Metrology for Bio Systems

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Abstract: The current paper addresses the advent of next generation bio system focussed Micro Nano Manufacturing Technologies (MNMT). These products and processes have placed significant new emphasis on specification and quality control systems, especially if these product and processes are to achieve economic scale up. Bio technology products and processes are a core element of MNMT and structured surfaces can be a key element in enabling bio system function. There examples of the application of such surfaces in bio systems for functions such as diverse as anti fouling and oseointegration. However a deficit exists in terms of metrology for bio structured surfaces and identifying suitable measurands and instrumentation remains a challenge for production engineers. Functional modelling would seem to point towards a better way of specifying metrology however for bio systems these are rare and often extensive function testing and clinical trials are used to inform the metrology selection. In the present paper the development of MNMT bio systems is discussed in the metrology context and several examples of developing metrology challenges. Four such bio related systems are discussed the solutions are outlined. The case studies cover traditional prosthetic implants, micro fluidic devices, cellular attachment and manufacture of cellular scaffolds.

1. Introduction

Advances in metrology and surface metrology over the last decade have taken the subject from one designed as a manufacturing quality control tool to a subject where the measured data can be used a diagnostic or forensic tool or as input to specific functional models across a ranges of disciplines. The discipline can be thought of as taking a philosophical shift from being a reactive process to a proactive technology. An aim for modern surface metrology is to efficiently measure surfaces using a minimum set of quantitative parameters that relate directly to the intended function of the surface. Clearly to achieve this in a "smart" way the surface function needs to be fully understood and ideally modelled. This then allows the key functional attributes of the surface to be quantified in the context of how they affect the surface functionality. The case of bio systems is no different and in the age where we are increasingly commercialising bio systems, efficient metrology for manufacture is essential.

The present paper seeks to contextualise the current position with regard to metrology for bio systems. The metrology need is presented through the increasing use of structured surfaces combined with bio related products. The need for functional models to aid specification is outlined and several case studies are presented.

2. Micro Nano Manufacturing Technologies

Many products are beginning to exploit MNMT (Micro Nano Manufacturing Technologies) enabled structured surface geometries to beneficially affect functionality. There are examples from electronics, photonics, fluidics, bio-engineering, energy and consumer product fields. However, the reality for commercial enterprises is that in the absence of reliable, easily applicable models and specification tools, many different surfaces must be trialled on an ad-hoc basis until the process variables achieve the desired functionality. Furthermore, the choice of geometries is often seriously limited by the MNMT techniques available; both for measurement techniques and manufacturing technologies.

Consequently, the implementation of micro/nano-scale surface structures is clearly hindered and in many cases prevented. A further limitation to MNMT research exploitation is that the up-scaling to real products or processes is prevented by the lack of knowledge, design and specification rules and limited measurement techniques, particularly in-process methods

The MINAM Roadmap[1] "Nano-manufacturing", Nanofutures Roadmap[2] and Manufacturing Forsight[3] highlight a range of micro/nano-manufacturing technologies forecast to be ready for industrial application in the short to medium term, where design, specification, quality and traceable measurement will be critical for innovative product development. The focus of these roadmaps is the combination of both traditional macro and micro/nano-manufacturing equipment and processes. Bio systems have been highlighted in these reports as an area ripe for exploitation. Optimised design rules allied to a defined, traceable and function related measurement processes are essential to attain the functionality of the MNMT enabled engineered surfaces and are critical to up-scaling research. Large scale manufacture of a new range of high-value products incorporating micro/nano scale structured surfaces with "designed in" functionalities from the micro- to nano-scale can facilitate strategic in high value manufacturing. A step-change in the scientific understanding of the product surface functionality, the design and traceable specification of surfaces as well as the surface structuring capability during the manufacturing process, is required to translate these in to innovative manufacturing processes which advanced economies can exploit.

