# PURITY TESTING OF SILICONE TUBING FOR BIOPHARMACEUTICAL APPLICATIONS

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Key Words: Particulate, Endotoxin, Bioburden, Fluid Contact, Purity

Silicone tubing products are widely used in critical biopharmaceutical processes including final fill and other aseptic operations that require high purity product contact components.

At Dow we have developed reliable and rigorous methods based on the industry standards for particulates, endotoxins and bioburden to test our Platinum-catalyzed silicone tubing family. The tests were conducted per the USP ‹788› Particulate Matter in Injection, the USP ‹85› Bacterial Endotoxins Test, and the ISO 11737-1 Sterilization of medical devices — Microbiological methods — Part 1: Determination of a population of microorganisms on products.

- Method 2 Microscopic Particle Count Test of USP ‹788› was used to count particulates collected by extraction from the inner lumen of the tubing followed by membrane filtration: results indicate very low particulate counts well below the compliance limits of USP ‹788›.

- The gel clot method of USP ‹85› involved a LAL reagent with low sensitivity (< 0.125 EU/ml) and validated that the endotoxin level of the silicone tubing products is well below this limit.

- The bioburden method per ISO 11737-1 used to measure aerobic bacteria, yeast, molds and spores was validated with excellent recovery efficiency and correction factor. There were no micro-organisms detected on the surface of any of the silicone tubing products tested by this rigorous method.

The results obtained demonstrates a high level in cleanliness, exceeding the industry standards, with respect to particulates, endotoxins and bioburden on the surface of the silicone tubing products tested.

This purity is driven by Dow’s vertically integrated supply chain for silicone tubing that controls the sourcing of raw materials, and the clean formulation of elastomer involved in the manufacture of the tubing.

# EMBEDDED PARTICLES IN SINGLE-USE FILMS: COSMETIC DEFECT OR INTEGRITY RISK?

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Key Words: Films, Gels, Particles, Integrity

Single-use films make up a large fraction of the surface area of single-use systems, and thus must meet stringent requirements not required for typical packaging films: high mechanical integrity and low levels of chemical leachables. Consequently, typical single-use films are relatively thick and contain much reduced levels of chemical additives (processing aids and stabilizers). Reduction of additives may result in a higher probability for finding gel particles embedded within the film. Gel particles, described as translucent unmixed or “un-melted” polymer resin perhaps with increased cross-linking or molecular weight, appear as “fish eye” shaped defects in the film. High temperatures within the extrusion process may chemically degrade gel particles, which then become amber, brown or black in color. In addition, the industrial scale and complex nature of film extrusion processes increases the risk for embedded foreign particle contamination in the film.

Are embedded particles in single-use films cosmetic defects, or do they represent significant risk to process reliability (process integrity) or risk to product purity? In an attempt to quantify risk to integrity, tensile testing, flexural durability testing, and a unique pressure burst test were applied to single-use films with varying type and size of embedded particles. For embedded gels, the results show that only extraordinarily large gels impact tensile test results, and only very large gels impact burst test results. Limited evidence shows similar effects for embedded foreign particles. After flexural durability testing, no pinholes were found even when multiple embedded gel particles were present in the film.

The test methods applied generate extreme stresses and strains compared to those found in real applications. In addition, the effects appear only with gels much larger than the detection capabilities of on-line inspection systems. Thus the risk of embedded particles to single-use film integrity appears low. Risk of embedded particles in film to product purity is addressed in a separate paper in this conference addressing overall particle contamination risk factors.

# TUFLUX TPE TUBING FOR PHARMA PROCESSING

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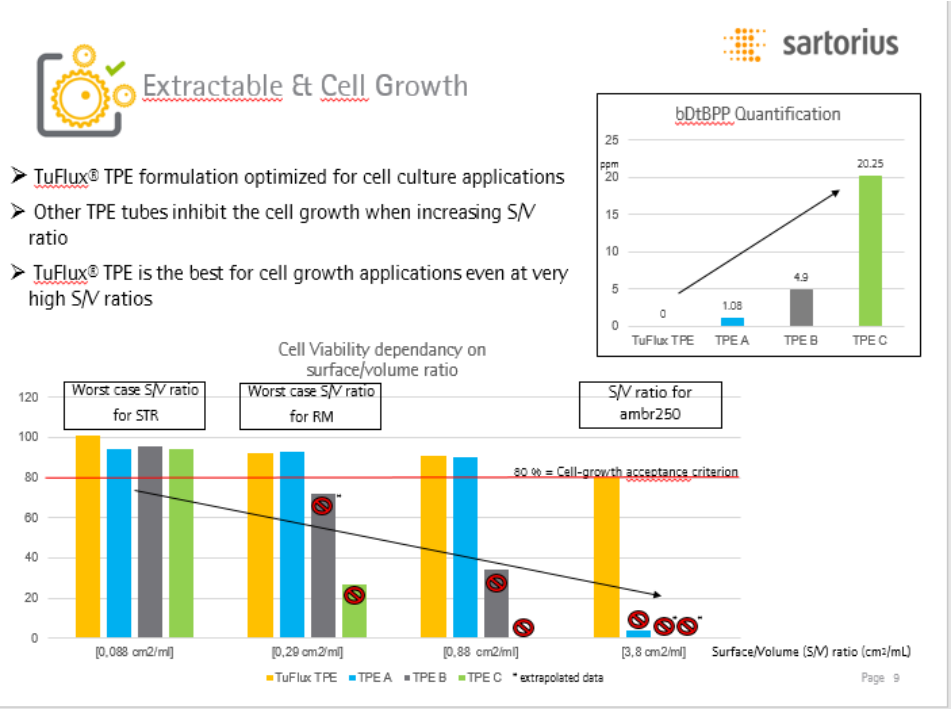
Key Words: TPE, Tubing, Pharma Processing

The guidelines for launching new products and manufacturing those approved products are becoming more stringent on a routine basis. Quality certifications, clean room requirements, forwards and backwards traceability on all components are the new standard for providing FDA-approve products. Part of insuring that quality criteria are being met is to understand how the raw material is created, how it is processed in to a technologically useful processing aid (tube) and then how is that tube tested. Thermoplastic resin is the key ingredient in creating TuFlux tubing. The broad chemistry footprint allows specialized compounders to create unique blends of components (using plasticizers, oils, finishing aids etc) to manipulate the material so that it meets USP Class VI standards but still provides the lower levels of leechables and extractables as well as minimize unwanted biological cell growth.

The raw resin is melted via combination of time, temperature and pressure and formed into a specific shape (single lumens, multi-lumens, oblong geometries) and wall thicknesses to meet the design requirements for the processing equipment of the OEMs. Stripes can be embedded or material can be reinforced with wires or fabric braids to offer further support for high pressure applications as well as reduce cross equipment contamination (due to the colored stripes). Thermoplastic has the unique ability to be re-melted to customize connectors and design solutions in production sites with unique vessel shapes or limited real estate. The combination of proprietary resin and the manufacturing process help to keep the E/L data well below industry limitations as well as reduce the potential growth of bDtBPP.



# THE PROPER USE OF EXTRACTBALES DATA - ASPECTS BEYOND EXTRACTABLES-MEASUREMENT

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Key Words: Extractable/Leachables, Single Use Devices, Single Use Assemblies, Risk Assessment

Appropriate extraction techniques for SUS/SUT and methods for analysis of extractables have been intensively discussed over the last years. Today, several proposals for common methods are available and used to conduct extractables studies in the bio-pharmaceutical industry. Therefore it is expected that the number of available extractables data will significantly rise over the next years and it is worth to (re-)consider their proper use for materials and device qualification and risk assessment. While this exercise is straightforward for container closure systems (CCS), for SUS/SUT the situation is more complex. In CCS applications, a single drug product, in contact with a well-defined container system for long term storage is studied. In contrast to a CCS the number of materials, their dimensions and combinations are highly flexible in SUS/SUT applications. Additionally, SUS/SUT are used under dynamic process-conditions of variable solvents, dwell-times, temperatures, flow-rates etc.

