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Determining the relative importance of titania nanotubes characteristics on bone implant surface performance: a quality by design study with a fuzzy approach

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Abstract

TiO2 nanotubes (TNTs) are suggested as an ideal bone/dental implant surface modification strategy with tailorable cellular modulation and therapeutic functions. However, inconsistencies related to the understanding of the influence of various TNTs characteristics on Ti implant functions, and the multi-factorial inter-dependence of such characteristics, requires an in-depth quality by design (QbD) analysis to derive optimized TNTs-modified implants. To this end, an extensive systematic literature search was undertaken to identify the various TNTs characteristics that may influence implant performance. Subsequently, in order to facilitate a QbD analysis, an expert questionnaire survey was carried out to determine the perceived contribution of the various TNTs characteristics on an implants' biological, physicochemical, and mechanical performance. To achieve this goal, the quality function deployment method was employed using symmetrical triangular fuzzy numbers to translate qualitative expert opinion into meaningful quantitative information. The results show that pore diameter, inter-nanotube distance and wall thickness were considered to be the TNTs characteristics with the most influential effects on the overall implant performance. This study is the first to evaluate the percieved importance of various parameters contribiting to TNT functionality, and represents a step forward in the implementation of QbD strategies towards nanoscale Ti implant surface modification approaches.

Keywords: Quality by Design, Titania Nanotubes, Quality Function Deployment, Triangular Fuzzy Numbers, Bone/Dental Implants.

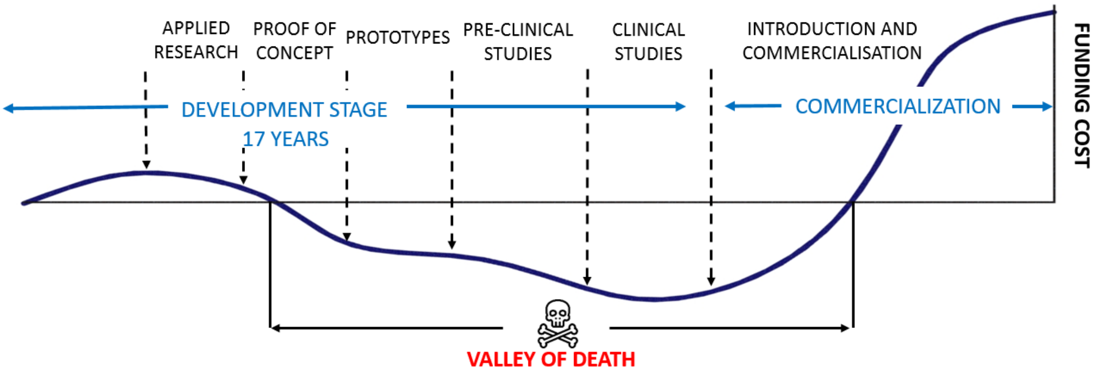

Figure 1. The "valley of death" in the product development life cycle of medical devices.

1. Introduction

Titanium (Ti) and its alloys are the ideal material choice for orthopaedic and dental implant applications, owing to their biocompatibility and corrosion resistance [1]. However, despite the excellent biocompatibility and mechanical properties of Ti and Ti alloys, they usually require long healing periods to create a stable interface with the surrounding bone [2], with insufficient implant osseointegration a potential outcome [3].

Hence, to further augment its bioactivity, Ti surface modifications have been performed in the macro, micro and nano-scales [4-6]. Briefly these include hydroxyapatite/polymer coating, protein immobilization and various topography modifications achieved by anodization or acid-etching [7]. Numerous in-vitro and in-vivo investigations have established that nanoscale topography outperforms macro and micro-scale surface features towards augmenting cellular functions [8]. Additionally, studies have also revealed immune-modulatory and antibacterial effects from various nano-scale surface modifications [9].

Among the various nano-topographic modifications of Ti, electrochemically anodized (EA) TiO2 nanotubes (TNTs) stand out due to their enhanced bioactivity, and ability to achieve local drug elution [10-12]. EA is a cost effective strategy to fabricate highly ordered TiO2 nanostructures with a great degree of control over their dimensions [13]. Moreover, TNTs have been explored extensively to investigate and optimize their bioactivity and therapeutic functions [14-17]. The ability to load and locally elute various therapeutics from nanotubes has demonstrated customizable anti-bacterial, immune-inflammatory and osteogenic functions [14-17].

Nonetheless, for TNTs based Ti surface modifications to survive the implant product development "valley of death'', a number of issues must be addressed [18]. The "valley of death", as shown in Fig 1, is the stage of the product development life cycle where the majority of products and new ventures fail due the large investment necessary for pre-clinical and clinical trials, necessary for subsequent clinical translation and commercialization [19]. In the case of TNTs-Ti implants, these challenges include the optimized fabrication of nanotubes on commercial implant surfaces via controlled EA parameters, in-depth mechanical stability investigations, long-term in vivo studies under mechanical loading, , and quantification of local drug release inside the bone micro-environment [20, 21].

Furthermore, for such novel implant technologies, rigorous regulatory requirements and the need for costly pre-clinical/clinical studies further exacerbates the translational challenges [22, 23]. Nevertheless, the recent advent of Quality by Design (QbD) has the potential to assist with the integration of novel medical technologies into current industries [24].

The QbD system was first created by Joseph Juran in 1985 and adopted by the US Food and Drug Administration (FDA) in 2004 [25, 26]. In relation to this study, QbD aims to facilitate the design of commercially viable nano-engineered implants by integrating different technologies within the same framework, thus considering all aspects of their life cycle [27]. The result is a flexible regulatory framework designed to optimize the product development and manufacturing processes, which in turn aims to enhance the overall quality [26]. QbD focuses on acquiring process control through a deep understanding of products and processes using science, engineering and quality risk management [28]. Moreover, QbD accelerates research timelines and reduces development costs, by avoiding trial-and-error studies, and focuses on utilizing methods aimed towards product development [29-31]. For example, a systematic implementation of the QbD system can lead to a reduction of experimental runs by up to 90% [19]. As a result, the implementation of QbD can help to reduce the regulatory burden that forces many product engineers to purposely design their products to fit within existing approved thresholds in order to avoid seeking further time consuming approvals for minor variations [32]. The QbD system comprises of eight steps that should be followed in a systematic feedback forward loop to achieve its benefits and allow for continuous improvement, as presented in Fig 2.

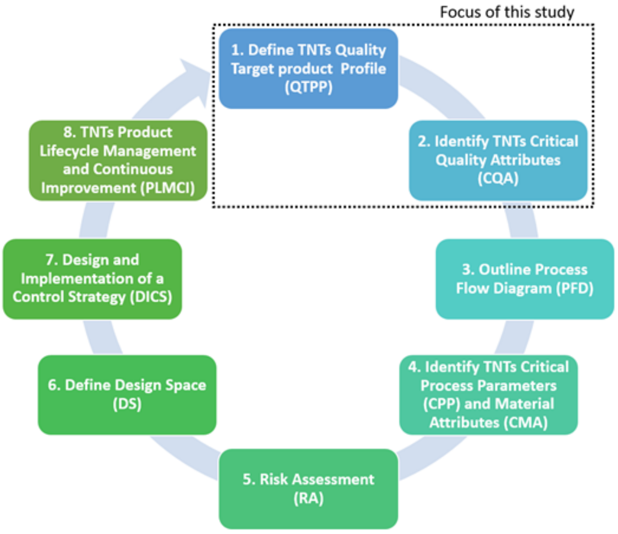

Figure 2. QbD systematic product and process design development flow chart, showing the focus of this study. TNTs: titania nanotubes.

2. Purpose and objectives

The purpose of this research is to provide researchers and industry with an in-depth adaptation of the QbD framework for the fabrication of TNTs-Ti bone/dental implants. QbD considers the ICH Harmonised Tripartite Guideline Pharmaceutical Development Q8(R2) [33] and existing quality engineering management tools. The current study has the following three main objectives:

1. Identify and rank the critical quality attributes (CQA) of TNTs-Ti surfaces that can enhance bone/dental implant performance.
2. Identify the relationships between the CQA of TNTs-Ti surfaces and TNTs characteristics for bone/dental implant performance.
3. Identify the level of perceived contribution of TNTs characteristics to the overall implant performance from the biological, physicochemical, and mechanical perspective.

3. Scope

The QbD approach comprises of eight main steps (Fig 2) that need to be systematically followed to acquire a complete comprehension of the product and its manufacturing process, including the identification and control of all variables to achieve the desired quality. Specifically, the scope of this present study was limited to the implementation of the first two steps of the QbD framework for TNTs fabrication via electrochemical anodization (EA). Details on how TNTs characteristics can be influenced by the various EA parameters is outside the scope of the present study.

4. Materials and methods

Taking into consideration the nature of this study (engineering management research in the field of nano-fabrication) a constructive research approach was adopted [34, 35]. The rationale behind this strategy was to formulate the QbD system for TNTs-Ti implants in order to qualitatively identify the level of contribution of TNTs characteristics to implants' biological, physicochemical, and mechanical performance. The solution for the first two steps of the QbD system was performed by applying two engineering management problem-solving techniques, namely QFD method and the HoQ matrix. Moreover, this constructive research approach requires both a systematic search and a questionnaire survey. The systematic search follows the premises of a systematic review according to the Prisma statement [36] to acquire an in-depth understanding of the problem and to extract key information necessary to design the questionnaire survey. Next, the questionnaire aims to extract industry and academic expertise to reveal specific information. The six phases of the constructive research approach were followed [34], to achieve the study's three main objectives, namely:

1. Find a challenge that has significant research potential (i.e. addressing research gaps in knowledge by formulating the QbD system for the development of TNTs).
2. Perform a systematic search, followed by tailoring the QbD system for TNTs.
3. Extend the QbD system into the emerging field of nanoengineered surfaces with TNTs for bone/dental implants.
4. Demonstrate that a systematic implementation of the QbD system for TNTs-Ti implants can assist in the qualitative identification of the most influential TNTs characteristics that can affect implant functions.
5. Show the connections between the research contribution and published literature. The newly proposed QbD approach is supported by both primary and secondary data from various datasets of peer-reviewed publications, and the questionnaire survey completed by the experts in the field of study (TNTs-based Ti bone/dental implants).
6. Examine the applicability of the QbD solution. This study will facilitate the design of TNTs-based Tibone/dental implants through a qualitative

identification of the relationship of TNTs characteristics with implant's mechanical, biological and physicochemical performances.

4.1 Data collection

The data collection for this study involved a systematic literature search as well as an online-questionnaire, which were integrated to develop the adaptation of the QbD system for the fabrication of TNTs implants. The systematic search was designed following the Prisma statement 27-item check list [36], and performed using the Google Scholar and Elsevier databases until the 2nd of April 2020. The main goal of the systematic search was to identify the CQA of TNTs-Ti implants that may influence implant functioning.

The online-questionnaire was designed to identify the significance of the CQAs of TNTs-based Ti implants, including the level of influence of TNTs characteristics in relation the CQAs. The design of the questionnaire was based on the principle the "wisdom of crowds" (WOC) which is widely used to solve complex forecasting problems in different research fields such as economics, molecular biology, neurocomputing, and robotics [37-40]. It has been demostrated in the forecasting literature that using the WOC method often perform as well as more sophisticated statistical methods [41, 42]. According to Budescu and Chen [43] the claim behind WOC is that "the judgments of a group of individuals will be more accurate than those of the average individual by exploiting the benefit of error cancellation". However, when a surveyed group is composed of uninformed judges can lead to inaccurate forecasts [44]. Therefore, it is noteworthy that the evaluation of this study is inherently reliant on expert judgment [43, 45]. It is therefore important that such assessments are based on all reliable and relevant scientific data, and that assessment principles and assumptions are transparently applied [37]. For more details about the data collection methodologies refer to the Electronic Supplementary Material.

4.2 Study selection and survey participation

The studies from the systematic search were selected according to the following inclusion criteria: (1) peer-reviewed papers with full-text published in the last 20 years (2000-2020); (2) studies describing methodologies and experimental factors used to control the mechanical, biological, and physicochemical properties of TNTs; (3) published in the English language; (4) articles within the first 30 pages of the search results; and (5) sort the search results by relevance.

The criteria to select the participants for the questionnaire was based on their experience and expertise in the field of study (TNTs-based Ti bone/dental implants) [46]. Moreover, taking into consideration that TNTs technology has not been translated into the commercial implant industry, only researchers in the field of TNTs fabrication via EA, orthopaedic/dental implants, bone biology and Ti implant-bone interaction were selected. It is noteworthy that the sample size of this study was limited by the number of researchers in this domain. In addition, the snowball sampling method was selected since it allows for further study participants to be suggested or introduced from the existent participant network [47].

4.3 Data extraction and analysis

For the systematic literature search, full-text screening was independently performed by D.M. and K.G. Any discrepancy between the reviewers was resolved by a consensus meeting. The articles were thoroughly reviewed, analysed and classified based on the following information: nano-engineered surfaces and TNTs' mechanical, biological, and physicochemical properties. Additionally, the reference list from collected papers was systematically reviewed to find further related research items. Once all applicable literature was identified the QbD system was specifically formulated for the fabrication of TNTs-Ti implants. More details are provided in the Electronic Supplementary Material.

