2D and 3D Inkjet Printing of Biopharmaceuticals - A Review of Trends and Future Perspectives in Research and Manufacturing

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Abstract
There is an ongoing global shift in pharmaceutical business models from small molecule drugs to biologics. This increase in complexity is in response to advancements in our diagnoses and understanding of diseases. With the more targeted approach coupled with its inherently more costly development and manufacturing, 2D and 3D printing are being explored as suitable techniques to deliver more personalised and affordable routes to drug discovery and manufacturing. In this review, we explore first the business context underlying this shift to biopharmaceuticals and provide an update on the latest work exploring discovery and pharmaceutics. We then draw on multiple disciplines to help reveal the shared challenges facing researchers and firms aiming to develop biopharmaceuticals, specifically when using the most commonly explored manufacturing routes of drop-on-demand inkjet printing and pneumatic extrusion. This includes separating out how to consider mechanical and chemical influences during manufacturing, the role of the chosen hardware and the challenges of aqueous formulation based on similar challenges being faced by the printing industry. Together, this provides a review of existing work and guidance for researchers and industry to help with the de-risking and rapid development of future biopharmaceutical products.

Table of Contents

Section 1. Introduction

Biopharmaceuticals are inherently biological in nature and manufactured by or from living organisms involving bioprocessing techniques [1] for the prevention, treatment and diagnosis of diseases. They are categorised most commonly by the product type or therapeutic category. The product-based categories include cell, gene, and antisense therapies, monoclonal antibodies (mABs), recombinant proteins and vaccines, with mABs being the most substantial subcategory in terms of number of products in the development pipeline [2]. More generally, protein-based therapeutics, encompassing and not limited to mABs, enzymes, polyclonal antibodies and hormones are the dominant product category within the biopharmaceutical industry and will therefore be the focus of this review as we consider the shift towards digital fabrication techniques.

The biopharmaceutical industry was valued at $186M in 2017 and is forecast to grow rapidly to $526M by 2025 [3]. 6 of the 10 highest grossing drug products in 2017 are mABs, 2 are small molecule drugs and there was 1 vaccine, and 1 insulin product meaning 8 of the 10 are biopharmaceuticals [4]. Yet unlike their small molecule counterparts, high revenues are driven by higher unit costs rather than increased sales volumes enabling the biopharma sector to be more fragmented in nature than the small-molecule pharma counterpart. This is noted in Figure 1, which also highlights the comparative complexity of biologics and their production processes, which can result in greater batch-to-batch variability and microheterogeneity [5].

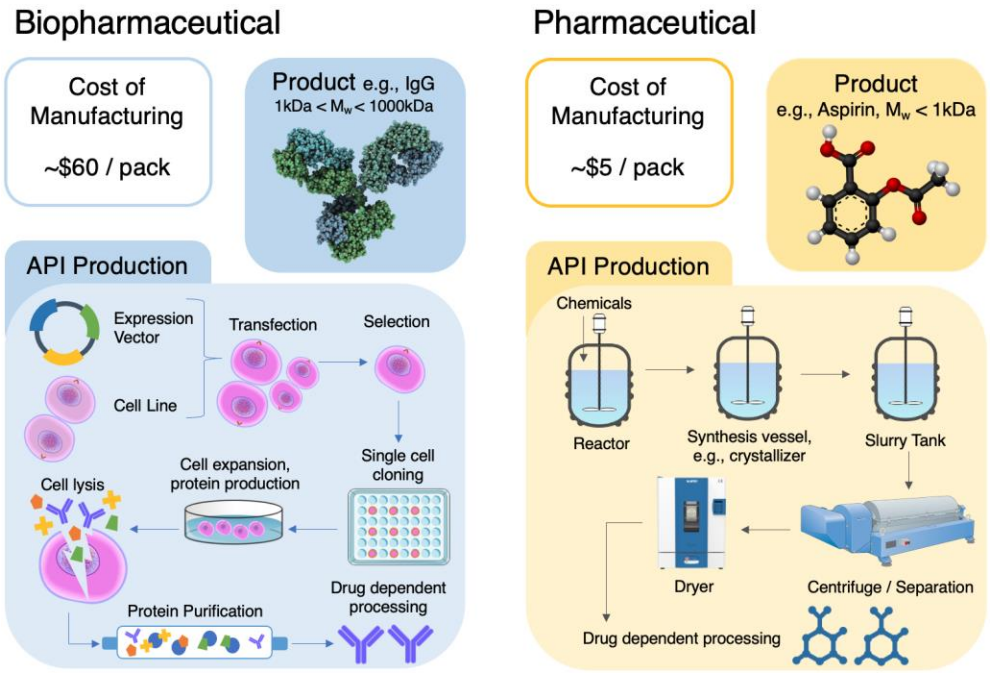

Figure 1: A comparison between manufacture, cost of manufacture [6] and product complexity within the biopharmaceutical and pharmaceutical industry for protein therapeutics and small molecule drugs.

Within the small-molecule sector, adoption of 2D and 3D printing technology has been explored for a variety of applications from system discovery through to production of pharmaceutics. It has been noted previously that for small molecules, printing technologies are most appropriate for use with relatively lower volume, higher variety products [7] and are frequently cited as a platform technology for dispensing active pharmaceutical ingredients (APIs) with control over the dosage that can be fully customised for the individual patient [8]. However, advantages for the use of 2D and 3D printing technology for the deposition of protein therapeutics must also be explored and the future challenges of technology to deliver this advanced therapy must also be highlighted to direct further research and help drive a rapid adoption. In this review, recent developments in the biopharmaceutical sector will be presented, linking with the observed manufacturing trends. This will provide context and justification for a shift to exploring digitally enabled 2D and 3D printing techniques, with a focus on protein therapeutics in particular. We then draw on multiple disciplines to explore the shared challenges facing researchers and firms aiming to develop biopharmaceuticals, allowing us to highlight a pathway for future research.

Section 2. Biopharmaceutical market developments

As indicated in Figure 1, there is a significant shift in manufacturing complexity and product cost when comparing small-molecule and biological pharmaceuticals. This review firstly examines the shift in business model that is enabling this move and how this is enabling a move to 2D and 3D printing of pharmaceutics.

2.1 A shift in business models - from small molecules to biologics

While the business challenges facing the pharmaceutical industry are wide-ranging, especially in supply chain management [9]–[12], the key issue for firms is how to address declining revenues in conjunction with increasing costs. While Ehrhardt *et al*. (2012) forecast that $400 billion of revenue could be exposed to generic competition [13], other sources estimate that the industry would lose $150 billion to

generics between 2012 and 2018 alone [14]. Pharmaceutical companies are strategically re-assessing ways of managing costs to direct necessary investments towards specific pipelines while improving confidence in target and candidate drug selection [15]. It has been argued that firms should strategically shift their R&D investments from small to large molecule (biologic) compounds [16] to address this main challenge. One primary reason for this is the average biologic offers a greater return on investment owing to higher average peak sales and a reduced drop-off in sales following a loss of exclusivity [17]. Guarding against such drop-offs and losses of exclusivity has become a defining business case requirement for the sector, which has typically experienced 80% volume losses in the first year off-patent. In the case of Prozac, for example, there were 70% volume losses in just 45 days [18]. This shift in business model was quantified by the Tufts Center for the Study of Drug Development, finding that 15.2% of products in clinical development were biologics in 2000, but this had increased to 40% by 2012. However, from 2010-2017, the U.S. Food and Drug Administration (FDA) approved a total of 262 new molecular entities (NMEs), excluding several diagnostic imaging agents and 1 insulin analogue, with 76% (199) of these being small molecules and only a quarter being biologics [19].

This 'patent cliff' which has come to signify the small molecule segment is now being felt in biologics, with 7 of the twenty largest biologic products having gone off patent in Europe [20]. Currently, monoclonal antibodies dominate the biologic market sales, and remains the largest technology class within the biologic pipeline [20]. These high revenue products would be expected to be top priority targets for biosimilar manufacturers. However, as of November 2016, biosimilars have been launched for only three of the seven (infliximab, insulin glargine, etanercept) with evidence of delays after patent expiry [20]. The reasons for this were identified as: cost; market opportunity; patent uncertainty; regulatory difficulties and complexity in development. It is not yet clear what will be the impact of more personalized medicines. It is anticipated this will lead to increasing manufacturing costs due to expensive (complex) processes for biologics, significant reductions in batch sizes, and a requirement to reassess traditional 'make-to-stock' supply chain models, all of which remain to be addressed.

With the current market for high volume therapeutics at saturation point, characterised by many existing options available to patients [9], it is important to note that environmental conditions are also shifting. An ageing population has seen an increase in the prevalence of chronic illnesses, such as diabetes, cardiovascular diseases and cancer [21]. There is an increasing need for new biologic-related rare disease treatments and the threat of COVID-19 type pandemics will likely spur the development of biologic vaccines [22]. With its many specificities, (niche and personalised, low volume – high variety, targeting for sub-populations) it is argued that the oncology segment may best exhibit the characteristics of next-generation therapeutics [23]. The segment is also representative of growing complexity – with more than 300,000 new cancers (excluding skin cancers) diagnosed annually in the UK, across over 200 different cancer types [24]. This is also reflected in the oncology segment fast becoming one of the major therapeutic areas in the sector, capturing 31% of all R&D effort [25], and experiencing the fastest growth compared to other therapy areas [26]. The emergence of more targeted molecular therapies has contributed to accelerated growth within the oncology market, with forecast spends expected to be circa $74–84 billion by 2018 [23].