3. Structured geometry surfaces

The process of creating a surface leaves a "finger print" on the surface of a part that is a unique result of the creation process. However surfaces are increasingly "structured", where global geometric processing imparts pre-determined surface functional property [4]. With the increasing application of MNMT components, surfaces and their associated properties are recognised as **the critical factor** dominating function, for example in optimising cellular attachment to a surface. Consequently to maximise the component functionality there has been a large focus on the component surfaces and designing the surface structures to optimise a particular surface-related function. This had led to a reclassification of surface types as shown in figure 1 in this classification structured surfaces are considered to be engineered towards a desired functional outcome. A critical consideration for structured surfaces is that at present there is limited understanding of the sensitivity of the functional behaviour in relation to changes of relevant and critical structure parameters and additionally to the sensitivity of instruments to measure such critical parameters including their measurement uncertainty.

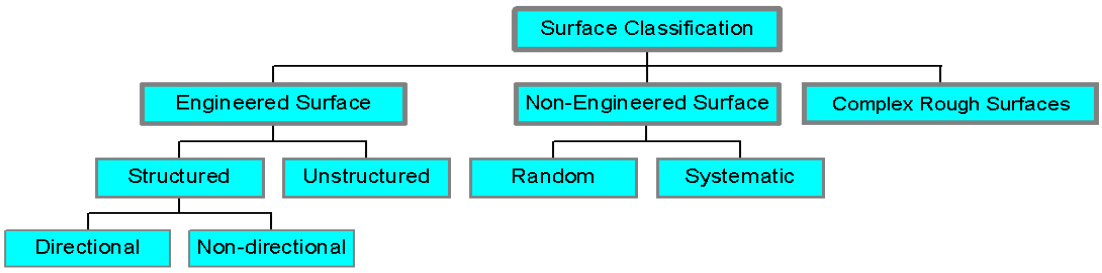

Figure 1 Classification of Structured Surfaces[4]

In traditional surface manufacturing processes the surface resulting from traditional machining processes are usually termed as "non-engineered". However, components, which have surfaces with features organised as deterministic patterns are becoming both technologically and economically critical in field such as biotechnology. The deterministic patterns on these surfaces include tessellations, rotationally symmetric patterns and linear patterns and cannot be quantified conventionally [5]. These structured surfaces whilst new to engineering products have been observed

in natural bio systems; from the self-cleaning abilities of the lotus leaf to the optimised fluid flow over shark skin[6,7].

The scale of surface structures can vary from the nano-scale to the millimetre scale. What is critical is that the structure operates at the scale of the unit event of the function that the structure is trying to influence. For example, where osseointegration is the desired function, manufactured porous structures optimised to cellular scale have been produced on titanium dental implants[8]. Bio structured surfaces comprise a dominant surface feature pattern, where the feature and properties are specifically designed to address a desired function of the surface. The range of functions is varied and across many scales, for example based on a biomimetic approach surface structures have been developed which mimic for example shark skin scales and at the appropriate scale, these surfaces show anti-fouling abilities figure 2 [9 10]. Another effect copied from nature is self-healing and many "wounds" in plants and animals "heal" themselves. This extraordinary ability is now being developed for automotive paint finishing. Self-healing coatings in the automotive industry applications lead to a better appearance and durability of the lacquer. Surface damage (abrasion, impact) from the nano-scale to the micrometre scale, now have the capability for self-healing [11].

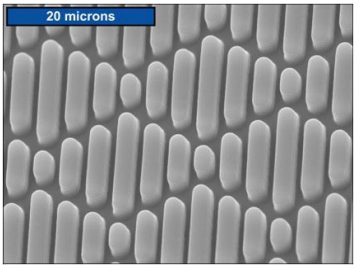

Fig 2 SEM image of sharklet™ surface produced on a polymer film

The current state of the art is that virtually all of these applications of structured surfaces are applied without specified guidelines, where the function is not fully understood or modelled and this is particularly problematic in the bio system field as few quantitative models for structured surfaces exist. Additionally the means of production and quality assurance and the standards infrastructure has yet to be fully developed. Consequently the underlying problem for wider application of bio structured surface products has always been the incorporation of function models within a design and specification tool, combined with inadequate measurement and characterization of the structured surfaces.

3. Surfaces for Bio Systems

3.1 Friction

Bio system models relating the functionality to surface geometry are rare. Bio systems are not easily quantified due to the extensive variables involved and empirical approaches are often used to develop functionally related surface topographies.

