Technical Brief

Demonstrating NovaSeal™ Crimping Tool Virus Integrity

Introduction

The separation of one part of a disposable assembly from the other is often required during operation of equipment in the pharmaceutical industry. When it comes to biopharmaceuticals, separation and isolation of compartments can be a cause of cross-contamination.

The patented NovaSeal™ crimping tool crimps and cuts a metallic pinch-pipe on the exterior of tubing in one simple motion, without requiring heat. This metal pinch pipe/tube combination, along with the crimping tool, provides a sterile means for separating a disposable assembly by crimping and cutting a plastic tube at a chosen location. It ensures that sterility is maintained and that the fluid is securely protected from the outside environment at all times.

NovaSeal™ crimpers are offered in two formats; the manual crimping tool can be used on 3 mm ID x 6 mm OD tubing and the battery-powered crimping tool can be used on ½-inch to ¾-inch OD tubing. The design of the manual NovaSeal™ crimping tool has been evaluated previously for mechanical robustness of the separation, as well as for cleanliness and integrity of the tubing crimp to bacteria.

This technical brief will evaluate crimper performance when the fluid in the tubing contains virus. Crimp cleanliness and integrity to virus outgress is demonstrated for several tubing types, sterilized by multiple methods under multiple operating conditions.

Objective

The NovaSeal™ crimping tool crimps and cuts the metallic pinch-pipe, pre-mounted on the tubing of your disposable assembly, maintaining sterility. The NovaSeal™ crimper enables disconnection of two compartments linked by plastic tubing, allowing further separated processing. A study was performed with the manual NovaSeal™ crimping tool to demonstrate the cleanliness and integrity of the tubing crimp to viral outgress. Crimping was performed on 3 mm ID x 6 mm OD tubing containing a high titer bacteriophage suspension to model a viral vaccine sample. Crimp integrity was tested for both silicone and thermoplastic elastomer (TPE) tubing, sterilized by either high-dose gamma or beta irradiation, with an additional autoclave sterilization step (silicone tubing), storage at both ambient and frozen (-80°C) temperatures, and with sample tubing crimps generated with both a new and fatigued (>1000 actuations) NovaSeal™ crimping tool. Contamination of the exterior of the crimp or virus outgress following tubing storage was measured by submerging the crimped tubing sections in *Escherichia coli (E. coli)*-containing broth for recovery and amplification of bacteriophage particles. Crimp cleanliness and integrity to virus outgress was demonstrated for each of the tubing types and conditions tested.

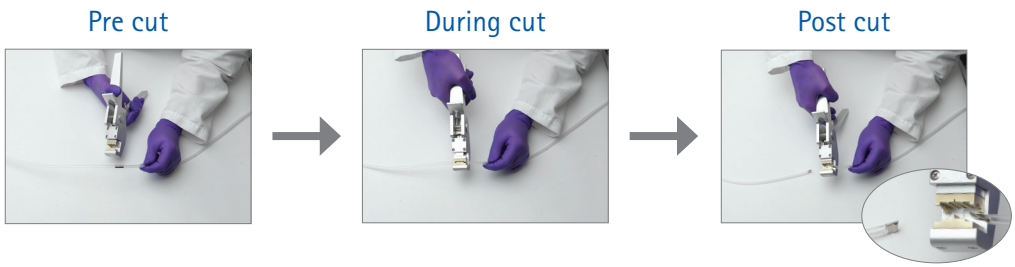

Figure 1.
Operation of the NovaSeal™ Crimping Tool.

Table 1.
Summary of performance characteristics for the NovaSeal™ manual crimping tool.

Pinch pipe	Nickel plated brass
Location of separation	Operator choice - Any place along the tubing
No outgress of virus	Pass
Microbial cleanliness of separation	Pass

Procedure

Overall Study Design

A sterilized tubing assembly set with 17 pinch pipes spaced approximately 2 inches (5 cm) apart with Luer connectors (on each end) was installed on a peristaltic pump and filled with a high titer Phi X174 bacteriophage challenge solution. With the manual crimping tool, the pinch pipes were crimped and cut, see Figure 1. Each crimped section was then placed into a tube containing an *E. coli* bacteriophage host culture. All operations were performed aseptically, inside a biosafety cabinet to minimize any contamination on the exterior of the crimped tubing sections prior to placement inside the tube containing *E. coli* culture. Following overnight incubation, the cleanliness and integrity of each crimp was assessed by plating the *E. coli* culture to determine the presence of Phi X174 bacteriophage.

