible to integrate manufacturing knowledge with big data for automating quality control processes, decision making systems, predictive modelling, supply chain management, job scheduling, storage and retrieval systems, and sustainability [163,164]. This marriage between advanced manufacturing and information technologies is known within industry as smart manufacturing. Massive data will be generated during biofabrication which may include—patient records, manufacturing reports, pheno-genomic data, scaffold designs, medical imaging, microscopic imaging, process parameters, sensors, etc [165,166]. These data can eventually be leveraged as training data to develop deep neural networks for automating and monitoring manufacturing processes. Biomanufacturing tissue products effectively requires Internet of Things (IoT) for manufacturing, which integrates sensors, cameras, and machines ("things") into cloud data centers. IoT supports bidirectional communication among plant machines resulting in real-time delivery of high-value information during manufacturing. Monitoring and predicting key performance indicators (KPIs) can be performed automatically and in real-time using cloud computing for assessing production processes, identifying opportunities for improvement, and sending alerts. Smart inventory also allows materials and products to be tagged and tracked for easy localization and inventory management. Previously mentioned quality monitoring systems can be managed by cloud computing services to minimize labor costs. Nanosensors and biosensors are emerging for real-time and non-invasive inline monitoring of stem cell-based products [167-169]. Paired with the advances in bioreactors and future bioprinters, this technology can provide feedback controls for controlling important environmental conditions (e.g. temperature, CO_2 and O_2, pH, humidity) during stem cell expansion, printing and tissue maturation phases [170]. As an example, research groups are working on automated setups that provide label-free and real-time monitoring of metabolic parameters such as pH and oxygen levels within 3-D bioprinted constructs [171]. Laboratories should look to integrate these sensors into IoT to create more robust quality systems.

With the advent of sensors and cloud computing, robotic cloud laboratories are beginning to emerge in the pharmaceutical industry and offer greater experimental control and process execution for smart manufacturing [172]. This is achieved using robotic workcells, which automate lab instrumentation (pipetting robots, reagent dispensers, PCR applications, etc.) and lab infrastructure (custom-modified freezers, refrigerators, incubator units) and integrate them using software and automated storage and retrieval containers for streamlining and scaling protocols [172]. Robotic cloud labs can be controlled remotely and minimize the variables contributing to poor reproducibility rates such as mismanaged reagents and materials, contaminations, and poorly defined protocols [164]. In addition, digitally connected plant units simplify facility management by notifying of device deviations from prescribed parameters, and plant unit variations (e.g. temperatures, vibrations) [173]. Real time analysis can be performed on data collected from storage environments, samples, and instruments using sensors [171]. IoT sensors can also track and categorize bioprinted products awaiting delivery according to duration-of-stay policy [173,174]. Production flow monitoring and inventory management eliminate unnecessary work and reduce production variability by helping managers oversee the work in progress, available materials, and estimated time of arrival for incoming materials [175]. Installing a robotic cloud laboratory is recommended for commercial biofabrication centers looking to make productivity gains. Safety can also be improved using low-cost sensors (e.g. gas, radiation) to measure exposure within the facility, alert workers to hazardous materials and reinforce safety compliance among personnel [176]. Lastly, asset tracking is crucial in the healthcare environment, because it ensures the right product gets to the right patient. Many health systems employ Radio-Frequency Identification (RFID) tags to identify, record, and monitor the movement of products through their manufacturing lifecycle. RFID labelled objects can be localized by deploying battery-powered beacons systematically throughout the facility for room-level discernment. Active RFID tags and QR codes can be printed by handheld printers to label incoming shipments and/or packaged therapies for verifying their location and that they reach the correct destination. Integrating employee smart phones into the cloud servers provides advanced solutions for localizing objects and broadcasting alerts to users. These capabilities are enhanced by improving localization algorithms and system accuracy should be tested with LIDAR. Additionally, RFID tags can also be IoT-enabled and monitored within the cloud server for their positions and environmental conditions as well. Administrators can search the cloud web interface for asset information, position, and manufacturing lifecycle. Laboratories can presently install cloud enabled, asset localization packages with the aforementioned features [177].

To realize the potential of smart manufacturing, a cyber-physical system (CPS) architecture will need to be developed that assimilates the physical laboratory components (e.g. bioprinters, robots, sensors, computers, and