2.2 A shift in manufacturing processes - from batch to continuous and printing

In this section, we briefly outline how and where printing technologies may enable a shift from batch to continuous processing in biologics, and in turn may offer process patents or patent extension opportunities to help ensure competitiveness through technological disruption, such as in a recent application involving the Integrated Continuous Manufacturing of Therapeutic Protein Drug Substances [27]. It is important to note that there are a range of dosage forms, and looking at oncology as an example there is a clear segmentation between injectables/vial options and oral formats, with a notable shift in recent years towards the latter [28]. When analysing the potential impact of printing on this sector, these will have different considerations. Production processes involving injectables currently involves a series of technological and process challenges (e.g., freezing and sublimation steps), that may lead to

opportunities for radical disruptions involving printing technologies within the process [23]. Oral dose forms, on the other hand will benefit greatly from a technology disruption both in continuous (primary) manufacturing and the manufacturing of pharmaceutics.

In their review of future pharmaceutical manufacturing, Rantanen and Khinast (2015) outlined that the key enabling factor for cost-effective personalised therapies is the development of new manufacturing principles [29]. Future re-distributed and sustainable manufacturing scenarios were explored in the context of smaller, more cost-effective facilities ('continuous' processing; micro-factories, 'lab-on-a-chip' systems), using smaller quantities of expensive ingredients and less energy, with more control over final product quality and performance [30], [31]. Printing is a key tool called upon when developing flexible processing solutions for continuous operations and these are expected to enable personalised drug delivery systems with tailor-made dose, drug release characteristics and combinations of drug compounds based on individual needs [32]. Progress has been made with automated control via detailed mathematical models on the bench scale, and there is growing evidence that continuous bioprocessing, while still lagging behind small molecule continuous processing, is starting to catch up on the commercial scale. In 2019, Sanofi opened a US-based facility that will use digital technology for the continuous production of its Genzyme portfolio of products, which includes biologics for rare diseases, immunology, and oncology [33], [34]. With further rolls out planned in five other legacy sites, this test-bed facility aims to use end-to-end data to optimise performance and use 'real time' data to digitise supply forecasting. As well as using digital twin concepts to make process adjustments, they also plan to use these as virtual training tools, in a sense building confidence, in their processes. This $400 million investment in digitalisation is part of its focus on biologics-based therapies, which is a reflection of Sanofi's future R&D pipeline [34].

Making medicines in less time, for twice the number of patients, and all within smaller environmental footprints is particularly relevant for unmet patient needs and affordability for low volume, high variant products. In this space, alternative processing models may be used to serve existing markets more effectively or those that have been previously considered uneconomical to serve (delivering unmet end-user needs) driven by new capabilities (such as 2D and 3D printing) that create new markets [35]. This is particularly true when treatment regimens are complex and increasingly difficult to retain profitability in manufacturing, such as for some specialised products (e.g. antiretrovirals [36]). Currently, certain volume demands are required to 'trigger' a production campaign. This process can be lengthy, with 8-month delays commonly recorded between initial orders and the initial batch production. In addition to antiretrovirals, the growing oncology segment also experiences frequent supply issues when it comes to essential drugs (e.g. Methotrexate, Paclitaxel), ranking second in terms of shortages [37]. Hence, eliminating such lead-times and improving security of supply, through adopting smaller volume runs through continuous-type routes, is an area of focus when assessing attractive business propositions and technological feasibility [35]. Advances in diagnostics, information technologies and digitisation are enabling disaggregated value chains to enable personalised medicines or niche product markets for patient populations [35]. Printing processing in the biopharmaceutical sector is anticipated to enable a range of linked disruptions [30], including (1) the rapid scale-up of new niche drug products in smaller volumes, (2) novel delivery formats, with the option of late-stage personalisation and customisation, and (3) agile supply chains designed to manage the potential of significantly increased stock-keeping-units (SKUs). However, before considering the technical challenges to deliver this, it is important to review regulatory challenges.

2.3 Regulatory considerations for 2D and 3D printing of biologics

As noted already, biologics are produced through complex processes, but it is important to note that they are also difficult to certify [38]. When developing precision-medicine, the costs of meeting safety and efficacy requirements of regulatory agencies have been steadily increasing [15]. Considering the complexity of biologics and limited clinical and regulatory experience with biosimilars, a more cautious and conservative approach has been common for all product classes [39]. In line with the Genzyme

example in section 2.2, one key area currently lacking is the enabling manufacturing and testing technologies that will allow effective integration of the upstream and downstream processes to support real-time release testing (RTRT) for, as one example, therapeutic proteins. To implement a complete RTRT solution, biologic manufacturers need to consider critical attributes, such as sterility and the measurement of viral and microbial contamination. Despite these challenges, continuous and printing technologies have the potential of informing 'proof-of-concepts' that may allow the rapid test and validation of new and existing treatment approaches (e.g. controlled drug release) across a variety of disease settings and patient cohorts. Case studies for the implementation of continuous platforms have also been presented covering perspectives such as scale-down mimics, control strategies, and cost of goods analysis [40].

While advances in digital production contexts have delivered value in terms of enabling data and information exchanges, implementing analytics linked to Quality-by-Design (QbD) principles for the 'real-time' release of products has long been an ambition. Continued advances in on-line and in-line sensor technologies are key for biopharmaceutical manufacturing in achieving regulatory approval of RTRT [41], which if achieved, will be have a dramatic impact on the market especially as targeted therapies and immunotherapies are expected to make up more than 70% of treatments by 2020 [42]. For example, predictive capabilities enabling 'real-time' comparisons against 'ideal' process states could enable reduced quality control resources (no post-manufacturing testing) and potentially eliminate or reduce end-product testing times.

Interestingly, the challenging regulations also offer another opportunity to benefit from drop-on-demand printing technologies. The majority of commercially produced recombinant protein therapeutics are through mammalian host cells, particularly Chinese hamster ovary cells and murine myeloma lymphoblstoid-like (NS0 and Sp2/0-Ag14) cells [43]. Regulatory guidance references the need for recombinant proteins to be produced from a single cell source in multiple guidance documents, including the European Medicines Agency (EMA) ICH Topic Q 5 D guidance [44], in the FDA guidance on manufacture and testing of monoclonal antibody products for human use [45] and in more recent legislation from the EMA from 2016 [46]. Yet it is not only a regulatory imperative to adhere to single cell clonality, from a manufacturing perspective it is advantageous to reduce variability in the final product quality. Phenotypic drift occurs during cell culture impacting upon characteristics of certain attributes within a cell population, through single cell sourcing the variation of a given attribute is minimised [47]. For compliant production of protein therapeutics, the isolation and manipulation of single cells is a necessary manufacturing competency. Inkjet printing technology and piezo-electric actuation has been used to isolate single cells. For the specific application of ensuring single-cell cloning Cytena's Single Cell printer ensures cells are from a single source with >99.99% assurance with an automated system based on piezo-electric dispensing of cell containing droplets [48]. Further studies using piezoelectric inkjet printers have successfully isolated single cells, although not necessarily for this specific application. Adult rat ganglian and glia cells were ejected with a microfab print system, a glass capillary piezoelectric nozzle and high speed images of the cell containing droplets were presented showing a single cell in each droplet with no evidence of cell deformation upon ejection [49]. Another relevant application is the production of single cell arrays. Instead of direct dispensing of cells into a specific position, a surface is modified digitally to provide sites for cell attachment. This method removes the need to expose cells to the potentially damaging effects of inkjet printing [50], [51] but relies on the reliability of single cell attachment, with examples showing yields from 60% [52] to 80% [53].

In summary, there are a range of exciting opportunities to build capabilities in manufacturing for biologics using printing technologies, but they have more complex production processes than small molecule drugs, and tend to yield much smaller quantities with less uniform batch-to-batch equivalence and so regulatory challenges are to be expected. It is also difficult currently to scale biologics from laboratory quantities used for early analysis and pre-clinical testing to larger-scale batches while

maintaining product purity [54]. The rest of this review will examine the technical challenges identified as critical to delivering improved reliability for printing in biopharmaceuticals and show that there is a complex journey to consider from the formulation, to the processing hardware and the printing flows to ensure a final active material.

Section 3 Developments and challenges in printing for biopharmaceutical applications

In this section we examine specific applications where 2D and 3D printing are already leading to rapid developments in biopharmaceuticals, namely in biologics discovery, drug delivery by microneedles, buccal drug delivery and oral administration. After this, we will review challenges that are shared both when developing pharmaceutics or when developing printing techniques for integration into distributed or continuous manufacturing.

3.1 Brief overview of 2D and 3D printing by drop-on-demand and extrusion techniques

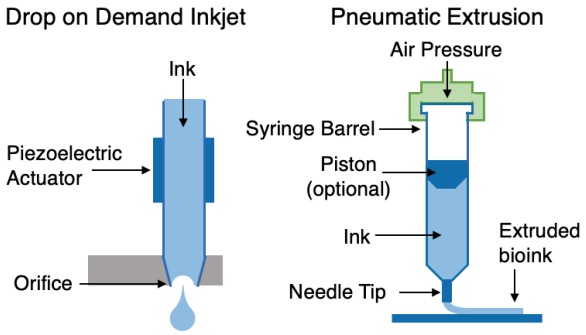

Figure 2: A simple representation of the key features of a drop on demand inkjet printhead and a pneumatic extrusion system.

