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Paper – a potential platform in pharmaceutical development

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Paper is predominantly composed of cellulose fibers that have an inherent ability to wick fluids by capillary action; it provides an interesting diagnostic platform that is inexpensive, easily obtained, and eco-friendly. Paper has been used in various types of biologically relevant applications including paper-based molecular assays, paper-based ELISA (P-ELISA), paper-based nucleic acid assays, and paper-based cell assays. Based on recent successes with the use of paper as a platform, we contend that paper is not only very suitable for diagnostics but could provide a more advantageous platform than current plastics-based platforms for drug discovery, and would be useful for accomplishing *in vitro* pre-compound screening steps while offering a possible solution to several economic obstacles inherent in the pharmaceutical industry.

Paper – a potential platform to adjust the resource structure of pharmaceutical development

Pharmaceutical companies develop well-established workflows to keep pace with the timelines for unmet medical needs. They usually devote all their resources to developing new drugs for prolonging life, improving quality of life, avoiding the adverse effects of drug treatment, and increasing convenience for people who require medications [1]. Launching a new drug to market is widely known to cost more than a billion dollars from concept to execution. Companies have refined the drug-development process to take a drug from benchtop to the market, while minimizing any waste in funding or resources as a result of trial and error. The drug-development process can usually be divided into six stages: target identification and validation, hit selection, lead identification [2], lead optimization [3], drug nomination, and clinical trials. The early stages of research and development focus on the molecular targets of disease, pre-compound synthesis, and screening, which are costly and very important in the pursuit of a successful product [4–6]. In early drug-screening stages such as hit selection, pharmaceutical companies have developed and have long employed a variety of molecular or cell based assays to test amounts of pre-compounds. They have also attempted to minimize cost and economize manpower with the use of automatic, high-throughput systems. However, the automatic high-throughput system is a costly instrument that wastes considerable resources and materials, including but not limited to the use of expensive reagents [e.g., monoclonal antibodies (mAbs), enzymes, and substrates]. These disadvantages make biological assays very expensive and create a high threshold for launching pharmaceutical industry research.

Paper has been successfully used for biological assays in recent years [7–19], and many review articles have summarized how well these versatile paper-based platforms perform in biological assays, and several advantages over conventional platforms have been demonstrated [20,21]. For instance, paper-based platforms can replace current *in vitro* assays including cell assays, and thus can overcome the high cost of microtiter plates. Furthermore, such assays can be implemented not only for hit selection but also for lead selection, lead optimization, candidate nomination, and even clinical trials. Based on these promising results, we will try, in this manuscript, to expand upon the benefits of paper-based assays and provide a perspective on using paper-based assays as an alternative to existing tools in the pharmaceutical industry (Figure 1). While we highlight assay advantages, it is also important to note that paper-based platforms are economically advantageous for use in developing and underdeveloped countries.

Benefits of paper-based diagnostics in current translational-medicine applications

Paper is affordable, abundant, disposable, and compatible with large-scale manufacturing processes for the production of microfluidic devices. Paper, essentially a thin sheet of cellulose fibers, possesses advantages that are compatible with many bioassays [20,22]. Paper is thin, lightweight, amenable to long-term storage, and of low cost [22]; paper is also easily disposed of by incineration, which is more eco-friendly than plastics. Paper is usually white, and is therefore suitable for colorimetric assays. Paper can be easily modified chemically and can be conjugated with many biomolecules, including peptides and nucleotides, and

