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Review

Microfluidic Advancements in Classical Biotechnology – A Review

Ishtiaq Ahmed [1*], Zain Akram [1*], Mohammed Hussen Bule[2] and Hafiz M. N. Iqbal [3]

[1] School of Medical Science, Gold Coast campus, Griffith University, Southport QLD 4222, Australia; i.ahmed@griffith.edu.au ; z.akram@griffith.edu.au

[2] Department of Pharmacy, College of Medicine and Health Sciences, Ambo University, Ambo, Ethiopia; mohammed2bule@gmail.com

[3] Tecnologico de Monterrey, School of Engineering and Sciences, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., CP 64849, Mexico. hafiz.iqbal@itesm.mx

* Correspondence: i.ahmed@griffith.edu.au ; Tel.: +61-435993196

Abstract: Micro-technology has played a substantial role in bioscience, biomedical and biotechnological research due to its core advantages in modern science and engineering. It has created unique development in various sectors of bio-research and upsurges the efficacy of research at the molecular level in recent years. Microfluidic technology makes it possible to manipulate sample volumes at the micro- and nano-level (called nanofluidics) with terrific control outside *in vivo* cellular microenvironment, enabling the reduction of discrepancies between *in vivo* and *in vitro* environments as well as reducing reaction time and cost. In this review, we discuss various effective integrations of microfluidic technologies into biotechnology and its paradigmatic significance in bio-research, supporting mechanical and chemical *in vitro* cellular micro-environment. Specific innovations relating to the application of microfluidics to advance microbial life, solitary and co-cultures along with a multiple-type cell culturing, cellular communications, cellular interactions and population dynamics are discussed.

Keywords: Microchannel; Micro-array, Microstructure, Biofilms; Polydimethylsiloxane; Micro-PCR; Reynolds number; Micro Electro Mechanical Systems.

1. Introduction

Eternal aspiration of inquiring biology at a single subsistence level has contrived microfluidic noticeably into the organic (living) space [1]. Invariably, due to its miniaturised length scale, rapidity in analysis, the selective capacity to minimize the volume of reagent, and reduction in turnaround, small scale fluidics has facilitated exceptional and approaching scene in the walled in area of natural science [2,3]. Microfluidic scaffolds are micro-fabricated devices that have gained much research prominence for cellular interactions [4]. Cultured cells are worn in an array of a framework like a cell science, tissue culturing, bacterial biofilms, biomedical designing and additionally pharmacokinetics for medication improvement [5,6].

In vitro cell culture model makes it reasonable to culture different cells in required volume and change continuous animal tests for medical screening. Cells comprise of these structures and communicate along with various cells [7]. On the other hand, in an *in vitro* model of cell culture, no polymer casings or structures are available to be held fast instead of cell culture dish. Not all of cell categories with which to associate or convey cells do not demonstrate their character as organ components do, except proliferation. Therefore, cultured cells conventionally change their properties practically corresponding to development frequency, moreover metabolic rate and morphology [8-10].

On the premise of the oddity among *in vivo* and *in vitro* situations, innovative techniques are obliged to set up *in vivo* alike microenvironment. The utilisation of innovative microfluidic approaches to transposition of culture media, structures and oxygen despite the fact that the unwanted items by cells exercise are exhausted like a parallel to the human circulatory framework [11]. Moreover, numerous studies have devoted on systematic microstructures brought together into a microfluidic stage that executed specimen blending, cell movement and separation in a micro-channel [12,13]. Microfluidic structures can be a response domain for cell assay and the wellspring of an *in vivo* resembling setting for cell culture. Previous studies illustrated a basic two-dimensional (2D) microstructure utilised for cells cultivation and mainly employed to fabricate a microfluidic framework exhibiting required characteristics [14,15]. Although microfluidic tools attained sophisticated advancement with an end goal to understand impeccable *in vivo* conditions on a chip, tools have been adjusted for utilisation with polymer platforms and three dimensional (3D) microstructures, guaranteeing different layers for co-cultures or 3D cell development [1,16]. Hence, the present study covers the advantages and challenges of using microfluidics in cell culture. Also, the potential developments of microfluidic cells cultivation techniques and their associated bio-applications are presented.

2. Micro-arrays: Encaging cell trapping concepts

Practice to progression cells online in microfluidic frameworks crop up with the development of more propelled microfabrication advancements and configuration. The grasp of aerially controlled low volume elastomer microvalves with the progression of the analogous sample and cells preparing frameworks were elaborated by Araci and Brisk [17]. A particular cell catching hydro-dynamically at T-intersection in a microfluidic channel favored draft exchanging of required fluids, so that omitted the settled cell. It was approved that time determined filtering of ionomycin-intervened calcium flux from Jurkat T-cells [18]. Irima and Toner [19] explained an analogies way as Thorsen that pneumatically actuated polydimethylsiloxane (PDMS) valves engaging cells inside.

Lee et al. [20] developed a centrifugal power to drive microfluidic framework for trapping and stimulation of responsive single cell investigations. The cell catching spots included smart hollows in the side wall of molded capillary that exchanged cells to the catching hollows as well as confined them in a position within the applied pressure. Efficiency of entrapping cells was significant in the region of 50 and 80%. Capillary valves were unified and facilitated the allocation of fluids and samples [21]. The survival rate increased 20-30% higher in comparison to the 96-well plate assay for single cells analysis.

Another design configuration is based on the confinement of cells for seeding in a densely parallel configuration i.e. micro-well cages. Using a chip surface, individual cells were allowed to settle in each position in micro-wells [11]. Rettig and Folch [22] reported that PDMS micro-wells with variable diameter and depth of wells were reserved for ideal single cell confining of rabasophilic leukemia (RBL) and fibroblasts cells. After that, Yamamura et al. [23] described an analogy approach with 3000 wells on each polystyrene chip. Single cell occupancy up to 80% was reported for human blood or mouse spleen lymphocytes. The microarray scanning was practiced checking temporary alteration of Ca^{2+} level in antigen-specific cells individually when defined anti-mouse IgM antibody, setting off receptor of the B-cell antigen. A course to a swift increase of new monoclonal antibodies is recommended prompting the selection of the antigen specific B-cells from the array, magnifying the DNA recovery by RT-PCR and antibody c-DNA determination [24].

