Microfluidic Bioreactors for Cell Culturing: A Review

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Abstract: The use of microfluidic bioreactor platforms for cell culturing holds considerable promise for a range of fields which include drug discovery, tissue engineering, bioprocessing optimisation and cell based screening studies. Microfluidic bioreactor systems have length scales that are well matched to the physical dimensions of most cells and microorganisms. In view of this, microfluidic bioreactors have attractive features which make them ideal to study the behaviour of cells and their internal organisation in their native microenvironment. Due to their small footprint microbioreactor platforms offer a number of advantages over conventional macroscale systems including improved biological function, higher quality cell-based data, reduced volume of reagents, ease of integration and lower cost. This review highlights the basic concepts, designs and operational requirements of microbioreactors for cell based studies. An illustrative outline of different applications of microbioreactors and some indication of new trends and progress in recent years are provided. Specific examples of applications of microbioreactors are drawn for cytotoxicity assays, tissue engineering, stem cells, microbial fermentations, single cell analysis and *in vitro* fertilisation.

Keywords: Bioprocessing, fabrication, microbioreactor, microenvironment, microfluidics, *in vitro*, *in vivo*.

1. INTRODUCTION

An effective *in vitro* cell culturing bioreactor platform should reliably and reproducibly mimic the *in vivo* microenvironment of the cell. To achieve this goal requires the identification and understanding of the role played by the *in vivo* cellular microenvironments (*also known as the* "cell niche") which regulate the cell functions such as proliferation and differentiation. The greatest challenge of *in vitro* cell culturing is the ability to recreate the physical characteristic of the cells' or tissue's native environment and to be able to manipulate the factors that govern the cell function. The *in vivo* cellular "microenvironment" is a complex set of physical, chemical, and biological conditions that surround the cell and enable it to perform its desired functions with great efficiency. The *in vivo* cellular microenvironment is made up of a complex blend of various components which include (i) extracellular matrix (ECM) [1-2], (ii) biochemical factors such as cell-cell and cell-matrix interactions, (iii) concentration gradient of soluble factors such as cytokines and glucose [3], (iv) O_2 availability and (v) a combination of physical factors (hydrodynamic shear, mechanical compression or stretch) and electrical signals exerted on the cells [2,4]. In the *in vivo* cellular microenvironment, cells continually sense the inputs that have been mentioned, process the information through signal transduction, communicate with other cells, perform genetic regulation, and execute behaviours that determine the cell fate. In their native microenvironments, animal cells exist within complex and organised three dimensional (3D) cell communities that form tissues under the support of the ECM [5-7]. The ECM is the extracellular part of the tissue which provides structural support and anchorage for cells and tissues. It is made up of collagen, elastin, vitronectin, fibronectin, tenascins, laminin, glycosaminoglycans (GAGs) and proteoglycans. In addition to providing support and anchorage, the ECM is involved in regulating the intracellular communication, normal homeostasis function and segregating tissues from one another [8].

Cell culture of bacterial and mammalian cells has been traditionally carried out in conventional bioreactor devices such as bench scale bioreactors, culture flasks, culture-dishes and microplates. However, these tools have fundamental limitations in that they (i) are not amenable to high throughput screening (ii) are bulky and consume a lot of resources (iii) are labour intensive and time consuming to operate and maintain (sterilizing, cleaning, assembling and disassembling of the bioreactor components) (iv) they generate large volumes of metabolic waste which may be toxic to natural ecosystems and costly to manage (iv) the sampling process is prone to contamination due to the number of manipulations that are made. Conventional tools also have unpredictable time scales and process variables, such as temperature, pH and partial pressure of oxygen (pO_2), are all difficult to control. Culturing cells in 2D monolayer environments do not necessarily produce results that are a true reflection of the *in vivo* microenvironment. For example, cells cultured in 2D monolayer format such as petri dishes lack the 3D matrices that are found in cells when they are in their natural microenvironment.

In response to these challenges, there has been a drive during the last decade to develop microfluidic devices, commonly known as microbioreactors that operate at the time and length scales proportionate with cellular phenomena and that are inexpensive to fabricate and ideal for high throughput screening, to specifically study the biological behaviour of cells. Microfluidic technology has the potential to facilitate the creation of the *in vivo* like microenvironment, since the scale of operation is similar to the dimensions of most cells.

Microbioreactors (biochips or cell-chips) are a scaled down version of conventional bioreactors, where cell based assays or biochemical active substances derived from such cells are carried out [9]. Microbioreactor systems have monitoring and control features similar to those found in macroscale bioreactor systems.

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Microbioreactor platforms enable low cost high-throughput screening in contrast to their macroscale counterparts. In the development of growth medium formulations and strain improvements cells can be cultured in parallel microbioreactor platforms to find the best combination of nutrients, including sugars, amino acids, vitamins, minerals and hormones. While in strain selection, clones can be screened against various media formulations and process variables to determine the optimal conditions [10]. Thus using microbioreactors in cell based assays experimental data can be generated in parallel, real time at low cost and in a high throughput manner. HTS platforms have the advantage of allowing large numbers of statistically designed experiments to be conducted in parallel such as in the production of biopharmaceuticals, which requires extensive optimisation of processes before scaling up.

The small size of microbioreactors allows low power consumption, portability and reduced space requirements. Microbioreactors utilise smaller volumes of reagents and samples (cells) than their macro scale counterparts. Consequently, this makes it cheaper to manage and manipulate small populations of cells and to study their behaviour in greater detail. Small volumes are also particularly important for minimizing waste when working with hazardous biological materials [11].

Microbioreactor platforms have structures with length scales that are similar to the intrinsic dimensions of eukaryotic and prokaryotic cells and the length scales of diffusion of oxygen and carbon dioxide in tissues [11]. An important characteristic of using microbioreactors is that fluid flow in the channels is laminar. Laminar flow systems are ideal for cell analysis as cells can be exposed to controlled chemical gradients and their biochemical and morphological responses studied *in vitro* [12]. Laminar flow based linear gradient generation in microbioreactors have been demonstrated in several publications as a way of studying cellular response to chemical stimulation [13-18]. Laminar flows are described in Section 3.2 of this review.

The small transport distances and low volume requirements in microbioreactor platforms enable fast responses to environmental stimuli in studies involving spatial and temporal gradients of factors [19]. The characteristic short distances in the microchannels results in the reduced transport times of mass and heat which is ideal for local transport of growth factors secreted by growing cells in the cell's microenvironment in a manner similar to the *in vivo* microenvironment [20-21]. Details of the effects of scaling on microbioreactors are discussed in Section 3.2.

Microbioreactor platforms are manufactured from relatively low cost polymer materials using techniques such as soft lithography. Many component elements, including fluidic, mechanical, electronic and optical components, can be integrated on microbioreactor platforms for more functional systems. The advantages of microfluidic systems over macroscale systems have been previously described [21-23]. These advantages offer the potential for microbioreactors to become useful tools for studying cell biology, biomedicine and drug screening [24].

Over the last decade, the application of microbioreactor platforms in bacterial and animal cell culture studies and in studies of tissue morphogenesis has increased at a very dramatic rate [25]. Zhu *et al*. [26] has described developments in microbioreactors up until 2005, covering aspects of designs, operation and application. Most of the research has focused on applications in: (i) microbial bioprocessing [27-28] (ii) stem cells [29-30] (iii) single cells [31] (iv) drug development [32] and (v) cytotoxicity [33]. In this review we consider some basic concepts, designs and operational requirements for microbioreactors for cell-based studies. Specific examples of the applications of microbioreactors are discussed in Section 5 and summarised in Tables **1-3**.

This review will provide an overview of the operation modes of microbioreactors and description of the key design and operation requirements that are necessary for effective microbioreactor operation. A description of microfabrication approaches for the manufacture of microbioreactors will be provided along with microbioreactor applications and the future outlook.

2. OPERATION OF MICROBIOREACTORS

Like their macroscale counterparts, microbioreactors can be operated in a variety of modes, such as batch feed, semi-batch feed and continuous feed. In a bioreactor, cell growth takes place in three successive stages, such as lag, exponential and stationary phases.

In a batch-operated microbioreactor, an inoculum of known concentration is introduced into the bioreactor at the start of the batch cycle, with the removal of the product at the end. During the cultivation period no additional growth media or cells are added to the bioreactor. Batch feed bioreactors represent a semi-closed system with static culture conditions. The majority of microbioreactors reported in the literature for microbial fermentations such as those reported by [34-37] are operated as batch fed mode. Batch bioreactor systems provide a number of advantages, including minimised contamination (due to short times of growth) and higher raw material conversion levels, resulting from a controlled growth period [38]. A major drawback of this type of operation is that as the cells grow, nutrients become depleted and the environment around the cells becomes flooded with metabolic end-products which are toxic and retard cell growth.

In semi-batch fed (also known as semi–continuous fed) operation, the bioreactor is inoculated with a known cell density, the cells are allowed to grow for a certain period of time until the culture approaches the early stationary phase. At this point, a large proportion of the culture broth is harvested and the bioreactor is replenished with fresh growth medium and the cycle repeated.

Continuously fed microbioreactors are characterised by the addition of culture medium at constant rate at the inlet of the microbioreactor and removal of the medium with cells at the same rate at the outlet. A chemostat is a good example, here cells are continuously removed and a steady state is maintained in continuous perfusion of culture, whilst metabolised cell free medium is removed through the outlet. In contrast to closed batch fed operations, continuously operated microbioreactors systems have the advantage of eliminating the lag and stationary growth phases; cells remain in

steady state of growth and thus the cell biomass, substrates and product concentrations remain constant and the chemical environmental conditions can be adequately defined and maintained independently of growth rate [39-41].

3. REQUIREMENTS FOR MICROBIOREACTORS

In the design and development of effective microbioreactor platforms, a number of aspects need to be considered. These include; material compatibility, mechanical (shear stress), mass and heat balance, physicochemical factors (oxygen tension, pH, CO_2, temperature), fluidic, sensing and control elements. Some of these designs and operational attributes are described in this section. A summary of the factors that contribute to successful microbioreactor design are shown in Fig. (**1**).

3.1. Material Compatibility

In biological processes, the primary requirement of any material substrate used in the construction of functional devices is that it must be biocompatible. The biocompatibility of a material can be defined in terms of its surface properties, these properties will affect the adherence of the cells and cytotoxicity. Thus, there is a need for the microbioreactor substrate to be chemically inert between itself and the fluid sample so as not to affect cellular growth [42]. The choice of the substrate material depends on a number of other requirements of the integrated system. For example, in optical measurements the substrate material is required to be optically transparent in the relevant region of the spectrum.

