

METHODS OF PRODUCING AND DETERMINING PROPENSITY FOR BACTERIAL ADHESION TO PARTS FOR BIOPROCESSING SYSTEMS

FIELD

- 5 The present disclosure relates to a method of producing a part for use in a bioprocessing system, and a method of determining propensity for bacterial adhesion to a surface of a part for use in a bioprocessing system.

BACKGROUND

- 10 3D printing technology (also referred to as additive manufacturing (AM)) has been in existence since the 1980s when it was primarily used for rapid prototyping for product development within certain industries. The technological growth and possibility of mass production of the different technologies within AM have proven their potential to complement and even replace conventional manufacturing techniques. Some of the
15 advantages that AM offers to the bioprocessing industry are the possibility of increasing geometry complexity and reducing costs and material waste while requiring low manufacturing skills.

- Among the existing AM technologies, powder bed fusion (PBF) is the most developed
20 and mature platform able to provide models with different shapes and sizes by using powder-based materials. However, various technical and regulatory challenges prevent the implementation of PBF technologies in the bioprocessing field. In particular, technical aspects related to cleanability, sterility, surface finish and dimensions should be designed according to good engineering principles to minimize bacterial adhesion on
25 the surface of the components. More specifically, an inherent challenge in PBF lies in its "stair-stepping" layering approach, which can impact surface quality. Additionally, post-processing steps are necessary to smooth as-printed components and achieve surfaces similar to traditional manufacturing methods, limiting the applicability of PBF-manufactured components in the bioprocessing field.

- 30 A primary concern for additively manufactured parts for bioprocessing equipment is the control of biofilms during biopharmaceutical production. Apart from its health impact, biofilm formation has enormous economic consequences in different fields. For instance, in the biopharmaceutical field, bacterial adhesion, which leads to biofilm formation in the
35 interior of bioprocessing equipment, results in an economic loss of billions in revenue. At present, there is a lack of understanding of how 3D-printed surfaces interact with bacteria and how to prevent bacterial growth and reduce biofilm formation to meet the

high microbiological requirements on components for use in contact with biological systems.

5 Biofilms are three-dimensional microbial communities that are embedded within a self-produced extracellular matrix (ECM) and have the capacity to adhere to surfaces. As biofilms mature, the extracellular polymeric substance (EPS) matrix boosts cell adhesion and coherence, facilitating the accumulation of microbes on a surface and the creation of closely packed cell clusters. This leads to the formation of a well-structured and firmly attached biofilm. Consequently, once biofilms take hold, eradicating the bacteria within
10 or dislodging the biofilm from surfaces becomes an arduous task.

Examining the interaction between the surface of bioprocessing equipment and microbial organisms reveals numerous variables affecting early bacterial adhesion and biofilm formation. These variables include bacterial traits such as cell wall composition, mobility,
15 exposure duration, and initial bacterial concentration. Surface properties of the bioprocessing equipment also play a significant role, incorporating properties like surface charge density, wetting characteristics, roughness, topography, and stiffness. Particularly, the influence of surface texture and wettability on the initial adhesion of bacteria and the subsequent formation of biofilms has gained considerable attention,
20 highlighting their significant impact on the biofilm establishment process. However, there is not yet clear evidence how these surface properties impact bacterial surface adhesion.

There is thus a need for an improved method of validating the suitability of a 3D-printed part for use in a bioprocessing system. In particular, there is a need for a method that
25 provides a more thorough validation of whether a 3D-printed part is likely to result in biofilm formation. In addition, there is a need for an improved method of manufacturing 3D-printed parts to ensure their suitability for use in a bioprocessing system.

Hence the present invention, as defined by the appended claims, is provided.
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SUMMARY

This summary introduces concepts that are described in more detail in the detailed description. It should not be used to identify essential features of the claimed subject matter, nor to limit the scope of the claimed subject matter.
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According to a first aspect of the present disclosure, there is provided a method of producing a part for use in a bioprocessing system, the method comprising:

manufacturing the part using an additive manufacturing process; and postprocessing a surface of the part, wherein the surface is intended to be wetted in use; wherein the postprocessed surface has one or more of the following areal roughness parameter values that may be measured in accordance with ISO 25178-2:2022, wherein the one or
5 more areal roughness parameter values are measured for the surface after filtering the surface to remove surface features having long wavelengths (e.g. above about 55 μm , such as above 54 μm): a reduced areal height, S_{vk} , of less than about 5 μm ; an arithmetical mean height, S_a , of less than about 2.5 μm ; a areal void volume, V_{vv} , of less than about 0.5 $\mu\text{m}^3 \mu\text{m}^{-2}$; and/or a five-point pit height, S_{5v} , of less than about 15 μm .

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The method of the first aspect allows for the manufacture of additively manufactured parts with surfaces having low propensity for bacterial adhesion. In particular, by tailoring the postprocessing to include one or more of the areal roughness parameter values listed above, an additively manufactured part can be validated as having low propensity for
15 bacterial adhesion without the need for inefficient and time-consuming validation methods. Current processes for validating bacterial adhesion to additively manufactured parts involve destructive testing of a certain number of prototypes, leading to additional waste and reduced manufacturing efficiency. In particular, alternative methods for validating bacterial adhesion to additively manufactured parts would involve
20 contaminating a certain number of prototypes using specific bacteria, in order to establish the extent to which such bacteria adhered to the surfaces of the prototypes over time. Such methods are time-consuming, owing to the need for establishing bacterial adhesion over time (e.g. 24 hours). Such methods also involve manual work and involve the potential for additional contaminants to be introduced as a consequence of the manual
25 handling of the selected prototypes being tested. Such methods require the manufacture of additional parts that are to be tested and may require many parts to be tested in order to provide statistical validity. Each surface would also need to be measured using a profilometer or microscope in order for a determination to be made that the part has low propensity for bacterial adhesion.

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In contrast, the method of the first aspect allows for verification that an additively manufactured part produced using the method has low propensity for bacterial adhesion, by tailoring the postprocessing of the surfaces of the additively manufactured part in order to provide one or more areal surface roughness values that correspond to surface
35 roughness values of surfaces with low propensity for bacterial adhesion.

According to a second aspect of the present disclosure, there is provided an additively manufactured part for use in a bioprocessing system, wherein the additively manufactured part comprises a surface intended to be wetted in use, wherein the surface has one or more of the following areal roughness parameter values that may be measured in accordance with ISO 25178-2:2022, wherein the one or more areal roughness parameter values are measured for the surface after filtering the surface to remove surface features having long wavelengths (e.g. above 54 μm): a reduced dale height, S_{vk} , of less than about 5 μm ; an arithmetical mean height, S_a , of less than about 2.5 μm ; a dale void volume, V_{vv} , of less than about 0.5 $\mu\text{m}^3 \mu\text{m}^{-2}$; and/or a five-point pit height, S_{5v} , of less than about 15 μm .

According to a third aspect of the present disclosure, there is provided a method of classifying a surface of a part as being suitable for use in a bioprocessing system, the method comprising: manufacturing the part, wherein the part comprises a surface that is intended to be wetted in use; and one or more of: measuring a reduced dale height, S_{vk} , of the surface after filtering the surface to remove surface features having long wavelengths, and classifying the surface as being suitable for use in the bioprocessing system if the reduced dale height, S_{vk} , of the surface is less than about 5 μm ; measuring an arithmetical mean height, S_a , of the surface after filtering the surface to remove surface features having long wavelengths, and classifying the surface as being suitable for use in the bioprocessing system if the arithmetical mean height, S_a , of the surface is less than about 2.5 μm ; measuring a dale void volume, V_{vv} , of the surface after filtering the surface to remove surface features having long wavelengths, and classifying the surface as being suitable for use in the bioprocessing system if the dale void volume, V_{vv} , of the surface is less than about 0.5 $\mu\text{m}^3 \mu\text{m}^{-2}$; and measuring a five-point pit height, S_{5v} , of the surface after filtering the surface to remove surface features having long wavelengths, and classifying the surface as being suitable for use in the bioprocessing system if the five-point pit height, S_{5v} , of the surface is less than about 15 μm , for example.

The method of the third aspect allows for the determination of whether surfaces of parts (and in particular, surfaces of additively manufactured parts) are suitable for use in a bioprocessing system by virtue of having low propensity for bacterial adhesion. Current processes for determining the propensity for bacterial adhesion to additively manufactured parts involve destructive testing of a certain number of prototypes, leading to additional waste and reduced manufacturing efficiency, as explained above.

In contrast, the method of the third aspect allows for a determination of whether additively manufactured parts are suitable for use in a bioprocessing system by virtue of having low propensity for bacterial adhesion. The determination is based on measurement of one or more areal surface roughness values that have the strongest association with surfaces with low propensity for bacterial adhesion. This allows for validation of additively manufactured parts as being suitable for use in a bioprocessing system by virtue of having low propensity for bacterial adhesion in a more efficient manner.

BRIEF DESCRIPTION OF FIGURES

Specific embodiments are described below by way of example only and with reference to the accompanying drawings, in which:

FIG. 1 shows a flowchart of a method of producing of a part for use in a bioprocessing system.

FIG. 2 shows a flowchart of a method of classifying a surface of a part as being suitable for use in a bioprocessing system.

FIG. 3 shows a schematic diagram of a peg sample used for evaluating bacterial adhesion according to the present disclosure.

FIG. 4 shows a plot of static contact angles for sample surfaces comprising a surface produced by CNC milling, a surface produced by injection moulding, and surfaces produced by PBF that have been subjected to different postprocessing steps, according to a first example.

FIG. 5 shows a plot of developed interfacial area ratio versus scale for sample surfaces according to the first example.

FIG. 6 shows a plot of complexity versus scale for sample surfaces according to the first example.

FIG. 7 shows results of principal component analysis for sample surfaces according to the first example.

FIG. 8 shows variable importance of projection for roughness parameters of sample surfaces according to the first example.

FIGS. 9a to 9e show *E. coli* and *S. aureus* attachment and biofilm growth on sample surfaces according to the first example.

- 5 FIGS. 10a to 10l show field emission-scanning electron microscopy (FE-SEM) images of 48 h *Escherichia coli* biofilms on sample surfaces according to the first example classified as class I sample surfaces.

- 10 FIGS. 11a to 11q show field emission-scanning electron microscopy (FE-SEM) images of 4 h *Escherichia coli* biofilms on sample surfaces according to the first example classified as class II sample surfaces.

- 15 FIGS. 12a to 12i show field emission-scanning electron microscopy (FE-SEM) images of 48 h *Staphylococcus aureus* biofilms on sample surfaces according to the first example classified as class I sample surfaces.

- 20 FIGS. 13a to 13o show field emission-scanning electron microscopy (FE-SEM) images of 48 h *Staphylococcus aureus* biofilms on sample surfaces according to the first example classified as class II sample surfaces.

FIGS. 14a and 14b show *Escherichia coli* and *Staphylococcus aureus* attachment and biofilm growth on sample surfaces according to the first example.

- 25 FIGS. 15a to 15j show values of the ten most relevant roughness parameters obtained from the variable importance in projection plot in FIG. 8 for sample surfaces according to the first example.

DETAILED DESCRIPTION

- 30 Implementations of the present disclosure are explained below with particular reference to manufacturing and determining propensity for bacterial adhesion to surfaces of parts used in bioprocessing systems. It will be appreciated, however, that the methods described herein may also be used to manufacture and determine the propensity for bacterial adhesion to surfaces of parts used in other settings. Moreover, implementations of the present disclosure are explained below with particular reference
35 to determining propensity for bacterial adhesion to surfaces of additively manufactured parts. It will further be appreciated, however, that the methods described herein may

also be used to determine the propensity for bacterial adhesion to surfaces of parts manufactured using other manufacturing techniques.