3. Microbial whole-cell arrays

The current development in microfluidic technologies revealed that there is a growing era of biosensors and an array of disciplines providing a base for combined future progress in both fields [25,26]. Microbial cell array system is distinctive as compared to old cell culture techniques such as multi-well plate methods. The microfluidic system significantly enhances the signals and enrichment of target [27,28]. These disciplines include immobilisation of biological components in different

polymers, genetic engineering, advancements of solid surfaces along with cellular viability for cells patterning and deposition [29]. Entire sensors have been recommended as potent devices to recognize a specific class of toxins, established upon bioavailability and biological activity [11].

4. Cell array biochips

4.1. Prokaryotic array biochips

Eukaryotic arrays, mainly engineered on mammalian frameworks, have the selective advantage of clearly simulating human cell reactions; prokaryotic cell frameworks have different balancing advantages. The accessibility of cells for growth and maintenance promptly available from huge homogenous populations and appropriate techniques are used for cell immobilisation and cell preservation. Prokaryotic cells are likewise most powerful and additionally have lower sensitivity to the biochemical and physical environment. They are vulnerable to biochemical infection [30,31].

Moreover, microscopic species are also susceptible to biological and physiochemical stimuli mandatory during the array formatting. Precisely, bacterial species can transmit their signals either chemically or physically in the established environment upon the availability of the target compounds or any change sensed in the surrounding environment. In general, this condition is attained by the combination of a detecting components and particular promoter to a described molecular system [32-35]. Couple of expressed independent reporter system has been reported in single organism [36-38]. In addition, reported sensors can identify particular analyte in distinctive media including soil, gas as well as water [39-41].

4.2. Eukaryotic array biochips

Although most research is on prokaryotes, eukaryotic cell arrays provide an adequate pattern to engineer high-throughput screening in the region of the whole cell array. It has been recommended that eukaryotic cell arrays can be utilised in different bio-applications such as micro-psychometry, gene and single cell analysis, bio-sensing and bio-identification of therapeutic agents [42-46].

The techniques utilised for developing cell arrays including various cells positioning that is different from lithographic approaches to inkjet technology [47-49]. Peculiar patterns involve utilising microcontact printing for designing of self-assembled monolayers and cell adhesion [50], photo- as well as electro-patterning of hydrogel-encapsulated fibroblasts arrays [51] and inkjet printing of sustainable mammalian cells [52]. Pishko and co-workers fabricated a three-dimensional array by introducing polyethylene glycol (PEG) hydrogel to test multi-phenotype of encapsulated cells (hepatocytes, fibroblasts and macrophages). The equal distribution of cells in each PEG hydrogel microstructure controlled via changing the cell density [53].

5. Microfluidics approaches in bio-applications

The microfluidic framework consists of the manipulation as well as control of fluid near to the micron level (10^{-9} to 10^{-18}). Along microchannels near to sub-millimeter extension, fluid flowing action is relatively different from a macro-scale system with complete characteristics of laminar flow that can influence shear forces as well as mixing near to the surface. Shear stress of fluid that designates the degree of applied force practiced to a surface parallel or tangentially is an essential characteristic of biofilm adhesion mechanism [54]. The simple microfluidic system holds straight channel that exhibits perfect laminar flow with no active or molecular based mixing determined by Stoke's drag as well as general effects surface tension. With an extensive series of daily life applications, the fabrication of devices is considered as an emerging technology, stabilising various micro-machining techniques, soft and polymer-based lithography such as PDMS devices. Moreover, the assimilation of

multi-functional Micro Electro Mechanical Systems (MEMS) devices: micropumps and valves as well as other dynamic mixers [55,56]. The active and passive mixing flow the integration of mini-actuators can easily achieve velocity and control over the reagents into microchannels. The development of chemical composition and spatiotemporal gradients of velocity in the microenvironment of laminar flow provides exceptional control over the flow. The different concentration gradients in the passive devices with no actuator can be developed in the microchannel by using dilution or mixing media in various logarithmic or linear levels [57,58].

The advantages of diffusion based microfluidic system to create steady and random chemical gradients outweighs the disadvantages while freely moving single cell bacteria analysis. The bacterial chemotaxis quantification, efficient analysis of dynamics of single or multicellular colonies in laminar flow as well as extraordinary throughput makes microfluidics an innovative application. As developments of *in vivo* bacterial biofilms intensify the protection as well as persistence of infections, the fabrication of microenvironment and utilisation for in *vitro* study should be preferred over traditional approaches to assessing antimicrobial agents. Minimum biofilm eradication concentration (MBEC) of yeast embedded biofilms can be 10 to 10,000 than minimum inhibitory concentration (MIC) of their planktonic counterparts [59]. However, antimicrobial susceptibility analyses of phenotypes of biofilm isolates are yet regularly assessed with classical methods covered by standard protocols. The dilution titer method is generally used for MIC calculation which helps to determine the antimicrobial agent's precise concentration required for growth inhibition or killing of planktonic microbial cells [54].

5.1. Microfluidic study of microbial biofilm

Biofilm characterisation has been carried out through various systems since the determination of "animalcules" by Van Leeuwenhoek in his teeth plaque in the 17th century. That notwithstanding, until 1978, not even a single common biofilm theory was published. According to the concept, the maximum bacterial growth took place in the matrix embedded cells which are adherent to nutrient rich surfaces of aquatic ecosystems. The sessile nature of these matrix embedded bacterial cells distinguished them from their planktonic cells [60].

Aquatic bacteria, in turn, stick to solid surfaces and construct bacterial community [61]. These bacterial communities are supported by extracellular polymeric substances (EPS). Microbes possess a protective shield provided by the biofilm matrix and apply mainly to various clinical trials, comprising (1) Symptomatic inflammation, (2) antibiotic resistance as well as (3) the proliferation of dangerous emboli [61-65]. Cells can exist within critical conditions, for example at elevated temperatures or antibiotics availability in biofilms. The biofilm matrix boasts a microbe's chance to spread within the structure. The dental hygiene and nosocomial diseases can be elaborated by such applications [66,67].