There is also a requirement for the substrate material to be sterilisable so as to eliminate bacteria, fungi and other foreign organisms that may interfere with the experimental work. Sterilisation can be achieved by using a variety of techniques, such as heat, chemical, filtration, and irradiation. The choice of the sterilisation method should not interfere with the material composition of the devices. For example, sterilisation of microbioreactors by heat may result in deformation that may affect the optical quality of the material. Sterilising microbioreactors with chemicals, such as passing 70% ethanol through the microchannels, can be effective. Another common approach for sterilisation includes irradiation with UV light, at 254 nm [43-44]. Exposure of bacteria, viruses and other microorganisms to UV radiation, results in the damage of their DNA leading to cell death.

Microbioreactor devices may be fabricated from a variety of materials, including polymers, ceramics, metals silicon, glass and wax. Polymer substrates are broadly classified as thermoplastic, elastomers and duroplastic polymers [45]. Thermoplastics and elastomers have emerged as the preferred substrates for microbioreactor devices due to their thermal stability, biocompatibility, ease of fabrication, transparency, gas exchange and their potential to be used with high replication for low cost devices [42,46]. The other significant advantage of polymer materials is the ease with which their surfaces can be modified using relatively inexpensive methods. Material surfaces in microbioreactors can be modified to promote cell attachment and or to prevent adsorption of proteins on the surfaces. For example, surface patterning techniques, such as standard photolithography liftoff techniques, photoreactive chemistry and soft lithography (microcontact printing and fluid patterning) are increasingly useful to engineer materials for cellular studies [47]. Polymers have advantages over glass and silicon substrate in respect of cost and compatibility but are often less attractive in terms of optical properties as compared with glass substrates and the difficulty of integration with electronic circuitry in comparison with silicon. Hybrid devices, which use a multi-material combination of polymer, glass and silicon can offer some advantages over monolithic devices but at the cost of some potential fabrication complexity. A summary of the properties of materials used for making biomicrofluidic chips is described in [48].

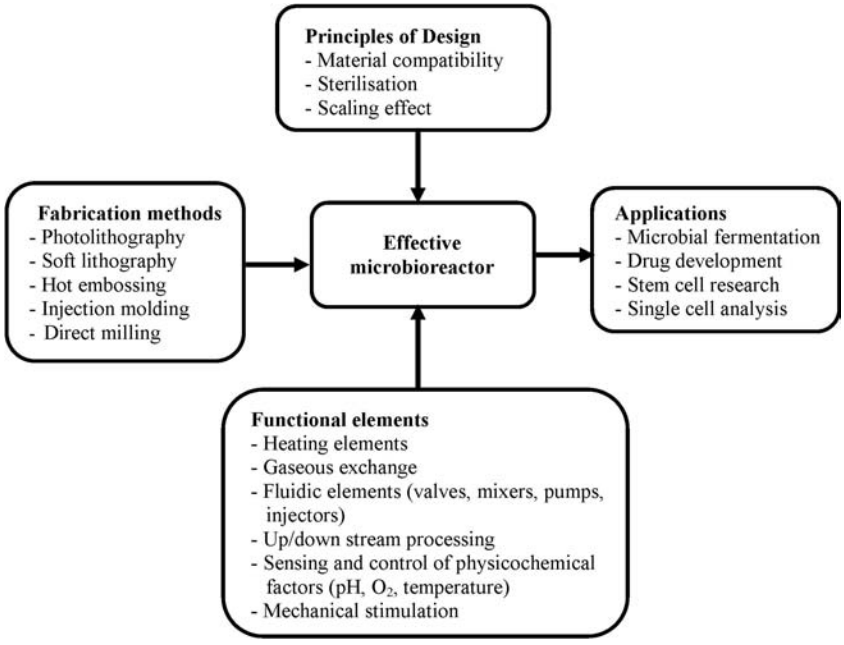

Fig. (1). Summary requirements for effective microbioreactor platforms.

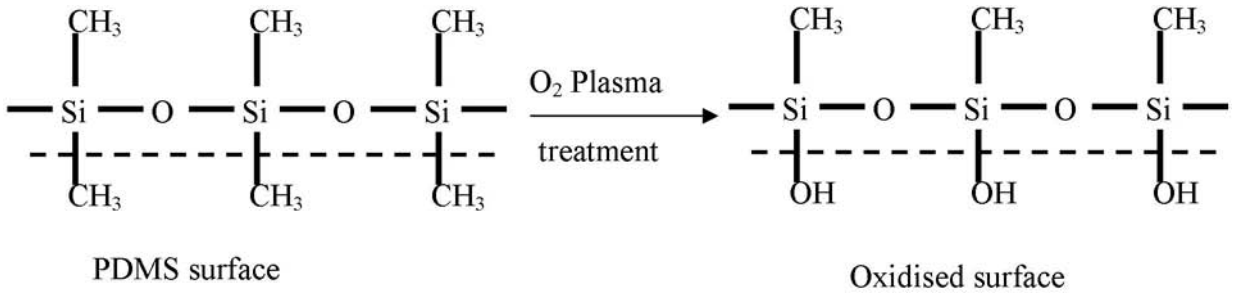

Fig. (2). Surface modification of PDMS by oxygen-plasma treatment [51].

Amongst all the polymers reported to date, PDMS is the most extensively used material for the fabrication of microfluidic devices for biological application. This can be attributed to numerous advantages including permeability to O_2 and CO_2, elasticity, ease of fabrication using prototyping approaches, creation of structures with high fidelity, integration with other system components and non-toxicity. PDMS has good optical transparency from the UV to the IR (230-1100 nm) regions of the electromagnetic spectrum, which makes it suitable for the integration of optical detection as part of microbioreactor platforms [49-50]. The PDMS surface can be easily modified by plasma treatment to make it hydrophilic (Fig. **2**). PDMS consists of repeating units of O-Si $(CH_3)_2$. By exposing PDMS to O_2 plasma, the CH_3-groups on the PDMS-surface are removed and substituted by polar groups (OH) to create silanol (Si-OH) groups on the surfaces, thereby rendering them hydrophilic [51].

The presence of the silanol (Si-OH) groups on the PDMS surface makes it more reactive to other silanes. When the oxidised surfaces are brought into contact, irreversible bonding occurs between the PDMS-PDMS layers [52- 55]. The elastomeric nature of PDMS makes it ideal for the production of integrated pumps, micromixers, valves as well as allowing easy incorporation of fluidic interconnects for the macro-world interface to the microfluidic device [55-57].

Zhang *et al.* [58] developed a microbioreactor fabricated from poly(methylmethacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS). The microchannels of the microbioreactor were coated with poly(ethylene glycol) (PEG)-grafted poly(acrylic acid) (PAA) copolymer films to prevent chemotaxisial back growth of bacterial cells, i.e. unwanted upstream flow of cells with a risk of contamination of parallel microchannels. The modified surfaces of the microbioreactor effectively reduced cell wall growth of *E. coli* for a long period of cultivation. Huang *et al.* [59] described preventing non-specific adsorption of proteins to PDMS channels by coating the walls with n-dodecyl-β-D-maltoside. The limitation of this approach is the potential for the disruption of the coated material during perfusion of medium. Protein and enzymes can also be immobilised on the surfaces as a way to promote cell growth. Monomolecular thin films such as self assembled monolayers (SAMs) or functionalised polymers with appropriate binding end groups can be used to coat the surfaces of microbioreactor channels [60].

3.2. Scaling Effects

The small microfluidic channels mean that microbioreactors are governed by physical effects. As the length scale of the microbioreactor decreases the surface to volume ratio increases. As a consequence, the fluidic dynamics behave in a non-intuitive way and become dominated by surface tension, fluidic resistance and capillary forces. Diffusive mixing becomes more important than turbulence, convective mixing and gravitational forces [61]. This has resulted in entirely new ways of obtaining biological, and physical information as well as enabling the creation of new types of assays [62].

In the small microchannel widths of microbioreactors, fluid flow tends to be laminar characterised by Reynolds numbers (Re) less than 2300. The Reynolds number is a dimensionless quantity which measures the ratio of inertial to viscous forces *(Equation 1)*.

$$Re = \frac{\rho dc}{\mu} \qquad \text{Eq. 1}$$

Where ρ *(kg* m^{-3}) is the density of the fluid, $\boldsymbol{d}$ (*m*) is hydraulic diameter of the channel, $\boldsymbol{c}$ (m s^{-1}) is fluid velocity and $\boldsymbol{\mu}$ **(**N s m^{-2}**)** is kinematic viscosity of the fluid. Laminar flows patterns have characteristic steady streamlines which are easy to predict and control. A Reynolds number (Re) higher than 2300 corresponds to turbulent flow [61-63]. In laminar flow, the viscous forces are dominant over the inertial forces and there is little macroscopic advection between the fluid layers.

Furthermore, the high surface to volume ratios are useful in facilitating fast and controlled thermal energy transfer effects and high diffusion rates to provide much greater control over cellular microenvironments. Due to these fundamental properties of fluids in miniaturised devices, microbioreactor platforms can easily be heated and cooled rapidly, hence as a consequence there is a need to integrate reliable temperature control systems to avoid such abrupt fluctuations. A comprehensive description of the important physical phenomena in microfluidic systems can be found elsewhere [64-65].

3.3. Physicochemical Factors

3.3.1. Temperature

Temperature is an important environmental factor for the growth of cells. Cell doubling time as well as enzymic processes are dependent on temperature. Scapper *et al.* [27] and Geschke *et al.* [63] have thoroughly reviewed temperature and pH control in microbioreactors. The main sensing elements for temperature in microbioreactors are thermocouples, thermistors and resistance temperature detectors (RTD). Platinum RTDs are often the preferred

sensing elements because of cost, size, ease of integration, accuracy and reproducibility. They are also sufficiently robust to allow operation for long periods of time.

Temperature control in microbioreactors is a challenging task due to the characteristic high rates of heat transfer when compared with macroscale systems. Microbioreactor platforms have a tendency to gain and lose heat very rapidly, leading to abrupt fluctuations in temperature. This requires the integration of precise control systems. Temperature control efficiency in microbioreactors is often affected by the position of the heating element in the system. Thus there is a need to position the heating elements in areas where they do not create high temperature gradients [66]. This method is particularly useful for materials with low thermal conductivities such as PMMA and PDMS [67]. Temperature can also be controlled in the microbioreactors by using programmable temperature controlled incubators [68-69]. Although, temperature control using an incubator is simple it is more difficult to carry out parallel experiment under different operating conditions and with a small device footprint. Another method of temperature control commonly used by scientists is the use of a controlled water bath where the base of the microbioreactor can be connected to a water bath and thermostated water circulated in and out from the base. This approach has been used in microfluidic bioreactors for microbial bioprocessing developments [28,35,58]. Microheaters are an important tool for controlling temperature in miniaturised devices due to their size and the ease with which they can be integrated in such microfluidic devices e.g. using photolithography [103-104].