Drop-on-demand (DoD) inkjet printers produce a single drop of ink only when required and in response to a trigger signal. Most commonly these are either piezoelectric or thermally actuated printheads, with either case providing energy to the volume of fluid close to the nozzle and enabling a drop to be created and fired towards a surface. There are a range of different actuation modes for piezoelectric driven printheads. However, in all cases they rely on the voltage applied to a piezoelectric element to drive a deformation of the actuator. This can, for example, change the volume of the ink reservoir to propagate a pressure wave and generate a droplet. Thermally actuated printers form droplets through rapid heating of the microscale resistance heaters close to a nozzle. The rapid pulsed heating drives bubble formation and collapse to create firstly a positive pressure wave through the ink resulting in droplet ejection, and a subsequent negative pressure to draw in fresh ink.

There are three commonly used actuation techniques for extrusion-based printing: pneumatic, mechanical and solenoid. Firstly, pneumatic extrusion relies on pressurised air being applied to a reservoir of ink and forcing it through a nozzle to the surface. Secondly, mechanical extrusion relies on a motor to drive a piston that then forces ink through the nozzle. Finally, there is solenoid extrusion, where a combination of pressurised air and actuation with a ferromagnetic plunger enables the ink to flow through the nozzle. Further information on inkjet [7], [55] and extrusion [56] can be found in the references detailed.

Inkjet printing or electrospray are predominantly used when printing 2D layers of biopharmaceuticals and involve the deposition of a functional material onto a substrate in an x and y axis, whereas 3D printing is used to produce layered structures that have a significant dimension. 3D printing of biologics is mostly carried out by ejecting continuous filaments of highly viscous fluids or gels, using pneumatic,

mechanical or solenoid valve actuation. Figure 2 illustrates the elements of inkjet and extrusion and Table 1 shows an overview of the key features compared for each approach. In summary, inkjet printing enables smaller feature sizes and is highly scalable by increasing the number of printheads or the number of nozzles in the selected printhead. Extrusion techniques are slower, have a lower resolution, but can manage to print with a much wider range of fluids, most commonly viscous fluids or gels containing the biologics.

Table 1: Features of Drop on Demand Inkjet Printing and Extrusion.

	DoD Inkjet	**Extrusion**	**Refs.**
Ink Viscosity	5 – 20 mPas	30 to > $6x10^7$ mPas	[57]–[59]
Print Speed	Industrially, ~200m / minute. Droplet ejection up to 10kHz.	Slow, in range of cm / s	[60] [61]
Nozzle Size	20 - 120 µm	>150 µm	[58] [62]
Resolution	1 to > 300pl, down to ~5 µm droplet spacing.	Spatial resolution down to 1 µm, but filaments typically >100 µm wide.	[62]–[64]
Scalability	High, nozzle packing density is high due to low crosstalk between nozzles.	Lower due to print speed and lower nozzle packing density.	[65]
Cost	Relatively lower, in the order of $10,000 for industrial systems	Relatively higher, $73,000 average industrial system cost. Systems can exceed $200,000	[66], [67]

3.2 Printing for pharmaceutics

3.2.1 Discovery

The pharmaceuticals industry quickly adopted the combination of 96-well plates and robotics as a drug discovery staple, with microplates containing up to 9600 wells today. The advantages were clear: miniaturisation in both size and volume, and a reduction in processing steps to find drug candidates [68]. High throughput screening (HTS) is used extensively for protein therapeutic and small molecule drug discovery to determine the efficacy or toxicity of a drug or drug candidate. The majority of cells cultured for drug screening are in two-dimensions on stiff plastic surfaces which are not representative of in vivo conditions [69], yet drug screening based on 2D cell culture systems is used pervasively within industry as it is an economical, well-established method with high throughput capacity. In contrast, 3D cell culture, such as spheroids or scaffold-based systems [70] are more representative of the in vivo environment but are more expensive and have a relatively lower throughput capacity. For example, the results of drug screening using 3D liver models have been better at detecting in vivo drug induced toxicity [71] and with greater reliability than their 2D counterparts [72].

Inkjet printing and extrusion based bioprinting are complementary technologies that enable 2D and 3D systems to be produced for high throughput screening to support discovery. The motivation for using both systems in parallel is to reach a balance between cost, throughput and results fidelity. 2D systems are important for identifying hits, compounds that bind to a specific target, whereas 3D systems are more important for moving from hit to lead, where the binding affinity of the hit is optimised and the

in vitro efficacy and toxicity is tested [73]. It takes on average 8% longer for biopharmaceuticals to reach the market compared to traditional pharmaceuticals [74] highlighting the importance of early data collection through HTS to reduce the sunk costs. Inkjet printing, utilised predominantly for 2D systems, offers the advantage of being low cost, enabling it to be deployed in a low resource setting as demonstrated by the examples of high throughput screening platforms produced with converted desktop printers [75]–[77]. Commercial microarraying systems based on inkjet technology include the sciFLEXARRAYER utilising glass capillary nozzles [78] and the Arrayjet which uses a Xaar XJ126 piezoelectric printhead, 126 nozzles and boasts 640 features per second [79].

Protein microarrays, another 2D system, are of particular importance for biopharmaceutical drug discovery and can be split into two broad categories – functional protein microarrays and protein detecting microarrays with the former being particularly useful for the discovery of protein therapeutics when probing protein-protein interactions [80]. Alternatively, other interactions with a target protein can be investigated. For example, glycan – protein interactions were probed using an array produced with a HP Deskjet 1010 inkjet printer, this array could also be used for the synthesis of glycoconjugates that can be used for vaccines [81]. The non-contact nature of inkjet printing makes it an ideal manufacturing technique for protein microarray production because the substrates used, typically nitrocellulose or hydrogel coatings [82] [81] are fragile and production of microarrays is 10 time faster than its contacting counterpart, micro-spotting [83]. A further crucial element of protein micro arraying is to ensure that the proteins function and integrity remains unchanged. Research from 2000 demonstrated that it was possible to deposit IgG, IgM and HRP without any degradation for a protein microarray produced with a thermal inkjet printer but it raised the concern that not all biomolecules may be suitable for use with this production technique [84].

High throughput screening utilising 3D extrusion based bioprinting is particularly prevalent for anticancer drug screening demonstrating a clear application for the biopharmaceutical sector as therapeutic proteins, particularly mABs are the fastest growing anticancer treatment option [2], [85]. A variety of 3D cancer cell models intended for drug screening have been produced with extrusion based bioprinting including a co-culture of human breast adenocarcinoma (MDA-MB-231) and mouse macrophage (RAW 264.7) suitable for culture in a 96-well plate [86], spheroids of MCF-7 human breast cancer [87] and scaffolded Hela cells for a cervical tumour model where the 3D model showed greater resistance to the chemotherapy drug paclitaxel than the 2D monolayer [88]. In all of these examples the reported cell viability was >90% yet similar studies utilising extrusion based bioprinting (EBB) for cell deposition report a greater drop in cell viability. 3T3 fibroblast cell viability dropped to 60% [89] and HepG2 liver cell viability dropped to 40% immediately after extrusion recovering to 85% after 24 hours [50] which also highlights that the relative importance of when the viability measurement is taken. This further supports the need for standardised 3D culture protocols to improve results reproducibility and reliability [90] as strict regulatory requirements and extensive published evidence is required for a 3D model to be validated for pharmaceutical use and to replace an animal model [73].

For industrial adoption of EBB 3D models for high throughput screening there is a trade-off between cost, quality and speed. Smaller volumes of high value reagents can be used with 2D systems with a higher reaction throughput, whereas academic research repeatedly shows drug screening with 3D models to be more representative of cell in vivo conditions yet it is more costly and time consuming to produce [91]. More extensive use of bio-printed cell models will help alleviate the attrition of drug candidates which is estimated to be as severe as 95% failure rate for new cancer medicines [92]. A better understanding of the impact printing processes has on the resulting cell or protein function will be required to increase industrial adoption, and so will be examined later in Section 4 along with other challenges shared across applications.

3.2.2 Delivery by microneedles

The delivery of biopharmaceuticals to a patient poses many challenges due to the high molecular weight and instability of the biologic. Protein based therapeutics are at risk of structural degradation caused by physical instabilities such as mechanical stress [93] and chemical instabilities, commonly oxidation, reduction or hydrolysis [94]. Accordingly, most biopharmaceuticals are delivered intravenously to ensure high bioavailability yet this route of administration is fraught with challenges that reduce patient compliance including aversion to needles or experiencing adverse events [95]. Moreover, high viscosity drug formulations, due to the drug concentration required to achieve a therapeutic dose within volume constraints set out by the FDA, reduce the syringeability. Consequently many researchers and pharmaceutical companies are looking into alternative delivery mechanisms for biopharmaceuticals, an overview of the strategies being investigated is detailed by Mitragotri *et al*. [96]. Specifically, 2D and 3D printing techniques have been utilised for drug delivery using microneedles as well as buccal and oral delivery.

