



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Applications of paper as a support material in biomedical sciences: A decadal review



Bimalendu Deka^a, Rima Kalita^a, Dinesh Bhatia^{a,*}, Animesh Mishra^b

^a Department of Biomedical Engineering, North Eastern Hill University, Shillong, 793022, Meghalaya, India

^b Department of Cardiology, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Shillong, 793018, Meghalaya, India

ARTICLE INFO

Keywords:

μPAD
Paper
Diagnosis
Paper-based-culture
Scaffold

ABSTRACT

In human history, the paper has long been used as a platform to record and preserve information. However, over the decades, paper has found its application in Biomedical Sciences, too. Both paper-based microfluidic devices (μPADs) and paper-based cultures and scaffolds have shown immense potential to be used as a sensor as well as a supporting material for in vitro tissue engineering. μPADs can be used to perform low-cost and fast biomolecular assays at Point-Of-Care (POC). They are being used to detect various biomarkers like viral proteins, metabolites, oncogenes, and antigens; and conditions like Venous Thromboembolism (VTE). On the other hand, the paper has also been used to develop paper-based 3D cultures and scaffolds to test drugs, and monitor cytotoxic effects in vitro cell microenvironments and also as implantable tissues. In this review, we intend to enumerate the development in the field of μPADs, paper-based cell cultures, and paper-based scaffolds and their plethora of applications over the last decade.

1. Introduction

Historically, paper has been used as a tool to record and preserve information [1], but over time it has found its application in the field of Biomedical Sciences [2]. Paper being a cellulose-based substrate, has a long history to perform biomolecular assays. Due to its affordability, wide availability, and its hydrophilic nature, it is considered to be one of the most promising materials for the fabrication of paper-based biosensors [3]. The first paper-based device for the semi-quantitative detection of glucose in urine was demonstrated in 1956 that further developed into immunochromatographic paper-based lateral flow test strips, with the well-known example of pregnancy test kits [4]. Later on, George Whitesides introduced the method of fabricating microchannels on paper for multiplex analyte detection based on colorimetry; thus giving rise to microfluidic paper-based analytical devices or simply, μPAD [5]. A μPAD can be defined as a paper-based analytical device used to perform biomolecular assays, consisting of a sample-loading zone and

a testing zone with varying architecture of channels concerning its application. Thin, light and flexible nature of cellulose paper makes it an excellent raw material to fabricate μPAD with applications ranging from blood-plasma separation to detecting viral, cardiac and oncological biomarkers [6,7]. These low-cost devices can be used in developing economies in Asia and Africa, where deploying sophisticated detection devices like spectrophotometers [85] are not economically viable. Moreover, the population in these economies is higher; thus increasing the chances of the prevalence of epidemics like Ebola and Severe Acute Respiratory Syndrome (SARS) in Africa and China, respectively. Hence, the development of low-cost paper-based analytical devices can help in quarantining those infected by such viruses as well as facilitate early detection of diseases and conditions like Hepatitis-C virus (HCV) infection and Venous Thromboembolism (VTE).

However, μPADs on their own are not efficient enough to perform the biomolecular assay, hence their surfaces are modified to increase the detection of an analyte as well as amplify the signal generated. Surface

* Corresponding author.

E-mail addresses: bimaldbdeka@gmail.com (B. Deka), kalitarima36@gmail.com (R. Kalita), bhatiadinesh@rediffmail.com (D. Bhatia), animesh.shillong@gmail.com (A. Mishra).



Production and hosting by Elsevier

<https://doi.org/10.1016/j.sintl.2020.100004>

Received 8 January 2020; Received in revised form 15 March 2020; Accepted 16 March 2020

Available online 9 April 2020

2666-3511/© 2020 The Authors. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-

NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

modification is done using nanomaterial [75,76] like carbon nanotubes (CNT), graphene nanosheets, metal nanoparticles, metal oxide nanoparticles, and their nano-conjugates, which are synthesized using a plethora of methods [8]. For example, arc discharge, laser ablation and chemical vapour deposition in case of CNT or, chemical reduction in case of Graphene Nanosheets and metal nanoparticles [9–11].

On the other hand, paper is also revolutionizing the field of Tissue engineering, viz. paper-based cell cultures and scaffolds [77]. For almost a century, cell cultures were being developed on surfaces of Petri dishes and glass plates in the form of 2D monolayers and tests were performed on them. However, they had major limitations as cell microenvironments were 3D. Hence scientists started to engineer 3D-substrates to make cell cultures. Eventually, they succeeded in doing so using artificial polymers; but the polymers were difficult to fabricate and were mostly non-biodegradable. Thus, the focus shifted to paper-based materials and the substrates developed so far, showed promising potential [12,13]. Similarly, synthetic polymers were used to make scaffolds. Fabrication of these scaffolds was neither convenient nor feasible, but now research is ongoing to replace it with paper-based-scaffolds [14], which are low-cost and can mimic the biological Extra-Cellular-Matrix (ECM). The paper-based scaffolds can provide a valuable platform for basic stem cell research to study material effects and also to perform drug test [14–16]. Generally, for tissue engineering fibrous nature, porosity and flexibility of scaffolds are desired and possessing all these characteristics paper [78] has the potential to replace conventional scaffolding materials like polydimethylsiloxane (PDMS), glass fiber, chitosan, which are difficult to fabricate and are not feasible for low-income economies. For the proper proliferation of cells [83,84] in vitro, a microenvironment that mimics the in vivo characteristics of the body is necessary [17]. However, the challenge with paper is that it is 2D in structure, hence researchers stacked the cell-laden papers on top of one another to make the 3D microenvironments. Using this technique, researchers developed "a beating heart on paper", which was beating for about 3 months; and in another instance, researchers developed a 3D liver model on paper scaffold which showed similar response, like in a real liver in vivo [15,16]. Similar to μ PADs, paper substrate used in the fabrication of cultures and scaffolds were surface modified to enhance cellular growth.

This review intends to enumerate the development in the field of μ PADs, paper-based cell cultures, and paper-based scaffolds over the last decade. We have explained their structure, composition, surface modification, and functionality. Hopefully, this review will encourage more research in these fields and the development of more commercially viable paper-based devices. Also, may this review be helpful to the newcomers in this field and may demonstrate the importance of such low-cost paper-based analytical devices and scaffolds.

2. Applications of paper as supporting material in Biomedical Sciences

2.1. Biomarker-based disease diagnosis using microfluidics

Biomarkers are indicators of the body's functioning, and their levels in the blood can indicate the occurrence of certain diseases [18,19]. To estimate the levels of biomarkers in the body, blood plasma is separated from whole blood using techniques such as centrifugation. This procedure is both tedious and requires the help of technicians. Hence, μ PADs like those developed by Yang et al can be used to separate blood plasma from whole blood [6]. Before this, researchers devised numerous techniques like electro-osmotic flow, bifurcation (Zweifach-Fung effect), membrane filtration and cross-flow filtration for blood plasma separation [20]. However, these methods come with disadvantages like the requirement of external force for the flow of substances through the microfluidic channel but paper can be used to make microfluidic systems that can work without the requirement of such forces. The μ PAD developed by Yang et al used the principle of RBC agglutination induced by anti-bodies. The plasma separation zone in the center was made

functionalized by spotting anti-A, B onto it, and the reagents of the colorimetric assay onto the test readout zones in the periphery. To perform the assay, a drop of whole blood was placed at the plasma separation zone, which leads to agglutination of RBC and separation of plasma. The plasma obtained using this process was significantly high to perform the colorimetric assay. Similarly, a year later, Songjaroen et al developed a different method of separating blood plasma from whole blood [20]. They fabricated a μ PAD consisting of two types of Paper, i.e. blood filter paper and patterned Whatman No.1 filter paper to separate blood plasma from other components by agglutination of the components and penetration of plasma through the paper by capillary action. By wax dipping method, various layers were incorporated onto the paper for separation of particles greater than 2–3 mm including red cells and platelets from whole blood. Once the plasma is separated from the other components of blood, biomarkers of interest can be analyzed using various biomolecular reactions. In the following paragraphs, we have divided the detection of biomarkers into blood-based and non-blood-based; i.e. biomarkers present in blood and those present in other body fluids like saliva, tears, and urine.

2.1.1. Blood-based detection

In this method, biomarkers present in the blood are analyzed using μ PAD. For example, in 2016, Hu et al. developed a μ PAD with combined blood serum [73,74] extractor with dual-mode iron health tests: fluorescent analysis for Fe³⁺ and colorimetric ELISA for ferritin, which is a blood protein containing iron [21]. The μ PAD was fabricated by sequentially patterning the filter paper with in situ growth of Carbon-Dots (CDs) and Au-Nano-Particles. For CDs rendered different reaction rates for paper and consequently led to two distinguished regions on paper simultaneously: the fluorescent CDs (F-CDs), for serum iron analysis and the non-fluorescent CDs (nF-CDs) for chemical deposition of Au. The high triggering ability for blood coagulation was shown by the surface, which enabled automatic and highly effective serum extraction on the μ PAD. Sensing of serum ferritin was enabled by the bio-modification of AuNPs. Subsequently, μ PADs were developed to detect viral, cardiac and oncological biomarkers in blood.

2.1.1.1. Viral biomarkers. According to a report published by Economic Times India, 80% of the population in some villages in the North-Western region of India, especially Punjab, Haryana, and Uttar Pradesh, is suffering from Hepatitis C [22]. Hence early diagnosis is necessary to cure this disease. Generally, the Hepatitis-C virus (HCV) [86] is diagnosed in a two-step process; first, detection of IgG against anti-HCV using enzyme-linked immunosorbent assay (ELISA) and second, recombinant immunoblot assay (RIBA) to confirm the diagnostic result, as ELISA might give a false-positive result. However, in 2014, Mu et al developed a multiplex microfluidic paper-based immunoassay to detect the human IgG antibody against HCV (anti-HCV) [23]. They used a technique called Craft Punch Patterning (CPP) to fabricate the multiple test zones on Nitro-Cellulose paper substrate; as NC is highly flammable. Hence, the developed microfluidic paper-based immunoassay harnessed the 'irreplaceable' merits of paper to integrate the lengthy, costly, and segmented assays rapidly and economically. Then, in 2016, Chen and Xinyu developed a much advance μ PAD to detect HCV and Human Immunodeficiency Virus (HIV) together in under 20 min with high accuracy and sensitivity as well as low Limit-Of-Detection (LOD) to existing sensors [24]. They claim to be the first to integrate μ PAD for low-cost diagnosis of HIV/HCV co-infection. It had eight electrochemical immunosensors capable of carrying out "ELISAs in parallel and testing multiple serum samples for both HIV and HCV antibody marker". Moreover, it could transmit the data to a host computer or smartphone via wireless transmission. In another instance, Bedin et al. to develop a wax printed MF1-based- μ PAD for the detection of the dengue and Zika NS1 (Non-Structural) viral proteins in blood plasma. The presence of glass fiber enables the separation of plasma from red blood cells [25]. The test

zone of the μ PAD was functionalized by adding an anti-NS1 antibody. If NS1 is present, it is captured by the anti-NS1 antibody and a blue band appears in the test. If NS1 is absent, there is no signal. The μ PAD take 4–6 min to analyze the sample and a smartphone application is used to interpret the results. The μ PAD performed better than a commercially available Lateral-Flow-Assay (LFA), both in terms of Limit-Of-Detection (LOD) and of time-to-result.