3.3.2. pH

The ability to control pH is one of the most important functional requirements of any bioreactor system. An optimum pH is critical to achieving high cell density growth, and efficient enzymic activity [70]. Also, protein configuration and activity are pH dependent, similar to cellular transport processes, reaction rates and growth rates. When the reaction is not within the optimal pH range, the reaction rate declines drastically.

Fluorescence indicator dyes have been used to monitor pH and dissolved oxygen in cell cultures [35, 71-73] and have advantages of high sensitivity and low cost but suffer from photobleaching and a narrow pH range [27]. Effective pH control in microbioreactors can be achieved by using buffer but can suffer from limited buffering capacity.

3.3.3. Oxygen

Oxygen availability to cells is a critical parameter that must be addressed adequately in all designs of microbioreactor that are based on aerobic fermentation. Oxygen is required for the metabolic processes in many biological systems such as fermentation and the production of ECM. Mammalian and microbial cells require constant replenishment of dissolved oxygen into the medium because of the low solubility of oxygen in aqueous solution (7.36 mg/mL at 25°C in distilled water) [74]. The consequences of hypoxia, or inadequate oxygen, in cells and tissues include reduced metabolic rates and vasodilatation [75]. Maintaining suitable oxygen concentrations is of particular importantance in the culture of highly metabolic cells, such as hepatocytes, in microfluidic devices [21,76]. Due to the importance of oxygen in cellular events, precise control and monitoring of oxygen is critical for cell culture developments in microbioreactor systems. Measurement of oxygen can be carried out using by fluorescence quenching of an indicator dye or electrochemical sensing.

The availability of oxygen in conventional bioreactors systems can be increased by implementing a number of mechanisms such as surface aeration, bubble aeration and shaking [77]. Membrane aeration is the most commonly used method for the supply of oxygen to microbioreactors (volumes 100-500 μL) [28, 34-35], this is not the case for macroscale bioreactors. The oxygen demand of cells in microbioreactors is therefore met by the diffusion of oxygen through an oxygen–permeable membrane such as those made from PDMS, diffusivity of oxygen in PDMS is $3.4 \times 10^{-5} cm^2/s$ [78]. One of the advantages of microfluidic systems is the reduced mixing times that result from small diffusion lengths. The high surface to volume ratio of microbioreactor systems creates a large interfacial area over which sufficient oxygen can diffuse and meet the requirements of the cell. Membrane aeration therefore facilitates high diffusibility of O_2 and CO_2 into and out of the microbioreactor systems.

3.4. Mechanical Elements

In their *in vivo* microenvironment, eukaryotic cells are under constant mechanical influences which dictate the metabolic and functional pathways of a number of specialised cells in muscles, heart, lungs and other tissues [79-80]. The effects of mechanical forces on ECs in microfluidic cell culture flow systems have been reviewed by Younga and Simmons [81]. The ability to simulate the conditions that cells experience inside the body such as creation of mechanical strain due to shear, in the physiological range, is an attractive aspect of using microbioreactors [21]. When cells are cultured under microbioreactor environments they are under constant perfusion of nutrients, oxygen, gradient of chemicals and exposure to mechanical shear stress [82]. Due to the smaller channel dimensions, cells are subjected to a higher stress gradient when cultured in microbioreactor devices. Shear stress is defined as a tangential force that is applied to the surface of an object [7]. The shear stress present in a microfluidic channel can be represented by a mathematical relationship with the Navier-Stokes equation for Newtonian fluid flow between parallel plates *(Equation2)* [83].

$$\tau = \frac{6\mu Q}{h^2 w} \qquad \text{Eq. 2}$$

where τ is the shear stress, μ is the dynamic fluid viscosity, Q is the fluid flowrate, h is the channel height and w is the channel width.

Fluid-dynamic stresses have been observed to influence the adhesion of leukocytes to the endothelium and the tendency of the blood to clot [84]. Yamamoto *et al.* [85] reported on the effect of shear stress on inducing differentiation in mouse embryonic stem cell (mESC), while endothelial cells, which make up the cardiovascular system, are thought to require a certain amount of laminar shear stress for their normal function [86]. In designing microbioreactors it is important to take into consideration the high shear stress

gradients, given in *Equation 2,* and the effects it produces on the cells that are cultured under continuous flow. The consequences of poorly designed microbioreactors are that intense mechanical forces may deform cells in the microchannels [7]. The main types of cells that are cultured in microbioreactors are microbial and mammalian cells, each of which posses unique characteristics that influence the type of microbioreactor used [71]. Mammalian, fish and insect cells are susceptible to shear stress due to the absence of a defined cell wall and they require more delicate handling than bacterial cells [38]. The high shear forces generated in microchannels can be overcome by the addition of shear protectant liquids such as serum or pluronic F-68 to the culture medium used for the cultivation of mammalian cells [87]. Shear forces generated in microfluidic channels can also be reduced by modifying the channel geometry [88-89].

3.5. Fluidic Elements

An integrated microbioreactor system consists of a number of key functional fluidic components including micropumps, microvalves, injectors, micromixer and sensing elements. These components can be used to create effective, complex and powerful integrated microfluidic networks. Detailed descriptions of microfluidic components have been reviewed previously [90-91].

3.5.1. Micromixers

Mixing is regarded as a mass transfer process for species, temperature and phases to reduce inhomogenity and it may lead to secondary effects such as reaction and change in properties [92-93]. Mixing in microbioreactors is necessary for a number of reasons including increasing enzyme activity and maintaining pH. Adequate mixing of species ensures an even temperature distribution profile and better oxygen transfer rates. Mixing in traditional macroscale bioreactors is simple and achieved through sparging and use of impellers to promote agitation and turbulence. In the case of microbioreactor systems, the inertial effects associated with turbulence to facilitate mixing of adjacent streams of fluids is absent and so the mixing of fluids requires special attention. Fluid flow in microbioreactor channels is entirely laminar, characterised by small Reynolds numbers (Re = 0.01-100) [94]. The adjacent streams of fluids with different chemical composition remain distinct except for diffusive mixing at their interface [95-96]. Molecular diffusion, advection and Taylor dispersion are the main transport phenomenon used in microbioreactors. However, mixing by diffusion is a very slow and inefficient process, due to the convection of the fluid in the microchannels, hence they need to use micromixers. The total mixing time in the microchannel can be estimated by rearranging Ficks law [97].

$$T \sim \frac{d^2}{D} \qquad \text{Eq. 3}$$

where ***T***, *is* the time needed to obtain whole mixing, ***d*** is the thickness of the lamellar structure and ***D*** is the diffusion coefficient.

Mixing in microbioreactors can be achieved by active and passive means. Active mixing involves the addition of energy into the system and the use of external forces to stir the fluids thereby inducing chaotic mixing. Examples of such micromixers include electrokinetic, ultrasonic, magnetic, thermal, magneto hydrodynamic centrifugal forces or electro-hydrodynamic stirrers, peristaltic driving and piezoelectric (PZT) actuation [98-102]. Active mixers are not always suitable for microbioreactor applications simply because when delicate cells or tissues are subjected to intense hydrodynamic shear stress, due to stirring, they are likely to be damaged and not suitable for further study. Most of the active mixers reported in the literature are used in microbial based microbioreactors [34,103-105]. Recently, a microbioreactor for the fermentation of yeast cells and their mixing was achieved by using a free floating micro magnetic stirrer bar (3 mm length, 1.2 mm diameter) [106]. The stirrer was actuated from above to create random chaotic motion inside the reactor chamber which facilitated effective mixing. This arrangement has the advantage of eliminating dead zones in the reactor whilst the microbial cells are kept in suspension.

Passive micromixers use channel geometry and energy provided by the flow to stir, stretch and fold fluids so as to increase the material interfacial area over which molecular diffusion occurs [107]. In passive mixers no external energy is applied and mixing is achieved solely by diffusion and chaotic advection. With chaotic advection the mixing path between culture medium and other solutes is reduced and this enhances mixing. The small diffusion distances for Brownian motion of molecules are ideal for fast and effective mixing. Several designs of passive micromixers working at low Reynolds numbers (Re) and using various channel geometries have been reported, these include tesla [108], thin layer crossing mixer [107], serpentine mixer [109], F-type mixer [97], gradient diffusion mixer [17], and herring bone type mixer [110]. More detailed reviews on micromixers can be found elsewhere [93,111-112].

3.5.2. Microvalves

The ability to control the flow and interaction of fluids in microbioreactor systems is important to increasing their functionality. A microvalve is a fundamental element of a microfluidic system, whose purpose is to precisely control the flow path of fluids. Apart from obstructing and directing flow, microvalves can be partially closed and used to filter out particles [113]. They can also be used to create peristaltic pumps, *i.e.* three valves in a row can be operated in a peristaltic motion to pump fluids. Microvalves are either active or passive [114]. Active microvalves are classified by their external actuation principles such as; pneumatic, thermopneumatic, thermomechanical, piezoelectric, electrostatic, electromagnetic and electrochemical. The high degree of control over the timing, rate and direction of fluid flow are considered to be the main advantages of using these types of microvalves. Although active microvalves have been successful for macroscale applications, their integration to microscale devices such as microbioreactors is still a challenge, due to a number of factors such as material incompatibility and the requirement of large external systems for actuation.

In a passive microvalve, the obstructed flow does not employ any external actuation. The absence of moving parts,

lower complexity and fabrication costs as well as being less prone to breakdown due to fatigue are considered as the key advantages of using passive microvalves in microfluidic application [26]. These types of microvalves restrict the flow of fluids in a single direction and can take the form of polymer based check valves, passive valves based on surface tension and hydrogel based biomimetic valves [115].

Passive valves based on surface tension are characterised by the use of forced motion of the membrane or flap to control the flow of fluids. These valves have no moving parts and flow is controlled by their physical structure and surface tension effects of the substrate. The high degree of susceptibility of the valves to clogging and mechanical wear and tear makes them less attractive for microbioreactor application. Hydrogel microvalves consist of a stimuli responsive hydrogel material that swells in response to a variety of inputs such as pH [116], temperature [117], electric fields [118], light [119], carbohydrates [120], and antigens [121]. The key feature for these microvalves is their ability to undergo rapid changes in volume in response to a stimulus without energy inputs. Hydrogel valves are often called as intelligent or adaptive valves.

Amongst all passive microvalves, PDMS monolithic microvalves developed by the Quake group, using multilayering soft lithography, have become widely used in microfluidics [55]. Several thousands of these valves can be created in parallel and integrated into complex networks with high number density [122]. The design of the microvalve consists principally of a cross channel architecture, where two microchannels at right angles are separated by a thin membrane (10-40 μm). One of the channel acts as a control channel and the other as a fluidic channel. When the control channel is pressurized, the membrane is deflected into the fluid channel functioning as a valve. The advantage of these monolithic valves is that they require very low actuation pressure and can be combined to form peristaltic micropumps and mixers respectively due to the low Young's modulus of the elastomeric PDMS. The valves have a small foot print and they occupy minimal space with minimal dead volume [123].

