3D Printed Biosensor Arrays for Medical Diagnostics

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Abstract

While the technology is relatively new, low cost 3D printing has impacted many aspects of human life. 3D printers are being used as manufacturing tools for a wide variety of devices in a spectrum of applications ranging from diagnosis to implants to external prostheses. The ease of use and availability of 3D design software and low cost has made 3D printing an accessible manufacturing and fabrication tool in many research laboratories. 3D printers can print materials with varying density, optical character, strength and chemical properties providing platforms for a huge number of strategies that can be chosen for user's needs. In this review, we focus on applications in biomedical diagnostics and how this revolutionary technique is facilitating development of low cost, sensitive and often geometrically complex tools. 3D printing in fabrication of microfluidics, supporting equipment, optical and electronic components of diagnostic devices is presented. Emerging diagnostic 3D bioprinting as a tool to incorporate living cells or biomaterials into 3D printing is also discussed.

Keywords

3D printing; diagnostics; optics; bioprinting, electronics, microfluidics

1. Introduction

First described by Charles W. Hull in 1986 as stereolithography [1], 3D printing has evolved into a multifunctional fabrication tool that offers unique advantages for biomedical applications including diagnostics [2], scaffolds for 3D implants [3], prosthesis [4] and tissue engineering [5]. In recent years, the ability to convert computer assisted design (CAD) files into 3D-printed pieces, also known as additive manufacturing, has sparked significant progress in the field of diagnostics [6]. 3D printing has been utilized in a wide spectrum of applications with excellent design and performance. As an additive manufacturing technique, production costs are much lower compared to traditional subtractive manufacturing techniques like photolithography. This same low-cost 3D printer can be used to produce different devices and parts without the need for pre-fabrication changes usually required in traditional subtractive manufacturing techniques [7,8]. These criteria make 3D printing a valuable tool in prototyping, testing and production of tools and equipment for analytical and diagnostic laboratories. In principle, CAD files of previously reported devices can be downloaded and printed in any laboratory, so that advanced diagnostic tools can be directly utilized by researchers without the need for purchase from a commercial vendor. This approach has the potential to bring advanced diagnostic tools more rapidly to the research lab than ever before.

3D printing impacts many design aspects including high resolution, low cost and fast fabrication of complex microfluidic devices in a continuous process [9,10,11]. 3D printed microfluidic devices have been used to fabricate semi and fully automated diagnostic approaches for diseases like cancer [12,13], infectious diseases [14,15,16], and xenobiotic genotoxicity [17]. 3D printing also can make tailored supporting devices that improve performance of existing diagnostics like spectrophotometers [18] and PCR devices [14,19] and to support smartphone integration for remote sensing [20,21]. The ability to print materials with special properties allows for the creation of new equipment that can dramatically reduce the cost of diagnostic devices like SPR [22]. All these applications used 3D printing for cost-effective multifunctional production to integrate several functions in one device [23].

Fabrication of diagnostic devices with embedded electronics and circuits have also been completed by 3D printing. The ability to print different materials simultaneously permitted the fabrication of electrodes incorporated into insulator plastic matrices with subsequent

electrochemical detection of metals [24,25,26], organic compounds [27,28] and biologically active molecules [29]. 3D printing avoids disadvantages associated with screen printing like the need for masking and drying steps and allows better resolution and faster fabrication [30].

3D bioprinting is another emerging modification to traditional 3D printing where cells, enzymes or proteins may be encapsulated or loaded into printable photocurable bio-ink solutions [31]. A major focus of this technique is to provide cell growth medium for tissue and organ repair and regeneration, but it also has been explored as a tool for diagnostic applications [32]. Bioprinting offers an opportunity to fabricate 3D printed implantable sensors that are biocompatible, geometrically complex, and cheap. There is a limited need for specialized training and devices can be tailored to users' needs [33,34]. In this review, the most common techniques for 3D printed diagnostics are briefly described with several examples of diagnostic platforms incorporating microfluidics, device supports, optical components, electronics and biomaterials.

2. Additive manufacturing techniques

2.1. Fused Deposition Modeling (FDM)

This technique utilizes thermoplastic polymeric material extruded to print objects layer-by-layer from a heated nozzle onto a surface or platform where it is cooled to below its thermoplastic temperature. Several materials have been utilized in this printing technique like acrylonitrile butadiene styrene (ABS), polycarbonate (PC), PC-ABS blend, and polylactic acid (PLA) [35]. Single, double and triple print-head machines are available for FDM which make it a good choice for simultaneous multi-material 3D printing [36]. The ability to incorporate conductive materials like pyrolytic graphite, graphene, carbon nanotubes and metal nanoparticles into the thermoplastic matrix enables FDM printing of conductive inks to fabricate electrodes and circuits [37,38,39,40]. FDM is good for rapid prototyping and fabrication of holders and supporting devices, but still suffers from several limitations including mechanical strength, roughness and shape integrity of the final product. Microfluidic devices printed using FDM can show leakage and shape deformation if printing parameters and thermoplastic polymer are not carefully tuned [37]. FDM has also been successfully used for 3D bioprinting living cells in thermoplastic matrices without loss of cell viability [41,42].

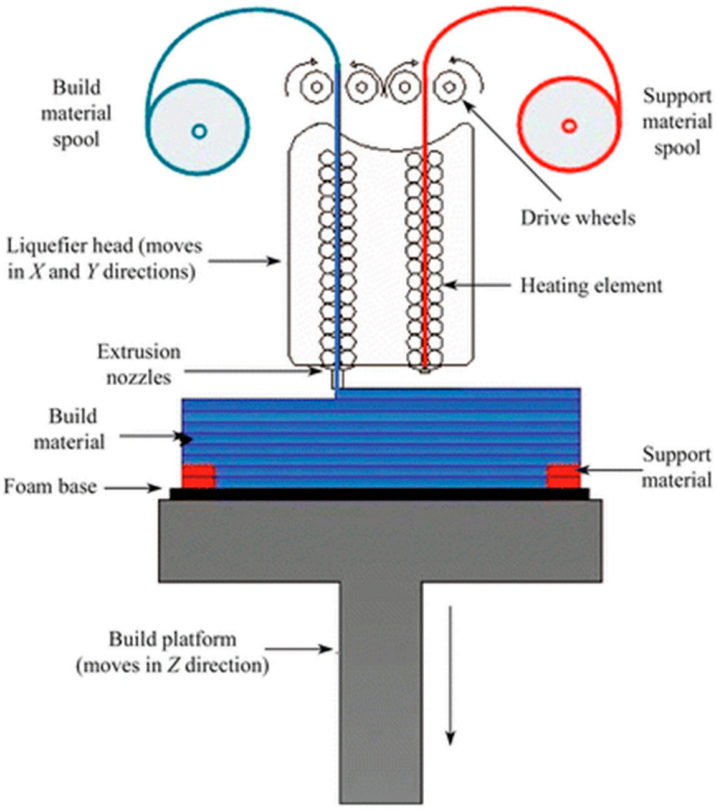

Figure 1: Schematic representation of dual head fused deposition modeling 3D printer. Thermoplastic polymer is extruded from a heated nozzle into a printing platform where it is cooled below its thermoplastic temperature. Reproduced with permission from [35]. Copyright (2015) Springer Nature.

2.2. Stereolithography

Stereolithography or digital light processing employs photocurable polymeric resin which cures into solid when exposed to light. Initially, curing was only possible with UV light, but recently polymers cured with visible wavelengths have been introduced. Highly focused lasers or LED beams with high intensity are used, and the spot size of the light beam determines printing resolution [43]. Each layer of the object is printed as a point-by-point 2D cross section cured by the scanning focused beam onto a printing platform immersed in a photocurable tank that holds the liquid resin [44]. Recently, projection-based stereolithography has been introduced with promise to decrease print time while maintaining almost the same resolution as line-based stereolithography. Projection based lithography replaces point-by-point curing with whole entire layer curing under one single UV or visible light exposure [45,46]. Stereolithography resin

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materials have been extensively studied to produce devices with different properties including transparency, color, flexibility and thermal stability [47]. Stereolithography has been also used for printing cells using biocompatible resin maintaining >90% of cell viability after printing [48].

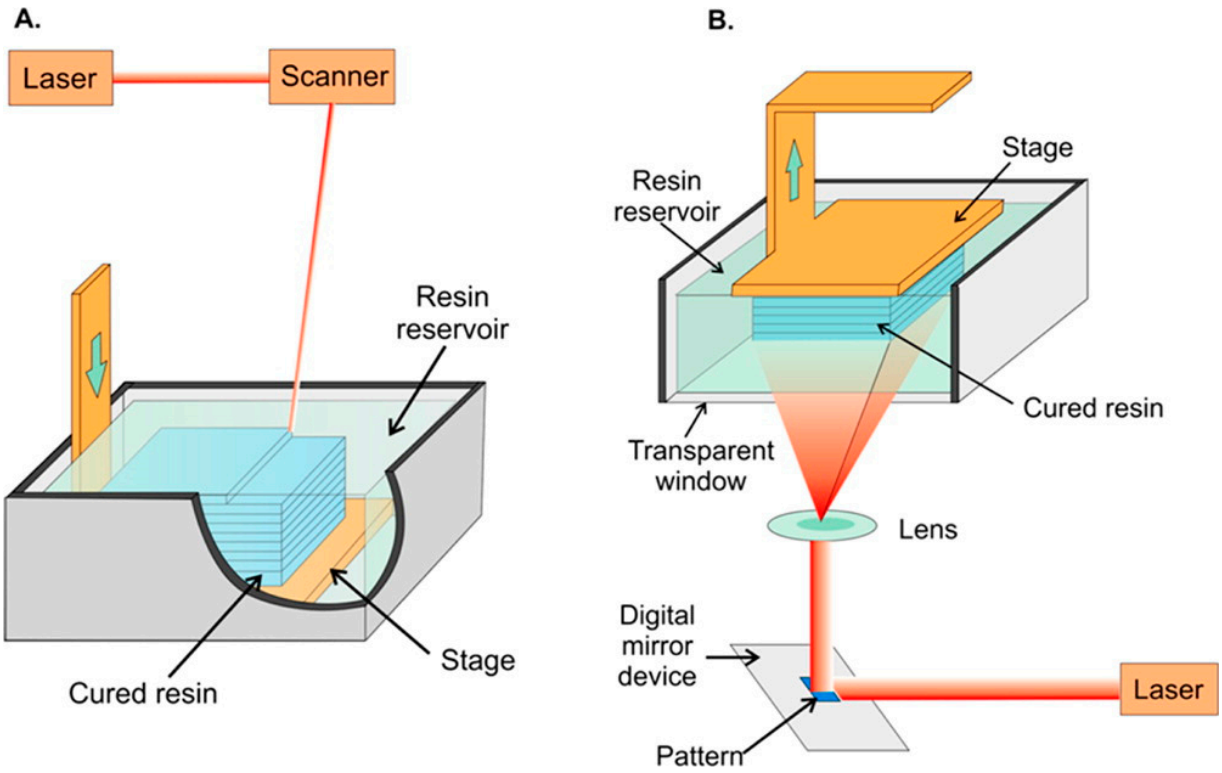

Figure 2: Schematic representation of stereolithographic 3D printing. (A) Scanning laser stereolithography where focused laser beam scans point-by-point to cure a layer of resin on top of a previously fabricated layer. (B) Projection stereolithography where an entire layer is printed in a single step by projecting the entire layer on top of previous layer. In both strategies a printing platform is immersed in a tank filled with liquid photocurable resin. Reproduced with permission from [43]. Copyright (2014) American Chemical Society.