Looking firstly at microneedles, these are used for transdermal drug delivery. Arrays of narrow, short needles only penetrate the stratum corneum to prevent nerve stimulation that would cause the patient pain. Beyond being pain free, microneedles also offer the advantage of faster healing at the site of injection, reduced infection risk and increased patient compliance, particularly with needle-phobic patients. This transdermal delivery technique can increase bioavailability through avoiding first-pass metabolism observed through oral administration [97] and enabling drug uptake through the capillaries and lymphatic system. The most commonly used method to produce microneedle arrays is with a reusable micro-mould [98], however fabrication of the master, of which the mould is formed from, often utilises expensive manufacturing techniques that require specialised equipment such as UV lithography [99], micro-milling [100], chemical wet etching [101] or electrical discharge machining [102]. Additive manufacturing techniques can also be used to produce a master mould [103], [104], often at a relatively lower cost without compromising on microneedle performance. For the active delivery of IgG antibody, used as a model drug system, a positive mould was produced with stereolithography 3D printing and the release kinetics resulted in enhanced outcomes in vivo for mouse models with melanoma compared to diffusion-based microneedles [105].

To avoid the use of a mould altogether, a combination of 2D and 3D printing techniques can be used for direct fabrication of microneedles for biopharmaceutical drug delivery. Stereolithography 3D printing (SLA) has been used to produce hollow microneedles that enable hydrodynamic mixing of drug solutions and transdermal drug delivery in a single device [106]. Solid microneedles can also be produced with SLA printing and are then coated with a drug solution, typically with piezoelectric drop-on-demand inkjet printing. Four different formulations of insulin were inkjet printed onto microneedles and the dissolution profile of the insulin into porcine skin in vitro was rapid for formulations with soluplus and gelatin suggesting feasibility for this delivery technique, yet circular dichroism indicated possible structural alterations of insulin for the gelatin formulation [107]. A similar fabrication technique investigated microneedles of different geometries with inkjet printed insulin sugar drug formulations, observing faster drug dissolution for larger surface area microneedles and lower blood glucose levels over 4 hours compared to conventional subcutaneous injections in mice [108]. However, positive results in animal models do not guarantee efficacy in a patient population. A clinical trial of transdermal drug delivery with microneedles of Abaloparatide, a parathyroid hormone related protein analogue drug used to treat post-menopausal osteoporosis, produced positive results for increasing bone density but more substantial results were observed for subcutaneous injections (ClinicalTrials.gov Identifier:NCT01674621).

Dissolvable microneedles encapsulate a drug within a polymer matrix that dissolve upon injection to the patient. Piezoelectric inkjet printing was used to dispense clinically relevant flu vaccines into a mould. Through the use of small droplet additive manufacturing, complete wetting of the mould was

observed ensuring the resulting microneedles had sharp tips. Lower voltage jetting parameters were selected because a decrease in the vaccine integrity was observed at 80V, possibly attributable to a greater force exerted on the biomolecule with higher ejection voltages [109]. The importance of careful consideration of fabrication parameters for droplet based manufacturing of dissolvable microneedles was highlighted when the polymer drug formulation, temperature and drying conditions were optimised to retain 99.8 ± 3.8% lysozyme activity in dissolvable microneedles [110].

The production of solid and dissolvable microneedles using 2D and 3D printing technology has been demonstrated at the research level, further research should select printheads appropriate for the production throughput required at an industrial scale, and additional work is needed to understand the impact on biologic integrity. Both of these areas will be explored further in Section 4 along with other shared challenges.

3.2.3 Buccal drug delivery

Buccal drug delivery enables drugs to diffuse through the oral mucosa directly into the bloodstream. This delivery method often utilises orodispersible films (ODFs), drug-loaded polymer films rapidly dissolving upon contact with saliva, which are of particular benefit to patients that have difficulty swallowing such as stroke patients or in paediatrics. Moreover, it is estimated that 28% of the general population have difficulty swallowing conventional tablets and medical practitioners frequently underestimate the gravity of this problem [111]. Compared to suspensions or drugs in solution, films can retain the drug in a stable format for a longer duration taking pressure off supply chains and enabling easier transportation and handling of drugs [112].

Orodispersible films are predominantly manufactured with solvent casting, but inkjet printing is an alternative production method that has been used to produce cellulose films containing clonidine hydrochloride that have better mechanical properties, tensile strength and Youngs modulus than solvent cast films [113]. Although there are no biologics delivered using ODFs with FDA approval to-date, there are many small molecule drugs delivered with ODFs including donepezil, used to treat the symptoms of Alzheimer's disease and fentanyl, a strong opioid pain reliever. Notwithstanding, many researchers are investigating the delivery of biologics with orodispersible films including insulin films produced with solvent casting [114] and films containing three model proteins lysozyme, β-galactosidase and ovalbumin produced with air and freeze drying to establish the feasibility of this delivery method for biologics [115].

Inkjet printing offers the advantage of depositing the API without making contact with the film and enables high levels of control of the dosage amount due to the picolitre-scale volume control of droplets. The deposition of lysozyme as a model protein with thermal inkjet printing has shown that the mechanical properties of the polymeric films are not affected as compared to the film prior to printing [116]. Further research is required to investigate the in vivo and in vitro dissolution profiles of biologics delivered with ODFs. A concern with using inkjet printing for protein deposition is the loss of activity of the printed protein which was observed when lysozyme was deposited for ODF production which was as severe as 70% for the lower lysozyme concentration [117]. However, as drug loading has previously been cited as a concern for the use of ODFs [113], it is not inconceivable a higher protein concentration in the ink formulation would need to be utilised, reducing the activity loss as observed with lysozyme. Similar to the concept of depositing the API onto a film as used with inkjet printing, spray drying was used to develop a novel delivery method for microparticles of the Edmonston Zagreb strain of the measles vaccines which showed promising preclinical results [118].

3.2.4 Oral Drug Delivery

The preferred route of administration for the majority of patients is oral drug delivery as it is non-invasive, pain free, and does not require professional intervention or equipment. It is the most convenient drug delivery method for patients, especially for long term, repeated dosing. The major

hurdle to oral delivery of protein and peptide therapeutics is the low bioavailability, typically around 1% for this delivery method [119]. Such low bioavailability is caused by degradation due to low pH in the stomach, proteolysis in the gastrointestinal tract, poor permeability through the gastrointestinal mucosa and first pass metabolism. For instance, despite a clinical trial of orally delivered insulin showing no difference in glycaemic control for diabetic patients compared to subcutaneously delivered insulin and slightly fewer adverse events reported, there was no further development of the product due to questions regarding the commercial viability of this delivery method as a greater dose of API was required to achieve the required bioavailability [120].

Many examples of 3DP tablets exist which contain small molecule drugs as the API [121], [122]. Some notable examples include UV curable inkjet printed ropinirole hydrochloride tablets [123] and extruded bilayer tablets of Mucinex [124] with printing technologies seen as a platform for personalised medicine by tailoring the dosage to the individual [125]. Moreover, these tablets extend beyond research as Aprecia Pharmaceuticals produced the first FDA approved 3DP tablet containing levetiracetam to treat epilepsy in 2015. The Zipdose technology enables high drug loading, up to 1000mg showing a potential benefit for biopharmaceutical applications [126].

The novelty of 3DP tablets could be a cause for concern in regard to patient acceptability. However, a study examining patient acceptability of 3DP tablets showed that the greatest preference was for torous shaped tablets, even above conventional discs [127]. Not only the views of adults have been examined but also patient acceptability within a paediatric population who would particularly benefit from the greater dosage flexibility 3DP tablets offer and the potential to easily produce chewable tablets [128]. Such studies have shown encouraging results that paediatric patients would accept 3DP tablets, particularly in the case of polypharmacy, where a single tablet contains more than one API [129]. These are promising indicators for the wider acceptance of 3DP medicines.

Research has focused on strategies to improve the bioavailability of tablets and oral delivery devices through improving the release profile of the therapeutic or extending the circulation lifetime in vivo [130]. 2D and 3DP can be used to produce oral dosage forms for biopharmaceuticals with most studies demonstrating a feasibility for these manufacturing techniques to be used for this application. Microcontainers filled with 100ng doses of insulin have been produced with inkjet printing and >94% of the deposited insulin remained intact [131]. The study demonstrated the ability to change the release profile of the API, insulin, by modifying the formulation of the microcontainers and the polymer, Eudragit S 100 was able to withstand a pH below 7, however bioavailability data was not present. A novel 3DP technique based on extrusion of two liquid co-polymers examined the release profile of two model systems, prednisone and BSA. Although the results demonstrated a feasibility of producing customisable pills containing a protein therapeutic, only 40% of the BSA contained within the pill was released and modifications to the ink formulation need to be made to achieve a higher printing resolution and improve the drug release kinetics [132]. Table 2 gives an overview of some of the achieved dosages of printed drug delivery devices detailed in Section 3.

Table 2: Details of printed drug delivery devices and the respective API dosage achieved.