Although, PDMS microvalves have been widely reported they have some drawbacks including the need for ancillaries, to operate the pneumatic valves, which occupy significant space both on and off the chip. They also require sophisticated interfacing with the device, and consume more power, thereby making portability difficult. The lifetime of the microvalves and the additional complexity for fabrication needs to be given some consideration for specific applications. Despite the significant progress made in recent years, microvalve technology has yet to become widely integrated in microbioreactor systems. The complexity of the valve architecture for highly multiplexed systems will mean that they face difficulties with respect to fabrication yield but clearly this challenge can be addressed. For widespread practical implementation, consideration needs to be given to the complexity of the valve architecture, the fabrication approach and yield as well as volume and cost of device and the benefit that can arise for the particular application. Innovations in materials, actuation principles and fabrication approaches mean that there is considerable potential for highly multiplexed fluidic operations.

3.5.3. Micropumps

The development of micropumps is still regarded as a key issue in realizing a fully integrated and functional microfluidic device [124]. Thus, the development of fully integrated microbioreactor systems requires an efficient and reliable system capable of pumping a wide range of fluids and gases. On a microbioreactor platform, a micropump actuates and provides pressure to pump growth media, cells in the system and transports the samples from one compartment of the fluidic bioreactor chip to another. The use of micropumps is also ideal for the multiplexing of microbioreactors [27]. The majority of micropumps that have been developed to date are either mechanical (moving parts), or non-mechanical with no moving parts. Mechanical micropumps can be further categorised based on their actuation principles such as piezoelectric, thermopneumatic, electrostatic, electromagnet and shape memory alloys (SMA), whilst subcategories of non-mechanical micropumps are determined by the method of transforming available non-mechanical energy into kinetic motion, these include electro-kinetic, magneto hydrodynamic (MHD), electrochemical, and electro hydrodynamic (EHD).

Mechanical pumps are also described as active, in that they employ energy to provide higher control over average flow rates. Flow patterns in these devices are often pulsed and their fabrication is relatively complex [55, 125]. The majority of publications that have been reported to date use macroscale external syringe and peristaltic pumps to pump culture media in microbioreactor chambers. This is a simple, low cost and practical approach but there is more difficulty in using this for more complex and parallelised fluidic operations.

Non mechanical pumps are characterised by non pulsed flows and they provide a wide range of flow rates at low pressure. The fabrication of non mechanical pumps is often less complex than mechanical pumps, which makes them suited for low cost mass production and easy to dispose. Several types of passive micropumps have been reported, the most common types include osmotic pressure [126], evaporation [127-128] and, surface tension [129-130]. Electro-osmotic flow (EOF) is a popular means of pumping liquids in microfluidic devices and is based on the application of a potential difference across a microchannel to induce the flow of a liquid [131]. The use of EOF has several advantages over pressure driven pumps in that they are simple, fast and can be operated without the need for mechanical pumps or valves but they have the disadvantage of having to rely on high voltages. A further type of passive pump that has been reported for microbioreactor devices uses cellular energy, e.g. use of intrinsic pulsatile mechanical functions of cardiomyocytes [132-133]. An overview on micropumps can be found elsewhere [134-136].

3.6. Elements for Sensing and Control

A key requirement for microbioreactors is the ability to measure process parameters such as optical density (OD), pH, and dissolved oxygen (DO) and flow rate data in real time [26]. The basic metabolic processes occurring within cells are determined by physicochemical variables which in turn determine the by-products that are produced during cel-

lular growth. As a consequence, there is a need for integrated effective miniaturised sensors for monitoring important cellular events and other interactions. A variety of microsensing techniques exist for quantifying analytes in microbioreactors systems and the most common techniques are optical and electrochemical methods (Fig. **3**).

3.6.1. Optical Methods

Optical methods can include fluorescence, absorbance, refractive index, colorimetry, Near Infrared and Raman spectroscopy, chemiluminescence and bioluminescence, (Fig. **3**). These methods are versatile in that they allow simultaneous recording of information such as wavelength and intensity. Fluorescence is the most sensitive and popular technique used for the detection of biomolecules, cancer progression in cells and biochemical activities in microfluidic systems. Laser induced fluorescence detection in single cell microbioreactors has been reported in a number of publications [137-140].

Fluorescence can also be used to measure the viability of cells which can be achieved by tagging cells with fluorophores such as calcein, propidium, ethidium bromide. Calcein AM and propidium can be infused directly into the microbioreactor and cells can be imaged using a fluorescence microscope. In this assay, the abundant esterase enzymes in the cytoplasm of live cells, convert calcien AM to calcien which is highly green fluorescent when excited with blue light. Dead cells can be stained red due to the propidium iodide which does not penetrate the membrane of live cells. Other fluorescence assays that can be carried out in microbioreactors include the coupling of reporter genes to detect and track specific cells events using reporter proteins, such as green fluorescent protein (GFP) [141], luciferase [130] and galactosidase.

Unlike other fluorescent tags, such as luciferase, β-galactosidase, or fluorescent-tagged antibodies, GFP and its variant mutants are the most commonly used reporter proteins. The advantages of GFP are that it does not require intrinsic or extrinsic cofactors to fluoresce, it is photostable, species independent and can be monitored non–invasively in situ, and in real time, by monitoring culture fluorescence of living cells using on-line optical sensors [142-143]. Cells labelled with GFP can be tracked with real time fluorescent imaging, e.g. in the identification of cancer cells in metastatic locations which is critical to understanding the molecular components that contribute to cancer progression. More recently, fluorescent nanoparticles have been used as labels for cell based assays. Fluorescent quantum dots nanomaterials are useful probes for many types of cell labeling in that they can be used for measuring cell viability by fluorescence techniques [26]. However, some studies have demonstrated that nanomaterials induce stress responses in some mammalian cells. For example, the work by Richter *et al.* [144], has shown that silver and gold nanoparticles induce stress, leading to reduction in collagen production in primary human fibroblast cells.

Microbial fermentation in microbioreactors can also be estimated by absorption and fluorescence techniques [28,35]. However, both absorption and fluorescence measurements suffer from a decreased sensitivity at high biomass due to inner filter effects [69,145]. Thus, an increase in growth by the microbial population in the microbioreactor results in the

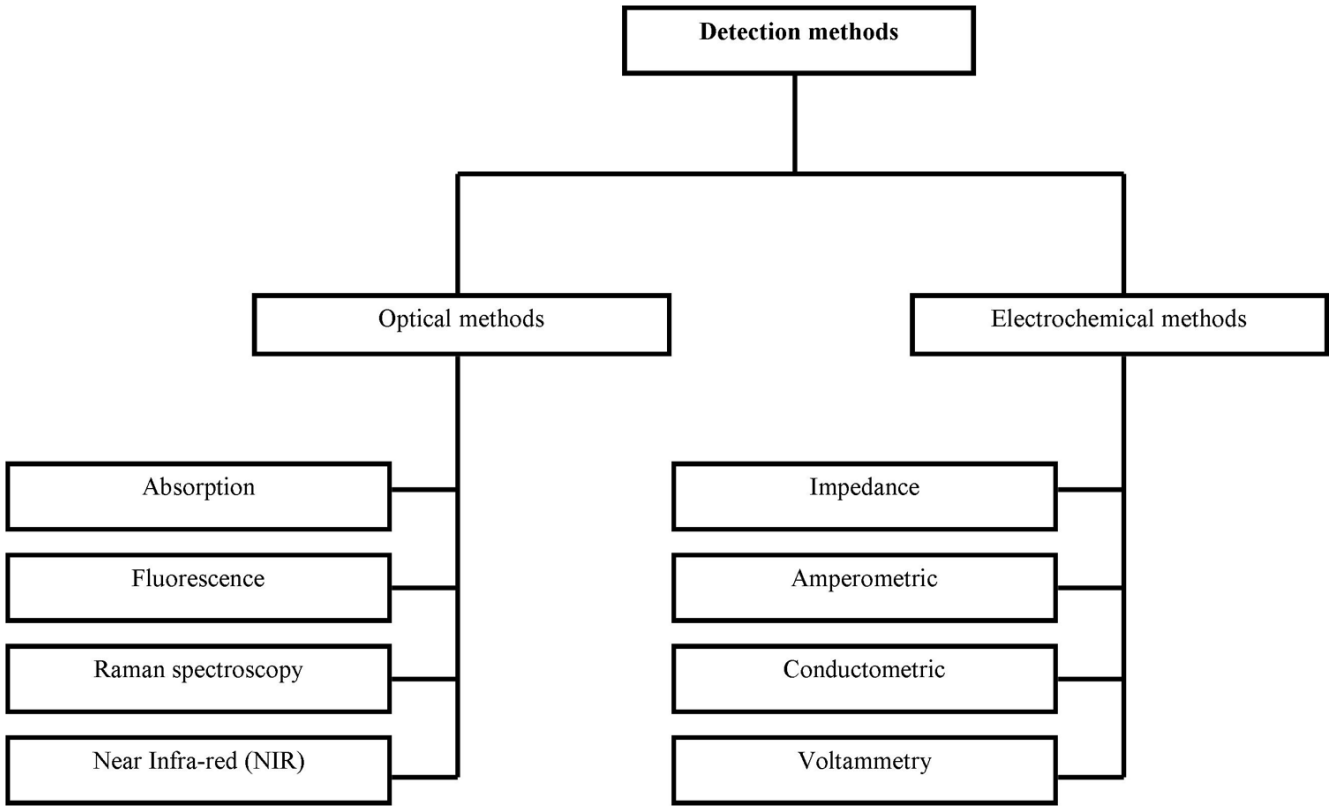

Fig. (3). Common detection methods used in microbioreactor platforms.

scattering of light by cells. There is also utility in considering bioluminescence and chemiluminescence that are sensitive techniques with reported detection limits in the femto molar range [28,35]. Optical sensors offer interesting opportunities for applications in process monitoring in that they are non invasive, thus data about the cell state and concentration can obtained directly from the microbioreactor chambers without the need for sample removal and direct contact. The non invasiveness, lightweight, low cost, robustness, ruggedness, high signal-to-noise ratio, and sensitivity are considered to be advantages of using optical sensors in microfluidic applications which require detection schemes that are small and portable.

Although, widely used optical sensing techniques have some limitations in some aspects. For example, most of the biological culture media used in fermentation and cell culture studies are highly fluorescent, with broad emission spectra which tend to interfere with the fluorescence of the analyte of interest. Optical fibres which are used as waveguides have a tendency to attenuate both excitation and output fluorescence emission which results in reduced sensitivity. A major limitation of using optical sensors is the need to focus and align the optical elements such as lenses, filters, detectors and light sources. Despite these draw backs optical sensing elements still dominate over other detection systems such as electrochemical methods. These optical detection methods have been discussed in a number of reviews [146-148]. For future, we are likely to see increasing integration of optical elements, e.g. light source and detector, as part of the microfluidic component.