2.1.1.2. Cardiac biomarkers. According to another article published by Economic Times India, Venous Thromboembolism (VTE) is becoming a leading cause of death along with heart disease, Brain stroke and cancer [26]. It is an under-diagnosed disease among many and most common preventable cause of death in India. VTE leads to long term complications like post-thrombotic syndrome (PTS) and chronic thromboembolic pulmonary hypertension (CTEPH). PTS can cause swelling and pain in the affected leg and CTEPH can cause the right side of the heart to work harder than normal owing to abnormal high-blood pressure. In both the condition, early diagnosis is necessary. It can be achieved by measuring the levels of thrombin or thrombin content in blood serum. The available methodologies to measure thrombin levels require both time and technicians. Recently, Xue et al constructed a Microfluidic paper-based photo-electrochemical device (μ PECD) to detect thrombin at point-of-care (POC) [27]. μ PECD employs the complete separation of the detection signal and excitation source, thus, providing high sensitivity with a low background signal. The high sensitivity in μ PECD can be achieved by the production of large photocurrent signals; which was achieved by incorporating Titanium Dioxide in the paper substrate, due to its good biocompatibility, photoelectric activity, and chemical stability. Again to gain more sensitivity, aptamers were used, instead of antibodies, due to their stability and reusability. To check its real-world feasibility, four human serum samples were tested with the μ PECD and ELISA. The results had no significant differences, thus demonstrating the excellent potential practical application of μ PECD. However, a year before, Lim et al developed a μ PAD for simultaneous detection of multiple cardiac biomarkers, viz. glycogen phosphorylase isoenzyme BB (GPBB) for early diagnosis; and troponin I or T (cTnI or cTnT), creatine kinase-MB (CK-MB) for late diagnosis of Acute Myocardial Infarction (AMI) which is an indicator of Acute Coronary Syndrome (ACS) [28]. Early detection of AMI upon onset of heart attack-like situations is crucial for patient survival. It is generally done by observing elevation of ST-segment in Electro-Cardiogram (ECG), which accounts for 23–40% of misdiagnosed myocardial infarction cases. The developed μ PAD could give accurate, reliable and quantitative results.

2.1.1.3. Oncological Biomarker. In the current medical scenario, cancer can be diagnosed using either of the three methods: Medical Imaging, or Endoscopy or Biopsy and Cytology Tests [29]. For early detection of cancer cells, medical imaging is used which is a non-invasive method involving the use of X-Rays or radionuclides for imaging specific parts of the human body. Endoscopy is used as a diagnostic method for Gastro-Intestinal Oncology related complications. It is used to detect lesions, facilitate macroscopic classification of tumors and enable biopsy [30]. In a biopsy, a tissue sample is taken out from the lump of cells, which might have been found by imaging tests or physical examination [31] and different cytological tests are done to examine the growth of cancerous cells.

However, all the above-mentioned methods are tedious, time-consuming and need expert assistance. Moreover, the methods are not efficient enough to detect cancer cells at an early stage. In the case of imaging tests, it can find large groups of cancer cells but not single cancer cells or even a small cluster of cells [32]. Also, endoscopy, even though being a safe method to visualize the colonic mucosa, however, the “sensitivity of the capsule endoscopy for detecting colonic polyps, advanced adenomas, and colorectal cancer was relatively low” [33]. μ PADs being a low-cost, non-invasive device can be a possible solution of

detecting cancer cells at an early stage. In 2014, Li et al. proposed a novel idea to detect oncogenes on target DNA on a μ PAD in vitro [34]. They developed a porous Au-paper working electrode (Au-PWE) which combined the porosity of paper and high conductivity of Au-nano-particles. The DNA sensor showed tremendous specificity and also had a Limit-Of-Detection (LOD) as low as 8.5×10^{-18} M.

Among the many antigens [79] found in blood, Carcinoembryonic antigen (CEA) can be used as oncological biomarkers which is one the most widely used tumor marker in the clinical diagnosis of colorectal, gastric, pancreatic and cervical carcinomas [35]. CEA levels directly correspond to the stage of the tumor, the outcome of therapy and the prognosis. In 2016, Wang et al developed a highly sensitive label-free paper-based electrochemical immunosensor employing a screen-printed working electrode (SPWE) which was modified with graphene nano-composite for detection of CEA [35]. It demonstrated high efficiency and high sensitivity to the samples it was tested against. Hence, it could provide a low-cost, sensitive Point-Of-Care diagnosis. However, in 2017, Fan et al demonstrated the detection of Neuron-specific Enolase (NSE), a tumor marker essential for monitoring effect and judging prognosis and diagnosis of small cell lung cancer using a wireless POCT (Point-Of-Care-and-Testing) device comprising of a μ PAD-based measuring system [36]. The detection results of NSE were comparable to those measured by a commercial electrochemical workstation. The data generated were hence, transmitted over to the smartphone using Bluetooth and displayed in real-time. Again in 2018, Draz et al. developed a hybrid Paper-Plastic Microchip (PPMC) for flexible and High-Performance Point-Of-Care (POC) Diagnostic device, which was tested for the detection of alpha-fetoprotein (AFP) and CEA as biomarkers of hepatocellular carcinoma (HCC) and colorectal cancer (CRC), respectively [37]. The microchip comprised of three-layer hybrid substrates made of cellulose paper substrate assembled with a transparent plastic sheet using double-sided adhesive. The fabrication process of the microchip took <1h and leveraged the advantages of layer-by-layer assembly. The PPMC had electrodes printed within the cellulose paper, which had better signal resolution compared to the common protocol of screen-printing electrodes on the surface of the chip. The results showed a good correlation between the expected and measured values with a maximum error of <7% (see Fig. 1).

2.1.2. Non-blood-based detection

In non-blood-based detection, biomarkers present in body fluids like, saliva, urine, or tear are used. The advantage that the methods using non-blood-based biomarkers are the fact that there’s no need for any purification methods, unlike blood-based detection where blood plasma needs to be separated from whole blood. Thus, rendering it better than blood-based methods. Nitrates are compounds naturally occurring in the human body and some foods like green leafy vegetables [38]. In the human body, nitrate after absorption in the upper gastrointestinal tract, it reaches the salivary gland via blood circulation and is secreted in the oral cavity where the microflora partially reduces it to nitrite [39]. In 2014, Bhakta et al developed a μ PAD for detection of nitrite in saliva, which used Griess reagent for detection, which could indicate chances of developing periodontitis with clinical symptoms like gingival redness, swelling, and bleeding of gums, as it is associated with the concentration of nitrite (NO_2^-) which is an antimicrobial agent produced from nitric oxide released by endothelial NO-synthases in saliva [40]. The selected prototype for the μ PADs had a main channel with four identical arms and four round “Testing zones”, designed using black lines and shapes on a white background. The dimensions of the finished μ PADs (Fig. 2) were 24 mm by 24 mm, with a 2 mm width for the main channel and a 3 mm diameter for the testing zones. The sample was up taken on the μ PAD through capillary action and the solution was driven into the branched channels and the testing zone of the μ PAD. In an acidic medium and the presence of nitrite, the two components of the Griess reagent led to the formation of a magenta azo compound, in the presence of nitrite. Later, to maximize the color development, the acetic acid of the Griess reagent

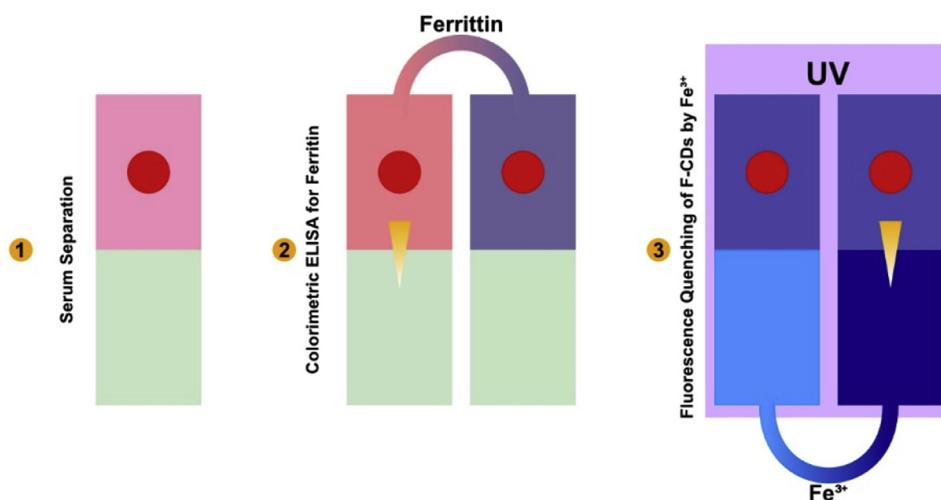


Fig. 1. μ PAD with combined blood serum extractor with dual-mode iron health tests.

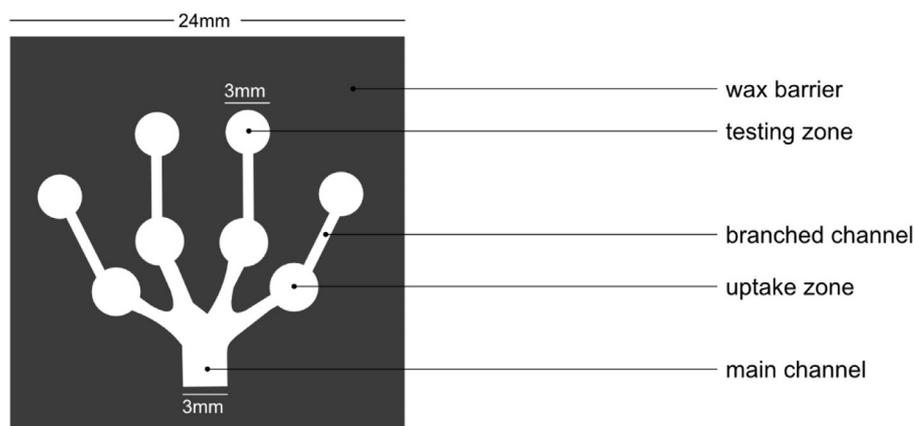


Fig. 2. μ PAD for the detection of Nitrite in saliva.

was replaced by 5% H_3PO_4 . A quick, easy, and inexpensive analytical method of quantifying nitrite levels in human saliva was offered by the optimized configuration. According to the researchers, this methodology could aid research leading to establish a definitive relationship between salivary nitrite levels and periodontitis.

Nitrite (NO_3^-) is one of the metabolic wastes excreted out with urine [41]. The detection of Nitrite (NO_2^-) in urine analysis indicates some Urinary Tract Infection (UTI). It is due to the conversion of nitrate to nitrite by gram-negative bacteria species like *Escherichia coli*, that Nitrite is detected in urine analysis [42]. Using this concept, Chen and Dong developed a peel-able μ PAD attached to diaper for elderly patients, in which the urine sample is collected and analyzed [24]. The μ PAD was made of filter paper with patterned wax with varying concentrations of nitrite in eight segregated chemical reagent zones. A color change from white to red-violet can be interpreted as a positive result. The intensity determines the concentration of nitrite in the sample.