Various studies on biofilm evolution have observed genetically as well as ecological factors, comprising shearing stress [68], the topography of surface [69], signals of quorum sensing [70,71], thermodynamics and nutritional level [72,73]. Nevertheless, precise insights of the mechanism associated to an evaluation of biofilm and along that alloying consequence of all factors have not been properly determined. Such conditions favor, microfluidic analytical techniques lead to an auspicious scaffold for the research of bacterial biofilm. Microfluidics provide specific control over real time-flow, online observation accessibility, high-throughput trials as well as *in vivo* explicitly biological environments. An understanding to the protocol of development of bacterial biofilm and along with that, the utilisation of microfluidic assay might help to determine keys therapeutic approaches for biofilm infections or biofilm-concerned issues.

Biofilm formation by *Pseudomonas aeruginosa* has been explained by various researchers via a sequence of levels as seen in Figure 1b [74]. The inceptive two stages are distinguished by loose planktonic bacterial cells adhesiveness to a surface top as well as the development of EPS. Planktonic

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cells motility under the surface helps to determine the physical forces, such as van der Wall and gravitational forces, hydrophobic coordination as well as Brownian motion and electrostatic surface charge respectively. When cells of bacteria reach another cell or come near to the surface, explicit coordination among the respective bodies turns noteworthy [75]. The planktonic cells accumulation and biofilm maturation during biofilm formation process carried out at third and fourth steps. Biofilm structure is dependent on the nutritional levels and can be figured as a slab or mushroom fit as a fiddle [76]. At fifth level, detachment and dispersion of planktonic cells from the matrix of biofilm takes place [77,78]. However, many investigations are carried out to examine the preliminary steps involve in biofilm evolution, particularly the studies about detachment reveal that it is the largest source of biofilms associated diseases which would be helpful to develop better strategies for therapy [79].

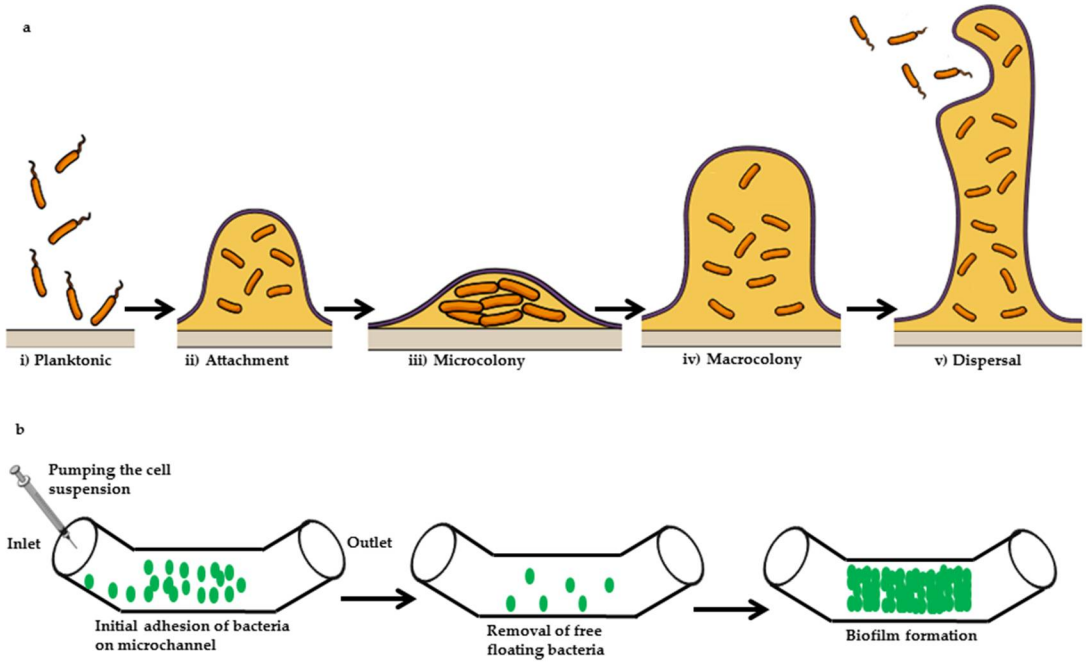

Figure 1. (**a**) Illustration of a general biofilm formation; (**b**) Microfluidic approach of biofilm formation. Initially bacterial cell suspension is injected into the inlet of microchannel of microfluidic device. Cells are allowed to adhere on the surface of microchannel and nonadhere cells are removed from the microchannel via using continuous flow of cells grown media. In subsequent final stage cells are allowed to form biofilm inside the microchannel.

Fluids, consumption in microfluidic devices at sub-millimetre scale that commonly strained to the full environment. Conveniently, devices are fabricated of wafers, glass, elastomers, plastics and papers. Various studies enforced microfluidic technology because of its significant compatibility; low volume liquid control, cells confining along with particles in a structural geometry, control of temperature and along with that specific gradient development, allowing moderate cost, fast and additionally accurate analysis. Bacterial biofilm studies are a promising stage given by microfluidic devices with an illustration presented in Figure 2. The closed system provided by microfluidic devices where biofilms of bacteria could coordinate with hydrodynamic settings. It permits formulating mathematical configuration that explain effects of such coordination and disclosing the consequences of the hydrodynamic environment (e.g., shear stress) on biofilms formation [80]. In such devices, the flow of fluid is relatively steady. It generates rapid response times because of the less Reynolds number and develops a gradient of chemical attractant as well as monitors bacterial chemotaxis. Concentrations, as well as translucency, allow online observation of the biofilm development by utilising high-throughput arrays. The limited diffusion time at limited scale supports planktonic cells to develop biofilm because dynamically favorable conditions are arranged. In

microfluidic devices, the conditions can be utilised to develop structures of the *in vivo* conditions in 3-D developmental stage [81]. Such characterisations are valuable not only for analysing the benefactions of all moving elements in the development of a biofilm, yet for reporting the allowed responses. An approach based on microfluidic can probably disclose the procedure in the biofilm development to fix various problems related to biofilm.