3.6.2. Electrochemical Methods

Some electrochemical detection systems with examples of their application include (i) amperometric, measurement of electrical responses of electrogenic cells (ii) conductometric, measurement of the conductivity of mechanical contacts between cells and substrate and (iii) potentiometric, measurement of chemical signals such as pH resulting from changes in metabolic by-products such as lactic acid or substrate such as glucose [148-149]. The application of microfluidic electrochemical sensing for biological applications has been reviewed [150,151].

Unlike optical methods where the signal output is dependent on detection volume, electrochemical detection systems are dependent on the electrode surface area [152]. As a consequence, the limit of detection in concentration terms does not degrade rapidly in electrochemical chemical detection as they would be for optical methods. In view of this, electrochemical sensing systems are becoming acceptable tools in biomicrofluidic analysis owing to their small sizes which are compatible with micron scale devices. Electrochemical microsensors can be integrated in microbioreactors by using a variety of approaches. The most common approach uses standard soft lithographic methods, where grooves or channels are created to house the electrodes in position followed by sealing the electrodes with another polymer layer. Other approaches involve the deposition and patterning of metal layers by using micromachining methods. A major drawback of electrochemical microsensing is their inability to provide information on specific cellular activities that are directly related to certain cell functions, biomarkers or signalling pathways. Previous reviews have described issues of integration of electrochemical detectors within microfluidic devices [153-154].

3.7. Up and Down Stream Processing

Depending on the application, some microbioreactors require components for up or down stream processing such as separation, filtration, lysis and purification. Cell separation, is increasingly becoming an important tool for researchers in studying the behaviour of single cells or homogeneous cell populations to understand their behaviour and functions in different situations e.g. separation of cells is important for clinical diagnostics, therapy and biotechnology applications [155]. Separation of cells requires the removal of one cell type from another by physical means and a variety of approaches, which includes filtration, centrifugation, fluorescence cell sorting (FACS) and magnetic sorting, is currently being used.

Lysis refers to the breakdown or disruption of cellular activities by rupturing the cell membrane. This can be achieved by various techniques such as sonication, mechanical, electric, heating, chemical, optical and osmotic shock. In microbioreactors, lysis is generally accomplished by using either chemical detergents, mechanical and electrochemical means [156]. Chemical lysis involves the use of enzymes (lysosomes) or non ionic detergent such as sodium dodecyl sulphate (SDS) [157] or Triton X-100 [158]. Chemical lysis has the advantage of easy integration in microbioreactors, in that chemicals can be dispensed to the cells from reservoirs within the device. Mechanical lysis, is a less common but alternative approach for lysis of cells in microfluidic devices. For example, shear forces can be generated from microfabrication of nanoscale knife-like ridges or "barbs" in a filtering region where cells can be made to pass through [159]. Potential limitations may include complexity of fabrication and the fouling that is likely to arise from the cell debris. An alternative approach is electroporation which uses integrated microelectrodes to generate high intensity electrical pulses that induce instabilities on the cell membrane and cause cell poration. Since its inception in 1982, by Neumann *et al*, [160] electroporation has been widely used due to its ability to achieve rapid high electrical lysis with disruptions times as low as 33ms, about eight times as faster than SDS [161]. Recently Xu *et al*. [162], developed a cell arraying-assisted electroporation (CAE) chip which uses both the positive dielectrophoresis and electroporation techniques to provide a simple and efficient method for gene transfer. The CAE chip in microelectrode array format is covered with SU-8 microwell structures to facilitate both cell positioning and electroporation. The authors envision the application of the device in high throughput screening of compounds in parallel and potential applications in cellular and molecular research. Filtration is a physical technique that is used to separate solid particles or cells in suspension by passing them through a barrier that retain them. With the power of microfabrication processes, microbioreactors with filtration components have been created to eliminate undesirable cell debris during fermentation processes.

4. MICROFABRICATION TECHNIQUES FOR MICROBIOREACTORS

The ultimate goal for the development of miniaturisation is to create integrated systems, capable of performing various functions on a single chip such as mixing, cell sorting, lysis, pumping, fluidic control (microvalves) and micro sensing elements among many others. To achieve this goal, microfabrication processes provide the needed tools. Microfabrication allows the creation of a variety of structures to be created that are well matched to the physical dimensions of most cells organisation [163]. There is a clear potential to perform several thousands of parallel experiments with single or group of mammalian and microbial cells that can be carried out under controlled conditions in a manner not possible for standard tissue culture techniques [158]. The microbioreactor approach offers advantages of greatly increased amount of biological information at reduced cost.

Microstructures can be created within the microbioreactor to study the behaviour of cells under controlled environmental conditions. Zhang *et al.* [164] demonstrated a microbioreactor with microporous fluidic barriers incorporated in the microchannels to form sieved-pockets to concentrate cells during loading. The microbioreactor environment was capable for mimicking the physiological liver mass transport and enabled the long term culture of hepatocytes cells without loss of viability. Microfabrication methods that are applicable for the creation of microfluidic devices are described in detail elsewhere [63,165-168].

4.1. Photolithography

Photolithography is a widely used technique for fabricating microstructures with roots in the semiconductor and subsequently the microelectromechanical systems (MEMS) sectors. Photolithography offers advantages of high precision, reproducibility and potential for large volume production with reduced cost [26]. In photolithography, a photosensitive resist on a substrate is used to transfer geometric designs/pattern from a mask to the surface of a photosensitive substrate. A photomask with the desired pattern either block or allow UV light to pass onto the photoresist coated substrate and exposed to UV radiation. The geometric pattern on the photomask is subsequently transferred to the photoresist during the exposure process.

For rapid prototyping of microfluidics structures the mask is a transparent plastic film with structures printed on it, using a high resolution image setter or printer. This approach is relatively fast and low cost compared with the use of a transparent glass and chromium mask. The exposed patterned photoresist is developed by etching of the resist pattern in the developer. Depending on the type of photoresist used, exposed areas may become soluble (positive photoresist) or insoluble (negative photoresist) in the developer [169]. A positive photoresist has photoactive elements which weaken the polymer matrix, and allow them to be dissolved during development. In case of a negative photoresist, such as SU-8, the area exposed to UV radiation is polymerised and hardened so that the unexposed area is more soluble in developer solution. In the case of SU-8, the patterned photoresist that remains after developing can be used as a master mold for subsequent production of microdevice. Although, photolithography has been extensively used, for fine structures there is a requirement for a clean room with high capital infrastructure that is beyond the reach of many laboratories.

4.2. Replicative Techniques

Current microbioreactors are largely fabricated using microfabrication approaches which have been adapted for use with polymer substrates. Several reviews on these microfabrication approaches have been described in the literature [45, 48, 63].

4.2.1. Soft Lithography

Soft lithography, developed by Xia and Whitesides [170], is one of the most commonly used approaches for cell culture studies (Table **2**). The approach is amenable to rapid prototyping, is low cost and can be used for the creation of highly complex microstructures with diverse functionalities (e.g. filters, valves, pumps, 3D scaffolds and mixers) [49,70,156,171-175]. This allows a microbioreactor chip to include, microvalves, pumps and multiple arrayed chambers which can be individually addressed for cellular analysis. Soft lithography (SL) uses the process of casting to create micro and nano structures [170] and is so coined because it uses soft elastomers, such as polydimethylsiloxane (PDMS) to replicate patterns of micron scale devices from master molds. The master molds are produced by either photolithography using SU-8, micro milling or e-beam lithography methods [55,170]. Fabrication of a master mold using SU-8 is common since thicknesses of 100 μm or less can be achieved and the process is fast and cost effective. SL employs several sets of techniques which includes replica molding, micro-contact printing, micro-transfer molding, micromolding in capillaries, and solvent assisted micro-molding [176].

Soft lithographic techniques make use of patterned elastomers with relief structures as a stamp, molds or mask. In SL a prepolymer PDMS is cast against the master mold and cured by heating in an oven or on a hot plate to produce an elastomeric replica. The replica PDMS stamp can be sealed hermetically by plasma bonding against another PDMS or glass material to create channels or reservoirs (Fig. **2**). Alternatively, the PDMS stamp can be used to create further copies of the microstructure without using the master mold, a process referred to as replica molding. The PDMS stamp can also be used to transfer protein or ink molecules to a substrate using microcontact printing [26]. Microbioreactors can also be fabricated from polymer substrate using other replicative techniques such as hot embossing, injection molding, and direct micro-milling techniques.

4.2.2. Hot Embossing

In hot embossing heat, compression is used to imprint microstructures on a polymer substrate using a master mold. The mold containing the negative relief is pressed against the polymer substrate heated at its glass transition (T_g) to define the desired pattern using well designed heat and pressure cycles. The mold and the thermoplastic are cooled below T_g of the thermoplastic to harden it. The mold is then separated from the substrate, leaving the desired pattern imprinted on

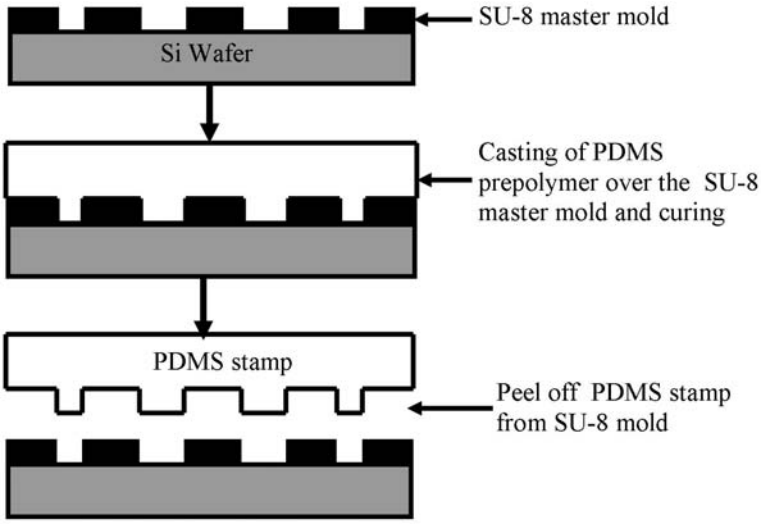

Fig. (4). Schematic illustration of the fabrication of a PDMS mold/stamp by casting and curing an elastomeric against an SU-8 mold [176].

the polymer surface. Hot embossing allows replication of microstructures with high fidelity and aspect ratio in the micron and nanoscale range for a wide variety of polymers including PMMA and polycarbonates [177]. The features created are dependent on a number of parameters including pressure, temperature, the chosen thermoplastic, viscosity in the melt and the adhesion of the polymer to the mold. The technique has longer cycle times than micro-injection mold and is most appropriate for the creation of hundreds of devices rather than the very large numbers that is achievable with injection moulding.