In urine, other compounds such as Creatinine (CRN) and uric acid are also released by the body. Creatinine is spontaneously produced in the metabolism of muscle contraction from creatine and phosphocreatine and is excreted by the kidneys. It is an important clinical marker for renal function since the rate of excretion of creatinine is relatively constant. Similar to this design developed by Bhakta et al, Edwardo et al, constructed a μ PAD and developed two optimized methods for the simultaneous quantification of two biomarkers in urine, namely creatinine and uric acid, using Jaffe's method and a Phenanthroline method respectively [43]. The proposed analytical device can be used to evaluate renal

function and help prevent kidney failure. The analytical device used was 19×19 mm, with 4 mm width for the main channel, 2 mm width for the arms, and 4 mm diameters for the testing and uptake zones. Using a wax printer, the design was printed on a Whatman No. 1 filter paper with wax toner following the description given by Carrilho et al [44].

Then, in 2016, Gabriel et al. showed that tears can also be used for glucose and uric acid estimation. They used Sensitive colorimetric detection for chitosan incorporated paper surface resulting in better solid support to adsorb enzymes ensuring a more effective reactive area in the whole detection zone [45]. These devices were used for the quantitative analysis of glucose and uric acid which yielded an accuracy at the range of 87 to 114%. The remarkable improvements in analytical sensitivity allowed the colorimetric detection of glucose in human tear samples. No statistical difference was found at a confidence level of 95%, in the performance of chitosan-modified μ PADs for the detection of glucose when compared to the analysis carried out by the spectrophotometry technique, thus proving the analytical reliability for quantitative analysis. In another instance, Jia et al developed a μ PAD for direct detection of glucose from saliva (artificial) using a smartphone-based colorimetric assay, having a LOD of 0.02 mM [46]. They modified the cellulose fiber of the paper with Graphene-Oxide prohibiting any possible enzyme cross-examination, and also enhanced reagent absorptivity, reactive efficiency, and homogeneity of color distribution. They claimed it to be universally applicable as the modification of the μ PAD with Graphene-Oxide can be achieved without the use of any linker, binder or retention aid. Thus, the developed device was disposable and could

perform rapid and reliable analysis of samples, when compared with other paper-based sensors, owing to automatic smartphone image analysis. Table 1 summarizes the above applications in chronological order.

2.2. Tissue engineering applications

Cells are best cultured in microenvironments *in vitro*, which can mimic the original environment where the cells grow. Previously, cell cultures were done on 2D substrates monolayers of cells on a glass plate [47,48] but had limitations as cell microenvironments were 3-Dimensional (3D). 3D microenvironments can bridge the gap between animal models and monolayer cell cultures [49] Hence, to develop 3D microenvironments with functional phenotype were developed using micro-engineering techniques like photolithography, replica modeling and micro-contact printing [50–52]. The material used in these techniques was synthetic polymers which were limited and required complicated fabrication processes and expensive instruments [52]. To prepare cell culture scaffolds, smooth polymers are used, but to develop such scaffolds with topographical features, time-consuming methods such as hot embossing and soft lithography had to be used. One such example of the 3D microenvironment is spheroids. Spheroids are hydrogel scaffolds that are widely used to assess therapeutic efficacies and toxicities. These microenvironments mimicked the poorly vascularized tumor tissue due to the presence of chemical gradients. These chemical gradients formed zones *viz.*, necrotic cells at the core, quiescent cells in the interior and proliferating cells along the exterior [49]. However, spheroids had certain limitations: Firstly, histological sections had to be made to study these spheroids; Secondly, they offered limited experimental control over the formed chemical gradients; and lastly, only a small subset of cell lines are capable of forming aggregates [80,81,82]. Hence, paper-based-cultures (PBCs) emerged as a promising alternative to polymer-based-cultures. Fig. 3 shows a pictorial representation of the different zones of cells in tumors, spheroids, and Cells-in-Gels-in-Paper (see Fig. 4).

2.2.1. Paper-based cultures

Paper is a flexible, porous, low-cost, biocompatible biomaterial offered as a good choice for making scaffolds [14]. Due to the flexible nature of paper [53], rapid and profitable methods like roll to roll fabrication methods, like printing can be used for applying chemicals (i.e. pharmaceutical ingredients or Toxic chemicals) in a controlled way and on a large scale. Paper is composed of a cellulose network that acts like the Extra-Cellular-Matrix (ECM). Paper is a 2D substrate, hence they are stacked on top of one another to create a 3D cell microenvironment. In 2009, Derda et al developed a technique of “stacking and de-stacking layers of paper impregnated with suspensions of cells in extracellular matrix hydrogel making it possible to control oxygen and nutrient gradients in 3D and to analyze molecular and genetic responses” called ‘Cells-in-Gels-in-Paper’ or CiGiP [54]. Using the same technique, in 2011, a group demonstrated that rapid generation and analysis of 3D cultures is achievable by stacking sheets of paper that contain 96 cell-containing zones [55]. Before this technique, the majority of 3D cultures based on high throughput assays which were only able to detect the average behavior of cells present in the culture. However, using CiGiP, it was easier to culture arrays of thin planar sections of tissues, either alone or stacked to create complex tissue models and thus, it was simpler to isolate cells from a specific region of the culture and analyze their behavior. This opened new possibilities where paper can be used as a substrate-material to mimic cell microenvironments. For instance, in 2014 Frederique et al developed a similar 96-well cell culture using CiGiP to examine the cytotoxic effects of apoptosis inducer, Phenyl-Arsine-Oxide (PAO) and alkylating agent, Cyclo-Phospho-Amide (CPA) on human breast cancer cells [56].

Again in 2016, Simon et al developed a CiGiP 3D culture system to evaluate the metabolic response of lung cancer cells to ionizing radiation within gradients of oxygen and other nutrients [12]. These cell-laden

scaffolds composed of the paper-plastic composite were placed in an acrylic holder that enabled the exchange of fresh medium with the stack and generate a decreasing gradient of oxygen. This arrangement mimicked the environment of a poorly-vascularized environment of tissues like that of cancerous tumors: top stack was well-oxygenated whereas the bottom stacks were experiencing hypoxic conditions and an increase in expression of HIF-1 α and CAIX. A similar model was used to study the chemoresistance of colorectal carcinoma cells [49]. Herein, stainless steel holders were used instead of an acrylic frame which ensured the scaffolds were in conformal contact throughout the experiment. In 2018, Wang and his group developed a multi-layer CiGiP to analyze tumor cell migration in 3D cultures [16]. To monitor the progression of malignant tumors, the motility of the cells is measured. The developed multi-layered 3D culture was seeded with prostate cancer cell in the middle layer and their heterogeneous motility to the top and bottom layers was measured. Fig 4 shows a timeline of the development of CiGiP-based paper cultures. Research Labs, across the globe, use Polystyrene (PS) Dishes to prepare 2D cell cultures [57].

However, in 2013, Juvonen, et al. developed a 2-dimensional printed paper-based array, which was successfully used for 2-D cell cultures and performed better than PS-dishes. Hydrophobic polydimethylsiloxane (PDMS) ink was used to print patterned structures on paper substrates for the preparation of arrays used for two-dimensional cell culture and a lipophilic staining agent was preferred for cell imaging [53]. They considered four different types of coated paper substrates with characteristic roughness and surface energy for analyzing the substrate surface factors that influence the 2-D cell culture. The main components in the top coating layer of the paper Substrates were: (P-1) precipitated calcium carbonate pigments and Latex binder; (P-2) a mixture of two different kaolin pigments and Latex binder; and (P-3) kaolin pigments and a latex binder. The sample P-4 was coated with an aqueous latex blend consisting of a 3:2 wt ratio of low-Tg film-forming (“soft”) styrene-butadiene and high-Tg non-film-forming (“hard”) polystyrene. It was found that the PDMS film had the lowest surface energy whereas the Polystyrene (PS) dish had the highest surface energy. Thus, paper proved to be a better alternative to prepare 2D cell cultures, too.

2.2.2. Organ-on-chip

Other than using paper to make paper-based-cultures to monitor chemoresistance of cells or metabolic responses; it has also been used to make 3D cell cultures that not only mimic the tissue environment but also develop working implantable tissues. Moreover, they showed similar activity and response to drugs and other chemicals. For instance, in 2014, Park et al. demonstrated the feasibility of paper materials for stem cell culture, differentiation, and implantation to repair tissue defects [14]. They developed a paper scaffold made of Weighing Paper (WP) which significantly enhanced osteogenic differentiation and *in vivo* bone [71, 72] regeneration of human-Adipose-Derived-Stem-Cells (hADSCs). In their research, Papers laden with osteogenically differentiated hADSCs and human endothelial cells, when stacked together led to vascularized bone formation *in vivo*. Fabricating such a paper-based scaffold was met with challenges as papers can be easily damaged by heat or organic chemicals when applied to modify the surface. Hence, they used a method called initiated-Chemical-Vapour-Deposition (iCVD), in which the conformal coating of functional polymer films can be applied over the paper substrate without any damage. Similarly, in 2015, Petersen et al. investigated the applicability of single layer paper-based scaffolds for the three-dimensional (3D) growth and osteogenic differentiation of equine adipose-derived stem cells (EADSC) and achieved a viable culture EADSC [58]. Likewise, Kim et al developed a paper scaffold, whose surface was conformally modified with poly(styrene-co-maleic anhydride) layer supported the prolonged culture of chondrocytes. It was versatile and allowed area-selective cell seeding. When transplanted properly into a three-ring defect trachea in rabbits [87,88], it replaced the native trachea without stenosis after 4 weeks and provided a tight seal without the need for sutures [59]. In the same year, Li Wang and his group claim to have

Table 1
Development of μ PAD to detect different biomarkers (Blood-based, and Non-Blood-based) over the last decade.

