Evaluating Cell Viability: A Crucial Step in Health Care Research and Development

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ABSTRACT

Bacterial inactivation using bacteriophages (or phages) has emerged as an effective solution for bacterial infections, but the screening methods used to evaluate the effectiveness of the phages to inactivate bacteria are not fast, reliable or precise enough. The efficiency of bacterial inactivation by phages has been evaluated by monitoring bacterial concentration either by counting colony-forming units (CFU), a laborious and time-consuming method, or by monitoring the optical density (OD), a less sensitive method. In this study, the resazurin cell viability assay was used to monitor the viability of bacteria from different genera during the inactivation by different phages, and the results were compared with the standard methods used to assess bacterial inactivation. The results showed that the resazurin colorimetric cell viability assay produces similar results to the standard method of colony counting and giving, and also more sensitive results than the OD method. The resazurin assay can be used to quickly obtain the results of the cell viability effect profile using two different bacterial strains and several different phages at the same time, which is extremely valuable in screening studies. Moreover, this methodology is established as an effective, accurate and rapid method when compared to the ones widely used to monitor bacterial inactivation mediated by phages.

INTRODUCTION

The increased emergence of antibiotic-resistant bacteria over the last years is a major public health problem. Alternative strategies must be developed to reduce the risk of development and dissemination of microbial resistance.Phage therapy is currently resurging as a potential complement/alternative to antibiotic treatment effectively killing even multidrug-resistant bacteria .[1]With this in mind, it is essential to obtain a method that allows us to rapidly evaluate the efficiency of bacterial inactivation by phages and to test a great number of

different phages at the same time to easily and rapidly preselect phages for further studies. A great variety of methods to evaluate the efficiency of bacterial inactivation are already available and include viable plate count methods, turbidity measurements, biluminescence assays and colorimetric test systems.[2]Optical density (OD) measurement has also been used in several studies as a tool to evaluate in real-time the process of bacterial inactivation by phages. However, despite this method being low cost, fast and non-destructive, it does not allow for the reliable evaluation of bacterial viability since it may be influenced by the aggregation of themicroorganisms, light scattering caused by anti-foam agents, dispersed gases, dead cells and cell debrisand unaccounted light scattering from dispersed inorganic salts and protein aggregates.