In our contribution we will discuss two major questions that persist and cannot be solved by means of analytics alone:

1. How can we obtain extractables data for SUS/SUT devices of different sizes and for complex device combinations (assemblies)? This aspect is critical for the device industry, because a high number of different devices and combinations are requested by our customers. Further, assembled products from Configured to Order (CTO) or Engineered to Order (ETO) processes utilizing various compounds, even such from various suppliers can increase their amount and combinations nearly infinitely. It is easily conceivable that it is technically impossible to conduct individual extractables studies for each possible combination.
2.
3. Another aspect which has to be taken into account in the future, is the proper use of extractables data for extrapolations toward potential leachables required for quality risk-assessment. In this context a publication from Jenke & Rabinow (2017) has to be considered, where they showed that the validity of the “intuitive” approach to scale extractables data just by surface area is questionable.

We will show how we can develop methods to overcome these - so far - unsolved problems. The proposed methods will be based on basic physical chemistry principles rather than “intuitive” worst-case assumptions. We will show illustrative examples on how extractables data, obtained by different protocols can be used heuristically in scaling and combination exercises. The limits of the conventional scaling by surface areas are discussed in terms of the influence of equilibrium versus diffusion controlled conditions in long versus short term contact.

Furthermore, an example will be shown for a calculation of potential leachables solely built on physical chemistry considerations and avoiding any generic worst-case assumption.

Jenke & Rabinow: Proper Accounting for Surface Area to Solution Volume Ratios in Exaggerated Extractions PDA J Pharm Sci and Tech (2017), 71 225-233

**WITHDRAWN**

# QUALIFICATION OF LOW DRIFT SINGLE-USE PH SENSORS FOR USE IN SINGLE USE BIOREACTOR PLATFORMS

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Key Words: Single-use, bioreactor, PAT.

The biopharmaceutical industry is currently limited by access to low drift, gamma stable, easy-to-integrate single-use pH sensors for application in single-use bioreactors (S.U.B.). Hamilton Company has developed the OneFerm VP 70 sensor, a unique single-use glass electrode, which provides an additional option for customers wishing to implement reliable single-use pH sensing capabilities in the S.U.B. platform. In order to demonstrate the efficacy of these devices, a series of qualification experiments were carried out using a Thermo Fisher Scientific 50L bioprocess container (BPC) custom-fitted with Hamilton OneFerm sensors. BPCs were manufactured in a cGMP facility and allowed to age for a pre-established period (either 30 or 180 days). A 14 day fed-batch cell run was executed using an in-house CHO-S cell line (mAb producing clone) and standard operating conditions. Online pH was controlled with a Hamilton EasyFerm pH sensor; reactor pH was controlled using $CO_2$ without acid or base. Each BPC was built with 6 OneFerm sensors (containing 2 each from 3 different production lots), which were monitored using stand-alone transmitters. Offline samples were evaluated every 24 hours using an Oakton pH sensor.

The results from the initial 30 day aged BPC evaluation demonstrate functional activity of the OneFerm sensors over a 14 day fed-batch cell run using a TruBio DeltaV S.U.B. controller. Functional stability of these sensors was demonstrated by maintaining the devices in sterile culture conditions for a period of 60 days; 5 out of 6 sensors met all manufacturers' specifications during this hold period. To determine if BPC storage time contributes to loss in sensor functionality, a 14 day fed-batch cell run was repeated using a 180 day aged BPC. All 6 OneFerm sensors met manufacturer's specifications after the cell run with no sensor exhibiting a gross pH drift greater than 0.11 during the entire 14 day period. Additionally, all 6 sensors also met manufacturer's specifications during the 60 day hold period with no sensor exhibiting an average gross pH drift greater than 0.15 during this extended time. Furthermore, all sensors demonstrated an average response time of less than 10 seconds following the 60 day hold period.

The results of these experiments demonstrate the effectiveness of Hamilton OneFerm sensors in the Thermo Fisher Scientific HyPerforma S.U.B. platform. Thermo Fisher Scientific has since developed a custom polycarbonate probe port adapter to robustly integrate the OneFerm sensor in a S.U.B. BPC. Future work will continue to evaluate the performance of sensors aged in BPCs for 24 to 36 months. We are excited to share this growing body of data with the bioprocess industry as probe drift, ionic strength sensitivity, and shelf life have greatly limited implementation of SU pH over the past decade. These results appear to indicate a viable technology is now available and is suitable for cGMP bio manufacturing.

# ONE-PAGE ABSTRACT TEMPLATE AND GUIDELINES –TITLE CENTERED AND ALL CAPS

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Key Words: shake flask, online sensor, metabolism, optical sensors, non-invasive

Shake flasks have been the work horse for microbial small-scale fermentations for decades. Recently, single use versions are becoming more and more popular -for both microbial and cell cultivation.

Typically, these vessels are still used as black boxes because no online measurement is integrated. The non-invasive measurement of oxygen and pH using chemical-optical sensors has already been commercially available for several years. With the recently added ability to measure biomass online, the metabolism is even more visible. This presentation discusses the use of a multitude of sensors in the small scale of shake flasks. It also presents a prototype sensor for the online measurement of CO2 that was developed recently and integrated into a multi-parameter platform. Applications are various: Although the CO2 sensor is only a prototype it is possible to follow a diauxie of E. coli cultivations online, while small changes in the growth curve detected by the biomass sensor indicate the exact time of limitations which was shown for different organisms.

This presentation includes research supported by the Federal Ministry for Economic Affairs and Energy (BMWi) via AIF within the ZIM-Initiative #KF2516104FR2.

# AUTOMATED MIXING PROCESSES FOR 50 L TO 2000 L SINGLE-USE MIXERS USING NEXT GENERATION TOUCHSCREEN CONTROL PANEL: SCALEABLE UPSTREAM MIXING EFFICENCIES AND AUTOMATED DOWNSTREAM VIRAL INACTIVATION

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Key Words: single-use mixer, mixing, viral inactivation, Touchscreen Control Panel, upstream, downstream, T95, hyperforma

Mixers are employed throughout all parts of bioprocessing. Single-use mixers (S.U.M.) are commonly used for upstream operations including media formulation and hydration, media holding, sterile filtration and downstream operations including media and product storage, viral inactivation, buffer preparation, and resin/slurry preparation for column packing. This study presents the automation of both simple and complex mixing procedures using the Thermo Fisher Touchscreen Control Panel. Also investigated are the selection method for scalable mixing parameters allowing normalized mixing performance across all single use mixer sizes. The following applications are demonstrated in this study:

- Characterization of scalable mixing parameters by comparing power input per volume and T95 blend time criteria
- Automation of a basic mixing process control variables including temperature, agitation , pump rate, flow valves, line pressure, and filtration effeciency using the Touchscreen Electrical Panel
- Automation of a complex viral inactivation process including multiple pH and temperature shifts

This work demonstrates best practices for mixing in bioprocessing unit operations including use of the Touchscreen Control Panel for automation thus minimizing operator intervention and process variance while maximizing the quality and traceability of data gathered during the mixing process thus improving overall lab efficiency.

# STUDY OF ACCURACY AND SELECTIVITY OF A HYDROGEL-BASED SENSOR ARRAY BY DESIGN OF EXPERIMENTS (DOE)

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Key Words: Process analytical technology, design of experiments, selectivity, accuracy, biosensor

Reliable continuous sensors are salient to achieving advanced Process Analytical Technologies in the bioproduction industry. Sensors provide information on key parameters in a bioreactor such as physical variables (temperature, pressure, speed of stirrer), chemical variables (pH, $pO_2$, $pCO_2$, nutrients, metabolites), and biological variables (biomass, cell metabolism).[1-2] Simultaneously, chemometric analysis using multivariate data analysis, bioprocess modeling, and design of experiments (DOE) have become important in developing advanced biosensors because of the need to clean the complex raw data from biosensors to provide repeatable, robust, and reliable information.[3-4] In this work, the first step of the chemometric analysis process, DOE was performed with a prototype biosensor developed to simultaneously monitor glucose, lactate, pH, and osmolarity to understand the accuracy and selectivity of this sensor.
Sets of experiments for the DOE were designed with 4 factors (glucose, lactate, pH, and osmolarity) and 3 levels of interaction of each factor in the continuous level mode using JMP® (SAS Institute). A total of four sensor probes were tested to confirm reproducibility and repeatability. Other environmental conditions such as temperature, type of media, speed of stirring, and depth of the sensor probe in the bioreactor were controlled. HPLC (Rezex ROA-Organic Acid H+, 150 x 7.8 mm ID, refractive index detector), pH electrode (Mettler Toledo), freezing point osmometer (Precision system, INC.) were used for independent measures for evaluation of the test-sensor data. Key results were: (a) the response trend of the pH hydrogel sensor showed a good correlation with the commercially available pH electrode data (figure 1). The accuracy of pH prediction by the pH hydrogel sensor was 92 % from this study, (b) glucose hydrogel sensor showed a cross-sensitivity to lactate (60% contribution of magnitude changes, figure 2), (c) 70 % of prediction accuracy can be achieved by using data from lactate responsive hydrogel (figure 3). Glucose response data analysis reflects the importance of an experiment set up and chemometric analysis process to obtain meaningful data from newly developed biosensors. The sensitivity of the osmolality sensor was less than expected from the prototype sensor. Since this study, a new osmolarity responsive hydrogel has been formulated that has improved osmolarity sensitivity.