FIG. 1 is a flowchart of a method 100 of producing a part.

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At 110, the part is manufactured using an additive manufacturing process. As one example, the part may be manufactured using an additive manufacturing process such as powder bed fusion (PBF), laser powder bed fusion (LPBF) or electron beam melting (EBM). In particular, a PBF process may include selective layer sintering (SLS). In one example, the manufactured part is intended for use in a bioprocessing system and comprises a surface that is intended to be wetted when the part is used in the bioprocessing system. More particularly, the surface may include a plurality of boundaries between adjacent layers of the part.

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At 120, the surface of the part is postprocessed to provide one or more areal roughness parameter values within a certain range (e.g. areal roughness parameters defined in ISO 25178-2:2022, as explained below). In one example, postprocessing the surface includes chemical vapour smoothing of the surface.

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In one example, the one or more areal roughness parameters can be measured by analysing images of the surface obtained using a confocal laser scanning microscope (CLSM), such as a VK-X1000 confocal laser scanning microscope available from Keyence Corporation of Osaka, Japan. The areal roughness parameters relate to a scale-limited surface, so measurement of the one or more areal roughness parameters firstly involves filtering one or more CLSM images of the surface of the part according to an appropriate scale range. In the examples described herein, features of the surface are filtered to remove surface features having long wavelengths, such as those that are not relevant for microbial/bacterial adhesion (e.g. above 54 μm , etc.). It will be appreciated, however, that the choice of filter affects the values of the areal roughness parameters. Accordingly, implementing a different filter may alter the values of the areal roughness parameters set out below.

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The postprocessing of the part at 120 may include postprocessing the surface to provide one or more surface wettability values (e.g. static contact angle values) within a certain range.

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The values of the areal roughness parameters provided by the postprocessing of the surface at 120 relate to areal roughness parameters that have been identified as providing a correlation with low propensity for bacterial adhesion to a surface. As described in more detail in the example below, these areal roughness parameters have

5 been determined by comparing two classes of surface – a first class (class I), comprising surfaces produced by CNC milling, injection moulding and PBF with chemical vapour smoothing (VS) postprocessing; and a second class (class II), comprising surfaces produced by PBF as-printed and surfaces produced by PBF and postprocessed using tumble surface finishing. The first class of surfaces are representative of surfaces used

10 in the bioprocessing industry today and that are proven not to cause significant bacterial adhesion and biofilm formation. The determination of the relevant one or more roughness parameters does not form part of the method 100. Instead, it will be appreciated based on the discussion below that a comparison of first and second classes to identify the most significant roughness parameters that describe the differences

15 between the classes yields the roughness parameters of greatest relevance to low propensity for bacterial adhesion. The postprocessing of the surface at 120 is carried out in order to provide one or more areal roughness parameter values for the roughness parameters of greatest relevance.

20 As described further in the example below, class I surfaces and class II surfaces were compared in order to determine areal roughness parameters with the highest degree of divergence between the two classes of surfaces.

Standardised areal roughness parameters are defined in ISO 25178. In particular,

25 roughness parameters that are used to describe surface topography are defined in ISO 25178-2:2022. Most existing studies on the influence of surface roughness on biofilm formation focus only on the arithmetical mean deviations, Ra and Sa, which describe respectively the average height from a 2-dimensional profile and a 3-dimensional surface. In contrast, the present disclosure involves consideration of a number of

30 different areal roughness parameters, which have been found to be of greater relevance to a surface's propensity for bacterial adhesion than solely the arithmetical mean deviations, Ra and Sa.

Specifically, the surface of the part may be postprocessed at 120 to provide one or more

35 areal roughness parameter values relating to one or more of the following 10 areal roughness parameters measured in accordance with ISO 25178-2:2022: material ratio of the dales, Smrk2 (%); reduced dale height, Sv_k (μm); dale void volume, V_{vv} (μm³ μm⁻²);

²); maximum pit depth, S_{vkx} (μm); arithmetical mean height, S_a (μm); maximum pit depth, S_v (μm); root mean square height, S_q (μm); five-point pit height, S_{5v} (μm); material ratio height difference, S_{dc} (μm); and maximum height, S_z (μm). These areal roughness parameters are identified in the example below as being the parameters of greatest significance based on a comparison between classes of surface with a high degree of difference in propensity for bacterial adhesion.

As explained above, the values of the areal roughness parameters listed above are dependent on the scale over which the surface is limited. The surface of the part may be postprocessed at 120 to provide one or more areal roughness parameter values within the ranges set out in the following paragraphs. The areal roughness parameter values listed below result from filtering the surface features to remove surface features having long wavelengths (e.g. above 54 μm).

The postprocessing of the surface at 120 may provide one or more of:

- (i) a reduced areal height, S_{vk} , of less than 5 μm , preferably less than 4 μm , more preferably less than 3 μm , more preferably less than 2 μm , and most preferably less than 1 μm ;
- (ii) an arithmetical mean height, S_a , of less than 2.5 μm , preferably less than 2 μm , more preferably less than 1.5 μm , and most preferably less than 1 μm ;
- (iii) a areal void volume, V_{vv} , of less than 0.5 $\mu\text{m}^3 \mu\text{m}^{-2}$; more preferably less than 0.4 $\mu\text{m}^3 \mu\text{m}^{-2}$, more preferably less than 0.3 $\mu\text{m}^3 \mu\text{m}^{-2}$, more preferably less than 0.2 $\mu\text{m}^3 \mu\text{m}^{-2}$, and most preferably less than 0.1 $\mu\text{m}^3 \mu\text{m}^{-2}$;
- (iv) a five-point pit height, S_{5v} , of less than 15 μm ; preferably less than 12.5 μm , more preferably less than 10 μm , more preferably less than 7.5 μm , and most preferably less than 5 μm ;
- (v) a maximum pit depth, S_{vkx} , of less than 20 μm , preferably less than 15 μm , more preferably less than 10 μm , and most preferably less than 5 μm ;
- (vi) a material ratio of the areal, S_{mrk2} , of between 85% and 95%, preferably between 86% and 93%, and more preferably between 87% and 91%;

- (vii) a root mean square height, S_q , of less than 4 μm , preferably less than 3.5 μm , more preferably less than 3 μm , more preferably less than 2.5 μm , and most preferably less than 2 μm ;
- 5 (viii) a maximum height, S_z , of less than 20 μm , more preferably less than 17.5 μm , more preferably less than 15 μm , and most preferably less than 12.5 μm ;
- (ix) a maximum pit depth, S_v , of less than 20 μm , preferably less than 15 μm , more preferably less than 10 μm , and most preferably less than 5 μm ; and
- 10 (x) a material ratio height difference, S_{dc} , of less than 7 μm , preferably less than 6 μm , more preferably less than 5 μm , more preferably less than 4 μm , and most preferably less than 3 μm .
- 15 The postprocessed surface may have more than one of the areal roughness parameter values listed at (i) to (x). In one example, the postprocessed surface may have all of the areal roughness parameter values listed at (i) to (x).
- In light of the strong correlation of these parameters with low propensity for bacterial
- 20 adhesion, a surface of a manufactured part is likely to have low propensity for bacterial adhesion (and therefore a low propensity for biofilm development) if the values of one or more of the above roughness parameters are similar to corresponding values for class I surfaces. Therefore, postprocessing the surface to include one or more of these values is likely to result in the surface having low propensity for bacterial adhesion.
- 25 As explained above, the postprocessing of the part at 120 may include postprocessing the surface to provide one or more surface wettability values (e.g. static contact angle values) within a certain range. Specifically, the postprocessing of the surface at 120 may provide a static contact angle of the postprocessed surface is between 45 and 105
- 30 degrees, preferably between 50 and 100 degrees, more preferably between 55 and 95 degrees, and most preferably between 60 and 90 degrees.

Once a part has been manufactured using the method 100, then, depending on the postprocessing carried out at 120, the surface of the part will have one or more of the

35 areal roughness parameter values listed at (i) to (x), and optionally the surface wettability values listed above.

The part manufactured using the method 100 is an additively manufactured part. The additively manufactured part may be formed of a material such as polymer (e.g. polypropylene). Moreover, the additively manufactured part may be formed using layers of powder material that have been bonded together using heat using a process such as PBF (e.g. SLS). This means that the additively manufactured part may include a first region (or a first plurality of regions) in which the material has not been melted, and a second region (or a second plurality of regions) in which the material has been melted and has resolidified.

10 The method 100 allows for the manufacture of additively manufactured parts with surfaces having low propensity for bacterial adhesion. In particular, by tailoring the postprocessing applied at 120 to include one or more of the areal roughness parameter values listed above, an additively manufactured part can be validated as having low propensity for bacterial adhesion without the need for inefficient and time-consuming validation methods. Current processes for validating bacterial adhesion to additively manufactured parts involve destructive testing of a certain number of prototypes, leading to additional waste and reduced manufacturing efficiency. In particular, alternative methods for validating bacterial adhesion to additively manufactured parts would involve contaminating a certain number of prototypes using specific bacteria, in order to establish the extent to which such bacteria adhered to the surfaces of the prototypes over time. Such methods are time-consuming, owing to the need for establishing bacterial adhesion over time (e.g. 24 hours). Such methods also involve manual work and involve the potential for additional contaminants to be introduced as a consequence of the manual handling of the selected prototypes being tested. Such methods require the manufacture of additional parts that are to be tested and may require many parts to be tested in order to provide statistical validity. Each surface would also need to be measured using a profilometer or microscope in order for a determination to be made that the part has low propensity for bacterial adhesion.

30 In contrast, the method 100 allows for verification that an additively manufactured part produced using the method 100 has low propensity for bacterial adhesion, by tailoring the postprocessing of the surfaces of the additively manufactured part in order to provide one or more areal surface roughness values that correspond to surface roughness values of surfaces with low propensity for bacterial adhesion.

35 In light of the low propensity for bacterial adhesion to the surface of the part, the surface of the part may be considered suitable for use in a bioprocessing system. The surface

of the part may be classified as being suitable for use in the bioprocessing system despite having an arithmetic mean height (line roughness), Ra, that exceeds a threshold value typically used for determining whether part surfaces are suitable for use in the bioprocessing system. For example, the surface of the part may be classified as being
5 suitable for use in the bioprocessing system if it has one or more of the areal roughness parameter values listed at (i) to (x), even though an Ra value of the surface is greater than 0.5 μm . The value of 0.5 μm is a threshold Ra value currently used in industry to determine whether part surfaces are suitable for use in a bioprocessing system.

10 FIG. 2 is a flowchart of a method 200 of classifying a surface of a part as being suitable for use in a bioprocessing system.

At 210, a part is manufactured. The part comprises a surface that is intended to be wetted in use. The part may be manufactured using an additive manufacturing process
15 such as powder bed fusion (PBF). Manufacturing the part at 210 may also comprise postprocessing the part.

At 220, one or more areal roughness parameters of the surface are measured (e.g. from CLSM images as described with reference to method 100). The method may include
20 filtering the CLSM images using an appropriate scale range (such as by filtering the images to remove surface features having wavelengths above 54 μm).

At 230, a determination is made as to whether the surface has a low propensity for bacterial adhesion, based on the one or more areal roughness parameters measured at
25 220.

