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Single-step flask to 250 L cell culture with a hybrid mixing single-use bioreactor

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The process of scaling suspended cell cultures from frozen stocks through a large single-use bioreactor (SUB) includes numerous processing steps and operations. These steps, or seed train, involve vessel transfers and associated connections that increase the potential for contamination and human error. Of the multiple methods available for inoculating a 250 L SUB with sufficient cell quanitites, including multiple shake flasks, tabletop stirred tank bioreactor, or a 50L SUBs. Over the past decade tilting-type single-use bioreactor has been particularly attractive. Such wave-inducing systems implement tilting or rocking motion to agitate cultures which provides gas transfer and maintains cell suspension. Additionally, these rocking systems capitalize on single-use bioprocess containers (BPCs) which facilitate rapid deployment and cleanup when compared to reusable alternatives. Unfortunately, while rocking systems are simpler than other options, they still require connection and transfer steps.

In an effort to further streamline the scale-up process, a tilting mechanism and bottom heating jacket have been added to a standard 250 L SUB to provide rocking motion and bottom temperature control to the BPC. This hybrid system can thus utilize rocking motion to mix small volumes of liquid (10-20L) and maintains standard impeller-based stirring functionality for volumes around 50 L and above. As designed, proliferating cells in 500 mL of media from flasks could be directly added to small volumes of media in the tilting SUB. Additional expansions with media addition are supported with rocking and bottom heating. An example expansion sequence would take cells in 500 mL media in a flask to 1.5 L of fresh media to form 2 L in the hybrid SUB. This would be followed by 8 L of fresh media addition after sufficient growth to form 10 L of media. 40 L of fresh media could then be added after cell growth and once media volume has reached 50 L. At this point, mixing via rocking will be discontinued and operation would follow well defined 5:1 turndown settings using a standard pitched blade impeller and drilled hole sparger (DHS) for the remaining SUB culture process.

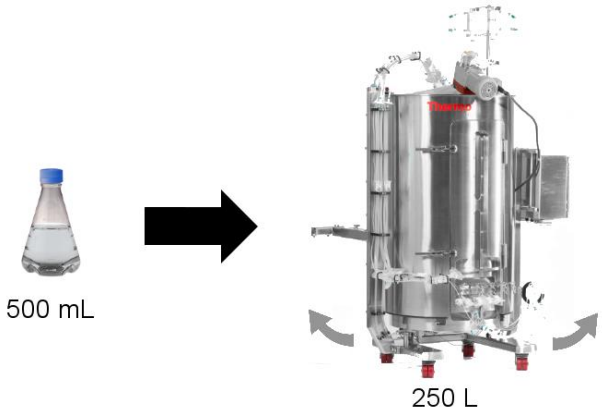

Figure 1. Single-step seeding of a rocking hybrid SUB for simplified cell culture.

Currently, overhead gassing coupled with liquid rocking is used primarily for expanding cultures. As low cell density shake flask and scale-up process do not often feature active feedback control, future cell-specific processing could be optimized as the 250 L SUB already equipped with integrated monitoring and controls for gassing, mixing, pH, dO2, dCO2, cell mass, and/or other emerging process analytical technologies (PAT). Implementation of such a hybrid SUB would reduce expenses, manual operations, and potential risk of process contamination. The 250L working volume SUB cultured to high density is sufficient for seeding multiple 1000L or 2000L SUBs in a production environment using a 5:1 turn-down. Alternatively, this concept should be of particular interest to those possessing high productivity cell lines that have need to generate clinical grade and quantities of materials, while only investing in only one piece of bioreactor equipment or they desire a scale-up solution that optimizes limited facility work space.