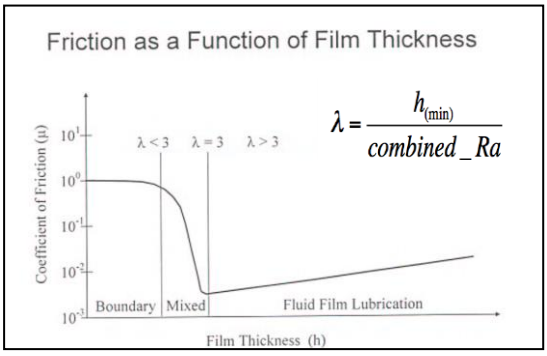

Figure 3 Stribeck Curve

However one of the most widely models adopted for use in the bio system field is the Stribeck curve as applied to prosthetic knee and hip joint bearing surfaces [12]. The Stribeck curve figure 3, can relate the coefficient of friction to the lubrication film thickness and the dominant lubrication regime pertaining to the given conditions can be described. Minimising wear in metal on metal hip joints for example is critical to their life in vivo if failure by aseptic loosening [13] is to be avoided and the 20 year lifespan target for such devices is reached. It is generally accepted that good fluid film lubrication occurs when the fluid-film thickness is three times larger than the combined average surface roughness of the articulating surfaces.

Theoretical calculations using equation figure are used to determine the lubrication regimes generated between the bearing surfaces. Research and clinical follow up data shows that both the knee and hip systems have a longer "life in service" if they are manufactured with nanoscale surface roughness on the bearing surfaces and micron level form/size tolerance. Fisher et al [12] showed an "increase of 0.1um in surface roughness resulted in a 13 fold increase in wear. The metrology approach taken by the industry in the implementation of this model is fairly basic and is used in component design. Manufacturing specifications for roughness are of the order of Sa=5nm. The surface can be measured at a suitable nanometer resolution using for example an optical interferometer and the average surface roughness Ra or Sa and the maximum peak to valley roughness Sz recorded.

3.2 Fluid Flow

The lab on a chip (LoC) concept has been a fertile area in for exploitation of microfluidics. Figure 4a. shows the Self-powered Integrated Microfluidic Blood Analysis System SIMBAS[15]. In this device relatively heavy white and red blood cell are separated using small trenches within the 100µm channels. Handling of and separating of blood cells to allow specific diagnostic process to be carried out and requires highly accurate devices with specific flow characteristics through accurately produced reactor channels [4,13]. The powered LoC shown, figures 4 b and c, is designed to separate blood products. The reactor channels here are clearly visible from the surface measurement. The channels are arranged such that human blood platelets (thrombocytes 2um diameter) can be separated from red cells, (erythrocytes 6-8um) and white cells leukocytes (7.5um). The flow through the reactor channels is governed by the Hagen Poiseuille Equation:-

$$Q = \frac{\Delta P.\omega.h^3}{12L\mu} \quad \ldots\ldots\ldots (1)$$

where Q= flow rate m^3/s, L = Length of micro channel m, ω = channel width m, h = channel height m, µ = dynamic viscosity Pa.s, ΔP = pressure drop along channel Pa. Total flow rate calculations assume all channels are free flowing. The channels are densely distributed and are greatly influenced by the defects from the moulding process used for manufacture, it is difficult and time consuming to manually evaluate each channel for blockages. In this case surface metrology followed by segmentation has been shown to have the capability to assess channel dimensions and to highlight blockages automatically.

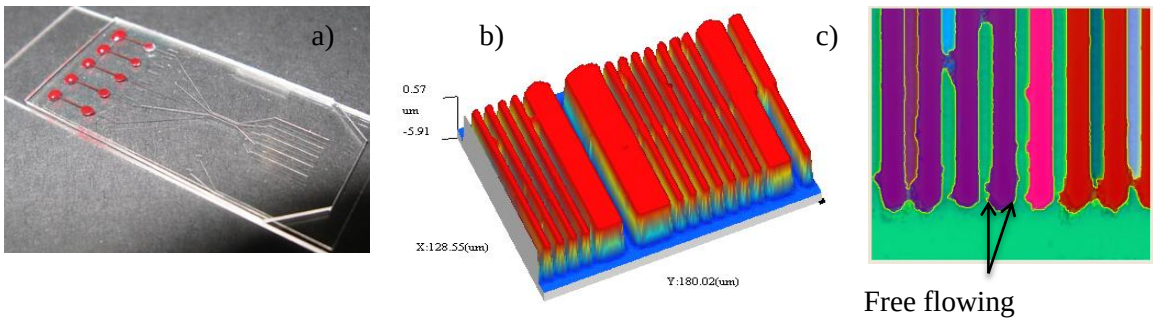

Figure 4 Microfluidic device for separating human blood cells; Self-powered Integrated Microfluidic Blood Analysis System 4b) measured reactor surface 4c) segmented surface data showing open and closed channels [4]

