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Development of a Novel Cold-Chain Tubing, FP-FLEX™, and Single-Use Freezing Bag

For Working Cell Banks Enabling Closed-System Processing to Temperatures as Low as -196ºC

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Abstract

Working cell banks (WCBs) are commonly applied to initiate cell culture manufacturing campaigns to produce therapeutic proteins. Those campaigns typically begin with the inoculation of cells previously cryopreserved in vials. Although vials are typically used to establish WCBs and initiate manufacturing campaigns, they are not optimal for the growing demands of commercial production.

Vials are small, and filling and removal of their contents are performed through an open cap. That process leads to numerous manual operations and culture vessels, resulting in contamination risks and potential campaign-to-campaign variability. Single-use bags would be ideal for WCB applications, but adoption has not been observed because currently available bags and tubing don't hold up to the demands (e.g., they break and/or can't be welded) when stored and transported at cryogenic (-196ºC) temperatures.

To overcome such challenges, novel thermoplastic tubing was developed to balance both the flexibility and robustness demands of cryogenic storage and tube welding characteristics necessary for sterile closed-system processing. The new FP-FLEX™ tubing can be frozen and maintained at cryogenic temperatures, thawed, and sterile welded to other thermoplastic tubing (such as C-Flex® tubing).

Material and Methods

Studies were designed and performed to assess durability and functional utility of the new FP-FLEX™ tubing for frozen storage and processing applications. A novel manufacturing method also was developed to enable unitized welding of FP-FLEX™ tubing directly to Freeze-Pak™ bags.

Handling and Transportation Testing

Testing was carried out using 500 mL bags with either FP-FLEX™ tubing or PVC tubing (standard). Bags were filled to 140 ml (±5 ml) with water, placed into storage cassettes and frozen in LN2 (-196ºC) with tubing attached. For the handling test bags were pulled from liquid nitrogen (LN2) storage, immediately dropped horizontally 4X from 1 ft., thawed and evaluated. Ten of 10 bags with FP-FLEX™ were completely intact, whereas 10 of 10 bags with PVC tubing were broken. A single drop from 2 ft. resulted in similar results. For the transportation test, bags (in cassettes) were placed in LN2 shippers followed by simulated ASTM transportation testing. After testing, bags were removed, thawed, and evaluated for damage.

Welding/ Functional Testing

To evaluate sterile welding capabilit ies of FP-FLEX™ tubing postthaw, tubing was frozen, thawed, and welded to C-Flex® tubing. Testing included integrity, flow rate, and weld strength. Tubing was capable of welding directly to C-Flex® tubing using standard sterile welders with flow rates up to 1L/min achieved successfully.

Summary

Freeze, Thaw, Weld: Making The Cold Chain Link

WCBs are commonly used for seed-train manufacturing of therapeutic products. Traditional vials represent an open manufacturing process and are also limited to small volumes, which contribute to lengthened production campaigns. The new FP-FLEX™ tubing has been designed and shown herein to meet the critical processing requirements for WCBs, including

- freezing/storage/transport to as low as -196ºC
- weldable to C-Flex® postfreeze/thaw
- compatible with standard tube welding and sealing devices
- closed-system aseptic transfer through tube-to-tube connection.

FP-FLEX™ tubing and Freeze-Pak™ Bag represent a closed-system solution, enabling frozen storage, sterile connection, and reduced scale-up time for therapeutic production.

Table 1: FP-FLEX™ Handling/Drop Test

Product	Drop Height	Drops/Bag	Result
PVC Tubing	12 inches	4 Times	0/10 Pass
FP-FLEX™	12 inches	4 Times	10/10 Pass
FP-FLEX™	24 inches	1 Time	5/5 Pass
FP-FLEX™	36 inches	1 Time	5/5 Pass

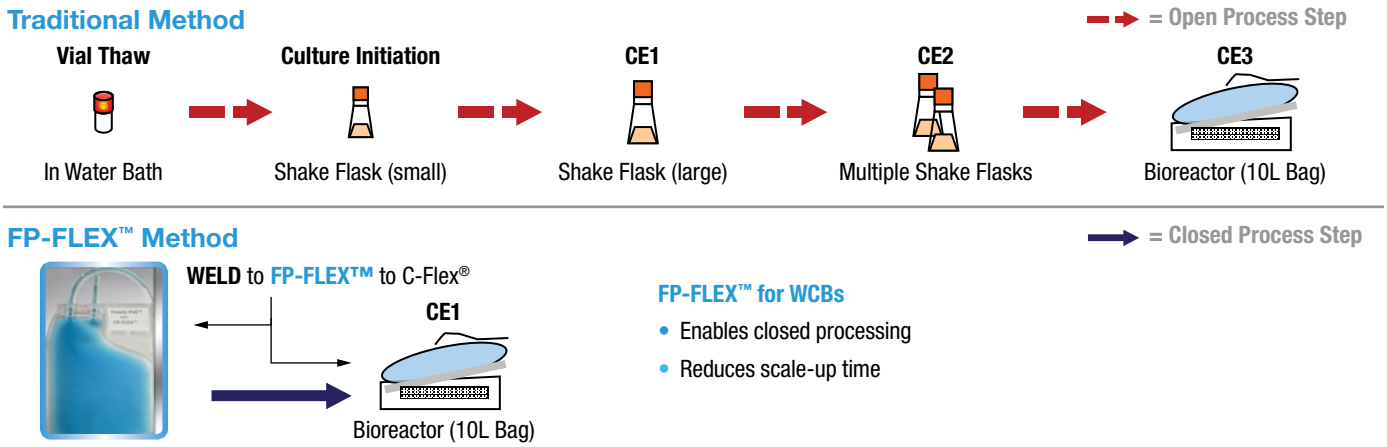
Table 2: FP-FLEX™ Transportation Test (Modified)

Modified	
Frequency(Hz)	**PSD (g2/Hz)**
5	0.0005
10	0.02
16	0.02
40	0.002
80	0.002
200	0.00002
Overall (grms)	0.675
Duation (min)	360

Table 3: Post Freeze/Thaw Welding FP-FLEX™

Property	Test Protocol	Result
Integrity	Pressure leak test (1psi)	21 Samples/Pass
Flow Rate	Welded junction flow rate ≥ 500mL/minute	21 Samples/Pass
Weld Strength	Freeze/thaw **FP-FLEX™** welded to C-Flex	Ave = 12.85 lbf

Figure 1: FP-FLEX™ Method Compared with Traditional Method



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