If, at 230, it is determined that the surface has a low propensity for bacterial adhesion, then the method 200 may comprise classifying, at 240, the part as suitable for use in the bioprocessing system.
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If, at 230, it is determined that the surface does not have a low propensity for bacterial adhesion, then the method 200 may comprise modifying, at 250, a design process relating to the design of the surface. Alternatively, or additionally, if it is determined at 230 that the surface does not have a low propensity for bacterial adhesion, then the
35 method may comprise modifying, at 260, a manufacturing process relating to the manufacture of the surface. Modifying the manufacturing process may include incorporating one or more postprocessing operations (such as chemical vapour

smoothing) into the manufacturing process, and/or modifying one or more postprocessing operations carried out at 210.

5 The one or more areal roughness parameters measured at 220 may include one or more of: material ratio of the dales, $Smrk2$ (%); reduced dale height, Svk (μm); dale void volume, Vvv ($\mu m^3 \mu m^{-2}$); maximum pit depth, $Svkx$ (μm); arithmetical mean height, Sa (μm); maximum pit depth, Sv (μm); root mean square height, Sq (μm); five-point pit height, $S5v$ (μm); material ratio height difference, Sdc (μm); and maximum height, Sz (μm).

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It may be determined at 230 that the surface has a low propensity for bacterial adhesion if, after filtering the surface features to remove surface features having wavelengths above $54 \mu m$, one or more of the following conditions are satisfied:

15 (i) a reduced dale height, Svk , of the filtered surface is less than $5 \mu m$, preferably less than $4 \mu m$, more preferably less than $3 \mu m$, more preferably less than $2 \mu m$, and most preferably less than $1 \mu m$;

(ii) an arithmetical mean height, Sa , of the filtered surface is less than $2.5 \mu m$, preferably less than $2 \mu m$, more preferably less than $1.5 \mu m$, and most preferably less than $1 \mu m$;

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(iii) a dale void volume, Vvv , of the filtered surface is less than $0.5 \mu m^3 \mu m^{-2}$; more preferably less than $0.4 \mu m^3 \mu m^{-2}$, more preferably less than $0.3 \mu m^3 \mu m^{-2}$, more preferably less than $0.2 \mu m^3 \mu m^{-2}$, and most preferably less than $0.1 \mu m^3 \mu m^{-2}$;

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(iv) a five-point pit height, $S5v$, of the filtered surface is less than $15 \mu m$; preferably less than $12.5 \mu m$, more preferably less than $10 \mu m$, more preferably less than $7.5 \mu m$, and most preferably less than $5 \mu m$;

30 (v) a maximum pit depth, $Svkx$, of the filtered surface is less than $20 \mu m$, preferably less than $15 \mu m$, more preferably less than $10 \mu m$, and most preferably less than $5 \mu m$;

(vi) a material ratio of the dales, $Smrk2$, of the filtered surface is between 85% and 95%, preferably between 86% and 93%, and more preferably between 87% and 91%;

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- (vii) a root mean square height, S_q , of the filtered surface is less than 4 μm , preferably less than 3.5 μm , more preferably less than 3 μm , more preferably less than 2.5 μm , and most preferably less than 2 μm ;
- 5 (viii) a maximum height, S_z , of the filtered surface is less than 20 μm , more preferably less than 17.5 μm , more preferably less than 15 μm , and most preferably less than 12.5 μm ;
- (ix) a maximum pit depth, S_v , of the filtered surface is less than 20 μm , preferably less than 15 μm , more preferably less than 10 μm , and most preferably less than 5 μm ; and
- 10 (x) a material ratio height difference, S_{dc} , of the filtered surface is less than 7 μm , preferably less than 6 μm , more preferably less than 5 μm , more preferably less than 4 μm , and most preferably less than 3 μm .
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- It may be determined at 230 that the surface has a low propensity for bacterial adhesion if, after filtering the surface features to remove surface features having wavelengths above 54 μm , more than one of the conditions listed at (i) to (x) is satisfied. In one example, the surface may be determined as having a low propensity for bacterial
- 20 adhesion at 230 if all of the conditions listed at (i) to (x) are satisfied. As described above, the surface may have an arithmetical mean height, R_a , value, greater than a threshold value typically used for classifying part surfaces as suitable for use in a bioprocessing system (e.g. greater than 0.5 μm).
- 25 The method 200 allows for the determination of whether surfaces of parts (and in particular, surfaces of additively manufactured parts) are suitable for use in a bioprocessing system by virtue of having low propensity for bacterial adhesion. Current processes for determining the propensity for bacterial adhesion to additively
- 30 manufactured parts involve destructive testing of a certain number of prototypes, leading to additional waste and reduced manufacturing efficiency. In particular, alternative methods for determining the propensity for bacterial adhesion to additively manufactured parts would involve contaminating a certain number of prototypes using specific bacteria, in order to establish the extent to which such bacteria adhered to the surfaces of the
- 35 prototypes over time. Such methods are time-consuming, owing to the need for establishing bacterial adhesion over time (e.g. 24 hours). Such methods also involve manual work and involve the potential for additional contaminants to be introduced as a consequence of the manual handling of the selected prototypes being tested. Such

methods require the manufacture of additional parts that are to be tested and may require many parts to be tested in order to provide statistical validity. Each surface would also need to be measured using a profilometer or microscope in order for a determination to be made that the part has low bacterial adhesion.

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In contrast, the method 200 allows for a determination of whether additively manufactured parts are suitable for use in a bioprocessing system by virtue of having low propensity for bacterial adhesion. The determination is based on measurement of one or more areal surface roughness values that have the strongest association with surfaces with low propensity for bacterial adhesion. This allows for validation of additively manufactured parts as being suitable for use in a bioprocessing system by virtue of having low propensity for bacterial adhesion in a more efficient manner.

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Example

This example describes a comparison of two classes of surfaces that are identified as having a high degree of difference in terms of their characterisation using roughness parameter values. Relevant roughness parameters with the highest degree of difference between a first class of surfaces (with a low propensity for biofilm development) and a second class of surfaces (with higher propensity for biofilm development) are identified. Postprocessing a surface to provide surface roughness parameters that correspond to the roughness parameter values of the first class of surfaces can therefore be expected to yield a surface with a low propensity for biofilm development.

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In order to study bacterial attachment and biofilm development, individual pegs 300 were manufactured. As shown in FIG. 3, each peg 300 had a length of 17.8 mm, a top diameter of 4.4 mm and a surface area of 45 mm². As reported in Zaborskyte et al, "Modular 3D-Printed Peg Biofilm Device for Flexible Setup of Surface-Related Biofilm Studies", Frontiers in Cellular and Infection Microbiology 11 (2022), the entire contents of which are herein incorporated by reference, biofilm growth primarily occurs at a depth of 4 mm from the peg's base at the air-liquid interface.

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Reference pegs were fabricated through two different methods: axis computer numerical control (CNC) milling manufacturing using a VF-8 milling machine available from Haas Automation Inc. of Oxnard, CA, USA for PP_CNC samples, and injection moulding using an ES 80/50 HL machine available from Engel of Schwertberg, Austria for PP_IM samples. Additively manufactured peg samples were produced by laser-based powder bed fusion (PBF-LB/P, or simply referred to herein as "PBF"). The layer thickness was

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0.1 mm with a printing tolerance of $\pm 0.3\%$. The sample orientation during the printing process was horizontal (0°). From FIG. 3, it will be appreciated that for additively manufactured peg samples, the outer peg surface includes a plurality of boundaries between adjacent layers.

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In this example, three grades of polypropylene (PP) were used as received. These three grades were: medical-grade isotactic PP homopolymer (used for reference samples produced using CNC milling, and identified herein as PP_CNC), medical grade ethylene-propylene copolymer (used for reference samples produced using injection moulding,
10 and identified herein as PP_IM), and industrial-grade ethylene-propylene copolymer (used for samples produced using PBF, and identified herein as PP_PBF). Each grade exhibited different characteristics in terms of degree of crystallinity (X_c) and melting point (T_m). The PP grade used for PP_CNC samples had an X_c of 43% and a T_m of 164°C ; the PP grade used for PP_IM samples had an X_c of 36% and a T_m of 151°C ; and the
15 PP grade used for PP_PBF samples had an X_c of 30% and a T_m of 146°C . PP grades copolymerised with ethylene were used for the PP_IM and PP_PBF samples to ensure dimensional stability during processing by reducing the X_c value (as higher X_c values can lead to increased material shrinking, which can negatively impact dimensional stability during processing).

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Different surface textures of the PP_PBF samples were obtained using six different types of postprocessing: (i) no postprocessing (i.e. as-printed PBF samples), identified herein as or PEG_PBF_0h; (ii) PBF samples tumbled for 5 h, identified herein as PEG_PBF_5h; (iii) PBF samples tumbled for 10 h, identified herein as PEG_PBF_10h; (iv) PBF samples
25 tumbled for 15 h, identified herein as PEG_PBF_15h; (v) PBF samples tumbled for 13 h and subsequently polished for 3 h, identified herein as PEG_PBF_13h_3P; and (vi) PBF samples postprocessed using chemical vapour smoothing (CVS), identified herein as PEG_PBF_VS. Reference peg samples are identified herein as PEG_CNC (for CNC milled samples) and PEG_IM (for injection moulded samples).

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Postprocessing approaches (ii) to (v) involved tumble surface finishing using abrasive polishing media with coarse grit, matte finish and medium media attrition rate. Due to the small size of the samples, the as-printed pegs were suspended on a porous bucket filled with the surface finishing media. This resulted in a surface finish characterised by
35 a matte appearance. Postprocessing approach (v) involved a subsequent operation using polishing media with fine grit, semi-gloss finish and very low media attrition rate, resulting in a surface finish with a semi-glossy appearance. For postprocessing

approaches (ii) to (v), the detergent concentration and dose was constant for each experiment, and it did not provide chemical energy to the surface finish process.

5 Postprocessing approach (vi) involved the use of a highly volatile solvent that is vaporised and injected onto the polymer surface of the sample. The solvent partially dissolves the polymer surface, resulting in the dissolved polymer flowing into surface features such as pores, holes and crevices. This flow of dissolved polymer diminishes the surface texture and increases smoothness after the solvent evaporates from the surface.

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FIG. 4 is a plot of static contact angles for the samples under consideration. Static contact angles were measured by depositing distilled water droplets (e.g. Milli-Q (RTM) water provided by a Milli-Q (RTM) water purification system available from Merck KGaA of Darmstadt, Germany) on a Theta Lite optical tensiometer available from Biolin Scientific of Gothenburg, Sweden. Calculation of the contact angle results were measured using OneAttension software (Version 1.8) available from Biolin Scientific of Gothenburg, Sweden. A 2 μ L droplet size was used on the curved surface of the pegs. The values shown in FIG. 4 are the averages of three measurements.

20 As shown in FIG. 4, reference samples (PEG_CNC and PEG_IM) and vapour smoothed samples (PEG_PBF_VS) exhibited the most hydrophilic behaviour, with contact angle values of $60^\circ \pm 2^\circ$, $82^\circ \pm 3^\circ$, and $88^\circ \pm 4^\circ$, respectively. The as-printed and surface-tumbled surfaces, especially those postprocessed with the abrasive media, displayed a higher degree of hydrophobicity, with contact angle values ranging from $118^\circ \pm 3^\circ$ (PEG_PBF_0h) to $114^\circ \pm 10^\circ$ (PEG_PBF_15h). The effect of using the polishing media decreased slightly the static contact angle to $111^\circ \pm 4^\circ$. The increased surface roughness of as-printed and tumbled pegs contributed to enhanced hydrophobicity, primarily due to the trapped air and increased surface area. In contrast, vapor smoothing yielded smoother surfaces, making the pegs more hydrophilic and yielding similar contact angle values to the reference samples.