Ref	API dosage	Ink	Dosage Dispensed	Application
[107]	Insulin ~ 40 units / day = ~ 0.0347 mg * 40 = 1.388mg / day	4 formulations: POX(poly(2-oxazoline) 5mg /Insulin 10ml gelatin (porcine skin) 5mg/Insulin 10ml trehalose dehydrate 5mg/Insulin 10ml Soluplus 5mg/Insulin 10ml.	110 ± 10µg per needle and 50 needles per cm^2. 6 droplets of 300pl every 50µm dispensed, 50 cycles. Would need ~ 12 needles to supply dosage.	IJ coated microneedles for transdermal insulin delivery
[108]	Insulin ~ 40 units / day = ~ 0.0347 mg	3DP microneedles: biocompatible Class I resin, Dental SG IJ: insulin (10mg/ml) sugar - insulin:xylitol (5:1 wt/wt), insulin: mannitol (5:1 wt/wt)	350µg, 10 units, insulin per array (48 needles).	3DP printed microneedles with IJ API coating for

	* 40 = 1.388mg / day	and insulin:trehalose (5:1 wt/wt) as 2% solid content.		transdermal insulin delivery
[109]	Hemagglutinin H1 A/California/7/2009 15µg dosage, twice 4 weeks apart	Aqueous solutions of trehalose dihydrate (Cargill), PVA 13–88 (Kuraray), polysorbate 80 purchased from Sigma Aldrich	Microneedles did not contain any active vaccine.	Inkjet printing into moulds to form dissolvable microneedles.
[133]	Insulin ~ 40 units / day = ~ 0.0347 mg * 40 = 1.388mg / day	Inkjet - three coating formulations were used, consisting of insulin:xylitol (5:1 wt/wt), insulin: mannitol (5:1 wt/wt) and insulin:trehalose (5:1 wt/wt) as 2% solid content 3DP - biocompatible Class I resin, Dental SG, procured by Formlabs.	350µg, 10 units, insulin per array (48 needles).	3DP printed microneedles with IJ API coating for transdermal insulin delivery
[134]	Cyclosporine A = 10mg/kg for oral dosage in rats.	CsA (100 mg) and HPC-SSL (1900 mg) were dissolved in 1,4-dioxane. The solute concentration was 2% (w/v).	100% dosage for weight of rat dosed for.	Preparation of amorphous solid dispersion for oral delivery.

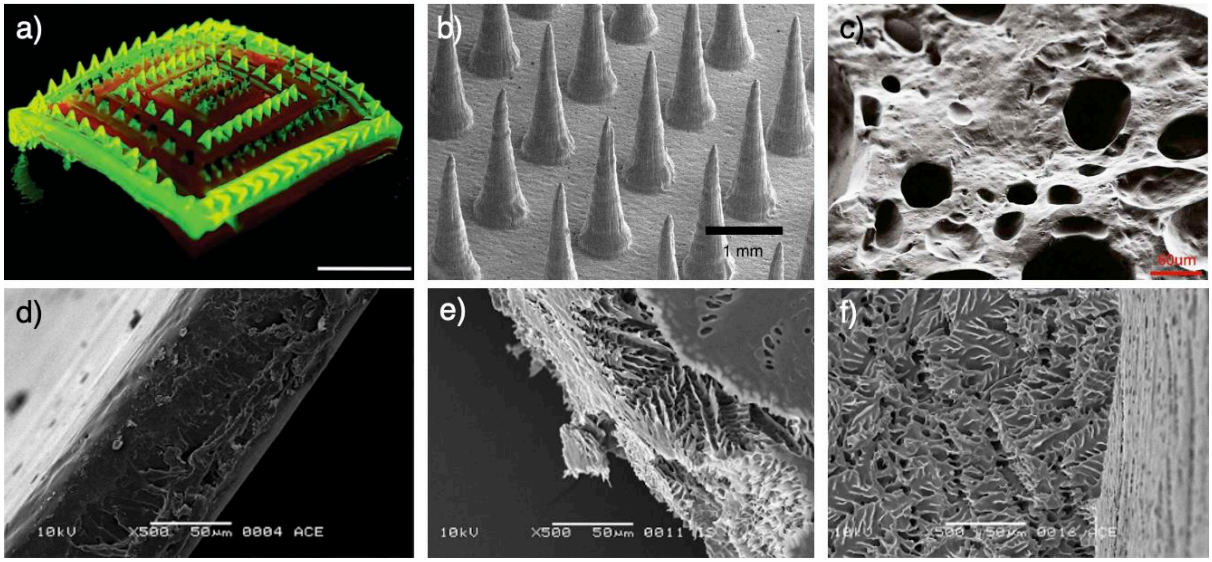

Figure 3: a) Digital photograph of a combinatorial dissolvable microneedle patch loaded with FITC (passive delivery compartment) and Rh6G + Mg particles (active delivery compartment). Scale bar, 5 mm [105] *(reprinted with permission from John Wiley & Sons, Inc. b) SEM image of 1.5 mm needle-to-needle distance microneedle array basin before filling* [103] *(open access) c) SEM image of 3d printed PEG4-PCL-SC gel pill incorporating prednisone and bovine serum albumin* [132] *(reprinted with permission from Elsevier) d) SEM of air dried pullulan ODF, e) freeze dried pullulan ODF and f) 40/60 trehalose/pullulan weight ratio freeze dried ODF* [115] *(open access).*

Some examples of printed drug delivery devices are shown in Figure 3. As noted throughout Section 3, there are a range of challenges that are commonly appearing. The potential mechanical effects on large molecules during printing, the chemical effects upon interaction with surfaces or during drying, the hardware selection and the challenges in formulation are all noted. And will be examined in turn in Section 4.

Section 4 Shared challenges when printing biopharmaceuticals

The application of 2D and 3DP technology within the biopharmaceutical industry is still predominantly at the research stage for pharmaceutics with a few industrial examples within the aforementioned applications. To accelerate industrial adoption, technical challenges must be overcome and researched further. These can broadly be categorised into mechanical effects, chemical effects, printhead and ink formulation considerations.

4.1 Mechanical effects

The effect of hydrodynamics shear forces on biomolecules has been a recurring concern in the bioprocessing industry [135]. Such effects can be categorised in three types: denaturation, aggregation and scission. Flow-induced denaturation occurs by misfolding after unfolding by viscous forces. Aggregation can be caused by partial unfolding exposing hydrophobic groups or can be a long-term consequence of denaturation. In addition, extremely high strain rates can induce molecular mechanical scission.

4.1.1 Flow-induced denaturation and aggregation

In nature, some proteins are very sensitive to hydrodynamic shear. For example, the van Willebrand Factor (vWF) is a high molecular weight protein in excess of 20 MDa which unfolds in blood stream in an event of a cut and helps the formation of platelet plugs [136]. *In-vitro* experiments report unfolding at elongational strain rates below 10^3 s^{-1} [137]. On the other hand, studies have shown shear-induced aggregation of pathogenic amyloids [138]–[140]. In addition, other flow-induced aggregation pathways have been considered for the biomimetic production of silk [141], [142].

Nevertheless, the role of hydrodynamic shear on protein unfolding or aggregation is still a subject of debate [143], [144]. Interfaces such as liquid-solid or liquid-air interfaces have also been shown to account for protein degradation, while decoupling this effect from the flow field is difficult. In recent studies carried with elongational flows, some authors report a negligible role of shear [145], while others emphasise that role [146]–[149]. Strain rates in excess of 10^4 s^{-1} are investigated, however this is still an order of magnitude below strain rates encountered in inkjet.

Even if no aggregation is observed, a conformation change could imply activity loss for enzymes. Studies of enzymes activity after high mechanical stress are also contradictory and depend strongly on the type of enzyme and type of shear [150]. Lencki *et al*. (1993) [151] and Ouyang and Barati (2014) [152] have reported a loss in enzymatic activity as shear stress increases, Edwards *et al*. (2010) [153] no effect of shear, and Ohno *et al*. (1995) [154] an enhanced activity upon shear.

Similar contradictory results are observed for studies investigating the impact inkjet printing has on the retained activity of inks containing enzymes. Two notable examples of such discrepancies relate to the ejection of horse radish peroxidase (HRP) and lysozyme. One custom piezoelectric actuated printer made of poly(tetrafluoroethylene) tube resulted in 30% retained activity of HRP [155], whereas ejection with a Dimatix DMP 2800 printhead saw a statistically insignificant loss in activity [57]. With lysozyme, using a thermal inkjet printer retained activity was as low as 30% [117] but up to 85% with a Dimatix Sapphire QS–256/80 AAA 80pL printhead [156].

4.1.2 Flow-induced scission

For random coil molecules such as DNA-RNA polymers, strong fluid flows can cause scission. This phenomenon has been extensively studied for DNA flowing though microfluidic contractions [157] [158], but is also encountered with high molecular weight polymers in general [159].

Yet, both continuous and drop-on-demand inkjet can be linked to polymer scission experiments, due to a contraction flow through the nozzle. There is also a purely extensional flow in the filament when a

drop is formed. Small nozzle diameters are typically in the range of 20 µm to 60 µm, and the velocity of the droplet is of the order of 10 m/s. A-Alamry *et al*. (2011) [160] reported the degradation of PMMA and PS during a DOD print. Molecular weights between 10^5 Da and 10^6 Da led to chain scission. [161] further analysed this result and showed that the polymer could break only in the nozzle and not in the filament where the extensional rate is too low. Finally, Wheeler *et al*. (2014) [162] compared CIJ and DOD in terms of polymer degradation. They concluded that, even though CIJ displayed the same strain rate magnitude, degradation occurred in the recirculation system of the ink and not in the nozzle.