interconnecting devices) with the software components (e.g. machine learning algorithms). This process can be performed via four enabling technologies: Data, Analytics, Platform, and Operations technologies [178]. Data Technologies (DT) allows IoT devices and manufacturing devices to interact, resultant data to be transferred from the factory floor to the cloud, and bilateral communication between cyberspace and the physical-space. IoT devices require basic internet infrastructure (Wi-Fi, 3G/4G/5G, National Broadband Network) and typically possess plug-and-play functionality. The devices capture data and communicate via cloud services. Platform Technologies (PT) consist of the hardware and software architecture that enables big data analytics (collection, storage, analysis, and visualization) and its delivery for enterprise applications [178]. Biofabrication laboratories can choose stand-alone, cloud, and/or edge configurations for their platform. Stand-alone databases include RDBMS [179], No Structured Query Language (NoSQL) [180], and NewSQL [181]. These categories refer to the programming languages and models utilized to communicate with the database. Cloud computing services are now hosted by large companies such as Amazon, IBM, Apple, Google, Microsoft, Alibaba, and Facebook for commercial data storage. Cloud data centers have an advantage over stand-alone configurations since they provide essentially unlimited computing power without significant investment in computational processing and storage infrastructure [182]. This greatly reduces expenditures on maintenance and hardware for in-house information technology infrastructure. Laboratories should consider combining their cloud services with edge data centers for more efficient data processing and laboratory control. Edge data centers provide increased processing and storage capacity locally without processing from the centralized cloud data center. This minimizes communication delays and unnecessary data transfers, while maintaining access to the remote cloud data center for more complex analysis. Data managers will need to consider their bandwidth and energy efficiency when uploading to remote cloud data centers. This can be achieved by taking advantage of cloud services at the edge (smart gateways) and network function virtualization solutions [183].

Analytic Technologies (AT) implement the techniques described in the previous section to process data using models that improve operations [184]. Computation and data-driven modeling generate analysis results which can be visualized and used to produce user-friendly visualizations reports for prognosing and diagnosing minute variabilities during production. Operations Technology can then recognize the process variabilities and correct them by enabling machine-to-machine communication and collaboration. Analytics is the process of extracting information from data. This process employs two separate methods—1) data mining and machine learning algorithms and 2) On-Line Analytic Processing. Operations Technology recognizes these process variabilities and corrects them by enabling machine-to-machine communication and collaboration. Selecting the appropriate computation framework for optimal processing speeds is crucial for analytica technologies. These options can include Hadoop, Spark, Flink, and Storm on High-Performance Computing systems for batching, micro-batching, and streaming varying volumes of data. Commercial cloud data centers provide these computational frameworks on their platforms. Operations technology (OT) is the final step for cloud manufacturing and requires AI algorithms to implement [178]. Enabling technologies (DT, PT, AT, OT) form the architectural foundation for the Industrial AI System (see GE Predix, Siemens MindSphere, IndustrialAi). Resources are available for laboratories wanting to scale to an Industrial AI system. The Center for Intelligent Maintenance Systems (IMS) is a resource for many members developing Industrial AI and Big Data Analytics. IMS has developed a collection of intelligent software tools (Watchdog Agent®) that can monitor equipment for performance, diagnose faults, and predict and prevent failures. LabVIEW by National Instruments is a system-design platform and developmental environment that many manufacturers use to automate hardware, testing, measurement, and control systems. The VI Package Manager by JKI allows developers to download and manage LabVIEW Add-ons. Building a CPS Platform for smart manufacturing will require programmers and application designers to generate an architecture for specific manufacturing needs. Simulation is an important aspect for architecture design because it integrates hardware more effectively for particular applications, models data and control flow, enables capacity planning, reviews energy requirements, and evaluates performance metrics. Popular simulation programs have been reviewed in previous studies [185]. Biofabrication technology is still in its infancy and biological products, bioprinting equipment, robotics, and sensors need to mature before cloud manufacturing can be considered. That being said, this highlights a need for developments and laboratories should design their biofabrication spaces with these developments in mind.

An obvious downside to using cloud data centers is encountering privacy and confidentiality conflicts when uploading sensitive healthcare data. Administrators will need to maintain compliance with regulations (e.g. GDPR, HIPAA, etc.) prior to uploading any biomedical data to the cloud. A workaround to this problem is to make use of unique identifiers to protect patient identities and sensitive information, thus enabling the laboratory to take full advantage of the advances in data analytic tools. Cloud computing service providers are also willing to form HIPAA business associated agreements (BAA) to share exposure risk with medical facilities. As a general rule, the minimum necessary should be uploaded to the cloud (e.g. data minimization rule), and uploading derivative data is preferred over source data [186]. Another option is for laboratories to

install their own private server and create their own analysis workflows using platforms that operate independent to public cloud services [187]. Laboratories would lose the advantages of cloud service capabilities, but lower liability concerns by uploading biomedical data to private servers.

9.3 Data Protection, Cloud Security, and IoT Regulations

Biofabrication laboratories in hospital or medical technology company settings will work with large volumes of personal data. Article 12 of the Universal Declaration of Human Rights [188] and Article 8 of the European Convention on Human Rights [189] provide the foundation for the regulatory systems found in Europe and USA. Figure 2 provides an overview of data regulations adopted in other countries. Hospitals and large healthcare systems should aim to establish a robust data governance framework to effectively process data for cloud manufacturing. A data governance framework consists of sequential operations that correspond to a particular phase of data processing. These phases include data protection, risk management, sharing, quality control, and analytics (Medical Data, Sharing, and Harmonization). At the protection stage—data must be de-identified followed by standardized audit trails. Directive 95/46/EC of the European Parliament and of the Council requires the assignment of strict roles such as data subject, recipient, controller, processer, and the data protection officer [190]. The data controller conducts risk management and establishes security levels to protect sensitive data from breaches. Data processors oversee data controllers and determine the appropriate level of access for processing personal data. Both data controllers and processors develop codes of conduct governing data collection, sharing, and processing [190-192]. Lastly, Data protection officers (DPO) are legal personnel with expertise in data protection laws and policies. DPO are responsible for monitoring compliance according to government regulations [191]. In the United States, The Health Insurance Portability and Accountability Act (HIPAA) is the legislative regulation providing national standards for protecting healthcare electronic medical records and transactions for healthcare providers. Healthcare settings should have internal systems already in place to be compliant with HIPAA standards. These internal systems and procedures will likely need to adapt these frameworks to encompass data sharing, QC, and data analysis functions.