2.3. Photopolymer inkjet printing (Multi-jet modeling- MJM)

This technique utilizes multi-head printers with print heads similar to inkjet printers that extrude layers of photocurable resin or molten wax usually with a second head printing support material to maintain the shape of the design until cured. After printing, the object is cured by UV

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irradiation or heat and support material can be removed by heating or dissolving in a specific solvent [49]. Researchers have been able to utilize this printing technique to print metal nanoparticles for printed electronics [50], preceramic polymers for 3D printed ceramics [51] and even metallic electrodes on flexible substrates [52]. The ability to print multiple materials with varying chemical and physical properties simultaneously made MJM a good candidate for diagnostic devices fabrication. Microfluidic channels integrated with porous membranes that contain viable cells for drug studies or electrodes for electrochemical signal detection have been printed using this technique [53,54]. MJM is ideal for 3D bioprinting especially with photocurable resins as printing is done at room temperature and aqueous resins that support cell viability are available. Most printing resins and materials are proprietary which make the cost of using MJM relatively higher than other 3D printing techniques [55].

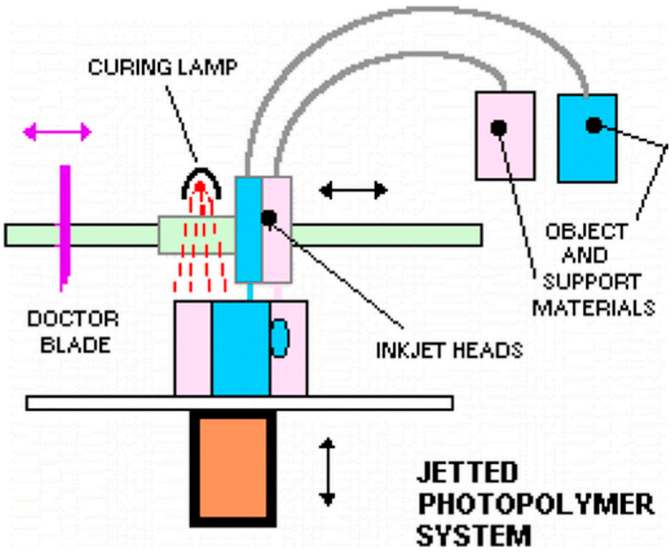

Figure 3: Schematic representation of multi-jet printing technique, a photocurable resin is printed simultaneously with a support material that can be removed after curing. Up to 10 printing heads can be used. Reproduced with permission from [49]. Copyright (2014) American Chemical Society.

2.4. Selective laser sintering (SLS)

A focused IR laser beam supplies enough localized energy required to sinter fine powdered polymer into layers of solid. IR laser scans through the surface of powder in the shape of each layer of the sliced 3D design. Due to the high energy required to sinter powders, high energy CO2 of Nd:YAG laser sources are usually used [56]. SLS can be divided into two distinctive subcategories based on the printing temperature, (i) solid-state sintering: binding happens at a temperature lower than the melting temperature and is usually used with polymers like polycarbonate and (ii) full melting: used for metals and ceramics where sintering requires a high

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temperature above the melting temperature [57]. Printing resolution is affected by powder particle size and can be controlled by scan speed and intensity of the laser beam which also affect the density and strength of the printed parts [58]. SLS has utilized several printing substrates including natural and synthesized polymers like cellulose and polycarbonate which make it compatible with bioprinting for tissue engineering and cartilage repair [59]. Other printing substrates include metals, ceramics and polymer/ceramic composites. It is important to notice the printing resolution with polymers is much lower compared to metals and ceramics [60]. Due to the high energy laser source required and substrate specifications, SLS is currently considered to be the most expensive 3D printing technique [61].

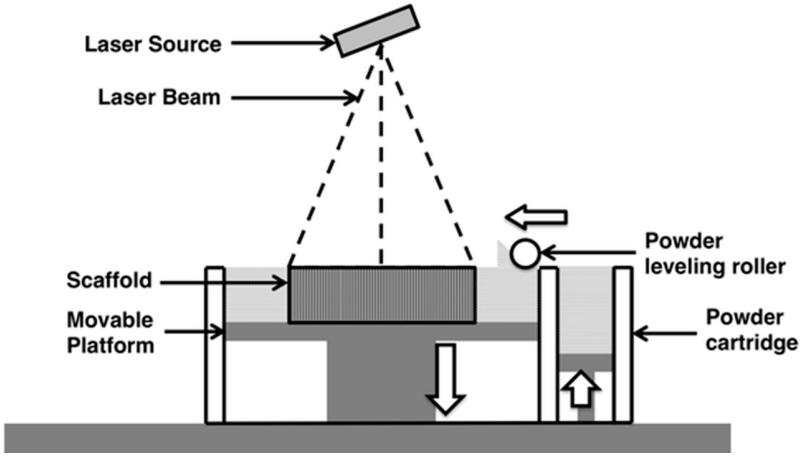

Figure 4: Schematic representation of Selective Laser Sintering, a rolling ball pushes powdered substrate to the surface of the printing platform. High energy focused laser beam scans the surface where it sinters the powder particles into a solid layer. Reproduced with permission from [60]. Copyright (2015) Springer Nature available under Creative Commons Attribution.

3. Application of 3D printing in Diagnostics

3D printing offered a boost to biomedical diagnostics on several aspects mainly due to the advantages gained by ease of onsite design and fabrication of different components researchers may need to develop or modify devices and equipment. Here we focused on the main areas where biomedical diagnostics research has been concentrated recently.

3.1. 3D printed Microfluidics

The most representative use of 3D printing technology in diagnostics is the design and development of microfluidic devices. The ability to fine tune geometrically complex structures at the micrometer level is an attractive feature 3D printing can offer while maintaining a low cost and time efficient processing. Several applications that have used 3D printed microfluidic devices are discussed.

3.1.1. Sample pretreatment

Sample pretreatment is an essential step in many diagnostics as it helps reduce the complexity of the matrix and improve the sensitivity of the assay. Rafeie et al utilized 3D printing to fabricate an ultrafast microfluidic blood plasma separator, an essential sample pretreatment step in most of the assays requiring blood samples. They were able to fabricate a spiral microfluidic device (Figure 5A) where cells would flow close to the inner wall of the channel and concentrate in a narrow band near the outlet allowing the separation of cell/platelet free plasma. They also multiplexed up to 16 channels in order to tune the separation rate from 1.5 mL/min to 24 mL/min [62]. Lee et al separated pathogenic bacteria, E. coli, from milk using a 3D printed helical channel [63]. They first used antibody labeled magnetic nanoclusters to capture bacterial cells, then collected the magnetic nanoclusters using a permanent magnet and finally flowed them through the helical microfluidic channel (Figure 5B) where free magnetic nanoclusters are separated from bacteria-bound clusters. Yan et al proposed a portable hand operated microfluidic device that can specifically separate platelets from peripheral blood mononuclear cells [64]. Their device is composed of a microfluidic channel equipped with a groove (Figure 5C) that effectively sorts platelets from blood samples with 100% purity where the user pumps the fluid manually with a hand-held syringe. While fluctuation in the flow rate did not affect the platelet purity, the percentage recovery of blood mononuclear cells varied. A microfluidic pre-concentrator for detection of E. coli was also proposed by Park et al [65]. Magnetic nanoparticles labeled with E. coli specific antibodies were allowed to capture bacteria from blood samples. The microfluidic device was equipped with a magnet to separate (Figure 5D) magnetic nanoparticles from blood matrix which then transferred with buffer for ATP luminescence analysis. Although these devices are interesting applications for 3D printing in sample pretreatment, they still

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require manual transfer of the treated samples to be detected which may affect assay sensitivities and reproducibility required for good diagnostic approach.