4.2.3. Micro-Injection Molding

In micro-injection molding polymer material in the form of pellets is fed into the injection molding machine and melted to liquid plastic (Fig. **5**). After injection of molten polymer into the mold, the melt cools and hardens into the mold shape and is removed. Injection molding is ideal for replicating polymeric microstructures at low cost using shorter cycle times unlike in hot embossing. Injection molding can produce thousands of structures with features in the microscale range. However the high costs of the fabrication of the mold make it less attractive than hot embossing for relatively small batches [178]. The microinjection molding process has been reviewed elsewhere [179] and aspects of device design, machine capabilities, mold manufacturing, material selection and process parameters are covered.

4.3. Direct milling method

Micro-milling is a mechanical process that is used to produce microstructures in hard materials such as metals (aluminium, steel) and polymer (PMMA, polycarbonate) substrates which are easy to cut. It uses a small revolving cutting tool, which removes areas of the polymer substrate to create structures on its surface. A computer is used to numerically position and move the cutting tool, hence it is commonly known as computer numerical control or CNC milling. Micro-milling has the its own advantage that the polymer substrate is not chemically degraded by heating or UV radiation but it does suffer from tensions and stress marks left behind during the milling process. These marks can cause problems when a smooth surface is required, e.g. on chip optical measurements, as they can distort accuracy and precision.

5. APPLICATION OF MICROFLUIDIC BIOREACTORS

Microbioreactor systems are increasingly beginning to find wide range of applications in various fields such as drug discovery, high-throughput bioprocessing, single cell analysis, stem cell research, genetic analysis, (Tables **1-3**). We provide here indicative examples of some microbioreactor applications. The examples here are not exhaustive, since the use of microbioreactors is a rapidly expanding area with a continuous emergence of new architectures for different applications.

5.1. Optimisation of Bioprocesses

Microbioreactors have found diverse applications in bioprocessing operations such as fermentation, where a number of high value products such as antibiotics, enzymes, vaccines and therapeutic proteins have been realized. Microbioreactors platforms integrated with highly sensitive detection systems to monitor key variables (pH, dissolved oxygen and biomass) have been applied to screen and optimise conditions for high-throughput fermentation processes. During the last decade, several designs of such microbioreactors have been demonstrated for high-throuput bioprocessing [27,35,37,58,73,180]. The performance of these microbioreactors compares favourably with their conventional macroscale counterparts in terms of the measurement profiles of key physicochemical variables (pH, dissolved oxygen and optical density). For example, Szita *et al.* [181], demonstrated reproducible performance of the parallel fermentation of *E.coli* in a multiplexed microbioreactor system. The microbioreactors are fabricated using PMMA and PDMS, with a working volume of 150 μL.The process variables such as optical density (OD), dissolved oxygen (DO) and pH, are monitored using optical sensors in-situ and in real time. A

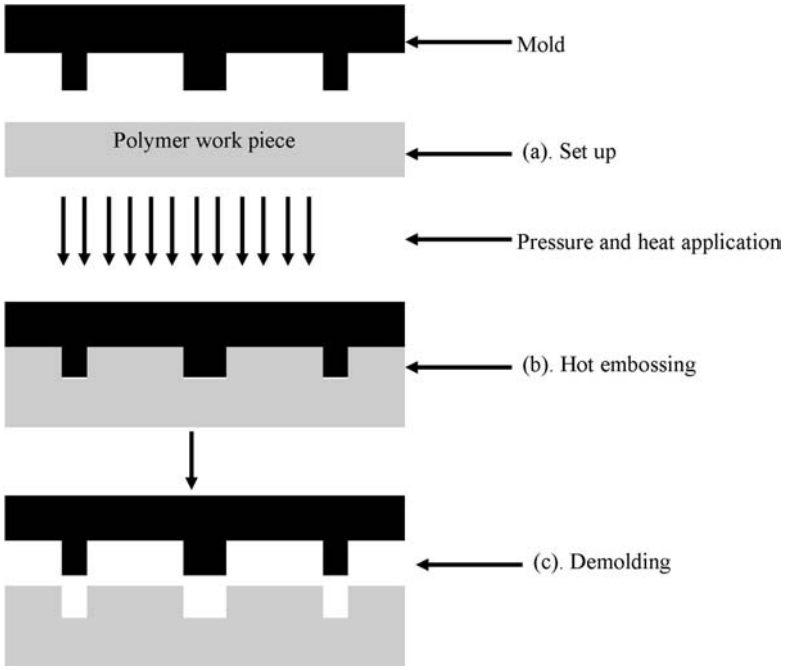

Fig. (5). Schematic of hot-embossing. (**a**) the mold and thermoplastic polymer work piece are aligned. (**b**) the mold and polymer piece are clamped together with heat and pressure applied. (**c**) work piece and mold are cooled and separated [63].

recent review by Schäpper *et al.* [27], provides a detailed discussion of microbioreactors used in high-throughput fermentation processes. The major progress in the development of microbioreactor for bioprocess developments is shown in Table **1**.

5.2. Tissue Engineering in Microbioreactors

Tissue engineering is an interdisciplinary field that uses the principles of engineering and biology to develop functional tissues in a laboratory setting using cells [182]. Microfluidic systems have the potential to impact significantly on a wide range of biochemical applications particularly in the area of tissue engineering and drug screening [183-184]. Culturing tissues in microscale devices provides a more holistic model for detecting cellular response to stimuli and the capacity to reproduce the cellular microenvironments [185-187] , unlike in macroscale bioreactor systems. Global epidemics and high accident rates are important drivers for considering tissue engineering for a potential role in organ transplantation. There has been considerable effort in developing appropriate microfluidic platforms to quantitatively control the cell culturing parameters for tissue development. For example, the process of tissue development is dependent on the spatial and temporal gradients which regulates proliferation, migration and differention of cells [188-189].

Microfluidics allows microstructures that enable the generation of biological and physical gradients which makes it easier to study cells in modified environments. Several topical reviews covering microfluidic tissue engineering have been published [190-193]. The most recent review by Huang *et al.* [194] summarises the developments in the fabrication of microfluidic hydrogels for using in tissue engineering. In another development, Yeo *et al*, [157] have reviewed the microfluidic devices for bioapplications where aspects of biomaterials synthesis for tissue engineering, drug development and point of care diagnostics are discussed.

Chin *et al.* [195] reported a microfluidic bioreactor array for high throughput monitoring of stem cell proliferation. The microfluidic platform is capable of culturing cells for a long periods of time, perfoming live cell imaging of single cells and tracking of individual cells to determine their fate. The microfluidic bioreactor array reported in this work has great potential for high throughput screening in tissue engineering. Lee *et al*, [196] reported a novel three dimensional direct printing technique to construct hydrogel scaffolds containing fluidic microchannels for tissue engineering. This was achieved by printing collagen hydrogel precursors followed by bubbling a solution of sodium bicarbonate. Furthermore, a heated solution of gelatine was printed in between the collagen layers to form a 3D hydrogel block. The utility of the device was tested by culturing dermal fibroblasts and the results showed that cell viability was higher in the fabricated device than in microchannels without the scaffolds. This work demonstrates the importance of 3D scaffolds in cell proliferation and differentiation. Recently, a 3D hydrogel biomimetic vasculature device for tissue engineering comprising of 3D tubular constructs with multilevel interconnected lumens was developed [197]. This work demonstrates that microfluidics can be a powerful tool for building structures for effective tissue engineering or *in vitro* tissue models.

In order to understand the cell-matrix interactions, Yang *et al*, [198] fabricated a microfluidic device with microchannels consisting of nanopatterns for dynamic cell culturing. In this work, poly thin film technology was used to develop a novel stitching technique to generate a large area of nanopatterned surface and microtransfer assemble technique for PDMS microfluidics. The functionality of the device was

Table 1. Application of Microbioreactor Platforms in Microbial Bioprocessing Developments

Type of study	Mode of operation	Cell line	Parameters monitored	Detection method	Source
Cultivation of bacterial cells in a150 μL, membrane-aerated, well-mixed microbioreactor	Continuous	*E. coli*	OD, pH, DO in real time	Absorbance.	[58]
Long-term culture and monitoring of small populations of bacteria	Continuous	*E. coli*	pH, OD, DO	Absorbance	[212]
Growth conditions for methanogenic bacteria	Batch fed	*M.concilii*	Temperature and pH		[213]
Culturing bacteria in a 5-50μmL membrane aerated microbioreactor	Batch fed	*E. coli*	Temperature, pH, OD, DO	Absorbance and fluorescence	[35]
Co-culturing of bacteria, algae and yeast	Batch fed	*E.coli, S.cerevisiae* and *C.cryptica*	OD	Absorbance and fluorescence	[214]
Measurement of carbon dioxide production	Batch fed	*Candida utilis*	CO_2	Conductivity	[69]
Measurement of the oxygen transfer capacity and online monitoring of the dissolved oxygen	Batch fed	*E. coli*	O_2	Fluorescence	[215]
Cultivation screening of *Aspergillus ochraceus*	Batch fed and continuous	*Aspergillus ochraceus*	pH and temperature	Light microscope	[216]
High throughput screening of microorganism	Batch	*L. plantarum* *E.coli XL2* *C.albicans JBZ32*	-	Fluorescence	[217]

tested with hMSCs and results show that nanotopography and fluid shear stress were instrumental in facilitating the adhesion, spreading and migration of the hMSCs.

A microfluidic platform fabricated from a biodegradable elastomeric polymer Poly(glycerol sebacate) (PGS), was developed by Bettinger *et al.* [199]. The device geometry was stacked with single layered microfluidic structures to form 3D network of scaffolds to promote the adhesion and proliferation of a high density seeding of hepatocytes (HepG2) cells. There are a number of advantages of using PGS including being highly biodegradable, easy to synthesise from glycerol and sebacic acid and finally bonding to itself without the use of solvents or adhesives which may be toxic to cells. The results for cell seeding demonstrated successful adhesion and proliferation of the hepatocytes cells in the device without loss of viability. The utility of the device was further tested by measuring the production of albumin by the HepG2 cells. The results showed that albumin was produced at rate of 24.3 ± 5.5 μgcm^{-2} per day, a value which compares favourably with other published results [200-201].

In a recent study, Wang *et al.* [202] developed an improved novel rapid fabrication technique for microfluidic device using a biodegradable elastomeric polymer poly(ester amide), poly(1,3 diamino-2-hydroxy propane-co-polyol sebacate) (APS). APS was selected due to its low Young's modulus and a longer degradation half–life which makes it an ideal biomaterial for tissue engineering. The results show that APS is stronger and less elastic than the previously used PGS material.