Micro-channels are fabricated by utilising PDMS. They allow online checking on the hydrodynamic environment where planktonic cells are cultivated. According to Bahar et al. [82] chambers of the microfluidic array are fabricated to determine the adhesiveness of Acidovorax citrulli. However, Lee et al. [83] determined the spatial alterations under shear stress inflicted in a PDMS device exhibited by biofilms with a fundamental conventional channel. During the dispersion stages or biofilm development in mature biofilms shear stress impacts negatively; although, throughout the initial moreover adhesion periods, shear stress facilitates biofilm development by proposing more nutrients as well as chance for dispersion [84]. Lack of stress facilitates the growth of a bacterial cell; biofilms development gradually correlates to the growth of cells under stressful situations. The geometry of flow channel can be fabricated to regulate the distribution of shear stress on biofilm [85]. Near various sites inside a channel, cells of bacteria can encounter different levels of shear stress. It alters the coverage of biofilm, thickness and viability. Assembled microfluidic channels explicate the alloyed impact of various effectors on the development of biofilm. The impact on biofilm eradication or detachment of Dispersin B hydrolysing enzyme or rifampicin antibiotic is configured by utilising poly-channel devices. Dispersin B as well as rifampicin medication activated most of the biofilms removal. Although at the intersection, because of inadequate shear flow the biofilm remained unmarked. The alloyed impacts of the hydraulics and medicines provide a constructive tool for the eradication of biofilms [83]. A strategy based on microfluidic could be priceless for constructing arithmetical models with these experimental works. Arithmetical model formulated by Janakiraman et al. [80] imparted on the development of biofilm within a closed system, where the growth of biofilm, as well as hydraulics conditions, are interconnected. Prognosis of the model is endorsed by utilising chambers of microfluidic device like a closed system. Constructed on synergy among the development of a biofilm, mass transport, and hydraulics, the calculations of the model are compatible with trials calculations. When bacterial cells develop biofilms, they systematically interlinked with their habitat and this synergy lastly impact to biofilms development. Directing confined habitats, strategy based on microfluidic authorise analysts to consider a community of viable cells as a biofilm that changed vigorously with its habitats.

In microfluidic devices, steady flow environment enhances flow-free development, balance gradients of subjective figure. In the growth of biofilm, the chemotaxis of uncontrolled swimming or bacterial cells adhered to surface exhibit an essential role. In microfluidic channels different cases of gradient development for the schematic study of bacterial chemotaxis are dependent on the flow. Devices having analogous or collateral movement entitled T-sensors control relayed on the convergence of trio stream that attach into a distinct micro-channel [86-88]. At the end of the channel bacterial dispersion is figured, generating a collective reaction to the emerging on streamlined gradient accomplished alongside the micro-channel [89]. Generators having flow-free chemotaxis design a degree of a slope relayed only on molecular dissemination and no flow is occurring. In this example, to build a preliminary gradient a flow structure is fabricated then flow is switched off by permitting the gradient to emerge by diffusion alone [90]. On a brief countdown when operated, the gradient in these devices can be proximate as being in steady-state. Whenever the countdown of the gradient relaxation is extended than the times of the sensing as well as behavioral mechanisms, these gradients are beneficial for chemotaxis quantification [89]. Integration of perforate substances like hydrogels or membranes allows the assimilation of stable chemical gradients in a condition within microfluidic devices totally relinquish of flow or shear [90]. *P. aeruginosa* adhesion on a surface altered the concentration of oxygen, which was fabricated utilising a gas permeable membrane and the introduction of dyes to the microstructures as described by Skolimowski et al. [72].

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Figure 2. Advantages of microfluidic approach for biofilms formation and eradication. Microfluidics provide unprecedented control over the flow condition, accessibility to real time observation, high throughput testing and *in vivo* like environment. Microfluidic approach helps to understand the mechanism underlying biofilm formation and to identify the problem.

5.2. Microfluidic approach to antimicrobial synergism

The utilisation of unified microfluidics is a tempting option for antibiotic susceptibility testing due to its associated benefits such as: low sample volume requirements, high-throughput, single cell sensitivity, the ability to obtain accurate bacterial cells information, and the propensity to quantify bacterial interactions in polymicrobial cultures. Various microfluidic platforms have supported these advantages for mono-microbial antibiotic susceptibility [5]. Moreover, microfluidic platforms have been used for studying co-cultures of cells in addition to cell interaction in 2D and 3D environment for several applications such as tissue engineering [91].

The autoinducer conciliated cell-cell signaling method is Quorum sensing (QS) among bacteria. It supports the development of a biofilm, resentment as well as various phenotypes of other multicellular. As antimicrobials, QS inhibitors are being examined due to their compatibility to lessen the manifestations of infectious disease and in the meantime minimising the development of resistant strains. Inhibition of genotypic QS responses has been explained by auto-inducer-2 (AI-2) analogs between various bacteria. Firstly, Roy et al. [92] explained the potential of C1-alkyl AI-2 analogs and isobutyl-DPD, particularly inhibition to maturation of *in vitro* cultivated *Escherichia coli* biofilms. Utilising a peculiar microfluidic system that integrates versatile, real-time computations, density and an alloyed method of biofilms is identified. According to this method, the combination of gentamicin and isobutyl-DPD is extremely implicated in yielding near total eradication of already available *E. coli* biofilms. Eradication of already available biofilms has lasted a serious issue of health care. These

computations approve meditations of a new protocol established on the alloying of "quenching" QS signal transduction methods accompanied by classy antibiotic therapy.