By processing the collected surface data using the Sobel edge operator, the step planes can be distinguished from the base plane. When applying the operator the step edges are transformed to a

“fence” forming a close contour around an isolated feature. Shown in Figure.4(c), the edges of the step planes form clear boundaries with the base plane and dimensions of the step planes can be easily extracted. In this case the whole measured surface was segmented into 9 sections, 8 for the channels and 1 for the base and channel path plane. The edge lines are perfectly consistent with the edge of the channel walls. It is clear that 5 channels are partially blocked by defects and 2 channels are visibly free fluid paths. The channel dimensions can be extracted by using standard step height algorithms once the data is segmented. In conclusion the techniques developed for feature parameters, with a minor modification(edge detection) can be applied to features on the component surface and allow for standard surface metrology in this case optical interferometry, as well simultaneously facilitating dimensional analysis.

3.3 Cell Culture

Cell culture is widely used for both diagnostic, therapeutic purposes and for the production of biological agents, such as vaccines or pharmaceuticals. In general, cell culture is divided into two sub-groups, adherent cell culture and suspension cell culture. Adherent cell culture requires cells to adhere to surfaces, whereas suspension cell culture requires that cells are kept in suspension in order to obtain the right cell behavior. Traditionally, suspension cell culture has been conducted in large bulk reactors, where the surface was negligible compared to the volume of production. However, many applications such as stem cell culture require less cells to be cultured in small individual volumes, where large bulk reactors [16]. are not a feasible solution. In this case, the best solution is to have a completely non-adherent surface on the surface of small containers. Most low-adherence surfaces are made through a proper selection of chemistry, where polypropylene is the most commonly chosen material. However, by designing the surface topography, a decrease in adherence of as much as a factor of 50 may be obtained by utilizing the lotus-effect, where micro or nanostructures allows the formation of a completely non-adherent air-layer to form in between the structures, and the only remaining adherence is the points where the structures support the liquid cell culture medium Although, the lotus effect has been known for 50 years, and many demonstrations of the effect has been made on polymeric surfaces, large scale manufacture and characterisation/quality control is difficult.

Non-adherent surfaces are important because in-vivo cells do not normally experience any hard (artificial) surfaces. Within stem cells or multi-type-cell colonies, properties such as cell communication, differentiation and enzyme complexing will be affected by cell adherence. In order to study cells that behave as naturally as possible, non-binding surfaces are required. Currently polypropylene (PP) or poly-ethylene-glycol (PEG) grafted surfaces that form a hydrogel on the surface are used for non-binding cells. Nevertheless, proteins will adhere on PP, and if the PEG grafted surfaces is not dense enough, proteins will also adhere on these surfaces. A new innovative solution [17] has been developed using a roll to roll (R2R) extrusion coating method, which has proven to be particularly efficient in the manufacturing of lotus-effect structures. This surface structuring method combines PP surfaces with nanoscale structures and the technique is able to reduce cell-surface interaction by a factor of 30-50. Significant demands on characterisation methods are encountered especially where in process measurement is required.

Metrology in this case needs to be fast in-line optical characterisation. Possible solutions additionally need to be insensitive to environmental mechanical noise, have the ability to auto focus on the measurement substrate and the characterization software needs to be able to detect and map departures form the nominal structure and produce a running defect map. Recently reported solutions to such metrology requirements in the R2R field have been reported as part of two large research programs NanoMend (EU) [18] and Vitriflex USA) [19]. In the Nanomend project a dual path Wavelength Scanning Interferometer is employed where an IR based interferometer is used for environmental stabilization feedback and an autofocus sytem is used to position the optics. This is combined with an air bearing substrate holding system and a software analysis capability that can handle large amount of

data (200Mb for a single 500mm measurement stripe) and extract salient features such as deviations from nominal and catalogue isolated defects.