5.3. Stem Cells in Microbioreactors

Recently, there has been a growing interest by researchers in studying stem cells using microfluidic technology. Stem cell research is one of the most promising areas of biotechnology, which offers the prospect of developing new methods to repair or replace tissues or cells damaged by injuries or diseases such as leukaemia as well as a possibility to study early human development [203-205]. Stem cells are classified into two broad categories namely pluripotent embryonic stem cells (ESC) that originates from the earliest stages of embryo development from the inner cell mass of the blastocyst and adult stem cells that are found in differentiated tissues. Adult stem cells act as a repair system for the body and maintain normal turnover of regenerative organs by replacing replenished specialized cells. Adult stem cells are found in various location of the body such as bone marrow, brain and skin [206-207]. The ability of stem cells to renew themselves through normal cell division and differentiate into specialised cell types has made them an important resource in modern medicine. Stem cells are used for cell–based therapies in human disorders, biological discovery, drug development, cell replacement and tissue engineering [158, 208].

The controlled microenvironments in microfluidic bioreactors are conducive for stem cell self-renewal and differentiation. Thus the laminar flows generated in microbioreactor channels exhibit some physiological features, such as maintaining a constant soluble microenvironment and having a large surface to volume ratio which is found in biological systems [209]. Microbioreactors for stem cell research have been reviewed with a focus on control of soluble biochemical factors, cell to cell interactions and co-culture, mechanical interaction with microenvironment, ECM interactions and high throughput screening among many others [210]. Concentration gradients are useful in controlling biological and pathological processes, such as metastasis, embryogenesis, axon guidance and wound healing [211]. Due to their

small size microfluidic bioreactors enable the control of the *in vivo* microenvironment, where cells communicate and interact. Consequently, there has been a drive to use microfluidic platforms in studying the behaviour of cells when exposed to such concentration gradients of different growth factors.

Chung *et al.* [218] reported a microfluidic platform with a concentration gradient generator, to study the effect of growth factor concentration on proliferation and differentiation of human neural stem cells (hNSCs) under continuous flow. The hNSC cultured in the device were exposed to a stable gradient of the growth factor. The rate of hNSC proliferation in the device showed a linear dependence to the growth factor concentration, while the differentiation into astrocytes was inversely proportional.

Kim *et al.* [219], developed a microfluidic device for culturing hESC over a varying logarithmic range of flow rates and concentration gradients to study various biological conditions. The results from this study show that proliferation of cells was negligible at the slowest flow rate, whereas at higher flow rates, cell growth was very high and healthy. Using a similar concept but a different design, Park *et al.* [220], demonstrated a microfluidic device that utilised an osmotic driven pump to generate a stable concentration gradient of various signalling molecules. The device was used to culture progenitors derived from hESC for eight days under continuous cytokine gradients (sonic hedgehog, fibroblast growth factor, and bone morphogenetic protein 4). The device was capable of sustaining the differentiation of neural progenitors at a rate directly proportional to sonic hedgehog concentrations. Given these examples, microfluidic gradient concentration devices are clearly useful in studying the spatial gradients of signalling molecules which are important in controlling the differentiation of stem cells.

Korin *et al.* [221], demonstrated the long term co-culturing of undifferentiated colonies of human embryonic stem cells (hESC) on foreskin fibroblast (HFF) in a microchannel bioreactor of height 100 μm. Numerical simulations were applied to examine the design parameters, mass transport and shear stress. The device was capable of sustaining the co-culture (hESC-HFF) for a long period without the loss of viability. Recently, Wang *et al.* [222] developed a microfluidic patterned co culture system for mouse mesenchymal stems cells (mMSCs) and neural cells. In this work, the effect of paracrine produced by the neural stem cells in facilitating the transdifferentiation from hMSCs to neuron cells was investigated. Neural cells and hMSCs were patterned in the device in an orderly manner without direct contact. Higher transdifferentiation ratios were observed in the microfluidic platform when compared with the traditional transwell co-culturing system.

The concept of using an integrated microfluidic bioreactor platform for stem cell analysis has been developed by Gómez-Sjöberg *et al.* [223]. The versatile automated microfluidic platform with 96 independent culturing conditions in 60nL chambers was used to study the proliferation, differentiation and motility of human primary mesenchymal stem cells (hMSCs). Each culturing chamber was individually addressed and cells were loaded precisely by successive loading cycles. The inoculation of each culture chamber is followed by feeding a mixture of 16 different components. The operation of the device e.g. the feeding cycles and optical measurements is highly automated using software based programmes. The results obtained show that cell motility was reduced in chambers that were simulated with osteogenic medium. Furthermore, hMSCs were observed to undergo full differentiation after a minimum of four days stimulation with osteogenic medium. The advantage of using such a microfluidic system is the ability to study multiple cell reactions on a single platform. This study clearly demonstrates how integrated microfluidic platforms can be used to optimise culture conditions for application in cell culture studies.

Wu *et al.* [224], developed a microfluidic device with several components which include cell seeding reservoirs, culture areas, micropumps microgates, waste reservoirs and fluidic microchannels for long term culture and differentiation of mesenchymal stem cells (MSC). An integrated microfluidic platform for the culturing of human embryonic stem cells has been reported [225]. Here, the microfluidic device comprises a serpentine microchannel which facilitate the prescreening of dissociated hESC clusters and six individually addressable chambers. The Wu-H group [226], further developed an integrated microfluidic system for isolating, counting and sorting of hematopoietic stem cells (HSC) from cord blood. The isolation component of the device comprises of a four membrane type micromixer, two pneumatic micropumps and a S-shaped microchannel. The purpose of the micromixer is to allow for the binding of HSC with magnetic microbeads, the pump was used for transporting the sample, while the S-shaped channel is for isolating the stem cells using a permanent magnet. The authors reported a separation efficiency of 88% of the HSC from the blood in a record time of 40 mins using a sample volume of 100 μL in contrast to 5h when using traditional systems. Microfluidic systems have also been applied to control the mechanical interactions of stem cells with their environment. *Ruiz and Chen* [227], used microfabricated stencils to create islands of hMSCs of various shapes and demonstrated that cells cultured in high stress region differentiated into osteocytes, while those in low stress region differentiated into adipocytes. This work demonstrates the role of mechanical forces in stem cell differentiation which could be exploited in stem cell based therapies. The recent developments of microfluidic bioreactors in stem cell research are shown in Table **2**.

5.4. Drug and Toxicological Screening

Drug and toxicological screening are part of the drug discovery process, where a variety of drug candidates are tested to establish their toxicological effect and therapeutic efficacy before making them available to the consumers. Effective toxicological screening assays require *in vitro* systems that are mirror images of the *in vivo* microenvironments of the cells or tissues [21].

Culturing cells in microfluidic bioreactors is a promising technology for applications in the pharmaceutical industry because of the associated benefits it brings which includes among other things; improved biological function, higher quality cell-based data, reduced reagent consumption, and lower cost [174, 246].

Table 2. Application of Microfluidic Bioreactors in Stem Cell Developments

Type of study	Cell line	Source
Stem cell differentiation	Human embryonic stem cells (hESC) in 2D and 3D culture formats	[228-229]
Proliferation and differentiation of neural stem cells NSCs) in a microfluidic gradient generator	Neural stem cells(NSCs)	[218]
High-throughput 3-D cell-based proliferation and cytotoxicity assays	Murine embryonic stem cells and colon cancer HT-29 in 3D scaffolds	[230][a]
Co-culturing of spheroids of various geometries and compositions	Mouse embryonic stem (mES) cells and hepatocytes	[231]
Stem cell culture for toxicity testing in 3D	Human bone marrow cells (hBMCs) in 3D scaffolds and in 2D monolayer	[232]
Perfusion culturing of foetal hepatocytes in microfluidic environment	Fetal human hepatocytes (FHHs) and human hepatocarcinoma(HepG2) cells	[200]
Human Foreskin Fibroblasts (HFF) cells as feeder cells for hESCs culture	hESCs	[233]
Patterning of mammalian cells in an integrated microfluidic device	Mouse embryonic stem (mES) cells	[234-235]
Optical monitoring the chemotaxis movement of neural stem cells	Neural stem cells	[236][b]
Study of stem cells in 3D microenvironment in real time	Mouse embryonic stem cells and mouse embryonic fibroblasts	[237]
Stem cell behaviour	Mouse fibroblast cells and human mesenchymal stem cells	[220]
Culturing MSCs on micropatterned PDMS substrates	Mesenchymal stem cells (MSCs)	[238]
Culturing embryonic stem (ES) cells and regulating embryoid body (EB) formation	Embryonic stem cells (ESCs)	[239]
Differentiation of murine embryonic stem cells into cardiomyocytes	Murine embryonic stem cells	[240]
Control of soluble factors	hMSCs	[241]
Culturing of stem cells in polyester conical microwells	Murine embryonic stem cells, human hepatoblastoma	[242]
Comparison of Human mesenchymal stem cells (hMSCs) differentiation rate under different conditions	hMSCs	[243]
Optimisation of embryoid bodies (EBs) formation in embryonic stem cells	Murine embryonic stem cells	[3]
Differentiation of stem cells	embryonic stem cells (ESCs)	[244]
Development of microfluidic device for easy cell loading, culture and post-culture operation.	embryonic stem (ES)	[245]

All the microfluidic bioreactors are fabricated by soft lithography using PDMS except those marked with superscripts a and b which are manufactured by micromilling and agarose gel respectively.

Current toxicological screening methods involves the use of animals. Animals studies are expensive, lengthy and can raise ethical issues. Microbioreactors have the potential to reduce the need for animal testing. Numerous examples of microbioreactor designs for toxicity and drug testing assays using various cell lines have been demonstrated in several publications [232,247-249].

In toxicological studies, the toxic effect of any drug candidate on a target tissue is dependent on another tissue particularly the liver. The liver plays various important roles particularly the liver. The liver plays various important roles in the mammalian body such as metabolism, detoxification, protein synthesis, glycogen storage, hormone production and bile secretion. Thus when performing toxicological studies there is a need to develop platforms that are capable of recreating the *in vivo* cellular conditions with high fidelity [250-251].

Miniaturised cell culture analog (CCA) of human and animal physiology holds great promise as metabolically ac-

curate models of complex biological systems. For example, a cell culture analog (CCA) can be created with miniaturised interconnected chambers and channels, where each chamber contains a different type of cell mimicking the activity of a particular tissue. Such physical models can be used as alternative methods to predict human response by exposure to chemicals or pharmaceuticals [252]. The basic concepts of CCA devices are described in [253-254]. Viravaidya *et al.* [255], developed a miniaturised CCA device for culturing liver, lung and fat cells in different interconnected compartments to mimic the physiological features such as residence time, of the circulation and exchange of metabolites in the body. In a similar development, a micro CCA device with 3-D hydrogel cell cultures to test the cytotoxic effect of anticancer drugs on colon cancer cells (HCT-116) and hepatoma cells (HepG2/C3A) encapsulated in matrigel was developed by Sung and Shuler [256]. The results obtained from using the miniaturised CCA devices showed that they are capable of providing a more physiological environment for pharmacokinetic-based drug screening.