The questionnaire consisted of two main sections, each divided into three subsections: biological, physicochemical, and mechanical. The first main section was for the rating of the CQA (biological, physicochemical, and mechanical) of TNTs-based Ti bone/dental implants. The second section was to identify the relationship of TNT characteristics with the CQA. Each participant had the option to choose the questionnaire sections accordingly to their field of expertise. The questionnaire design was based on the house of quality (HoQ) matrix, which is part of the Quality Function Deployment (QFD) method developed by Mitsubishi in 1972 and adopted by a variety of firms such as Toyota, Procter & Gamble, Ford, GM, Hewlett-Packard, and Puritan-Bennett to assist and accelerate the product development process [48, 49] (Fig 3).

The HoQ is a tool used to translate customer's requirements into product design and to identify the design

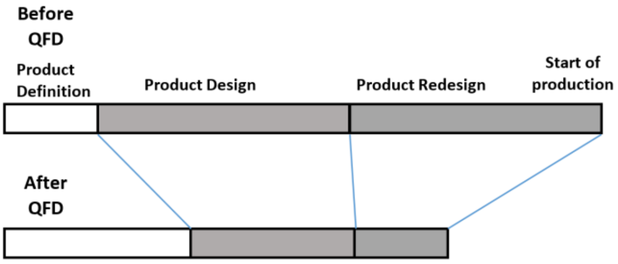

Figure 3. Effect of Quality Function Deployment (QFD) on product development phases.

characteristics that best satisfy the customer's needs [48]. Therefore, by using the HoQ, it is possible to find the TNTs characteristics that have the most impact on implant performance, thus facilitating the identification of fabrication factors which need more attention during the quality control process. At the top of the matrix is the roof of the HoQ where the correlation of the design characteristics are allocated to help to identify existing conflicts or constraints between them [50]. It has to be noted that changes in one engineering attribute may affect others. Therefore, the roof of the HoQ facilitates the identification of the relationships between design variables in order to eliminate negative relationships and contradictions [50]. Nevertheless, this section of the HoQ was not covered by this study.

Human subjects tends to interpret information and assess concepts in a subjective manner using linguistic terms, therefore it is not reasonable to use defined and precise numbers in the application the HoQ matrix. Moreover, the complexity of nano-scale interactions of biological systems makes the development of TNTs-Ti implants cumbersome, due to the limitations of precise mathematics for dealing with animated systems [51]. Therefore, fuzzy logic is an ideal approach to model, describe, and solve complex biomedical problems [52].

Zadeh [53] introduced the concept of fuzzy sets to deal with the "principle of incompatibility", which states that precision is inversely proportional to complexity. This principle can be seen in stochastic and uncertain soft systems inherent in medicine, biology, genetics, and agriculture [52]. Fuzzy logic was developed for solving problems in which descriptions of objects are subjective, vague and imprecise Chan and Wu [54]. It uses partial membership values to classify imprecise data, in which the transition from membership to non-membership is gradual rather than abrupt [53]. Subsequently, the assessment of the HoQ of this study was performed using symmetrical triangular fuzzy numbers (STFNs), so their vagueness can be captured, thereby allowing a more accurate description of the decision-making process. This in turn provides a suitable method to extract and summarise qualitative expert knowledge to be used in decision support systems, such as HoQ [55, 56]. Further details on the data extraction and analysis procedures are provided in the Electronic Supplementary Material.

5. Results

The systematic search identified a total of 181 studies which were qualitatively analysed to tailored steps 1 and 2 of the QbD system for TNTs implants. For the online questionnaire, a total of 57 expert participants were invited and 39 researchers and medical practitioners responded (response rate of 68.4%). From the total participants 37 responded the first section of the questionnaire, and 13 participants responded the second section. It is noteworthy to mention that this study is characterized by small samples, however the samples involved detailed and extensive work [57]. The participants' pool included post-doctoral researchers, professors, and medical practitioners with an average of 14 years of experience (minimum 3 years, maximum 45 years) in different fields such as: biomaterials; tissue engineering; nano-engineered titanium implants; dental implantology; hard-/soft-tissue regeneration; wound healing; oral and maxillofacial surgery; bone interfacing implants; and orthopaedic surgery. Moreover, the survey covered participants located in North America, South America, Europe, Asia, and Oceania.

5.1 QbD Step 1: ideal quality target product profile (QTPP)

The QTPP helps to formulate the ideal characteristics and desired features of the product, taking into account safety and performance [58]. To establish the QTPP it is important to understand the users' (clinicians) needs in order to direct the product development process to a clear goal, while minimizing risks throughout the various development phases [59].

A recent study by the authors recommended that the quality of the next generation of bone implants should be defined from three broad unique perspectives, namely product-based, manufacturing-based, and user-based [19]. Moreover, within these three perspectives of quality there are eight different dimensions of quality used to describe product characteristics: (1) performance, (2) features, (3) reliability, (4) conformance, (5) durability, (6) perceived quality, (7) serviceability, and (8) aesthetics [60]. However, in the context of TNTs-modified implants, the first six dimensions of quality are the most relevant, as presented in Table 1.

In the product-based approach, quality is based on product attributes focusing on performance, features, and reliability. It is assumed that products with more attributes are more expensive [61]. This quality perspective in the biomedical device market is supported by the belief that a product with better attributes requires increased research and development, as well as expensive and advanced technologies for its manufacturing. The Performance quality dimension refers to the product's basic functional attributes that can be measured. Features refers to the additional attributes that complement the product's basic functions. Reliability is the third dimension and relates to the probability of failure in a specified period of time [60]. The manufacturing-based approach is the engineering and production point of view which is concerned with the product's quality deviations from specifications. Thus, the focus of this approach is to minimize product failure resulting in lower costs. In the case of medical devices, the manufacturing-based approach is strongly emphasized by the strict regulations imposed by medical regulatory organizations such as the FDA.

[1] **Table 1.** The ideal six quality dimensions of titania nanotubes (TNTs) modified titanium bone/dental implants.

Quality Approach	Dimension	Definition	Description
Product	Performance	Relates to measurable product attributes	TNTs surfaces should upregulate bone cell functions resulting in long-term osseointegration, in both orthopaedic and dental implant settings.
	Features	Complementary product's attributes	Tailored cellular modulation and local drug elution can be achieved by TNTs modification of implants.
	Reliability	Probability of failure	Optimized fabrication of mechanically robust TNTs on commercial implant geometries with a high degree of reproducibility, minimum defects, and zero failure rates (within their life expectancy).
Manufacturing	Conformance	Meeting standards	TNTs surfaces must comply with or exceed current medical regulations and quality standards.
	Durability	Product life span	TNTs should withstand mechanical forces experienced during handling, implantation surgery and subsequent function in a bone microenvironment constantly under load.
User	Perceived quality	Product brand image	Clinicians should have access to relevant information through medical reports and independent research data whereby TNTs-Ti implant performance can be evaluated.

The quality dimensions associated with this approach are conformance and durability. Conformance is related to how a product or process characteristic meets established standards. Durability refers to the product's life span having economic and technical aspects, which are closely related to reliability Garvin [62].

The user-based approach defines quality from a consumers' perspective, thus the products with the highest quality are those which best satisfy their preferences Garvin [62]. The quality dimension of this approach relevant to TNTs is perceived quality. This dimension is closely related to brand reputation where product brand image, advertising and market trends are the main drivers. On the contrary, in the orthopaedic/dental implant markets the clinician is the client, as they usually lead the decision-making process and base their decisions on medical and scientific reports describing the product's technical information such as failure rates.

5.2 QbD Step 2.1: critical quality attributes (CQA) of nanotube modified implants

The CQAs are the product characteristics that should be within specific limits to ensure that they comply with desired quality standards defined in the QTPP [33]. The identification of CQA is derived from prior knowledge about the product and the fabrication process [63] based on a scientific and risk management rationale, taking into consideration industry and regulatory requirements [28]. In this study the identification of the CQA of TNTs-Ti implants was informed through a systematic literature review. According to our results, there are 21 CQAs of TNTs-Ti implants that can be categorized into three main groups: biological, mechanical, and physicochemical [64-67], as shown in Table 2.

Table 2. Summary of CQA of nano-engineered Ti implant surfaces for orthopaedic and dental applications.

Biological CQA	Physicochemical CQA	Mechanical CQA
Protein adhesion	Wettability/surface energy	Modulus of elasticity
Cell adhesion	Surface charge	Wear resistance
Cell migration	Corrosion resistance	Fatigue strength
Cell proliferation	Topography	Compressive strength
Cell differentiation	Micro roughness	Bond/shear strength
Mineralization	Nano roughness	Hardness
Immune-inflammatory responses		Toughness
Antibacterial properties		

These three CQA groups contain the essential properties that need to be considered for development of TNTs-Ti implants due to their likely influence on implant performance based on the QTPP defined in the step 1.

To develop an effective engineering strategy for the design of these nano-engineered implants, it is vital to first identify the relative degree of importance of each CQA. To achieve this, each participant in the online-survey was asked to indicate their perception on the importance of each CQA based on a qualitative five rating scale proposed by Chan and Wu [54], as shown in Fig 4.

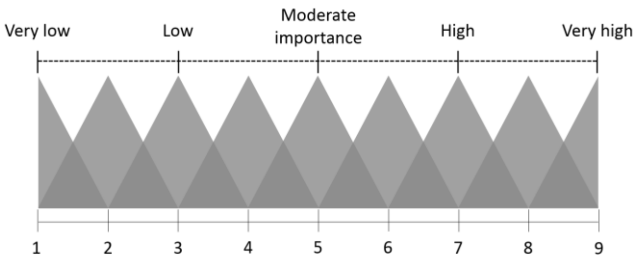

Figure 4. Qualitative five rating scale for CQA and its corresponding triangular fuzzy numbers.

5.2.1 Biological characteristics

Each implantable material inside the human body must satisfy the biocompatibility demands depending on the biochemical and physical environments of the implant location. In the case of bone/dental implants, the ultimate goal is to achieve osseointegration, which is the establishment and maintenance of a long-term bond between bone and the implant surface [68]. In the case of compromised osseointegration, a fibrous tissue encapsulating the implant may form leading to implant loosening and failure [69].

Based on our findings, there are eight main biological CQAs that need to be carefully addressed during the design of a nano-engineered implant surfaces. As perceived by the survey participants, immune-inflammatory response is the most important biological CQA with a fuzzy score between 7.16 and 8.24 out of 9 (Table 3). The high level of importance of this CQA is due to the fact that the regenerative healing process of tissues is initiated by the immune-inflammatory response which controls the initial response of the biological environment to the insertion of any biomaterial, and involves the conditioning of the material surface by the rapid adsorption of proteins [70, 71]. Notably, protein adhesion was identified as the fifth most important biological CQA. Moreover, during the bone healing process, the injury site is experiences variations including: oxygen tension, pH, growth factors, and mechanical stability [72]. Disturbances during this initial inflammatory process can potentially lead to impaired downstream bone healing [73]. Therefore, the inflammatory phase is the first step of the healing cascade that triggers the wound healing leading to bone formation and ultimately successful osseointegration [73-75].

Cell adhesion was rated as the second most important biological CQA with a fuzzy score between 6.38 and 7.97 out of 9, as shown in Table 3. According to Schneider et al. [76] osteoblast adhesion is a vital step towards [77-79] the deposition of a mineralized matrix [76, 78, 80] required to eventually achieve direct contact between the implant surface and bone tissue [81]. Poor cell adhesion on the other hand may result in implant failure [82]. Moreover, the quality of cell adhesion dictates a cell’s capacity to proliferate and differentiate [78, 81]. It is well established that the long-term success of the implant depends on the quality of osseointegration which is closely related to the mineralization of the bone’s extracellular matrix [83]. Therefore, bone mineralization is considered as one of the main performance indicators of bond strength between the implant and bone [81, 84, 85], and accordingly it was identified as the third most important CQA of TNTs for bone implants. This CQA has a very close fuzzy score to the one obtained for cell adhesion, meaning that these CQAs have the comparable importance. Following in importance as the 4th, 5th, 6th, and 7th rated biological CQA are cell differentiation, protein adhesion, cell migration, and cell proliferation, respectively, having crisp scores ranging from 6.46 to 6.78. It is noteworthy that the implant surfaces that can stimulate cell proliferation and differentiation may present better osteoinductive properties [72]. Additionally, adhesion of proteins onto the implant surface enables cellular attachment, and can facilitate mesenchymal-derived cells to migrate, proliferate, and differentiate into bone cells [86]. Moreover, cell migration is vital during the osteoconduction process for cell colonization of the implant surface [87].

Table 3. Relative importance rating (crisp and fuzzy) and ranking of physicochemical CQA of nano-engineered Ti implant surfaces.

Biological CQA	Crisp score	Fuzzy score	Ranking of CQA
Immune-inflammatory response	8.14	[7.16, 8.24]	1
Cell adhesion	7.38	[6.38, 7.97]	2
Mineralization	7.32	[6.32, 7.89]	3
Cell differentiation	6.78	[5.78, 7.46]	4
Protein adhesion	6.73	[5.73, 7.49]	5
Cell migration	6.57	[5.57, 7.30]	6
Cell proliferation	6.46	[5.46, 7.27]	7
Antibacterial properties	5.65	[4.65, 6.51]	8

It is noteworthy that the healing process can be disrupted by ingress of pathogenic bacteria [88] which requires extensive antibiotic therapy and can lead to complete implant failure requiring a revision surgery [89]. For example, more than 50% of infections in joint replacements are caused by Staphylococcus aureus, which leads to treatment costs that are five times higher in comparison to non-infected prosthesis [90]. As a result, one of the major challenges in the design of implant surfaces is the control of biofilm formation making it the eighth most important biological CQA, and it is notable that bacterial adhesion and colonization are also influenced by nano-topography [91].