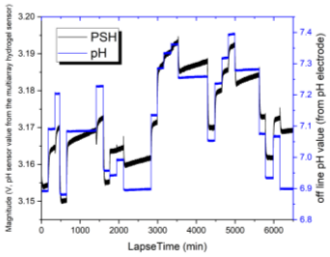


*Figure 1. Comparison of pH response from the pH hydrogel sensor and a pH electrode (raw sensor data)*

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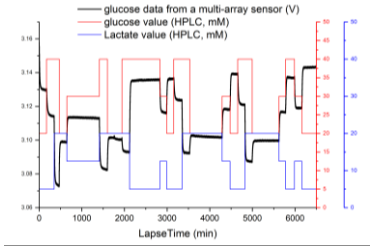


*Figure 2. Comparison of continuous hydrogel glucose sensor response to glucose & lactate samples measured by HPLC (raw sensor data)*

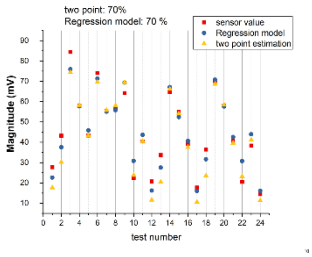


*Figure 3. Glucose sensor accuracy based on the DOE data (used regression & two point estimation model to predict sensor magnitude).*

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# SINGLE-USE PH SENSOR VIA A COPLANAR PH GLASS ELECTRODE DESIGN

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The elimination of high temperature and high pressure sterilization processes by the advent of Single-Use-Systems (SUS) opens up a new era for the glass electrode based pH sensor. Without the requirement for pretreatment of a pH glass electrode under high temperature and pressure, the classic pH sensing technology delivers a more reliable, more rugged, and more accurate pH measurement. However, the innovative and unique SUS practice also posts new challenges, such as the long shelf-life requirement and the limitation of access to the sensor for calibration once on site,.

At Broadley-James Corporation, a new manufacturing technology has been developed to allow a coplanar pH glass electrode design. This innovative sensor design will allow the sensing surface to transfer from a storage/calibration position to a measurement position while maintaining a fully closed and sterile system status. A single use pH sensor based on this design exhibits features such as shelf life up to 2 years post gamma, on site calibration capability, post use validation capability, and 2 – 12 pH range coverage. This poster will present a coplanar tubular glass electrode design for upstream (bag) installation, as well as a coplanar flat glass electrode design for downstream (flow path) installation. Sensor accuracy verification, design details, and preliminary test results will also be presented.

**WITHDRAWN**

# EXTRACTABLES AND LEACHABLES IN CONTINUOUS PROCESSING SYSTEM

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Single-use system and continuous processing are two significant trends in biopharmaceutical production. The two techniques are parallel and complementary to each other thus together it can provide significant improvement on drug manufacturing quality assurance as well as efficiency. While single-use system had been widely implemented for decades, adoption of continuous processing in pharmaceutical production is still in its early stage. The rapid adoption of single use technologies complements the implementation of continuous bioprocess, providing facile and enclosed systems for bioprocess manufacturing. A new generation of single-use system has been developed to fit in a continuous processing platform. Since extractables and leachables remain a major concern for single-use system adoption, each individual key component of the continuous processing system was evaluated for extractables following BPOG protocol. Risk and toxicology assessment has been performed on the extractables from critical components particularly in downstream processing. Provision of comprehensive, BPOG-aligned extractables packages for each single use bioprocessing component helps frame what extrinsic compounds and degradants may potentially leach into the process flow. However, the robust nature of these studies coupled with high surface to volume ratios may exaggerate the number and level of compounds expected to leach and persist throughout the bioprocess. From a risk assessment perspective, many of these compounds may be expected to be diluted or readily cleared during typical continuous bioprocess application steps. To evaluate the capability of the downstream purification steps to remove extractables from upstream components, samples were collected after each step in continuous processing for extractables studies. This study tracks the emergence and clearance of extractables in model fluids observed at various stages throughout a typical continuous bioprocess implementation. Few extractables in model fluids from upstream components survived after downstream purification and diafiltration steps. Subsequent evaluation of extractables in drug product in continuous processing will be in future studies.

**SINGLE USE COMPACT SETTLER FOR CLARIFYING CELL CULTURE BROTH, SELECTIVE REMOVAL OF DEAD CELLS AND AFFINITY CAPTURE OF ANTIBODIES**

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Key Words: Compact Plastic Settler, Cell culture clarification device, Selective cell retention device, Direct affinity capture of antibodies from cell culture broth.

We have redesigned the successful "inclined settler" technology used as a selective cell retention device in mammalian cell perfusion bioreactor cultures into a more compact and easily scalable design using cylindrical and conical geometries. Through this novel settler design, we have achieved 6 – 10 x more settling area for the same footprint, compared to the traditional multi-plate or lamellar rectilinear scale up design. Using this compact settler design, we have demonstrated significant clarification of the smaller yeast *Pichia pastoris* cells in continuous harvest or settler effluent stream and high cell densities (700 – 1000 O.D.) in perfusion bioreactors operated over several months.

We have now fabricated this compact settler as a single use disposable plastic settler at 6 inch diameter scale and are planning to fabricate it in two larger sizes: 12" diameter and 24" diameter over the next six to twelve months. Recent experimental data with this compact settler as the selective cell retention device for achieving high cell densities in mammalian perfusion bioreactor cultures (operated for over a month of culture) will be presented. Our industrial collaborators are testing this device as a single use disposable device for clarification of cell culture broth from fed-batch bioreactor, for potential replacement of centrifuge for this operation.

Another exciting application of this compact plastic settler is the affinity capture of antibodies from cell culture broth directly onto protein A beads suspended inside the settler, while the cells and unbound host cell proteins are easily washed away in the settler top effluent, followed by elution, cleaning and regeneration steps on the beads suspended inside the settler. This integrated bioprocessing application can potentially replace the current unit operations of centrifugation, depth filtration and affinity column chromatography. Reproducible data from the preliminary experiments on this novel bioprocess application will also be presented.

# OPTIMIZATION OF THE SINGLE USE BIOREACTOR FOR GROWTH AND BEAD-TO-BEAD TRANSFER OF VERO CELLS CULTURED ON MICROCARRIERS

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Key Words: Single-Use, Vero, Microcarrier, Scale-up

Scale up and vaccine production processes of adherent cells, such as Vero cells face many challenges. The fundamental steps of equipment selection and chosen operating parameters have a significant impact upon the detachment and reattachment of cells through the scale up process. Microcarriers greatly increase the surface area for adherent cells and offer flexibility for expansion to bioreactors, but scale-up methods require optimization of the mixing within the vessel and also optimization of how the cells are transferred from bead to bead at each step in the seed train. In this study we take a process previously shown to a work in spinner flasks (<1L)[1] and demonstrate how the 50L Thermo Scientific™ HyPerforma™ Single-Use Bioreactors (S.U.B.) can be optimized for growing and scaling adherent cells on microcarriers, methods for bead-to-bead transfer of the cells at each scaling step, and final cell isolation using the Harvestainer single use bead capture bag.

References

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# SCALABLE, HIGH PERFORMANCE SINGLE-USE TECHNOLOGY TO MEET GENE THERAPY PRODUCTION DEMANDS

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Key Words: Single-use, gene therapy, viral vectors, scalability, process development.