4.1.3 Models and scaling laws

Regarding polymers including DNA strands, models and scaling laws have been developed for flow-induced scission, based on the concept that a critical strain rate can be defined above which scission occurs. This critical strain rate has been found to be a function of the backbone strength, molecular weight, concentration, solvent quality and turbulence or laminar nature of the flow [163]–[166]. On the other hand, modelling and theoretical aspects of flow-induced unfolding of proteins have been limited to lower molecular weights than experimental studies, detailed in Figure 4, and is still an active field of research. Even the concept of a critical strain rate has been questioned in experiments where strain instead of strain rate seemed to dominate the unfolding behaviour [167]. Unravelling random coiled polymer requires overcoming entropic forces which are primarily agnostic to the chemistry of the polymer, hence the possibility of modelling general trends. By contrast, folded proteins are stabilised by strong internal enthalpic interactions which depend on their amino acids sequence. This makes general interpretations a very difficult task. Nevertheless, simulations using Molecular Dynamics (MD) methods have been used to study specific proteins under flow. Unfolding simulations are commonly done in a steered approach, where the two ends of the proteins are pulled apart. The resulting force-extension curve can be compared to force spectroscopy experiments [168]. To be more relevant to flow-induced unfolding, other methods have been proposed, such as tethered MD, where one end of the protein is anchored while solvents molecules are flowing past, and free flow MD, where a shear flow in induced in the simulation box and the protein is free to move [169]–[173]. However, only few studies have analysed the refolding outcome after the flow is switched off, although in the context of manufacturing it would be crucial to quantify the degree of irreversibility and production of non-native states after a shearing event [170].

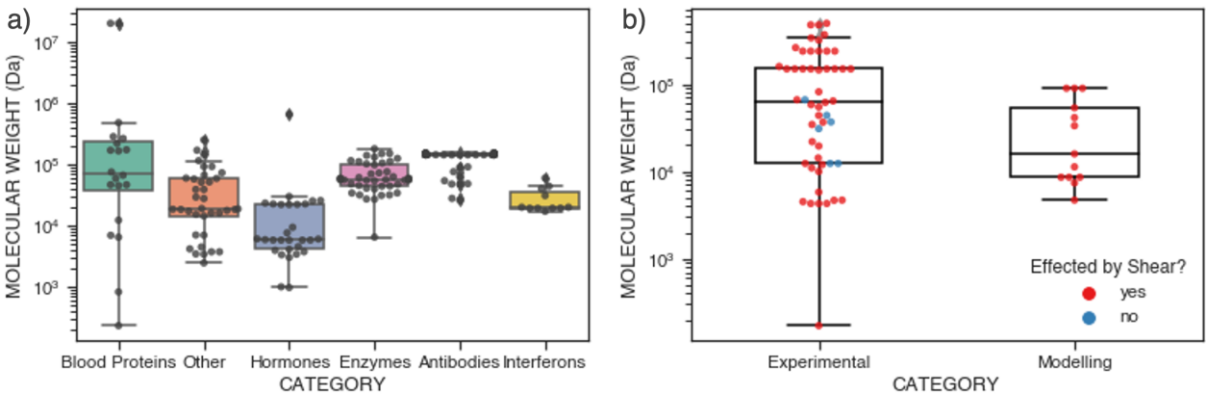

Figure 4: a) the molecular weights of approved protein therapeutics, data from - [174] *b) The molecular weights of proteins examined in experiments or molecular dynamics simulations detailed in Section 4 indicating whether shear flow had a detrimental effect on the protein (yes) or the protein was unaffected (no).*

4.2 Chemical effects

Protein therapeutics are susceptible to degradation due to chemical effects which must be carefully considered to ensure preservation of expensive biopharmaceutical APIs. This section will firstly briefly consider the detrimental impact the material choice of printing equipment can have on protein therapeutics through adsorption then briefly examine the impact of ink drying after deposition.

4.2.1 Protein Adsorption

Proteins will adsorb to surfaces when the Gibbs free energy of the system, G, decreases as a result of the adsorption. Equation 1 states the conditions for protein adsorption to occur, H is the enthalpy, T is temperature and S is entropy.

$$\Delta_{adsorption}G = \Delta_{adsorption}H - T\Delta_{adsorption}S < 0 \quad (1)$$

Proteins can absorb to surfaces of the ancillary equipment or printhead in an IJ system resulting in a reduction in concentration of the final printed solution. Poncin–Epaillard *et al*. (2012) stored the recombinant human prion protein in tubes of different materials for 24 hours at 4°C. Each of the polymers tested experienced a reduction in the amount of protein recovered with poly(tetrafluoroethene) performing the worst with 33% recovery compared to storage in an ultra–low temperature freezer [175]. To reduce protein losses, a coating of a sacrificial protein, such as bovine serum albumin (BSA), can be used [176]. Alternatively, ancillary equipment material should be coated with hydrophilic polymers, such as PEG or poly(methyl methacrylate), PMMA, to reduce electrostatic forces and hydrophobic interactions between the proteins and the surface [177], [178]. As protein therapeutics are costly losses due to adsorption on the printhead or ancillary equipment can be a deterrent to using printing processes in manufacturing.

4.2.3 Drying

The majority of FDA approved protein therapeutics are in the liquid state and required cold chain storage between 2-8ºC which has major implications on supply chain costs [179]. Through lyophilisation, drug products can be stored within a more favourable temperature range, 0 – 25ºC and remain stable for a greater duration, such as the mAB Nucala sold under the trade name Mepolizumab. However, drying processes can result in structural damage to the dried protein despite the gains in storage stability [180]. 2D and 3D printing of protein therapeutics for buccal and transdermal delivery dried the active protein, careful consideration of the ink formulation is required to prevent structural damage. In a broader sense, because printing technologies can dispense picolitre scale volumes through droplet-based manufacturing, air drying will be rapid. The use of sugars and glycerol as a humectant aim to stabilise the protein and ensure moisture is retained but the impact these excipients have on the rheology of the inks must also be considered and how they affect the printability.

4.3 Printhead selection for printing biopharmaceuticals

There is a discontinuity between the review of industry and literature presented so far. There are clear needs in the biopharmaceutical industry that can be addressed with a shift towards inkjet printing in manufacturing. However, the vast majority of technological developments in 2D and 3D printing described so far, that are hoping to enable such progress, are carried out through rigorous scientific studies at the lab scale using research-scale printheads. Supporting infra-technologies have to develop in parallel if digital fabrication is to become a reality. In this section we first look at where industrial printing technologies have successfully been employed in biopharmaceutical printing before, secondly, looking at the current direction of industrial printhead development, before concluding with comments about what research and activities need to be considered now to enable a smooth transition to full scale biopharmaceutical printing applications.

The most advanced area for printing biopharmaceuticals is in microarray printing applications. The contactless printing devices found in industrial use are based on a variety of techniques, ranging from piezoelectric drop-on-demand to acoustic or solenoid-valve actuation. Firms have developed their own solutions and not relied on original equipment manufacturers (OEM), who dominate other areas of digital printing. Some examples of these companies include Cellink, Biodot and Arrayjet.

Cellink printing devices use proprietary technologies; I-DOT™ (Immediate Drop on Demand Technology) for liquid handling and BIO X™ for bioprinting. Their I-DOT system [181], developed by Dispendix GmbH (acquired by Cellink in 2018) uses a pressure-based dispensing technology. There are nozzle sizes available in the range of 60-200μm, 8 individual channels firing at 100 Hz and it can handle liquids of viscosities ranging from 1 to 100 mPa.s. Cellink has also recently acquired Scienion AG and their dispensing portfolio, which includes piezoelectric DoD technology [182]. This has individual channels with inert glass capillaries that are easily exchangeable, with sizes featuring drops from 100 to 800 pL (viscosity limited to 5 mPa.s) and 25nL -1μL (viscosity limited to 22mPa.s). Cellink integrates these printheads into various manufacturing scales, such as reel-to-reel operation systems like the sciFLEXARRAYER S100 [183]. Cellink's selection of bioprinting devices are mostly based on pneumatic control, with its closest analogous to inkjet being its BIO X EMD (Electromagnetic Droplet).

BioDot also employs proprietary technologies, including the BioJet™ Non-Contact Nanoliter to Microliter Solenoid Dispenser for single droplet volumes of 1.3 nL- 1 μL, based on a high-speed micro solenoid valve, and PolyJet™ with pneumatic actuation for viscous fluids (stated up to 1,000 mPa.s). In addition, they have developed piezoelectric-based technologies for lower volume deposition. Their Ultra™ Picoliter to Nanoliter Piezo Dispenser, described as a "true non-contact picoliter liquid handling and spotting solution with drop-on-demand capability", reaches volumes from 100 pL to 1.0 nL [184]. Their Rainmaker system allows higher frequencies than their Ultra Piezo Dispensers, not being very specific with "a wide range of volumes as a single drop or a high frequency burst" [185]. These are all designed to integrate into a wide range of motion platforms, conveyor belt and reel to reel systems, making them suitable for continuous production.

Arrayjet microprinters took a different approach and mounted a Xaar XJ126 piezoelectric print head into their system. This range of printheads was originally designed with printed graphics in mind and are relatively mature printhead technologies, having launched in 2003 [186]. Arrayjet states a dispensing volume of 100 pL to 10 nL with 100 pL increments [187].