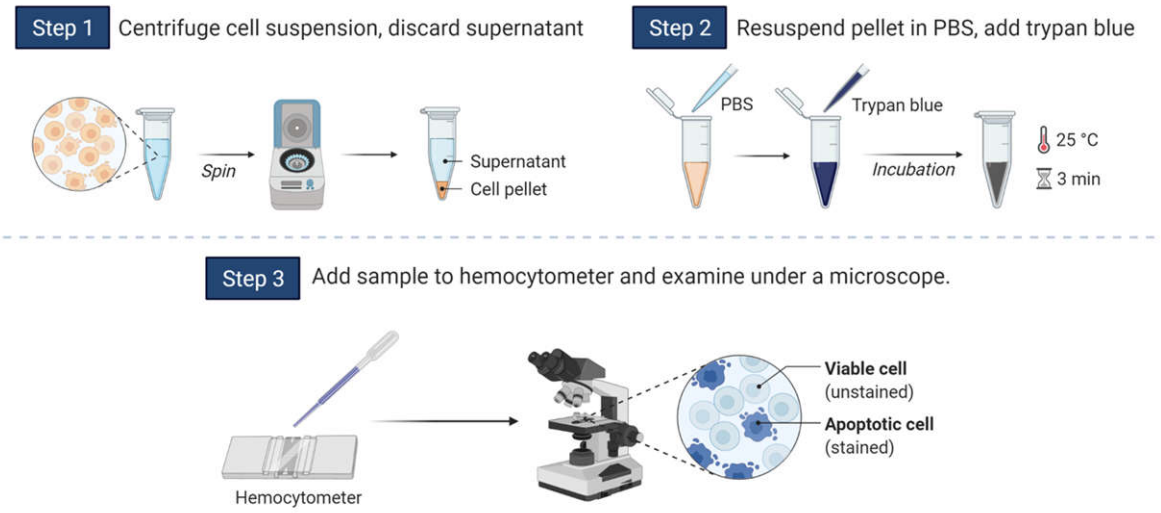

Figure-1-Steps involved in cell viability

The use of genetically modified bacteria with bioluminescence as a timesaving, real-time, cost-effective and simple operation for bacterial killing monitoring has been used, avoiding the laborious and time-consuming method of colony counts.[3]However, the optimum bacterial light emission conditionslimit the application of this method. The bioluminescence signal of bacteria depends on temperature, oxygen concentrationand the detection limit of the luminometer recorder. These factorsmake this methodology limited to bacterial inactivation by phage's, which is, in general, long.[4]Moreover, this method can only be used for genetically modified or naturally bioluminescent bacteria. Consequently, this approach is more adequate to study, for instance, the phage-host interaction or the impact of physical and chemical conditions on the efficiency of phages to inactivate bacteria.[5]TheMTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) tetrazolium assay is a popular colorimetric assay used to estimate the metabolic activity of living cells. Originally devised to be used in eukaryotic cells lines, it was later applied in bacteria and fungi. It consists of the spectrophotometric quantification of the intense purple-blue-collar of formazan, an enzymatic reduction of the lightly colored tetrazolium salt.[6]This colorimetric assay has already been used to determine phage screening, assess bacterial viability after phage treatmentand monitor biofilm inactivation by phages. How-ever, the absorption wavelength of tetrazolium salt reduction products is between 500 and600 nm, which coincides with the wavelength at which the bacterial optical density is read.[7-8]This factor can lead to the misinterpretation of the results since both absorbances of bacterial turbidity and the purple-blue color of formazan

will be read in the spectrophotometer. Other variants, like the XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-carboxanilide-2H-tetrazolium), can be used to overcome some of the MTT drawbacks by being more soluble and having a different absorption wavelength (450–500 nm).

2. Cell Viability and Cell Proliferation Assays

2.1. Cell Viability Assays

Morphological methods (including ultrastructural studies) require highly skilled personnel, expensive equipment and results are not usually quantifiable; cells must also be destroyed so continuous monitoring or kinetic studies are ruled out. Various cell viability and cell proliferation assays are used to determine the effect of a test compound on cells propagated in vitro. Indirect techniques that assess cell viability by monitoring cell membrane integrity after drug exposure, i.e., dye exclusion and preferential dye uptake, also destroy or interfere with the cell's functioning and hence are terminal assays. Other assays that measure cell viability, indirectly, by quantitation of reduction of the intracellular environment using indicator metabolic markers are useful and offer fewer limitations than other methods. Assays that quantify intracellular ATP (the ATP status of cells reflects the cells' energy capacity and viability) have also been developed where the concentration of ATP in indicative of the number of viable cells in that culture.

3. The Alamar Blue Assay

3.1. Assay Chemistry and Redox Principle

Alamar Blue monitors the reducing environment of the living cell. The active ingredient is resazurin (IUPAC name: 7-hydroxy-10-oxidophenoxazin-10-ium-3-one), also known as diazo-resorcinol, azoresorcin, resazoin, resazurine, which is water-soluble, stable in culture medium, is non-toxic and permeable through cell membranes. Continuous monitoring of cells in culture is therefore permitted. Resazurin was first used to assess bacterial or yeast contamination in milk by Pesch and Simmert in 1929.[9]It is a blue non-fluorescent dye that is reduced to the pink-colored, highly fluorescent resorufin. Resazurin solution is highly dichromatic based on Kreft's dichromaticity index (DI).The dye acts as an intermediate electron acceptor in the electron transport chain without interference of the normal transfer of electrons. Fluorescencesignals are measured at an excitation wavelength at 530–560 nm and an emission wavelength at 590 nm.