DEVICE DESCRIPTION/ARCHITECTURE	SENSING MECHANISM	TARGET MOLECULE	ANALYTICAL PERFORMANCE	REAL SAMPLE	REFERENCE
Plasma separation μ PAD <ul style="list-style-type: none"> • With the plasma separation zone in the center and four testing zones on the periphery • Hydrophobic barrier was printed using a wax printer onto a chromatography paper • The patterned paper was heated on a hot plate to melt the wax and to create the hydrophobic barrier spanning the entire thickness of the paper substrate. 	Colorimetric Assay. Colour change of potassium iodide starch (from colorless to brown) in the presence of hydrogen peroxide was produced by oxidation of glucose by glucose oxidase.	Plasma glucose	NR	Venous blood	[6]
Plasma Separation μ PAD <ul style="list-style-type: none"> • With a bottom separation zone and two top detection zones • Connected through a y-junction. • Patterned using wax dipping methods. 	Plasma separation simultaneously with a colorimetric assay. Protein determination based on Bromocresol Green (BCG) method was performed.	Plasma Protein	LR 1.6–5.3 g dL ⁻¹	Blood	([20]
Nitrite detecting μ PAD (in saliva) <ul style="list-style-type: none"> • With the main channel with four identical arms and four testing zones • Designed using black lines and shapes on a white background • Printed with a wax toner onto grade 1 thin chromatography paper using a laser printer. 	Colorimetric Assay. Griess reagent was used, which lead to the formation of a magenta-azo-compound whose color intensity relates to the concentration of nitrite	Nitrite	LR 10–1000 μ mol/L LOD 10 μ mol/L	Saliva	[40]
DNA detecting μ PAD <ul style="list-style-type: none"> • A PDDA-GR (Poly-(Diallyldimethylaluminium-chloride)-functionalized Graphene) modified Au paper working electrode (PWE). • An Au paper working electrode modified by PDDA-GR and hybridized by incubation with a varying concentration of target DNA and then with reporter probes (S₃-CaCo₃/cma@ AgNPs bioconjugates). 	Electro-chemiluminescence. Signals related to the target DNA (s2) concentrations were measured from the Paper Working Electrode (PWE).	DNA	LR 4.0 $\times 10^{-17}$ – 5.0 $\times 10^{-17}$ M LOD 8.5 $\times 10^{-18}$ M	Human Serum	[34]
A multiplex microfluidic (nitro-cellulose) paper-based immunoassay <ul style="list-style-type: none"> • With eight channels and a central zone. • Patterned by Craft Punch Patterning (CPP) in a die-cast mechanism under ambient temperature. 	Antigen-Antibody reaction. Based on the quantitative detection of mouse IgG, qualitative detection of anti HCV by using an equal mixture of individual recombinant HCV antigens, including Core, NS4, and NS5 (except NS3) was demonstrated.	Anti-HCV (Core, NS5, AND NS4)	LOQ ~40 pg LOD ~1 pg	Human Serum	[23]
UTI biomarker detecting μ PAD <ul style="list-style-type: none"> • With eight distribution channels and eight square zones with a dimension of 5 mm at the ends of the channel with a width of 3 mm. • Printed on a PMMA plate • Patterned using CO₂ laser engraving method 	Colorimetric Assay. Sample reacted with 50 mm sulfanilamide, 10 mm n-(1-naphthyl)-ethylenediamine 330 mM citric acid in methanol to produce a red-violet color	Urinary Tract Infection biomarkers. (NITRITE)	NR	Urine	[24]
A handheld, eight-channel potentiostat <ul style="list-style-type: none"> • With an E-μPIA in its electrical connection slot. • The eight reaction zones were patterned on a piece of cellulose chromatography paper via wax printing • Electrodes were formed on the reaction zones via stencil printing 	Indirect ELISA. Indirect ELISA of antibodies to HIV p24 and HCV core antigens were realized on an E- μ PIA handheld potentiostat with a Bluetooth module that was developed for readout of output signals from the device.	HIV AND HCV antibodies	LOD 300 pg/mL, for HIV 750 pg/mL, for HCV	Mouse Serum	[24]
μ PAD for sensitive electrochemical detection of carcinoembryonic antigen	Electrochemical Sensing	Carcinogenic agents	LR 50–500 pg/mL LOD 10 pg/mL	Human Serum	[6]
Glucose detecting μ PAD <ul style="list-style-type: none"> • With eight zones interconnected by microfluidic channels with a central zone for the sample inlet • Fabricated by a stamping process. 	Colorimetric Assay. Performed with the help of a selected chromogenic agent (4-AAP/DHBS, KI and TMB FOR glucose detection and 4-AAP/DHBS and OD for uric acid) with an office scanner using 600 dpi resolution. The recorded images were converted to the RGB scale and analyzed.	Glucose and Uric Acid	LOD: 23 μ M, for Glucose 37 μ M, for Uric Acid	Artificial Serum Sample Human Tear Sample	[45]
Ketamine detecting E- μ PAD <ul style="list-style-type: none"> • Integrated with Nano-crystal Zeo-GO • The working and counter electrode was ornated on the paper using commercial conducting carbon ink • Hydrophilic channels with hydrophobic barrier were printed using wax printing followed by heating 	Electrochemical Sensing. Electro-oxidation of drugs amplified by the use of Nano-crystalline Zeo-GO (Zeolite-graphene oxide)	Ketamine (Anaesthetic)	LR 0.001–5 nM/mL DL 0.001nM/mL	Ketamine samples	[64]
Blood Iron analyzing μ PAD	For Fe ³⁺ : 5 ml of ascorbic acid was added to 1 ml	Fe ³⁺ and Ferritin	NR	Blood	[21]

(continued on next page)

Table 1 (continued)

DEVICE DESCRIPTION/ARCHITECTURE	SENSING MECHANISM	TARGET MOLECULE	ANALYTICAL PERFORMANCE	REAL SAMPLE	REFERENCE
<ul style="list-style-type: none"> Made by masking filter paper by PDMS which bonded by plasma bonding Followed by hydrothermal deposition of Carbon Dots (CD) followed by Au-NP plating. Two regions were formed –one with Au-NPs on NF-CDs and the other with F-CDs 	<p>OF human blood sample mixed with 2 mL of red blood cell lysate buffer and incubated at room temperature for 5mins. A fluorescence quenching test was done on the supernatant of this mixture.</p> <p>For Ferritin: Colorimetric ELISA For Ferritin Analysis</p>				
<p>The μ-PAD had a pattern</p> <ul style="list-style-type: none"> Constituted of a channel with a larger area on top for loading of sample and four small areas all separated by lines of yellow wax. The pattern was wax printed. 	<p>On AuNPS/NF-CDs region was done. Antigen-Antibody Reaction. P122 and P82 polyclonal antibodies were used to detect specific antigens.</p>	Dengue and Zika NS1 Detection	LOD (whole blood) 20 ng/mL, for Dengue and ZIKV	Whole Blood	[25]
<p>NSE detecting μPAD</p> <ul style="list-style-type: none"> With two circular zones –one sample zone with a diameter of 3 mm and the other one is for an auxiliary zone with a diameter of 3.5 mm adopting three electrodes system (working, reference and counter electrodes) Printed by wax printing and screen printing techniques. 	Electrochemical Sensing	Neuron Specific Enolase	LR 1–500 ng/mL LOD 10 pg/mL	Serum	[36]
<p>Creatinine detecting μpad</p> <ul style="list-style-type: none"> Consisting of the main channel and three identical arms each containing a circular testing zone Circular uptake zone printed using a wax printer 	<p>FOR CREATININE: 2 μL aliquot of a solution of 0.1 mol/l picric acid in 1 mol/l NaOH was spotted onto the uptake zone.</p> <p>For Uric Acid 2 μL aliquot of a solution containing 1,10-phenanthroline(0.06 mol/l)and acetate (0.53 mol/l)was added to the uptake zone and a 2 μl aliquot of a solution of Fe^{3+}(0.45 mol/l)in H_2SO_4(0.25mol/l)was spotted onto the testing zone</p>	Creatinine Uric Acid	LR 50–600 mg/L, for Creatinine 50–500 mg/L, for Uric Acid LOD 15.7 mg/L, for Creatinine 16.5 mg/L, for Uric Acid	Urine	[43]
<p>A paper plastic microchip (PPMC)</p> <ul style="list-style-type: none"> Comprising of three-layer hybrid substrates prepared of a cellulose paper substrate assembled with a transparent plastic sheet by double-sided adhesive Laser cutting was used for patterning Screen printing to print electrodes on the surface of the paper. 	<p>FOR ZIKV DETECTION, Viral lysate prepared using a 1% Tritonx-100 solution was used after capturing the ZIKV particles using magnetic beads modified with anti-ZIKV envelope monoclonal antibody(anti-ZIKV mab), followed by impedance testing with different virus concentrations. (with the increase in viral concentrations, the impedance decreased; which could be attributed to the increase in charged particles released during the lysis step.</p> <p>FOR HPV, Loop-mediated isothermal amplification was used, which resulted in the loop like amplicons for both HPV16 and 18.</p> <p>FOR MULTIPLEXED DETECTION OF ALPHA FETOPROTEIN AND CARCINOEMBRYONIC ANTIGEN (CEA). PPMC designed with two semi-circular electrodes and two detection zones modified with biomarker specific monoclonal antibodies were used (the increase in concentrations resulted in a decrease in impedance)</p>	Zika Virus, HPV, Alpha-Fetoprotein And Carcinoembryonic Antigen (CEA)	LOD For cancer protein biomarkers, 100 ng/mL For viral particles, 1000 particle/mL For HPV nucleic acid, 100 copies/mL	Serum	[37]
<p>A μPAD for multiplex detection of cardiac biomarkers with three reaction zones and one sample zone at the center.</p>	<p>Colorimetric detection of multiplex cardiac markers by using only an available phone camera or desktop scanner for quantification.</p> <p>For GPBB detection. The target analyte was sandwiched between a capturing antibody and a detecting antibody and color signals were observed from the labeled nanoparticles conjugate –detecting antibody. (yellow for GPBB, purple for CK-MB and red for cTnT) For a particular analyte. Three different colored NPS (gold, silver</p>	GPBB (Glycogen Phosphorylase Isoenzyme BB) with CK-MB (Creatine Kinase-MB)And cTnT Cardiac Troponin T	LOD For GPBB, 0.5 ng/mL For CK-MB, 0.5 ng/mL For cTnT, 0.05 ng/mL	SERUM	[28]

(continued on next page)

Table 1 (continued)

DEVICE DESCRIPTION/ARCHITECTURE	SENSING MECHANISM	TARGET MOLECULE	ANALYTICAL PERFORMANCE	REAL SAMPLE	REFERENCE
GO-modified μ pads coupled with smartphone-based colorimetric detection for direct quantification Fabricated by methods of photolithography	and gold urchin) were used as detecting indicators. Graphene Oxide enhanced colorimetric assay	Glucose	LOD 0.02 mM LR 0–1 mM	Artificial Saliva	[46]
Paper-based electrochemical sensing platform μ PEC for Thrombin detection <ul style="list-style-type: none"> The hydrophobic barriers were fabricated through wax printing The Ag/AgCl reference electrode and a carbon counter electrode were obtained through screen printing. 	Paper-based TiO ₂ Nano-sheets (PTNs) and CeO ₂ were employed as an electron transporting material and photoactive material respectively. Upon illumination, the photogenerated electrons of CeO ₂ promptly transferred into PTNs increasing in photocurrent intensity. By regulation of electron-transfer tunneling distance between PTNs and CeO ₂ sensitive detection of Thrombin was realized.	Thrombin	LR 0.02pM- 100 pM DL 6.7 fM	Human serum	[27]

NR - not reported | LOD – Limit OF Detection | LR – Linear Range | LOQ – Limit Of Quantification | DL – Detection Limit.

