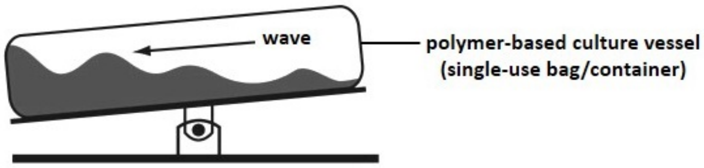
A MINIATURE HYBRID WAVE-INDUCED MIXING BIOREACTOR SYSTEM: STUDIES AND MODELING OF ANIMAL CELLS GROWTH

Increasingly, single-use (also called disposables) have been pushing forward in modern bioprocesses and bioengineering. Such simple and ready-to-use devices replace their reusable counterparts made from stainless steel or glass whenever possible. Nowadays, the users have possibilities to choose among a multitude of products from microscale, then laboratory, and finally production scale. In fact, the design of fully disposable production facilities for simple miniaturized as well as small- and middle-volume-scale production processes such as cell-inoculated or even tissue-like implants development has recently been possible.

Mixing constitutes one of the most critical process operations used in cell cultures or biomanufacturing and is utilized to achieve homogenization, suspension dispersion (in liquid-liquid as well as gas-liquid systems), and heat exchange. Conventional mixing devices fabricated with stainless steel currently represent the gold standard in bioprocess engineering and bioproducts manufacturing. However, the increasing interest in single-use technology within the implants development, due to its numerous advantages and the fewer constraints compared with conventional steel-made apparatus, has resulted in robust and prosperious development of miniaturized (i.e. mililiter-scale volume) plastic bag or container wave-induced mixing systems.

Instead of rotating or tumbling a stirrer to induce fluid flow, continuously oscillating devices can be utilized, and mixing is achieved by horizontal and/or vertical oscillation of the bag, which is fixed in a rocker unit. The rocking motion is very efficient in generating waves, and the wave-induced motion in the culture bag/container causes large volumes of fluid to move and facilitates dispersion of components of culture microenvironment, i.e. respiraty gases, nutrients, extracellularly secreted metabolic waste bioproducts. The optimum operating parameters depends on the combination of container geometry, its support, filling volume, rocking angle, frequency and rate, and the characteristic of the utilized culture medium.



Synthetic liquid perfluorochemicals (synonym: fluorocarbons) can be used as efficient liquid carriers of respiratory gases (both O_2 and CO_2), which has raised much interest in medical and technical applications. The oxygen solubility in perfluorochemicals is approximately 20 times higher than in water; the solubility of carbon dioxide in liquid perfluorochemicals can even be up to 3 times higher. Importantly, liquid PFCs are immiscible with aqueous media and they create a separate liquid lying below the medium, at the bottom of a culture dish. The lack of toxicity and negative side effects of liquid perfluorochemicals on living cells has been confirmed by in vitro experiments and also in clinical investigations, and by the Food and Drug Administration (USA). An innovative bioengineering application of liquid perfluorochemical is the liquid-liquid culture system for in vitro cultures of 3-D aggregated mammalian and human cells. Such aggregates can be grown on the liquid-liquid interface created between a perfluorochemical and a culture medium. Very advantageously, in difference to other existing technologies, multicellular aggregates or sheets of mammalian and human cells can be easily collected and passaged. Thus the 3-D structure of them can be preserved during subcultivation. However, data on mammalian and human cell cultures at perfluorochemical-medium surface are very limited.

The basic aims of the research project are: (I) to develop and prototype the innovative miniaturized hybrid single-use bioreactor system which incorporating a hydrophobic liquid perfluorochemical as a liquid gas carrier to enhance supplying/removing of respiratory gases (O_2 and CO_2); (II) determination of effects between two contacting liquid phases (perfluorochemical and medium), as well as (III) modeling of the growth of mammalian cells cultured in the proposed system. The proposed bioreactor system will be characterized by low hydrodynamic shear forces due to application of the continuous wave-induced mixing system for obtaining homogenous gas concentration in both contacting liquid phases of the culture system. From the bioprocess engineering point of view, the quantitative investigation of growth kinetic of cultured mammalian and human cells, as well as the modeling of mass transfer effects in the designed system will be significant scientific achievements of the proposed project.

If such technology could be designed and its prototype be manufactured, this would be <u>a new worldwide milestone</u> in the improvement of bioprocess engineering and bioengineering practical applications. The researchers and the laboratories in the field will benefit from results of the proposed project.