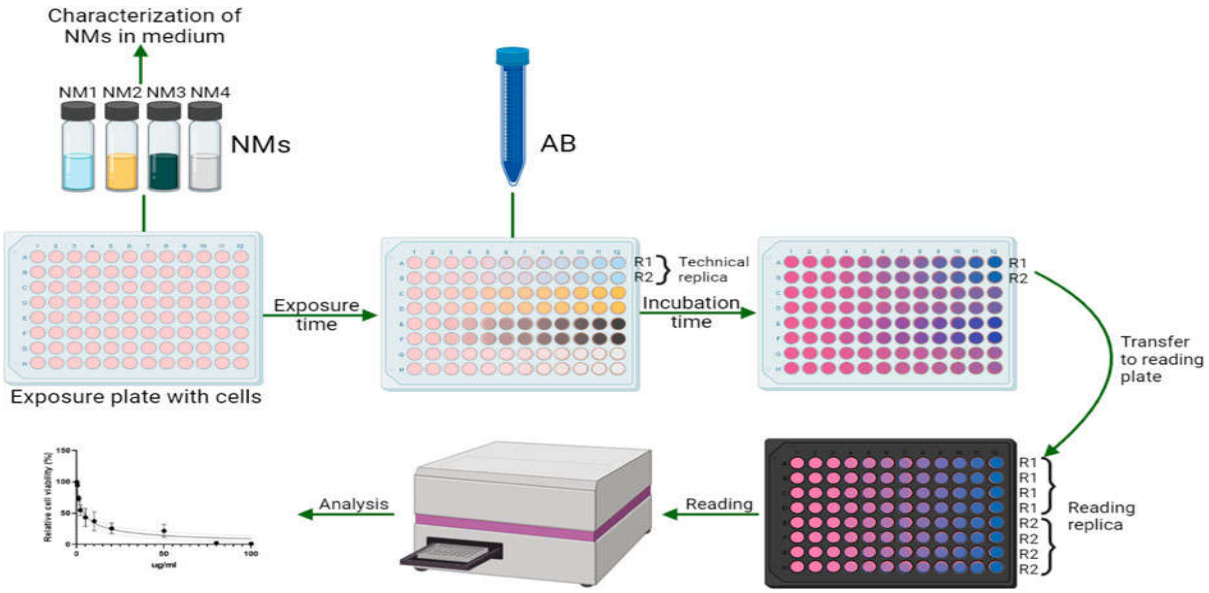

Figure-2-Alamar Blue Assay

3.2. Optimization Parameters and Assay Responsiveness

The optimum pH of the assay is ranges between 7.0 and 7.4, therefore, culture medium must have buffering capacity. The optimum incubation temperature is 37 °C and plates must therefore, be sealed to prevent evaporation. Assays must be carried out at a uniform temperature to ensure reproducibility of all wells and across a single plate. Alamar Blue is photosensitive and the incubations must be done in the dark.[10]The culture medium and the test compound themselves should not interact with the assay chemistry. Negative and positive controls must be empirically determined to ensure that there are no non-specific interactions with assay chemistry which would result in artifacts or false positive signals. The incubation time and cell density must be empirically determined and standardized as low cell density means slower growth and lower than expected levels of dye reduction. The end-point of the assay depends on the cell density used. Generally, it is recommended that the cells should be in the exponential stage of growth.

4. Specific Studies in Relation to Suitability of the Alamar Blue Assay and Comparison with other Bioassays

The various parameters that influence assay responsiveness are particularly important in studies of anti-microbial sensitivity in different micro-organisms. Alamar Blue has been used extensively in biomedical research to assess the relative susceptibilities of a number of pathogens to anti-microbial compounds, including multi-drug screening of various clinically important pathogenic yeasts and filamentous fungi (including Aspergillus spp., Candida spp.) with high levels of agreement with the recommended reference methods of the NCCLS.[11]Similarly, there have been numerous publicationsthat describe the successful use of Alamar Blue in drug screening for bacterial pathogens including but not limited to Mycobacterium spp., Staphylococcus spp., Enterococcus spp. and Pseudomonas spp . Methods for high-throughput screening of anti-biofilm compounds are needed. In such assays, Alamar Blue has been shown to be a more reproducible and cheaper redox indicator

than XTT and is useful in identifying resistance-compromised mutants and identification of compounds with anti-biofilm activity.

5. Alamar Blue Compatibility and Assay Chemistry Interactions

As with any bioassay, suitability must be determined for each application and cell model. While Alamar Blue has been used extensively in cell viability and cytotoxicity studies, its use in monitoring cell proliferation may be limited to studying specific cell models.

5.1. Selecting a Cell Model

The effects of indomethacin on tendon-derived cell proliferation using the Alamar Blue assay was studied. It was demonstrated that tendons appear to contain two subpopulations of cells; one subpopulation with apparently normal metabolic activity and a second subpopulation of cells with low levels of mitochondrial enzymes and subsequently low levels of oxidative metabolism.[12]Although it was also concluded that the Alamar Blue dye had no toxic effect on these cells, because of the differential metabolic rate of tendon-derived cells, reduction of Alamar Blue dye may appear to be non-linear. As indicated earlier, one of the more critical assumptions of such quantitative viability assays is that cell number and drug concentration share a linear and inversely proportional relationship.

5.2. Interaction Effects

Some of the reductases involved in reduction of Alamar Blue are present in subcellular components and not only in the mitochondria which implies that Alamar Blue reduction may not be entirely due to changes in mitochondrial function. Depending on the objectives of any given study, it may be necessary to determine the importance of mitochondria-restricted Alamar Blue reduction for each cell model.[13]Knowing the contribution of mitochondrial reductases to Alamar Blue reduction may be important to establishing the predictive ability of in vitro data. Interaction effects and assay compatibility problems have been specifically demonstrated in screening bioengineered nanomaterials. Engineered nanomaterials vary in diversity and complexity of the types of materials and have different physicochemical properties. As a result, it has been recommended that a diverse portfolio of cell viability and cytotoxicity assays (including the Alamar Blue assay) with different chemistries and detection mechanisms should be used for the assessment of nanoparticles or nanomaterials.