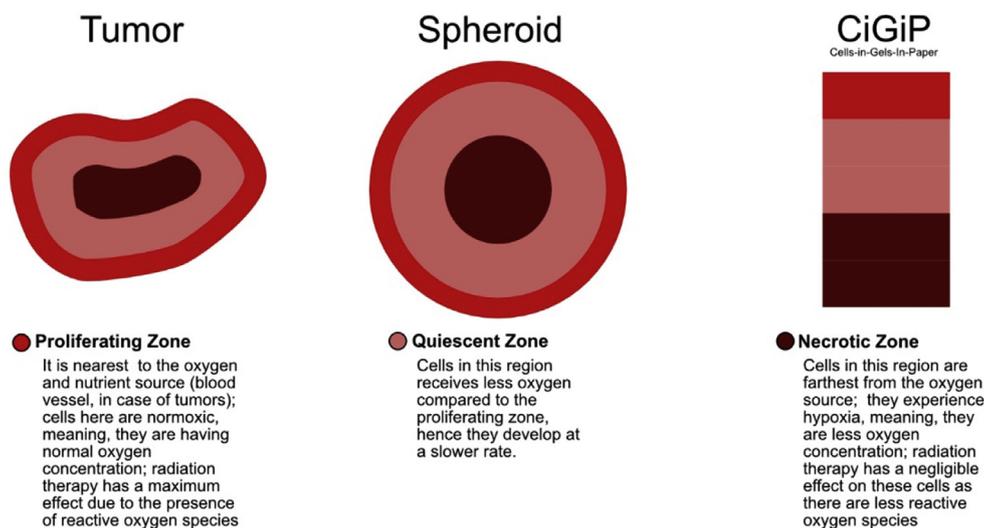


Fig. 3. Different zones of cells in a tumor, spheroids, and Cells-In-Gels-In-Paper.

made a ‘heart on a paper’. It was the first attempt to fabricate a paper-based-array for the culture, proliferation, and direct differentiation of human-induced-Pluripotent-Stem-Cells (hiPSCs) into functional beating cardiac tissue. The hiPSC-derived cardiac tissue was able to maintain a long-term spontaneous beating activity of about 3 months [15]. A year later, Whitesides et al. showed the use of paper to guide the deposition of minerals like Calcium Phosphates by Osteoblasts. The porous and fibrous structure of paper enables the transport of nutrients and oxygen across the entire thickness of the paper, which thereby allows cellular remodeling, proliferation, and differentiation of cells [60] (see Fig. 5).

In another model, Yaqing Wang and his group developed a 3D liver model (Fig. 6), which was formed by the co-cultures of human-induced-Hepatocytes (hiHEPs) and Human-Umbilical-Vein-Endothelial-Cells (HUVECs) on the paper scaffold. It was able to maintain a long-term stable morphology and hepatic functions assessed by secretion of albumin, synthesis of urea and CYP450 gene expression which is responsible for the oxidation of steroids, fatty acids, and xenobiotics, for 60 days. Also, the 3D culture showed hepatotoxic characteristics of well-known drugs like Acetaminophen (APAP) and Pioglitazone [16].

3. Discussion and challenges

Paper being a thin, light and flexible material, has long been used as a substrate to perform biomolecular assays using the conventional technique of Lateral Flow Immunoassay (LFA) [3,61,62]. However, the devices were only able to give the user, a qualitative response; rather than a quantitative one; i.e. a yes or no, rather than an estimation of the concentration of the analyte. Microfluidic- Paper-based-Analytical-Device (μ PAD) sensors brought a paradigm shift in the field of biosensors and showed its potential to revolutionize the diagnosis [21,24,25] and therapeutics [14,59] sectors of Biomedical Sciences. These sensors were not only able to give a colorimetric response but also give an estimation of the concentration of the analyte. On the other hand, the paper has also found its applications in developing paper-based-cultures and scaffolds which mimicked the 3D cellular environment of tissues. Both these technologies have to overcome many hurdles to become a mainstream technology to be used as biosensors and tissue scaffolds. Also, μ PADs are being used as sensors to test milk quality, or to test the presence of drugs like Ketamine or to detect and quantify nucleic acid [63–65]. In this review, we examined the progress done in the last 10 years in both these technologies.

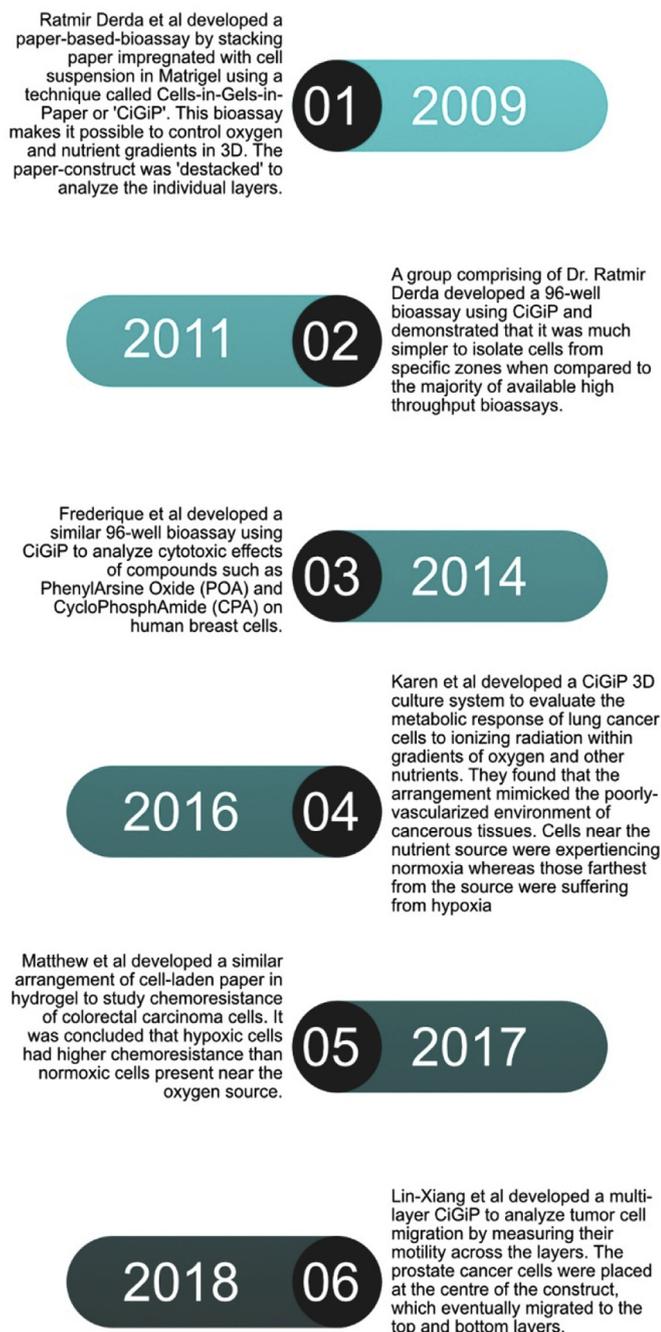


Fig. 4. Timeline of CiGiP-based 3D cultures.

A major challenge with paper-based sensors is the composition of paper, which is mostly cellulose and hemicellulose. It is inherently, a non-conducting material; hence paper was primarily used for colorimetric tests. Hence, modification was done in the paper substrate using carbon nanotubes, graphene nanosheets, metal nanoparticles, metal oxide nanoparticles, and their nano-conjugates, to fabricate μ PADs, like those of Electrochemical- μ PAD (E- μ PAD); thus improving the performance of the sensor system [66]. E- μ PADs are being given prime attention due to its ability to provide low cost, and precise estimation of target molecules. Another challenge was that of amplifying the signals detected using μ PADs. Hence, in 2018 Wu et al. developed a μ PAD with ultra-sensitive and high-throughput detection of cancer biomarkers by introducing graphene onto the surface of the immune-device which efficiently accelerated the electron transfer and enhanced the detection signal [7]. Moreover, they provided a solution for accomplishing

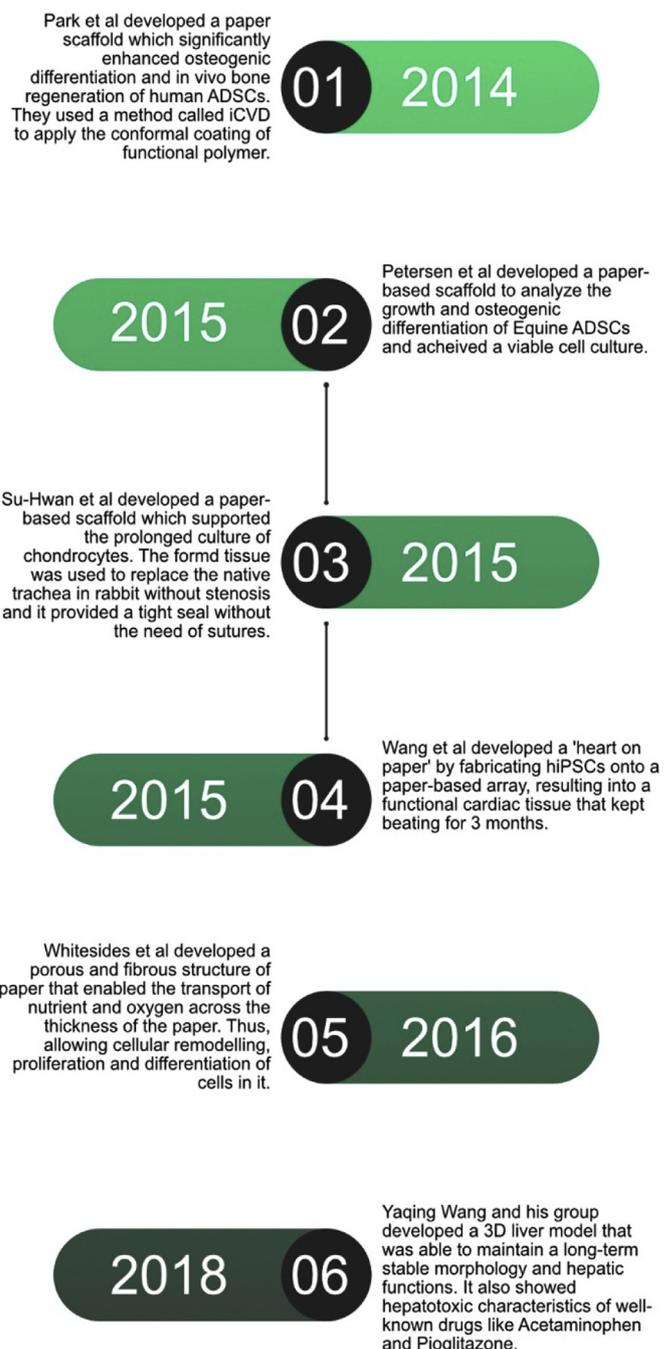


Fig. 5. Timeline of the development of Organ-On-Paper.

controlled radical polymerization reaction, Activator Generated Electron Transfer for Atom Transfer Radical (AGET ATRP), on a μ PAD.