The biotech world is currently living through an exciting time of discoveries, making use of gene-base therapies for the treatment of diseases that previously had no cure. There is currently a large number of gene therapies in both R&D and clinical stages, and it is expected that demand for technologies able to address the industrial manufacture of these will boom in the coming years and decades.

The main challenges associated with scaling up gene therapies from their currently R&D and clinical stage to the market will be to ensure robust and scalable technology is available through the complete process. Gene therapies typically require high doses per patient, while the patient pool size on average is much smaller than for traditional medicines. As a result, technology needs to be adapted to produce such high titers at reasonable costs. Additionally, technology also needs to be flexible enough to accommodate a range of therapies and vectors with ease.

Univercells will present a case study on how it has developed single-use cell culture technology to address the challenges cited here above, and how it has as a result improved reproducibility, reduced both capital and operating costs and removed the scalability bottleneck between clinical trials and full commercial production.

# MANUFACTURING HUMAN MESENCHYMAL STEM CELLS AT CLINICAL SCALE: PROCESS AND REGULATORY CHALLENGES

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Key Words: Single-use devices, human mesenchymal stem cells, Microcarrier, Advanced therapeutic medicinal product

There is an obvious increasing interest in human mesenchymal stem cell (hMSC)-based therapies for regenerative medicine (e.g. neurology, cardiology, immunology, orthopaedics). At the beginning of May 2018, there were 253 registered clinical trials using hMSCs (www.clinicaltrials.gov). Despite the large number of current clinical studies, only 13 hMSC-based products have received regulatory approval. In order to efficiently manufacture hMSC-based products, not only must the targeted cell quantity and quality be taken into account, but the production costs must also be considered. In general, autologous and allogeneic stem cell products are characterized by similar upstream processing (USP), downstream processing (DSP), formulation, and fill & finish operations. Typical USP operations are manufacturing of the Master Cell Bank (MCB) and the Working Cell Bank (WCB), seed cell production, and subsequent cell expansion. The DSP steps include cell harvesting, cell detachment, cell separation, washing and concentration procedures, and medium exchange. However, before hMSCs can be administered as an Advanced Therapeutic Medicinal Product (ATMP), additional formulation, and fill and finish steps have to be carried out. The main differences between allogeneic and autologous manufacturing approaches are the number of therapeutic doses generated in each batch and the number of patients treated. Therefore, it is unsurprising that allogeneic therapies are the more cost-effective method in terms of hMSC production. Furthermore, various economic studies have demonstrated that USP and in particular, hMSC expansion, represent the main cost drivers when examining the entire manufacturing process. In order to achieve the high cell numbers of up to $10^{13}$ cells per batch needed in allogeneic hMSC manufacturing processes, manufacturers have to move away from traditional planar cultivation systems. Many reports over the last years have shown that instrumented, single-use bioreactors in combination with microcarriers are promising systems for this task.

Even though different procedures and equipment for USP and DSP are already available and established for allogeneic production of hMSCs, various challenges still exist. Therefore, the authors intend to highlight the current state of the art of allogeneic hMSC manufacturing and show the current main process and regulatory challenges for USP and DSP operations.

**BIOPROCESSING AND ENGINEERING CHARACTERISATION OF T-CELL THERAPY MANUFACTURE IN AN AMBR® 250 BIOREACTOR**

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Key Words: T-cell manufacturing, ambr® 250, Dissolved Oxygen, metabolites

The use of engineered CAR-T cells in clinical trials has been growing over the last years. The recent approval of Kymriah® (Novartis) and Yescarta® (KitePharma) made CAR-T cell treatments available to a broader public. However, despite the recent successes and significant improvements, there are different aspects that need to be further assessed in order to develop a reproducible, cost-effective manufacturing process for the production of personalized T-cell therapies. This requires an approach, which generates sufficient quantities of patient-specific cells at the appropriate quality required for clinical application, overcoming the challenge imposed by significantly different starting material.
The work carried out is focused on the growth of T-cells in stirred tank bioreactors. In order to do so, experiments were carried out in an ambr® 250 (Sartorius) single use bioreactor. The ambr® 250 has already demonstrated significant success for suspension-based mammalian cell culture applications, both as a product development and scale-up tool. Both commercially available vessels were characterised in terms of cell yield, viability, metabolites profile and T-cell subpopulations after expansion. The comparison between the two vessels was performed based on stirring speed and power per unit volume.
T-Flask expansion of primary T-cells was carried out as a static control and results were compared with the dynamic culture conditions. Results revealed a higher final cell density in the ambr® 250 bioreactor compared to the static platform (Figure 1). Moreover, the final product composition was not significantly affected by the stirring regime.
Small scales bioreactors, as the ambr® 250, are a big resource for autologous therapies, where a volume of 250ml is enough for a single dose. A 24 way ambr® 250 system has the potential to produce 24 patient-specific treatments in parallel. On the other hand, this results can be used for scaling-up the manufacturing process to 1-5l stirred tank bioreactors. This will be of uttermost importance for allogeneic therapies, where single donor material is expanded in larger volumes in order to reach the number of cells needed multiple doses.
Investigating and optimising the manufacturing process will improve the consistency, yield and quality of T-cells and facilitate more cost effective production for both autologous and allogeneic CAR-T cell therapies.

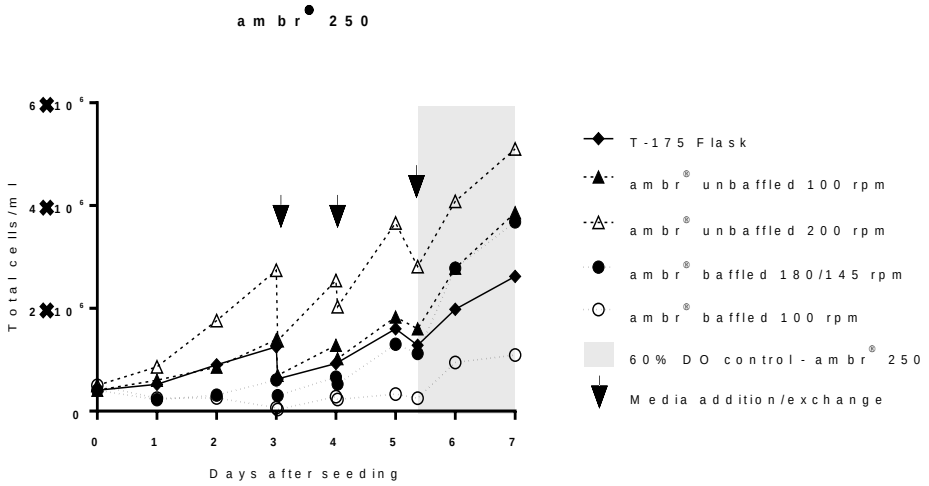


*Figure 1 – Growth curve (Total cells/ml) for primary T-cells isolated form healthy donors. The growth in T-175 Flasks was compared to ambr® 250 vessels (baffled and unbaffled) at different speeds. It can be seen how the baffled vessels performed better that the T-Flask in all tested conditions, while the baffled vessel at 100 rpm was the only one to have a lower yield when compared to the static control.*

# STERILE MEDIA HOLD SCALE-UP USING MOBIUS® SINGLE-USE TECHNOLOGY

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Key Words: Single-Use Mixing, Sterile media hold, Scalable process

The benefits of single-use systems (SUS) in biopharmaceutical manufacturing are well understood, and their use is widespread in the manufacture of mAb, rProteins, and related therapies. New frontiers in medicine such as cell and gene therapy present an opportunity for SUS to enable speed to the clinic, however some unique hurdles must be overcome. This poster outlines the collaborative development of a single-use process to address the challenge of supplying sterile media to a bioreactor for inoculation and growth of human tissue cells. To alleviate time constraints, cleaning concerns, and contamination risks, the biopharmaceutical manufacturer chose to employ single-use technology when conducting a 5-fold scale up from a glass bottle process. A significant challenge with this human tissue cell culture process is the 60-day sterile media hold at the cell culture temperature of 36ºC, during which time the bioreactor is intermittently perfused with fresh media. The Mobius® Mix50 single-use mixer (SUM) solves this challenge by first beginning with a sterile, gamma-irradiated mixer bag to eliminate concerns over validation of CIP and SIP cycles. Next, a low-pressure overlay is maintained with a carefully-sized hydrophobic vent filter, to prevent contaminants from entering the sealed mixer container. Process variables requiring assessment for this application include the air overlay pressure and flow rate, the liquid (media) flow rate during filling and draining of the SUM, sizing of the vent filter area, the liquid volume in the SUM, and the sterile condensate collection rate. A series of experiments provided a repeatable and scalable single-use solution for implementation into the manufacturing process. This novel application demonstrates the flexibility of single-use in the rapidly expanding clinical market of products derived from human cells with the unique challenges they present.