Using microfluidic platforms, the efficiency of an antimicrobial candidate has been investigated against wound model methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) biofilms. Dispersin B, a glycoside hydrolase enzyme, has been shown to constrain biofilm development in *Staphylococcus* species effectively, particularly *S. epidermidis* [83,93]. Dispersin B can be an effective entity for analysing the wound infection prototypes viability; obtained results can be confirmed by utilising well-recognised methods of micro-titter plate assay [94,95]. The efficacy of Dispersin B was previously analysed utilising classical approaches to investigate wound associated bacterial species viz *Klebsiella pneumoniae* and *Staphylococcus epidermidis* [96]. Nevertheless, the associated impact has not been determined on an alternative wound infection triggering MRSP bacterium. Multidrug-resistant pathogens may across at wound location, leading to huge indisposition and mortality rate as well as healthcare expenses for humans and animal respectively. The development of biofilm has been assumed the presence of exceedingly resistant as well as MRSP pathogenic clones [97].

Terry and Neethirajan, [98] assembled a microfluidic array to investigate antimicrobial agents' effect on wound biofilms associated microorganisms including MRSP. However, they were incapable of reviewing prior results that Dispersin B- gentamycin is greatly effective towards biofilms of *Staphylococcal* by using this array and capable of confirming its consequence in a micro-titter plate assay. Different experimental settings (e.g. strains, flow rate, exposure time, etc.) for analysis of biofilms are greatly accounted for better results.

Traditional approaches for analysing antibiotic resistance comprises of disk diffusion and broth dilution methods [99]. Microfluidic devices have the capability to develop infectious diseases management at point-of-care as well as the implementation of antimicrobial susceptibility test (AST) at clinics [55,100]. An essential condition for the prompt growth of bacterial species is the availability sufficient oxygen in the microenvironment [101]. In traditional orbital shaking bacterial cultivation approaches, bacterial growth sustained via the supply of oxygen in the media through vigorous shaking. Sufficient oxygen supply to cell and tissue cultures carried out by oxygenator usually comprised of bioreactors and perfusion circuits [102]. On the contrary, microfluidic systems have a larger surface to volume ratio due to the small length scale. This gives an easy, still effective, approach for oxygenation within a microfluidic cell culture system [103,104].

Leibovitz et al. [105] analysed that *P. aeruginosa* colonized in the oropharynx of those elderly patients which are disposed to feed with nasogastric tubes (NGT). Antibiotic susceptibility comparison was carried out between isolates of oropharyngeal derived *P. aeruginosa* and mucus cultures acquired from their hospital's bacteriologic research centre. Isolates derived from oropharyngeal *P. aeruginosa* were found highly resistance; noteworthy differences were found for amikacin antibiotic ($p<0.03$). The profile of pulsed-field gel electrophoresis for these microorganisms was analogues to oropharyngeal isolates of *P. aeruginosa*. Patients fed with (NGT) might behave as carrier of *P. aeruginosa* resistant strains.

5.3. Microfluidic approaches for in vitro fertilisation (IVF)

Notably, most research has centered on the upgrading progression of an embryo *in vitro* relying on the chemical composition of culture media. Although such studies have been verified to be highly fruitful and have doubtlessly participated mainly to enhance progress level subsequently supported reproduction. The sequential pair as well as systems comprising monoculture media has been improved. The growth of better quality blastocysts *in vitro* is currently an interested area [106,107]. Although the chemical demands of the growing embryo do not only require being valued, yet potential physical demands can also be major characteristics in the pursuing of upgraded *in vitro* environment. It is imperative that movement of the embryo via the reproductive tract of female additionally results in disclosure of the embryo to an alternating liquid, yet it gives steady mechanical

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stimulation, that can affect progression of embryo [108,109]. Moreover, physical characteristics of the culture platform have impact on the chemical composition of media through synchronising the chemical gradients that create about the progressive embryo. Understanding of the pre-implantation embryo enhances as well as recent methods of analysis and technologies evolve, testing of different unique platforms of culture to identify the consequence of physical along with motorised refinements resting on the embryo can assist in *in vitro* sequence of supplementary adaptations [110,111]. In addition, such platforms can lead to a likely way of upgrading different common strategies utilised among the laboratory of IVF and somewhere else.

The method used to restrict embryos to a limited area from long been are micro-drops, to acquire the assistance of the probable gain of factors: trophic autocrine or else paracrine. Conventional magnitudes of such drops primarily dimensioned from; 10 to 50 microliters [112], however, few can be less and can also be active along group and individual culture of the embryo. Solitary limitation in this method, although, is that fragment as well as coalesce within drops, that may lead to embryos being cultured in various concentrations of the medium along with being robust to categorise. Efficient micro drop plates are newly accessible to exaggerate this feature: drop/embryo disarticulation. They may be substantial in only some laboratories for a succession of the embryo by supporting revelation with the supervision of the embryos [112]. Additional to these, some other scaffold-free 3D cell culture methods are illustrated in Figure 3 [113]. Some of these such as the conventional hanging drop culture method has been used for initiating the development of embryonic bodies.