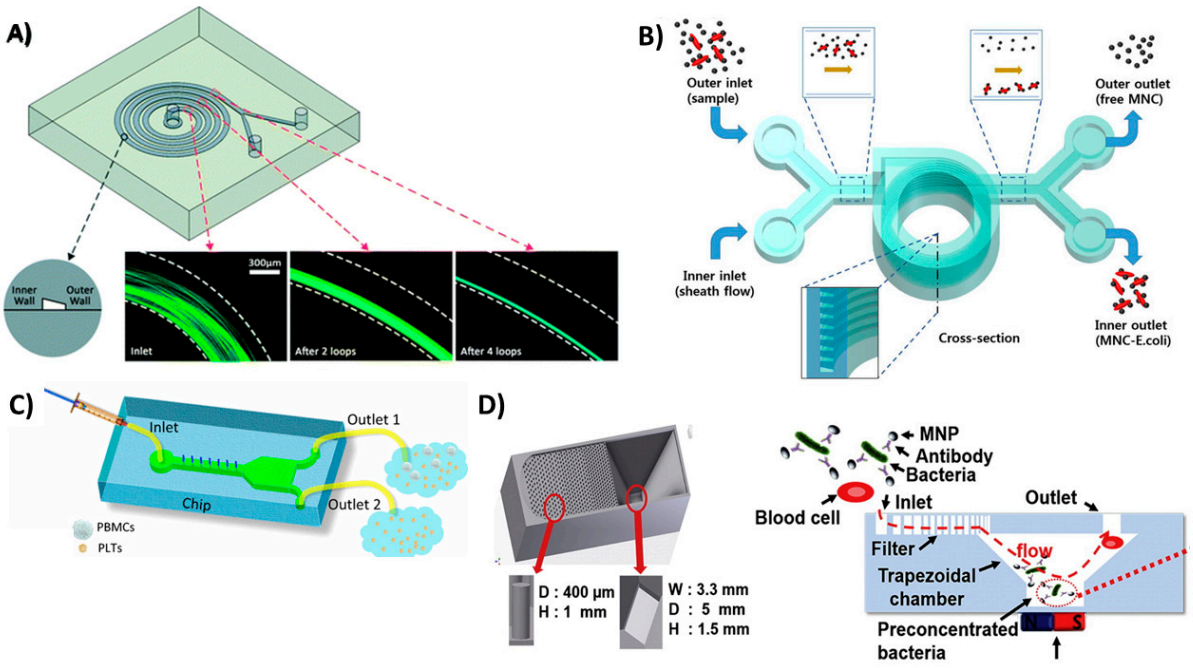

Figure 5: 3D printed devices for sample pretreatment. (A) Spiral microfluidic device to separate blood cells and platelets from plasma, as the cells and platelets tend to flow in a narrowing band near the inner wall of the spiral channel. Reproduced with permission from [62]. Copyright (2016) Royal Society of Chemistry. (B) Helical microfluidic device to separate magnetic nanoclusters coupled to E. coli from free magnetic nanoclusters. Reproduced with permission from [63]. Copyright (2015) Springer Nature available under Creative Commons Attribution. (C) A hand driven microfluidic channel with a groove like structure to separate platelets from blood mononuclear cells. Reproduced with permission from [64] Copyright (2018) Springer Nature. (D) Trapezoidal filter equipped microfluidic channel for the preconcentration of E. coli captured on magnetic beads. Reproduced with permission from [65] Copyright (2017) Elsevier.

3.1.2. Flow control

Microfluidic devices offer the most promising approach for miniature fluid control devices because of their ability to handle very small sample volumes and assay reagents in a controlled manner. 3D printing has pushed prototyping and development of microfluidics forward by

supporting fast and easy design with lower production costs compared to traditional microfabrication techniques. 3D printing also offers an efficient tool to generate geometrically complex microfluidic devices designed easily on any computer with aid of 3D design software eliminating the hassle associated with such designs in traditional manufacturing tools. Utilizing these advantages, Oh et al designed and fabricated a 3D printed blood viscosity analysis capillary circuit [66]. They designed a hand-held device that can be operated and read manually that measures blood viscosity using the same principle as commercial viscometers which are very expensive and complex (Figure 6A). Even with the great advances in 3D printing resolution, their device did not make use of that instead they added Tygon tubing with inner diameter of 0.508 mm to build a capillary circuit inside 3D printed channel. Santangelo et al proposed a highly sensitive 3D printed continuous flow microfluidic device for quantification of adenosine triphosphate (ATP) molecules (Figure 6B). The device comprised two main functions; first was mixing of ATP sample with the luminescence reagent mixture (Luciferin/Luciferase mixture). The second function was to provide a detection chamber that can bring the produced luminescence close to a silicon photomultiplier detector. Using 3D printing they were able to analyze cell lysate samples with a detection limit of 8 nM and 5 orders of magnitude dynamic range [67]. Tang et al utilized 3D printing to fabricate a unibody ELISA inspired chemiluminescence assay to detect and quantify prostate specific antigen (PSA) and platelet factor-4 (PF-4) as cancer biomarker proteins (Figure 6C) [68]. They proposed a design that can reduce the assay time to 30 min while approaching an ultra-low sensitivity of 500 fg/mL and dynamic range of four orders of magnitude. Their design is divided into three connected compartments, first was a mixing chamber to accelerate the interaction between detection antibodies, antigens and poly-HRP labels. The second compartment consisted of sample and reagent reservoirs and the third was the transparent detection compartment. The ability to 3D print transparent objects allowed them to directly detect chemiluminescence signal in their device using a CCD camera without the need for complex processing. Recently, a Lego-like modular microfluidic capillary-driven 3D printed flow device was introduced by Nie et al [69]. This approach proposed a strategy to build microfluidic devices tailored to different applications. Flow in such devices was driven by capillary forces with improved flow rate programmability and biocompatibility. They were able to design different modules assembled in different designs and utilized them in different applications like degradable bone scaffolds and cell culture.

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Kadimisetty et al proposed a 3D printed microfluidic unit that manually controls the flow of sample and assay reagents for electrochemiluminescent detection of PSA and PF-4 in addition to prostate specific membrane antigen (PSMA) in human serum [12]. The printed device had a slot to incorporate a screen-printed carbon electrode labeled with detection antibodies for each of the selected protein biomarkers (Figure 6D). Also, it was equipped with reservoirs for washing buffers and electrochemiluminescence reagents required to generate the signal from Ru(bpy)32+ labeled silica nanoparticles. In these discussed examples, 3D printing was the key for better diagnostic performance through providing a tool for low cost incorporation of multiple fluidic functions easily without the need for laborious manufacturing procedures.

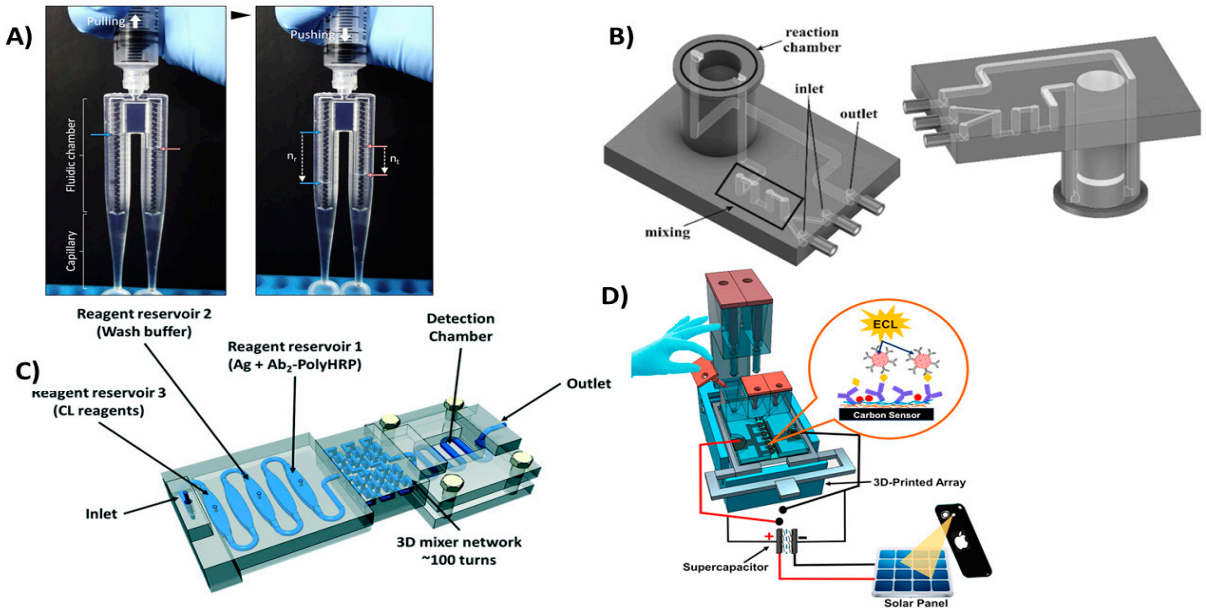

Figure 6: 3D printed microfluidic devices for flow control. (A) Viscometer like 3D printed syringe attachment for blood viscosity measurement. Reproduced with permission from [66]. Copyright (2018) Elsevier. (B) Mixing and detection microfluidic device for luminescent detection of ATP. Reproduced with permission from [67]. Copyright (2018) Elsevier. (C) Unibody 3D printed microfluidic chip for detection of PSA and PF-4. Reproduced with permission from [68]. Copyright (2017) Royal Society of Chemistry. (D) Manually controlled flow regulatory system for electrochemiluminescence detection of PSA, PSMA and PF-4. Reproduced with permission from [12]. Copyright (2016) Elsevier.

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3.1.3. Microfluidic mixers

Efficient mixing is a successful approach to improve diagnostics by enhancing the interaction kinetics between reactants. Microfluidics has been the method of choice for efficient mixing as it can enhance the chaotic convection in solutions increasing the chance of interactions between solutions' components [70]. Plevniak et al proposed a 3D printed microfluidic mixer for diagnosis of anemia (Figure 7A). In their work they were able to integrate the device with smartphone-aided colorimetric signal detection to overcome distance barrier for efficient anemia screening [71]. The device can analyze a finger prick of blood (~5µL) driven by capillary force into the mixing chamber where it is mixed with an oxidizing agent in less than 1 sec with cost 50 cents/chip. Due to the fast and easy prototyping with 3D printing, they were able to compare the performance of different mixer designs in a time efficient manner. Another mixing device was introduced by Mattio et al [72], where a complex valve design was fabricated using 3D printing (Figure 7B). The device was comprised of two inlets equipped with solid phase extraction columns to selectively separate cadmium and lead from water samples. The device also had eight other reagent inlets connected to a valve where they were mixed with samples through a coil equipped with baffles. The fluorescence of the two metals was generated using fluorescence reagent Rhod-5N™ with a detection limit of approximately 0.2 µg/mL obtained in less than 18