30 Preprocessed images derived from surface topographies measured by CLSM were used for scale-sensitive fractal analysis. Surface texture of the samples was measured with a VK-X1000 confocal laser scanning microscope available from Keyence Corporation of Osaka, Japan. Each surface image had an area of $275.3 \times 206.5 \mu\text{m}^2$ (2048×1536 pixels). Four images per specimen and three specimens per sample resulted in a total of 12 images which were taken at 50x magnification and lateral point spacing of 0.134

μm , using the Multi-File Analyser software (Version 2.1.2.17). During image acquisition, each specimen was rotated around its axis 90° to capture the surface texture characteristics of the full peg sample.

- 5 Image preprocessing involved the following steps: (i) topography channel extraction (i.e. extracting the relevant channel (topography) from the three channels of CLSM data), from which outliers were removed, and missing points (if any) were filled in; (ii) surface denoising by application of a spatial filter with a window size of 5×5 pixels; (iii) a form removal operation to remove large (i.e. high wavelength) surface features from the
10 surface; and (iv) levelling of the surface through the least-squares plane method (e.g. to reduce any convexity or concavity of the surface).

Scale-sensitive fractal analysis was then carried out on the preprocessed images. Scale-sensitive fractal analysis uses area-scale relations which have been developed from
15 fractal geometry and is based on the assumption that the observed area is dependent on the scale of observation. The “relative area” parameter can be calculated for different scales of observation and used for characterization. Relative area describes how much surface area that is added by the roughness of the surface texture. A nominally smooth and flat surface has a relative area of 1. If a texture is added, the relative area increases,
20 and the increase will be different depending on the scale of observation. The area-scale method is further described in the ISO 25178-2 standard. In the present disclosure, a version of the area-scale method is employed that calculates the areal field parameter Sdr (developed area ratio) at different scales. Similarly to relative area, Sdr is a measure of how much surface area is added by the roughness of the surface texture, but it is
25 normally only calculated at the sampling scale. Here, it is calculated at different scales to provide ‘Sdr area ratio’ as a function of scale. Complexity is the measure of the slope of the Sdr area plot at each scale. This measure captures the intricacy of the peg surface at various scales of observation, thereby facilitating an understanding of the surface’s complexity. In this example, scales below $150 \mu\text{m}^2$ are of interest, on the assumption
30 that colonies of a few hundred bacterial cells will colonise a triangular area approximate to this threshold within a few hours. The triangular area of $150 \mu\text{m}^2$ was based on a length of $20 \mu\text{m}$ and a height of $15 \mu\text{m}$, representing a cluster of approximately 100 *E. coli* cells that had adhered to a surface following a 6-hour incubation period. Accordingly, the scales of observation are similar to the size of standard microorganisms such as
35 bacteria that exist on the bioprocessing equipment.

FIG. 5 shows a plot of the Sdr area ratio (developed interfacial area ratio) versus scale for each sample. At a scale of $0.1 \mu\text{m}^2$, the Sdr area ratio was highest for the PEG_PBF_0h samples, followed by the PEG_PBF_5h samples, the PEG_PBF_10h samples, the PEG_PBF_15h samples, the PEG_PBF_13h_3P samples, the PEG_IM samples, the PEG_CNC samples, and finally the PEG_PBF_VS samples. The threshold of $150 \mu\text{m}^2$ is indicated in FIG. 5. Values close to one were observed for PEG_CNC, PEG_IM and PEG_PBF_VS samples, indicating that the surfaces are smooth. As explained above, the maximum Sdr area ratio is observed for PEG_PBF_0h samples, with values ranging from 2.2 to 1.5 for scales from 0.01 to $150 \mu\text{m}^2$, respectively. FIG. 5 also shows the effect of tumble surface finishing on the samples, with progressively lower Sdr area ratios being associated with longer tumble surface finishing times.

FIG. 6 shows a plot of the complexity versus scale for each sample. At a scale of $10 \mu\text{m}^2$, the complexity was highest for the PEG_PBF_0h samples, followed by the PEG_PBF_5h samples, the PEG_PBF_10h samples, the PEG_PBF_15h samples, the PEG_PBF_13h_3P samples, the PEG_IM samples, the PEG_CNC samples, and finally the PEG_PBF_VS samples. FIG. 6 helps distinguish the scales at which various manufacturing processes yield distinct surface features on the peg surfaces. As-printed (PEG_PBF_0h) and tumbled pegs (PEG_PBF_5h, PEG_PBF_10h, PEG_PBF_15h) exhibited the highest complexity variation, ranging from values close to zero at low scales to values exceeding 80 at scales of $150 \mu\text{m}^2$. Conversely, CNC (PEG_CNC) and vapour smoothed (PEG_PBF_VS) samples showed a constant trend at both low and high scales. For IM samples (PEG_IM), complexity remained low at low scales, but increased at higher scales, likely due to the presence of welding lines in the surface topography.

Subsequent to the identification of a relevant scale threshold of $150 \mu\text{m}^2$, a length of $18 \mu\text{m}$ was calculated using the equation $L \approx \sqrt{2 \cdot a}$. For the final step of analysis, the surface was filtered to remove surface features having wavelengths above $54 \mu\text{m}$ (e.g. by using a high-pass robust Gaussian filter with an order of 1 and a nesting index of $54 \mu\text{m}$). This filter was implemented to achieve S-L filtered surfaces for parameter evaluation. The determination of this filter range involved considering a cutoff wavelength of three times the length determined earlier, which has been identified in the literature as providing good results. Finally, according to the ISO-25178-2:2022 standard, a comprehensive set of 85 areal roughness parameters was calculated. Image preprocessing, area-scale analysis, areal roughness parameter calculation and 3D-image surface filtering were performed using MountainsLab (RTM) Premium surface analysis software (Version 9.3.10281) available from Digital Surf of Besançon, France.

Principal component analysis (PCA) was implemented to address the high dimensionality of the initial dataset containing the roughness parameters for each sample, with the aim of retaining its most critical information. By employing PCA statistical tools, the surface roughness information coming from the different manufacturing processes was condensed in a set of three principal components (PC), facilitating a clearer visualisation and interpretation of the complex data patterns. As shown in FIG. 7, the score plot representation of the first ($t[1]$) and the third principal component ($t[3]$) indicated similarities between PEG_CNC samples (green circles – all located between -2.5 and -7.5 on the $t[1]$ axis and between -3 and 4 on the $t[3]$ axis), PEG_IM samples (grey circles – all located between -2.5 and -12.5 on the $t[1]$ axis and between -4 and 4 on the $t[3]$ axis) and PEG_PBF_VS samples (pink triangles – all located between -5 and -12.5 on the $t[1]$ axis and between -4 and 2 on the $t[3]$ axis), while the as-printed and tumbled samples were grouped with high dispersity on the right side of the graph (where: PEG_PBF_0h samples are shown using maroon triangles located between 0 and 10 on the $t[1]$ axis and between -5 and 2 on the $t[3]$ axis; PEG_PBF_5h samples are shown using orange triangles located between 2.5 and 15 on the $t[1]$ axis and between -8 and 6 on the $t[3]$ axis; PEG_PBF_10h samples are shown using yellow triangles located between 0 and 12.5 on the $t[1]$ axis and between -3 and 4 on the $t[3]$ axis; PEG_PBF_15h samples are shown using purple triangles located between 0 and 10 on the $t[1]$ axis and between -3 and 5 on the $t[3]$ axis; PEG_PBF_13P3 samples are shown using blue triangles located between -2.5 and 5 on the $t[1]$ axis and between -2 and 5 on the $t[3]$ axis). Using a confidence ellipse calculated based on Hotelling's T^2 with a significance level of 0.05, three outliers were found for the PEG_PBF_5h samples (orange triangles).

Using this information, two different classes were identified to determine the most relevant surface roughness parameters. The first class encompassed the reference samples (PEG_CNC and PEG_IM) and the vapor smoothed surface samples (PEG_PBF_VS), while the second class included the rest of the samples (including all tumble surface finished samples). By implementing partial least squares discriminant analysis (PLS-DA), the variable importance of projection (VIP) was plotted, showing the most significant roughness families and parameters that should be used to describe the differences between both classes (FIG. 8). From the calculation of 85 roughness parameters, ten were selected as the most interesting for further implementation to describe surface differences between the two classes. Functional (stratified surfaces) and height parameters were the families with the highest importance in the ten selected

parameters. The information provided by the parameters in the ten selected parameters are mainly related to the description of the dales of the surface. As shown in FIG. 8, the ten roughness parameters corresponding to greatest surface differences between the two classes were: material ratio of the dales, Smrk2 (%); reduced dale height, Svk (μm);
 5 dale void volume, Vvv ($\mu\text{m}^3 \mu\text{m}^{-2}$); maximum pit depth, Svkx (μm); arithmetical mean height, Sa (μm); maximum pit depth, Sv (μm); root mean square height, Sq (μm); five-point pit height, S5v (μm); material ratio height difference, Sdc (μm); and maximum height, Sz (μm).

10 In order to investigate bacterial adhesion, two bacterial models were employed. These were *Escherichia coli* (*E. coli*) CFT073 (DA47112) and *Staphylococcus aureus* (*S. aureus*) subsp. *aureus* Rosenbach (DA78720). These are two standard bacterial models extensively employed in the bioprocessing industry. These Gram negative and Gram positive microorganisms are particularly relevant as representatives, as they are
 15 recommended by regulatory authorities for assessing and controlling bioburden impact in biopharmaceutical production.

Bacterial cultures were grown in liquid and solid media during the experimental procedures. The liquid media employed were Luria-Bertani Broth (LB) (Sigma Aldrich,
 20 USA), and M9 media with 0.2% of glucose. M9 media consisted of 0.1 mM CaCl_2 , 1 mM MgSO_4 , 1x M9 salts (Sigma Aldrich, USA) and 0.2% (w/v) glucose. Solid media consisted of LB agar, high salt (LA) (Sigma Aldrich, USA) and Mueller-Hinton (MH) agar (Beckton Dickinson, USA). Overnight (O/N) liquid cultures (1 ml media in a 10 ml tube)
 25 were initiated from a single colony and incubated at 37°C under continuous shaking at 180 rpm, whereas cultures employed in the biofilm experiments were cultivated at 37°C without shaking. Agar plates harbouring the bacterial colonies were incubated without shaking at 37°C or 30°C.

Biofilms were cultivated on the surface of manufactured pegs utilising the FlexiPeg
 30 biofilm device identified in Zaborskyte et al, "Modular 3D-Printed Peg Biofilm Device for Flexible Setup of Surface-Related Biofilm Studies", *Frontiers in Cellular and Infection Microbiology* 11 (2022). Inoculation was performed in microtiter 96-well plate with flat bottom wells (using a Nunc (RTM) 96-well plate available from Thermo Fisher Scientific of Waltham, MA, USA), with 200 μl of diluted O/N bacterial cultures. For experiments
 35 with LB media or M9 media, O/N cultures were diluted 100,000-fold (approx. $2-4 \times 10^4$ bacteria per well) in the respective media before inoculating the well. For experiments in phosphate-buffered saline (PBS) the O/N cultures were diluted only 10,000-fold

(approx. $2-4 \times 10^5$ bacteria per well) in the PBS. The wells were covered with sterilized (autoclaved) lids with inserted pegs and the plate lids were put in plastic boxes to prevent media evaporation. Boxes were put in 37°C incubators without shaking for a set amount of time before harvesting the biofilm from the pegs. Pegs in LB or M9 media were grown for 4 h or 48 h (media was changed after 24 h), while pegs in PBS were grown for 4 h, 6 h, 16 h or 24 h. After removing the device from the incubator all pegs were washed in PBS by dipping them in a new microtiter plate (flat bottom) containing 240 µl of PBS/well for 1 minute. The washing step was repeated 3 times with changes of PBS in between. Washed pegs were pushed (with sterile tweezers) into glass tubes containing 600 µl of PBS and vortexed (Vortex-Genie 2T, 230V available from Scientific Industries, Inc. of Bohemia, NY, USA) for 2 minutes to disperse all bacterial cells attached to the pegs. To measure the number of bacteria grown on pegs, the extracted bacteria in PBS were diluted 10, 100, 1,000, 10,000, 100,000 and 1,000,000x, and 5 µl drops from every dilution were transferred onto MH agar plates (LA, Sigma-Aldrich), dried and grown at 30°C overnight. If low colony forming units (CFU) numbers were expected, 50 µl of undiluted sample was plated on half of a plate with a sterile inoculation loop and grown at 37°C overnight. Formed colonies were then counted, CFU/peg calculated and plotted in graphs.