However, μ PADs face other difficulties like complexity of cells migration from loading zone to testing zone [67], lack of proper adhesion sites [68] lack sensitivity to amount of analytes and complexity in integration of functional reagents to substrates [21]. To tackle the problems different scientist groups proposed different solutions. To tackle the problem of transporting suspensions of discrete objects like cells or microparticles laser micromachined hybrid open/paper microfluidic chips were developed in which the liquid carrier was transported in a Mylar microchannel but driven by a paper channel, with quantitative flow control [67]. Then, Ng et al. developed the ES-PCL/FP (Electro-spin-PCL over Filter Paper) which showed incredible cell adhesion of human-Foetal-Osteoblastic cells (hFOB). Lack of sensitivity was tackled

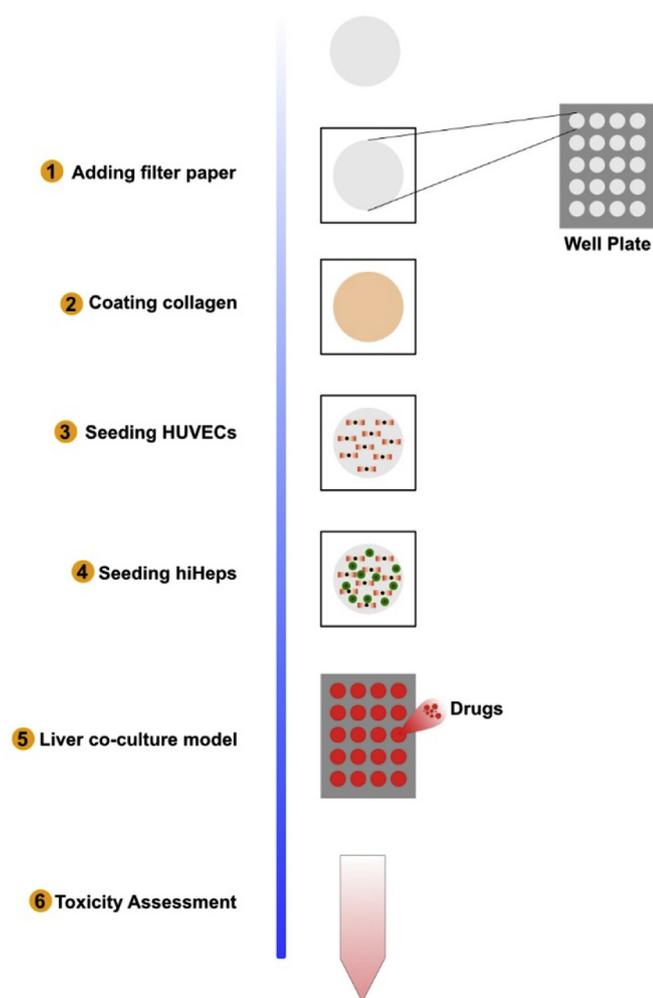


Fig. 6. Schematic diagram depicting the procedure of fabricating the paper-based array in the well-plate and establishing the liver co-culture model.

by concentrating targets from a larger volume by speeding up intrinsic thermodynamic partitioning in aqueous two-phase systems [67]. Lastly, the integration of functional groups or surface modification can be controlled by using techniques like initiated-Chemical-Vapour-Deposition (iCVDs). Also, these paper-based-sensors are sensitive to temperature and humidity, as the detection zones are dependent on the temperature and relative humidity of the environment [69]. Even though the rise in temperature improves enzyme activity, they also evaporate the water from the spots faster and even denature enzymes. The thermal stability can be increased by adsorption of enzymes on paper with conjunction with a humidifier substance.

On the other hand, paper on its own is not an optimal material for culturing cells, as it lacks cell adhesion moieties [68]. To develop the 'heart on paper', Li Wang and his group cultured the hiPSCs on the paper array pre-coated with gelatine and Matrigel to facilitate the proper proliferation of cells. Similarly, Yaqing Wang and his group pre-coated their paper-substrates with a thin layer of 100 $\mu\text{g}/\text{ml}$ rat tail collagen type I for better cell proliferation in the substrate. Hence, optimum surface modification was required for proper cell adhesion and proliferation. In a recent study, Ng et al compared three paper-substrate modification techniques, namely dip coating, hydrogel modification, and electro-spinning over Filter Paper (FP) (Whatman grade 114) using human foetal osteoblast (hFOB) [68]. Dip coating provided the least favorable cellular response, also resulted in reduced porosity; thus decreasing the number of anchoring spots for the cells and limiting the overall cell penetration. In the case of hydrogels; the high molecular

weight polymers did not obstruct cell penetration and distribution within the scaffold. But, Electro-spin- Polycaprolactone coating over FP (ES-PCL/FP) proved to be superior in cell viability and mechanical properties due to better surface morphology and higher porosity of ES-PCL/FP. PCL and paper complemented each other, resulting in the highest metabolic and ALP activity. Also, these culture systems must be disassembled before analysis, thus preventing the taking of measurements as gradients formed. To this, a potential solution was developed Matthew et al to measure spatial and temporal information about oxygen gradients, without disassembling the layers by using a luminescent thin-film sensor [13]. Oxygen readily diffused into these films resulting in changes in luminescence intensity with changes in the surrounding oxygen tension.

Lastly, a challenge that remains with paper-based scaffolds is that of imaging them to check cell adhesions, proliferation or interaction with chemicals. Currently, such scaffolds are unfolded layer-by-layer and then imaged [70]. It is both laborious and can give conflicting results. Again, most laboratory techniques are for testing and evaluating drug responses on 2D cultures, hence new methods have to be invented to overcome the same [17] Hence, more research is required to improve paper-based sensors and scaffolds.

4. Conclusion and future directions

Paper has been used as supporting material in Biomedical Sciences and has shown immense potential for the development of biosensors and scaffolding-material in the field of Biosensors and Tissue Engineering, respectively. This literature review enumerated the applications of paper as a supporting material over a decade and the research and development going on in the field of Biomedical Sciences. Earlier, LFAs were used for diagnostic purposes but they gave only qualitative results; but with further research paper-based devices were prepared which were able to give qualitative as well as quantitative results. For diagnosis purposes using paper, various biomarkers are considered, viz. viral proteins, metabolites viz. NO_3 , NO, Creatinine, Uric Acid which can be detected using a μPAD . Moreover, paper has also been used for oncological diagnosis and prognosis and proved to be efficient and easy to handle platform which gave an edge to cancer therapy.

Paper-based scaffolds have shown the immense capability to revolutionize the field of Tissue engineering. It has been used to develop 'heart on a paper' and co-cultures of Liver cells where drug testing showed promising results. The porous, and fibrous structure of paper enabled the transport of nutrients and oxygen across the entire thickness of the paper, which thereby allows cellular remodeling, proliferation, and differentiation of cells. The scaffolds developed were also used as an implantable material on rabbits to replace a damaged trachea. However, out of the various laboratory experiments and researches on μPADs , presented in this paper, none have found commercial utility till now. Therefore, current and future researches need to be more market-oriented and be commercially viable to the masses. For that, the researchers have to overcome the aforementioned challenges in the writing of the paper.

A future can be imagined in which people use a paper for diagnosing malicious diseases like cancer at home in a simple kit, similar to the pregnancy test kits available in today's market. Moreover, these devices will be cheap compared to the ones available now, and also easily disposable. On the other hand, scaffolds used in Tissue Engineering can be used to test drugs using cells obtained from the person requiring the drug. Drugs then will be patient-specific with negligible side effects. Also, the development and deployment of these drugs to the market will be faster compared to today's standards, as clinical trials could be performed faster using the cells from the patients. Thus, reducing the development time and cost at the same time.

In conclusion, more research is required to access the change in analytical performance of the various surface modification done on the paper substrate; the effect of temperature and other environmental factors on the biosensors and non-enzymatic approaches must be explored

in near future. In the field of paper-based cultures and scaffolds, more research in cell viability in the cultures and scaffolds must be done. Also, more research is required to compare the available cytotoxic results done on 2D cell cultures with the new 3D cell cultures.

Declaration of competing interest

No conflicts of interest.