Cloud consumers should be informed and versed in their national cloud security guidelines. This ensures laboratories remain compliant with government standards when selecting security objectives, security controls and performing security assessments with providers. Specific to cloud computing regulations, several entities outline best practices for data security. Figure 2 lists the guidelines for cloud providers to tailor their services for laboratory operations (e.g. CSA, IEEE-SA, ENISA, NIST, ISM etc.) [193-197]. Cloud Security Alliance (CSA) outlines cloud provider responsibilities and the relationship between the providers and end users. The CSA has legal entities in Asia Pacific, Europe, and USA and is seen as a world leader in cloud security regulations. Cloud computing possesses a layered architecture made up by a hardware/IaaS layer (e.g. CPU, RAM, etc.), back-end/PaaS layer providing the development environment for creating the applications and services, and front-end/SaaS layer – the cloud-based applications and services [198-207]. CSA defines the security measures for each layer. For example, CSA recommends Software Defined Networking (SDN) within the IaaS layer as opposed to Virtual Local Area Networks (VLANs). SDN provides security isolation and supports multiple users (or tenants) using the same IP address via physical network segregation [208], whereas VLANs are more widely used for single-tenant networks [193]. CSA advises two conventional methods for storage security, specifically, Network-Attached Storage and Storage Area Network to encrypt storage units and prevent exposure [209]. Vulnerability testing will need to be performed, routinely. Static Application Security Testing (SAST) and Dynamic Application Security Testing (DAST) should be used in combination. DAST checks for web vulnerabilities for API executions, while SAST scans for API calls and credentials to prevent system damage. Cloud Access and Security Blockers, IP filtering, and Data Loss Prevention are all viable options for continuously monitoring cloud API connections [210,211]. These cloud-based security brokers monitor user activities to enforce security policies, prevent malware, and alarm administrators of dangerous actions. Multifactor authentication is the preferred form of user authentication for all layers within the cloud [212]. Cloud service providers should perform these security procedures as part of their service.

With regard to IoT standards, there is not a single authoritative regulatory body that laboratories can refer to at this time. Industrial IoT generally refer to a variety of regulatory and standards groups contingent on the type of industry. Figure 2 provides an overview of the standards and regulations laboratories should consult before deploying cloud computing and IoT systems. Laboratories should also be aware of transformative technologies (e.g. blockchain, machine learning) on the horizon that may strengthen data protection strategies [213,214].

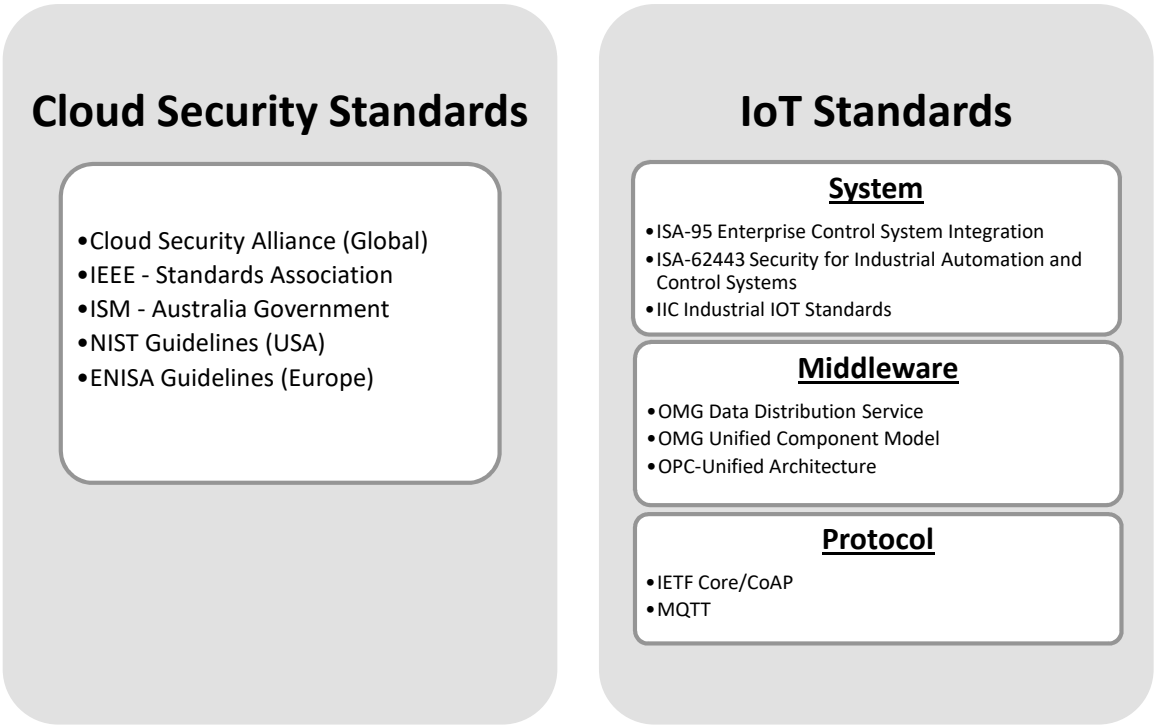

Figure 2. Industrial Security Standards: Left panel lists organizations providing cloud security guidelines. Right panel provides industrial security standards for IoT systems.

