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Microspectroscopic investigation of the membrane clogging during the sterile filtration of the growth media for mammalian cell culture

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ABSTRACT

Growth media for mammalian cell culture are very complex mixtures of several dozens of ingredients, and thus the preparation of qualified media is critical to viable cell density and final product titers. For liquid media prepared from powdered ingredients, sterile filtration is required prior to use to safeguard the cell culture process. Recently one batch of our prepared media failed to pass through the sterile filtration due to the membrane clogging. In this study, we report the root cause analysis of the failed sterile filtration based on the investigations of both the fouling media and the clogged membranes with multiple microspectroscopic techniques. Cellular particles or fragments were identified in the fouling media and on the surfaces of the clogged membranes, which were presumably introduced to the media from the bacterial contamination. This study demonstrated that microspectroscopic techniques may be used to rapidly identify both microbial particles and inorganic precipitates in the cell culture media.

1. Introduction

Preparation of liquid media is one of the initial steps during the cell culture process for the production of biotherapeutics in the biopharmaceutical industry. Depending on the specificity of cell lines, properly formulated cell culture media (CCM) are needed for healthy cell growth that is critical to the viable cell density (VCD) and the product titers. For mammalian cell culture, the growth medium is typically a very complex mixture and may contain up to nearly one hundred ingredients, which include amino acids, inorganic salts, carbohydrates, vitamins, fatty acids, trace metal elements and other constituents, such as buffering agents and surfactants [1–3]. Each class of these ingredients has specific and important roles [2] to grow healthy cells in the media: amino acids are the key protein building blocks; inorganic salts help retain osmotic balance and regulate membrane potential; carbohydrates in the form of sugars are the major source of energy; vitamins are essential for growth and proliferation of cells; fatty acids are important in serum-free media; and trace elements (copper, zinc, etc.) are needed for proper cell growth. In the present biomanufacturing industry, culture media may be supplied commercially in three forms: powder, concentrated liquid and working solution [1,2]. Although less expensive, the powdered media ingredients need to be compounded properly and then sterilized prior to use. The media sterilization can be carried out with sterile filtrations which can remove microbial particles and precipitates but allow dissolved ingredients to pass through.

During our sterile filtration of a batch of growth media prepared from powdered ingredients, the membranes became clogged in the middle of the filtration process. The clogged filter was replaced with a new one, but it was clogged again. However, when a new batch of media was prepared with the same sets of ingredients, it passed through the sterile filter smoothly and no membrane clogging was observed. This indicated that the raw materials were not an issue in this case and the preparation and storage of this specific batch of media were questionable. To aid for risk assessment and mitigation, root cause study was initiated to determine the identity and possible origin of the clogging substances in the CCM.

In this study, we report the root cause analysis of the clogged sterile filtration based on multiple microspectroscopic techniques [4–6], including optical microscopy (OM), fluorescence microscopy (FM), scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) microscopy and energy dispersive *x*-ray spectroscopy (EDS). OM was used for visual inspection and imaging of targeted samples. FM was used to provide higher contrast images

Abbreviations: ATR, attenuated total reflectance; CCM, cell culture media; DNA, deoxyribonucleic acid; EDS, energy dispersive X-ray spectroscopy; FM, fluorescence microscopy; FT-IR, Fourier-transform infrared; GCM, gold-coated membrane; IR, infrared; NA, nucleic acids; OM, optical microscopy; PES, polyethersulfone; RNA, ribonucleic acid; SEM, scanning electron microscopy; SVP, sub-visible particles.

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of samples that fluoresce differently from their background when appropriate excitation light was used. SEM was used to obtain detailed information about particle sizes and morphology in higher resolutions. The infrared analysis was used to obtain molecular IR 'fingerprints' to identify unknown samples. The IR analysis were carried out in the reflection mode for the particles isolated from the media, and in the attenuated total reflection (ATR) mode for the particles retained on the membranes. The ATR-IR technique has been used extensively in the studies of membrane filters [7–10] for separation and purifications. The EDS can provide elemental compositions of the samples, which are complementary to the infrared data and aid in the analysis of low-levels of inorganics [4,5].

By taking advantages of the above complementary techniques, we have identified cellular particles in the fouling culture media, and cellular debris or fragments on the surfaces of the clogged membranes. Based on the root cause analysis, we concluded that the bacterial contamination was responsible for the failure of the sterile filtration.

2. Materials and methods

2.1. Media preparation

The liquid culture media were prepared by compounding and dissolving the powdered ingredients following the established procedure. The prepared liquid media (~30 g/L, pH 7.2) were then sterilely filtrated using an Opticap XL 10 capsule filter (Millipore Express SHR cross-flow filter, polyethersulfone or PES membranes with pores sizes 0.5 μm/0.1 μm), and one batch of the liquid medium had clogged the membrane during filtration. However, the filtered media (filtrate) passed through a new filter smoothly without clogging, indicating the clogging substances were in the fouling medium and were also retained by the clogged membranes. In this study, both the fouling medium (before sterile filtration) and the clogged membranes were used for root cause analysis. Numerous particles were observed inside the fouling medium under the microscope.