# ENGINEERING SCALABLE MANUFACTURING OF HIGH-QUALITY HUMAN MSC FOR CELL THERAPY: FROM UP TO DOWNSTREAM PROCESSING INTEGRATION TO CELL PROTEOME CHARACTERIZATION

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Key Words: cell therapy, product characterization, scale-up, single-use technology mass spectrometry,

Human mesenchymal stem cells (hMSC) are relevant cell therapy products for autologous and allogeneic therapies. To deliver the required cell numbers and doses to therapy, scaling up production and purification processes (at least to the liter-scale) while ensuring high purity, viability and maintaining cells' critical quality attributes (CQA) and functionality is essential.

Therefore, the aim of this work was to prove scalability of an integrated streamlined bioprocess compatible with current good manufacturing practices (cGMP) comprised by cell expansion, harvesting, volume reduction and washing unit operations using human mesenchymal stem cells (hMSC) isolated from bone marrow (BM-MSC) and adipose tissues (AT-MSC). Single-use technologies were adopted at different steps of the manufacturing workflow to support process integration and scale-up.

BM-MSC and AT-MSC expansion and harvesting steps were scaled-up from spinner flasks to 2 L single-use stirred tank bioreactor using synthetic microcarriers and xeno-free medium, ensuring high cellular volumetric productivities (50 x $10^6$ cell.$L^{-1}$.$day^{-1}$), expansion factors (14 - 16 fold) and cell recovery yields (>80%).

For the volume reduction and washing steps, flat sheet cassettes (FSC) and hollow fiber cartridges (HF) were compared showing a fairly linear scale-up, with a need to slightly decrease the permeate flux (30 - 50 LMH, respectively) to maximize cell recovery yield. Nonetheless, FSC performed better allowing recovering 18% more cells after a volume reduction factor of 50 without compromising cell's CQA of viability, identity and differentiation potential.

"Omic" tools in combination with standard analytical assays allow for a better cell characterization, increasing product and process understanding and are thus fundamental for process development. Thus, alongside the standard quality assays for evaluating hMSC's CQA, a proteomics workflow based on mass spectrometry tools was established to characterize the impact of processing on hMSC' CQA. Overall, through sensitivity, robustness and throughput, this type of workflow provided the identification of specific signatures of the final product. Therefore, it proves to be essential to understand the cells' final quality as well as to evaluate the impact of manufacturing at different stages of processing.

The authors acknowledge UniMS – Mass Spectrometry Unit team (ITQB-NOVA/iBET, Oeiras, Portugal), iNOVA4Health Research Unit (LISBOA-01-0145-FEDER-007344), and Fundação para a Ciência e Tecnologia (FCT, Portugal) for funding the project CARDIOSTEM (MITP-TB/ECE/0013/2013), and the grants SFRH/BD/51940/2012 (MIT-Portugal), SFRH/BD/52302/2013, SFRH/BD/52481/2014, SFRH/BPD/86513/2012.

# IS IT EVER TOO EARLY TO CLOSE AND /OR AUTOMATE MANUFACTURING OF CELL THERAPIES?

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Key Words: Automation, Single-use, Manufacturing, Closed-System, Cell Therapy

The rapidly growing field of cell therapy is providing very promising treatments for conditions that previously were considered untreatable or whose treatments were not considered very effective. These opportunities however do not come without their own set of unique challenges. Patient Specific Cell Therapies (PSCT) can be increasingly difficult to manufacture on a large scale. Each manufactured PSCT is a batch of one, meaning that it is intended for one patient and is not for off the shelf purposes. This can lead to issues with donor variability, time, cost of goods, and ultimately quality of product. These major issues can be greatly remedied if a proper automation system is implemented. This however requires a large amount of time, man power, development, and most importantly money. All these things must be considered when deciding to transition your given therapy from the lab bench toward closed, single use, automated manufacturing.

During my presentation I will discuss the factors that will determine if a cell therapy product is ready for and can benefit from incorporating automation into the manufacturing process. The decision on when to move to a more automated process will depend on several variables. One of these variables is what stage of the development process the product is in. It may not be necessary to implement costly technology when there is little proof of concept and the product is not fully developed, however if a major change in the manufacturing process occurs too late in the clinical testing process it would cause major complications with the regulatory approval process. Patient population is also a deciding variable for when to move to a more automated manufacturing process. For example, if a condition qualifies for orphan designation (less than 200,000 cases per year) the costs and time needed to implement process automation may not be exceeded by the benefits of the technology itself. On the other hand, if a product treats a condition like lymphoma it will require a much larger scale of manufacturing where the return on investing in automation is going to be much higher. The number of steps and difficulty of the steps can also determine if automation is beneficial. A product with multiple processing days and manipulations can be heavily improved by automation by decreasing the number of skilled workers needed, the number of hours to complete the manipulation, and the risk of human error during each step. Lastly if the technology available is not sufficient to manufacture a cell therapy on a consistent and repeatable basis it may be beneficial to hold off on automating your process to avoid machine failures or inconsistency with product characteristics.

This work will discuss the above variables in detail alongside other risks that occur that may influence the decision point on when and how to automate and close a manufacturing process. This work will highlight studies that have been performed by HCATS, taking on-board over 18 years of experience with over 150 cell therapy companies. As this industry takes off such questions need to be answered with the current data set and the end goal in mind. A walk through of the questions, answers and case studies will help showcase when and how closed, automation solutions should be implemented.

# HARVESTING EXOSOMES FOR THERAPEUTIC APPLICATIONS

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Key Words: Exosomes and extracellular vesicles; Harvesting; Therapeutic applications

Exosomes are membrane nanovesicles secreted by most cells. The uptake of an exosome by a local or distant cell transfers the molecular cargo derived from a secreting parent to a recipient cell. Biologically active molecules exchanged by such mechanism of cell-to-cell signaling include surface and luminal proteins, membrane-bound microRNAs, and other compounds. A growing number of studies implicated exosomal signaling in tumor metastasis, drug resistance, and modulation of immune response. As an intrinsic molecular delivery system in health and disease, the exosomes are beginning to attract interest for their therapeutic potential to deliver various biologically active compounds and educate adaptive immune responses.

In this presentation, we review early results on therapeutic applications of exosomes and their harvesting from biofluids, excluding bioreactor cell growth medium. The utility of the exosomes in monitoring cell growth conditions in bioreactors is discussed.

# CASE STUDY: LEVERAGING AUTOMATION AND CUSTOM SINGLE-USE SYSTEMS TO STREAMLINE MEDIA PRODUCTION AND ENABLE SCALABILITY FOR CAR-T MANUFACTURING

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Key Words: cell therapy, closed processing, scalability, automation, media production

In cell therapy, historical media production methods have typically utilized off-the-shelf, single-use assemblies from blood management suppliers with open connection devices. The use of these assemblies requires open processing in ISO 5 biological safety cabinets (BSCs), with significant manual labor required to aseptically access and transfer media components. Manual and open media production processes create capacity challenges that are only partially alleviated with efficiency improvements to the manual methods. Furthermore, these methods also create potential contamination risks.

An automated media production system has been developed to dispense individual components, mix the components, and fill the compounded media into a large quantity of small-volume bags for use in manufacturing operations. The process has been closed using custom single-use assemblies with aseptic connectors and weldable TPE tubing, enabling the relocation of the operation to a less stringent ISO 8 cleanroom and eliminating the ergonomic strain associated with prolonged BSC operations. Additionally, legacy blood management bag films have largely been replaced with next generation biopharmaceutical bag films, reducing extractables and leachables risk.