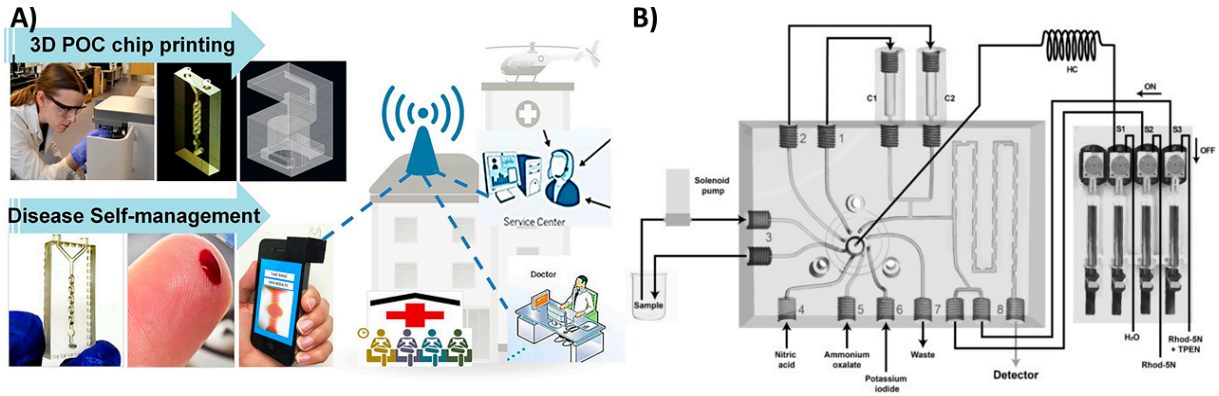

Figure 7: 3D printed microfluidic mixers. (A) Microfluidic mixer for tele-diagnosis of anemia. Less than one second of mixing allowed of the blood sample with the oxidizing agent; generated colorimetric signal detected with a smartphone. Reproduced with permission from [71]. Copyright (2016) AIP Publishing. (B) A lab on valve complex 3D printed microfluidic chip for quantification of lead and cadmium in water samples. Reproduced with permission from [72]. Copyright (2018) Elsevier.

min.

3.1.4. Multifunctional microfluidics

In previous examples, 3D printing was utilized to fabricate microfluidics that served only one purpose to improve diagnostic techniques. With huge advances in 3D printing, several researchers have proposed multifunctional microfluidic devices capable of performing several tasks simultaneously. Kadimisetty et al introduced a microfluidic device that can analyze extracts from e-cigarette vapors [17]. The device is equipped with sample and reagent reservoirs, in addition to an electrochemiluminescence signal detection compartment equipped with platinum counter and Ag/AgCl reference electrodes (Figure 8A). The device was powered by micropumps that were fully automated to reduce variation and make it a good point of care diagnostic approach. Another multifunctional microfluidic device was also introduced recently by Kadimisetty et al [9], where they were able to extract, concentrate and isothermally amplify nucleic acids in different body fluids as an approach for microfluidic point of care diagnostics (Figure 8B). The microfluidic device was integrated with a membrane to isolate nucleic acids, then placed in a chamber where loop mediated isothermal amplification is induced. Finally, signal produced was either colorimetric detected by a mobile phone or fluorescence detected with USB fluorescence microscope. This proves utilizing 3D printing is very promising to improve microfluidic diagnostic devices for point of care applications that can be automated with low cost.

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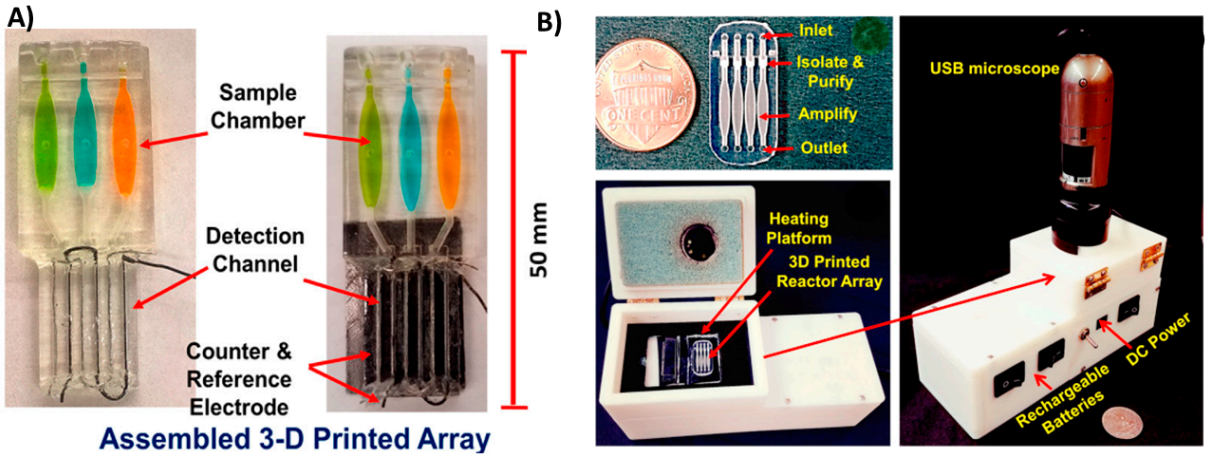

Figure 8: Multifunctional 3D printed microfluidics. (A) 3D printed chip to detect genotoxicity of metabolites from e-cigarette extracts. Device has sample and reagent reservoir compartment and detection compartment equipped with platinum counter electrode and Ag/AgCl reference electrode. Reproduced with permission from [17]. Copyright (2017) American Chemical Society. (B) 3D printed microfluidic array for isolation of nucleic acids equipped with separation membrane and heating compartment to amplify nucleic acids using loop mediated isothermal amplification that can be attached to USB microscope for fluorescence detection. Reproduced with permission from [9]. Copyright (2018) Elsevier.

3.2. 3D printed Electronics

In addition to the evolving application of 3D printing in microfluidic diagnostics, 3D printing has attracted researchers especially in the field of electrochemistry to design and fabricate sensing electronics. Supported by the versatility of printable materials 3D printing can utilize the ability of 3D printers to produce well defined shapes without complicated masking procedures. Thus, enabling 3D printing to be used to fabricate electrode biosensors and electronic sensors. Li et al used a home-made 3D printer to print a conductive polymer made by doping carbon nanotubes in polydimethylsiloxane (PDMS) or Ecoflex™ to fabricate stretchable electrode sensors [73]. They printed the conductive polymer on a glass substrate, then transferred the printed pattern onto either PDMS or Ecoflex™ to make it flexible (Figure 9A). Using this homemade 3D printer, they were able to get resolution of 400µm with electrode height of 1 mm. 3D printing provided them with an easy approach to change the shape and pattern of the electrodes design using the same machine without complex procedures usually encountered in traditional screen printing. They used the printed electrodes as a tactile sensor with good sensitivity and reproducibility and for electrochemical detection of sodium chloride with 1µM detection limit. Another approach for 3D printing electrodes using fused deposition modeling was proposed by Palenzuela et al [74]. A commercially available graphene/polylactic acid filament was used to print electrodes of distinctive shapes designed on CAD software (Figure 9B). The printed electrodes were characterized using different redox probes and utilized to detect picric acid and ascorbic acid in solution. In order to fabricate more complex electronics, Leigh et al used a triple head fused deposition modeling printer to impede conductive filament, composed of carbon black filler in matrix of polycaprolactone, within nonconductive ABS or PLA matrix [38]. Using this approach, they were able to fabricate a variety of complex functional objects like 3D flex sensor, capacitive buttons and a smart vessel (Figure 9C).

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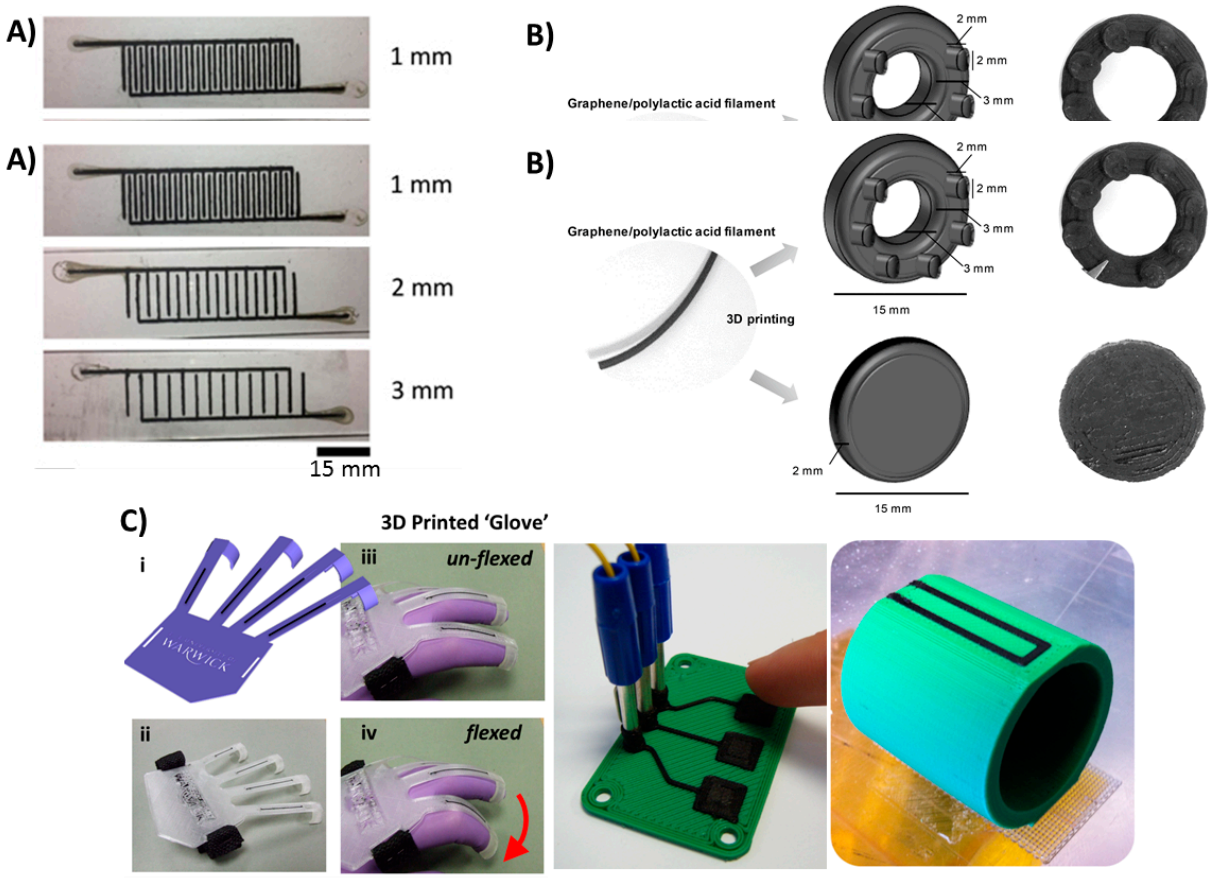

Figure 9: 3D printed electronics. (A) 3D printed tactile electrode sensor. Conductive PDMS doped with carbon nanotubes were printed on PDMS or Ecoflex™ to fabricate flexible electrode sensors. Reproduced with permission from [73]. Copyright (2018) IOP Publishing. (B) 3D printed graphene/polylactic acid electrode with ring or disc shaped. Reproduced with permission from [74]. Copyright (2018) American Chemical Society. (C) 3D printed conductive carbon black electrode in different objects from left to right: flexible glove sensor, capacitive buttons and smart vessel. Reproduced from [38]. Copyright (2012) PLOS available under Creative Commons Attribution.