5.2.2 Physicochemical characteristics

Material surface physicochemical properties such as surface roughness, topography, surface energy, corrosion resistance, are key factors that determine cellular behaviour on implant surfaces [92]. Furthermore, nano-scale modified surfaces can independently modulate these properties to enhance biological responses [93].

In this study a total of six physicochemical CQAs were identified and prioritized, as shown in Table 4. According to the completed survey, the participants indicated that micro-roughness is the most important physicochemical CQA, with a fuzzy score between 6.32 and 8.00 out of 9. Indeed, various clinical studies have suggested that micro-roughness (the 'gold standard') is one of the main factors that stimulates osseointegration in both bone and dental implants [94]. For example, osteoblasts attach, spread and proliferate faster on smooth and low energy surfaces [95, 96], whereas, those on rough and higher energy surfaces display accelerated differentiation, matrix mineralization and higher production of growth factors [97]. As a result, most of commercial orthopaedic and dental implants have a micro-rough topography [98]. It has been reported that the micro-roughness provides a better support for the initial fibrin clot extensions, thereby enhancing cell attachment and the strength of the ECM, and ultimately leading to enhanced osseointegration [99].

Table 4. Relative importance rating (crisp and fuzzy) and ranking of physicochemical CQA of nano-engineered Ti implant surfaces.

Physicochemical CQA	Crisp score	Fuzzy score	Ranking of CQA
Micro roughness	7.32	[6.32, 8.00]	1
Topography	7.22	[6.22, 7.95]	2
Corrosion resistance	6.95	[5.95, 7.59]	3
Nano roughness	6.84	[5.84, 7.59]	4
Wettability/surface energy	6.73	[5.73, 7.49]	5
Surface charge	6.03	[5.03, 6.92]	6

The second ranked physicochemical CQA was surface topography, which reflects the form, waviness, micro- and nano-scale features of its landscape (Fig 5). Roughness (Ra) is the quantification of the average of the measured microscopic peaks and valleys of these micro and nano-features. If these deviations are large, the surface is considered rough, and if they are small the surface is considered relatively smooth [100, 101]. These features can be either random or defined surface patterns that can create isotropic or anisotropic topographies [100]. The geometry, depth, spacing, width, pattern direction, and waviness of these features can affect tribological and chemical material surface properties such as friction, wear, surface energy and roughness [102-105], which will eventually dictate cellular responses and implant performance. Moreover, the direction of surface irregularities promotes cell orientation, showing a trend of cell elongation along patterns known as the contact guidance phenomenon [100, 106-108]. Additionally, appropriate surface topography coupled with surface wettability, could cause a synergistic effect on cell response [104, 109].

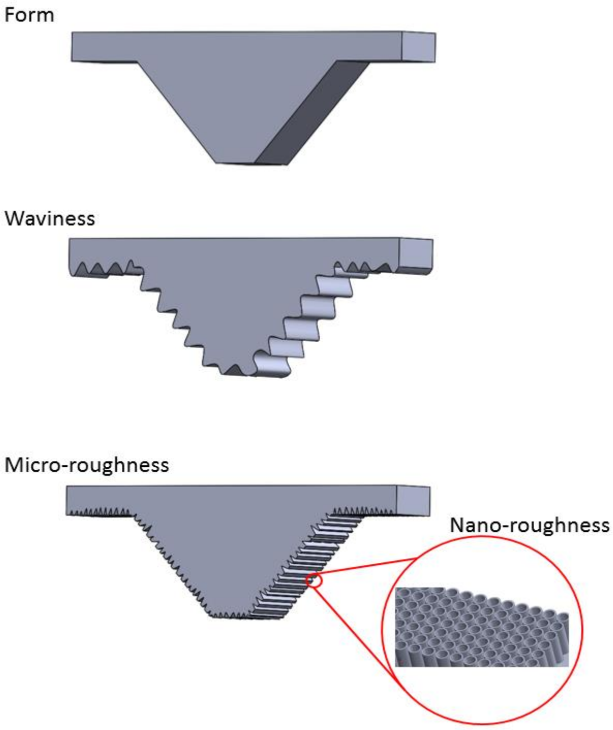

Figure 5. Schematic representation of the thread of an implant demonstrating form, waviness, micro-roughness, and nano-roughness (with titania nanotubes).

The third most important physicochemical CQA is corrosion resistance followed by nano-roughness, wettability, and surface charge. Once implanted, the implant surface undergoes electrochemical reactions with physiological fluids [110, 111]. It has been proposed that corrosion rates depend on the physical and chemical characteristics of the material surface such as roughness, surface crystal structure, and nanoporosity, including the physico-chemical state of the environment [112]. It is noteworthy that corrosion of implants (release of metal ions) can result in toxicity and implant rejection [113]. Additionally, corrosion leads to topological changes creating notches and pores that may affect the material's mechanical properties and reduce its fatigue resistance [114, 115]. Therefore, surface modification of Ti implants should not only enhance surface biocompatibility, but also improve their corrosion resistance without detrimental effects on mechanical properties [21].

It has been demonstrated by several studies that cell behaviour and attachment are directly influenced by nano-topography [106, 116], where cells move and sense their environment based on chemical and physical signals [64]. For example, it has been found that the smallest height of topographical features that human mesenchymal stem cells (MSCs) can sense is 8 nm [117]. While, no cell responses have been found (except red blood cells) for grooves greater than 20 µm [118]. Nonetheless, there is not a general trend that can describe cell adhesion of different cell types on micro/nanostructures. This means that each cell type will have a specific behaviour upon interaction with micro/nanostructures [116].

Protein adhesion is also affected by nano-rough surfaces [119]. Indeed, Gonzales-Garcia et al. [120] found that fibronectin adsorption can be increased by 50% with controlled fabrication of nano-topographies. This is possible because nanoscale modified surfaces can independently change surface energy, wettability and surface charge to attract low surface tension liquids thereby enhancing the cell adhesion [93, 96, 121, 122]. Moreover these physicochemical properties are closely interrelated [123]; however, their relationships are not always clear [124].

Furthermore, surface energy of implants is strongly correlated with cell adhesion and proliferation [124]. It is well established that Ti surface affinity for protein adsorption depends on its surface energy and charge [67, 111]. Surface energy affects the wettability of a surface, thereby playing a crucial role in the initial stage of wound healing [125, 126]. Different water contact angles (WCA) yield distinct cellular responses [64]. Additionally, wettability is strongly correlated with cell adhesion, proliferation, and bacterial adhesion [124]. However, there is an ongoing debate about which are the optimal WCAs [127] to enhance proteins adsorption for the most effective cell adhesion and proliferation. For example, hydrophilic surfaces enhance cell adhesion [125, 128]. Some authors suggested that the ideal WCA to facilitate protein adhesion, cell adhesion and proliferation will have moderate hydrophilicity [126, 129], with WCA ranging between 40◦ to 80◦ [64, 107, 130-133]. Nevertheless, a recent study demonstrated that super-hydrophilic (WCA of 0◦) and wetted super-hydrophobic surfaces (wetted SH, the Wenzel state with WCA between 140◦ to 160◦) displays a higher degree of fibroblast adhesion [134]. These results revealed that surface wetted states strongly influence the cytoskeletal organization and focal adhesion of cells, due to the increased nanoscale interaction between the cell surface and the nanostructured implant surface, resulting in enhanced cell adhesion performance [134]. An opposite cell response was found in another study where macrophage interaction with super-hydrophilic TNTs was studied. According to Gao et al. [135] macrophage proliferation rate and inflammatory response can be reduced by modification with super-hydrophilic TNTs. The findings confirmed that TNTs' wettability can be used to regulate macrophage responses to influence tissue repair and wound healing [135]. Moreover, it has been reported that TNTs surfaces have great potential to trigger the host inflammatory response through distinct pathways from conventional biochemical factors [136]. Taken together, the evidence suggests that there are three wettability states whereby cell adhesion and proliferation can be enhanced. These states are: super-hydrophilic (WCA 0◦), moderate hydrophilicity (WCA between 40◦ to 80◦), and wetted super-hydrophobic (WCA between 140◦ to 160◦).

The surface charge of metallic implants is also known to be a factor to guide bone cells adhesion and early stage bone mineralization [123]. As a result, strategies to directly modify an implant's surface charge has been gaining attention [123]. Surface charge, also known as Zeta potential (ζ-potential), plays a vital role in protein adsorption and initial cell adhesion [111, 137]. For example, positively charged biomaterials attract anions that repel molecules and proteins, negatively affecting osteoblast adhesion and proliferation [138]. However, Ti and its alloys have a negatively charged surface that initially attracts positively charged protein, thus forming a substrate that subsequently attracts negatively charged proteins such as fibrinogen and fibronectin [93, 139], as well as negatively charged osteoblasts [140]. Therefore, the electric field strength and the magnitude of the negative electric potential on the implant surface are crucial for the improvement of protein adsorption and cell adhesion [111, 139].

5.2.3 Mechanical stability

Appropriate mechanical properties of bone/dental implants are critical for their long-term functioning in a constant load-bearing application [21]. Consequently, nano-engineered surfaces of bone implants must overcome the concerns associated with mechanical stability before they can proceed to clinical trials [20]. According to our results it is perceived that the bond strength between the implant coating and the substrate is the most important mechanical CQA of TNTs modified Ti implants, with a fuzzy score between 6.89 and 8.33 out of 9 (Table 5). Inappropriate interfacial adhesion between Ti substrates and the TNTs film can lead to crack propagation and delamination, which may eventually lead to implant failure, thereby limiting its clinical applications [18, 141-147]. Consequently, the long-term success of modified Ti implants mainly depends on the adhesive and cohesive integrity of the modification [148, 149].

The second most important mechanical CQA was identified as the fatigue strength. In the case of load-bearing implants, fatigue resistance is critical in materials under constant in-vivo cyclic stress in order to avoid surface delamination and fractures [66, 150, 151]. For example, orthopaedic implants can experience up to six times the patient's weight during daily activities, during which the material stresses are just a fraction of the material's ultimate stress fatigue threshold. Nevertheless, in an implant with a life expectancy of 10 years (10^7 cycles), the accumulation of these small stresses can lead to coating fatigue failure [152]. Other factors that can affect the fatigue life of Ti-based implants are residual stresses and stress concentrators, caused by the high surface roughness of the coating, and by some manufacturing processes [153].

Table 5. Relative importance rating (crisp and fuzzy) and ranking of mechanical CQA of nano-engineered Ti implant surfaces.

Mechanical CQA	Crisp score	Fuzzy score	Ranking
Bond strength/shear strength	7.89	[6.89, 8.33]	1
Fatigue strength	7.22	[6.22, 7.94]	2
Wear resistance	6.44	[5.44, 7.33]	3
Compressive strength	6.44	[5.44, 7.22]	4
Modulus of elasticity	6.33	[5.33, 7.17]	5
Toughness	6.22	[5.22, 7.06]	6
Hardness	5.33	[4.33, 6.33]	7

In the third and fourth place are wear resistance and compressive strength, both with maximum fuzzy scores between 7.33 and 7.22 (out of 9) respectively. It is noteworthy that surface modifications with high wear resistance prevents fretting wear, which is caused at the bone-implant interface due to micromotions when an implant is loaded [154]. This is a destructive phenomenon that creates wear debris leading to osteolysis and eventually aseptic loosening [155, 156]. Therefore, surface modifications for bone/dental implants should be designed to consider the wear resistance at the bone-implant interphase. Bone tissue has evolved to mainly support compressive stress, it is 30 percent weaker under tensile stress, and 65 percent weaker under shear stress [157]. Similarly a biomimetic implant surface design for load bearing applications requires a high compressive strength to prevent fractures and improve functional stability [66].

The fifth, sixth, and seventh ranked mechanical CQA are modulus of elasticity, toughness, and hardness, respectively. Implant surfaces needs a modulus of elasticity close to natural healthy bone (3-30 GPa)[6, 158], in order to provide a uniform stress distribution [66], and prevent the stress shielding effect, which can cause severe bone resorption and promote implant micro-motion [159]. For the design of nano-scale implant surfaces, toughness must also be considered due to its close relationship with other mechanical properties. For example, adhesion of the surface modification can be enhanced with a higher coating toughness [160], and by reducing the material modulus of elasticity [161]. Enhanced toughness reduces the probability of crack initiation and propagation, and fatigue life [66, 162-164].