Crystal violet staining (CVS) was used for quantification of biofilm biomass. To achieve this, biofilms were grown in LB medium for 4 h, and 48 h with a media change after 24 h. Additionally, biofilms were also grown in PBS for 24 h, following the methodology described above. After incubating at 37°C, the pegs were thoroughly washed three times by transferring the peg lid to a 96-well plate containing 240 µl of PBS. Lids with washed pegs were turned upside down, transferred onto a tissue paper and dried for about 30 minutes at 37°C. To stain the dried pegs, they were inserted into microtiter wells containing 250 µl of 0.05% (w/v) CV solution and left to incubate for 15 minutes at room temperature. After the incubation period, the CV solution was discarded, and the pegs were washed three times for 1 minute each in 250 µl of PBS. Washed pegs were air dried for 10 minutes and visually inspected for presence of biofilm. To de-stain the pegs, they were transferred into a new microtiter plate with 200 µl of 10% (v/v) acetic acid. Absorbance of wells containing acetic acid with CV from pegs was measured at 540 nm in a microplate photometer (Multiskan (RTM) FC Microplate Photometer, available from Thermo Fisher Scientific of Waltham, MA, USA).

Eight biological replicates were conducted, and for each replicate, the average absorbance value of control pegs grown without any bacteria under identical media and

conditions was determined. These control pegs were subjected to the same staining, solubilization, and measurement process as the biological samples. To account for any background signal or non-specific absorbance, the average absorbance value of the control pegs was subtracted from the absorbance values obtained for the biological samples.

FIGS. 9a to 9e show *E. coli* and *S. aureus* attachment and biofilm growth on the surface of reference samples and additively manufactures samples. FIG. 9a shows the viable cell count of *E. coli* in LB media after 48 h, FIG. 9b shows the viable cell count of *S. aureus* in LB media after 48 h, FIG. 9c shows the viable cell count of *E. coli* in LB media after 4 h, and FIG. 9d shows the viable cell count of *S. aureus* in LB media after 4 h. In FIGS. 9a to 9d, the y-axis represents the colony forming units (CFU) per peg on a logarithmic scale, while the x-axis shows the different types of peg samples. Results from two independent experiments are presented, with a total of eight biological replicates. The median is visually indicated as a black line. Statistical analysis was conducted by Brown-Forsythe and Welch ANOVA, followed by Dunnett's T3 multiple comparison. The label 'ns' is shown for p-values higher than 0.0332, the label '**' is shown for p-values between 0.0021 and 0.0332, and the label '***' is shown for p-values between 0.0002 and 0.0021. FIG. 9e shows representative pictures of biofilms grown on different pegs for 48 h and stained with 0.1% crystal violet.

As shown in FIG. 9c and FIG. 9d, significant differences emerged between class I and class II surfaces in terms of colony-forming units per peg for both bacterial models after the 4-hour interval. Specifically, class I pegs exhibited a relatively low cell count, typically numbering in the tens, while as-printed pegs displayed substantially higher cell counts. In the case of *E. coli*, this ranged from 1000 to 10,000 cells, and for *S. aureus*, it spanned from 100 to 1000 cells.

For class II pegs, the use of the abrasive media at different times (5 h, 10 h, and 15 h) had negligible effects on reducing the number of cells per peg (FIG. 9c and FIG. 9d). However, pegs that underwent postprocessing with the polishing media for 3 hours showed a decrease in cell count, although not statistically significant. Interestingly, vapor-smoothed pegs exhibited a significant reduction in CFU/peg, displaying values lower than reference pegs processed by injection moulding for both *E. coli* and *S. aureus*.

The CV assay served as a method for quantifying biomass of the biofilm. The initial assessment was conducted after 4 and 48 hours in LB and after 24 hours in PBS for

both bacteria. While this assay does not directly measure the functional aspects of the biofilm, it offers valuable insights into the overall biofilm structure and biomass. Particularly, CV staining facilitates the visualization and quantification of both viable and dead cells within the biofilm, as well as the presence of extracellular matrix (ECM) components. Of particular interest, it was observed that only experiments carried out in LB for 48 hours resulted in a measurable biofilm through CV staining. The assessment was based on absorbance measurements at 540 nm obtained after dissolving the CV in acetic acid. Subsequently, the remaining postprocessed pegs were prepared under these specific conditions, utilizing both *E. coli* and *S. aureus*. Visual evidence revealed clear rings of stained biomass around all pegs at the liquid-air interface, as illustrated in FIG. 9e. Interestingly, the biofilm on PEG_IM with *E. coli* and PEG_PBF_VS with *S. aureus* appeared as small circular shapes, whereas a more uniform ring was formed on the remaining samples.

Although the numbers of CFU/peg were comparable for all surfaces after 48 hours in LB, a relevant distinction emerged in the biomass presence between class I and class II peg surfaces, approximately 5-fold in difference. For instance, the crystal violet absorbance for CNC and IM pegs was 0.110 and 0.090, respectively, while the PEG_PBF_0h pegs exhibited a significantly higher value of 0.559 for *E. coli*. By incorporating visual inspection, it can be inferred that a more evolved biofilm with a higher ECM content was formed on the as-printed pegs. Furthermore, the effect of surface tumbling with the abrasive media for 5, 10, and 15 hours revealed comparable biofilm growth with *E. coli* compared to the as-printed pegs. However, the additional 3-hour treatment with the polishing media resulted in a substantial 70% reduction in crystal violet absorbance. In addition, *S. aureus* samples postprocessed with the abrasive media displayed values 2-4-fold higher than as-printed pegs, whereas PEG_PBF_13hP3h showed values similar to PEG_PBF_0h. In accordance with CFU/peg results, PEG_PBF_VS displayed comparable results to those of reference pegs, with absorbances of 0.042 and 0.059 for *E. coli* and *S. aureus*, respectively. These findings therefore offer valuable insights into the biofilm development on different peg surfaces and the impact of postprocessing techniques on biofilm formation, contributing to a better understanding of the interplay between surface properties and bacterial attachment in bioprocessing equipment.

In addition, to elucidate the biofilm distinctions and gain insights into the observed surface differences, FE-SEM imaging was conducted on biofilms grown on LB media for 48 hours. Specifically, FE-SEM (JSM 7400F, JEOL, Japan) was used to analyse the surface morphology of the scaffolds. A chromium coating (~10 nm) was used to sputter

(SC7640, Polaron, United Kingdom) the strands before imaging at 1 kV. The image acquisition was performed on both class I and class II pegs for both *E. coli* and *S. aureus*.

FIG. 10 shows field emission-scanning electron microscopy (FE-SEM) images of 48 h
5 *Escherichia coli* biofilms on CNC milled pegs (PEG_CNC) in Luria-Bertani Broth (LB)
medium and imaged at (a) x1000 magnification, (b) x2200 magnification, (c) x5000
magnification, (d) x10000 magnification; on injection moulded pegs (PEG_IM) in LB
medium and imaged at (e) x1000 magnification, (f) x2200 magnification, (g) x5000
magnification, (h) x10000 magnification; and on vapor smoothed pegs (PEG_PBF_VS)
10 in LB medium and imaged at (i) x1000 magnification, (j) x2200 magnification, (k) x5000
magnification, and (l) x10000 magnification.

FIG. 11 shows field emission-scanning electron microscopy (FE-SEM) images of 4 h
15 *Escherichia coli* biofilms on as-printed powder bed fusion pegs (PEG_PBF_0h) in Luria-
Bertani Broth (LB) medium and imaged at (a) x1000 magnification, (b) x2200
magnification, (c) x5000 magnification, (d) x10000 magnification; PBF pegs
postprocessed by surface tumbling using the abrasive media for 5h (PEG_PBF_5h) in
LB medium and imaged at (e) x1000 magnification, (f) x2200 magnification, (g) x5000
magnification; PBF pegs postprocessed by surface tumbling using the abrasive media
20 for 10h (PEG_PBF_10h) in LB medium and imaged at (h) x1000 magnification, (i) x2200
magnification, (j) x5000 magnification; PBF pegs postprocessed by surface tumbling
using the abrasive media for 15h (PEG_PBF_15h) in LB medium and imaged at (k)
x1000 magnification, (l) x2200 magnification, (m) x5000 magnification, (n) x10000
magnification; and PBF pegs postprocessed by surface tumbling using the abrasive
25 media for 13h including three hours of polishing step (PEG_PBF_13hP3h) pegs in LB
medium and imaged at (o) x1000 magnification, (p) x2200 magnification, and (q) x5000
magnification.

FIG. 12 shows field emission-scanning electron microscopy (FE-SEM) images of 48 h
30 *Staphylococcus aureus* biofilms on CNC milled pegs (PEG_CNC) in Luria-Bertani Broth
(LB) medium and imaged at (a) x1000 magnification, (b) x2200 magnification, (c) x5000
magnification; on injection moulded pegs (PEG_IM) in LB medium and imaged at (d)
x1000 magnification, (e) x2200 magnification, (f) x5000 magnification; and on vapor
smoothed pegs (PEG_PBF_VS) in LB medium and imaged at (g) x1000 magnification,
35 (h) x2200 magnification, (i) x5000 magnification. FIG. 13 shows field emission-scanning
electron microscopy (FE-SEM) images of 48 h *Staphylococcus aureus* biofilms on as-
printed powder bed fusion pegs (PEG_PBF_0h) in Luria-Bertani Broth (LB) medium and

imaged at (a) x1000 magnification, (b) x2200 magnification, (c) x5000 magnification; PBF
 pegs postprocessed by surface tumbling using the abrasive media for 5h (PEG_PBF_5h)
 in LB medium and imaged at (d) x1000 magnification, (e) x2200 magnification, (f) x5000
 magnification; PBF pegs postprocessed by surface tumbling using the abrasive media
 5 for 10h (PEG_PBF_10h) in LB medium and imaged at (g) x1000 magnification, (h) x2200
 magnification, (i) x5000 magnification; PBF pegs postprocessed by surface tumbling
 using the abrasive media for 15h (PEG_PBF_15h) in LB medium and imaged at (j) x1000
 magnification, (k) x2200 magnification, (l) x5000 magnification; and PBF pegs
 postprocessed by surface tumbling using the abrasive media for 13h including three
 10 hours of polishing step (PEG_PBF_13hP3h) pegs in LB medium and imaged at (m)
 x1000 magnification, (n) x2200 magnification, and (o) x5000 magnification.