9.4 IoT Security

IoT platform security for local laboratories must focus on functionality (e.g. secure boots, key storage, cryptographic acceleration) and assurance (e.g. validating that functions work as intended). Laboratories will need to implement cybersecurity measures to protect IoT devices, intellectual property, networks, and the data acquired during manufacturing [215-217]. IoT can be divided into three distinct layers: application, network, and perception layers [218]. Security issues (see Figure 3) must be designed within the architecture of these layers. Securing IoT architectures must account for a large population of IoT devices, their ability to interact (communicate) with one another and with humans, and their relatively shorter life cycles (high turnover). These features make IoT systems more vulnerable to cyber-attacks [219,220]. Lab developers setting up IoT platforms should review resources that describe common threats encountered at each architectural layer and strategies to neutralize them [221-223]. Cryptography is a tool that shields data using a process of authentication, encryption and decryption. Cryptography algorithms and their keys (e.g. secret value) convert data into cipher text (encryption key) and the key-holder allows data to be converted back into plain text (decryption key). A message-authentication code (MAC) with an authentication key can be sent through the internet to prove that senders and recipients are legitimate and not impersonators. This allows data to be stored and transferred across legitimate senders/recipients (confidentiality), while keeping out intruders (integrity). Commercial laboratories should consider the cost of cryptography when deploying IoT systems for cloud manufacturing. Cryptography contributes to the overhead in the areas of memory, storage, computation, and network bandwidth. Cryptosystems have different trade-offs associated with them including diverging memory size, code size, storage size, and the ability to scale (increase the number of interacting nodes) [224]. Cryptosystems also have varying financial footprints with symmetric-key (secret-key) being the least, asymmetric key (public-key) moderately so, and certificate-based asymmetric cryptography having the most weight. Selecting between secret- and public-key cryptosystems narrows preferred cryptographic algorithms laboratories will likely implement. Secret-key systems typically employ Caesar cipher, Block cipher, AES (advanced encryption standard), and DES (Data Encryption Standard) algorithms [225]. Whereas, public-key systems predominantly use elliptic curve cryptography (ECC) [226], Diffie-Hellman, and Rivest-Shamir-Adelman (RSA) algorithms [227]. Many IoT standards prefer ECC algorithms over RSA for their smaller key sizes. Quantum computers will pose a greater security threat for cryptosystems when they become more common [228]. However, it is too early to determine which quantum-safe algorithms will become industry favorites. Laboratories can be confident that symmetric cryptography is sufficient for IoT, with doubling key sizes being the most feasible deterrent to quantum computers [229]. Novel encryption and signature algorithms are currently being developed to prepare for a post-quantum world including: code-based encryption [229], lattice-based encryption [230], lattice-based signatures [231], multivariate-quadratic-equation signatures [232], hash-based signatures [233], isogeny-based cryptography [234], and Kuperberg's algorithm [235]. A major component in IoT system security for

biofabrication laboratories will be the digital surveillance system (DSS). DSS will likely include the camera systems mentioned in cell production (e.g. microscopy), biomanufacturing facilities (e.g. bioprinting cameras), and storage unit cameras. These cameras are vulnerable to attacks and IoT security systems need to be established [236-238]. Network architectures should separate video data from other traffic using a protected VLAN. This does not encrypt the data, rather it creates a separate logical segment within the network for video traffic. Tags can be applied to the camera devices so that they share the same cabling with the network architecture. This would limit potential attackers to that device without exposing the entire data network.

IoT platforms must anticipate and accommodate IoT device key sizes, key infrastructures and cryptographic algorithms. The Open Connectivity Foundation (OCF) (see https://openconnectivity.org) is providing industry with IoT interoperability standards and architecture for connecting devices regardless of operating system, manufacturer, or chipset. IoTivity (see https://iotivity.org) is an open source reference that laboratories can access to design their IoT frameworks according to OCF standards.