Interestingly, the most popular choice for bone/dental implants are Ti and its alloys which are considered soft

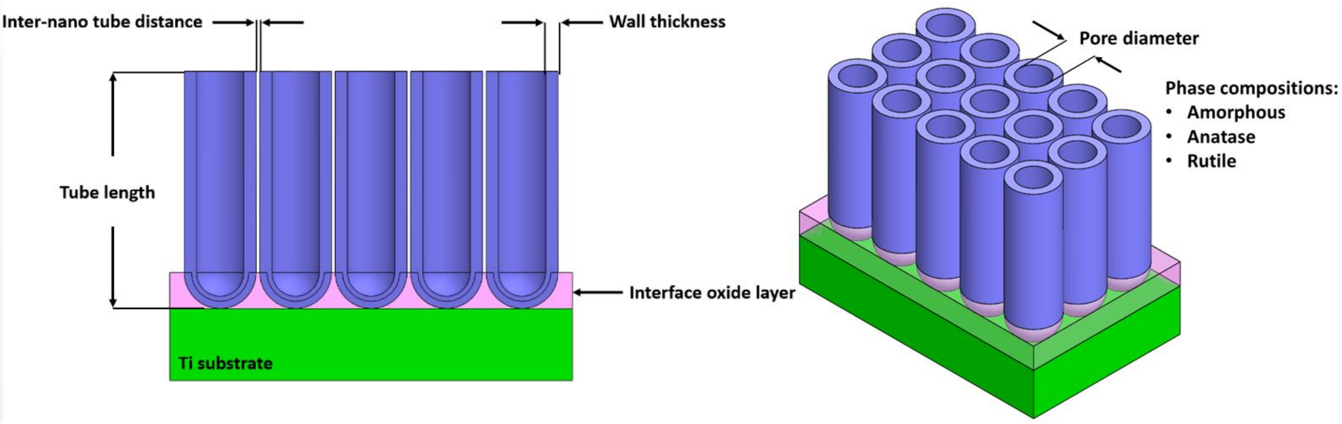

Figure 6. The eight titania nanotubes (TNTs) characteristics considered in this study: tube length; inter-nano tube distance; wall thickness; interface oxide layer; pore diameter; amorphous structure; and anatase and rutile crystal structures.

materials (200–350 HV) susceptible to abrasive wear as they cannot be hardened by martensitic transformation as the case with steel [154]. Therefore, hardness was found to be the seventh most important mechanical CQA. Hard materials provide a better protection to wear [165], and reduce the probability of corrosion [166]. However, increased hardness can reduce ductility and toughness [166].

5.3 QbD Step 2.2: Optimizing the CQA by varying TNTs characteristics

To design and manufacture the next generation of nano-engineered implant surfaces, a cost-effective engineering management approach such as QbD is required. Achieving this goal requires thorough understanding of the current body of knowledge to appreciate the underlying relationships of TNTs characteristics with the CQA of nano-engineered surfaces. Moreover, It is known that TNTs characteristics can be altered by controlling the different parameters of the EA process [93, 167]. Further, any variation of TNTs characteristics can directly affect the CQA (biological, physicochemical, mechanical) of the implant surface, and in turn the implant performance. Therefore, the objective of the QbD step 2.2 was to identify the level of influence of each TNTs characteristic on the CQA of nano-engineered surfaces. To achieve this objective, a section of the online-survey, which was responded by 13 participants, sought to capture the degree of influence of TNTs characteristics on each of the 21 CQA. Furthermore, eight TNTs characteristics were considered in this study (Fig 6); these include: pore diameter (internal diameter); inter-nanotube distance; tube length; wall thickness; barrier oxide layer; amorphous; anatase; and rutile crystalline structures. Moreover, the qualitative rating used in this section of the survey was based on a similar five-rating scale presented in section 5.2, but with different qualitative descriptions (Fig 7) [54]. Following this, the survey results were computed into the HoQ relationship matrix to identify the most influential TNTs characteristics on each CQA group. The results of the HoQ are crisp and fuzzy final ratings that in order to be comparable, are scaled to have maximum rating or upper limit of unity.

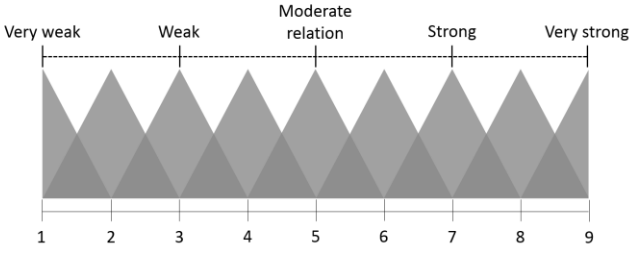

Figure 7. Qualitative five rating scale to rating for relationship of titania nanotubes (TNTs) characteristics and CQA with its corresponding triangular fuzzy numbers.

5.3.1 Optimizing biological CQA with TNTs

According to our HoQ results, pore diameter and inter-nanotube distance were considered the most influential TNTs characteristics towards the biological performance of the modified implants (Table 6). These two characteristics have a combined 35 percent influence on the surface biological performance. This is supported by several studies which have reported that TNTs diameter can directly influence protein adhesion, and consequently control cellular behaviour and biofilm formation [168, 169]. For example, the adsorption of plasma pro¬teins is enhanced by TNTs with pore diameter of 100 nm, while TNTs with smaller diameters significantly decreased protein binding [170]. Similarly, Yang et al. [171] reported that the amount of collagen type I adsorption is improved with 100 nm diameter TNTs. In another study, Oh et al. [172] observed that fibronectin and albumin aggregates are increased in TNTs with 30 and 50 nm diameters. Furthermore, different TNTs diameters influence various cell behaviours, such as adhesion, proliferation, migration, mineralization and apoptosis in different cells types, such as mesenchymal stem cells, endothelial cells, hematopoietic stem cells, fibroblasts, macrophages, osteoblasts, and osteoclasts [173-175]. For example, by controlling macrophage cell response it is possible to enhance the healing process, by promoting a reparative rather than a chronic inflammatory response [176] at bone/dental implants. Furthermore, it has been shown that cell adhesion, migration and proliferation are higher with TNTs between 15nm and 30 nm diameter [177-180], whereas cell apoptosis is induced with TNTs diameters between 50nm to 100nm [14, 177, 180, 181].

Table 6. Final ranking of titania nanotubes (TNTs) characteristics and relative importance (RI) to the biological CQAs.

Characteristic	Scaled rating	RI %	Ranking
Pore diameter	[0.62, 1.00]	18.08	1
Inter-nanotube distance	[0.58, 0.97]	17.05	2
Anatase crystal structure	[0.41, 0.74]	12.21	3
Rutile crystal structure	[0.39, 0.72]	11.70	4
Wall thickness	[0.37, 0.71]	11.50	5
Amorphous structure	[0.35, 0.65]	10.51	6
Barrier oxide layer	[0.33, 0.64]	10.06	7
Tube length	[0.29, 0.59]	8.90	8

However, contradictory results have been reported by other studies [172, 182-184] demonstrating that TNTs with diameters between 80nm to 470nm increase cell adhesion, migration and cell proliferation without inducing apoptosis. The divergent results of the studies indicate an inappropriate understanding of these phenomena [18, 185], possibly caused by the variation in fabrication/sterilization protocol (imparting changes to surface topography/chemistry of the nanotubes), use of different cell lines and distinct methods employed to assess cellular activities [180]. This implies that the existence of a threshold point of nanotube diameter for maximum biological performance may depend on a delicate balance of TNTs characteristics and adjacent tissue condition (specific to each patient). The beneficial effect of TNTs diameter in decreasing biofilm formation has also been reported by several studies [15, 186, 187]. For example, it has been reported that the density of Staphylococcus aureus and Staphylococcus epidermidis on implant surface can be decreased by changing TNT diameter [15, 182, 188-190]. Moreover, Peng et al.[182] and Kumeria et al. [191] demonstrated that is possible to decrease bacteria adhesion on TNTs surfaces, while simultaneously increasing cell adhesion.

In case of inter-nanotube distance, there is a lack of information with respect to its effect on bone regeneration. However, in a recent study Necula et al. [192] reported that inter-nanotube distance has the potential to induce osteogenic diferentiation. Hence, the inter-nanotube distance of TNTs cannot be ignored when fabricating TNTs-based bone implants. These findings correlate with the opinion of the experts surveyed in this study, who ranked inter-nanotube distance as the second most influential TNTs characteristic.

The third and fourth ranked influencing characteristics were anatase and rutile crystal structures with a contribution of 12.21, and 11.70 percent, respectively, to the biological CQA of TNTs-Ti implants. The material structure of TiO2 can influence cell behaviour. Indeed, TNTs biocompatibility can be significantly improved when their structure is changed from amorphous to anatase, and to a mixture of anatase and rutile [12, 93, 127, 193, 194]. For example, it was reported that using anatase TNTs, osteoblast adhesion speed and growth was increased by approximately 370% and 140% compared to untreated Ti and amorphous TNTs, respectively [195]. Moreover, it has also been reported that amorphous TNTs are not as effective in reducing bacterial adhesion compared to anatase TNTs [15]. It is believed that the presence of lower levels of residual fluorine in anatase TNTs could be the reason for their superior effects on cell adhesion [196]. The remaining four influencing TNTs characteristics (i.e. wall thickness, amorphous structure, barrier oxide layer and tube length) were considered to have a combined effect of almost 41 % on the biological CQA.

5.3.2 Optimizing physicochemical CQA with TNTs

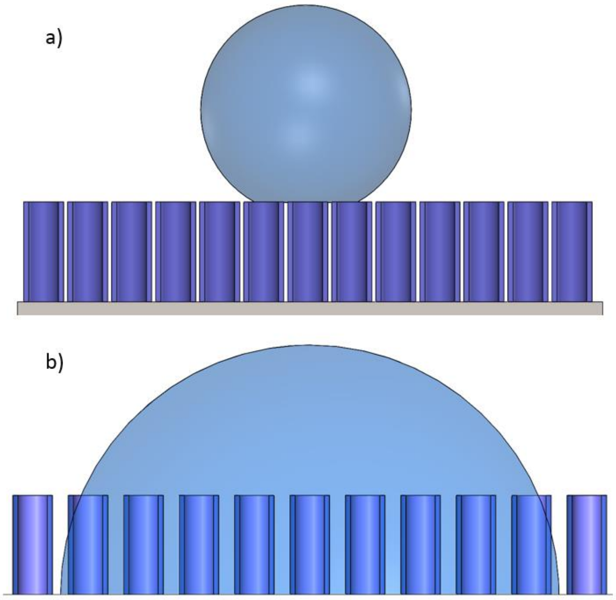

Figure 8. Schematic representation of wetting behavior of titania nanotubes (TNTs): a) Cassie-Baxter state (super-hydrophobic); and b) Wenzel state (super-hydrophilic). Wider inter-nanotube distance allows for fluid penetration and hence hydrophilicity.

Based on our survey evaluation, pore diameter, inter-nanotube distance, and wall thickness are the three most influential TNT characteristics over the most desired physicochemical CQA of TNTs-Ti implants, contributing 17.58, 16.93, and 12.8 percent, respectively (Table 7). It is known that Ti surface affinity for protein adsorption largely depends on its wettability and surface charge [67, 111], whereby different water contact angles (WCA) induce distinct cellular responses [64]. It has been reported that when TNTs pore diameter and Inter-nanotube distance are increased, surface wettability is improved by achieving a super-hydrophilic behaviour [122, 151, 197]. For example, Anitha et al.Anitha, Lee, Lee, Banerjee, Joo and Min [186] obtained similar results varying TNTs diameter and maintaining a constant surface roughness. According to the Wenzel model the wettability of a surface depends on the topographical interstitial spaces that draw the liquid into it, making the surface more wettable [198]. Therefore, TNTs with larger diameters have more capillary force and space for liquid penetration [197, 199] (Fig 8), thus increasing the interaction energies with proteins such as collagen type I [171]. On the contrary, when TNTs wall thickness is increased, it affects the average porosity of the film creating a non-wetted Cassie-Baxter state making the surface super-hydrophobic [122, 134] (Fig 8). Moreover, according to Kulkarni et al. [170] the ζ-potential of TNTs does not change significantly when varying the TNTs diameters.

In fourth and fifth place with a maximum combined effect of approximately 23 % in the overall physicochemical CQA are barrier oxide layer and tube length, respectively, as shown in Table 7. It has been shown that when a Ti surface is coated with TNTs, the Ti substrate is protected against corrosion by the TNTs themselves[200, 201] and by the barrier oxide layer located underneath the TNTs (interface of nanotubes and underlying implant substrate) [202, 203]. Longer TNTs provide a thicker barrier that improves material corrosion resistance [204, 205]. Moreover, the corrosion resistance of the barrier oxide layer mainly depends on its chemical composition and thickness, which is approximately equal to the pore radius [167, 200]. For example, a thicker barrier oxide layer with low levels of oxygen vacancies provides a lower rate of titanium release and improves long-term stability in biological environments [206]. Nonetheless, a large diameter and inter-nanotube distance provides more channels for body fluids to penetrate and corrode the material [200]. Therefore, for optimal corrosion protection it is imperative to achieve an adequate balance among TNTs' length, diameter, inter-nanotube distance, and barrier oxide layer.

Table 7. Final ranking of titania nanotubes (TNTs) characteristics and relative importance (RI) to the physicochemical CQAs.