2.2. Particle filtration

The fouling medium was kept at 2–8 °C overnight to allow the particles to concentrate in the lower portion of the solution. Then the concentrated particles were filtered onto both gold-coated membranes (GCM, 0.8 μm, rap. ID Inc., NJ 8852) and silver coated membranes (0.8 μm, STERLITECH Co. Kent, WA 98032). The larger pore sizes of the gold-coated and silver-coated membranes allowed for capturing sufficient number of particles before being clogged by the fouling media. The captured particles were rinsed with Milli-Q water and dried prior to analysis.

2.3. Membrane preparation

The fouling filter was flushed with purified water to remove residual media and then the cartridge was cut-open at the two ends. The clogged membranes along with the supporting fibers were removed from the cartridge and stored at 2–8 °C prior to examination. Representative pieces of membranes were sliced off with a stainless steel razor blade and transferred to adhesive carbon tapes for further microspectroscopic analysis.

2.4. Light microscopy

Both filtered particles and clogged membranes were examined and imaged with a Carl Zeiss Stemi 2000C optical microscope (Thornwood, NY 10594). Color micrographs were captured using an AxioCam MRC color digital camera attached to the microscope. The isolated particles on the GCM were also imaged with a Carl Zeiss Axio Imager Z2m fluorescence microscope (Thornwood, NY 10594) under filter set #2 (G365 nm excitation, LP420 nm emission) for improved contrast.

2.5. Infrared microscopy

The IR spectra of the isolated particles were obtained in the refection mode from 600 to 4000 cm^{-1} with a Bruker Hyperion 3000 IR Microscope attached to a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Billerica, MA 1821). The particle-free (blank) area on the GCM was used as the reference background. In the real-time mode, the particles across entire filtration area were scanned by the IR beam to check the particle heterogeneity based on the live spectra at different spots. Representative spectra were collected for 128 scans at 4 cm^{-1} spectral resolution and used for spectral library search. The ATR IR spectra of the particles on the membrane surface were collected from 600 to 4000 cm^{-1} using a LUMOS® (Bruker Optics, Billerica, MA 1821) stand-alone IR microscope equipped with a germanium ATR crystal. The diameter of crystal tip was approximately 100 μm. Multiple spots on the membranes were selected for spectral collection and comparison.

2.6. SEM/EDS

The SEM micrographs of the samples were captured with a Zeiss EVO MA 10 SEM. The EDS spectra were recorded with an Oxford INCA PentaFET-*x*3 EDS detector (Concord, MA 1742) attached to the Zeiss SEM. Both the SEM imaging and EDS analysis were carried out at a variable pressure mode with the electron beam acceleration voltage set to 20 kV. The SEM micrographs and EDS spectra of particles on both the GCM and silver coated membranes were obtained. The latter enabled the peaks of elements P and S from the particles to be visible at the EDS spectrum, but they were obscured by the strong Au peaks from the GCM.

3. Results and discussion

3.1. Particles inside the culture media

The particles from the fouling media were captured by a GCM via filtration, shown in Fig. 1 as the inset, which was imaged under a UV light. The isolated particles were sub-visible in sizes (in the order of several microns). A representative IR spectrum of the isolated particles on the GCM is shown in Fig. 1, which appears to be a protein spectrum at first glance. However, a careful examination of the spectrum reveals that it exhibits typical features of whole cellular IR bands where detailed assignments have been reported in a number of literatures [11–18]. In particular, the IR spectrum of the particles matches excellently with an IR spectrum of bacteria [17].

The most prominent bands in the spectrum (Fig. 1) were from proteinaceous components including amide I at 1646 cm^{-1}, amide II at 1544 cm^{-1}, and amide A (N—H) at 3295 cm^{-1}. The broad amide A band was also partly contributed from O—H bands from polysaccharides. Several C—H bands in the spectrum include $-CH_3$ asymmetric stretch at 2959 cm^{-1}, $-CH_2$ asymmetric stretch at 2928 cm^{-1} and symmetric stretch at 2855 cm^{-1}, as well as $-CH_2$ bending at 1455 cm^{-1}. These C—H bands were contributed mainly from lipids, protein side chains and polysaccharides. The band at 1397 cm^{-1} was the $-COO^-$ symmetric stretch and mostly contributed from protein side chains and lipids. The two distinct bands at 1237 cm^{-1} and 1083 cm^{-1} are characteristic of $-PO_2^-$ asymmetric and symmetric stretches, and both are mainly contributed from nucleic acids (NA) [11,13,15,18], and with some contributions from