The value of this implementation also includes increased capacity (see Figure 1) and decreased direct labor requirements. The large-volume lots produced with the new system reduce media waste and increase volume discounts on raw materials significantly.

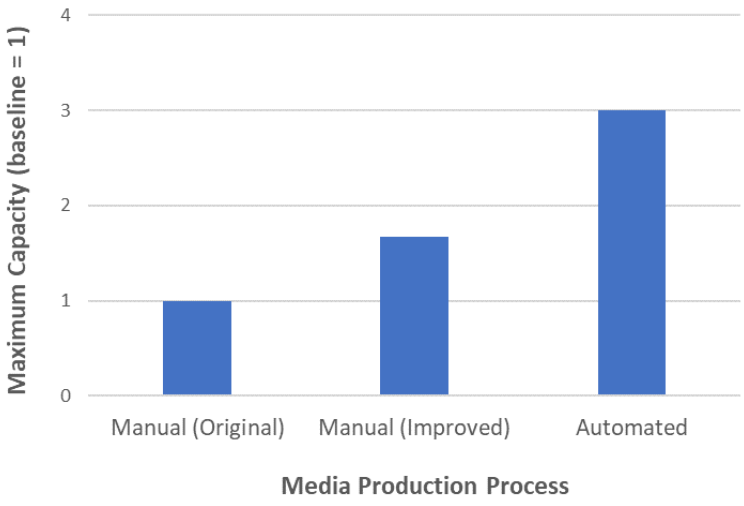


*Figure 1. Plant Capacity for Different Processes*

**PROCESS DEVELOPMENT FOR INCREASED MSC PRODUCTION IN SINGLE USE STIRRED TANK BIOREACTORS**

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Industry trends in regenerative medicine highlight a critical need for closed, single use cell culture systems that support scalable manufacturing of adherent cell therapies. Due to the limited downstream processing steps and shelf-life requirements for cell therapies, single use technologies are essential for cell therapy production. However, typical single-use static *in vitro* culture methods, are often too cumbersome and inefficient to support commercial scale production of mesenchymal stem/stromal cells (MSCs). Single-use stirred tank bioreactor systems are a platform that can address this scaling limitation by decreasing labor, footprint, and overall cost. When developing a stirred tank bioreactor process, bioreactor seeding and process control strategies, such as agitation, must be optimized to enable the process to scale for commercial manufacturing. Herein, case studies are presented illustrating solutions to this need. The first case study demonstrates the application of Zwietering's equation for suspension of solids to overcome scaling challenges often associated with microcarrier culture in stirred tanks. The second case study reviews strategies to further close the bioreactor seeding process. Identifying optimal seeding and process control strategies for microcarrier-based bioreactor expansion of adherent cells is paramount for the development of robust cell therapy manufacturing platforms.

**IMPROVED DYNABEAD REMOVAL USING DESIGNED-FOR-PURPOSE BIOPROCESS CONTAINERS**

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Key Words: CAR-T Cells, Dynabead, Cell Therapy, Single-Use

The recent FDA approval of a CAR-T cell based therapy has been an important milestone for progress in how we collectively can target and treat diseases. A key tool in these therapies have been Dynabeads for selection and activation of target cells. One important workflow step is the removal of Dynabeds after their use and prior to patient administration of cells.

To further improve Dynabead removal outcomes for users of the DynaMag CTS system, we have endeavored to create a bioprocess container (BPC) with improved Dynabead retention characteristics. This bag leverages existing film and manufacturing expertise from Thermo Fisher Scientific that have been extensively used in the biopharmaceutical industry over the past decade. Fundamentally, this designed-for-purpose BPC increases the flow path of fluid over the DynaMag to provide longer exposure to magnetic fields. This in turn provides for superior Dynabead removal from the fluid when compared to standard bags without this design as demonstrated in Figure 1.

These designs are easily incorporated into standard cell processing steps and compatible with existing single-use, closed-system workflows via sterile welding or aseptic connectors. The outlined BPC provides another tool for maintaining quality and repeatability in the inherently variable landscape of autologous T-cell therapy.

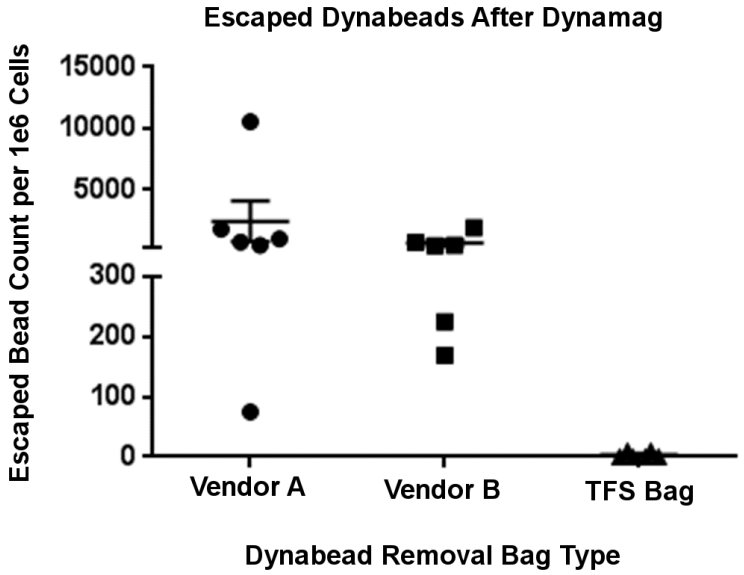


*Figure 2 – Dynabead removal performance of Thermo Fisher Scientific BPC compared to other vendors*

# GROWTH BEHAVIOR OF HUMAN ADIPOSE TISSUE-DERIVED STROMAL/STEM CELLS IN SINGLE-USE SPINNER FLASKS: NUMERICAL AND EXPERIMENTAL INVESTIGATIONS

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Key Words: Single-use spinner flasks, Computational Fluid Dynamics (CFD), Euler-Euler and Euler-Lagrange model, human adipose tissue-derived stromal/stem cells, MC-associated growth model

Human adipose tissue-derived stromal/stem cells (hASC) represent a valuable source of cells for clinical applications, especially in the field of regenerative medicine. Therefore, it comes as no surprise that interest in hASCs has increased greatly over the last decade. However, in order to use hASCs successfully in clinical applications, *in vitro* expansion is required. Single-use bioreactors in combination with microcarriers (MC) have been shown to be suitable systems for this task (1-3). However, hASCs are prone to higher shear sensitivity than conventional cell lines (e.g. CHO, BHK) that are normally expanded in these systems. Hence, the goal of this study was to investigate the influence of different shear stress levels on the growth of hASCs in small scale single-use spinner flasks. For this purpose, *Computational Fluid Dynamics* simulations based on a *Euler-Euler* and *Euler-Lagrange* approach were performed to predict the hydrodynamic stresses (0.06 – 0.87 Pa), the residence times (0.4 – 7.3 s) and the circulation times (1.6 - 16.6 s) of the MCs in various high shear zones. The numerical findings were combined with experimental data from cultivation studies (0.29 – $1.1 \cdot 10^6$ hASC/mL) in order to develop a segregated mathematical growth model for the prediction of MC-associated hASC growth in small scale single-use spinner flasks.

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# STUDY ON MIXING AND FLUID DYNAMICS IN SINGLE-USE SHAKEN SYSTEMS

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Key Words: Mixing, PIV, Microwells, Square reactors, Orbital shaking

Bioreactors are widely used in a range of applications, including the food/drink, pharmaceutical and medical industries. Stirred Tank Reactors (STRs) are the most widely used and rely on mechanical stirrers to achieve the optimal fluid motion, and their flow dynamics has been extensively studied for a broad combination of operating conditions (Ducci and Yianneskis 2005, 2007, Escudie and Line 2003). Orbitally Shaken Reactors (OSRs) promote agitation through the orbital motion of the bioreactor, which induces sloshing of the free surface. Their flow dynamics has been mainly assessed for lab scale reactors of cylindrical cross-section, while few studies in terms of mixing and fluid dynamics are available for unconventional shapes at limited scales. During early stages of bioprocess development, single-use ml-scale shaken multi-well plates are commonly used for scale-down studies as they allow a large number of experiments to be performed using small amounts of material. However, very few studies published on shaken bioreactors have thoroughly studied the engineering aspects and the hydrodynamics at such a small scale, thus resulting in a lack of accurate scaling correlations between shaken and large scale conventional bioreactors. The flow in orbitally shaken reactors has been characterised by Weheliye *et al* (2013) and Ducci and Weheliye (2014) and a scaling law has been developed for two cylindrical reactors with internal diameters of 10 and 13 cm. However, the understanding of reactors with square shape is still very limited despite they have a number of practical applications. For example, two different disposable shaken reactors with square cross-sections have been used by Stettler et al (2007) for transient gene expression. The aim of this work was twofold – (i) to estimate the mixing time in microwell plates of different geometry and determine an effective scaling parameter between micro-scale and lab-scale reactors and (ii) to determine the fluid dynamics in square reactors and identify analogies with baffled stirred tanks.