Characteristic	Scaled rating	RI %	Ranking
Pore diameter	[0.62, 1.00]	17.58	1
Inter-nanotube distance	[0.59, 0.97]	16.93	2
Wall thickness	[0.42, 0.79]	12.80	3
Barrier oxide layer	[0.39, 0.73]	11.60	4
Tube length	[0.36, 0.70]	10.94	5
Amorphous structure	[0.34, 0.67]	10.34	6
Anatase crystal structure	[0.33, 0.66]	9.98	7
Rutile crystal structure	[0.32, 0.65]	9.83	8

The final three ranked characteristic are related to TNTs crystal structure, namely, amorphous, anatase, and rutile with RI contributions of 10.34, 9.98, and 9.83 percent to the overall surface physicochemical performance, respectively (Table 7). It is noteworthy that changes in TNTs structure also effects its corrosion resistance. In several studies it has been shown that the corrosion resistance of TNTs is improved progressively when their structure is changed from amorphous to anatase and rutile (amorphous < anatase < rutile) [204, 207]. For example, anatase TNTs are more stable in Hank's solution than amorphous TNTs and smooth Ti surfaces [208]. This is because crystalline TNTs have higher stability, and thicker interface layer than amorphous TNTs leading to an increase in corrosion resistance [193, 207, 209]. Nonetheless, a mix of anatase and rutile crystal structures provides higher corrosion resistance than plain anatase structure [193, 204]. Mazare et al. [210] reported a linear trendline between an increase in rutile content and an improvement in corrosion resistance. The explanation for this is that a rutile crystalline structure has a more compact structural configuration and less electrical conductivity, thus enhancing surface corrosion protection [204]. Moreover, there is a general agreement between different studies that the water contact angle in TNTs decreases when their material structure changes from amorphous to anatase and rutile [211-213]. The most significant effects in wettability improvement were seen in TNTs with anatase structure and only small changes were obtained in rutile TNTs [122]. The results of these studies in relation to TNTs crystal structure suggests that the higher rating of the amorphous structure obtained in this study could signify that TNTs amorphous structure has a negative contribution to the physicochemical performance of Ti surfaces.

Another way to drastically modify surface wettability is by changing TNTs roughness through their crystal structure, tube length, and pore diameter. For example, TNTs with larger diameters can drastically increase surface energy providing a 11% to 23% [1] increase in surface area [151]. Wang et al.Wang, Wen, Hodgson and Li [214] demonstrated that disordered TNTs have higher surface energy and lower WCA than highly ordered TNTs. The reason for this is that disordered TNTs layers have more sharp edges and spikes that increase surface roughness. Nonetheless, results of different studies with TNTs have demonstrated that the combined effect of micro- and nano-scale roughness provides an outstanding synergistic effect on cell adhesion, and enhanced osseointegration by providing a more suitable surface topography that mimics natural bone dual micro- and nano-scale topographical features [5, 99, 215, 216]. For example, as previously discussed, dual micro-/nano-topography can augment osteoblast and fibroblast activity, while simultaneously reducing the proliferation of macrophages. Therefore, as Gulati et al.[217] demonstrated in their latest attempt at generating dual micro-nano surfaces, via the use of EA alone, that it is crucial to preserve underlying the micro-roughness already present in commercial dental implants. On the other hand, in other studies micro-roughness values (Ra) of 1,537 to 2,566 nm have been obtained by modifying TNTs crystal structure [212], while nano-roughness values ranging from 15 nm to 64 nm have been achieved through the modification of TNTs tubes length and pore diameter [158, 218, 219].

5.3.3 Optimizing mechanical CQA with TNTs

One of the major challenges for clinical translation of TNTs for implantation purposes is their poor mechanical properties [144]. Therefore, to improve TNTs mechanical properties it is important to first understand their main failure mechanisms. Based on our survey results, all eight TNTs characteristics were considered to have a similar contribution to the mechanical CQA, ranging from 11.22% to 13.27% (Table 8).

Nevertheless, from a structural point of view, it was noted that the first four ranked TNTs characteristics correspond to the factors (i.e. geometry and Young's modulus) that define the structural integrity of nanotubes. According to Hooke's law, the maximum compressive stress (σy) of a material is linearly proportional to its Young's modulus (E) and strain (ε) (Equation 1) [144]. This means that beside TNTs' geometry, their crystal structures are the ones that dictate TNTs maximum compressive stress (σy). In the case of titania, different crystal structures have different Young's moduli, and hence varied mechanical properties [220]. For example, amorphous TNTs present higher hardness and Young's modulus values than TNTs with a crystalline anatase structure [221, 222]. Additionally, a closely packed rutile structure has higher hardness and Young's modulus values, and a reduced friction coefficient value [209, 221, 223]. However, according to survey participants' opinion, changes in TNTs crystal structure does not significantly affect TNTs mechanical performance. This is shown in Table 8, where TNTs amorphous structure was rated with a similar Ri (12.89%) as anatase and rutile crystal structures (both accounting for 12.30%).

Table 8. Final ranking of titania nanotubes (TNTs) characteristics and relative importance (RI) to the mechanical CQAs.

Characteristic	Scaled rating	RI %	Ranking
Pore diameter	[0.57, 1.00]	13.27	1
Wall thickness	[0.56, 0.96]	12.91	2
Amorphous structure	[0.57, 0.96]	12.89	3
Tube length	[0.55, 0.96]	12.78	4
Barrier oxide layer	[0.52, 0.94]	12.34	5
Anatase crystal structure	[0.54, 0.92]	12.30	6
Rutile crystal structure	[0.54, 0.92]	12.30	6
Inter-nanotube distance	[0.47, 0.87]	11.22	7

Based on their geometry, TNTs behave as columns, and hence under the influence of an axial compressive force (such as in load bearing implants), TNTs may collapse via two different failure mechanisms: pure compressive stress and buckling [224], as shown in Fig 9. Failure by pure compressive stress requires that the axial load applied overcomes the material compressive strength, resulting in the brittle fracture of TNTs [224]. Further, assuming that TNTs behave as long columns, elasticity and not the compressive strength of the column material will determine the critical load necessary to cause the column to fail by buckling [144, 224, 225]. This may occur even though the stresses that are developed in the structure are below those needed to cause failure of the material [144]. Therefore, when considering TNTs as hollow columns, their critical load and critical stress for buckling depends mainly on their length (L), external diameter (d2), and pore diameter (d1), as shown in equations 2 and 3 [144]. These equations provide theoretical support for our results, whereby pore diameter, wall thickness, and tube length were ranked as the first, second, and fourth (respectively) for the most influential characteristics of TNTs mechanical properties. In summary, characteristics such as pore diameter, wall thickness, crystal structure, and tube length determines the TNTs' strength under compression [225, 226]. Furthermore, according to the results of other studies, these geometrical characteristics may affect other TNTs mechanical properties. For example, thicker walls in TNTs arrays substantially improves hardness [222]; Longer TNTs arrays have lower apparent Young's modulus and hardness, due to their densification and collapse when they

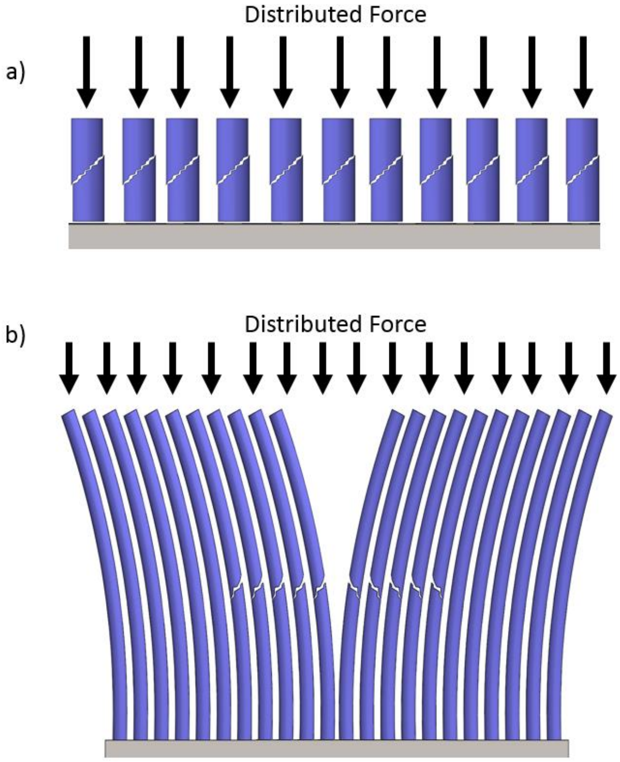

Figure 9. Scheme showing possible failure mechanisms of titania nanotubes (TNTs) under load-bearing bone/dental implant application: a) failure by pure compressive stress on shorter length TNTs, and b) failure by buckling on longer TNTs.

are under an external force [21, 155, 227]. Increments in porosity of the TNTs layer drastically reduces TNTs apparent modulus of elasticity [129, 221, 227]; and TNTs scratch resistance and film adhesion strength are enhanced by increasing the thickness of the TNTs layer, making the film more difficult to be penetrated [142, 155].

$$\text{(1)} \quad \sigma_y = E\varepsilon$$

$$\text{(2)} \quad P_{cr} = \frac{\pi E I_x}{2L} \rightarrow \frac{\pi^3 E (d_2 - d_1)^4}{256L}$$

$$\text{(3)} \quad \sigma_{cr} = \frac{2\pi E}{2L(d_2 - d_1)^2} \qquad \sigma_{cr} < \sigma_y$$

Moreover, the formation of TNTs during the EA process creates residual compressive stresses on the surface. These residual stresses are further increased during annealing when amorphous structure change into crystalline state. These residual stresses provide a mechanical protection to the implant surface and improve other mechanical properties, such as fatigue resistance, fracture toughness, interfacial shear strength, critical debonding stress, critical crack stress, and critical buckling stress [228-230]. On the other hand, the rutile structure negatively affects the fatigue performance of TNTs films [231]. This negative effect on fatigue resistance is not present in Ti-6Al-7Nb and Ti-6Al-4V alloys, whereby the elastic behaviour of TNTs on alloys is attributed to the compact barrier oxide layer present at the bottom of TNTs [114]. TNTs poor interfacial adhesion is also attributed to the ultra-thin fluoride-rich layer at the interface that is developed during the EA process [21, 145, 232]. These observations correspond to our study in which in which the barrier oxide layer (12.34%) was perceived to be the fifth most influential characteristic on TNTs mechanical properties. Consequently, several studies have focused on developing a modified anodization process in a fluoride-free electrolyte to remove the residual fluoride ions in the oxide barrier layer and to also slightly increase its thickness [145, 232, 233]. Moreover, as Gulati et al. [147] previously reported, cracks in TNTs on curved surfaces can be controlled by changing the thickness of the oxide barrier layer, which is directly related to TNTs wall thickness. Therefore, a thicker oxide barrier layer can improve the adhesion strength between TNTs and the underlying substrate [21]. Nevertheless, to achieve improvement in adhesion strength of TNTs, fabrication strategies should be designed in order to have a set of mechanical properties compatible with the base metal [234]. As reported by Gulati et al. [217] and Li et al. [149], the 1-step EA fabrication of aligned TiO2 nanopores (with preserved underlying micro-features) on micro-machined Ti implants (including commercial dental implants) had exceptional mechanical properties, as compared to conventional and mechanically-enhanced nanotubes. Gulati et al. [174] also found that these nanopores mechanically stimulate osteoblasts and fibroblasts, which were found to align parallel to the direction of the nanopores. Interestingly, such mechanically-superior structures have not been adequately explored towards implant applications.

5.4 QbD Step 2.3: Influence of TNTs characteristics on the combined performance of CQAs

In the previous QbD step (2.2) we qualitatively identified the TNTs characteristics that contribute to the overall surface performance of each CQAs group: biological, physicochemical, and mechanical. Nonetheless, an optimal nano-engineered surface should provide an adequate balance of biological, physiochemical, and mechanical properties to best satisfy customer requirements, which in this case were the participants of this study. For this purpose, we created a HoQ matrix containing all of the biological, physicochemical, and mechanical CQAs combined. Hence, the resulting HoQ allowed for identification of TNTs characteristics that contribute most to the combined overall performance of all CQAs, as represented in Fig 10.

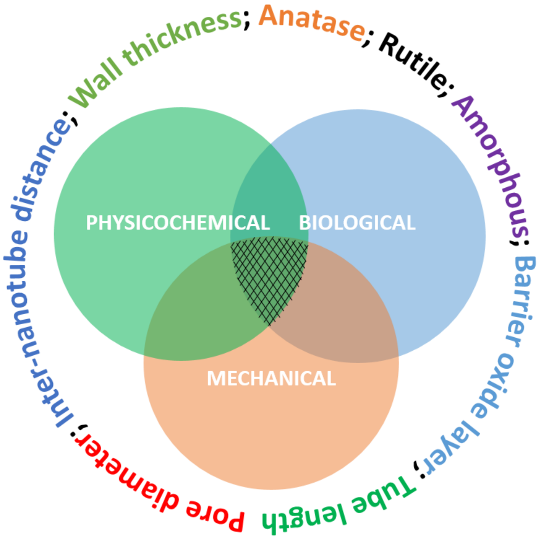

Figure 10. Graphic representation of the combined effect of titania nanotubes (TNTs) characteristics on the biological, physicochemical, and mechanical CQA groups obtained from the HoQ.

According to Table 9, the three most representative TNTs characteristics for implants applications are pore diameter, inter-nanotube distance, and wall thickness. Based on expert opinion and our modelling, these characteristics together contributes to approximately 44% to the overall surface implant performance. From the results presented in section 5.3, it can be seen that pore diameter is the most influential characteristic for the three CQAs groups. Clearly, pore size dictates protein adhesion and hence cell attachment, and can also influence water contact angle. Inter-nanotube distance was ranked second for the biological and physicochemical CQAs, while it was the lowest ranked for the mechanical CQAs. Following in ranking with a very similar RI rating ranging from 11.33% to 11.64%, were anatase crystal structure, rutile crystal structure, and amorphous structure. A similar situation can be seen with the barrier oxide layer, which was ranked in eighth place, having the same fuzzy scaled rating (0.41, 0.76) as the amorphous structure. The lowest ranked TNTs characteristic was tube length with a RI and fuzzy scaled ratings of 10.85% and [0.39, 0.74], respectively. These findings indicate that the geometrical characteristics of TNTs are considered to have the most influential effects on the preferred CQAs configuration provided by the experts.