Production and incorporation into creams

Many different techniques for the production of lipid nanoparticles have been described in the literature. Therefore, no regulatory problems exist for the production of topical pharmaceutical and cosmetic preparations using this production technique. It can be considered as being industrially the most feasible one. Lipid nanoparticles can be produced by either the hot or cold high pressure homogenization technique. shows schematically the steps of these two methods. The active compound is dissolved or dispersed in melted solid lipid for SLN or in a mixture of liquid lipid (oil) and melted solid lipid for NLC. In the hot homogenization method the lipid melt containing the active compound is dispersed in a hot surfactant solution of the same temperature (5–10 ◦C above the melting point of the solid lipid or lipid blend) by high speed stirring.

Pharmaceutical formulations and benefits

Topical treatment of skin diseases has the advantage that high drug levels can be achieved at the site of disease and systemic side effects can be reduced compared to oral or parenteral

drug administration. Topical drug administration is still a challenge in pharmaceutics due to the difficulties in controlling and determining the exact amount of drug that reaches the different skin layers. The drugs and the vehicles physicochemical properties are considered to be the main features responsible for the drug differential distribution in the skin. Lipid nanoparticles have been investigated to improve the treatment of skin diseases such as atopic eczema, psoriasis, acne, skin mycosis and inflammations. Apart from the treatment of skin diseases by topical application, e.g. gastrointestinal side effects of non-steroidal anti-inflammatory drugs can be decreased by topical antirheumatic therapy.

5.3. Topical glucocorticoids

Topical corticosteroids are the first-line therapy of acute exacerbations of atopic dermatitis and contact dermatitis. Prednicarbate is superior to the halogenated glucocorticoids because of an improved benefit/risk ratio. However, at the present the separation of desired anti-inflammatory effects and undesired antiproliferative effects is still not satisfying. Therefore, lipid nanoparticles were investigated as a delivery system for prednicarbate. Santosetal. reported an improved extent of prednicarbate uptake by human skin in vitro, if applied as SLN dispersion or cream containing prednicarbate-loaded SLN (Santos Maia et al., 2000). The authors found that a prednicarbate targeting to the epidermis occurred (Santos Maia et al., 2002). This is particular relevant because prednicarbate in the dermis is responsible for the induction of irreversible skin atrophy while the inflammatory process is most pronounced within the epidermis (Schäfer-Korting et al.,2007). Therefore, a better benefit/risk ratio is expected for the application of pretnicarbate in SLN containing topical formulations.

Methodology

A literature-based search, covering research reports that have been published the last five years, was performed to retrieve information on the chemistry, health effects, molecular pharmacology, herb–drug interaction, nanotechnology-based drug delivery, and safety of black cumin and TQ from accessible online databases, such as PubMed, Web of Science, Scopus, and Google Scholar, using the key search terms of ‘black cumin’ and chemicalconstituents, antioxidant, anti-inflammatory, immunomodulatory, neuroprotective, nephroprotective, anti-obesity, cardioprotective, hepatoprotective, anticancer, nanotechnology or toxicity, etc. This review covers those articles that demonstrate the health benefits of black cumin alone or its compounds or both.

6.Phytochemical Profiles

The phytochemical composition of black cumin varies, depending on the growing regions, maturity stage, processing methods, and isolation techniques. Bioactive phytochemicals of black cumin, comprising major and minor secondary metabolites, have been categorized into different chemical classes.

6.1. Electro-Osmotic Flow (EOF)

The fluid transport is generated through microfluidic channel walls of chargeable materials. An electric field is induced when a thin ion-layer of liquid is formed at the wall surface which is oppositely charged to the charge of the wall . The fluid will operate as an electric double layer; a thin layer (Stern layer) of counter ions at the wall and another thicker layer (diffuse layer) of excess charges with the same polarity as those in the Stern layer.[14]The ions within the Stern layer are fixed at the wall and the ions in the diffusion layer are movable. By

applying an electric field across the channel the fluid will move towards the electrode of opposite polarity, starting near the walls and transferring via viscous forces to the convective motion of Polymers the fluid. This method has been used with different microfluidic devices fabricated from different materials such as glass, polymers and PDMS (polydimethylsiloxane). The method is used widely in capillary electrophoresis. However, this method is not qualified for some electrophysiological investigations due to the noise arising from electromagnetic fields.