The reference pegs produced through CNC milling (FIGS. 10a and 10b) exhibited a
 uniform surface with visible lines resulting from the cutting tools. Additionally, samples
 15 incubated with *E. coli* formed localized and scattered thin layers of cell colonies where
 higher density was observed on the surface features produced during manufacturing
 (FIGS. 10c and 10d). No 3D structures or EPS were found. Similarly, samples incubated
 with *S. aureus* showed isolated cells and small colonies of 20-30 cells dispersed on the
 surface (FIGS. 12a, 12b, and 12c). In contrast, pegs manufactured using IM displayed
 20 the smoothest among all surfaces, showing only the presence of welding lines (FIGS.
 10e and 10f). Additionally, these surfaces showed the most variation between the two
 bacterial strains. Incubation with *E. coli* formed large circular colonies on the smooth
 peg surface (FIGS. 10e, 10f, 10g and 10h), whereas *S. aureus* exhibited a preference
 for adhering along the welding line, forming a continuous stream of cells, with only a few
 25 scattered colonies on the remaining surface area (FIGS. 12d, 12e, and 12f). Moreover,
 vapor-smoothed surfaces also demonstrated a high degree of smoothness compared to
 the as-printed ones. However, their surfaces also exhibited "volcano-like" structures
 formed during the postprocessing. In the context of bacterial adhesion, it was observed
 that *E. coli* (FIGS. 10i, 10j, 10k and 10l) cells exhibited a scattered distribution, occupying
 30 both the smooth surface and the "volcano" features. Conversely, *S. aureus* cells
 demonstrated a significantly higher density of attachment, uniformly colonising the
 surface, regardless of the presence of surface features (FIGS. 12g, 12h, and 12i). In
 summary, the surface topography analysis of the class I peg samples revealed distinct
 characteristics in terms of surface texture and bacterial adhesion behaviour. CNC pegs
 35 displayed a uniform surface with visible cutter lines, while the IM and VS pegs were the
 smoothest ones, but they exhibited surface features like welding lines and volcano-like
 structures, respectively. *E. coli* and *S. aureus* cells showed the highest adhesion on IM

pegs, while CNC and VS surfaces showed attachment of *E. coli* only on the surface features. Conversely, *S. aureus* cells densely adhered to the VS surfaces, with only small colonies found on CNC samples.

- 5 Class II pegs including as-printed and surface tumbled peg samples with the abrasive and polishing media at different tumbling times were incubated at identical conditions as class I pegs (FIGS. 11 and 13). As mentioned previously, PEG_PBF_0h (as-printed) pegs exhibited a highly rough surface with the presence of adhered PP powder particles (ranging in size from 20 μm to 100 μm) fused onto the outer of the peg's surface, known
10 as neck phenomenon. Biofilm studies demonstrated that *E. coli* (FIGS. 11a, 11b, 11c, and 11d) and *S. aureus* (FIGS. 13a, 13b, and 13c) cells were found concealed within the interstices of powder particles, rendering them imperceptible on the outer surfaces. The presence of crevices, holes and irregularities was observed as the favoured attachment sites for these cells. Nevertheless, their ability to colonise and generate extensive biofilm
15 patches, akin to what was observed in class I samples, was limited.

The impact of surface tumbling using the abrasive media for 5, 10, and 15 hours resulted in a minor reduction in the initial roughness of the as-printed peg samples. This surface treatment revealed the presence of certain smoother areas on the surfaces, yet overall,
20 they remained comparable to the PEG_PBF_0h samples. Subsequent analysis of the PEG_PBF_5h samples revealed that *E. coli* (FIGS. 11e, 11f and 11g) and *S. aureus* (FIGS. 13e, 13f and 13g) cells appeared as dispersed and small colonies. However, with increased postprocessing time, the degree of smoothness improved, leading to pegs with denser and larger cell patches for both bacterial species (FIGS. 11h, 11i, 11j, 11k,
25 11l, 11m, 11n, 13g, 13h, 13i, 13j, 13k, and 13l). The implementation of the polishing media provided the highest smoothness for the surface tumbling samples were also patches of potential biofilm was found on both intersection between particles and smooth regions (FIGS. 11o, 11p, 11q, 13m, 13n, and 13o).

- 30 To evaluate the impact of surface texture on bacterial growth and adhesion, a series of experiments were conducted using PBS as the medium. PBS was selected as it provides a nutrient-free environment, allowing to focus solely on the effects of surface texture without confounding factors from nutrient availability. The experiments were performed at four time points: 4, 6, 16 and 24 hours with both bacteria, *E. coli* and *S.*
35 *aureus* (FIGS. 14a and 14b).

FIG. 14 shows *Escherichia coli* and *Staphylococcus aureus* attachment and biofilm growth on the surface of reference pegs (PEG_CNC and PEG_IM) and additively manufactured pegs (PEG_PBF_0h, PEG_PBF_5h, PEG_PBF_10h, PEG_PBF_15h, PEG_PBF_13hP3h and PEG_PBF_VS). FIG. 14a shows viable cell count of *E. coli* in PBS at 4, 6, 16 and 24 hours. The y-axis represents the colony forming units (CFU) per peg on a logarithmic scale, while the x-axis displays the different time points. Results from two independent experiments are presented with a total of 8 biological replicates. Medians for all conditions are shown as symbols. At 24h, the values (in increasing order) correspond to: PEG_IM, PEG_CNC, PEG_PBF_VS, PEG_PBF_5h, PEG_PBF_15h, PEG_PBF_13h_3P, PEG_PBF_0h, and PEG_PBF_10h.

FIG. 14b shows viable cell count of *S. aureus* in PBS at 4, 6, 16 and 24 hours. The y-axis represents the colony forming units (CFU) per peg on a logarithmic scale, while the x-axis displays the different time points. Results from two independent experiments are presented with a total of 8 biological replicates. Medians for all conditions are shown as symbols.

In the *E. coli* PBS experiments (FIG. 14a), distinct behaviours were observed among the class I and class II samples in terms of bacterial attachment. Class I samples exhibited a maximum threshold of 100 CFU per peg, beyond which the attachment of cells increased significantly at each time point. As-printed and surface-tumbled pegs showed similar levels of *E. coli* attachment, regardless of the abrasive media used and the time point considered. No substantial differences were observed among these samples. Consistent with previous findings, only the PEG_PBF_VS pegs demonstrated the ability to reduce bacterial attachment at each time point. As expected, reference pegs (CNC and IM) also exhibited the lowest number of CFU per peg at each time point. Furthermore, the attachment of *E. coli* cells on the pegs reached saturation at 6 hours, with no significant changes in CFU observed up to 24 hours.

S. aureus experiments (FIG. 14b) displayed a different trend, with a decrease in bacterial attachment observed at 16 hours for all samples. The saturation point for *S. aureus* occurred earlier, at 4 hours, where the CFU per peg remained relatively constant before experiencing a notable decline at 16 hours. These findings clarified the contrasting behaviours of gram-positive and gram-negative bacteria with respect to attachment on various peg samples over time. The results highlighted the importance of surface characteristics and properties in influencing bacterial adhesion dynamics.

FIG. 15 shows values of relevant roughness parameters obtained from the Variable Importance in Projection (VIP) plot in FIG. 8. These roughness parameters describe the dale regions of the peg samples. FIG. 15a shows material ratio of the dales (Smrk2), FIG. 15b shows reduced pit depth (Svk), FIG. 15c shows maximum pit depth (Sv_{kx}), FIG. 15d shows arithmetic mean height (Sa), FIG. 15e shows root mean square height (Sq), FIG. 15f shows maximum pit depth (Sv), FIG. 15g shows maximum height, FIG. 15h shows dale void volume (V_{vv}), FIG. 15i shows five-point pit depth (S_{5v}), and FIG. 15j shows material ratio height difference (S_{dc}). The calculated averages are derived from a set of 12 specimens for each peg sample. FIGS. 15a to 15c show stratified surfaces' functional parameters, FIGS. 15d to 15g show height parameters, FIG. 15h shows a volume-based functional parameters, FIG. 15i shows a feature parameter, and FIG. 15j shows a functional parameter.

A visual inspection including FE-SEM imaging of the peg surfaces revealed notable distinctions in surface topography. Surfaces produced through conventional methodologies (CNC and IM) exhibited low dale roughness parameters (FIGS. 10, 12, and 15). Conversely, AM-generated samples presented a rugged surface characterized by fused powder particles, yielding a plethora of surface features ideal for attachment of the cells (FIGS. 11 and 13). Employing tumbling postprocessing with the abrasive media exhibited a minimal impact on the relevant roughness parameters, thereby leaving the dale region of the peg surfaces predominantly unaltered (FIG. 15). The use of the polishing media yielded superior results but was uneven in this effect, displaying both smooth regions and areas devoid of discernible impact (FIGS. 11 and 13). Solely vapor smoothing generated a substantial surface transformation, resulting in smooth surfaces interspersed with residual "volcano" surface features (FIGS. 10 and 12). Several explanations could account for this phenomenon; however, further research is needed to determine the main cause.

The next characterisation step involved in-depth surface texture analysis, focusing on various relevant roughness parameters. Robust correlations were determined between specific surface roughness parameters and the adhesion of *E. coli* and *S. aureus* during the first bacterial attachment phase (4 hours) in LB media (FIG. 9c and FIG. 9d, and FIG. 15). Significant differences on the surface of the different pegs were described by parameters from different families, including stratified surface parameters (Smrk2, Svk, Sv_{kx}), height-related parameters (Sa, Sq, Sv, Sz), volumetric functional parameters (V_{vv}), feature-specific parameters (S_{5v}) and functional parameters (S_{dc}). Moreover, the

surfaces with the highest hydrophilicity, belonging to class I pegs (as shown in FIG. 4), exhibited the lowest bacterial attachment after 4 hours.

5 Focusing on these set of roughness parameters, it is apparent that dale regions wield a more substantial influence than their peak and hill counterparts. Within class I pegs, encompassing PEG_CNC, PEG_IM, and PEG_PBF_VS, both reduced pit height and maximum pit depth exhibited discernibly lower values, as shown in FIG. 15. Vapor-smoothed pegs, within this classification, notably manifested the lowest values. This trend persisted consistently across all examined parameters, affirming the salience of
10 dale regions in mediating bacterial attachment phenomena. Additionally, this was corroborated by the analysis of the S5v parameter, which quantifies the mean of the five most prominent pit depths (FIG. 15i). Class I pegs exhibited an average pit depth of less than 5 μm , significantly contrasting with the range of 35 to 46 μm observed in class II pegs.

15 Expanding the analysis to encompass surface roughness, the efficacy of cultivating surfaces characterized by shallow features and non-deep pits in mitigating the adhesion of *E. coli* and *S. aureus* is apparent. This effect is remarkable during the initial hours of bacterial attachment, as evidenced by FIG. 9c and FIG. 9d. However, the count of CFUs
20 per peg increased substantially to approximately 10^7 after a 48-hour period, regardless the specific surface attributes of the pegs, as shown in FIG. 9a and FIG. 9b. It is conceivable that extended incubation periods would yield even greater biomass accumulation.

25 In correlation with the surface roughness findings, the static contact angles measured on class I pegs indicated higher hydrophilicity compared to class II pegs (as shown in FIG. 4). Specifically, those fabricated using CNC milling manufacturing exhibited the lowest degree of hydrophobicity with a value of $60^\circ \pm 2^\circ$. In the bioprocessing field, the use of highly hydrophobic surfaces holds significance to avoid water adhesion within equipment
30 interiors where complex geometries and unstable flows are present. However, contradicting results regarding the initial adhesion of bacteria based on surface wettability have been reported in the literature. In general, it is recognised that superhydrophobic and superhydrophilic surfaces tend to reduce bacterial adhesion, while no consensus has been reached for surfaces with medium wettabilities. The
35 present disclosure reveals that hydrophilic peg surfaces (class I pegs) showed lower bacterial attachment, whereas hydrophobic peg surfaces (class II pegs) displayed higher bacterial attachment for both *E. coli* and *S. aureus*. FIG. 4 illustrates how the surface

texture of the peg influenced the contact angle results, which were measured using an optical tensiometer.