Table 9. Final ranking of TNTs characteristics in relation to all CQAs groups.

Characteristic	Scaled rating	RI %	Ranking
Pore diameter	[0.60, 1.00]	16.20	1
Inter-nanotube distance	[0.55, 0.94]	14.90	2
Wall thickness	[0.45, 0.81]	12.36	3
Anatase crystal structure	[0.42, 0.77]	11.64	4
Rutile crystal structure	[0.41, 0.77]	11.42	5
Amorphous structure	[0.41, 0.75]	11.33	7
Barrier oxide layer	[0.41, 0.76]	11.30	8
Tube length	[0.39, 0.74]	10.85	9

6. Conclusions

TiO2 nanotubes (TNTs) modified titanium implants have favourable characteristics which make them an attractive customizable bioactive and therapeutic bone/dental implant modification. However, to bridge the gap between research and the implant industry it is imperative to implement product development strategies capable of avoiding the “valley of death”. Moreover, the complex nano-scale biological interactions, discrepancies among nanotube studies and research gaps with respect to mechanical stability, raise additional concerns for clinical translation and commercialization. Further complicating the situation is the fact that the multiple characteristics of nanotubes are interlinked, and the influence of individual parameter may be cumbersome to predict.

Hence, the present study semi-qualitatively estimated, with a simple but robust approach, the level of importance of the Critical Quality Attributes (CQAs) of TNTs modified titanium implants, including the most optimal configuration of CQAs that satisfied the participants’ expert opinion. Furthermore, through the use of the House of Quality (HoQ) it was possible to qualitatively rate the level of perceived contribution of each titania nanotubes characteristic to the overall implant performance from three different perspectives; biological, physicochemical, and mechanical. This in turn allowed us to arrive at the titania nanotubes characteristics that need more attention and control during the design and fabrication process of electrochemically anodized (EA) TNTs for implant applications.

Overall, it can be concluded that the Quality by Design (QbD) system in conjunction with tools such as the HoQ with a fuzzy approach can translate customers’ requirements and expert’s knowledge in a more meaningful manner. Subsequently, the main purpose of this application of the QbD system is to better direct the design of nano-engineered implant surfaces with TNTs in a targeted fashion, thus reducing the need for costly and time-consuming trial-and-error studies. Future work will focus on a systematic testing of TNTs charactestics to corroborate the result obtanied in this study by quantitatively identify the percentage contribution of each titania nanotubes characteristic to the overall implant performance. Nevertheless, for TNTs modified titanium implants to be able to reach the implant market, further studies are necessary to continue the implementation of the QbD system, including the identification of the current state of TNTs properties in relation to medical regulatory standards and the commercially available implant surfaces.

CRediT authorship contribution statement

Daniel Martinez-Marquez, Karan Gulati, Christopher P. Carty, Rodney A. Stewart, and Sašo Ivanovski: Conceptualization; **Daniel Martinez-Marquez and Karan Gulati:** Data curation; **Daniel Martinez-Marquez:** Formal analysis; **Daniel Martinez-Marquez and Christopher P. Carty:** Funding acquisition; **Daniel Martinez-Marquez:** Investigation; **Daniel Martinez-Marquez: Methodology; Karan Gulati, Christopher P. Carty, Rodney A. Stewart, and Sašo Ivanovski:** Project administration; **Rodney A. Stewart, and Sašo Ivanovski:** Resources; **Daniel Martinez-Marquez: Software; Christopher P. Carty, Rodney A. Stewart, and Sašo Ivanovski:** Supervision; **Daniel Martinez-Marquez, Karan Gulati, Rodney A. Stewart, and Sašo Ivanovski:** Validation; **Daniel Martinez-Marquez:** Visualization; **Daniel Martinez-Marquez:** Roles/Writing - original draft; **Daniel Martinez-Marquez , Karan Gulati, Christopher P. Carty, Rodney A. Stewart, and Sašo Ivanovski:** Writing - review & editing.

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Conflicts of interest

None of the authors have any conflicts to declare.

Ethics statement

The authors confirm that consent has been obtained by all the participants of this study. Moreover, the protocol and procedures employed in this study were reviewed and approved by the Griffith University Human Research Ethics Committee under the Ref No: 2018/322.

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References

[1] Geetha M, Singh A, Asokamani R and Gogia A 2009 Ti based biomaterials, the ultimate choice for orthopaedic implants–a review *Progress in Materials Science* **54** 397-425

[2] Strnad J, Strnad Z, Šesták J, Urban K and Povýšil C 2007 Bio-activated titanium surface utilizable for mimetic bone implantation in dentistry—Part III: Surface characteristics and bone–implant contact formation *Journal of Physics and Chemistry of Solids* **68** 841-5

[3] Zhao X, Wang T, Qian S, Liu X, Sun J and Li B 2016 Silicon-doped titanium dioxide nanotubes promoted bone formation on titanium implants *International journal of molecular sciences* **17** 292

[4] Le Guéhennec L, Soueidan A, Layrolle P and Amouriq Y 2007 Surface treatments of titanium dental implants for rapid osseointegration *Dental materials* **23** 844-54

[5] Gulati K, Prideaux M, Kogawa M, Lima- Marques L, Atkins G J, Findlay D M and Losic D 2017 Anodized 3D–printed titanium implants with dual micro- and nano- scale topography promote interaction with human osteoblasts and osteocyte-like cells *Journal of Tissue Engineering and Regenerative Medicine* **11** 3313-25

[6] Sabir M I, Xu X and Li L 2009 A review on biodegradable polymeric materials for bone tissue engineering applications *Journal of Materials Science* **44** 5713-24

[7] Yu W, Zhang Y, Jiang X and Zhang F 2010 In vitro behavior of MC3T3- E1 preosteoblast with different annealing temperature titania nanotubes *Oral diseases* **16** 624-30

[8] Staruch R, Griffin M F and Butler P 2016 Nanoscale Surface Modifications of Orthopaedic Implants: State of the Art and Perspectives *The open orthopaedics journal* **10** 920-38

[9] Puckett S, Pareta R and Webster T J 2008 Nano rough micron patterned titanium for directing osteoblast morphology and adhesion *International journal of nanomedicine* **3** 229

[10] Gulati K, Aw M S and Losic D 2011 Drug-eluting Ti wires with titania nanotube arrays for bone fixation and reduced bone infection *Nanoscale research letters* **6** 571

[11] Wang X, Chen X, Hodgson P and Wen C 2006 Elastic modulus and hardness of cortical and trabecular bovine bone measured by nanoindentation *Transactions of nonferrous metals society of china* **16** s744-s8

[12] Gulati K, Maher S, Findlay D M and Losic D 2016 Titania nanotubes for orchestrating osteogenesis at the bone-implant interface *Nanomedicine* **11** 1847-64

[13] Macak J, Tsuchiya H, Ghicov A, Yasuda K, Hahn R, Bauer S and Schmuki P 2007 TiO 2 nanotubes: self-organized electrochemical formation, properties and applications *Current Opinion in Solid State and Materials Science* **11** 3-18

[14] Bauer S, Park J, von der Mark K and Schmuki P 2010 TiO2 nanotubes for stimulated cell response: Control of cell-surface interactions at the nanoscale *Europ. Cells Mater* **20** 16

[15] Ercan B, Taylor E, Alpaslan E and Webster T J 2011 Diameter of titanium nanotubes influences anti-bacterial efficacy *Nanotechnology* **22** 295102

[16] Simi V S and Rajendran N 2017 Influence of tunable diameter on the electrochemical behavior and antibacterial activity of titania nanotube arrays for biomedical applications *Materials Characterization* **129** 67-79

[17] Kulkarni M, Mazare A, Schmuki P and Iglič A 2014 *Nanomedicine*, ed A Seifalian, *et al.* (Altrincham, UK: One Central Press (OCP)) p 111

[18] Li M and Yang Y 2017 Nanoscale TiO2 nanotubes as a basis for governing cell behaviors and application challenges *International journal of nanomedicine* **12** 575

[19] Martinez-Marquez D, Mirnajafizadeh A, Carty C P and Stewart R A 2018 Application of quality by design for 3D printed bone prostheses and scaffolds *PLOS ONE* **13** e0195291

[20] Gulati K and Ivanovski S 2016 Dental implants modified with drug releasing titania nanotubes: therapeutic potential and developmental challenges *Expert Opinion on Drug Delivery* 1-16

[21] Li T, Gulati K, Wang N, Zhang Z and Ivanovski S 2018 Understanding and augmenting the stability of therapeutic nanotubes on anodized titanium implants *Materials Science and Engineering: C* **88** 182-95

[22] Brown A, Dixon D, Eatock J, Meenan B J and Young T 2008 A survey of success factors in new product development in the medical devices industry. In: *2008 IEEE International Engineering Management Conference,* (Estoril, Portugal: IEEE) pp 1-5

[23] Hollister S J 2009 Scaffold engineering: a bridge to where? *Biofabrication* **1** 012001

[24] Larson E W and Gray C 2011 *Project management-The managerial process* (Irwin, New York: McGraw-Hill) p 211

[25] Juran J M 1992 *Juran on quality by design: the new steps for planning quality into goods and services*: Simon and Schuster)

[26] Fahmy R, Kona R, Dandu R, Xie W, Claycamp G and Hoag S W 2012 Quality by design I: application of failure mode effect analysis (FMEA) and Plackett–Burman design of experiments in the identification of "main factors" in the formulation and process design space for roller-compacted ciprofloxacin hydrochloride immediate-release tablets *AAPS PharmSciTech* **13** 1243-54

[27] Martinez-Marquez D, Gulati K, Mirnajafizadeh A, Carty C P, Stewart R and Ivanovski S 2018 Quality by Design towards Standardization of 3D Printed Bone Implants and scaffolds for Industry Translation. In: *4th World Congress on Electrical Engineering and Computer Systems and Sciences,* (Madrid, Spain: EECSS)

[28] Vogt F G and Kord A S 2011 Development of quality- by- design analytical methods *Journal of pharmaceutical sciences* **100** 797-812

[29] Naidu N V R, Babu K M, Rajendra G and ebrary I 2006 *Total quality management* vol 1 (New Delhi: New Age International (P) Ltd., Publishers)

[30] Lawrence X Y 2008 Pharmaceutical quality by design: product and process development, understanding, and control *Pharmaceutical research* **25** 781-91

[31] Krucoff M W, Brindis R G, Hodgson P K, Mack M J and Holmes J D R 2012 Medical device innovation: prospective solutions for an ecosystem in crisis. Adding a professional society perspective *JACC Cardiovascular interventions* **5** 790

[32] Sangshetti J N, Deshpande M, Zaheer Z, Shinde D B and Arote R 2017 Quality by design approach: Regulatory need *Arabian Journal of Chemistry* **10** S3412-S25

[33] ICH Harmonised Tripartite Guideline 2009 Pharmaceutical development Q8 (R2) *ICH Steering Committee, Step* **4**

[34] Oyegoke A 2011 The constructive research approach in project management research *International Journal of Managing Projects in Business* **4** 573-95

[35] Kasanen E, Lukka K and Siitonen A 1993 The constructive approach in management accounting research *Journal of Management Accounting Research* **5** 243

[36] Liberati A, Altman D G, Tetzlaff J, Mulrow C, Gøtzsche P C, Ioannidis J P, Clarke M, Devereaux P J, Kleijnen J and Moher D 2009 The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration *Annals of internal medicine* **151** W-65-W-94

[37] Ray R 2006 Prediction Markets and the Financial" Wisdom of Crowds" *The Journal of Behavioral Finance* **7** 2-4

[38] Savage N 2012 Gaining wisdom from crowds *Communications of the ACM* **55** 13-5

[39] Wang Q, Xu W and Zheng H 2018 Combining the wisdom of crowds and technical analysis for financial market prediction using deep random subspace ensembles *Neurocomputing* **299** 51-61

[40] Ershad M, Rege R and Fey A M 2018 Meaningful Assessment of Robotic Surgical Style using the Wisdom of Crowds *International Journal of Computer Assisted Radiology and Surgery* **13** 1037-48

[41] Armstrong J S 2001 *Principles of forecasting: a handbook for researchers and practitioners* vol 30: Springer Science & Business Media)

[42] Clemen R T 1989 Combining forecasts: A review and annotated bibliography *International journal of forecasting* **5** 559-83

[43] Budescu D V and Chen E 2014 Identifying expertise to extract the wisdom of crowds *Management Science* **61** 267-80

[44] Simmons J P, Nelson L D, Galak J and Frederick S 2009 Intuitive biases in choice vs. estimation: implications for the wisdom of crowds *Journal of Consumer Research*

[45] Mannes A E, Soll J B and Larrick R P 2014 The wisdom of select crowds *Journal of Personality and Social Psychology* **107** 276-99

[46] Mellers B, Ungar L, Baron J, Ramos J, Gurcay B, Fincher K, Scott S E, Moore D, Atanasov P and Swift S A 2014 Psychological strategies for winning a geopolitical forecasting tournament *Psychological science* **25** 1106-15

[47] Harrell M C and Bradley M A 2009 Data collection methods. Semi-structured interviews and focus groups. (Santa Monica, CA, USA: DTIC Document)