In summary these existing, commercialised printing devices process droplet volumes in the order of 100 pL - 50 nL and can achieve feature sizes of about 90 – 500 μm. Some of the ejecting devices can handle viscosities up to 400 mPa.s, as the nozzle sizes are quite large. They can access low frequencies (of the order of 600 Hz or 100 Hz per channel respectively for [187], [184]) and slightly higher frequencies (up to 1500 Hz [188]). Importantly, some of these dispensing heads are designed so that every nozzle has an individual liquid channel to minimize cross contamination [189], [190] This is not something observed in standard OEM drop-on-demand printheads.

Contrary to these technologies described above, the most recent trends for DoD commercial printheads manufactured by large format printer makers or by OEMs such as Fujifilm Dimatix, Xerox, Canon, Epson, Seiko, HP, Konica Minolta, Xaar, Kyocera, Ricoh, Panasonic, or Toshiba, show a move to features of finer resolutions with drop volumes of the order of 5-30 pL, closer packing density of the jet orifices, and homogenising inks with recirculation ink supply systems of large volumes.

There is not a clear approach visible in the literature from established inkjet printhead manufacturers towards the design of devices to match the range of characteristic features needed for printing biopharmaceuticals. Nozzle size, nozzle pitch or printing frequency seem to be going against the trends that we have described from manufacturers such as Arrayjet, Biodot or Cellink. Trends show an increasing number of nozzles and decreasing nozzle size to improve printing resolution. As noted earlier

in Section 4, this decreasing nozzle size will only increase the likelihood of detrimental stresses on biomolecules or shear-induced aggregation. Furthermore, most piezoelectric inkjet printheads (this concept does not apply for thermal inkjet where a tuneable waveform as in piezo- inkjet is not possible) are making the most of the versatility and fast response of the piezo crystals to develop greyscale capabilities, which, contrary to binary printheads, can produce droplets of various sizes by fine tuning the input electric signals. However, this mechanism does not currently seem to be needed in printing biopharmaceuticals, where a binary system should suffice, indicating that thermal inkjet can still be as good as a solution as piezo DoD.

Printing frequencies are also being continuously increased in the newest DoD printheads, with Ricoh or Xaar printheads suitable for handling aqueous inks with 14-30 kHz [191], and up to 100 kHz [192] respectively, which approach printing frequencies that may be damaging to some biomolecules [193]. Thermal inkjet printheads reach frequencies of the order of 10 kHz (12-18 kHz for 2.5 HP Technology [194]). There is one notable exception from HP, the HP D300e Digital Dispenser based on thermal inkjet that initially addressed microarray printing for high-throughput screening of pharmaceuticals and has since been improved to print water-based biomolecules such as proteins, DNA and lipids [195]. This product is commercialised by a partnering company of automated laboratory solutions, Tecan Group Ltd. The HP D300e Digital Dispenser can mount two types of printing cassettes, with which it can handle up to four or up to eight fluids (4 or 8 individual channels respectively). Its technical specifications state a minimum dispensing volume of 11 pL which should be the minimum drop volume [196].

There is some history in academic researchers using industrial printheads for bio-based 2D patterning and 3D scaffolding applications, where higher resolution and complex patterns are required. These applications range from the manufacture of orodispersable biopharmaceutical forms (HP thermal inkjet printers widely used for these, and detailed explanation of their modifications generally given [197], [198], [116]) and biosensors (with printers by Olivetti [199], Canon EPSON, Dimatix, etc. [200], [201]) to complex 3D structures for regenerative medicine [202]. However, while these techniques show good scalability, they prevent a shift to continuous manufacturing because of the need to refill or replace printing cartridges.

Finally, it is important to consider trends in throughput, if this technology is to reach an industrial scale. Arrayjet, Cellink I-DOT and Cellink sciDROP PICO appear to have throughputs on the order of 50-100 μL/s, 6.4 - 40 μL/s and 16nL/s - 9.6 μL/s respectively. The XaarXJ126, shows the highest ejected volume from these products but achieves this through the greatest number of nozzles. In the case of the thermal-based HP D300e Digital Dispenser, we estimate the throughput to be on the order of 8 μL/s. If we compare these values with graphics-focused industrial printheads, we can see the MH2620 Ricoh printhead under typical printing conditions obtains a flow rates on the order of 240 μL/s. In the case of industrial thermal inkjet printheads, the HP TIJ 4.0 family suitable for aqueous inks can print with throughputs of 456.2 μL/s.

During this review, there has not been evidence of detailed analyses of the interaction of printing devices with the biopharmaceutical inks and the tuning of formulations to deliver the level of printability required for reliable manufacturing. This is a key part of inkjet printing and so we have included here the main considerations when formulating an aqueous ink, before concluding how it may be impacted by the need to ensure no detrimental effects on the biopharmaceuticals.

4.4 Formulation for printability

The previous section noted the range of current printheads reported in the literature for printing biopharmaceuticals. Formulation of inks to ensure printability needs to be integrated into printhead choice activities. Narrowing the field to suitable printheads is based on application requirements and

especially the key factors of speed, resolution, print frequency and fluid type. In addition, when formulating non-standard inks or relying on inks not designed by printhead manufacturers, it is vital to consider the waveform licensing policy of the printhead suppliers. Some manufacturers have open policies, allowing users to create and optimise the drive waveforms that allow tuning of the drop printing. This is important for new formulations to broaden the operating window for reliable printing. When jetting aqueous fluids, as is almost entirely the case for biopharmaceuticals, it is important to consider compatibility of printheads, as these relatively high conductivity fluids can create issues internally where electrodes are exposed. Finally, when considering printheads, the pumping capacity needs to be considered in conjunction with the internal architecture. Within each printhead type, there will be a unique channel geometry feeding the nozzles. These architectures dictate the forces experienced by biopharmaceuticals and the potential for degradation, as noted in Section 4.1. This is especially important when considering if the fluid will pass through the printing system once or if there will be a recirculation, as it has been shown that large molecules do progressively breakdown with multiple passes through high shear environments.

With a shortlist of application-suitable printheads in mind, printability and formulation can be considered in more detail. The term printability is used when assessing the properties of the ink when predicting how suitable the formulation is for a given print system. There are limitations on the printable ink for inkjet and extrusion and printability is defined differently for both manufacturing techniques. For inkjet printing, widely accepted guidelines are based on the dimensionless Ohnesorge and Reynolds numbers, shown in Figure 5, however it must be stressed that the capabilities of inkjet printing often extend beyond the limits shown as demonstrated by studies that have printed water, which has Oh~0.012 [203].

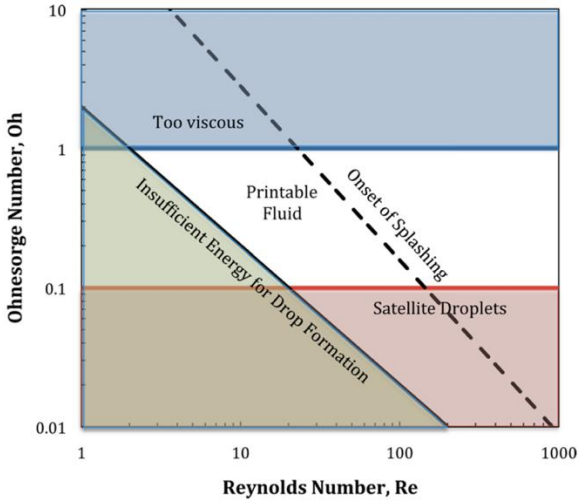

Figure 5: the region within which fluids are considered to be printable based on values of the dimensionless groups the Reynolds and Ohnesorge number [204]. *Repreinted with permission from Elsevier*.

However, more recent research has re–examined the region considered to be printable and defined it in terms of the Weber number and Z number, the inverse of the Ohnesorge number. The printable region has been expanded to $0.3 < Z < 700$, equivalent to $0.0014 < Oh < 3.33$ in comparison to the range shown in Figure 6 and data verifies printability in this range between $1 < Z < 40$ or $0.025 < Oh < 1$ [205]. This is promising for the adoption of inkjet printing technologies within the biopharmaceutical industry as this enables a greater range of ink formulations to be used. In practise, the requirements for the bulk fluid properties of an ink, such as viscosity and surface tension, are detailed within a specific printhead specification. Such properties will depend upon the printhead architecture and the fluid path of the ink. Even if an ink falls within these boundaries, the ink may not be able to produce stable jetting with the desired droplet volume, velocity and print frequency. Unlike the bulk property specifications, the

required dynamic properties, such as viscoelasticity of the ink, are not mentioned in the printhead specification. In the absence of this information, extensive reformulation and jetting optimisation are required for each ink and printhead to achieve satisfactory printing. Optimisation of the high frequency linear viscoelasticity and the non-linear viscoelasticity, shear and extensional behaviours are required and are vital for effective ink formulation. This is typically achieved through using mechanical and capillary rheometers to characterise the ink shear rheology, Piezo Axial Vibrator (PAV) and filament stretching tools to assess the high frequency viscoelasticity and extensional rheology to provide an efficient, fast screening of ink formulation.