System abstractions are logical representations of the set of physical devices comprising the IoT system. IoT devices are physical equipment, while IoT nodes are their logical abstractions. The core framework layer defines the abstraction model in the OCF architecture and contains built-in resources for security, permissions, identity, data transmission, data management, and device management. The OCF security architecture oversees three main features: encryption, access, and device lifecycle management. A variety of cryptographic algorithms are supported on the OCF architecture including symmetric, asymmetric and certified asymmetric. Effective IoT system management will entail device lifecycle management and requires rigorous inspection, configuration, updating, and proper decommission. Decommissioning ensures sensitive data (e.g. keys and credentials) are erased from the device. IoT system scalability can more easily be achieved by selecting flexible cryptosystems and designing adaptable IoT frameworks (e.g. middleware layers supporting IoT applications). IoT architects should document the principles, architecture, and connectivity choices when designing the IoT system to simplify maintenance and updates. Laboratories can experiment with other IoT frameworks (e.g. Universal Plug and Play, AllJoyn, Lightweight Machine 2 Machine, etc.) beyond OCF depending on their preferences. Blockchain architecture is being developed and incorporated into Industrial IoT/CPS applications [239]. Blockchain provides advanced cryptography, decentralized data sharing, more efficient data storage, and built-in cryptocurrency support [240]. Laboratories should be aware of these developments and the security issues unique to blockchain architectures [241].

Lastly, computer system validation (CSV) consists of procedural hardware and software tests to confirm consistent operation of AM systems. Smart manufacturing also requires regular network testing to verify its stability under normal and high load. Printed products are directly related to the processing software of the printer and the automated systems governing the manufacturing process [242]. ISPE GAMP 5 offers a set of guidelines for meeting cGMP regulations in these areas. In general, electronic records are another concern, and the FDA and Europe EudraLex provide rules and recommendations for proper management [243, 244]. The electronic records and signatures are considered equivalent to paper records. Computer systems and networks should be evaluated to ensure their accuracy, reliability, consistent performance, and their capability to recognize invalid or altered records (21 CFR 11.10). Standardized procedures should be used when creating, modifying, maintaining, or transmitting electronic records to certify their authenticity, integrity, and confidentiality (21 CFR 11.30). Annex 11 requires IT infrastructure to be qualified and data should be protected by physical and electronic means. Laboratories should validate these applications no matter they use their own server infrastructure or outsourced cloud platforms.

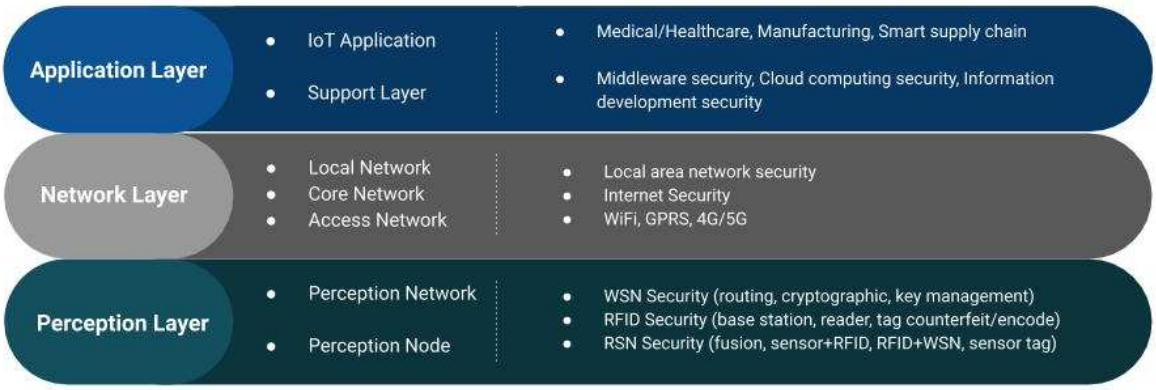

Figure 3. IoT Architecture of Security: IoT Application layer provides users with specific services along with their corresponding support layer for each application. The Network layer connects devices and divides into local, core, and access networks. The perception layer is the physical layer with their information sensors. Each layer identifies components making up the security architecture.

9.5 – The Hospital of the Future

The types of Biofabrication technologies that will exist in the Hospital of the Future will be driven by the marriage of such diverse disciplines ranging from advanced medical scanning, virtual and augmented reality, machine learning and 3D scanning modelling and printing. This unique multidisciplinarity dictates a special workforce skillset encompassing medical physicists, clinicians, materials engineers, big data experts, gamers, mechatronics and roboticists, biologists and health economics experts. This workforce must connect and communicate to push technology towards commercialisation, a critical gateway for Biofabrication technologies to see application in the clinic. The 3D-P Biofabrication laboratory is key to this vision and Universities are key players to upskill and train the next generation of Biofabrication technical staff and researchers. Alongside these facilities and workforces, the clinical teams should be well integrated to ensure that clinical problems can be well defined from the outset and commercialisation opportunities would ideally be supported within nearby spaces to take novel inventions from bench to bedside. The concept of having patient consultations with biofabrication active clinicians who are embedded within such a facility provides opportunity for rapid solutions using advanced imaging, modelling and printing and brings the technology ever closer to point of care manufacturing which is the holy grail of 3D printing in healthcare.

