Reducing the fungal footwear risk: Evaluating polymeric antimicrobial flooring for cleanroom entry

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Introduction

Several studies have demonstrated the effectiveness of antimicrobial polymeric flooring (such as the special flooring manufacturing by Dycem) (1). These studies have focused on particulate removal and reductions in microbial levels (2). What has been less-well studied is the suitability of this type of flooring for the reduction in fungal counts. This article presents a summary of a study conducted into the ability of Dycem flooring to reduce levels of fungi from footwear compared with conventional cleanroom flooring. The outcome showed the Dycem flooring was significantly better at lowering the fungal bioburden across a range of different fungal genera.

The contamination transfer problem

There are a number of contamination sources that present a risk to cleanrooms used for the manufacture or processing of pharmaceutical products, including air, water, people and surfaces. To reduce the risks presented an array of contamination control solutions are required and these include the need to assess and remediate what is happening at floor level.

Entry into cleanrooms is via airlocks and changing rooms. Given that most cleanrooms are operating to strict HVAC design parameters, the primary route of contamination is via people or via the transfer of materials. Given that contamination will either remain suspended in the air (prior to its eventual removal through air extracts) or settle onto a surface, transfer from surface to surface exists as an ever-present contamination control for the cleanroom manager. Contamination will either be present on the shoes worn by personnel or be deposited onto the floor area as a result of airborne particles being deposited onto the surface (through gravitational settling and other air current disturbances) (3). Contamination on shoes will exist even when shoes are captive to the facility (the manual disinfection of shoes is often not easy to achieve to a satisfactory level, which strengthens the need for an additional decontamination step). This is an important consideration for any contamination control strategy. A further concern arises through redispersal, where the activities of walking or pushing carts can cause deposited particles to be resuspended in the air and contaminate whatever is moving through the space (4).

Fungal challenges

The types of contamination present will include fungi (yeasts and molds). Fungi present a problem in pharmaceutical manufacturing due to the ability of fungal spores to travel across air currents; with fungi able to grow on a wide variety of substrates; and due to the risks from mycotoxins being produced within a product (5). Consequently, fungal contamination incidents make up a large proportion of pharmaceutical product recalls due to microbial contamination (6, 7).

Fungal contamination risks arise from a variety of sources. There are a number of common fungi that are either associated with people (as part of the human skin microbiome); present as part of the as-built environment; or associated with the urban or rural environment. Although

the types of external environmental fungi will vary geographically, there are some common genera found in most parts of the world (such as species of the filamentous fungi *Aspergillus*, *Fusarium* and *Penicillium*). Microbial risks exist as organisms in the airstream and attached to surfaces; the majority of microorganisms in the air are attached to larger particles (such as skin detritus).

Contamination solutions

For cleanroom managers the main options for seeking to control contamination at floor level include the use of standard flooring and regular disinfection; the use of tacky-mats; or the layering of antimicrobial control mats made of polymeric materials. The concern with standard flooring is that the disinfectants used to periodically treat the floor only have a short-lasting residual activity resulting in many times during the working day when the flooring will provide a resting place to a relatively high number of particles and microorganisms. With tacky-mats, these are rarely of a sufficiently large surface area to effectively pull contamination from footwear, and they present a contamination control issue when each layer is removed in terms of resuspending a portion of the collected particles into the airstream. Polymeric flooring has been shown to have a strong electrostatic charge and it can pull an equivalent (or better) levels of particles from footwear compared with the tacky-mat (8) (electrostatic forces are active across the optically flat, flexible surface, and this serves to pull away and to trap particles of varying sizes) (9). In addition, some manufacturers (such as Dycem) incorporate antimicrobial additives. In this case Biomster technology is incorporated into the polymeric flooring providing silver ion activity. Silver is a safe inorganic antimicrobial, effective at minute concentrations, exhibiting an 'oligodynamic' effect through the presence of toxic metal ions. The activity of silver ions (Ag+) occurs as ions deposit themselves into the cell walls and vacuoles of bacteria and fungi, damaging cell structures (10). Once inside the cell, silver ions bind to DNA and RNA molecules, causing them to condense (11). This makes it more difficult for ribosomes to transcribe or read the DNA and RNA, a process necessary to protein synthesis and cell division (12, 13).

Evaluating fungal remediation at floor level

To reduce the level of contamination entering changing rooms minimizing contamination levels on footwear is essential. This contamination will include fungi. Some of the fungal sources will be from the external environment, some from the built environment, and some will arise from the human mycobiome. One consequence of the take up of cleanroom socks is that personnel will expose their feet more often to undertake a sock change and care needs to be taken with hand disinfection given that the highest concentrations of fungi on the human body are with the plantar heel, toeweb and toenail (14).

To assess the effectiveness of Dycem flooring to reduce fungal numbers (as measured by colony forming units) a study was conducted at a pharmaceutical facility in the south-east of England. This study evaluated Dycme Cleanzone flooring located between a Controlled-Not-Classuified (CNC) space leading into an EU GMP Grade C / ISO 14644 Class 8 (in operation) cleanroom changing area.