References

- [1] i. A. Authority, The leon valley dead sea scrolls, Retrieved from Dead Sea Scrolls, https://www.deadseascrolls.org.il/learn-about-the-scrolls/introduction?local&equals=en_US, 2019, October 1.
- [2] T.C. history, A timeline of pregnancy testing, Retrieved from National Institutes of Health (NIH), <https://history.nih.gov/exhibits/thinblueline/timeline.html>, 2019, November 11.
- [3] A.J. Martin, R.L. Synge, A new form of chromatogram employing two liquid phases: a theory of chromatography. 2. Application to the micro-determination of the higher monoamino-acids in proteins, *Biochem. J.* 35 (12) (1941) 1358–1368, <https://doi.org/10.1042/bj0351358>.
- [4] N.H. Landmark, Al and helen free and the development of diagnostic test strips, Retrieved from American Chemical Society Chemistry For Life, <https://www.acs.org/content/acs/en/education/whatischemistry/landmarks/diagnosticteststrips.html>, 2010, May 1.
- [5] A.W. Martinez, S.T. Phillips, M.J. Butte, G.M. Whitesides, Patterned paper as a platform for inexpensive, low-volume, portable bioassays, *Angew Chem. Int. Ed. Engl.* 46 (8) (2007) 1318–1320, <https://doi.org/10.1002/anie.200603817>.
- [6] X. Yang, O. Forouzan, T.P. Brown, S.S. Shevkopyas, Integrated separation of blood plasma from whole blood for microfluidic paper-based analytical devices, *Lab Chip* 12 (2) (2012) 274–280, <https://doi.org/10.1039/c1lc20803a>.
- [7] Y. Wu, P. Xue, K.M. Hui, Y. Kang, A paper-based microfluidic electrochemical immunodevice integrated with amplification-by-polymerization for the ultrasensitive multiplexed detection of cancer biomarkers, *Biosens. Bioelectron.* 52 (2014b) 180–187, <https://doi.org/10.1016/j.bios.2013.08.039>.
- [8] A. Kumar, B. Purohit, K. Mahato, P. Chandra, Advance engineered nanomaterials in point-of-care immunosensing for biomedical diagnostics, in: U.A. Minhaz, Z. Mohammed, T. Eiichi (Eds.), *Immunosensors*, Royal Society of Chemistry, 2019.
- [9] P. Jan, D. Jana, C. Jana, H. Jaromir, J. Ondrej, A. Vojtech, K. Rene, Methods for carbon nanotubes synthesis—review, *J. Mater. Chem.* 21 (40) (2011) 15872–15884.
- [10] X. Zhang, K. Li, H. Li, J. Lu, Q. Fu, Y. Chu, Graphene nanosheets synthesis via chemical reduction of graphene oxide using sodium acetate trihydrate solution, *Synth. Met.* 193 (2014) 132–138.
- [11] D.D. Carla, R.N. Beatriz, E.C. Maria, Review of the methodologies used in the synthesis gold nanoparticles by chemical reduction, *J. Alloys Compd.* 798 (2019) 714–715.
- [12] K.A. Simon, B. Mosadegh, K.T. Minn, et al., Metabolic response of lung cancer cells to radiation in a paper-based 3D cell culture system, *Biomaterials* 95 (2016) 47–59, <https://doi.org/10.1016/j.biomaterials.2016.03.002>.
- [13] M.W. Boyce, R.M. Kenney, A.S. Truong, M.R. Lockett, Quantifying oxygen in paper-based cell cultures with luminescent thin film sensors, *Anal. Bioanal. Chem.* 408 (11) (2016) 2985–2992, <https://doi.org/10.1007/s00216-015-9189-x>.
- [14] H.J. Park, S.J. Yu, K. Yang, et al., Paper-based bioactive scaffolds for stem cell-mediated bone tissue engineering, *Biomaterials* 35 (37) (2014) 9811–9823, <https://doi.org/10.1016/j.biomaterials.2014.09.002>.
- [15] L. Wang, C. Xu, Y. Zhu, et al., Human induced pluripotent stem cell-derived beating cardiac tissues on paper, *Lab Chip* 15 (22) (2015) 4283–4290, <https://doi.org/10.1039/c5lc00919g>.
- [16] L.X. Wang, Y. Zhou, J.J. Fu, Z. Lu, L. Yu, Separation and characterization of prostate cancer cell subtype according to their motility using a multi-layer CiGiP culture, *Micromachines (Basel)* 9 (12) (2018) 660, <https://doi.org/10.3390/mi9120660>. Published 2018 Dec 14.
- [17] S.M. Cramer, T.S. Larson, M.R. Lockett, Tissue papers: leveraging paper-based microfluidics for the next generation of 3D tissue models, *Anal. Chem.* 91 (17) (2019) 10916–10926, <https://doi.org/10.1021/acs.analchem.9b02102>.
- [18] A. Abdelhak, A. Huss, J. Kassubek, H. Tuman, M. Otto, Serum GFAP as a biomarker for disease severity in multiple sclerosis, *Sci. Rep.* 8 (2018) 14798.
- [19] J.K. Pandey, D. Agarwal, Biomarkers: a potential prognostic tool for silicosis, *Indian J. Occup. Environ. Med.* 16 (3) (2012) 101–107, <https://doi.org/10.4103/0019-5278.111746>.
- [20] T. Songjaroen, W. Dungchai, O. Chailapakul, C.S. Henry, W. Laiwattanapaisal, Blood separation on microfluidic paper-based analytical devices, *Lab Chip* 12 (18) (2012) 3392–3398, <https://doi.org/10.1039/c2lc21299d>.
- [21] S.W. Hu, S. Qiao, B.Y. Xu, X. Peng, J.J. Xu, H.Y. Chen, Dual-functional carbon dots pattern on paper chips for Fe³⁺ and ferritin analysis in whole blood, *Anal. Chem.* 89 (3) (2017) 2131–2137, <https://doi.org/10.1021/acs.analchem.6b04891>.
- [22] S. Akhter, Some of the places in India, almost 80% of the population is suffering from Hep C. dr. Yogesh Batra, Retrieved from EHealthWorld, <https://health.economictimes.indiatimes.com/news/diagnostics/in-some-of-the-places-in-india-almost-80-of-the-population-is-suffering-from-hep-c-dr-yogesh-batra/65172694>, 2018, July 28.
- [23] X. Mu, L. Zhang, S. Chang, W. Cui, Z. Zheng, Multiplex microfluidic paper-based immunoassay for the diagnosis of hepatitis C virus infection, *Anal. Chem.* 86 (11) (2014) 5338–5344, <https://doi.org/10.1021/ac500247f>, published correction appears in *Anal. Chem.* 2015 Aug 4;87(15):8033.
- [24] C. Chen, T. Dong, Microfluidic paper-based analytical devices for colorimetric detection of urinary tract infection biomarkers on adult diapers, in: 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, IEMBS, Milan, Italy, 2015, pp. 5892–5893.
- [25] F. Bedin, L. Boulet, E. Voilin, G. Theillet, A. Rubens, C. Rozand, Paper-based point-of-care testing for cost-effective diagnosis of acute flavivirus infections, *J. Med. Virol.* 89 (9) (2017) 1520–1527, <https://doi.org/10.1002/jmv.24806>.
- [26] S. Akhter, Venous Thromboembolism is becoming a leading cause of disability and death in India : dr. Suviraj John, Retrieved from Economic Times Health World, <https://health.economictimes.indiatimes.com/news/industry/venous-thromboembolism-is-becoming-a-leading-cause-of-disability-and-death-in-india-dr-suviraj-john/55200566>, 2016, November 02.
- [27] J. Xue, L. Zhang, C. Gao, P. Zhu, J. Yu, Microfluidic paper-based photoelectrochemical sensing platform with electron-transfer tunneling distance regulation strategy for thrombin detection, *Biosens. Bioelectron.* 133 (2019) 1–7, <https://doi.org/10.1016/j.bios.2019.03.022>.
- [28] W.Y. Lim, T.M. Thevarajah, B.T. Goh, S.M. Khor, Paper microfluidic device for early diagnosis and prognosis of acute myocardial infarction via quantitative multiplex cardiac biomarker detection [published correction appears in *Biosens. Bioelectron.* 2019 Mar 15;129:299], *Biosens. Bioelectron.* 128 (2019) 176–185, <https://doi.org/10.1016/j.bios.2018.12.049>.
- [29] A.C. Society, Tests to find and diagnose cancer, Retrieved from cancer.org, <https://www.cancer.org/healthy/find-cancer-early/tests-to-find-and-diagnose-cancer.html>, 2019, November 17.
- [30] A. Costa, Endoscopy in gastrointestinal oncology, Retrieved from Cancer World, <https://cancerworld.net/e-grandround/endoscopy-in-gastrointestinal-oncology/>, 2016, April 16.
- [31] A.C. Society, How is cancer diagnosed?, Retrieved from cancer.org, <https://www.cancer.org/treatment/understanding-your-diagnosis/tests/testing-biopsy-and-cytology-specimens-for-cancer/how-is-cancer-diagnosed.html>, 2015, July 30a.
- [32] A.C. Society, Imaging (radiology) tests for cancer, Retrieved from cancer.org, <https://www.cancer.org/treatment/understanding-your-diagnosis/tests/imaging-radiology-tests-for-cancer.html>, 2015, November 30b.
- [33] A. Van Gossom, M. Munoz-Navas, I. Fernandez-Urien, et al., Capsule endoscopy versus colonoscopy for the detection of polyps and cancer, *N. Engl. J. Med.* 361 (3) (2009) 264–270, <https://doi.org/10.1056/NEJMoa0806347> [published correction appears in *N Engl J Med.* 2009 Sep 17;361(12):1220. Navas, Miguel Munoz [corrected to Munoz-Navas, Miguel]].
- [34] M. Li, Y. Wang, Y. Zhang, J. Yu, S. Ge, M. Yan, Graphene functionalized porous Au-paper based electrochemiluminescence device for detection of DNA using luminescent silver nanoparticles coated calcium carbonate/carboxymethyl chitosan hybrid microspheres as labels, *Biosens. Bioelectron.* 59 (2014) 307–313, <https://doi.org/10.1016/j.bios.2014.03.072>.
- [35] Y. Wang, H. Xu, J. Luo, et al., A novel label-free microfluidic paper-based immunosensor for highly sensitive electrochemical detection of carcinoembryonic antigen, *Biosens. Bioelectron.* 83 (2016) 319–326, <https://doi.org/10.1016/j.bios.2016.04.062>.
- [36] Y. Fan, J. Liu, Y. Wang, et al., A wireless point-of-care testing system for the detection of neuron-specific enolase with microfluidic paper-based analytical devices, *Biosens. Bioelectron.* 95 (2017) 60–66, <https://doi.org/10.1016/j.bios.2017.04.003>.
- [37] M.S. Draz, M. Moazeni, M. Venkataramani, et al., Hybrid paper-plastic microchip for flexible and high-performance point-of-care diagnostics, *Adv. Funct. Mater.* 28 (26) (2018) 1707161, <https://doi.org/10.1002/adfm.201707161>.
- [38] G. Kris, Are nitrates and nitrites in foods harmful?, Retrieved from Healthline, <https://www.healthline.com/nutrition/are-nitrates-and-nitrites-harmful>, 2020, February 10.
- [39] G. Eisenbrand, B. Spiegelhalder, R. Preussmann, Nitrate and nitrite in saliva, *Oncology* 37 (4) (1980) 227–231, <https://doi.org/10.1159/000225441>.
- [40] S.A. Bhakta, R. Borba, M. Taba Jr., C.D. Garcia, E. Carrilho, Determination of nitrite in saliva using microfluidic paper-based analytical devices, *Anal. Chim. Acta* 809 (2014) 117–122, <https://doi.org/10.1016/j.aca.2013.11.044>.
- [41] D.A. Nnate, N.K. Achi, Nitrate metabolism: a curse or blessing to humanity? *J. Sci. Res. Rep.* 1 (2016).
- [42] C. Honrado, T. Dong, A capacitive touch screen sensor for detection of urinary tract infections in portable biomedical devices, *Sensors (Basel)* 14 (8) (2014) 13851–13862, <https://doi.org/10.3390/s140813851>. Published 2014 Jul 30.
- [43] E.L. Rossini, M.I. Milani, E. Carrilho, L. Pezza, H.R. Pezza, Simultaneous determination of renal function biomarkers in urine using a validated paper-based microfluidic analytical device, *Anal. Chim. Acta* 997 (2018) 16–23, <https://doi.org/10.1016/j.aca.2017.10.018>.
- [44] E. Carrilho, A.W. Martinez, G.M. Whitesides, Understanding wax printing: a simple micro patterning process for paper-based microfluidics, *Anal. Chem.* 81 (16) (2009) 7091–7095, <https://doi.org/10.1021/ac901071p>.
- [45] E.F. Gabriel, P.T. Garcia, T.M. Cardoso, F.M. Lopes, F.T. Martins, W.K. Coltro, Highly sensitive colorimetric detection of glucose and uric acid in biological fluids