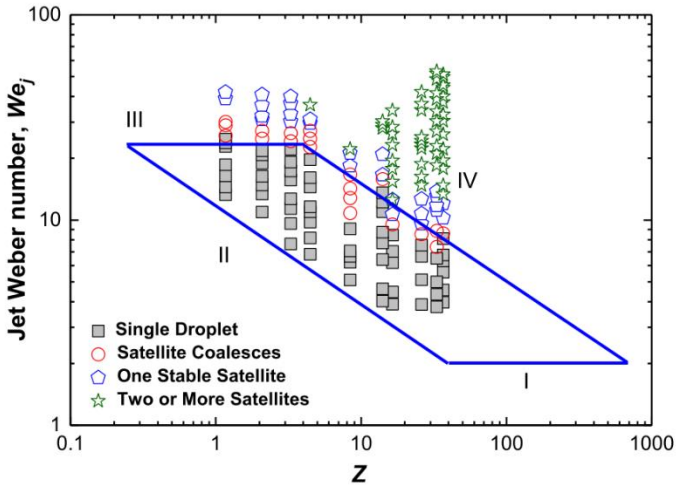

Figure 6: defines a region for the jetting parameters and fluid properties to be able to produce a stable single droplet during inkjet printing using the inverse of the Ohnesorge number and the Weber number [205]. *Repreinted with permission from AIP Publishing*.

For biopharmaceutical applications, inks are typically aqueous with surfactants added to reduce the high surface tension to 20-30s mN/m, and viscosity modifiers added to improve printability. The biggest challenge of jetting aqueous ink is the lower nozzle open time requiring frequent cleaning of the printhead nozzles. Humectants are used to improve nozzle open time by reducing ink drying on the nozzle. Humectants however also slow the drying of the ink on the substrate affecting the output and print quality. In formulation, it is a delicate balance of humectant concentration to achieve higher nozzle open time and at the same time faster drying on the substrate. Glycerol, a humectant, is frequently added to ink formulations containing proteins in a concentration exceeding 10 wt% [81], [116], [117], perhaps due to its extensive use as a protein stabiliser for solutions undergoing freeze-thaw cycles, yet a study examining the effect of viscosity modifiers on the model enzyme HRP shows a loss of activity for inks containing >10wt% glycerol concentration [57]. In low concentrations surfactants can enhance enzymatic activity [206], while at higher concentrations they impair activity and denature enzymes [207]. This emphasises that careful consideration must be given to viscosity modifiers used for biopharmaceutical ink formulations and alternative polymers can be used as cryoprotectants for protein solutions [208]. A balance must be struck between printability and the resulting bioactivity.

The novelty of EBB means that there is yet to be a standardised protocol to define printability which is ambiguous and makes it difficult to make cross–study comparisons. Notwithstanding the printability of EBB is often divided into the categories of 1) extrudability, (2) filament classification, (3) shape fidelity and (4) printing accuracy [209]. Extrudability concerns the force needed to push the bioink through the nozzle at a given flow rate. Filament classification considers the quality of the extruded filament before deposition. Shape fidelity looks at the entire print and assesses how much the printed structure deviates from the shape that was intended to be printed. Print accuracy assesses how well the chosen printing parameters perform for achieving the intended shape, size and location of a construct. Shape fidelity

and printing accuracy are similar metrics, but shape fidelity is often studied using multiple bioinks whereas printing accuracy will focus on the parameters for a specific bioink.

Section 5 Conclusions

In this review, the business case for pharmaceutical companies to shift their focus towards biopharmaceutical development and production has been explored. The use of 2D and 3D printing techniques as enabling technologies has been examined, specifically the opportunities and challenges for more extensive adoption of these technologies for uses surrounding protein therapeutics. The commercial motivation to focus more on biologics rather than small molecules is clear: greater returns on R&D expenditure and exclusivity in product sales, yet these benefits do not come without challenges. The increased complexity of biologics means their manufacture is less straight forward than their small molecule counterparts; increased batch to batch variation is of great concern and regulatory constraints are even more stringent. However, the shift towards continuous and digital manufacturing, seen in both the pharmaceutical and biopharmaceutical industries, enables increased product yields through elimination of batch-to-batch variation. This shift is complemented by 2D and 3D printing technologies, particularly through late-stage customisation and scale up of small volume drug products.

The adoption of 2D and 3DP technologies is evident from system discovery to pharmaceutics and smart packaging applications. Challenges that impede more extensive industrial usage of these technologies are not unique to specific applications and can be broadly categorised into considerations of the ink or printhead. There is a clear theme within academic research of ensuring the biomolecule integrity within the ink is retained across all applications. To address this concern, a more systematic understanding of the impact 2D and 3D printing techniques have on protein therapeutics structure and function is required rather than examination on a case-by-case basis, not neglecting the impact of excipients and carrier fluids as well. Moreover, research into ink formulation must focus on both printability of inks and the resulting efficacy of the printed protein therapeutic. The various proof of concepts of biopharmaceutical drug delivery devices seen in the literature are an important first step in progressing 2D and 3D printing uptake within the industry, but there are very few examples utilising printheads appropriate for use at scale. There is a need for more multi-printhead studies with biopharmaceutical inks to help bridge this gap between proof of concept and production at scale as each printing setup and ink formulation is tailored to a specific printhead. Moreover, the range of protein therapeutics deposited with 2D and 3DP is limited, typically insulin or low molecular weight peptides, despite the largest biopharmaceutical product category being mABs with molecular weights that are orders of magnitude greater. This has implications for the integrity of the biopharmaceutical which emphasises just how interconnected the challenges of ink formulation and printhead selection are. To transition from research to industrial adoption of printed biopharmaceuticals, a wider range of inks containing protein therapeutics must be examined and studies need to progress towards clinical trials. Further research in these areas is needed to advance 2D and 3DP from the lab to a commercial scale, and it is clear that from the many interdependencies that the future of printing biopharmaceuticals needs to closely link the application-focused manufacturing challenges with the lab-based experimental set-up at an early stage, to ensure successful translation.

From our perspective, 2D and 3DP of protein therapeutics is particularly important for enabling more efficient and productive system discovery, particularly due to the escalating costs of drug discovery. Printed biopharmaceutical drug delivery devices are still in their relative infancy, but through more extensive research in the field it is highly likely that these devices will follow in the footsteps of their small molecule counterparts once the key challenges surrounding ink formulation, printhead selection and protein integrity are researched further. Although proof of concepts pave the way to greater industrial interest and adoption, scaling up should not be dismissed at this early stage of research to ensure these manufacturing techniques are economically viable in the future.

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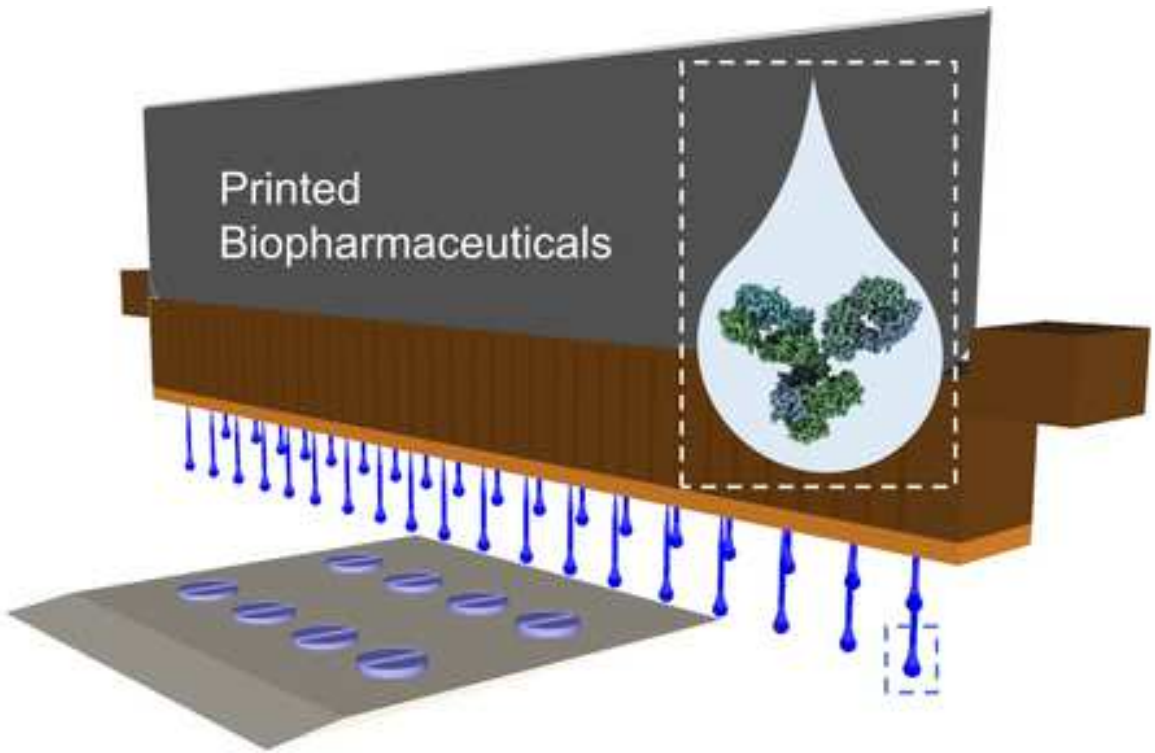
Manuscript (with changes highlighted)

Graphical Abstract (for review)



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Susannah Elizabeth Evans: Conceptualization, Methodology, Investigation, Writing - Original Draft, Review & Editing.

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Conflict of Interest

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: