In-situ quality monitoring during embedded bioprinting using integrated microscopy and classical computer vision

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Abstract

Despite significant advances in bioprinting technology, current hardware platforms lack the capability for process monitoring and quality control. This limitation hampers the translation of the technology into industrial GMP-compliant manufacturing settings. As a key step towards a solution, we developed a novel bioprinting platform integrating a high-resolution camera for in-situ monitoring of extrusion outcomes during embedded bioprinting. Leveraging classical computer vision and image analysis techniques, we then created a custom software module for assessing print quality. This module enables quantitative comparison of printer outputs to input points of the CAD model's 2D projections, measuring area and positional accuracy. To showcase the platform's capabilities, we then investigated compatibility with various bioinks, dyes, and support bath materials for both 2D and 3D print path trajectories. In addition, we performed a detailed study on how the rheological properties of granular support hydrogels impact print quality during embedded bioprinting, illustrating a practical application of the platform. Our results demonstrated that lower viscosity, faster thixotropy recovery, and smaller particle sizes significantly enhance print fidelity. This novel bioprinting platform, equipped with integrated process monitoring, holds great potential for establishing auditable and more reproducible biofabrication processes for industrial applications.

Introduction

Bioprinted organs promise to revolutionise medicine by tackling the organ transplant shortage and providing new animal-free platforms for drug screening and discovery. Despite significant advances, 3D bioprinting technologies, such as extrusion bioprinting, suffer from reproducibility challenges that will hamper deployment in clinical or industrial settings [1–3]. This is due to the multitude of complex process variables that must be optimised for specific bioink compositions and bioprinting processes [4–7], which makes it challenging to print anatomically accurate and viable constructs consistently. Furthermore, even when optimal conditions have been identified, bioink inconsistencies and environmental fluctuations can induce extrusion errors, leading to bioprinting failures. The reproducibility challenges with existing bioprinting technology can be attributed to its inherent open-loop nature, which lacks capabilities for monitoring extrusion outputs. This technological constraint impedes the in-process optimisation and correction of material deposition parameters, which is crucial for precision during additive manufacturing processes [8,9]. Overcoming this limitation will be important for advancing bioprinting from research to industrial and clinical applications, where producing consistent, scalable, and high-quality outputs under Good Manufacturing Practice (GMP) compliance is fundamental.

Early work in the field has shown the potential of using camera systems to monitor extrusion bioprinting outcomes [10–12]. Such approaches can be used for in-situ assessment of print quality on the bioprinting platform. For example, an offline vision approach has been developed with a camera attached to a bioprinter to investigate the creation of a deep learning model to detect anomalies in bioprinted grid structures to determine which anomalies were most common [13]. Similarly, integrated process monitoring and control strategies have been developed that measure feature error in bioprinted grids [12]. The control strategy was capable of intelligently updating the extrusion inputs in the g-code to improve accuracy for future layers [12]. While promising, cameras have typically been located at relatively large distances or oblique angles from the extrusion nozzle [10–12,14–16]. This limits image resolution and prevents monitoring of bioink flow, filament formation, and small features within the bioprinted construct. Similarly, camera-based systems are challenging to implement for embedded bioprinting in support hydrogels, an increasingly popular bioprinting modality where the bioink is extruded into a secondary support bath that prevents ink collapse under gravitational or surface tension effects [17–21]. Support baths are typically opaque in-nature and require containers that can obstruct imaging of the printing process.

Support baths fabricated using jammed hydrogel microparticles are increasingly employed for embedded bioprinting due to their shear-thinning and self-healing properties and relative ease of fabrication [22–24]. The earliest particle-based support hydrogels were fabricated using gelatin microparticles, now known as FRESH (freeform reversible embedding of suspended hydrogels)[23]. Several other groups have also developed particle-based support hydrogels using agarose, polyacrylamide, and alginate [20,22,23,25–27]. Interestingly, despite the increasing use of particle-based support hydrogels, general design criteria for their development have not yet been established. For example, a wide variety of manufacturing methods are used to create microparticles for support baths, which can result in significant differences in microparticle morphology and rheological behaviour [28–30]. It has been demonstrated that increased support hydrogel viscosity can improve print resolution, assessed by measuring the width of extruded filaments [16,18,20]. Lower viscosity supports have been shown to cause filament instability and buckling during extrusion, and this effect was exacerbated when the ink extrusion speed and printhead translation speed were not matched [16]. Overall, relatively few studies have investigated the impact of support bath rheology on print quality, and quality assessment metrics have primarily focused on simple outcomes such as filament width. For example, the fluidic behaviour of the support hydrogel will impact more complex aspects of the embedded bioprinting process, such as extrusion outcomes during direction changes and the disruption of previously deposited ink during successive deposition steps. Such outcomes are challenging to assess and quantify using existing bioprinting platforms that lack in situ processing monitoring capabilities.

Here, we present a novel 3D bioprinting hardware prototype with an integrated camera that tracks and monitors extrusion outcomes through a transparent glass platform. This enables high-resolution process monitoring of bioink flow and filament formation during embedded bioprinting. Additionally, we developed a classical computer vision software that can compare extrusion outputs to input points of the CAD model's 2D projections for offline and real-time assessment of print accuracy in terms of construct area and position. The platform's performance was evaluated using several bioink and dye combinations, as well as support baths with different opacities, including commonly used agarose and polyacrylamide particle-based supports [19–22,31]. Moreover, to demonstrate the platform's utility for process optimisation, we investigated how the rheological properties of agarose support baths can influence print quality, helping shed light on optimal design criteria for particle-based supports. This novel bioprinting platform with integrated process-monitoring functionality holds great potential for enhancing quality control and reproducibility in bioprinting. This continuous visual monitoring enables a consistent assessment of print accuracy, helping to ensure that each output aligns with design specifications and meets quality standards. By systematically recording visual data, our setup helps reduce print variability and provides objective evidence of process consistency. In GMP-compliant manufacturing settings, documentation and traceability are essential. The visual monitoring system in our platform creates a comprehensive record of each print, supporting process validation and traceability through a detailed visual history. This data can serve as a