As previously discussed, the differentiation in CFU per peg was evident in the 4-hour LB experiments, wherein pegs with high dae roughness parameters exhibited 100-fold more adhered cells compared to class I pegs (FIG. 9c and FIG. 9d). For pegs postprocessed with the abrasive and polishing media, intermediate values between reference pegs and as-printed pegs of CFU/peg were observed for both bacterial models (FIG. 9c and FIG. 9d). Nonetheless, it was only the pegs subjected to vapor smoothing that displayed noteworthy variations in bacterial attachment compared to PEG_PBF_0h. In contrast, all samples treated with tumbling demonstrated analogous counts for *E. coli* and higher counts for *S. aureus* when compared to the as-printed counterparts. FE-SEM imaging following a 4-hour incubation in LB media with both cell types was conducted, as this adhesion time unveiled relevant differences in CFU/peg across distinct surfaces.

The next step included the adoption of PBS-based experiments to simulate real-life conditions in the bioprocessing field where media often lack essential nutrients. Analysed at multiple time points (4, 6, 16 and 24 hours), the expected trend of increasing CFU/peg attachment over time did not align with the observed data patterns (FIG. 14). However, class I pegs exhibited a lower number of attached *E. coli* cells in comparison to class II samples, as previously observed from the LB media analysis.

With the ambition of observing clear discrepancies among the different surfaces, a 48 hour bacterial adhesion evaluation with *E. coli* on LB media was carried out for each sample. The results switched towards an equilibrium state with comparable CFU per peg values, except for the vapor smoothed samples (FIG. 9a). This observation demonstrates that bacteria in nutrient-rich media eventually adhere to and proliferate on various surfaces.

In the biofilm analysis performed using crystal violet staining, class II pegs (high dae roughness parameters) demonstrated a greater biomass accumulation. These pegs reached equilibrium earlier and exhibited an enhanced capability to generate ECM, resulting in the formation of a more mature biofilm in contrast to the reference pegs (FIG. 9e). In addition, crystal violet staining also revealed pronounced biomass accumulation at the air-liquid interface, forming a characteristic ring around pegs.

FE-SEM investigations including pegs with a bacterial load exceeding 10^6 CFU per peg were conducted over a 48 hour timeframe, confirming distinct degrees of bacterial adhesion across the different peg surfaces. For *E. coli* cells, the highest level of attachment was observed on IM pegs, characterised by the presence of voluminous and circular cell colonies distributed along the smooth region of the peg (FIGS. 10e, 10f, 10g, and 10h). Conversely, in the case of *S. aureus*, a denser attachment was witnessed along the welding line of the peg (FIGS. 12d, 12e, and 12f). In the latter scenario, a stratified bacterial layer manifested, involving cellular division and substantial attachment to the surface. The inverse scenario was observed for as-printed pegs, PEG_PBF_0h, where dispersed *E. coli* and *S. aureus* cells were found (FIGS. 11b, 11c, 13b, and 13c). Meanwhile, the remaining peg samples characterised by low dae roughness parameters (CNC and VS) showed scattered cluster of cells, dispersed in a random pattern across the surface. VS pegs displayed an elevated density of *S. aureus* cells (FIGS. 12g, 12h and 12i). In contrast to the results from crystal violet staining, SEM images suggest that high dae roughness parameter pegs present more difficulties for bacteria to attach. On smooth surfaces, once attachment occurs, bacteria experience less hindrance and proliferate more readily across the surface due to the absence of surface constraints. Nevertheless, the most mature biofilm with presence of ECM was observed for the pegs which were tumbled with the abrasive media, as well as was obtained from the crystal violet staining, and correlated with the characterisation of high dae roughness parameters.

From the above analysis, therefore, it can be appreciated that tailoring the ten relevant surface roughness parameters (identified in FIG. 8) to the values associated with Class I pegs (shown in FIG. 15) can be expected to yield surfaces that have low propensity for bacterial adhesion (i.e. in line with the findings in FIG. 9c and FIG. 9d). Specifically, surfaces of postprocessed additively manufactured parts can be expected to have low propensity for bacterial adhesion if they have one or more of the following areal roughness parameter values resulting from filtering the surface features to remove surface features having long wavelengths (e.g. above $54\text{ }\mu\text{m}$):

- (i) a reduced dae height, S_{vk} , of less than $5\text{ }\mu\text{m}$, preferably less than $4\text{ }\mu\text{m}$, more preferably less than $3\text{ }\mu\text{m}$, more preferably less than $2\text{ }\mu\text{m}$, and most preferably less than $1\text{ }\mu\text{m}$ (as shown from FIG. 15b, where the S_{vk} value for PEG_CNC is $0.8\text{ }\mu\text{m}$, the S_{vk} value for PEG_IM is $1.1\text{ }\mu\text{m}$, and the S_{vk} value for PEG_PBF_VS is $0.1\text{ }\mu\text{m}$, contrasting with values above $17\text{ }\mu\text{m}$ for class II surfaces);

(ii) an arithmetical mean height, S_a , of less than $2.5\text{ }\mu\text{m}$, preferably less than $2\text{ }\mu\text{m}$, more preferably less than $1.5\text{ }\mu\text{m}$, and most preferably less than $1\text{ }\mu\text{m}$ (as shown from FIG. 15d, where the S_a value for PEG_CNC is $1.0\text{ }\mu\text{m}$, the S_a value for PEG_IM is $0.5\text{ }\mu\text{m}$, and the S_{vk} value for PEG_PBF_VS is $0.1\text{ }\mu\text{m}$, contrasting with values above $4.5\text{ }\mu\text{m}$ for class II surfaces);

(iii) a daled void volume, V_{vv} , of less than $0.5\text{ }\mu\text{m}^3\text{ }\mu\text{m}^{-2}$; more preferably less than $0.4\text{ }\mu\text{m}^3\text{ }\mu\text{m}^{-2}$, more preferably less than $0.3\text{ }\mu\text{m}^3\text{ }\mu\text{m}^{-2}$, more preferably less than $0.2\text{ }\mu\text{m}^3\text{ }\mu\text{m}^{-2}$, and most preferably less than $0.1\text{ }\mu\text{m}^3\text{ }\mu\text{m}^{-2}$ (as shown from FIG. 15h, where the V_{vv} value for PEG_CNC is $0.06\text{ }\mu\text{m}^3\text{ }\mu\text{m}^{-2}$, the V_{vv} value for PEG_IM is $0.08\text{ }\mu\text{m}^3\text{ }\mu\text{m}^{-2}$, and the V_{vv} value for PEG_PBF_VS is $0.01\text{ }\mu\text{m}^3\text{ }\mu\text{m}^{-2}$, contrasting with values above $1.7\text{ }\mu\text{m}^3\text{ }\mu\text{m}^{-2}$ for class II surfaces);

(iv) a five-point pit height, S_{5v} , of less than $15\text{ }\mu\text{m}$; preferably less than $12.5\text{ }\mu\text{m}$, more preferably less than $10\text{ }\mu\text{m}$, more preferably less than $7.5\text{ }\mu\text{m}$, and most preferably less than $5\text{ }\mu\text{m}$ (as shown from FIG. 15i, where the S_{5v} value for PEG_CNC is $2.8\text{ }\mu\text{m}$, the S_{5v} value for PEG_IM is $4.2\text{ }\mu\text{m}$, and the S_{5v} value for PEG_PBF_VS is $0.6\text{ }\mu\text{m}$, contrasting with values above $35\text{ }\mu\text{m}$ for class II surfaces);

(v) a maximum pit depth, S_{vkx} , of less than $20\text{ }\mu\text{m}$, preferably less than $15\text{ }\mu\text{m}$, more preferably less than $10\text{ }\mu\text{m}$, and most preferably less than $5\text{ }\mu\text{m}$ (as shown from FIG. 15c, where the S_{vkx} value for PEG_CNC is $2.7\text{ }\mu\text{m}$, the S_{vkx} value for PEG_IM is $4.4\text{ }\mu\text{m}$, and the S_{vkx} value for PEG_PBF_VS is $0.8\text{ }\mu\text{m}$, contrasting with values above $41\text{ }\mu\text{m}$ for class II surfaces);

(vi) a material ratio of the dales, S_{mrk2} , of between 85% and 95%, preferably between 86% and 93%, and more preferably between 87% and 91% (as shown from FIG. 15a, where the S_{mrk2} value for PEG_CNC is 90.4%, the S_{mrk2} value for PEG_IM is 87.9%, and the S_{mrk2} value for PEG_PBF_VS is 87.8%, contrasting with values below 79% for class II surfaces);

(vii) a root mean square height, S_q , of less than $4\text{ }\mu\text{m}$, preferably less than $3.5\text{ }\mu\text{m}$, more preferably less than $3\text{ }\mu\text{m}$, more preferably less than $2.5\text{ }\mu\text{m}$, and most preferably less than $2\text{ }\mu\text{m}$ (as shown from FIG. 15e, where the S_q value for PEG_CNC is $1.4\text{ }\mu\text{m}$, the S_q value for PEG_IM is $1.2\text{ }\mu\text{m}$, and the S_q value for PEG_PBF_VS is $0.1\text{ }\mu\text{m}$, contrasting with values above $7\text{ }\mu\text{m}$ for class II surfaces);

(viii) a maximum height, S_z , of less than 20 μm , more preferably less than 17.5 μm , more preferably less than 15 μm , and most preferably less than 12.5 μm (as shown from FIG. 15g, where the S_z value for PEG_CNC is 11.8 μm , the S_z value for PEG_IM is 12.9 μm , and the S_z value for PEG_PBF_VS is 1.5 μm , contrasting with values above 68 μm for class II surfaces);

(ix) a maximum pit depth, S_v , of less than 20 μm , preferably less than 15 μm , more preferably less than 10 μm , and most preferably less than 5 μm (as shown from FIG. 15f, where the S_v value for PEG_CNC is 3.6 μm , the S_v value for PEG_IM is 4.8 μm , and the S_v value for PEG_PBF_VS is 0.8 μm , contrasting with values above 41 μm for class II surfaces); and

(x) a material ratio height difference, S_{dc} , of less than 7 μm , preferably less than 6 μm , more preferably less than 5 μm , more preferably less than 4 μm , and most preferably less than 3 μm (as shown from FIG. 15j, where the S_{dc} value for PEG_CNC is 3.0 μm , the S_{dc} value for PEG_IM is 0.7 μm , and the S_{dc} value for PEG_PBF_VS is 0.2 μm , contrasting with values above 14 μm for class II surfaces).

Such postprocessed surfaces can also be expected to have low propensity for bacterial adhesion if they have a static contact angle of between 45 and 105 degrees, preferably between 50 and 100 degrees, more preferably between 55 and 95 degrees, and most preferably between 60 and 90 degrees (as shown from FIG. 4, where the static contact angle value for PEG_CNC is 60 degrees, the static contact angle value for PEG_IM is 82 degrees, and the static contact angle value for PEG_PBF_VS is 88 degrees, contrasting with static contact angle values above 110 degrees for class II surfaces).

Variations or modifications to the systems and methods described herein are set out in the following paragraphs.