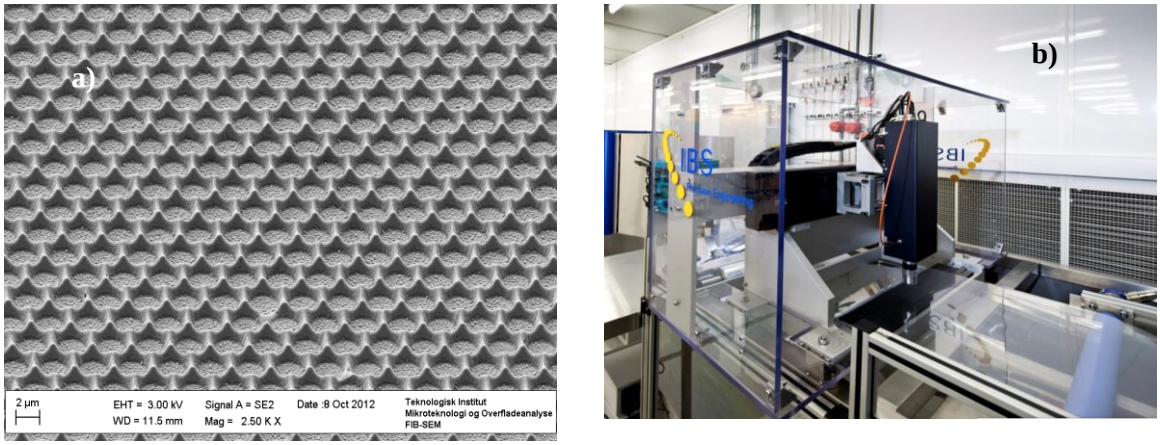

Figure 5. 5a) Superhydrophobic structures made by R2R extrusion coating 5b) Implemented WSI system in an R2R production environment.

3.4 Scaffolds and bio functionalised surfaces:

One of the major obstacles in scaffold based regenerative medicine is the lack of standardisation of materials and methods used for the realisation of tissue engineered constructs. None of the technologies: biomaterial development and processing, cell provision, cell cultivation, tissue characterisation are currently regulated and process measurement is lagging significantly. The dramatic increases in the need for bioactive materials for tissue regeneration on one side and the limited availability of transplantable bio inert materials on the other side requires fundamental approaches such new materials and their metrology to be investigated.

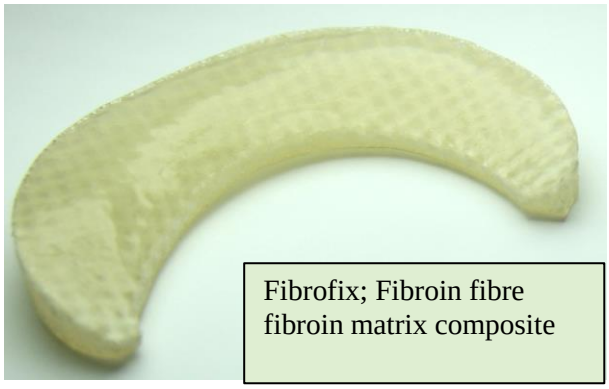

Figure 6 FibroFix meniscal implant, a fibroin/fibroin fibre composite material *courtesy of Orthox Ltd* "Supported by the Innovate UK Advanced Materials Award 101221"

The authors have recently taken part in a project to develop implantable scaffolds suitable for replacement of human knee meniscal cartilage [20]. This type of tissue replacement procedure is highly relevant since circa 1.7 million meniscectomies are annually performed worldwide. Fibroin is a bio compatible protein extracted from natural silk and shows structural homology to fibronectin, a constituent material in cartilage. Silk from the mulberry silk worm, *Bombyx mori*, is inexpensive and manufactured on a commercial scale, yet unlike spider silk it is not known for its inherent strength as it is spun in a different way. The UK based Oxford University spin out company Orthox manufacture regenerative cartilage tissue scaffolds FibroFix Meniscus and FibroFix Cartilage. Formed from a protein extracted from silk fibres, they are anatomically shaped, mechanically functional implants with smooth surface characteristics which are designed to restore the patients cartilage to full function whilst simultaneously supporting regeneration of the patients own cartilage tissue through the porous body of the implant.