3.3. 3D printed supporting devices

Versatility, ease of design and modification in a fast and economic manner, made 3D printing the method of choice to develop supporting equipment and pieces required for diagnostics. Shanmugam et al used 3D printing to fabricate a custom designed mobile phone microscopy support unit that perfectly aligns the sample compartment with simple optics and mobile phone

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camera [75]. The proposed design composed of a glass slide holder, where samples are spotted for testing. This holder is connected to a mobile phone holder equipped with a lens right above the camera. They were able to add a filter holder in between the sample compartment and lens in case of fluorescence detection (Figure 10A). They also proposed a holder that can incorporate a microfluidic chamber for analyzing flowing samples rather than stationary samples (Figure 10B). Using such equipment, they were able to perform screening of soil-transmitted parasitic worms in resource limited areas by either traditional or fluorescence imaging. Another supporting device for a paper based electrochemical sensor was proposed by Scordo et al [76]. Reagent free sensor was proposed to test butyrylcholinesterase activity by detecting thiocholine (byproduct of butyrylcholinesterase catalyze decomposition of butyrylcholine). A 3D printed support equipped with sample application hole was used to provide the supporting strength and insulation required for electric connections (Figure 10C).

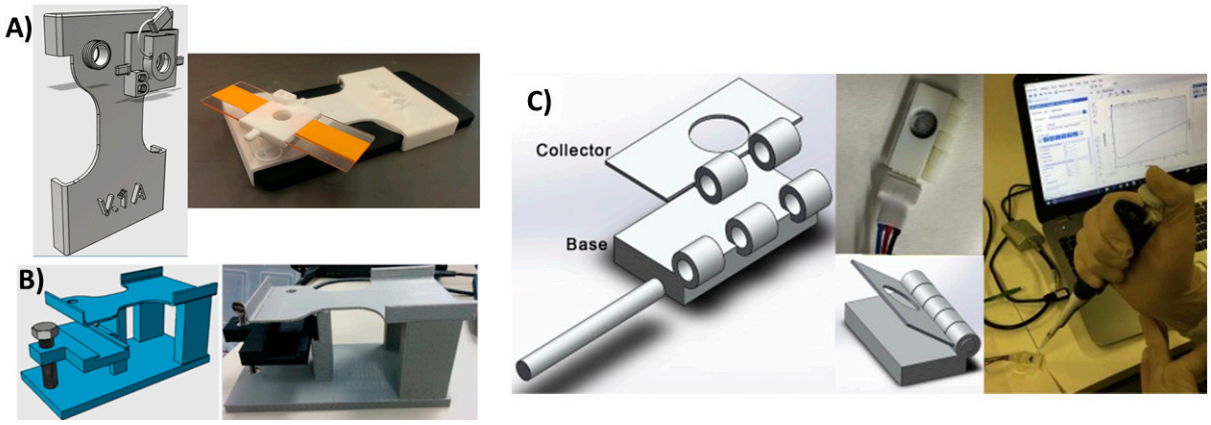

Figure 10: 3D printed support devices. (A) Soil analysis system with 3D printed mobile phone holder equipped with a glass slide holder where samples were fixed and lens in between mobile camera and sample holder. This jig had a replaceable filter just above the lens for fluorescence imaging. Reproduced from [75]. Copyright (2018) PLOS available under Creative Commons Attribution. (B) Alternate soil analysis system with the same support components, but modified to hold a microfluidic chip for flowing samples. Reproduced from [75]. Copyright (2018) PLOS available under Creative Commons Attribution. (C) Support device with sample application hole for paper based electrochemical detection of butyrylcholinesterase activity. Reproduced with permission from [76]. Copyright (2018) Elsevier.

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3.4. 3D printed optics

Despite the limitations of current 3D printing techniques in terms of printing fully transparent surfaces without defects that may affect light reflection and transmission, researchers still aimed to 3D print functional optical components that can be used to reduce the cost and improve performance of diagnostic devices. An interesting trial was done by Hinamn et al, where they 3D printed a prism that can be used for plasmonic sensing applications [22]. They used simple benchtop polishing to decrease surface defects and improve the light guiding performance of the prism (Figure 11A). In order to prove functionality, they were able to deposit a layer of gold on one side of the prism and use it after functionalizing with lipid membrane interface to detect cholera toxins. They were also able to print prisms with different geometries and use them to monitor nanoparticle growth (Figure 11B). This proves the versatility of 3D printing as a promising technique to develop multifunctional diagnostic devices tailored to each user's

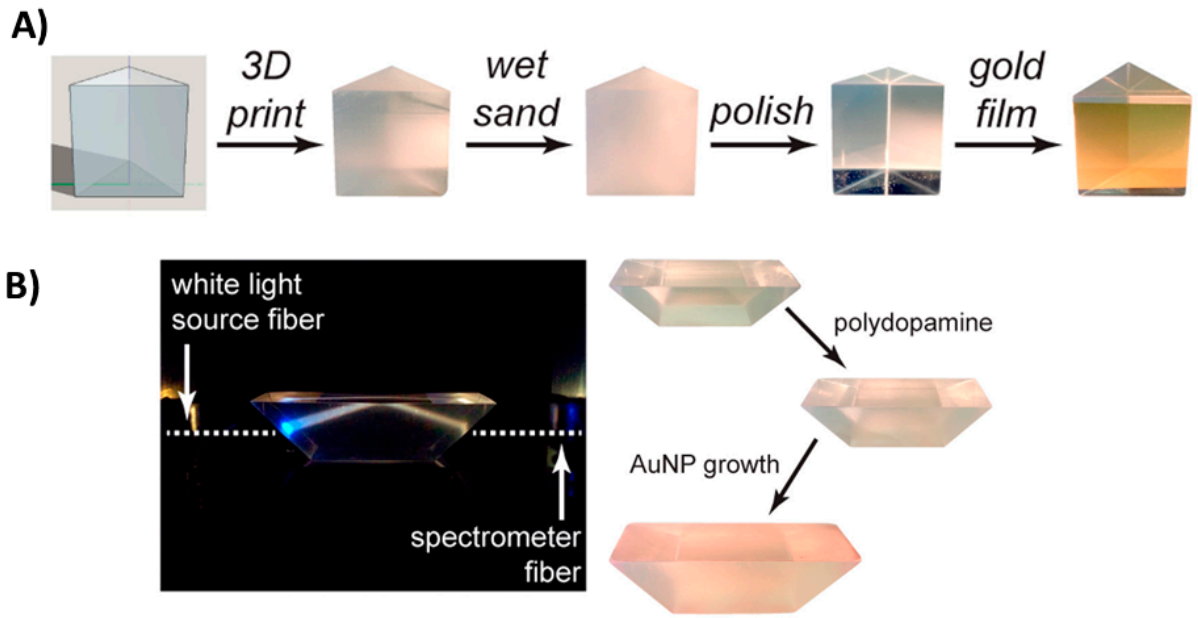

Figure 11: 3D printed optics. (A) 3D printed prism polished with simple benchtop polishing decorated with a layer of gold and used for plasmonic sensing of cholera toxins. Reproduced with permission from [22]. Copyright (2017) American Chemical Society. (B) 3D printed prism with different geometry than (A) used to monitor nanoparticle growth. Reproduced with permission from [22]. Copyright (2017) American Chemical Society.

specific needs.

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3.5. 3D Bioprinting

Recently, the ability to use biocompatible 3D printing substrates allowed the incorporation of biomaterials in 3D printed scaffolds for tissue-like construct fabrications and bioactive skeletons. This facilitate the further investigation of multifunctional 3D printed devices that can express biomimetic activity in diagnostic applications. A bioinspired microfluidic chip that can be attached to whole organs was proposed by Singh et al [77]. This microfluidic chip was fabricated based on structured light scanning of the whole organ followed by stereolithographic 3D printing using the scanned conformation. The as printed device has been attached to porcine kidney for biomarker extraction and profiling (Figure 12A) without any tissue removal. The microfluidic chip is perfused wit PBS buffer to allow the extraction of biomarkers and metabolites from organ cortex. This approach enables the study of metabolic activities of different organs in a living whole organ, paving the road for further investigation in drug toxicity screening and biomarker discovery. Another cell-laden bone matrix was proposed by Zhou et al [78] to study breast cancer metastasis. They printed gelatin-based methacrylate hydrogel with incorporated bone stromal cells (osteoblasts or human bone marrow mesenchymal stem cells) to study their interactions with breast cancer cells (Figure 12B). Using this 3D printed construct, they were able to visualize living cells with confocal microscope after staining and test biomarkers like vascular endothelial growth factor (VEGF) and alkaline phosphatase from cell cultures. In vivo alkaline phosphatase testing platform was introduced by Park et al [79] using 3D printed calcium deficient hydroxyapatite. The 3D printed scaffold was biocompatible and was labeled with a fluorescent probe that exhibits enhanced fluorescence in presence of alkaline phosphatase. The as manufactured device was implanted successfully in mice to monitor bone formation.