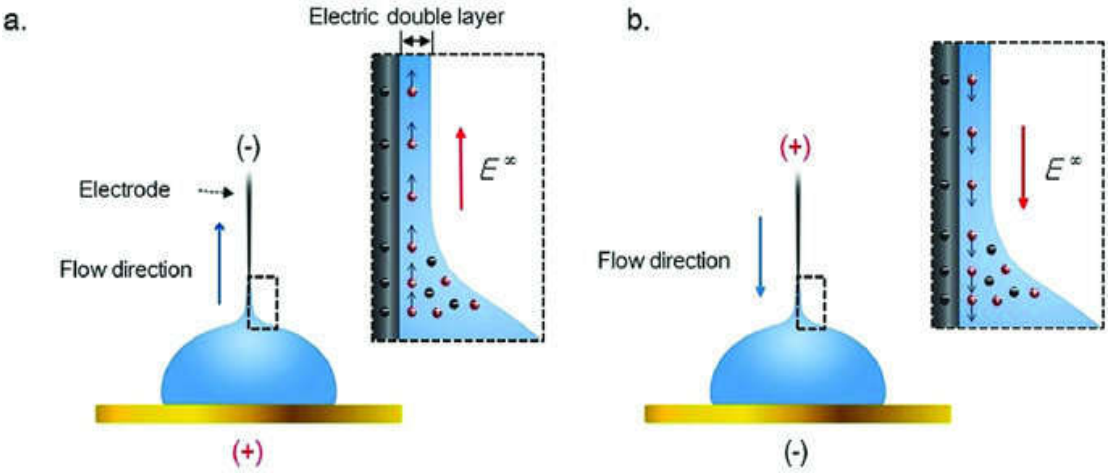

Figure-3-Electro-osmotic flow

6.2. Electrophoresis Flow

Electrophoresis flow results from the accelerating force due to the charge of a molecule in an electric field balanced by the frictional force. The induced forces accelerate the charged ions towards the cathode and the anode.[15]The movement velocity of the ions in an applied electric field is expressed by electrophoretic mobility $\mu = v/E$ where v is the movement velocity of ions and E is the electric field intensity. Hence the mobility is independent of particle size (for uniform surface charge and electrical field). One approach is to manipulate samples within an ionic buffer solution at a specific pH. The samples, cells or particles, will experience a different mobility that may be separated depending on their size and charge.

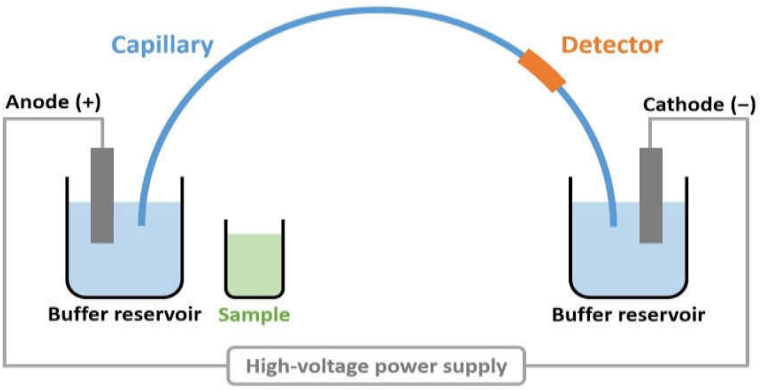

Figure-4-Electrophoresis Flow

7. Fabrication of Microfluidic Devices

Microfluidic devices were initially based on non-polymeric materials like silicon or glass, fabricated by the well-established integrated circuit (IC) production techniques such as photolithography and surface micro-matching due to the equipment availability and the possibility to be integrated with electronics.[16]Glass materials were preferred for their excellent biocompatibilityand the high tolerance of high temperature and strong solvents that was suitable for application with capillary electrophoresis. However, the drawbacks were mainly the non-optical transparency of silicon, the high cost and the micromachining complexity of silicon and glass materials. The reduction of fabrication costs and the optimization time have been improved by rapid prototyping techniques, where device geometries are quickly evaluated.[17]State-of-the-art fabrication techniques of microfluidic devices may be classified into direct techniques such as laser ablation or laser micromachining of polymers, photolithography or optical lithography, X-ray lithography and prototyping techniques, including hot embossing , injection moulding , and soft lithography.

7.1. Integrated Micromachined Devices

Micromachined devices have shown new advances to control the fluid flow within microfluidic systems, allowing high levels of microchip integration and analytical throughput. Many designs in various applications have been presented over the past ten years.[18]They show a considerable potential for integrated microfluidic devices or systems, including microvalves, micropumps, and micromixers, Embedded microvalves improve the function of microfluidic systems by controlling the direction of fluid flow over time. Micropumps for control and manipulation of fluid volumes on-chip are good options to replace external pumps. Microvalves are used to control the fluid flow within the microchannels by changing external parameters.