In the production process the outer layer of silkworm silk fibers is stripped off leaving the fibroin protein core, which is subsequently dissolved to give an aqueous fibroin solution. The aqueous silk fibroin is then processed using individualised moulds resulting in porous knee cartilage implants known as FibroFix™. The processing has been developed from an understanding of how a spider spins its silk into a highly ordered molecular structure, leading to implants with high compressive moduli. Further tensile strength is gained by incorporating fibroin fibres around which the porous fibroin is moulded, thereby forming a fibroin-matrix fibroin-fibre composite figure 6. The primary function of the material is to provide a good bearing surface, whilst providing structural support and enabling regeneration of *de novo* cartilage tissue through the porous body of the implant.

As human anatomy and physiology is variable, and as cartilage surfaces vary from person to person, it is difficult to establish the exact geometrical and surface characteristics of "healthy" cartilage from which to benchmark. In a studies by Shekhwat et al [21] Ghosh et al [22], the surface roughness of articular cartilage was measured using scanning white light interferometry and Atomic Force Microscopy (AFM), there was a large variation found in measurement dependent upon the technique used, and the magnification used with gave surface roughness (Sa) variations from 25nm to 1.25µm were reported. These studies highlighted the inherent variation in surface topography of "healthy" cartilage, and also the variation in metrology techniques. A more useful benchmark for the component developed was taken from ISO 7207-2:2011 Implants for surgery – Components for partial and total knee joint prostheses-Part 2: Articulating surfaces. Here the *Ra* maximum value of ≤ 0.2 µm is stipulated for polymer components. This reflects the conformal nature of the polymer surfaces, and was taken as the benchmark for the FibroFix surface. The additional metrology challenge for quantifying the surfaces is that they must be measured fully hydrated, clearly not a feasible possibility using interferometery though possible using AFM and a liquid cell tip. The solution developed was to use a silicone replica (Microset) and measure larger areas indirectly figure 6a and b.

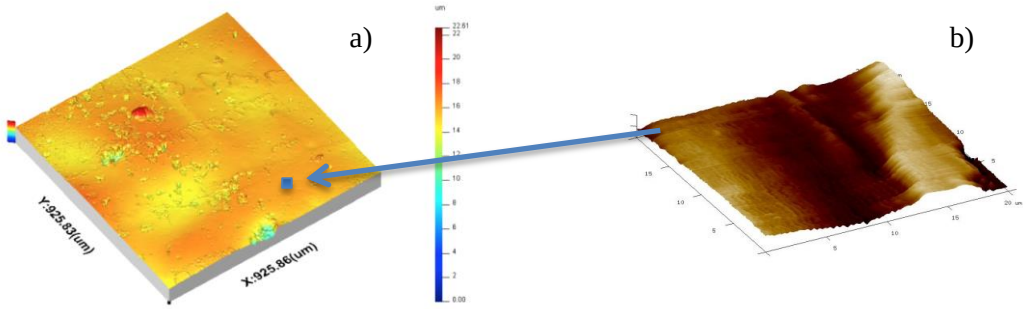

Figure 7 a) FibroFix articular cartilage Sq = 0.25µm measured by interferometry using silicone replica b) Direct AFM FibroFix surface measured using a liquid cell tip (note differing area size)

Extensive in vitro trials have indicated favourable results and approvals for human clinical trials has now been given.

4. Conclusions

Outside of the traditional manufacture and use of largely metallic prosthetic joints, the advent of MNMT enabled bio systems has highlighted a deficit in the tools for design, specification, quality and traceable measurement. This deficit can be traced to the lack functional models. Additionally in terms of scale up from research to product, traceable metrology for process development and quality control has also proved to be elusive. In the context of surface metrology, new paradigms have

become evident such as extensive use of structured surfaces, soft or living surfaces, in process measurement need and metrology in challenging (hydrated) environments. The present paper has attempted to highlight these new paradigms and has illustrated some of the issues and possible solutions through a series of exemplar cases. What is evident from these cases is that traditional surface metrology solutions are inadequate for bio systems and the case for functional models informing the characterisation protocol is critical.

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