[48] Park T and Kim K-J 1998 Determination of an optimal set of design requirements using house of quality *Journal of operations management* **16** 569-81

[49] Hauser J R 1993 How Puritan-Bennett used the house of quality *Sloan Management Review* **34** 61-70

[50] Akao Y 1990 *Quality function deployment: integrating customer requirements into product design* (Cambridge, Mass: Productivity Press)

[51] Maiers J E 1985 Fuzzy set theory and medicine: the first twenty years and beyond. In: *Proceedings of the Ninth Annual Symposium on Computer Application in Medical Care*, (Baltimore, MD, USA: American Medical Informatics Association) pp 325-9

[52] Torres A and Nieto J J 2006 Fuzzy logic in medicine and bioinformatics *BioMed Research International* **2006** 91908-

[53] Zadeh L A 1973 Outline of a new approach to the analysis of complex systems and decision processes *IEEE Transactions on systems, Man, and Cybernetics* 28-44

[54] Chan L-K and Wu M-L 2005 A systematic approach to quality function deployment with a full illustrative example *Omega* **33** 119-39

[55] Abbod M F, von Keyserlingk D G, Linkens D A and Mahfouf M 2001 Survey of utilisation of fuzzy technology in medicine and healthcare *Fuzzy Sets and Systems* **120** 331-49

[56] Center B and Verma B P 1998 Fuzzy logic for biological and agricultural systems *Artificial Intelligence Review* **12** 213-25

[57] Anderson C 2010 Presenting and evaluating qualitative research *American journal of pharmaceutical education* **74** 141

[58] Chavan S D, Pimpodkar N V, Kadam A S and Gaikwad P S 2015 Quality by Design *Journal of Pharmaceutical Quality Assurance* **1**

[59] Rathore A S 2009 Roadmap for implementation of quality by design (QbD) for biotechnology products *Trends in Biotechnology* **27** 546-53

[60] Garvin D A 1987 Competing on the eight dimensions of quality *Harvard Business Review* **65**

[61] Sebastianelli R and Tamimi N 2002 How product quality dimensions relate to defining quality *International Journal of Quality & Reliability Management* **19** 442-53

[62] Garvin D A 1984 What does product quality really mean *Sloan management review* **26**

[63] Charoo N A, Shamsher A A, Zidan A S and Rahman Z 2012 Quality by design approach for formulation development: a case study of dispersible tablets *International journal of pharmaceutics* **423** 167-78

[64] Idaszek J, Kijeńska E, Łojkowski M and Swieszkowski W 2016 How important are scaffolds and their surface properties in regenerative medicine *Applied Surface Science* **388** 762-74

[65] Puleo D and Nanci A 1999 Understanding and controlling the bone–implant interface *Biomaterials* **20** 2311-21

[66] Saini M, Singh Y, Arora P, Arora V and Jain K 2015 Implant biomaterials: A comprehensive review *World Journal of Clinical Cases: WJCC* **3** 52

[67] Albrektsson T and Wennerberg A 2004 Oral implant surfaces: Part 1-review focusing on topographic and chemical properties of different surfaces and in vivo responses to them *International Journal of Prosthodontics* **17** 536-43

[68] Vignoletti F, Johansson C, Albrektsson T, De Sanctis M, San Roman F and Sanz M 2009 Early healing of implants placed into fresh extraction sockets: an experimental study in the beagle dog. De novo bone formation *Journal of Clinical Periodontology* **36** 265-77

[69] Huo K, Gao B, Fu J, Zhao L and Chu P K 2014 Fabrication, modification, and biomedical applications of anodized TiO 2 nanotube arrays *RSC Advances* **4** 17300-24

[70] Lord M S, Foss M and Besenbacher F 2010 Influence of nanoscale surface topography on protein adsorption and cellular response *Nano Today* **5** 66-78

[71] Moursi A M, Globus R K and Damsky C H 1997 Interactions between integrin receptors and fibronectin are required for calvarial osteoblast differentiation in vitro *Journal of cell science* **110** 2187-96

[72] Dimitriou R, Tsiridis E and Giannoudis P V 2005 Current concepts of molecular aspects of bone healing *Injury* **36** 1392-404

[73] Schmidt-Bleek K, Schell H, Schulz N, Hoff P, Perka C, Buttgereit F, Volk H-D, Lienau J and Duda G N 2012 Inflammatory phase of bone healing initiates the regenerative healing cascade *Cell and tissue research* **347** 567-73

[74] Xing Z, Lu C, Hu D, Miclau III T and Marcucio R S 2010 Rejuvenation of the inflammatory system stimulates fracture repair in aged mice *Journal of Orthopaedic Research* **28** 1000-6

[75] Mountziaris P M and Mikos A G 2008 Modulation of the inflammatory response for enhanced bone tissue regeneration *Tissue Engineering Part B: Reviews* **14** 179-86

[76] Schneider G, Zaharias R and Stanford C 2001 Osteoblast integrin adhesion and signaling regulate mineralization *Journal of dental research* **80** 1540-4

[77] Bacakova L, Filova E, Parizek M, Ruml T and Svorcik V 2011 Modulation of cell adhesion, proliferation and differentiation on materials designed for body implants *Biotechnology Advances* **29** 739-67

[78] Biggs M and Dalby M 2010 Focal adhesions in osteoneogenesis *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine* **224** 1441-53

[79] Gulati K, Kogawa M, Prideaux M, Findlay D M, Atkins G J and Losic D 2016 Drug-releasing nano-

engineered titanium implants: therapeutic efficacy in 3D cell culture model, controlled release and stability *Materials Science and Engineering: C* **69** 831-40

[80] Rahman S, Gulati K, Kogawa M, Atkins G J, Pivonka P, Findlay D M and Losic D 2016 Drug diffusion, integration, and stability of nanoengineered drug- releasing implants in bone ex- vivo *Journal of Biomedical Materials Research Part A* **104** 714-25

[81] Anselme K 2000 Osteoblast adhesion on biomaterials *Biomaterials* **21** 667-81

[82] Zreiqat H, Howlett C R, Zannettino A, Evans P, Schulze- Tanzil G, Knabe C and Shakibaei M 2002 Mechanisms of magnesium- stimulated adhesion of osteoblastic cells to commonly used orthopaedic implants *Journal of Biomedical Materials Research* **62** 175-84

[83] Joos U, Wiesmann H P, Szuwart T and Meyer U 2006 Mineralization at the interface of implants *International Journal of Oral & Maxillofacial Surgery* **35** 783-90

[84] Rebaudi A, Koller B, Laib A and Trisi P 2004 Microcomputed tomographic analysis of the peri-implant bone *International Journal of Periodontics and Restorative Dentistry* **24** 316-25

[85] Rho J-Y, Kuhn-Spearing L and Zioupos P 1998 Mechanical properties and the hierarchical structure of bone *Medical engineering & physics* **20** 92-102

[86] Kalfas I H 2001 Principles of bone healing *Neurosurgical focus* **10** 1-4

[87] Davies J and Hosseini M M 2000 Histodynamics of endosseous wound healing *Bone engineering* **1** 1

[88] Gulati K, Atkins G J, Findlay D M and Losic D 2013 Nano-engineered titanium for enhanced bone therapy. In: *Biosensing and Nanomedicine VI*, (San Diego, California, USA: International Society for Optics and Photonics) p 88120C

[89] Ferraris S and Spriano S 2016 Antibacterial titanium surfaces for medical implants *Materials Science and Engineering: C* **61** 965-78

[90] Pérez- Jorge C, Conde A, Arenas M A, Pérez- Tanoira R, Matykina E, de Damborenea J J, Gómez- Barrena E and Esteban J 2012 In vitro assessment of Staphylococcus epidermidis and Staphylococcus aureus adhesion on TiO2 nanotubes on Ti–6Al–4V alloy *Journal of Biomedical Materials Research Part A* **100** 1696-705

[91] Anselme K, Davidson P, Popa A, Giazzon M, Liley M and Ploux L 2010 The interaction of cells and bacteria with surfaces structured at the nanometre scale *Acta biomaterialia* **6** 3824-46

[92] Vandrovcová M and Bacakova L 2011 Adhesion, growth and differentiation of osteoblasts on surface-modified materials developed for bone implants *Physiological Research* **60** 403

[93] Minagar S, Wang J, Berndt C C, Ivanova E P and Wen C 2013 Cell response of anodized nanotubes on titanium and titanium alloys *Journal of biomedical materials research Part A* **101** 2726-39

[94] Jimbo R, Wennerberg A and Albrektsson T 2015 *Implant Surfaces and their Biological and Clinical Impact*: Springer) pp 13-20

[95] Jäger M, Zilkens C, Zanger K and Krauspe R 2007 Significance of nano-and microtopography for cell-surface interactions in orthopaedic implants *BioMed Research International* **2007**

[96] Zhao G, Raines A, Wieland M, Schwartz Z and Boyan B 2007 Requirement for both micron-and submicron scale structure for synergistic responses of osteoblasts to substrate surface energy and topography *Biomaterials* **28** 2821-9

[97] Novaes Jr A B, Souza S L S d, Barros R R M d, Pereira K K Y, Iezzi G and Piattelli A 2010 Influence of implant surfaces on osseointegration *Brazilian dental journal* **21** 471-81

[98] Wennerberg A and Albrektsson T 2009 Effects of titanium surface topography on bone integration: a systematic review *Clinical oral implants research* **20** 172-84

[99] Gittens R A, Olivares-Navarrete R, Schwartz Z and Boyan B D 2014 Implant osseointegration and the role of microroughness and nanostructures: lessons for spine implants *Acta biomaterialia* **10** 3363-71

[100] Wennerberg A and Albrektsson T 2000 Suggested guidelines for the topographic evaluation of implant surfaces *The International journal of oral & maxillofacial implants* **15** 331

[101] Verran J and Boyd R D 2001 The relationship between substratum surface roughness and microbiological and organic soiling: a review *Biofouling* **17** 59-71

[102] Liu J, Hu Y Z and Ma T B 2016 *Reference Module in Materials Science and Materials Engineering*: Elsevier)

[103] Wyatt H, Elliott M, Revill P and Clarke A 2016 The effect of engineered surface topography on the tribology of CFR-PEEK for novel hip implant materials *Biotribology* **7** 22-30

[104] Tserepi A, Gogolides E, Bourkoula A, Kanioura A, Kokkoris G, Petrou P and Kakabakos S 2016 Plasma nanotextured polymeric surfaces for controlling cell attachment and proliferation: a short review *Plasma Chemistry and Plasma Processing* **36** 107-20

[105] Rosales-Leal J, Rodríguez-Valverde M, Mazzaglia G, Ramón-Torregrosa P, Diaz-Rodriguez L, Garcia-Martinez O, Vallecillo-Capilla M, Ruiz C and Cabrerizo-Vílchez M 2010 Effect of roughness, wettability and morphology of engineered titanium surfaces on osteoblast-like cell adhesion *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **365** 222-9

[106] Teixeira A I, Abrams G A, Bertics P J, Murphy C J and Nealey P F 2003 Epithelial contact guidance on

well-defined micro-and nanostructured substrates *Journal of cell science* **116** 1881-92

[107] Anselme K, Ploux L and Ponche A 2010 Cell/material interfaces: influence of surface chemistry and surface topography on cell adhesion *Journal of Adhesion Science and Technology* **24** 831-52

[108] Calzado-Martin A, Méndez-Vilas A, Multigner M, Saldana L, González-Carrasco J, González-Martín M and Vilaboa N 2011 On the role of RhoA/ROCK signaling in contact guidance of bone-forming cells on anisotropic Ti6Al4V surfaces *Acta biomaterialia* **7** 1890-901

[109] Gittens R A, Scheideler L, Rupp F, Hyzy S L, Geis-Gerstorfer J, Schwartz Z and Boyan B D 2014 A review on the wettability of dental implant surfaces II: biological and clinical aspects *Acta biomaterialia* **10** 2907-18

[110] Kirmanidou Y, Sidira M, Drosou M-E, Bennani V, Bakopoulou A, Tsouknidas A, Michailidis N and Michalakis K 2016 New Ti-alloys and surface modifications to improve the mechanical properties and the biological response to orthopedic and dental implants: a review *BioMed research international* **2016**

[111] Gongadze E, Van Rienen U and Iglič A 2011 Generalized stern models of the electric double layer considering the spatial variation of permittvity and finite size of ions in saturation regime *Cellular & Molecular Biology Letters* **16** 576-94

[112] Krajewski A, Piancastelli A and Malavolti R 1998 Albumin adhesion on ceramics and correlation with their Z-potential *Biomaterials* **19** 637-41

[113] Wu S, Liu X, Yeung K W, Guo H, Li P, Hu T, Chung C Y and Chu P K 2013 Surface nano-architectures and their effects on the mechanical properties and corrosion behavior of Ti-based orthopedic implants *Surface and Coatings Technology* **233** 13-26

[114] Campanelli L C, Bortolan C C, da Silva P S C P, Bolfarini C and Oliveira N T C 2017 Effect of an amorphous titania nanotubes coating on the fatigue and corrosion behaviors of the biomedical Ti-6Al-4V and Ti-6Al-7Nb alloys *Journal of the mechanical behavior of biomedical materials* **65** 542-51

[115] Hansen D C 2008 Metal corrosion in the human body: the ultimate bio-corrosion scenario *The Electrochemical Society Interface* **17** 31