In the first part of this study, mixing time was measured in microscale systems by adopting the Dual Indicator System for Mixing Time (DISMT) method, and the effects of fill volume, fluid viscosity and surface tension were investigated in 24-DSW and cylindrical geometries on a ThermoMixer with orbital diameter of 3 mm. The mixing time of the DSW showed in general the typical variation of a mixing number curve, however it was identified a range of rotational speed N=600-650 rpm, which was denoted by an increase of mixing time with speed. This phenomenon is caused by a reduced free surface oscillation over this range of speeds, which does not occur when a cylindrical geometry is considered. With a reduction of surface tension this phenomenon disappears also in the deep square wells. Mixing time measurements were also carried out in intermediate-sized reactors and compared to those obtained in lab-scale reactors by Rodriguez et al (2013, 2014). These data indicate that the natural frequency of a filled container can be used as an effective parameter to scale between microwells and larger scale shaken reactors.

Secondly, Horizontal PIV measurements were carried out in a squared shaken bioreactor with a diameter of 6.2 cm, which has the same cross-sectional area as the cylindrical reactor used by Rodriguez et al (2014). The flow in a square OSR is clearly different from what have been observed in cylindrical reactors previously. All the ensemble averaged velocity fields obtained in the square tank for a range of rotational speeds shown the effect of the four corners on fluid flow. The directions of the flow at a few phase angles were also investigated by phase-resolved PIV measurements and they were found in agreement with the flow directions in cylindrical shaken reactors. The average of the kinetic energy of a lower plane is smaller than that for a higher plane and the kinetic energy distribution also demonstrated the effects of the presence of four corners on the flow inside the square reactor.

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Weheliye *et al* (2013) *AIChE J.*, 59(1): 334-344.

# ADAPTIVE COMBINATION OF SSB AND SUB EQUIPMENT TO MASTER COMPLEXITY IN CLINICAL MANUFACTURING IN THE CLINICAL SUPPLY CENTER

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Key Words: COMBINATION, SINGLE-USE, SUB, STAINLESS STEEL, SSB, COMPLEXITY, ROBUSTNESS, FLEXIBILITY

Today's clinical manufacturing of complex monoclonal antibodies under GMP conditions needs to be highly adaptive in order to face the requirements of constant acceleration of processes.

The main challenge of the clinical supply center (CSC) in Penzberg (Germany) is to successfully master the complexity of producing different products in different phases (clinical phase I – III) with different process versions. New products for clinical phase I have different requirements than older products, as well as products in later phases or resupplies. In addition, the actual change to more intensified processes will increase the complexity even more.

The CSC is equipped with a variety of bioreactors in different sizes and types. Stainless steel as well as single use bioreactors, which were installed over the past 25 years. In order to face the main challenge, it is mandatory to create a framework that allows the adaptive combination of standard (SSB) and new technologies (SUB). To realize that, SUBs and SSBs are treated equally. SUBs have the advantage to be used more flexible than SSBs because of less preparation time and an easier adaption to intensified processes (like perfusion modules in the N-1 bioreactor). Furthermore, SUBs can be easily connected to every existing bioreactor type (SSB as well) via hose connections. This all offers the chance to utilize existing bioreactor racks more efficient with less slack time. An adaptive combination is also faster and more favorable than just replacing existing with new equipment. The poster shows the successful implementation of the adaptive combination in the CSC, by adding SUBs with increasing volume to the facility (starting with 250L and ending with 2000L production volume) and apply them for existing and new processes. The intensified usage of SUB equipment shortens the upgrade time to adapt to future needs (e.g. switch to perfusion technology).

With this flexible setup the multi-product-GMP facility in Penzberg is perfectly prepared for actual and upcoming challenges.

# COMPARISON OF ALTERNATIVE SINGLE USE HARVEST TECHNOLOGIES FOR LARGE SCALE HARVESTS OF MAMMALIAN CELL CULTURE PROCESSES

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In a fully disposable facility where the use of continuous disk-stack centrifuges are not preferred, harvest processes based on conventional depth filtration become more challenging with increasing single-use bioreactor (SUB) size and higher density culture. Here, several alternative single-use harvest technologies were evaluated. A disposable centrifuge and a range of different synthetic depth filters were tested. Results showed significant improvement in filterability and reduction of depth filter area compared to full traditional depth filtration train.

# NEW APPROACH FOR QUALIFYING LIQUID HANDLING IN SINGLE-USE BAGS

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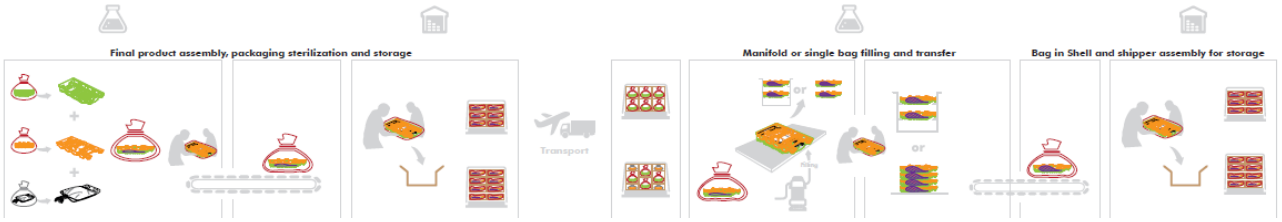
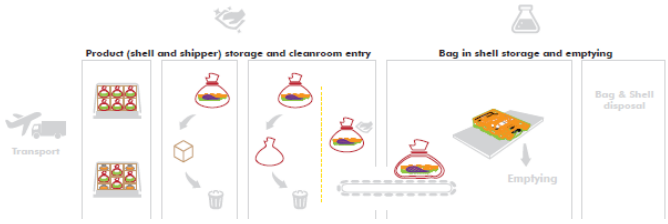
Key Words: Bulk Drug substance handling, process qualification, Operator handling, error free, safe handling, poka-yoke, risk assessment, life cycle, single-use.

The technical and economic benefits of Single-use in bio-manufacturing are well known and the industry is broadening their adoption. The increasing manual handling of bags in the biotech process involves higly trained operators and specific guidelines in order to secure the process, especially for bulk drug substances or even for drug products handling.

The lecture will focus on a new approach of the liquid handling process life cycle analysis that led to the development of specific shells for Flexsafe® 2D bags. This scientific approach supported by bioprocess engineers, packaging engineers and material experts provides a secured way for developing and qualifying the robustness of these liquid handling systems.




Based on real life and worse case conditions experienced over the whole life cycle of the product, this approach allows to provide a comprehensive testing program with different ASTM norms and a clear rationale about the protocol tests choices and the expected safety margin.




This process qualification approach allows to secure liquid handling and demonstrates the bag integrity for the best protection of the drug substances, finally saving cost for end-user process validation.

**UNDERSTANDING THE FUNCTIONAL LIMITS OF SINGLE USE COMPONENTS THROUGH PRESSURE TESTING**

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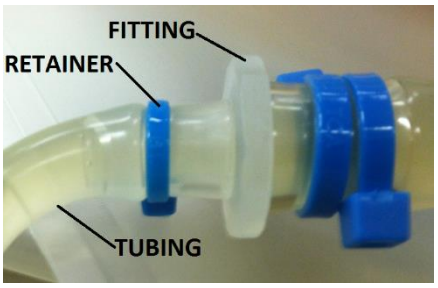
Summary:
Transferring fluid from one container to another is a very common bioprocessing operation. A variety of different components have been created for the purpose of fluid transfer, including bag ports, tubing, aseptic connectors, barbed fittings, tubing retainers, etc. In order to provide high quality, highly reliable single use assemblies, it is important to test the functional limits of fluid transfer components. Thermo Fisher has designed a versatile system to test the pressure limits of components.