A Hospital of the Future vision might incorporate the following spaces for Biofabrication research to deliver bench to bedside solutions for patients suffering tissue loss (Figure 4) [245]. The critical space is of course the 3-D printing and Biofabrication area which could span both floors including bioprinting to be located close to the cell biology and cell culture facility and generic 3D printing and associated workshops which are on the floor above, connected through a staircase. To ensure connectivity and creativity open and collaborative spaces for researchers, clinicians, students, industry partners and spin outs will be located in the central innovation hub. Co-lab spaces will house technology spin outs. 3D scanning using advanced medical imaging as well as optical scanning will be located closely to the patient interface zone where clinicians will have patient consultations and the patients are able to enter and leave the facility with a degree of discretion. The institute space should also be educational and support university students and high school students to undertake cutting-edge projects within the facility as well as being a hub for industry events and networking to enable partnering opportunities and exposure for early stage technology investment.

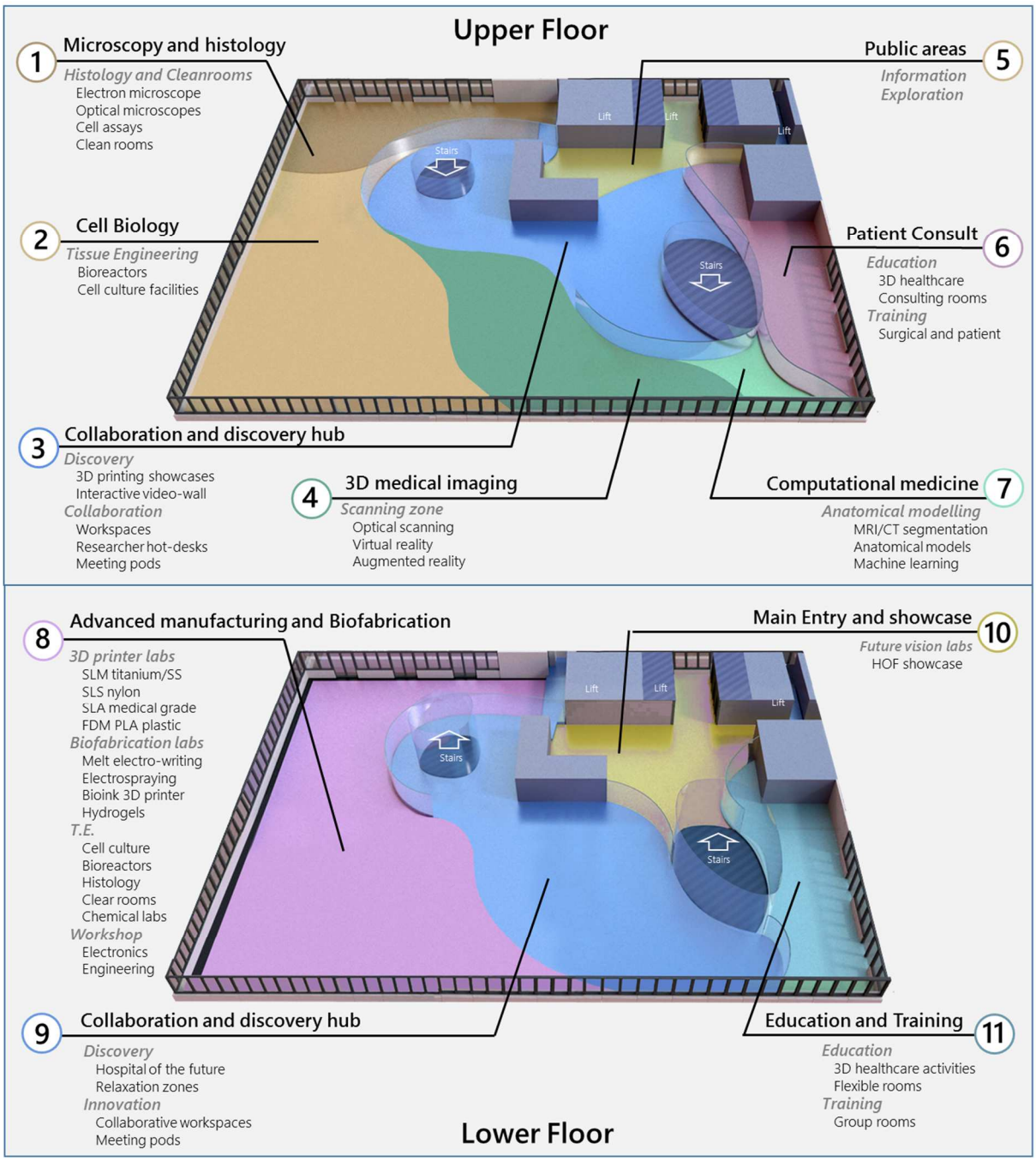

Figure 4. Floorplans of hypothetical biofabrication institute indicating the various zones.

10. Other Considerations

Local jurisdictions will not only affect the building requirements the biofabrication facility has to fulfill, it will also impact what sort of a regulatory framework the bioprinted product will have to comply with. Depending on where the product is sold and utilized, classification of bioprinted products currently vary widely. The regulatory bodies historically classify therapeutic products according to their use, which can be a medical device (classic example would be a hip implant), a pharmaceutical compound (a drug) or biological medical device (cell implant) [246]. Bioprinted constructs do not often fit into a single category and might be a medical device (the material scaffold), a pharmaceutical compound (releasing a drug) and a biological medical device (containing cells) at the same time. While in Australia the Therapeutic Goods Administration (TGA) would treat bioprinted products as a so called "borderline" or a "combination" treatment [247], in the US the FDA would classify them as a "combination product" [248]. In the EU on the other hand, bioprinted products might fall into one of several categories of so called "Advanced Therapeutic Manufactured Products" [249]. Operators of a biofabrication facility and manufacturers of the bioprinted products are well advised to consider the ever-changing regulatory landscape in the field of bioprinting and consult current guidelines relating to what

classification their intended bioprinted products would fall into [250]. For a more in-detail analysis on how the regulatory frameworks would apply to the bioprinted products we refer the reader to dedicated reviews on the topic (246,251).