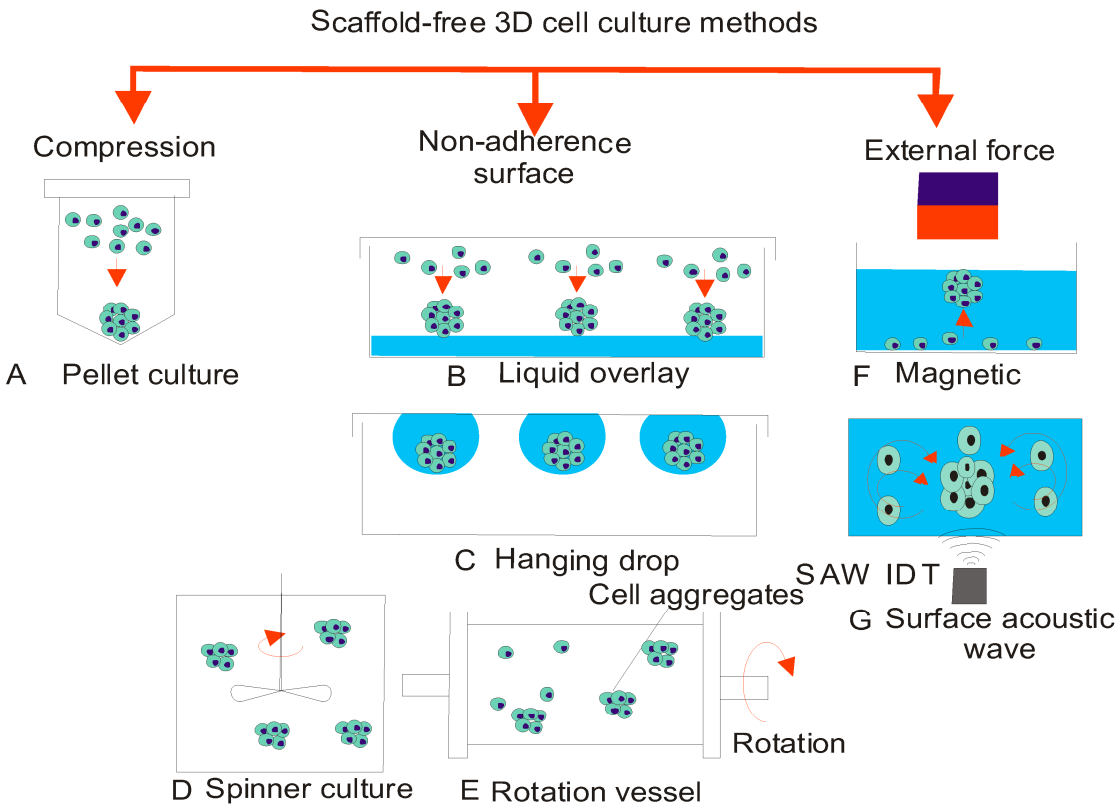

Figure 3. Images of platforms utilised for microfluidics-based culturing: (**a**) Test tubes; (**b**) Center organ culture dish; (**c**) Nuns culture plate having four wells; (**d**) Culture dish: GPS embryo; (**e**) Culture dish: Poultry Embryo. The reported platforms are fabricated with plastic cultivation, poly-styrene time, and has the potential to retain gametes and embryos [113]. Conventional methods for spheroid generation: (A) pellet culture; (B) liquid overlay; (C) hanging drop; (D) spinner culture; (E) rotating vessel; (F) magnetic force; and (G) surface acoustic wave. (Reproduced from Ref. [113], an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Moreover, the progression of the embryo, although much tempting is the ability of versatile systems of culture, major perfusional system and unifies different protocols of laboratory over a unit device. It was recently demonstrated by unifying IVF and the approaching culture of embryo promisingly over the correspondent microfluidic device retaining embryos of mouse [114,115]. Importantly perfusion culture platforms lead clearly by different media in the progression embryos, therefore by minimising interlinked pressure from persisted organisation of the embryos. Among dish on the way to the dish they are shifted and minimising stress from sudden alterations of varying media contents and concentration. According to this method, embryos would no longer be constrained to the demonstration to the isolated single unit or else progressive integer of culture media through 1 or 2 refilling in the culture duration. Such notion of manifesting gametes in addition to embryos gradually altering mediums can be beneficial for other ideas of cryopreservation, either steady rate freezing or verification. In two cryopreservation procedures, manual bit-by-bit alterations in the cryopreservation media indices activate shrinkage of the cell as well as re-expansion which may be lethal to later gamete or embryo cryosurgical plus/or else application. The potential to automatise deliberate cryosolution alteration in the cell, even if pondering the cell in an inactive stage, could have numerous bio-technical returns [110-112,116]. Low efficacy of Assisted Reproductive Technology (ART) stimulated researchers and technologists to explore new possible methods. The possible transformation of IVF primary mechanisms has been recognised in various studies. The superiority of microfluidic system during sperm isolation and collection illustrated through various advantages (short time analysis, high efficacy, online observation, natural microenvironment and automation of devices) over traditional assays. Although, it is considered that the evolution of advanced technology can compile entire experimental steps on a single device [117].

The non-invasive consequence of metabolism next to the single cell level will have numerous applications in organising cellular physiology. The solitary clinical application would be to compute the metabolic performance of embryos developed by way of assisted reproduction. There is validation that embryos with better developmental capability have distinct metabolic profiles [118]. Solitary of the criterion means pro evaluating embryonic metabolism has been to estimate consumption along with the production of various input active substrates (glucose, pyruvate, in addition to lactate) exploiting microfluorometric enzymatic assays. These assays are showed by hand exploiting pipes, which significantly confines the efficacy of this scheme. In the course of multilayer soft lithography, Urbanski et al. [117] have premeditated a microfluidic device that can disclose these assays in an automated manner. This scheme shows a sample with enzyme cocktail aliquotting, integration of reagents, and records acquisition in addition to facts investigation exclusive of operator intervention. Among the improved throughput along with flexibility of this scheme, numerous obstacles to the organising metabolism of embryos along with single cells are expelled. As a testimony of standard, metabolic actions of individual murine embryos were arranged via exploiting this device.

5.4. Microfluidic approaches for DNA analysis

DNA sequencing is an important step in genomics. Conversely, the Human Genome Project [108] was investigated many years back, DNA sequencing considers as a core course in studies designed at perceptive a range of diseases alongside by identifying prospective drug targets [119]. Diverse microdevices for DNA sequencing recognised ones that exploit microfluidic methodologies for Sanger sequencing, to facilitate the most uncomplicated conventional approach for *de novo* genomic sequencing [120-123]. Three stages including thermal cycling, sample purification, and capillary electrophoresis assimilated and practiced on the unique micro-device by Blazej et al. [124]. On the other hand, the sequencing length has got to be improved to accomplish the current sequencing stage; these micro-devices lead to the theory that assimilated microfluidic devices can be

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recognised for sequencing for additional uses such as low-cost proper sequencing or else for single-cell genome scrutiny [125,126].

Nucleic acid intensification techniques: polymerase chain reaction (PCR) plus the current thermal amplification is obligatory in every field of biology [127,128]. Pro-nucleic acid escalation, microfluidics leads to various recompenses while compared to conventional approaches such as reduced reagent expenditure, fewer escalation times, upgraded diagnostic throughput, with minimised risk of contamination, amplified sensitivity with assimilation. In the direction of plan an optimal taster in gene analysis scheme, micro-PCR has been recognised for utilising continuous-low, droplet-based microreactors with valve-actuated PCR micro-chambers [129,130]. The fabricated chip comprises 2.20cm in length with 90μm and 40μm width and depth of channels respectively. The individual channel frequently crossed via three precise temperature zones (95, 77, 60°C) utilising thermal settled copper blocks [129].