7.2. Direct Fabrication Techniques

7.2.1. Photolithography

Photolithography is a technique based on the exposure of light on a photoactive material that undergoes a chemical reaction to form a liquid-solid transition. The basic components needed for photolithographic fabrication are a light source, usually UV light of 254–365 nm, a spin coater with programmable speed and time, and a suitable substrate with a photo-reactive material.[19]Anon-polymeric solid substrate (glass or silicon) is spin-coated to apply a thin layer 1–500 μm of photoresist, usually SU-8, by centrifugal force. After evaporation of the solvents in the photoresist via soft baking, the substrate is exposed to UV light through a high-resolution mask of plastic or glass film with the desired microfluidic pattern. After baking, depending on which photoresist is used, the un-, or exposed layer of the photoresist is removed using chemical bath development.[20]The desired pattern remains on the substrate forming apositive or negative mold. The light-exposed areas on the positive photoresist are usually soluble. For the negative photoresist, the areas are insoluble.The photoresist is often used to obtain negative molds for PDMS-based microfluidic devices replicated by soft lithography.

7.3. Prototyping Techniques

7.3.1. The Hot Embossing Technique

The Hot Embossing technique is based on a mold of permanent microstructures that is bossed gradually by pressing with a specific force on a polymeric thermoplastic substrate while it is heated slightly above the glass transition temperature (Tg) of the substrate . After cooling, the thermoplastic substrate is released, and contains a glassy or semi crystalline microstructure. The method enables straightforward fabrication of microfluidic devices of single layers and thin channels. However, to produce devices with enclosed channels, bonding of multi thermoplastic layers with heat, glue or other techniques are necessary.[21]Also, the shallow channels make it challenging to achieve connections within the microchip. Moreover, undercut structures cannot be shaped; therefore assembly of separately fabricated layers is essential for enclosed structures. Recently, a broad range of improvements has been reported, related to hot embossing techniques.Large-scale hot embossing to create low cost and high quality microfluidic structures has been presented .

7.3.2. Computer Numerical Control (CNC) Micromachining

Computer numerical control (CNC) micromachining is used for fabrication of PMMA-based microchips. Most analytical microchips require features with dimensions in the order of 10–200 μm. Such tolerances are possible with CNC milling as a standard fabrication technique. The conventional CNC milling is used to fabricate prototype microchips for straight use or structured molds of harder materials for rapid generation of analytical microchip platforms via PDMS casting or hot embossing.[22]In some applications, the CNC machines may be combined with microscopic surveillance to yield microchips with accurate tolerances on milled structures in the order of 2–10 μm.Priorto any milling, drilling, or cutting, the desired tolerances of the vertical and horizontal positions of the mill should be adjusted relative to the surface of the block of PMMA or other materials.

8.Microfluidic Applications in Pharmaceutical, Biological and Biomedical Engineering

8.1. Manipulation of Biological Cells on-Chip

During the last few years, the manipulation of biological cells in microfluidic devices has increased widely in biology, medicine and biotechnology. The manipulation of biological cells makes it possible to move the sample without coming into physical contact within a closed microfluidic system. Most touch-free manipulation techniques can be easily integrated into existing setups, making them widely usable. The main methods are presented in the following sections, with some detaileddescriptions on the common applications in cell biology.

8.1.1. Magnetic Manipulation

Magnetic manipulation is a contact-free method and uses magnetic forces induced by external or embedded magnetic fields to manipulate magnetically labelled biological cells. The cells are labelled magnetically by attaching nano paramagnetic beads with high selectivity.[23]The magnetic beads can be functionalized with antibodies, peptides or lectins to interact with the biological cells. Commercialization of the technique has been presented as a Magnetically Actuated Cell Sorter (MACS) by Miltenyi Biotec GmbH, in Germany . In other designs, a quadrupole magnet surrounding the flowing cellular solution enables the deflection of magnetic-labelled cells in desired directions.

9.Tissue Engineering Models on Microfluidic Chips

The topic of tissue engineering includes mainly in vitro reconstruction of tissue or organ function. Microfluidic systems may offer opportunities to facilitate such applications. Lately, a generation of artificial lung tissue has been produced on a microfluidic chip with an immunological functional bi-layer of endothelial and epithelial cells on two sides of a porous PDMS membrane.[24]The system has been used as a model to investigate the toxicity of nanoparticles by imitating the deformation of the bi-layer that activates the dependent uptake of nanoparticles by the epithelium. 3-D tissue models, such as bone marrow, use advanced biomaterials that can be photo-polymerized by microfabrication techniques such as photolithography, to create complex patterned structures. Microfluidics can also allow precise configuration for micro-tissues on chip models or 3-D micro-tissues off chip using hydrogel microbeads.[25]The challenge to create functional artificial tissues is the 3-D patterning of manifold types of cells into a tissue-like structural design. The control of components of 3-D co-culture spatial systems by microfluidics is valuable . An approachusing a hydrogel bi-layer of hepatocytes and endothelial cells has allowed the imitation of interstitial flow for the formation of tissue-like structures by the hepatocytes.