The study set out to demonstrate that shoe soles can be vectors for fungal contamination and to investigate if polymeric flooring is an effective decontamination device and hence decrease the risk of transferring fungal contamination into a cleanroom changing room effectively.

To test this, a diverse array of different types of fungal morphologies was required. The following fungi were selected (as per Table 1):

Table 1: Fungi used in the study

Fungus	**Culture collection reference**	**Reason for selection**
Aspergillus brasiliensis	ATCC 16404	A representative filamentous fungus and one of the current QC strains for culture media testing. In terms of pathogenicity, *A. brasiliensis* can cause pulmonary infections. The organism is found throughout the environment within soil and water, on vegetation, and suspended in the air.
Botrytis cinerea	ATCC 11542	A common fungus found on vegetation and feeding on decaying plant matter. It has an association with the outdoor environment. It is not regarded as a human pathogen.
Fusarium graminearum	ATCC 46779	A fungal plant pathogen which causes fusarium head blight on wheat and barley. It is a significant spoiler of agricultural products.
Penicillium chrysogenum	ATCC 10106	This fungus is primarily found in indoor environments, especially in damp or water-damaged buildings.
Mucor circinelloides	ATCC 24905	A dimorphic fungus found worldwide, inhabiting soil. It can sometimes pose a pathogenic risk to humans.
Candida albicans	ATCC 10231	A representative dimorphic fungus (often yeast-like) and one of the current QC strains for culture media testing. It is detected in the gastrointestinal tract and mouth of the majority of adults. It can lead to candidiasis, which results from an overgrowth of the fungus in people who have weakened immune systems.

With the reason for selection, as well as being chosen on the basis of their presentiveness the fungi also needed to meet Biohazard Safety Level 1 (so that occupational safety criteria were met).

Each fungus was challenged, individually, onto standardized rubber-soled shoe soles. The shoes had previously been unworn and decontaminated with 6% hydrogen peroxide. One shoe in each study was unspiked and serve as a negative control. The target challenge in each case was 10 to 100 colony forming units (CFU). It was assumed that the fungi were largely be in the vegetative state, although some may have been in the spore state. Each shoe challenge for each fungus was carried out three times (using different personnel).

With each of the people, samples were taken from inoculated unworn shoes (which served as the controls to assess the change in count) and from inoculated worn shoes, sampled after walking across Dycem flooring or from walking across the standard vinyl flooring. An assessment of fungal survival on the sole of shoes under both test conditions were assessed. An assessment of the sole of new shoes that have not been challenged was also performed (to provide a negative control).

For the study, each operator took six steps (each foot contacted the flooring three times equaling 'three footsteps' as per the minimum recommendation made by Dycem) across both the Cleanzone surface and the standard flooring.

Assessment of fungal counts was by contact plate, using Sabouraud Dextrose Agar (SDA), with each plate incubated at 20-25ºC for 7 days (the optimal conditions for fungal growth).

The results from the Dycem Cleanzone assessment are shown in Figure 1 and Table 2.

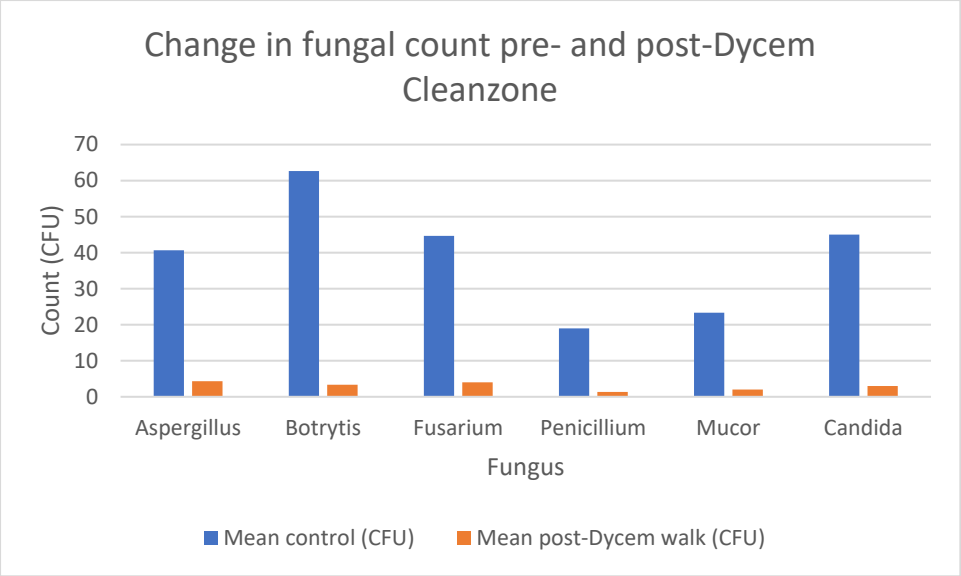

Figure 1: Chart showing change in fungal counts after walking across the Dycem Cleanzone flooring

Table 2: Changes in fungal count expressed as percentage change (in relation to Dycem Cleanzone):

Fungus	**Difference (CFU)**	**Percentage difference**
Aspergillus	Reduction: 36	89% reduction
Botrytis	Reduction: 59	95% reduction
Fusarium	Reduction: 41	91% reduction
Penicillium	Reduction: 18	93% reduction
Mucor	Reduction: 21	86% reduction
Candida	Reduction: 42	94% reduction

The results from the standard vinyl flooring assessment are shown in Figure 2 and Table 3.