Table 2.
Test Matrix.

Sample Tubing Type	Sterilization	24--Hour Hold Temperature
TPE	Gamma	--80°C
		37°C
	Beta	--80°C
		37°C
Silicone	Gamma + Autoclave	--80°C
		37°C
	Beta + Autoclave	--80°C
		37°C

Two temperatures, crimper age, two sample tubing types, including TPE and silicone (autoclaved after irradiation), and two types of irradiation, including maximum doses of either gamma or beta (E-beam), were assessed. Ten sections representing eleven individual cuts were tested for each of the eight combinations. The test matrix was conducted twice, with both a new crimper and a fatigued crimper that had performed >1000 cuts.

Tubing Preparation

TPE and silicone tubing assemblies were sterilized with gamma (40 kGy ± 5 kGy) or E-beam (70 kGy ± 5 kGy) irradiation. Following irradiation treatment, all silicone tubing assemblies were autoclaved at 134°C for 60 minutes.

Challenge Virus and Bacterial Host

Bacteriophage Phi X174 (cs70 α3, Promega Corporation I104) was used as a model virus due to its small size (25 – 35 nm diameter), rapid enumeration capability and ease of handling. *E. coli* C, ATCC® 13706™, was used as the bacterial host to recover/amplify in solution any bacteriophage present on the crimp exterior or outgressed through the tubing crimp. *E. coli* HF4714, ATCC® 49696™, was used for plating for plaque enumeration.

Virus Challenge Preparation and Bacterial Culture Preparation

The Phi X174 challenge solutions were prepared by diluting a stock of approximately $1x10^{12}$ pfu (plaque forming units)/mL in 500 mL of a sodium phosphate buffer for a final concentration of approximately $1x10^{7}$ or $1x10^{8}$ pfu/mL. The *E. coli* C bacterial culture was prepared by inoculating 50 mL of nutrient broth with 500 µL of an 18 - 24 hour culture. The inoculated broth was shaken at 200 rpm at 37°C until an OD_{650} of 0.4 – 0.5 was reached. Two mL of the *E. coli* C culture was aliquotted into sterile 15 mL polypropylene tubes immediately before insertion of the crimped and cut tubing sections.

Test Execution

Each tubing set was aseptically placed into a peristaltic pump. The pump was operated until the Phi X174 challenge solution filled the entire length of the tubing assembly. After filling, ~5 centimeter (~2 inch) sections of tubing were produced by aseptically crimping and cutting all individual pinch pipes. Ten crimped and cut sections, filled with the Phi X174 challenge solution, were alternately placed in opposite orientations inside 15 mL polypropylene tubes filled with 2 mL of *E. coli* C culture in order to evaluate both the upstream and downstream sides of the crimp. Tubes were then incubated at 37 ± 2°C (with shaking at 200 rpm) for 24 hours. For crimped sections that were first frozen at -80°C, sections were placed in empty sterile polypropylene tubes, frozen at - 80°C for 24 hours, and then thawed at room temperature. After thawing, the tubes were filled with 2 mL of *E. coli* C and incubated at 37 ± 2°C (with shaking at 200 rpm) for an additional 24 hours. After incubation, the

total volume of *E. coli* C culture in each tube was assayed for the presence/absence of bacteriophage using a double agar overlay plaque assay using *E. coli* HF4714 host. The bacteriophage challenge solution inside at least one filled section from each tubing set was titered after 24 hours of incubation to ensure Phi X174 viability throughout the duration of testing. Titers obtained at the end of each test ranged from $1x10^7$ to $4.5x10^4$ pfu/mL, reflecting some loss of phage recovery during test incubation and likely variable adherence to the inside tubing surface.

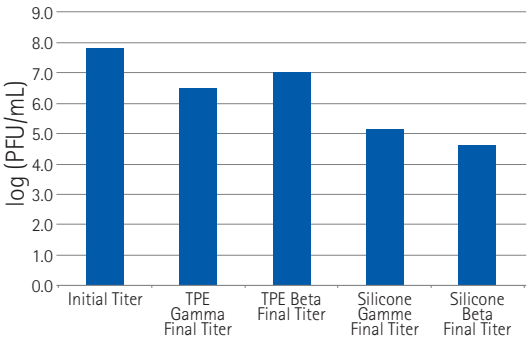

Figure 2.
Final bacteriophage titer inside tubing sections at the end of test.