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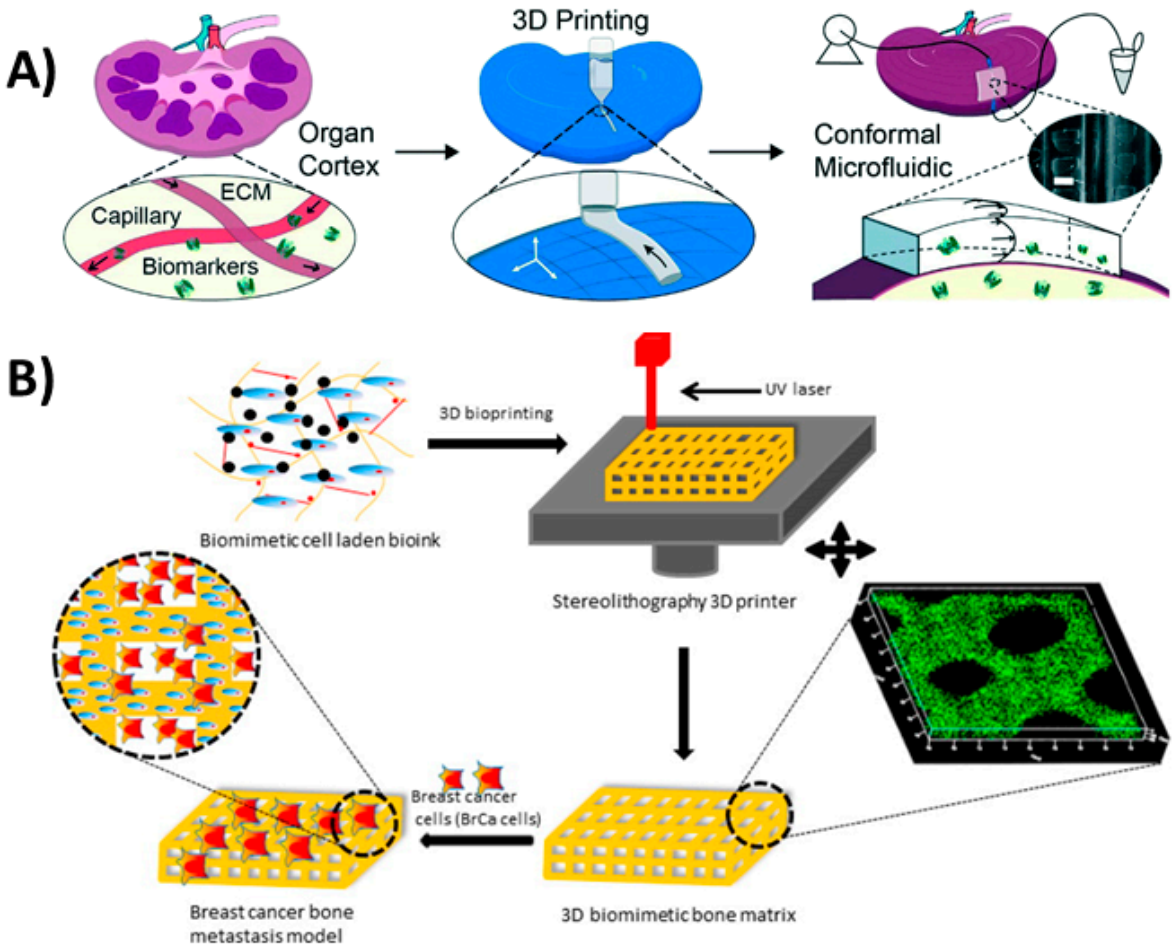

Figure 12: 3D bioprinting. (A) 3D printed perfusion chip for extraction of metabolites and biomarkers from whole organs. Reproduced with permission from [77]. Copyright (2017) Royal Society of Chemistry (B) 3D printed bone like scaffold carrying bone stromal cells to study their interactions with breast cancer cells. Reproduced with permission from [78]. Copyright (2016) American Chemical Society.

4. Conclusion

With all these evolving applications and developments, 3D printing has proved to be the next work horse for readily available, cheap, miniaturized, multifunctional and sensitive diagnostics. Researchers from different backgrounds have focused on the development of different aspects of diagnostic assays using versatile approaches due to facility granted by the 3D printing

technology. It has been used as a handy tool for devices prototyping and development with photolithography technique most commonly used because of availability of materials exhibiting different properties and high resolution. 3D printing has pushed biomedical diagnostics research toward multifunctional devices in one fabrication that can perform several functions like proteins and metabolites extraction, fluid flow control, photo and electrochemical signal detection. With the progressive nature of 3D printing technique, we expect in the near future to see more complex architectures. Recent research is focused on the development of microfluidic pumps [80], automated flow control valves [81], atomic force microscopy [82] and even sophisticated scanning electron microscope sample holders [83]. These are examples for very complex architectures that could not have been approached in lab easily without the use of 3D printing. This implies again the significance of incorporating 3D printing in bioanalytical and diagnostic research providing a platform for achieving what was believed to be imaginary in the pre-3D printing era.

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References

1- HULL, C. Apparatus for production of three-dimensional objects by stereolithography. *US Pat. No 4575330* **1986**.

2- Gross, B.; Lockwood, S. Y.; Spence, D. M. Recent Advances in Analytical Chemistry by 3D Printing. *Anal. Chem.* **2017**, *89*, 57–70, doi:10.1021/acs.analchem.6b04344.

3- Chia, H. N.; Wu, B. M. Recent advances in 3D printing of biomaterials. *J. Biol. Eng.* **2015**, *9*, 4, doi:10.1186/s13036-015-0001-4.

4- Diment, L. E.; Thompson, M. S.; Bergmann, J. H. Three-dimensional printed upper-limb prostheses lack randomised controlled trials: A systematic review. *Prosthet. Orthot. Int.* **2018**, *42*, 7–13, doi:10.1177/0309364617704803.

5- Bose, S.; Vahabzadeh, S.; Bandyopadhyay, A. Bone tissue engineering using 3D printing. *Mater. Today* **2013**, *16*, 496–504, doi:10.1016/j.mattod.2013.11.017.

6 - Waheed, S.; M. Cabot, J.; P. Macdonald, N.; Lewis, T.; M. Guijt, R.; Paull, B.; C. Breadmore, M. 3D printed microfluidic devices: enablers and barriers. *Lab. Chip* **2016**, *16*, 1993–2013, doi:10.1039/C6LC00284F.

7- Why 3D Printing Could Be A Manufacturing And Logistics Game Changer Available online: https://www.manufacturing.net/blog/2013/10/why-3d-printing-could-be-manufacturing-and-logistics-game-changer (accessed on Apr 30, 2018).

8- Au, A. K.; Lee, W.; Folch, A. Mail-order microfluidics: evaluation of stereolithography for the production of microfluidic devices. *Lab. Chip* **2014**, *14*, 1294–1301, doi:10.1039/C3LC51360B.

9- Kadimisetty, K.; Song, J.; Doto, A. M.; Hwang, Y.; Peng, J.; Mauk, M. G.; Bushman, F. D.; Gross, R.; Jarvis, J. N.; Liu, C. Fully 3D printed integrated reactor array for point-of-care molecular diagnostics. *Biosens. Bioelectron.* **2018**, *109*, 156–163, doi:10.1016/j.bios.2018.03.009.

10- Yazdi, A. A.; Popma, A.; Wong, W.; Nguyen, T.; Pan, Y.; Xu, J. 3D printing: an emerging tool for novel microfluidics and lab-on-a-chip applications. *Microfluid. Nanofluidics* **2016**, *20*, 50, doi:10.1007/s10404-016-1715-4.

11- Zhang, Z.; Xu, J.; Hong, B.; Chen, X. The effects of 3D channel geometry on CTC passing pressure – towards deformability-based cancer cell separation. *Lab. Chip* **2014**, *14*, 2576–2584, doi:10.1039/C4LC00301B

12- Kadimisetty, K.; Mosa, I. M.; Malla, S.; Satterwhite-Warden, J. E.; Kuhns, T. M.; Faria, R. C.; Lee, N. H.; Rusling, J. F. 3D-printed supercapacitor-powered electrochemiluminescent protein immunoarray. *Biosens. Bioelectron.* **2016**, *77*, 188–193, doi:10.1016/j.bios.2015.09.017.

13- Damiati, S.; Peacock, M.; Leonhardt, S.; Damiati, L.; Baghdadi, M. A.; Becker, H.; Kodzius, R.; Schuster, B. Embedded Disposable Functionalized Electrochemical Biosensor with a 3D-Printed Flow Cell for Detection of Hepatic Oval Cells (HOCs). *Genes* **2018**, *9*, 89, doi:10.3390/genes9020089.

14- Mulberry, G.; White, K. A.; Vaidya, M.; Sugaya, K.; Kim, B. N. 3D printing and milling a real-time PCR device for infectious disease diagnostics. *PLOS ONE* **2017**, *12*, e0179133, doi:10.1371/journal.pone.0179133.

15- Singh, H.; Shimojima, M.; Shiratori, T.; An, L. V.; Sugamata, M.; Yang, M. Application of 3D Printing Technology in Increasing the Diagnostic Performance of Enzyme-Linked Immunosorbent Assay (ELISA) for Infectious Diseases. *Sensors* **2015**, *15*, 16503–16515, doi:10.3390/s150716503.

16- Chudobova, D.; Cihalova, K.; Skalickova, S.; Zitka, J.; Rodrigo, M. A. M.; Milosavljevic, V.; Hynek, D.; Kopel, P.; Vesely, R.; Adam, V.; Kizek, R. 3D-printed chip for detection of methicillin-resistant Staphylococcus aureus labeled with gold nanoparticles. *ELECTROPHORESIS* **2015**, *36*, 457–466, doi:10.1002/elps.201400321.

17- Kadimisetty, K.; Malla, S.; Rusling, J. F. Automated 3-D Printed Arrays to Evaluate Genotoxic Chemistry: E-Cigarettes and Water Samples. *ACS Sens.* **2017**, *2*, 670–678, doi:10.1021/acssensors.7b00118.

18- Schäfer, M.; Bräuler, V.; Ulber, R. Bio-sensing of metal ions by a novel 3D-printable smartphone spectrometer. *Sens. Actuators B Chem.* **2018**, *255*, 1902–1910, doi:10.1016/j.snb.2017.08.207.