Results

Replicability was affected by suboptimal resazurin reduction assay and cell culture protocols.

To be able to compare drug potency estimates between different studies, long-established breast cancer cell lines (HCC38 and MCF7) and pharmaceutical agents (bortezomib, cisplatin, and carboplatin) in cancer research and drug screening were utilized. To investigate the effect of these chemotherapeutic agents on cell viability in MCF7 and HCC38 cells, we initially performed the resazurin reduction assay using standard experimental protocols and calculated dose-response curves and the half-maximal inhibitory concentration (IC50) after 24-hour drug exposure. Cells were plated at a density of 1.0×104 cells per 96-well in 100 µl HuMEC basal serum-free medium. Previous studies have shown that complete growth medium supplemented with FBS reduces the effect of proteasome inhibitor bortezomib on proteasome activity, thereby warranting the use of HuMEC medium for all drug treatments.

Discussion

Liposomes in themselves are relatively unstable delivery systems because of theirmembrane instability in aqueous solution, which can affect their bioavailability and pharmacological effect, the addition of adjuvants being necessary to bring more rigidity andstability. AST seems to enhance the liposomes' stability when on the membrane, whichhelps its delivery. The strategy of adding other adjuvants to improve membrane rigidity seems to be necessary to ensure stability, which should be taken into account when assessing production costs. Reducing the particle size with the development of nanoliposomes seems to be an excellent alternative method to improve their stability and consequently their penetration, solubility, and continuous release; however, it is still necessary to considerthe machinery and costs involved. Another innovative alternative method to deliver ASTon deep skin layers is the use of the iontophoretic technique with charged delivery systems, developed with liposomes, which makes it possible to reach the stratum corneum and act as a whitening agent.Emulsion delivery systems, especially nano emulsions, are an effective and widely applied form to

deliver bioactive compounds in medicines and cosmetics. Due to the hydrophobic character of AST, most of the nano emulsions prepared in the literature areoil/water systems. Studies based on experimental design allow researchers to identify the best conditions for nano emulsion preparation, with reduced time and costs of research. In addition, nano emulsions can be incorporated in other delivery systems, such as films, which can significantly improve their biological effects and their acceptance by the patentor customer. Unfortunately, only one article was found concerning the use of microemulsions in dermatological and cosmetics applications. More investment in research on thisnew product delivery system is desirable.

10. Colony-Counting Method

Since eight different phages and two bacteria were used, it was possible to observe different growth rates and killing curves through the use of the colony-counting method. This method has the main advantages of well-standardized protocols and usually higher sensitivity compared to other methods. Although very precise, like other authorssuggested , colony-counting in solid medium is too complex, laborious and time-consuming to test more than two samples with their correspondent control at the same time.

10.1. Optical-Density Method

OD measurement is a very fast method to monitor bacterial growth/inactivation in real-time, but it lacks accuracy. As seen in the results, when bacterial inactivation occurs in the first 4 h of the assays, even with high concentrations of bacteria (7 log CFU/mL), the absorbance is too close to the method's lower detection limit to observe any early inactivation and to efficiently discriminate the effectiveness of the different phages, mainly when the bacterial inactivation starts and when it reaches its real maximum. Some authors suggested that OD measurements are not suitable when substantial changes in cell size are expected to occur during microbial growth and that it is only applicable in specific concentration ranges.

10.2. Resazurin Method

The results with the resazurin cell viability method showed similar bacterial inactivation profiles to the ones observed in the colony-counting methodology for both E. coli and S. Typhimurium, demonstrating that the resazurin microplate method is as accurate as the plate colony-count technique. Despite the inactivation of E. coli by phage ELY-1 monitored by colony-forming units not showing a perfect match with the results achieved with the resazurin cell viability assay in the first 4 h of incubation, in general, all profiles are similar. It cannot be neglected that resazurin assays measure the cell viability of bacteria, which does not mean that when the fluorescence decreased the bacteria were inactivated, but only affected, showing a decrease in their viability. These small variations on the bacterial viability cannot be detected by colony-counting methodology. Similar findings were observed by Tapetal. where the authors developed a resazurin microplate method for the evaluation of the antimicrobial activity of antiseptics and disinfectants on several different bacteria and reported that this method was as precise as the plate count method, showing similar detection limits.