The exploitation of droplet-based microfluidics restrains the walls of channels from consequent by way of the polymerase with template DNA also apart from the bound DNA or enzyme that may lead to imitating the results, enhances reaction yield, also reduce contamination of samples. Hindson et al. [131] designed a digital droplet PCR (ddPCR) device for quantification of DNA. The structure was facilitated to process, in sync, 20,000 PCR reactions as of just about 20μl of reagent mixture. Ottesen et al., [132] fabricated a micro-PCR for single cell detection. Hindson et al. [131] fabricated a single step real-time PCR system so as to incorporate cell lysis with PCR on a chip to identify bacterial seeds. The assimilation of various cell lysis techniques in micro-PCR is exploiting thermal, chemical, physical and electrical methods [133].

PCR microfluidic chip exploits small volumes; appropriate consideration must be halted to restrain reagent evaporation while the thermocycling action [134]. Isothermal intensification techniques are economical and not labor intensive to restrain their exploitation in schedule investigations; first isothermal escalation approaches are an exceptional alternative to identify nucleic acids in microfluidics systems. Recently, different DNA-based microfluidic probes have been exploited along with dynamic dimension approaches such as surface plasmon resonance, imaging and fluorescence [135]. Hong et al. [136] designed a nanofluidic system for DNA recovery exhibiting three parallel processors. To get some useful forensic information, several steps can be distinguished, as shown in Figure 4 [137], in which the conventional techniques, as well as their existing microfluidic counterparts are listed.

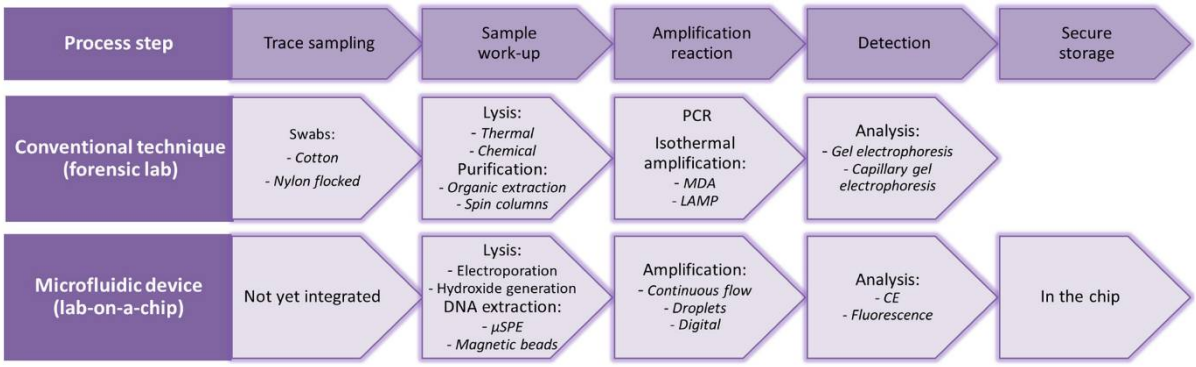

Figure 4. In the top row, the consecutive steps in the process of forensic DNA analysis are listed; within the middle row, the conventional technique with some examples. In the bottom row, microfluidic analogies can be found with some examples. (Reproduced from Ref. [137], an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

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The potential to create synthetic genes leads to a potent tool for genetic investigative fields: genomics plus transcriptomic also for small-molecule fabrication. Even if, high expenditure in recent times hinders *de novo* incorporation of DNA theories. Microfluidic oligonucleotide incorporation identifies a financial reserve to conventional approaches, need lesser amounts of reagents [138]. Kong et al. [139] have identified multi-chamber fabricated microfluidic coordination for synthesising a 1kb elongated gene to exploits small amount of oligonucleotides (1025nM) and reagents than expected techniques. Presently, a micro-device fabricated for 16 oligonucleotides that can gather into a 200bp elongated DNA [140]. The device is a valve-fabricated microfluidic device, coordinate to allow entity trial management and product assortment. Kosuri et al. [141] integrated a 35 kb of DNA encoding 47 genes containing 13,000 oligonucleotides.

In vitro genotoxicity, investigations categorise carcinogens that are measured to proceed mainly in the course of a method consisting straight genetic damage. Ahn and Gu [142] fabricated a Geno-Tox cell array chip for genotoxicity investigation; eight recombinant biolumi nascent bacteria were exploited to formulate a Geno-Tox cell array chip productively. Four well-characterised DNA damage chemicals were designated to set up the competence of this Geno-Tox array chip as well as all strain was typically alert according to the precise form of the genotoxic act. Even though, this Geno-Tox cell array chip could be exploited to categorise and value the genotoxic modes of impact; accordingly, it could be exploited to grant the genotoxic means of action for novel drugs or anonymous or recently formulated chemicals in cooking also the atmosphere.

In previous years, many gene expression characterising processes have established and also progressively applied to research. They comprise discrepancy show, subsequent investigation of gene expression and microarrays [143,144]. Sixteen prostate cancer specimens were differentiated from nine Benign prostatic hyperplasia (BPH) samples by Luo et al. [145] that was consisted gene expression modifications as tested on 6,500 cDNA microarrays. Different groups utilising subtractive hybridisation as well as microarray analysis have characterised many prospective immunomodulatory candidates for prostate cancer treatment: protein, six-transmembrane epithelial antigen of the prostate (STEAP), and prostate cancer antigen 3 (PCA3) [52,146]. Prostate cancer cell line is recruited by Virolle et al. [147] which exhibited a higher constitutive level of Egr1 protein, a transcription factor overexpressed mostly in advanced tumorigenic prostate cancer cells. They occupied a pair of consequent subtractive DNA as well as testing of the microarray to compute novel genes principally for squamous cell sarcoma of the head and also neck (HNSCC) as potential markers of tumor and antiserum entities. Nine recognised genes were acknowledged to be correctly overexpressed in HNSCC relatively to healthy tissue. Likewise, in sarcomas subset, four unique genes were overexpressed.