19- Mendoza-Gallegos, R. A.; Rios, A.; Garcia-Cordero, J. L. An Affordable and Portable Thermocycler for Real-Time PCR Made of 3D-Printed Parts and Off-the-Shelf Electronics. *Anal. Chem.* **2018**, *90*, 5563–5568, doi:10.1021/acs.analchem.7b04843.

20- Wang, L.-J.; Sun, R.; Vasile, T.; Chang, Y.-C.; Li, L. High-Throughput Optical Sensing Immunoassays on Smartphone. *Anal. Chem.* **2016**, *88*, 8302–8308, doi:10.1021/acs.analchem.6b02211.

21- Chen, Y.; Fu, Q.; Li, D.; Xie, J.; Ke, D.; Song, Q.; Tang, Y.; Wang, H. A smartphone colorimetric reader integrated with an ambient light sensor and a 3D printed attachment for on-site detection of zearalenone. *Anal. Bioanal. Chem.* **2017**, *409*, 6567–6574, doi:10.1007/s00216-017-0605-2.

22- Hinman, S. S.; McKeating, K. S.; Cheng, Q. Plasmonic Sensing with 3D Printed Optics. *Anal. Chem.* **2017**, *89*, 12626–12630, doi:10.1021/acs.analchem.7b03967.

23- Rusling, J. F. Developing Microfluidic Sensing Devices Using 3D Printing. *ACS Sens.* **2018**, *3*, 522–526, doi:10.1021/acssensors.8b00079.

24- Lee, K. Y.; Ambrosi, A.; Pumera, M. 3D-printed Metal Electrodes for Heavy Metals Detection by Anodic Stripping Voltammetry. *Electroanalysis* **2017**, *29*, 2444–2453, doi:10.1002/elan.201700388.

25- Rymansaib, Z.; Iravani, P.; Emslie, E.; Medvidović-Kosanović, M.; Sak-Bosnar, M.; Verdejo, R.; Marken, F. All-Polystyrene 3D-Printed Electrochemical Device with Embedded Carbon Nanofiber-Graphite-Polystyrene Composite Conductor. *Electroanalysis* **2016**, *28*, 1517–1523, doi:10.1002/elan.201600017.

26- Honeychurch, K. C.; Rymansaib, Z.; Iravani, P. Anodic stripping voltammetric determination of zinc at a 3-D printed carbon nanofiber–graphite–polystyrene electrode using a carbon pseudo-reference electrode. *Sens. Actuators B Chem.* **2018**, *267*, 476–482, doi:10.1016/j.snb.2018.04.054.

27- Cheng, T. S.; Nasir, M. Z. M.; Ambrosi, A.; Pumera, M. 3D-printed metal electrodes for electrochemical detection of phenols. *Appl. Mater. Today* **2017**, *9*, 212–219, doi:10.1016/j.apmt.2017.07.005.

28- Tan, C.; Nasir, M. Z. M.; Ambrosi, A.; Pumera, M. 3D Printed Electrodes for Detection of Nitroaromatic Explosives and Nerve Agents. *Anal. Chem.* **2017**, *89*, 8995–9001, doi:10.1021/acs.analchem.7b01614.

29- Liyarita, B. R.; Ambrosi, A.; Pumera, M. 3D-printed Electrodes for Sensing of Biologically Active Molecules. *Electroanalysis 0*, doi:10.1002/elan.201700828.

30- Lewis, J. A.; Ahn, B. Y. Device fabrication: Three-dimensional printed electronics. *Nature* **2015**, *518*, 42–43, doi:10.1038/518042a.

31- Derakhshanfar, S.; Mbeleck, R.; Xu, K.; Zhang, X.; Zhong, W.; Xing, M. 3D bioprinting for biomedical devices and tissue engineering: A review of recent trends and advances. *Bioact. Mater.* **2018**, *3*, 144–156, doi:10.1016/j.bioactmat.2017.11.008.

32- Park, C. S.; Ha, T. H.; Kim, M.; Raja, N.; Yun, H.; Sung, M. J.; Kwon, O. S.; Yoon, H.; Lee, C.-S. Fast and sensitive near-infrared fluorescent probes for ALP detection and 3d printed calcium phosphate scaffold imaging in vivo. *Biosens. Bioelectron.* **2018**, *105*, 151–158, doi:10.1016/j.bios.2018.01.018.

33- Knowlton, S.; Onal, S.; Yu, C. H.; Zhao, J. J.; Tasoglu, S. Bioprinting for cancer research. *Trends Biotechnol.* **2015**, *33*, 504–513, doi:10.1016/j.tibtech.2015.06.007.

34- Zhou, X.; Zhu, W.; Nowicki, M.; Miao, S.; Cui, H.; Holmes, B.; Glazer, R. I.; Zhang, L. G. 3D Bioprinting a Cell-Laden Bone Matrix for Breast Cancer Metastasis Study. *ACS Appl. Mater. Interfaces* **2016**, *8*, 30017–30026, doi:10.1021/acsami.6b10673.

35- Mohamed, O. A.; Masood, S. H.; Bhowmik, J. L. Optimization of fused deposition modeling process parameters: a review of current research and future prospects. *Adv. Manuf.* **2015**, *3*, 42–53, doi:10.1007/s40436-014-0097-7.

36- Dul, S.; Fambri, L.; Pegoretti, A. Fused deposition modelling with ABS–graphene nanocomposites. *Compos. Part Appl. Sci. Manuf.* **2016**, *85*, 181–191, doi:10.1016/j.compositesa.2016.03.013.

37- Wang, X.; Jiang, M.; Zhou, Z.; Gou, J.; Hui, D. 3D printing of polymer matrix composites: A review and prospective. *Compos. Part B Eng.* **2017**, *110*, 442–458, doi:10.1016/j.compositesb.2016.11.034.

38- Leigh, S. J.; Bradley, R. J.; Purssell, C. P.; Billson, D. R.; Hutchins, D. A. A Simple, Low-Cost Conductive Composite Material for 3D Printing of Electronic Sensors. *PLOS ONE* **2012**, *7*, e49365, doi:10.1371/journal.pone.0049365.

39- Cruz, M. A.; Ye, S.; Kim, M. J.; Reyes, C.; Yang, F.; Flowers, P. F.; Wiley, B. J. Multigram Synthesis of Cu-Ag Core–Shell Nanowires Enables the Production of a Highly Conductive Polymer Filament for 3D Printing Electronics. *Part. Part. Syst. Charact. 0*, 1700385, doi:10.1002/ppsc.201700385.

40- Lee, J.-Y.; An, J.; Chua, C. K. Fundamentals and applications of 3D printing for novel materials. *Appl. Mater. Today* **2017**, *7*, 120–133, doi:10.1016/j.apmt.2017.02.004.

41- P. Rimington, R.; J. Capel, A.; R. Christie, S. D.; P. Lewis, M. Biocompatible 3D printed polymers via fused deposition modelling direct C 2 C 12 cellular phenotype in vitro. *Lab. Chip* **2017**, *17*, 2982–2993, doi:10.1039/C7LC00577F.

42- Rosenzweig, D. H.; Carelli, E.; Steffen, T.; Jarzem, P.; Haglund, L. 3D-Printed ABS and PLA Scaffolds for Cartilage and Nucleus Pulposus Tissue Regeneration. *Int. J. Mol. Sci.* **2015**, *16*, 15118–15135, doi:10.3390/ijms160715118.

43- Gross, B. C.; Erkal, J. L.; Lockwood, S. Y.; Chen, C.; Spence, D. M. Evaluation of 3D Printing and Its Potential Impact on Biotechnology and the Chemical Sciences. *Anal. Chem.* **2014**, *86*, 3240–3253, doi:10.1021/ac403397r.

44- Bose, S.; Vahabzadeh, S.; Bandyopadhyay, A. Bone tissue engineering using 3D printing. *Mater. Today* **2013**, *16*, 496–504, doi:10.1016/j.mattod.2013.11.017.

45- Gauvin, R.; Chen, Y.-C.; Lee, J. W.; Soman, P.; Zorlutuna, P.; Nichol, J. W.; Bae, H.; Chen, S.; Khademhosseini, A. Microfabrication of complex porous tissue engineering scaffolds using 3D projection stereolithography. *Biomaterials* **2012**, *33*, 3824–3834, doi:10.1016/j.biomaterials.2012.01.048.

46- Zhang, A. P.; Qu, X.; Soman, P.; Hribar, K. C.; Lee, J. W.; Chen, S.; He, S. Rapid Fabrication of Complex 3D Extracellular Microenvironments by Dynamic Optical Projection Stereolithography. *Adv. Mater.* **2012**, *24*, 4266–4270, doi:10.1002/adma.201202024.

47- Macdonald, N. P.; Cabot, J. M.; Smejkal, P.; Guijt, R. M.; Paull, B.; Breadmore, M. C. Comparing Microfluidic Performance of Three-Dimensional (3D) Printing Platforms. *Anal. Chem.* **2017**, *89*, 3858–3866, doi:10.1021/acs.analchem.7b00136.

48- Lin, H.; Zhang, D.; Alexander, P. G.; Yang, G.; Tan, J.; Cheng, A. W.-M.; Tuan, R. S. Application of visible light-based projection stereolithography for live cell-scaffold fabrication with designed architecture. *Biomaterials* **2013**, *34*, 331–339, doi:10.1016/j.biomaterials.2012.09.048.

49- Hofmann, M. 3D Printing Gets a Boost and Opportunities with Polymer Materials. *ACS Macro Lett.* **2014**, *3*, 382–386, doi:10.1021/mz4006556.

50- Kamyshny, A.; Steinke, J.; Magdassi, S. Metal-based Inkjet Inks for Printed Electronics. *Open Appl. Phys. J.* **2011**, *4*.

51- Zocca, A.; Gomes, C. M.; Staude, A.; Bernardo, E.; Günster, J.; Colombo, P. SiOC ceramics with ordered porosity by 3D-printing of a preceramic polymer. *J. Mater. Res.* **2013**, *28*, 2243–2252, doi:10.1557/jmr.2013.129.