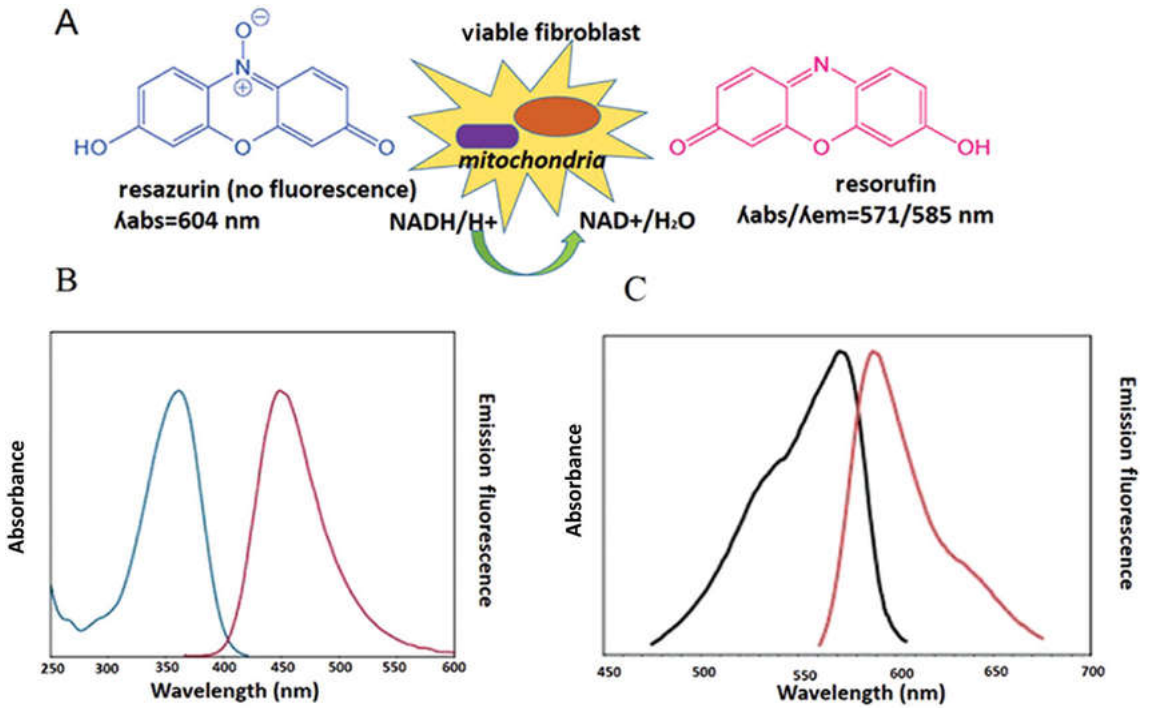

Figure-5-Resazurin Method

Application:

• Study objective: specific impairment of cytosolic vs. mitochondrial reductases important.
• Cell model: cell type, adherent/non-adherent cells, cell density, cell line and age of cells.
• Other studies demonstrated success with the cell model under investigation.
• Availability and type of instrumentation: fluorometric or colorimetric or both.
• Data recovery: indirect qualitative and/or quantitative.
• End-point determinations important.
• Need for a second assay to directly measure cell number.
• Time-course monitoring required.
• Storage and stability of the reagent.
• Reproducibility of the data and level of intra- and inter-assay agreement.
• Total cost of the assay.
• Can biological implications be reliably predicted from in vitro dataSLN and NLC are verywell-tolerated carrier systems for dermal application.

Conclusions

This work has shown that resazurin cell viability assay can effectively assess bacterial inactivation by several different phages at the same time. The decrease profiles given by the resazurin method are similar to those given by the colony counting method, making this colorimetric method a promising alternative to OD measurements as a fast and precise method to assess bacterial inactivation by phages. This method can provide a lower detection limit than the OD method and be used to assess the inactivation of different strains by several different phages at the same time, which is extremely valuable in screening studies and the preselection of phages. Accurate prediction of the adverse effects of test compounds on living systems, detection of toxic thresholds, and expansion of experimental data sets to include multiple toxicity end-point analysis are required for any robust screening regime. Important

Points to Consider with Respect to Suitability of the Alamar Blue Assay for a Specific.Delivery systems are useful for the improvement of the physicochemical profile of this compound, such as stability, water solubility, antioxidant properties, drug release, and in vivo and in vitro biological activities. Therefore, the delivery systems for loading AST described in this article create opportunities for industrial applications; however, other industrial issues for the development of new products must be evaluated.

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