The transcriptome in oral cavity squamous cell sarcoma was acknowledged by Alevizos et al. [148]. The identifications of six hundred participant genes that comprised transcription factors, malignant genes, differentiation markers, tumor suppressors, metastatic proteins also xenobiotic enzymes which were vigorously tinted in oral cancer, certifying merely a trio of these genes via PCR. The breast sarcoma technical heterogeneity, microarray system may be an excellent tool for accurate auxiliary presentation. Primal experiments exploiting microarray-based expression profiling discussed its capacity to organise estrogen receptor accurately- negative with breast sarcomas consisting estrogen receptor- positive and to categorise BRCA1- equivalence sarcoma commencing BRCA2- analogy also sporadic tumors [149-151].

Alizadeh et al. [152] studied eight hundred and sixty-four DNA constituents screened in resistant to ten ovarian cancer cells lines as well as 5 in the pink epithelial cell lines exploiting limited cell seeding to enlarge the epithelium surface of ovarian before the exclusion of RNA. They documented the 2 types of diffuse large B- cell lymphoma (DLBCL) comprised on gene expression protocols which are indicators of different levels of B-cell isolation. This categorisation of molecules has characteristic value independent of stratification by the uncomplicated scientific grading. In lymphoid malignancies

to elucidate gene expression, a strong coordinating community recognised a proficient microarray: Lymphochip. It is supplemented in genes which are correctly exhibited in lymphocytes auxiliary in adaptable lymphocyte function genes. Marcy et al. [153] have developed a conventional microfluidic device that permits the isolation as well as genome amplification of entity microbial cells, thus, permitting organism genomic investigation of complicated microbial environments' devoid of the need for culture.

5.5. Microfluidic outlook for protein analysis

In various life sciences related fields including drug discovery or pathogen detection, inspecting each cellular protein have central importance [154]. Within the cell, however, proteins are mostly found in intensively limited copy numbers that develop identification of the proteins in the adventurous single-cell analysis [155,156]. Protein analysis among multiple or single cells microfluidic reactors has been carried out [157]. Taniguchi et al. [158] have designed an automated image rotated microfluidic platform to compute the proteome of *E. coli* as well as in single cells transcriptome along with its single-molecule sensitivity. A chip of PDMS was encompassed of 96 separate channels allocated to clench 96 discrete samples in collateral channels [158]. All channel quantifications: (w)× (l) × (h) = 150μm × 10 mm × 25 μm and to immobilise bacteria within the micro-channels, these channels were pre-coated using poly-L-lysine [159]. Additional method is flow cytometry for organising protein expression with the purpose to count microscopic details [160]. At this time, micro-devices scrutinised antibody staining as well as transfection competency of the green fluorescent protein (GFP) [161,162]. For instance, a commercial lab on a chip device (Bio-analyzer, Technologies GmbH) exploited to ensure protein expression in cells [163].

Enzyme-linked immunosorbent assay (ELISA) plates all along concurrent with bacterial cells solid phase immunoassays immobilised at the same time as antigens have been with altered bacteria exploiting antigen–antibody dexterity on cell facade. Principally, whole cell assays are favorable when the antigens that respond all along with the antibodies in opposition to a definite pathogen are unidentified [164]. Likewise, pure antigens segregation is a bit complex and labor exhaustive [165]. Joint reimbursement of a cell-based immunoassay with the compensation of a restrain array configuration. A bacterial array biochip, exploited for the antibody revealing and also Gram-negative well as Gram-positive strains were pre-printed all along with a microarray copier on nitrocellulose-coated glass substrates [166].

In tissues, a protein expression acquired from squamous cell carcinomas of the oral cavity via an antibody microarray technique have been ascertained by Hanash and Schliekelman [167]. Micro-dissection ambushed by exploiting laser to prompt complete proteins driving out of microscopic cellular communities. They inspected potent expression of diversified proteins in stromal cells encompassing as well as in vicinity suburbs of diseased epithelium which are merely equated along with tumor progression of the epithelium. Paweletz et al. exploited an emphasising reverse arrangement of proteomics microarray that provoked proteins deriving out of patient specimen was pre-printed onto a facial and also in prostate cancer permitting disguise of biochemical signaling arrays [168]. Rapid investigations perceived autoantibodies amid peculiar intracellular as well as facial antigens which were attributable in the serum driving out of patients along with peculiar cancer categories [169]. One more technique for diagnosing tumor antigens which surpass an immune feedback exploiting a whimsical peptide-library arrangement was pre-printed [167]. Lastly, the discovery and modern-day innovated integration of modern technology in the fabrication of microfluidic devices will not only enhance the productive output as well as it will make an attractive target for different bio-applications.

6. Conclusion

Over the years, many researchers have proposed and developed unique technological tools, both at the micro and nano levels, for fabricating microfluidic-based network devices, cell-based analyses,

cell-based manipulation systems such as optical, electrical, mechanical and magnetic manipulation methods. However, there still less defined standard methods and the entire fabricating process is solely reliant on various critical aspects that are hinged on: (i) application, (ii) device channel geometry (dimensions), (iii) integration type (single or 3D), (iv) network integration with electronics or sensors and (v) material system. Microfluidics present is a wider spectrum of potential applications with opportunities for enhanced performance through automation, controlled flow speed, portability, cost-effective ratio, minimal energy consumption and little or no waste products. Microfluidic-based techniques for the study of cell bioprocessing e.g. cell separation and cell lysis are well developed and documented in the literature. However, many new developments such as organs-on-a-chip and lab-on-a-chip are taking place to revolutionise the current microfluidic technologies at an advance level. In this context, organs-on-a-chip and lab-on-a-chip program are likely to remain the subject of intensive investigations in the different sectors of research and material-based industries. In summary, microfluidic approaches have become significant in the foreseeable future of biotechnological innovations.

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