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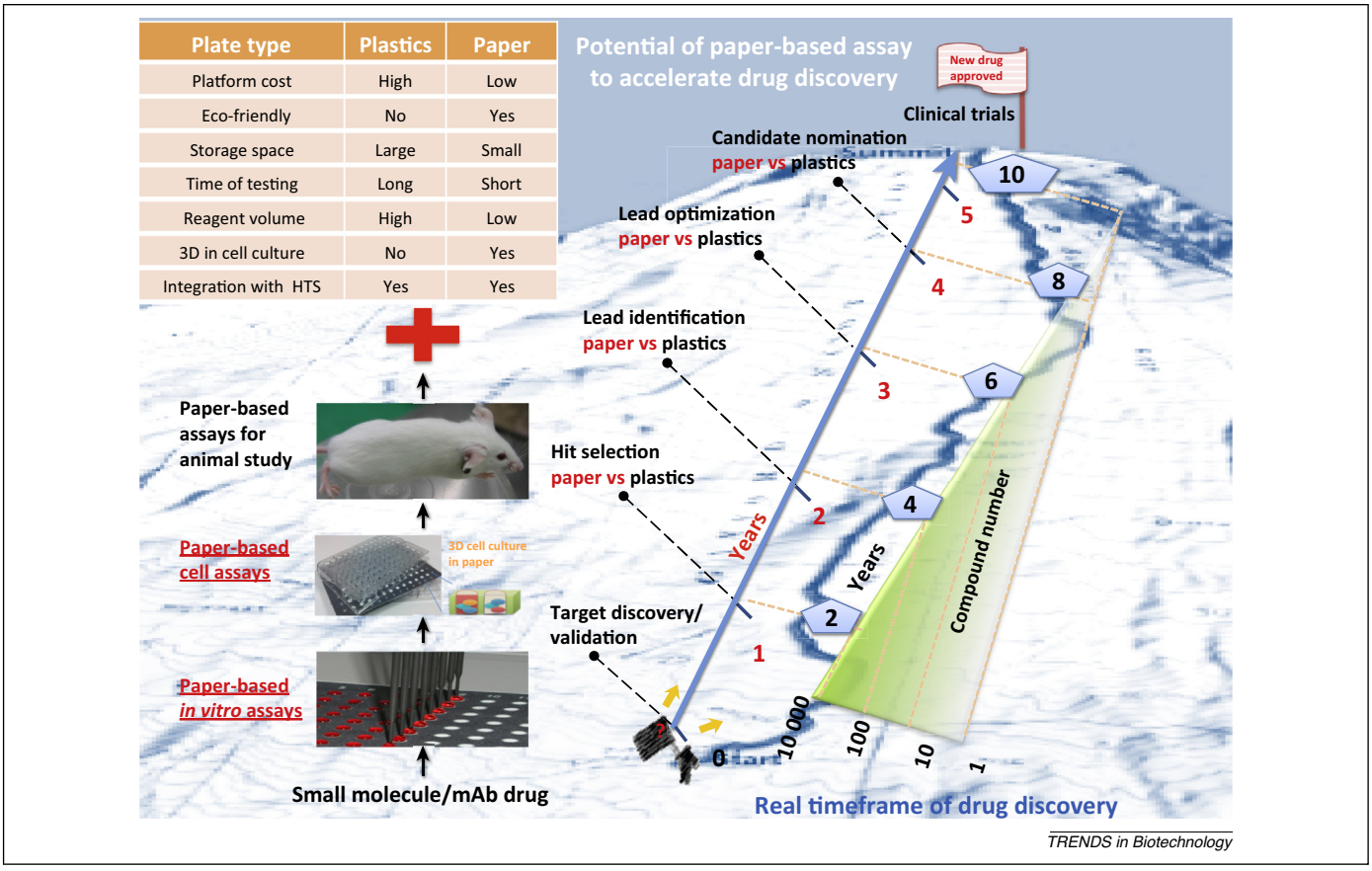

Figure 1. Adjusting the resource structure of drug development with paper-based platforms. Paper has distinct advantages over plastic (top left). P-ELISA, paper-based cell assays, and paper-based animal studies (bottom left) have the potential to accelerate the rate of drug discovery and thus decrease the amount of time required to achieve drug approval (right). Abbreviations: HTS, high-throughput screening; mAB, monoclonal antibody; P-ELISA, paper-based ELISA.

can be customized to meet special needs [21]. Patterned barriers (e.g., 96-well and 384-well formats for high-throughput use) can be easily created on paper using wax printing (Box 1) [23], microchannels can easily be fabricated on paper, and some multiplex and labor-consuming steps can be simplified by leveraging the micro-fluidic advantages of paper (Box 1) [24]. Based on these characteristics and advantages, paper can be used in many bioassays, including molecular assays, P-ELISA, cell culture studies, and more [8–17,19,21,25–27].

Diagnostic assays are the most achievable application for paper-based platforms, and these have already been used to examine clinical samples such as blood, saliva, tears, aqueous humor, seminal fluid, and more [10,11,17,26,28–32]. Highly versatile, paper-based micro-fluidic devices (μPADs) have several advantages because they require small sample and reagent volumes, and can be rapidly and conveniently integrated into portable instruments that use diverse analytical detection approaches including colorimetry, fluorescence and chemiluminescence, electrochemical methods, or transmittance [33]. We will highlight current studies that use paper bioassay platforms for ELISA (Table 1) and cell based assays, and discuss their benefits as superior replacements for current plastic-based bioassay platforms in the pharmaceutical industry.

Paper-based ELISA assays

Ligand-binding assays (LBA) have developed for the detection or quantification of molecules based on immunological affinity. ELISA, the most widely used LBA, employs signal amplification via a specific antibody combined with high-turnover catalytic enzymes and an enzymatic substrate that produces a detectable signal. ELISA is an efficient method for the routine assessment of large numbers of samples, for example in the quantification of drugs [34] and hormones [35], and provides a fundamental tool for measuring drugs and biomarkers in *in vitro* and *in vivo* samples during compound screening, animal studies, and clinical trials. ELISA assays are routinely used for drug screening of over 100 000 compounds. Although ELISA can provide high-throughput performance with rapid runs, the assays rely on high sample volume and use multiple unique reagents. P-ELISA provides several distinct advantages over conventional ELISA, such as sample conservation, economical use of reagents, and time and labor savings.

P-ELISA was first used to determine immunoglobulin G (IgG) and HIV antigen titers via colorimetric assay [16]. P-ELISA can reduce reagent requirements to 1/25 of the volume needed for current microtiter plate processes, and can reduce the reaction time to 1/5 of that required for conventional ELISA [16]. P-ELISA is also a powerful tool for quantifying drugs and biomarkers in living samples. For example, P-ELISA using antibody against vascular endothelial growth factor (VEGF) has been used to measure VEGF concentrations in aqueous humor. In humans, only 200 μL of aqueous humor can be collected from the anterior chamber before threat of anterior chamber collapse. For P-ELISA, only 2 μL of aqueous humor is