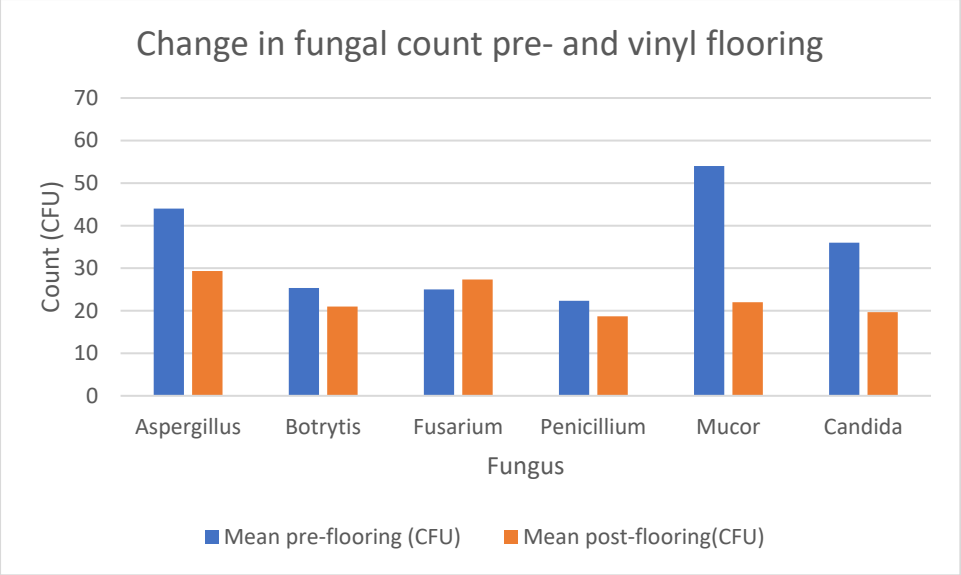

Figure 2: Chart showing change in fungal counts after walking across standard vinyl flooring

Table 3: Changes in fungal count expressed as percentage change (in relation to standard flooring)

Fungus	**Difference (CFU)**	**Percentage difference**
Aspergillus	Reduction: 15	43% reduction
Botrytis	Reduction: 5	18% reduction
Fusarium	Increase: 2	9% increase
Penicillium	Reduction: 3	17% reduction
Mucor	Reduction: 32	59% reduction
Candida	Reduction: 16	45% reduction

Discussion

The study assessed colonisation of a simulated cleanroom changing room entry environment and assess the level of fungal count change from Dycem Cleanzone flooring against controls and compared with standard flooring. This was designed to mimic how personnel could potentially transfer contamination into a controlled environment.

The data shows that the Dycem Cleanzone flooring reduced the fungal count significantly (a mean of 91%). This will be the consequence of two mechanisms: the electrostatic nature of the flooring and the presence of silver ions. Furthermore, the results obtained against the Dycem flooring were relatively consistent, indicating that fungal diversity was a not a particular factor.

A comparison was also made with standard vinyl flooring. Here there was some removal of contamination, as a result of physical removal (to a mean level of 32%). Given that standard vinyl flooring possesses no significance physiochemical pull and does not contain any antimicrobial agents (no residual disinfectant activity was considered likely), the microbial reduction step was not as great and hence a level of risk would continue to be presented to the cleanroom. With the standard flooring, the differences between fungal genera was more

apparent. Here reductions were relatively high for *Mucor* and relatively low for *Botrytis*, and the levels actually increased for *Fusarium*. The increase with *Fusarium* was within the margin of recovery error and represents 'no reduction'. Potentially the spores of this fungus are especially difficult to detach from footwear, although a greater understanding of fungal morphologies would be required in order to explore these data variations further.

The results showed a clear difference in the levels of reduction seen between the Dycem flooring and the standard vinyl flooring. There will be some variables that will influence the data relating to recovery error from the culture media and the different physiological states of the fungi. However, given this error will apply to all samples, the significant difference between the two surfaces is apparent. Areas of further work could include varying the length of time that the fungal challenge is in contact with the sole of the shoe; looking at different types of shoe and the age and any physical degradation of the shoe (where different physicochemical interactions between different fungi and the shoe surface could occur); considering differences between operators and the pressure applied when walking; and varying the number of footsteps taken. In addition, the study can be broadened to other microorganisms.

Conclusion

The study presented in this article shows a clear advantage with the use of antimicrobial polymeric flooring (such as the Dycem range) compared with standard flooring as evaluated against a range of common fungi. A microbial reduction step is apparent both in terms of the pulling away of particles from shoes and as a consequence of ionic activity. On this basis, such flooring can contribute towards a facility contamination control strategy and to support other contamination reduction measures relating to cleanroom air control, personnel gowning, and regular cleaning and disinfection.

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