Amid all the regulatory, technical and logistical challenges a 3D biofabrication laboratory poses, with every new technology, ethical questions are also undoubtedly raised. These questions vary from the source of the cells used in the printing approach to what happens with the final printed product. While autologous cells from the patients' own body might not raise ethical questions [252], other cell sources such embryonic stem cells (ESCs) already do [253]. With the advent of human induced pluripotent stem cells (hiPSCs) [254,255], the ethical issues have seemingly been bypassed, but not without presenting the scientific community with a new set of ethical concerns such as abnormal reprogramming, tumorogenicity, human cloning or the production of human germ cells [256]. On the other end of the biofabrication production line, we need to have a discussion about how we could potentially use these newly bioprinted products. While full organ printing might be a long-term goal, an early agreement of what is an acceptable use for these products would be beneficial for acceptance of the technology; could they be simply used to replace a diseased organ, or could we use them to enhance humans such as athletes or soldiers, effectively creating super-human power? How often can we replace a diseased organ i.e. when does it become unethical to prolong a human life beyond its natural lifespan? While biofabrication is an exciting technology which has the potential to save human lives, researchers in the field are already faced with these challenging questions [257-259]. It is vital for the creators of a 3D biofabrication facility to pay close attention to public perception to ensure transparent and accurate science communication creates a safe and innovative space, rather than one shrouded with secrecy or hype. Importantly, it is critical to ensure robust data, peer review and ethical work standards are enforced for optimal clinical outcomes. None of this is driven by technology advances, it relies heavily on scientific expertise, culture, collaboration and creating the right teams who are united in the single vision, in their pursuit of Biofabrication excellence to always strive to improve patient quality of life.

Tissue engineering overlaps with many of the prominent ethical conversations today such as privacy concerns (big data) and medical ethics as it relates to healthcare access, and the ethics pertaining to electronic repairs. As mentioned in previous sections, precision medicine and biofabrication will require obtaining personal data from a patient to customize products for their condition. This data will include traditional forms of data collected in electronic medical records along with new forms such as genomic data and tissue samples. These data will be collected more readily and may be vulnerable to cyberattacks and unethical sharing. Big Tech and genomic firms have faced cyberattacks or committed such violations with user statistics collected from their platforms. The maturation of blockchain technology may supersede the current cloud-based epoch and reestablish a decentralized platform with privacy. Until then, tissue engineering organizations will have to navigate these ethical tensions. Data sharing may be an impactful source to innovating the field of tissue engineering. The EU BioSHARE Project developed an initiative known as the Framework for Responsible Sharing of Genomic and Health-Related Data [260-263]. This initiative provides foundational principles for ethically sharing sensitive data. Laboratories can look to this framework for guidance if no other alternative frameworks are available locally.

Similar to most new products, the cost of production depreciates over time as the company and industry matures. The cost of production affects the market price, and ultimately the consumers who can afford them. For example, the first car ever assembled was the steam-powered automobile in 1769 [264]. The first vehicle powered by an internal combustion engine was 1803. The first gasoline-powered production vehicle was created in 1885. Yet, the automobile did not become mass-produced until 1908 nearly 140 years after the first car. A similar pattern is observed with the history of computers [265]. Public health ethics is focused on positive rights [266], population health, disparities, inequalities [267], access [268], affordability [269] and has become the dominant paradigm amongst most OECD countries [270]. Countries may accept disparities for certain technologies, but it will be difficult for countries with public health systems to accept unequally distributed technologies that greatly enhance quality of life or increase life expectancy (e.g. nerve regeneration and motor function restoration, patient-specific organ transplants etc.). The field of tissue engineering will eventually enter the cultural conversation on whether regenerative medicine services are a universal right or privilege (service). New economic, business, legal, or ethical systems may have to be improvised until tissue engineered products can be mass produced.

Finally, the ethical concerns related to legislation governing the ability to repair and modify consumer electronic devices is beginning to influence the 3D-P industry. Electronic manufacturers with large market shares in an industry have a vested interest in protecting confidential trade secrets and other intellectual property [271]. Many electronic manufacturers accomplish this by creating systems where repairs and repair parts can only be

obtained from authorized vendors or the original manufacturers. Companies have been successful in lobbying governments to create legislation that prevent consumers from repairing or tinkering with devices [271]. The right to repair movement has grown out of these conditions and many large corporations have resisted. Companies in several industries have used these tactics to drive up repair services for consumers and or throttling the speed of their products to encourage product upgrades. 3D-P and other medical technology companies have developed similar institutional practices to regulate software experimentation (experimental licensing), mandating company technicians to repair printers, and manufacturing printers to be more modular thus requiring entire subsystems to be replaced rather than the single damaged part to be replaced. These developments have drastic effects on the cost of 3D-P activities, 3D printer lifespan, industry dynamism, and opens 3D-P consumers to similar abuses found in other industries [272]. Laboratories with large investments in commercial bioprinters should consider forming an escrow agreement to ensure maintenance of software or hardware should the licensor go bankrupt or fail to update its product. Laboratories with smaller budgets can avoid these costs up front by building their own 3D bioprinters using the instructions in the following resources: McElheny; Kahl et al.; Kharel et al.; Lanaro et al. [273-276].