Tubing Connections:
Tubing, fitting, retainer. What are its functional limits?




Connection Test Unit:
- Pressure decay technology – quantifiable leak rate, long pressure hold durations, inexpensive operation
- Test temperature is controllable
- Tubing manipulation is controllable
- 80 psi maximum test pressure
- Complete range of fitting/tubing sizes

Case Studies:
Each of the case studies below have been investigated, with interesting and enlightening results.
1. If a tubing is bent or kinked, does it affect the leak resistance of the connection?
2. What is the optimal Barb Design?
3. Does location of cable tie or other retainer effect leak resistance?
4. How do different tubing materials compare to one another? (Silicone, PVC, TPE)
5. Can we make product improvement recommendations based on pressure testing?

Conclusion:
Testing of single use components exposes the functional limits of those components. Such testing is the foundation of fit-for-purpose single use design.
Thermo Fisher is currently working to better understand all components that are used on our products. A wide variety of test scenarios are being considered and tested.

# SMALL VOLUME SINGLE USE FACILITY STRATEGY – HARVEST CASE STUDY

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# USE OF THE AMBR 250 TO ENABLE RAPID CLONE SELECTION AND PROCESS DEVELOPMENT FOR LARGE SCALE MANUFACTURING PROCESSES

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Key Words: Ambr 250, scale-down modeling, automated microscale bioreactor, CFD modeling.

Currently, widely used bench scale bioreactor systems require much user manipulation, a large amount of raw materials, and have a long turnaround time for reactor cleaning and rebuilding. New technologies such as robotic disposable bioreactor systems provide a solution that is miniaturized, high throughput, and substantially automated. The Ambr® 250 offers such a solution, with 24x250mL bioreactors controlled independently. Although this new technology is rapidly being adopted by several groups as a way to increase efficiency and speed within upstream development, it remains to be proven that these systems are complete models for process characterization.

We have shown that Ambr 250 is a good scale down model for multiple cell line systems. The aim of this work is to further characterize the engineering environment of the Ambr® 250 with a view of defining its role in industrial cell culture process development and scale-up. CFD modeling of the Ambr 250 mammalian vessel with validation via Particle Image Velocimetry (PIV) was conducted to simulate the hydrodynamic environment in the vessel. These findings were evaluated against current benchtop models and manufacturing scales. Cultures were run utilizing different engineering parameters (vvm, P/V, $k_La$) to assess the scalability of the current system. Cell growth, production, and product quality were compared across to recommend operating conditions for the Ambr® 250 that best match manufacturing scale reactors. Multiple CHO host cell lines were examined in order to find optimal operating conditions for the Ambr® 250 system.

# CLOSED SYSTEM APPROACH TO CELL EXPANSION

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This poster describes the development of a novel closed system approach to cell culture expansion in shaker flasks. An insert with microporous membranes was created and combined with a cap closure with integral tubing attached to the cap for the aseptic input of media and inoculate and for the aseptic output of samples and finished product. The pH of the solution was measured as a function of carbon dioxide concentration and compared with traditional shaker flasks. Cell culture doubling times, cell viability, and total cell counts were measured for 500 mL, 1,000 mL, and 3,000 mL systems. Additive manufacturing was also used to speed up the evaluation of the technology. Sample caps were 3D printed for end user evaluations of the early prototypes. Closed system processing is now a viable option for cell culture expansions.

# TRANSITIONING TO FACILITY USING SINGLE USE TECHNOLOGY (SUT)

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Key Words: cell culture, single use, wave bioreactor, bag film testing

Establishing comparability in SUT facilities for processes that were previously run in stainless steel facilities can be a challenge. This poster will document a case study for demonstrating cell culture performance and product quality comparability for a process transitioning into a SUT facility. Experiments that were conducted to test rocking WAVE bioreactor systems to replace stirred tank bioreactors for seed stage will be described. Results from lab and pilot scale experiments to test bag films that will be used in the inoculum and production bioreactors will be presented. Results from preliminary experiments to test the effect of nanofiltration of cell culture medium will also be discussed.

# HOW TO DEVELOP HEALTH-PROMOTING FOOD SUPPLEMENTS BY USING SINGLE-USE BIOREACTORS

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Key Words: single-use bioreactors, plant cell cultures, cell culture chocolate, *Theobroma cacao*

There is currently considerable interest in alternative and sustainable production methods for healthy foods. The cultivation of plant cell cultures in suitable bioreactors instead of growing whole plants on the field may be a solution. In this way, the cell cultures of interesting plant species can be established independent of the location. Furthermore, secondary metabolism can be specifically controlled during mass propagation of the cells. In other words, the expression of compounds promoting health and wellbeing can be supported, and the formation of substances with adverse health effects can be suppressed. We used this approach to make cacao powder and to produce a 'cell culture chocolate' by growing suspension cells from *Theobroma cacao* in a Flexsafe RM 20L bag with a screw cap from a BIOSTAT RM 20/50. The cell line (dark culture) was established from a well-growing and friable callus clone, and has a doubling time of 4 days. It provided up to 40% higher concentrations of the polyphenols epicatechine, procyanidine B1, B2 and C1, and cinnamtannine A2 than cocoa beans from pods grown in Puerto Rico. The alkaloids caffeine and theobromine were absent in the cell culture grown in MS-medium. On day 16, about 300 g biomass (fresh weight) was harvested from the wave-mixed single-use bioreactor operated in feeding mode. Addition of an antifoam agent and pH-regulator was not required. The biomass was freeze-dried, resulting in in vitro cacao powder that was roasted and milled before adding sugar, lecithin and cocoa butter. 3 blocks of dark chocolate (70%) were produced, which provided the experts on the ZHAW`s sensory panel with a unique taste experience. The flavour was intensive and complex, citric and berry flavours being predominant. The results demonstrate the suitability of wave-mixed bioreactors for the development of plant cell-based health-promoting food and food ingredients. Subsequent studies will focus on the influence of power input and shear stress on polyphenol formation, and the development of a scalable low-cost bioreactor.

# MANUFACTURING SINGLE USE SYSTEMS WITH QUALITY IN MIND: HOW TO ASSURE PERFORMANCE, ROBUSTNESS, AND STERILITY OF SINGLE USE SYSTEMS

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Key Words: single use, quality, sterility, particles, integrity

Quality control during the manufacturing of single use systems is critical. With traditional stainless-steel systems, the end user has significant control over the design, construction, qualification, validation, and maintenance of the system. When implementing a single-use system, the supplier of the single use product takes responsibility for many of these functions from the user. It is therefore important that the single use supplier has established and uses a robust quality control system. This presentation will highlight the quality systems, processes, facilities, and personnel required to assure the performance, robustness, and sterility of single use systems.

The following topics will be covered:

- Single-use assembly validation
  - Qualification of components
  - Sterilization qualification
  - Manufacturing processes
- Quality control
  - Release testing
  - Certification
- Risk mitigation practices
  - Process particulate control
  - Operator training
- Leachables & Extractables
  - Patient safety evaluation, study design
  - Support by the supplier

# A CHALLENGE WITH SINGLE-USE TECHNOLOGY: PROTECTING BULK DRUG SUBSTANCE (DS) DURING COLD CHAIN HANDLING, STORAGE, AND TRANSPORT

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Key Words: cold-chain, frozen_storage, bio-container, fluoropolymer, bulk_drug_storage

The issue of single-use bag durability during cold chain handling, transport and storage of bulk drug substance is well-known in the biopharmaceutical market, particularly after freezing at -86°C (-123°F). This poster will address market trends driving the growth of temperature-sensitive pharmaceuticals and the challenges of using disposable containers, principally at frozen temperatures. Gore will present the durability performance of its new GORE STA-PURE Flexible Freeze Container System, highlighting key fitness-for-use tests including frozen durability (impact resistance, freeze/thaw and long term storage). In addition, the extractables profile of Gore fluoropolymer film will be compared to other single-use frozen storage containers' film.