

## Appraisal of Microcarrier Suspension in a Single-use Shaken Bioreactor with Conical Bottom

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The pharmaceutical industry is at the forefront of the production of drug products using mammalian cell-based cultures, some of which rely on anchorage dependant cells, with single-use technology bioreactors gaining prominence. Whilst often adapted to suspension cultures, hybridoma and other cells give increased product yields when cultured attached to a substrate, such as microcarriers, resulting in smaller fermentation runs at higher densities. Microcarriers are also used in tissue engineering and cell for therapy culture. Stem cells are adherent-dependant cells and traditional 2-dimensional static cultures rely on disposable multilayer vessels, which have become the common route for stem cell expansion. It has been a recent industry trend to use shaken bioreactors at large scale in the upstream process, performing mammalian cell culture in single-use bags which eliminate the need for cleaning in place, offer flexibility and lower production down-times. Whilst currently still to be fully developed, large scale production of stem cells would require the use of 3-dimensional dynamic culture methods by employing microcarrier technology, as demonstrated by Frauenschuh et al. (2007). Microcarriers consist of spherical beads with a size of 100-300  $\mu\text{m}$ , and can be made of a wide variety of materials, amongst others: plastics, glass, acrylamide, silica, silicone rubber, cellulose dextran and collagen. Medium to large scale single-use devices would greatly benefit the consistency of stem cell culture whilst maintaining potency.

Whilst most studies have focused on investigating optimal compositions of microcarrier materials, their concentration and cell culture media, little research has been undertaken on the engineering aspects of microcarrier use. The aim of the work is to characterize the suspension dynamics of microcarriers in a cylindrical orbitally shaken bioreactor (OSR) with conical bottoms of different heights. This study builds upon previous works of the research group (1-4) for a flat bottom reactor, where increases in Froude number were found to determine a mean flow transition which was found to be instrumental in determining the just-suspended speed.

The dynamics of solid suspension is studied using commercially available Cytodex-3, stained with trypan for improved visual contrast and image acquisition. The experimental procedure allows to estimate not only the speed required for the solids to lift from the vessel bottom, but also the conditions at which a homogenous distribution of microcarriers is obtained. Preliminary results indicate that the presence of the conical bottom improves solid suspension by requiring lower agitation rates for the microcarriers to lift from the bottom completely. The critical Froude, which determines the flow type controlling the bioreactor, can be used to scale the suspension of microcarriers in OSRs.