11. Conclusions

Many of the recommendations listed as part of this review are the product of prior pitfalls experienced while establishing a medical 3-DP lab. These pitfalls are often known to engineers and professionals working at industrial labs which have previously been the home to such technologies. However, given the recent decreases in cost of technologies, as well as interest from the medical and educational communities in 3D-P, it is important to develop a set of guidelines and best practices for those individuals not familiar with setting up such technologies within a new facility. By addressing potential pitfalls in a systematic way as outlined in this review, one can implement the appropriate procedures and decrease the risk and cost of preventable equipment failure, while "future proofing" biofabrication laboratories for eventual upgrades in 3D-P technology. Table 6 summarizes many of these recommendations for professionals engaged in planning and designing biofabrication laboratories. Laboratories should consider the cost of implementation, cost of ownership (maintenance and operation), ease of implementation, and scale of implementation before investing in any strategy. Firms in academic, community, or commercial environments will have different priorities and abilities to invest in a given laboratory capacity. For sustainability, laboratories should prioritize their investment in market proven technologies with widespread adoption. Many inexperienced laboratories will invest heavily into the latest equipment generation (e.g. bioprinter, microscope, bioreactor, etc.) with short product lifecycles without considering the appropriateness of the facility needing to support it. Finally, laboratories should consider the ease of transition before investing in any given technology. For instance, laboratories with FDM printer expertise will find the transition to extrusion-based bioprinters much easier than SLA/SLS printing technology. Laboratories can avoid incurring tremendous costs for training and additional equipment by considering these issues.

We anticipate the hospital of the future will develop revolutionary technologies that will transform healthcare to deliver highly automated, personalized, and customized patient solutions. These advances will provide lower health costs, accelerated implementation of optimised clinical treatments, and deliver significantly better health outcomes for individuals and society [245]. 3D-P plays a key role in this revolution, among these approaches; biofabrication is a growing area of interest which requires specialised spaces, teams, organisation and culture to realise the true clinical impact [245]. Advanced technology of 3D-P combined with advanced medical imaging and modelling promises to produce patient-specific replacement tissue constructs and restore biological function and health in a rapid, tailored manner. As an alternative approach to current bone grafting and permanent implants, biofabrication combines the body's own regenerative capacity with bioactive factors and biodegradable biomaterials. These are formed into the complex shapes required to restore tissue form and function [240]. Not surprisingly, the promise of biofabrication is driving significant research activity as teams progress this new technology toward routine clinical use and the guidelines for establishing world-leading facilities to support this promising new era become increasingly important.

Design Consideration	Potential Pitfall	Mitigation Strategy
Site Regulations	GTP compliance	BSL2 Laboratory
Power Considerations	Energy inefficiency Emergency power system	Power consumption devices Diesel Generator + UPS
HVAC System	Environmental control Inadequate filtration	BAS + agar plating Pre-filter + HEPA filter
Vibration	Print defects	Install printers on floating surface
Microscopy	Inadequate equipment	Large-scale: Laser-autofocusing, confocal microscopy; small-scale: Phase-contrast microscopy
	Automated cell profiling	Segmentation with CellProfiler, DeepCell, CDeep3M, U-Net Feature Extraction: CellProfiler, PhenoRipper, CellCognition Classification with CellProfiler, Micropilot, Cell Cognition Explorer Image Resolution: DenseDeconNet
	Cell sorting	Intelligent image-activated sorting
Bioprinters	3D bioprinting system cost	Build in-house 3D bioprinter units
	Print parameter optimization	Test parameter settings with second printer
	Print contamination	Perform trial runs on second printer. Final prints performed within BSC.
	Maintenance	Develop bioprinter validation plans; Follow recommendations of manufacturer
Quality Control	Cell preparation	SOPs, Cell characterization/sterility tests, and Batch records
	Bioink preparation	Automated pipetting workstation
	Print validation	Shape fidelity: Microscopic & OCT Imaging Construct maturation: Spectroscopic analysis & Quantitative Ultrasound
	Post processing	Automated bioreactor system
	Storage, delivery and tracking	Automated Storage and Retrieval RFID Tags on products Cloud enabled asset localization
Data Management	Storage and processing	Commercial cloud data centers
	Security	Virtual-LAN Tagging for IoT devices Unique Identifiers for cloud data

Table 6. Design considerations, potential pitfalls and mitigation strategies for bioprinting within a medical 3-DP laboratory.

Declaration of interest

None.

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