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Characterisation of a shaken cylindrical 12 L bottle regarding mixing times and microcarriers

Abstract/ Introduction

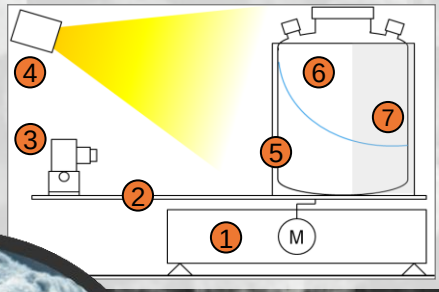
Human **stem cells** (adherent) have gained increased importance for therapeutic applications in recent years [1]. To meet the market's need for stem cells, robust expansion methods are required [2].
The **microcarrier culture** is one of the most effective techniques to cultivate **adherent cells** under dynamic conditions and has proven suitable for the cultivation of stem cells [3]. However, stem cells are fragile and **shear stress** can drastically affect the way the cells grow and differentiate [4].

pic. 1: 12L culture vessel (Nalgene)

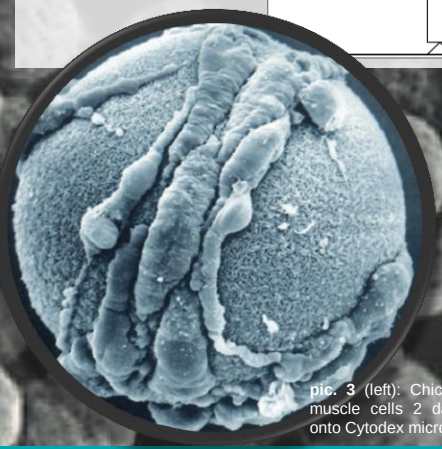
Orbitally shaken vessels expose cells to low shear stress, making them suitable for the cultivation of shear sensitive organisms [5,6].
In this work the influence of microcarriers on **mixing times** in an orbitally shaken **disposable 12 L cell culture bottle** (pic.1) was investigated. Furthermore **microcarrier's distribution** was analysed to determine the minimal shaking frequency needed to achieve homogeneous microcarrier distribution without unnecessary shear stress.

Materials and methods

- **Microcarriers (MCs):** Untreated Cytodex 3 (GE Healthcare) with a conc. of 3 g per L deionised water, swelled for 24 h.
- **Shaker:** ES-X (Kühner AG) with 50 mm shaking diameter.
- **Vessel:** 12 L disposable polycarbonate bottle (Nalgene).
- **Mixing times**: Determined using the decolourisation method (Lugol's iodine in a 0.4 vol.% starch solution, decoloured with 1M sodium thiosulfate).
- **Microcarrier distribution:** The MCs were stained in deion. water with blue food colouring (Trawosa) and shaken for 24 h (swelling of MCs and avoiding of aggregation). The bottle was filmed with a high-speed camera (Casio EX-FH20) with 210 fps and the video was modified (increasing of blue colour intensity and contrast) with the video-modification software virtual dub (filter MSUColorEnhancement 1.0b).



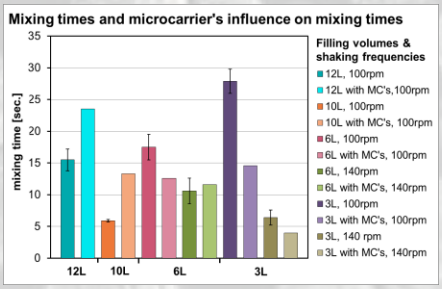
pic. 2: Experiment's setting
1) Shaker
2) Shaker tray
3) High-speed camera
4) Light source
5) Lucent acrylic glass cylinder
6) Disposable 12L bottle
7) White paper background



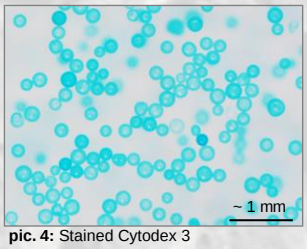
Background: Scanning electron micrographs of Cytodex microcarriers [7].

pic. 3 (left): Chicken embryo skeletal muscle cells 2 days after inoculation onto Cytodex microcarrier [7].

Results and Discussion

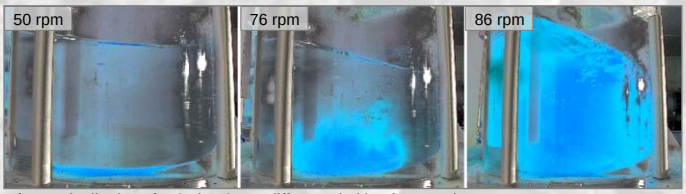


Mixing times: The tests without MCs were performed in triplicate, the mixing times with MCs once. The addition of MCs in the volumes of 10 L and 12 L significantly increased the mixing times. In contrast, at lower filling volumes, mixing times were somewhat reduced. Hence, MCs influence the mixing times but with no clearly predication.



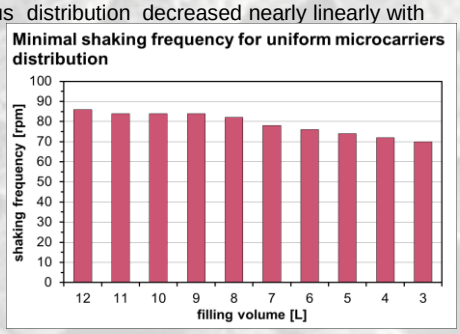
pic. 4: Stained Cytodex 3

Microcarrier distribution: To check if the colour promotes the forming of MC aggregates, the MCs were analysed microscopically (pic. 4). Regarding pic. 4 it seems to be that aggregates occurred, but softly poking the petri dish with the MCs in solution, showed free motion of all MCs, indicating that no aggregates were formed.



pic. 5: Distribution of MCs in 12 L at different shaking frequencies

The distribution of the stained MCs was visually observable (pic. 5). It was shown, that the minimal shaking frequency needed to get a (visually) homogeneous distribution decreased nearly linearly with declining filling volumes. With 12 L, 86 rpm are necessary to achieve uniform distribution. With 3 L, 70 rpm are required. Below the approved minimal shaking frequencies, accumulations in the centre of the vessel were observable.



Conclusion

The general MC distribution is observable with the presented method, but exact conc. gradients aren't analysable with it. Mixing time experiments showed that MCs possess the potential to influence engineering parameters, which we speculate to be due to increased turbulent flow, surface changes and suspension characteristics. But mixing times are still acceptable, thus orbital shaken bioreactors promise to be a suitable technique for the stem cell cultivation.

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