52- Bucella, S. G.; Nava, G.; Vishunubhatla, K. C.; Caironi, M. High-resolution direct-writing of metallic electrodes on flexible substrates for high performance organic field effect transistors. *Org. Electron.* **2013**, *14*, 2249–2256, doi:10.1016/j.orgel.2013.05.002.

53- Munshi, A. S.; Martin, R. S. Microchip-based electrochemical detection using a 3-D printed wall-jet electrode device. *Analyst* **2016**, *141*, 862–869, doi:10.1039/C5AN01956G.

54- Anderson, K. B.; Lockwood, S. Y.; Martin, R. S.; Spence, D. M. A 3D Printed Fluidic Device that Enables Integrated Features. *Anal. Chem.* **2013**, *85*, 5622–5626, doi:10.1021/ac4009594.

55- Bhattacharjee, N.; Urrios, A.; Kang, S.; Folch, A. The upcoming 3D-printing revolution in microfluidics. *Lab. Chip* **2016**, *16*, 1720–1742, doi:10.1039/C6LC00163G.

56- Williams, J. M.; Adewunmi, A.; Schek, R. M.; Flanagan, C. L.; Krebsbach, P. H.; Feinberg, S. E.; Hollister, S. J.; Das, S. Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials* **2005**, *26*, 4817–4827, doi:10.1016/j.biomaterials.2004.11.057.

57- Shirazi, S. F. S.; Gharehkhani, S.; Mehrali, M.; Yarmand, H.; Metselaar, H. S. C.; Kadri, N. A.; Osman, N. A. A. A review on powder-based additive manufacturing for tissue engineering: selective laser sintering and inkjet 3D printing. *Sci. Technol. Adv. Mater.* **2015**, *16*, 033502, doi:10.1088/1468-6996/16/3/033502.

58- Kolan, K. C. R.; Leu, M. C.; Hilmas, G. E.; Velez, M. Effect of material, process parameters, and simulated body fluids on mechanical properties of 13-93 bioactive glass porous constructs made by selective laser sintering. *J. Mech. Behav. Biomed. Mater.* **2012**, *13*, 14–24, doi:10.1016/j.jmbbm.2012.04.001.

59- Yeong, W. Y.; Sudarmadji, N.; Yu, H. Y.; Chua, C. K.; Leong, K. F.; Venkatraman, S. S.; Boey, Y. C. F.; Tan, L. P. Porous polycaprolactone scaffold for cardiac tissue engineering fabricated by selective laser sintering. *Acta Biomater.* **2010**, *6*, 2028–2034, doi:10.1016/j.actbio.2009.12.033.

60- Wu, G.-H.; Hsu, S. Review: Polymeric-Based 3D Printing for Tissue Engineering. *J. Med. Biol. Eng.* **2015**, *35*, 285–292, doi:10.1007/s40846-015-0038-3.

61- Olakanmi, E. O.; Cochrane, R. F.; Dalgarno, K. W. A review on selective laser sintering/melting (SLS/SLM) of aluminium alloy powders: Processing, microstructure, and properties. *Prog. Mater. Sci.* **2015**, *74*, 401–477, doi:10.1016/j.pmatsci.2015.03.002.

62- Rafeie, M.; Zhang, J.; Asadnia, M.; Li, W.; Ebrahimi Warkiani, M. Multiplexing slanted spiral microchannels for ultra-fast blood plasma separation. *Lab. Chip* **2016**, *16*, 2791–2802, doi:10.1039/C6LC00713A.

63- Lee, W.; Kwon, D.; Choi, W.; Jung, G. Y.; Au, A. K.; Folch, A.; Jeon, S. 3D-Printed Microfluidic Device for the Detection of Pathogenic Bacteria Using Size-based Separation in Helical Channel with Trapezoid Cross-Section. *Sci. Rep.* **2015**, *5*, 7717, doi:10.1038/srep07717.

64- Yan, S.; Tan, S. H.; Li, Y.; Tang, S.; Teo, A. J. T.; Zhang, J.; Zhao, Q.; Yuan, D.; Sluyter, R.; Nguyen, N. T.; Li, W. A portable, hand-powered microfluidic device for sorting of biological particles. *Microfluid. Nanofluidics* **2018**, *22*, 8, doi:10.1007/s10404-017-2026-0.

65- Park, C.; Lee, J.; Kim, Y.; Kim, J.; Lee, J.; Park, S. 3D-printed microfluidic magnetic preconcentrator for the detection of bacterial pathogen using an ATP luminometer and antibody-conjugated magnetic nanoparticles. *J. Microbiol. Methods* **2017**, *132*, 128–133, doi:10.1016/j.mimet.2016.12.001.

66- Oh, S.; Kim, B.; Lee, J. K.; Choi, S. 3D-printed capillary circuits for rapid, low-cost, portable analysis of blood viscosity. *Sens. Actuators B Chem.* **2018**, *259*, 106–113, doi:10.1016/j.snb.2017.12.025.

67- Santangelo, M. F.; Libertino, S.; Turner, A. P. F.; Filippini, D.; Mak, W. C. Integrating printed microfluidics with silicon photomultipliers for miniaturised and highly sensitive ATP bioluminescence detection. *Biosens. Bioelectron.* **2018**, *99*, 464–470, doi:10.1016/j.bios.2017.07.055.

68- K. Tang, C.; Vaze, A.; F. Rusling, J. Automated 3D-printed unibody immunoarray for chemiluminescence detection of cancer biomarker proteins. *Lab. Chip* **2017**, *17*, 484–489, doi:10.1039/C6LC01238H.

69- Nie, J.; Gao, Q.; Qiu, J.; Sun, M.; Liu, A.; Shao, L.; Fu, J.; Zhao, P.; He, Y. 3D printed Lego ® -like modular microfluidic devices based on capillary driving. *Biofabrication* **2018**, *10*, 035001, doi:10.1088/1758-5090/aaadd3.

70- Suh, Y. K.; Kang, S. A Review on Mixing in Microfluidics. *Micromachines* **2010**, *1*, 82–111, doi:10.3390/mi1030082.

71- Plevniak, K.; Campbell, M.; Myers, T.; Hodges, A.; He, M. 3D printed auto-mixing chip enables rapid smartphone diagnosis of anemia. *Biomicrofluidics* **2016**, *10*, 054113, doi:10.1063/1.4964499.

72- Mattio, E.; Robert-Peillard, F.; Vassalo, L.; Branger, C.; Margaillan, A.; Brach-Papa, C.; Knoery, J.; Boudenne, J.-L.; Coulomb, B. 3D-printed lab-on-valve for fluorescent determination of cadmium and lead in water. *Talanta* **2018**, *183*, 201–208, doi:10.1016/j.talanta.2018.02.051.

73- Li, K.; Wei, H.; Liu, W.; Meng, H.; Zhang, P.; Yan, C. 3D printed stretchable capacitive sensors for highly sensitive tactile and electrochemical sensing. *Nanotechnology* **2018**, *29*, 185501, doi:10.1088/1361-6528/aaafa5.

74- Manzanares Palenzuela, C. L.; Novotný, F.; Krupička, P.; Sofer, Z.; Pumera, M. 3D-Printed Graphene/Polylactic Acid Electrodes Promise High Sensitivity in Electroanalysis. *Anal. Chem.* **2018**, *90*, 5753–5757, doi:10.1021/acs.analchem.8b00083.

75- Shanmugam, A.; Usmani, M.; Mayberry, A.; Perkins, D. L.; Holcomb, D. E. Imaging systems and algorithms to analyze biological samples in real-time using mobile phone microscopy. *PLOS ONE* **2018**, *13*, e0193797, doi:10.1371/journal.pone.0193797.

76- Scordo, G.; Moscone, D.; Palleschi, G.; Arduini, F. A reagent-free paper-based sensor embedded in a 3D printing device for cholinesterase activity measurement in serum. *Sens. Actuators B Chem.* **2018**, *258*, 1015–1021, doi:10.1016/j.snb.2017.11.134.

77- Singh, M.; Tong, Y.; Webster, K.; Cesewski, E.; P. Haring, A.; Laheri, S.; Carswell, B.; J. O'Brien, T.; H. Aardema, C.; S. Senger, R.; L. Robertson, J.; N. Johnson, B. 3D printed conformal microfluidics for isolation and profiling of biomarkers from whole organs. *Lab. Chip* **2017**, *17*, 2561–2571, doi:10.1039/C7LC00468K.

78- Zhou, X.; Zhu, W.; Nowicki, M.; Miao, S.; Cui, H.; Holmes, B.; Glazer, R. I.; Zhang, L. G. 3D Bioprinting a Cell-Laden Bone Matrix for Breast Cancer Metastasis Study. *ACS Appl. Mater. Interfaces* **2016**, *8*, 30017–30026, doi:10.1021/acsami.6b10673.

79- Park, C. S.; Ha, T. H.; Kim, M.; Raja, N.; Yun, H.; Sung, M. J.; Kwon, O. S.; Yoon, H.; Lee, C.-S. Fast and sensitive near-infrared fluorescent probes for ALP detection and 3d printed calcium phosphate scaffold imaging in vivo. *Biosens. Bioelectron.* **2018**, *105*, 151–158, doi:10.1016/j.bios.2018.01.018.

80- Alam, M. N. H. Z.; Hossain, F.; Vale, A.; Kouzani, A. Design and fabrication of a 3D printed miniature pump for integrated microfluidic applications. *Int. J. Precis. Eng. Manuf.* **2017**, *18*, 1287–1296, doi:10.1007/s12541-017-0152-y.

81- 3D Printing: A New Era for Valve Manufacturing? Available online: http://www.valvemagazine.com/web-only/categories/trends-forecasts/7689-3d-printing-a-new-era-for-valve-manufacturing.html (accessed on May 8, 2018).

82- Building Atomic Force Microscope with 3D printing, electronics and LEGO Available online: http://www.robaid.com/tech/building-atomic-force-microscope-with-3d-printing-electronics-and-lego.htm (accessed on May 8, 2018).

83- Meloni, G. N.; Bertotti, M. 3D printing scanning electron microscopy sample holders: A quick and cost effective alternative for custom holder fabrication. *PLOS ONE* **2017**, *12*, e0182000, doi:10.1371/journal.pone.0182000.