In a similar development, Ma *et al.* [257] developed an integrated multi-layered microfluidic device composed of a quartz substrate with embedded separation microchannels and a perforated three-microwell array containing sol-gel bioreactors of human liver microsome (HLM), and two PDMS layers. The aim of the study was to simultaneously characterise drug metabolites and carry out a cytotoxicity assay. The feasibility of drug metabolism on the bioreactor platform was validated by first studying UDPglucuronosyltransferase (UGT) metabolism of acetaminophen (AP) and its cytotoxic effect on HepG2 liver cell line. This was followed by a further study on the metabolism based drug-drug interaction between AP and phenytoin (PH). The reported cell viabilities in the device for the three conditions tested (PH only, AP only and co-administration of PH and AP) was 90%, 86% and 58%, respectively. Furthermore, the toxicity of AP on the HepG2 was shown to increase significantly in the presence of PH. This work is of interest because it combines both drug interaction and toxicological testing on the same platform and demonstrates the potential of microfluidic bioreactors in clinical based drug interaction research. Toh *et al.* [258], developed a multiplexed 3D microfluidic chip consisting of a concentration gradient generator for drug cytotoxicity testing using primary hepatocyte cells. The IC_{50} results obtained using the device correlated well with the reported *in vivo* LD_{50} values for 5 model drugs tested.

Recently, Baudoin *et al.* [251] developed a microfluidic bioreactor to study the behaviour of a model liver cell line hepatocarcinoma (HepG2/C3A) with respect to variations of two culture parameters that is the inoculated cell density (0.35×10^6, 0.45×10^6, 0.65×10^6 cells per bioreactor) and flow rate (0, 10, 25 μL/min). The microbioreactor consists of a large cell culture chamber made from two PDMS layers. The bottom layer consists of microstructures with a series of microchambers and microchannels to support the attachment of cells. The top layer consists of inlet and outlet channels and sits on top of the bottom layer to close it. The capacity volume of each growth chamber is 40μL with a cell growth surface area of 2 cm^2. The effect of an environmental pollutant modelled with the ammonia concentrations (0, 5 and 10 mM) was also investigated. The proliferation rates for the HepG2/C3A in the device was found to be dependent on flowrate and inoculation density. It was also demonstrated that metabolic rates were higher in dynamic conditions than in static conditions. Furthermore, cell proliferation at low cell density was inhibited at high concentration of ammonia chloride, whereas at higher cell densites there was no effect. This work demonstrates the applicability for microfluidics to be used in larger *in vitro* toxicological studies.

5.5. Single Cell Analysis

The ability of microfluidic devices to manipulate, handle and analyse small volumes of samples precisely, has opened up new opportunities for the analysis of intracellular constituents [158]. Performing single cell analysis in microbioreactors holds great potential to studying the biochemistry and biophysics of individual cells leading to a better understanding of their genetic make up and diseases progression. The first approach for single cell analysis was demonstrated with micro-column separation techniques and capillary electrophoresis [259]. Since then, single cell analysis has become a field of intensive research with many proofs of principle devices being reported by several laboratories. Single cell microbioreactors have been applied in a number of different contexts including intracellular research, gene and protein content expression, cytotoxicity and fluorescence screens, antibody secretion, clone formation, trapping and sorting among many others [250].

The methods used for single cell analysis have been reviewed by Brown *et al.* [260], while the advantages and disadvantages of microfluidic devices for single cell analysis have been described by Chao *et al.* [261]. Single cells can be manipulated using a variety of methods including: hydrodynamic flow and focusing [249], the use of on chip microvalves and pump to direct cell transport [212], incorporation of cells into microfluidic droplets [262], optical and optoelectronic [263], trapping of cells and dielectrophoretic trapping of cells [264]. The majority of the work reported on single cells has shown that they can be studied by either destructive or non destructive methods. In destructive analysis, cells are lysed and their contents extracted and analysed. In non destructive analysis, the cell is studied according to a detectable signal arising from a specific cell response [261. Hong *et al.* [265] developed a microfluidic device integrated with pneumatic valves to isolate and lyse single cells using chemical methods to extract messenger RNA from a single cell. In a similar study, Zhong *et al.* [266], developed a multilayered PDMS device capable of processing 20 single cells simultaneously. The microdevice was used to extract RNA from single hESC and convert the mRNA to cDNA. The rate of converting mRNA to cDNA in the microdevice was five times more efficient than using the conventional bulk systems.

Hsiao and colleagues [267] developed a microfluidic device fabricated from glass and PDMS substrates, patterned with a pair of electrodes, for capturing and isolation of single cells. Positive dielectrophoretic forces were used to capture and lyse the single cells using the pair of electrodes, whereas cells were isolated into nanoliter compartments using the pneumatically actuated PDMS valves. The chip was capable of trapping, isolating and lysing individual cells in a parallel

Table 3. Specific Examples of Microfluidic Bioreactors for Single Cell Analysis

Description of study	Type of cell line	Detection method	Source
Analysis of reduced glutathione (GSH) and reactive oxygen species (ROS) in single erythrocytes	Single erythrocytes	Fluorescence	[271-272]
DNA isolation from the human whole blood sample and analyzing the Rsf-1 gene	Human whole blood	-	[273]
Quantitative analyses of protein and mRNA expression in individual cells	*E. coli*	Fluorescence	[274]
Development of an optofluidic system for performing absorbance-based flow cytometric analysis	T-lymphocyte cells (Jurkat)	Absorbance	[275]
Trapping of single bacteria cells in spatially well-defined locations without the use of chemical surface treatments	*E. coli*	Fluorescence	[276]
Integration of cell impedance analysis into a single-cell trapping microfluidic structure	HeLa	Impedance	[277]
Handling of cells in microfluidic platform using dielectrophoresis methods	*S.cerevisiae* and sheep red blood		[278]
Continuous differential impedance analysis of single cells held by a hydrodynamic cell trapping	HeLa	Impedance	[279]
Determination of hydrogen peroxide (H_2O_2) in individual HepG2 cells	HepG2	Fluorescence	[280]
Single cell quantification using microwell-based docking and programmable live cell imaging	*S. cerevisiae*	Fluorescence	[281]
On-chip low power piezoelectric actuated micro-sorter for deflecting single particles and cells at high-speed.	*E. coli*	Fluorescence	[282]
Molecular analysis of single human embryonic stem cells.	hESCs	Fluorescence	[283]
Encapsualtion of single cells	HL-60	Fluorescence	[284]
Immobilisation and culturing of cells	CHO-K1	Fluorescence	[285]

manner. A high throughput microfluidic imaging system capable of tracking single cells over multiple generations in 128 simultaneous experiments with programmable and precise chemical control has been described [268]. To achieve the high resolution imaging, the authors immobilised yeast cells in the device using a combination of mechanical clamping and polymerisation in an agarose gel. The complexity of the device which includes microvalves allows for the system to analyse yeast pheromone signalling response across 8 genotypes and 16 conditions. The above examples show that integrated microfluidic systems hold great promise in single cell analysis.

An electrical approach to single cell analysis in a microfluidic device fabricated from PDMS was developed by Jao *et al.* [269]. The group used a coplanar waveguide electrode inside the channel of the device to measure the impedance of a single human cervical epithelioid carcinoma (HeLa) cells. Single cells were characterised by a two port vector network analyser in the frequency range of 1 MHz –1GHz.

Kobel *et al.* [270] used fluid dynamics simulations in combination with particle image velocimetry to optimise trap architectures. The group developed a microfluidic chip with enhanced single cell trapping and on-chip culture performance. To demonstrate the utility of the device, an automated process was used to separate two daughter cells generated from a single division. The authors reported trapping efficiency of 97% and the device was capable for sustaining growth of non adherent cells for a long term without loss of viability. The selected examples show that integrated microfluidic platforms provide exciting insights into single cell analysis. Table **3** provides a summary of the most recent application of microbioreactors in single cell analysis.

5.6. *In Vitro* fertilisation

Microfluidic bioreactors have also been useful in artificial reproduction. Beebe *et al.* [286] developed a microfluidic device for manipulation of embryos and oocytes in the microchannels. In some other studies, Yamanish *et al.* [287 demonstrated the removal of the zona pellucid of a swine oocyte using magnetically driven microtool in a microfluidic bioreactor chip. The system was capable of manipulating a multiple of oocytes at one time with high stability. In contrast to conventional systems which use manual means of pipetting to remove the zona pellucida, the authors claim that the developed system is more efficient and ideal for high throughput and effective manipulations and has a great potential in the field of cloning and fertility treatment. Han *et al*, [288] reported a novel integrated microwell–structured microfluidic device that is capable of trapping single oocytes, fertilisation and subsequent embryo culturing. The device in an array format was used to capture and hold individual oocytes during the flow-through process of oocyte

and sperm loading, medium substitution and debris cleaning. To establish the effectiveness in oocyte trapping and removal of debris, computational and flow washing experiments were carried out to compare the difference in the sizes of the microwells. The results of the fertilisation process using the device compared favourably with the rates of the standard oil-covered drops in petri-dish method and demonstrate the potential to IVF practices for oocyte handling and manipulation. Krisher *et al.* [289], have reviewed the advantages of microfluidics for *in vitro* embryo production. Other reviews have dealt with assisted reproduction technology and embryonic stem cell growth and differentiation [29, 290].

6. FUTURE OUTLOOK

Different types of microbioreactors have been reported that have demonstrated their value as novel *in vitro* biomimetic engineered tools for a variety of different applications including high throughput cell culture analyses, understanding cell physiology and behaviours, drug development, protein production, therapeutics production, growth medium development, enzymatic processes, mRNA amplification and strain improvement [26, 130, 234, 242, 250].

Microbioreactors are increasingly opening up new opportunities especially in the area of cell biology. Despite this rapid progress in the use of microbioreactors there are several areas that need to be addressed for wider use and applicability. There will clearly be increasing efforts on development of commercial applications for microbioreactors. Efforts in this area have already been made by a number of companies including Cellsaic, Micronit, Fluidigm, Alipine, Aldagen, Evotec and Innovative Microtechnology (IMT). Future work on microbioreactor is likely to continue the integration of an increasing number of elements to create devices with higher functionality with a reduced footprint. Current microfluidic systems have a number of limitations in that they are not designed to screen multiple compounds simultaneously. Apart from downsizing bioreactors to microscale there is likely to be much more emphasis on creating microbioreactors using the design rules derived from the physics of fluid mechanics, diffusion in the microchannels and material behaviour [62, 291]. Despite the increase in the number of microbioreactor platforms, a major challenge in creating a microbioreactor for cell analysis is to understand the *in vivo* microenvironment of different cells. Different cells or tissues have slight different microenvironments which influence their phenotype. The utility and potential for microbioreactors is clear but a number of challenges remain and there is a need to combine scientific disciplines such as mechanics, fluidics, biology and chemistry to address these challenges.

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