It will also be appreciated that the methods described herein are not limited to the evaluation of surfaces of parts produced by PBF, and may also be applied to surfaces of parts produced by other AM techniques. Moreover, it will be appreciated that the methods described herein are not, in fact, limited to the evaluation of surfaces of parts produced by an AM technique, and may alternatively or additionally be applied to surfaces of parts produced using other manufacturing techniques.

In addition, the methods described herein are not limited to the evaluation of peg-shaped surfaces. In particular, the method of FIG. 2 may be used to validate the propensity for bacterial adhesion to other features of manufactured parts, such as pockets, cavities and corners. The findings described herein have particular applicability to surfaces that
5 include boundaries between adjacent layers of the additively-manufactured part.

The described methods may be implemented using computer executable instructions. A computer program product or computer readable medium may comprise or store the computer executable instructions. The computer program product or computer readable
10 medium may comprise a hard disk drive, a flash memory, a read-only memory (ROM), a CD, a DVD, a cache, a random-access memory (RAM) and/or any other storage media in which information is stored for any duration (e.g., for extended time periods, permanently, brief instances, for temporarily buffering, and/or for caching of the information). A computer program may comprise the computer executable instructions.
15 The computer readable medium may be a tangible or non-transitory computer readable medium. The term "computer readable" encompasses "machine readable".

The singular terms "a" and "an" should not be taken to mean "one and only one". Rather, they should be taken to mean "at least one" or "one or more" unless stated otherwise.
20 The word "comprising" and its derivatives including "comprises" and "comprise" include each of the stated features, but does not exclude the inclusion of one or more further features.

The above implementations have been described by way of example only, and the
25 described implementations are to be considered in all respects only as illustrative and not restrictive. It will be appreciated that variations of the described implementations may be made without departing from the scope of the invention. It will also be apparent that there are many variations that have not been described, but that fall within the scope of the appended claims.

30

CLAIMS:

1. A method (100) of producing a part (300) for use in a bioprocessing system, the method comprising:
- 5 manufacturing (110) the part (300) using an additive manufacturing process; and postprocessing (120) a surface of the part (300), wherein the surface is intended to be wetted in use;
- wherein the postprocessed surface has one or more of the following areal roughness parameter values (optionally measured in accordance with ISO 25178-10 2:2022), and wherein the one or more areal roughness parameter values are measured for the surface after filtering the surface to remove surface features having long wavelengths:
- a reduced dale height, S_{vk} , of less than about 5 μm ;
- an arithmetical mean height, S_a , of less than about 2.5 μm ;
- 15 a dale void volume, V_{vv} , of less than about 0.5 $\mu\text{m}^3 \mu\text{m}^{-2}$; and/or a five-point pit height, S_{5v} , of less than about 15 μm .
2. The method (100) according to claim 1, wherein the surface includes a plurality of boundaries between adjacent layers of the part (300).
- 20 3. The method (100) according to claim 1 or claim 2, wherein the part (300) is manufactured using powder bed fusion.
4. The method (100) according to any of claims 1 to 3, wherein postprocessing the surface comprises chemical vapour surface smoothing of the surface.
- 25 5. The method (100) according to any of claims 1 to 4, wherein the reduced dale height, S_{vk} , of the postprocessed surface is less than about 4 μm , preferably less than about 3 μm , more preferably less than about 2 μm , and most preferably less than about 1 μm .
- 30 6. The method (100) according to any of claims 1 to 5, wherein the arithmetic mean height, S_a , of the postprocessed surface is less than about 2 μm , preferably less than about 1.5 μm , and more preferably less than about 1 μm .
- 35 7. The method (100) according to any of claims 1 to 6, wherein the dale void volume, V_{vv} , of the postprocessed surface is less than about 0.4 $\mu\text{m}^3 \mu\text{m}^{-2}$, preferably less than

about $0.3 \mu\text{m}^3 \mu\text{m}^{-2}$, more preferably less than about $0.2 \mu\text{m}^3 \mu\text{m}^{-2}$, and most preferably less than about $0.1 \mu\text{m}^3 \mu\text{m}^{-2}$.

8. The method (100) according to any of claims 1 to 7, wherein the five-point pit height, $S5v$, of the postprocessed surface is less than about $12.5 \mu\text{m}$, preferably less than about $10 \mu\text{m}$, more preferably less than about $7.5 \mu\text{m}$, and most preferably less than about $5 \mu\text{m}$.

9. The method (100) according to any of claims 1 to 8, wherein the postprocessed surface has a maximum pit depth, $Svkx$, optionally measured in accordance with ISO 25178-2:2022, of less than about $20 \mu\text{m}$, preferably less than about $15 \mu\text{m}$, more preferably less than about $10 \mu\text{m}$, and most preferably less than about $5 \mu\text{m}$, wherein the maximum pit depth, $Svkx$, is measured for the surface after filtering the surface to remove surface features having long wavelengths.

10. The method (100) according to any of claims 1 to 9, wherein the postprocessed surface has a material ratio of the dales, $Smrk2$, optionally measured in accordance with ISO 25178-2:2022, of between about 85% and 95%, preferably between about 86% and 93%, and more preferably between about 87% and 91%, wherein the material ratio of the dales, $Smrk2$, is measured for the surface after filtering the surface to remove surface features having long wavelengths.

11. The method (100) according to any of claims 1 to 10, wherein the postprocessed surface has a root mean square height, Sq , optionally measured in accordance with ISO 25178-2:2022, of less than about $4 \mu\text{m}$, preferably less than about $3.5 \mu\text{m}$, more preferably less than about $3 \mu\text{m}$, more preferably less than about $2.5 \mu\text{m}$, and most preferably less than about $2 \mu\text{m}$, wherein the root mean square height, Sq , is measured for the surface after filtering the surface to remove surface features having long wavelengths.

12. The method (100) according to any of claims 1 to 11, wherein the postprocessed surface has a maximum height, Sz , optionally measured in accordance with ISO 25178-2:2022, of less than about $20 \mu\text{m}$, more preferably less than about $17.5 \mu\text{m}$, more preferably less than about $15 \mu\text{m}$, and most preferably less than about $12.5 \mu\text{m}$, wherein the maximum height, Sz , is measured for the surface after filtering the surface to remove surface features having long wavelengths.

13. The method (100) according to any of claims 1 to 12, wherein the postprocessed surface has a maximum pit depth, S_v , optionally measured in accordance with ISO 25178-2:2022, of less than about 20 μm , preferably less than about 15 μm , more preferably less than about 10 μm , and most preferably less than about 5 μm , wherein
5 the maximum pit depth, S_v , is measured for the surface after filtering the surface to remove surface features having long wavelengths.
14. The method (100) according to any of claims 1 to 13, wherein the postprocessed surface has a material ratio height difference, S_{dc} , optionally measured in accordance
10 with ISO 25178-2:2022, of less than about 7 μm , preferably less than about 6 μm , more preferably less than about 5 μm , more preferably less than about 4 μm , and most preferably less than about 3 μm , wherein the material ratio height difference, S_{dc} , is measured for the surface after filtering the surface to remove surface features having long wavelengths.
15. The method (100) according to any of claims 1 to 14, wherein a static contact angle of the postprocessed surface is between about 45 and 105 degrees, preferably between about 50 and 100 degrees, more preferably between about 55 and 95 degrees, and most preferably between about 60 and 90 degrees.
16. The method (100) according to any of claims 1 to 15, wherein the part (300) is formed of polypropylene.
17. The method (100) according to any of claims 1 to 16, wherein an arithmetical
25 mean height, R_a , along the surface is greater than about 0.5 μm .
18. An additively manufactured part (300) for use in a bioprocessing system, wherein the additively manufactured part (300) comprises a surface intended to be wetted in use, wherein the surface has one or more of the following areal roughness parameter values optionally measured in accordance with ISO 25178-2:2022, wherein the one or more areal roughness parameter values are measured for the surface after filtering the surface to remove surface features having long wavelengths:
- a reduced dale height, S_{vk} , of less than about 5 μm ;
 - an arithmetical mean height, S_a , of less than about 2.5 μm ;
 - 35 a dale void volume, V_{vv} , of less than about 0.5 $\mu\text{m}^3 \mu\text{m}^{-2}$; and/or
 - a five-point pit height, S_{5v} , of less than about 15 μm .

19. The part (300) according to claim 18, wherein the surface includes a plurality of boundaries between adjacent layers of the part (300).
20. The part (300) according to claim 18 or claim 19, wherein the reduced dale height, S_{vk} , of the surface is less than about 4 μm , preferably less than about 3 μm , more preferably less than about 2 μm , and most preferably less than about 1 μm .
21. The part (300) according to any of claims 18 to 20, wherein the arithmetic mean height, S_a , of the surface is less than about 2 μm , preferably less than about 1.5 μm , and more preferably less than about 1 μm .
22. The part (300) according to any of claims 18 to 21, wherein the dale void volume, V_{vv} , of the surface is less than about 0.4 $\mu\text{m}^3 \mu\text{m}^{-2}$, preferably less than about 0.3 $\mu\text{m}^3 \mu\text{m}^{-2}$, more preferably less than about 0.2 $\mu\text{m}^3 \mu\text{m}^{-2}$, and most preferably less than about 0.1 $\mu\text{m}^3 \mu\text{m}^{-2}$.
23. The part (300) according to any of claims 18 to 22, wherein the five-point pit height, S_{5v} , of the surface is less than about 12.5 μm , preferably less than about 10 μm , more preferably less than about 7.5 μm , and most preferably less than about 5 μm .
24. The part (300) according to any of claims 18 to 23, wherein the part (300) is formed of polypropylene.
25. The part (300) according to any of claims 18 to 24, wherein an arithmetical mean height, R_a , along the surface is greater than about 0.5 μm .
26. A method (200) of classifying a surface of a part (300) as being suitable for use in a bioprocessing system, the method comprising:
manufacturing (210) the part (300), wherein the part (300) comprises a surface that is intended to be wetted in use; and
one or more of:
measuring (220) a reduced dale height, S_{vk} , of the surface after filtering the surface to remove surface features having long wavelengths, and classifying the surface as being suitable for use in the bioprocessing system if the reduced dale height, S_{vk} , of the surface is less than about 5 μm ;
measuring (220) an arithmetical mean height, S_a , of the surface after filtering the surface to remove surface features having long wavelengths, and

- classifying the surface as being suitable for use in the bioprocessing system if the arithmetical mean height, S_a , of the surface is less than about $2.5\ \mu\text{m}$;
- measuring (220) a dale void volume, V_{vv} , of the surface after filtering the surface to remove surface features having long wavelengths, and classifying the surface as being suitable for use in the bioprocessing system if the dale void volume, V_{vv} , of the surface is less than about $0.5\ \mu\text{m}^3\ \mu\text{m}^{-2}$; and
- measuring (220) a five-point pit height, S_{5v} , of the surface after filtering the surface to remove surface features having long wavelengths, and classifying the surface as being suitable for use in the bioprocessing system if the five-point pit height, S_{5v} , of the surface is less than about $15\ \mu\text{m}$.

27. The method (100, 200) or part (300) of any preceding claim, wherein long wavelengths are: above about $10\ \mu\text{m}$; above about $20\ \mu\text{m}$; above about $30\ \mu\text{m}$; above about $40\ \mu\text{m}$; above about $50\ \mu\text{m}$; above $54\ \mu\text{m}$; above about $54\ \mu\text{m}$; above about $55\ \mu\text{m}$; above about $60\ \mu\text{m}$; above about $70\ \mu\text{m}$; above about $80\ \mu\text{m}$; above about $90\ \mu\text{m}$; or above about $100\ \mu\text{m}$.