- using chitosan-modified paper microfluidic devices, *Analyst* 141 (15) (2016) 4749–4756, <https://doi.org/10.1039/c6an00430j>.
- [46] Y. Jia, H. Sun, X. Li, et al., Paper-based graphene oxide biosensor coupled with smartphone for the quantification of glucose in oral fluid, *Biomed. Microdevices* 20 (4) (2018) 89, <https://doi.org/10.1007/s10544-018-0332-2>. Published 2018 Oct 12.
- [47] A.S. Yap, S.W. Manley, Thyrotropin inhibits the intrinsic locomotility of thyroid cells organized as follicles in primary culture, *Exp. Cell Res.* 214 (1) (1994) 408–417, <https://doi.org/10.1006/excr.1994.1274>.
- [48] C.A. Ollis, R. Davies, D.S. Munro, S. Tomlinson, Relationship between growth and function of human thyroid cells in culture, *J. Endocrinol.* 108 (3) (1986) 393–398, <https://doi.org/10.1677/joe.0.1080393>.
- [49] M.W. Boyce, G.J. LaBonia, A.B. Hummon, M.R. Lockett, Assessing chemotherapeutic effectiveness using a paper-based tumor model, *Analyst* 142 (15) (2017) 2819–2827, <https://doi.org/10.1039/c7an00806f>.
- [50] H. Ye, K. Zhang, D. Kai, Z. Li, X.J. Loh, Polyester elastomers for soft tissue engineering, *Chem. Soc. Rev.* 47 (12) (2018) 4545–4580, <https://doi.org/10.1039/c8cs00161h>.
- [51] F. Baino, E. Fiume, Elastic mechanical properties of 45S5-based bioactive glass-ceramic scaffolds, *Materials (Basel, Switzerland)* 12 (19) (2019) 3244, <https://doi.org/10.3390/ma12193244>.
- [52] N. Tanaka, H. Ota, K. Fukumori, J. Miyake, M. Yamato, T. Okano, Micro-patterned cell-sheets fabricated with stamping-force-controlled micro-contact printing [published correction appears in *Biomaterials*. 2015 Jan;38:109], *Biomaterials* 35 (37) (2014) 9802–9810, <https://doi.org/10.1016/j.biomaterials.2014.08.043>.
- [53] H. Juvonen, A. Määttä, P. Laurén, et al., Biocompatibility of printed paper-based arrays for 2-D cell cultures, *Acta Biomater.* 9 (5) (2013) 6704–6710, <https://doi.org/10.1016/j.actbio.2013.01.033>.
- [54] R. Derda, A. Laromaine, A. Mammoto, et al., Paper-supported 3D cell culture for tissue-based bioassays, *Proc. Natl. Acad. Sci. U. S. A.* 106 (44) (2009) 18457–18462, <https://doi.org/10.1073/pnas.0910666106>.
- [55] R. Derda, S.K. Tang, A. Laromaine, et al., Multizone paper platform for 3D cell cultures, *PLoS One* 6 (5) (2011), e18940, <https://doi.org/10.1371/journal.pone.0018940>. Published 2011 May 6.
- [56] F. Deiss, A. Mazzeo, E. Hong, D.E. Ingber, R. Derda, G.M. Whitesides, Platform for high-throughput testing of the effect of soluble compounds on 3D cell cultures, *Anal. Chem.* 85 (17) (2013) 8085–8094, <https://doi.org/10.1021/ac400161j>.
- [57] M.J. Lerman, J. Lembo, S. Muramoto, G. Gillen, J.P. Fisher, The evolution of Polystyrene as a cell culture, *Material. Tissue Eng Part B Rev.* 24 (5) (2018) 359–372, <https://doi.org/10.1089/ten.TEB.2018.0056>.
- [58] G.F. Petersen, B.J. Hilbert, G.D. Trope, W.H. Kalle, P.M. Strappe, A paper-based scaffold for enhanced osteogenic differentiation of equine adipose-derived stem cells, *Biotechnol. Lett.* 37 (11) (2015) 2321–2331, <https://doi.org/10.1007/s10529-015-1898-x>.
- [59] S.H. Kim, H.R. Lee, S.J. Yu, et al., Hydrogel-laden paper scaffold system for origami-based tissue engineering, *Proc. Natl. Acad. Sci. U. S. A.* 112 (50) (2015) 15426–15431, <https://doi.org/10.1073/pnas.1504745112>.
- [60] G. Camci-Unal, A. Laromaine, E. Hong, R. Derda, G.M. Whitesides, Biomimetic mineralization guided by paper templates, *Sci. Rep.* 6 (2016) 27693, <https://doi.org/10.1038/srep27693>. Published 2016 Jun 9.
- [61] J. Bogdanovic, M. Koets, I. Sander, et al., Rapid detection of fungal alpha-amylase in the work environment with a lateral flow immunoassay, *J. Allergy Clin. Immunol.* 118 (5) (2006) 1157–1163, <https://doi.org/10.1016/j.jaci.2006.07.004>.
- [62] R. Verheijen, P. Stouten, G. Cazemier, W. Haasnoot, Development of a one step strip test for the detection of sulfadiazine residues, *Analyst* 123 (12) (1998) 2437–2441, <https://doi.org/10.1039/a804695f>.
- [63] K. Mahato, P. Chandra, Paper-based miniaturized immunosensor for naked eye ALP detection based on digital image colorimetry integrated with smartphone, *Biosens. Bioelectron.* 128 (2019) 9–16, <https://doi.org/10.1016/j.bios.2018.12.006>.
- [64] J. Narang, N. Malhotra, C. Singhal, et al., Point of care with micro fluidic paper based device integrated with nano zeolite-graphene oxide nanoflakes for electrochemical sensing of ketamine, *Biosens. Bioelectron.* 88 (2017) 249–257, <https://doi.org/10.1016/j.bios.2016.08.043>.
- [65] S. Roy, N.F. Mohd-Naim, M. Safavieh, M.U. Ahmed, Colorimetric nucleic acid detection on paper microchip using loop mediated isothermal amplification and crystal violet dye, *ACS Sens.* 2 (11) (2017) 1713–1720, <https://doi.org/10.1021/acssensors.7b00671>.
- [66] A. Kumar, B. Purohit, P.K. Maurya, M.L. Pandey, P. Chandra, Engineered nanomaterial assisted signal-amplification strategies for enhancing analytical performance of electrochemical biosensors, *Electroanalysis* 31 (9) (2019) 1615–1622.
- [67] Chueh-Yu Wu, Oladunni Adeyiga, Jonathan Lin, Di Carlo, Dino, Research highlights: increasing paper possibilities, *Lab Chip* 14 (17) (2014a), <https://doi.org/10.1039/C4LC90067G>.
- [68] K. Ng, P. Azari, H.Y. Nam, F. Xu, B. Pingguan-Murphy, Electrospin-coating of paper: a natural extracellular matrix inspired design of scaffold, *Polymers (Basel)* 11 (4) (2019) 650, <https://doi.org/10.3390/polym11040650>. Published 2019 Apr 9.
- [69] G.G. Morbioli, T. Mazzu-Nascimento, A.M. Stockton, E. Carrilho, Technical aspects and challenges of colorimetric detection with microfluidic paper-based analytical devices (μPADs) - a review, *Anal. Chim. Acta* 970 (2017) 1–22, <https://doi.org/10.1016/j.aca.2017.03.037>.
- [70] D. Rodenhizer, E. Gaude, D. Cojocari, et al., A three-dimensional engineered tumour for spatial snapshot analysis of cell metabolism and phenotype in hypoxic gradients [published correction appears in *Nat Mater.* 2016 Feb;15(2):244], *Nat. Mater.* 15 (2) (2016) 227–234, <https://doi.org/10.1038/nmat4482>.
- [71] D. Bhatia, Bone-Cement: the new medical quick fix, *Chronicles Young Sci.* 1 (2010).
- [72] D. Bhatia, B. Rajak, M. Gupta, A. Mukherjee, Application of micro-electro-mechanical systems as neural interface, *J. Adv. Biomed. Eng. Technol.* 2 (2015) 1–2.
- [73] R.G. Bowler, The determination of thiocyanate in blood serum, *Biochem. J.* 38 (5) (1944) 385–388, <https://doi.org/10.1042/bj0380385>.
- [74] L.A. Carlson, L.B. Wadstrom, Determination of glycerides in blood serum, *Clin. Chim. Acta* 4 (2) (1959) 197–205, [https://doi.org/10.1016/0009-8981\(59\)90130-5](https://doi.org/10.1016/0009-8981(59)90130-5).
- [75] P. Chandra, E. Segal, Nanobiosensors for Personalized and Onsite Biomedical Diagnosis, *Institution of Engineering and Technology*, 2016.
- [76] S.S. Evans, D.B. Lee, T. Han, T.B. Tomasi, R.L. Evans, Monoclonal antibody to the interferon-inducible protein Leu-13 triggers aggregation and inhibits proliferation of leukemic B cells, *Blood* 76 (12) (1990) 2583–2593.
- [77] F.J. O'Brien, Biomaterials & scaffolds for tissue engineering Mater, *Today Off.* 14 (2011) 88–95, [https://doi.org/10.1016/S1369-7021\(11\)70058-X](https://doi.org/10.1016/S1369-7021(11)70058-X).
- [78] K. Mahato, A. Srivastava, P. Chandra, Paper based diagnostics for personalized health care: emerging technologies and commercial aspects, *Biosens. Bioelectron.* 96 (2017) 246–259, <https://doi.org/10.1016/j.bios.2017.05.001>.
- [79] T. Motoyama, H. Watanabe, S. Takeuchi, T. Watanabe, S. Gotoh, E. Okazaki, Cancer Antigen 125, Carcinoembryonic Antigen, and Carbohydrate Determinant 19-9 in Ovarian Tumors, *Cancer*, 1990, pp. 2628–2629.
- [80] T.W. Navaraj, H. Heidari, A. Polishchuk, D. Shakhthivel, D. Bhatia, R. Dahiya, Upper limb prosthetic control using toe gesture sensors, in: *IEEE Sensors. Busan, South Korea: IEEE*, 2016.
- [81] J. Ozhikandathil, S. Badilescu, M. Packirisamy, A brief review on microfluidic platforms for hormones detection, *J. Neural. Transm.* 124 (1) (2017) 47–55, <https://doi.org/10.1007/s00702-016-1610-x>.
- [82] L.B. Rajak, M. Gupta, D. Bhatia, Growth and advancements in neural control of limb, *Biomedical Science and Engineering* (2015), <https://doi.org/10.12691/bse-3-3-1>.
- [83] J.L. Tullis, R.J. Tinch, J.G. Gibson 2nd, P. Baudanza, A simplified centrifuge for the separation and processing of blood cells, *Transfusion* 7 (3) (1967) 232–242.
- [84] Y. Wang, W. Su, L. Wang, et al., Paper supported long-term 3D liver co-culture model for the assessment of hepatotoxic drugs, *Toxicol Res (Camb)* 7 (1) (2017) 13–21, <https://doi.org/10.1039/c7tx00209b>. Published 2017 Sep. 28.
- [85] J.B. Willis, The determination of metals in blood serum by atomic absorption spectroscopy—I: Calcium, *Spectrochim. Acta* (1960) 259–261.
- [86] C. Zhao, X. Liu, A portable paper-based microfluidic platform for multiplexed electrochemical detection of human immunodeficiency virus and hepatitis C virus antibodies in serum, *Biomicrofluidics* 10 (2) (2016), 024119, <https://doi.org/10.1063/1.4945311>. Published 2016 Apr 12.
- [87] P. Zhao, S. Liu, Y. Bai, et al., hWJECM-derived oriented scaffolds with autologous chondrocytes for rabbit cartilage defect repairing, *Tissue Eng Part A* 24 (11–12) (2018) 905–914, <https://doi.org/10.1089/ten.TEA.2017.0223>.
- [88] P. Zhao, S. Liu, Y. Bai, et al., hWJECM-derived oriented scaffolds with autologous chondrocytes for rabbit cartilage defect repairing, *Tissue Eng Part A* 24 (11–12) (2018) 905–914, <https://doi.org/10.1089/ten.TEA.2017.0223>.



Bimalendu Deka is doing his Bachelor's in Technology in Biomedical Engineering from the School of Technology, North-Eastern Hill University. His areas of interest are Nanomedicine, Biosensors, Microfluidics, Tissue Engineering, and Bio-MEMS.



Rima Kalita is doing her Bachelor's in Technology in Biomedical Engineering from the School of Technology, North-Eastern Hill University. Her areas of interest are Nanomedicine, Microfluidics, Tissue Engineering, and Bioelectronics.



Dinesh Bhatia received his Ph.D. in the field of bio-rehabilitation engineering from Motilal Nehru National Institute of Technology (MNNIT), Allahabad. His specific interests lie in the development of bio-rehabilitative devices and the study of EMG signals. He is currently an Associate Professor in the Department of Biomedical Engineering, North Eastern Hill University, Shillong, Meghalaya, India. He was the recipient of the BOYSCAST Fellowship, INAE Fellowship and was also the International Young Biomedical Scientist ICMR award. He has several publications in reputed journals, books and members of different engineering and life sciences societies.



Animesh Mishra received his Post-Doctoral Degree DM Cardiology from G.B.Pant Hospital and associated MAMC, New Delhi, Delhi University in 2004. His specific interests lie in the Interventional Cardiology, particularly Heart Failure Management. He is currently a Professor in the Department of Cardiology, NEIGRIHMS, Shillong, Meghalaya, India with teaching and research experience of more than 23 years. He was the recipient of the Indian Society of Cardiology Fellowship (2011), and many other fellowships. He has several publications in reputed journals, books and members of different Medical and professional bodies.