Bacteriophage Recovery and Contamination Controls

Four sections were aseptically crimped and cut from each tubing assembly prior to filling with the challenge organism. Two of these cut sections were placed in polypropylene tubes filled with 2 mL of *E. coli* C spiked with Phi X174, targeting a low spike of <100 pfu/mL (Positive Control). This control confirms bacteriophage recovery at low concentrations in the presence of the pinch pipe and tubing. The additional two empty cut sections were placed in polypropylene tubes filled with 2 mL of *E. coli* C, to detect the presence of bacteriophage on the exterior of the crimped sections due to environmental contamination (Negative Control). Controls were conducted for each tubing set to assess the presence or absence of bacteriophage contamination on the exterior of the crimp after the initial cut. Both ends of two crimped sections filled with bacteriophage were dipped in a polypropylene tube containing 2 mL of *E. coli* C, and then removed. This control would confirm a lack of bacteriophage contamination on the exterior of the crimp after the initial cut (Dip Control). An additional study for recovery of bacteriophage present on the exterior of the crimp was performed by pipetting ~2 µL of the high titer Phi X174 challenge solution on the exterior of a crimp, allowing the solution to dry, and then placing the tubing section into the *E. coli* C host solution, followed by 37°C incubation.

Results and Discussion

For all tubing sections for each condition, bacteriophage were not amplified following incubation of the filled, crimped tubing sections in *E. coli* C host, resulting in 0 pfu (plaque forming units) on all enumeration plates, indicating there was no detectable bacteriophage contamination or outgress from either side of the tubing crimp for each tubing type, storage temperature and sterilization method. Contamination of the crimp/cut exterior with a very small volume (down to nanoliter volumes) of the sample tubing fill solution would have been detected, based on amplification of bacteriophage spiked at very low levels in the recovery tube (Positive Controls). These controls, conducted to ensure recovery of extremely low levels of bacteriophage present in the *E. coli* C host solution, showed amplification to high concentrations of bacteriophage from an average initial concentration of ~5-10 pfu/mL amount of bacteriophage that would represent exterior contamination by nanoliter amounts of the tubing fill solution in the *E. coli* C host culture. Storage and freeze/thaw of a crimped sample tube at -80°C did not impact the integrity of the crimp. Bacteriophage applied to the exterior of a crimp and allowed to dry was recovered, ensuring that if bacteriophage had been present on the crimp exterior following crimping, these would have been detected.

Tubing Type	Sterilization Mode	Sample Description	37°C New (pfu/mL)	37°C Fatigued (pfu/mL)	-80°C New (pfu/mL)	-80°C Fatigued (pfu/mL)
		Positive Control Initial	2.5	< 1	15	1
TPE	Gamma	Positive Control Final	TNTC,TNTC[1]	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
		Negative Control	0,0	0,0	0,0	0,0
		Dip Control	0,0	0,0	0,0	0,0
		Crimped Sections	All 0	All 0	All 0	All 0
	Beta	Positive Control Final	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
		Negative Control	0,0	0,0	0,0	0,0
		Dip Control	0,0	0,0	0,0	0,0
		Crimped Sections	All 0	All 0	All 0	All 0
Silicone	Gamma	Positive Control Final	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,25
		Negative Control	0,0	0,0	0,0	0,0
		Dip Control	0,0	0,0	0,0	0,0
		Crimped Sections	All 0	All 0	All 0	All 0
	Beta	Positive Control Final	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
		Negative Control	0,0	0,0	0,0	0,0
		Dip Control	0,0	0,0	0,0	0,0
		Crimped Sections	All 0	All 0	All 0	All 0

Table 3.
Experimental matrix results.

[1] Too Numerous To Count

Conclusion

The NovaSeal™ crimper with #A100/104 jaws produces a clean cut on the pinch pipe, without contamination by virus from the sample tubing solution on the crimp exterior. The crimp maintains an integral barrier to viral outgress during all conditions tested in this study, when the crimp and cut is produced with either a new or a fatigued crimper. The NovaSeal™ crimping tool is ideally suited for sterile product sampling for viral vaccine production applications, ensuring cleanliness and integrity of the sampling crimp and cut, protecting the product and ensures operator safety during bioprocess sampling and handling.

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