[116] Martinez E, Engel E, Planell J and Samitier J 2009 Effects of artificial micro-and nano-structured surfaces on cell behaviour *Annals of Anatomy-Anatomischer Anzeiger* **191** 126-35

[117] McNamara L E, Sjöström T, Seunarine K, Meek R D, Su B and Dalby M J 2014 Investigation of the limits of nanoscale filopodial interactions *Journal of tissue engineering* **5** 2041731414536177

[118] Wilkinson C, Riehle M, Wood M, Gallagher J and Curtis A 2002 The use of materials patterned on a nano-and micro-metric scale in cellular engineering *Materials Science and Engineering: C* **19** 263-9

[119] Rupp F, Liang L, Geis-Gerstorfer J, Scheideler L and Hüttig F 2018 Surface characteristics of dental implants: A review *Dental Materials* **34** 40-57

[120] González-García C, Sousa S R, Moratal D, Rico P and Salmerón-Sánchez M 2010 Effect of nanoscale topography on fibronectin adsorption, focal adhesion size and matrix organisation *Colloids and Surfaces B: Biointerfaces* **77** 181-90

[121] Yao C, Slamovich E B and Webster T J 2008 Enhanced osteoblast functions on anodized titanium with nanotube- like structures *Journal of Biomedical Materials Research Part A* **85A** 157-66

[122] Prakash C J, Raj C C and Prasanth R 2017 Fabrication of zero contact angle ultra-super hydrophilic surfaces *Journal of Colloid and Interface Science* **496** 300-10

[123] Guo C Y, Matinlinna J P and Tang A T H 2012 Effects of surface charges on dental implants: past, present, and future *International journal of biomaterials* **2012**

[124] Hallab N J, Bundy K J, O'Connor K, Moses R L and Jacobs J J 2001 Evaluation of metallic and polymeric biomaterial surface energy and surface roughness characteristics for directed cell adhesion *Tissue engineering* **7** 55-71

[125] Lavenus S, Ricquier J-C, Louarn G and Layrolle P 2010 Cell interaction with nanopatterned surface of implants *Nanomedicine* **5** 937-47

[126] Bandhyopadhya A and Bose S 2013 *Characterization of biomaterials* (Amsterdam: Elsevier)

[127] Gulati K, Maher S, Chandrasekaran S, Findlay D M and Losic D 2016 Conversion of titania (TiO2) into conductive titanium (Ti) nanotube arrays for combined drug-delivery and electrical stimulation therapy *Journal of Materials Chemistry B* **4** 371-5

[128] Lampin M, Warocquier C, Legris C, Degrange M and Sigot-Luizard M F 1997 Correlation between substratum roughness and wettability, cell adhesion, and cell migration *Journal of biomedical materials research* **36** 99-108

[129] Crawford G, Chawla N and Houston J 2009 Nanomechanics of biocompatible TiO 2 nanotubes by interfacial force microscopy (IFM) *Journal of the mechanical behavior of biomedical materials* **2** 580-7

[130] Vogler E A 2012 Protein adsorption in three dimensions *Biomaterials* **33** 1201-37

[131] Narayanan R, Kwon T-Y and Kim K-H 2009 TiO2 nanotubes from stirred glycerol/NH4F electrolyte: Roughness, wetting behavior and adhesion for implant applications *Materials Chemistry and Physics* **117** 460-4

[132] Dowling D P, Miller I S, Ardhaoui M and Gallagher W M 2011 Effect of surface wettability and topography on the adhesion of osteosarcoma cells on plasma-modified polystyrene *Journal of biomaterials applications* **26** 327-47

[133] Oliveira S M, Alves N M and Mano J F 2014 Cell interactions with superhydrophilic and superhydrophobic surfaces *Journal of Adhesion Science and Technology* **28** 843-63

[134] Meng J, Yang G, Liu L, Song Y, Jiang L and Wang S 2017 Cell adhesive spectra along surface wettability gradient from superhydrophilicity to superhydrophobicity *Science China Chemistry* **60** 614-20

[135] Gao S, Lu R, Wang X, Chou J, Wang N, Huai X, Wang C, Zhao Y and Chen S 2020 Immune response of macrophages on super-hydrophilic TiO2 nanotube arrays *Journal of Biomaterials Applications* **34** 1239-53

[136] He Y, Luo J, Zhang Y, Li Z, Chen F, Song W and Zhang Y 2020 The unique regulation of implant surface nanostructure on macrophages M1 polarization *Materials Science & Engineering C* **106** 110221

[137] Eriksson C 1976 Bone morphogenesis and surface charge *Clinical orthopaedics and related research* **121** 295-302

[138] Ohgaki M, Kizuki T, Katsura M and Yamashita K 2001 Manipulation of selective cell adhesion and growth by surface charges of electrically polarized hydroxyapatite *Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials* **57** 366-73

[139] Cai K, Frant M, Bossert J, Hildebrand G, Liefeith K and Jandt K D 2006 Surface functionalized titanium thin films: zeta-potential, protein adsorption and cell proliferation *Colloids and Surfaces B: Biointerfaces* **50** 1-8

[140] Kabaso D, Gongadze E, Perutková Š, Matschegewski C, Kralj-Iglič V, Beck U, van Rienen U and Iglič A 2011 Mechanics and electrostatics of the interactions between osteoblasts and titanium surface *Computer methods in biomechanics and biomedical engineering* **14** 469-82

[141] Bauer T W 1995 Hydroxyapatite: Coating controversies *Orthopedics* **18** 885-8

[142] Crawford G, Chawla N, Das K, Bose S and Bandyopadhyay A 2007 Microstructure and deformation behavior of biocompatible TiO 2 nanotubes on titanium substrate *Acta Biomaterialia* **3** 359-67

[143] Roy M, Bandyopadhyay A and Bose S 2011 Induction plasma sprayed nano hydroxyapatite coatings on titanium for orthopaedic and dental implants *Surface and Coatings Technology* **205** 2785-92

[144] von Wilmowsky C, Moest T, Nkenke E, Stelzle F and Schlegel K A 2014 Implants in bone: Part I. A current overview about tissue response, surface modifications and future perspectives *Oral and maxillofacial surgery* **18** 243-57

[145] Zhang Y, Han Y and Zhang L 2015 Interfacial structure of the firmly adhered TiO 2 nanotube films to titanium fabricated by a modified anodization *Thin Solid Films* **583** 151-7

[146] Ai J, Biazar E, Jafarpour M, Montazeri M, Majdi A, Aminifard S, Zafari M, Akbari H R and Rad H G 2011 Nanotoxicology and nanoparticle safety in biomedical designs *International journal of nanomedicine* **6** 1117

[147] Gulati K, Santos A, Findlay D and Losic D 2015 Optimizing anodization conditions for the growth of titania nanotubes on curved surfaces *The Journal of Physical Chemistry C* **119** 16033-45

[148] Tsui Y C, Doyle C and Clyne T W 1998 Plasma sprayed hydroxyapatite coatings on titanium substrates Part 1: Mechanical properties and residual stress levels *Biomaterials* **19** 2015-29

[149] Li T, Gulati K, Wang N, Zhang Z and Ivanovski S 2018 Bridging the gap: Optimized fabrication of robust titania nanostructures on complex implant geometries towards clinical translation *Journal of Colloid And Interface Science* **529** 452-63

[150] Alvarez K and Nakajima H 2009 Metallic scaffolds for bone regeneration *Materials* **2** 790-832

[151] Brammer K S, Oh S, Cobb C J, Bjursten L M, van der Heyde H and Jin S 2009 Improved bone-forming functionality on diameter-controlled TiO 2 nanotube surface *Acta biomaterialia* **5** 3215-23

[152] Smith T 1994 The effect of plasma-sprayed coatings on the fatigue of titanium alloy implants *JOM* **46** 54-6

[153] Apachitei I, Leoni A, Riemslag A C, Fratila-Apachitei L E and Duszczyk J 2011 Enhanced fatigue performance of porous coated Ti6Al4V biomedical alloy *Applied Surface Science* **257** 6941-4

[154] Dong H 2010 *Surface Engineering of Light Alloys*: Elsevier) pp 58-80

[155] Tang X and Li D 2009 Fabrication, geometry, and mechanical properties of highly ordered TiO2 nanotubular arrays *The Journal of Physical Chemistry C* **113** 7107-13

[156] Richard C, Kowandy C, Landoulsi J, Geetha M and Ramasawmy H 2010 Corrosion and wear behavior of thermally sprayed nano ceramic coatings on commercially pure Titanium and Ti–13Nb–13Zr substrates *International Journal of Refractory Metals and Hard Materials* **28** 115-23

[157] Misch C E 2007 Stress treatment theorem for implant dentistry *Contemporary Implant Dentistry. 3rd ed. India: Mosby, Elsevier* 68-91

[158] Chaves J, Escada A, Rodrigues A and Claro A A 2016 Characterization of the structure, thermal stability and wettability of the TiO 2 nanotubes growth on the Ti–7.5 Mo alloy surface *Applied Surface Science* **370** 76-82

[159] Bosco R, Van Den Beucken J, Leeuwenburgh S and Jansen J 2012 Surface engineering for bone implants: a trend from passive to active surfaces *Coatings* **2** 95-119

[160] Su Y, Cockerill I, Zheng Y, Tang L, Qin Y-X and Zhu D 2019 Biofunctionalization of metallic implants by calcium phosphate coatings *Bioactive Materials* **4** 196-206

[161] Hahn R, Kirnbauer A, Bartosik M, Koloszvári S and Mayrhofer P H 2019 Toughness of Si alloyed high-entropy nitride coatings *Materials Letters*

[162] Dossett J and Totten G 2013 Introduction to Surface Hardening of Steels. DOI)

[163] Ranade A N, Rama Krishna L, Li Z, Wang J, Korach C S and Chung Y-W 2012 Relationship between hardness and fracture toughness in Ti–TiB2 nanocomposite coatings *Surface & Coatings Technology* **213** 26-32

[164] Family R, Solati-Hashjin M, Nik S N and Nemati A 2012 Surface modification for titanium implants by hydroxyapatite nanocomposite *Caspian Journal of Internal Medicine* **3** 460-5

[165] Kim H-J, Yoo S-S and Kim D-E 2012 Nano-scale wear: a review *International Journal of Precision Engineering and Manufacturing* **13** 1709-18

[166] Rocha L, Oliveira F, Cruz H, Sukotjo C and Mathew M 2013 *Bio-tribocorrosion in biomaterials and medical implants*: Elsevier) pp 223-49

[167] Mor G K, Varghese O K, Paulose M, Shankar K and Grimes C A 2006 A review on highly ordered, vertically oriented TiO 2 nanotube arrays: fabrication, material properties, and solar energy applications *Solar Energy Materials and Solar Cells* **90** 2011-75

[168] Tan A, Pingguan-Murphy B, Ahmad R and Akbar S 2012 Review of titania nanotubes: Fabrication and cellular response *Ceramics International* **38** 4421-35

[169] Bhardwaj G and Webster T J 2017 Reduced bacterial growth and increased osteoblast proliferation on titanium with a nanophase TiO2 surface treatment *International journal of nanomedicine* **12** 363

[170] Kulkarni M, Flašker A, Lokar M, Mrak-Poljšak K, Mazare A, Artenjak A, Čučnik S, Kralj S, Velikonja A, Schmuki P, Kralj-Iglič V, Sodin-Semrl S and Iglič A 2015 Binding of plasma proteins to titanium dioxide nanotubes with different diameters *International Journal of Nanomedicine* **10** 1359-73

[171] Yang W, Xi X, Ran Q, Liu P, Hu Y and Cai K 2014 Influence of the titania nanotubes dimensions on adsorption of collagen: an experimental and computational study *Materials Science and Engineering: C* **34** 410-6

[172] Oh S, Brammer K S, Li Y S J, Teng D, Engler A J, Chien S and Jin S 2009 Stem Cell Fate Dictated Solely by Altered Nanotube Dimension *Proceedings of the National Academy of Sciences of the United States of America* **106** 2130-5

[173] Gulati K, Aw M S, Findlay D and Losic D 2012 Local drug delivery to the bone by drug-releasing implants: perspectives of nano-engineered titania nanotube arrays *Therapeutic delivery* **3** 857-73

[174] Gulati K, Moon H-J, Li T, Kumar P S and Ivanovski S 2018 Titania nanopores with dual micro-/nano-topography for selective cellular bioactivity *Materials Science and Engineering: C* **91** 624-30

[175] Li J, Mutreja I, Tredinnick S, Jermy M, Hooper G J and Woodfield T B F 2020 Hydrodynamic control of titania nanotube formation on Ti-6Al-4V alloys enhances osteogenic differentiation of human mesenchymal stromal cells *Materials Science & Engineering C* **109** 110562

[176] Neacsu P, Mazare A, Cimpean A, Park J, Costache M, Schmuki P and Demetrescu I 2014 Reduced inflammatory activity of RAW 264.7 macrophages on titania nanotube modified Ti surface *The international journal of biochemistry & cell biology* **55** 187-95

[177] Park J, Bauer S, von der Mark K and Schmuki P 2007 Nanosize and vitality: TiO2 nanotube diameter directs cell fate *Nano letters* **7** 1686-91

[178] Bauer S, Park J, Faltenbacher J, Berger S, von der Mark K and Schmuki P 2009 Size selective behavior of mesenchymal stem cells on ZrO 2 and TiO2 nanotube arrays *Integrative Biology* **1** 525-32

[179] Park J, Bauer S, Schlegel K A, Neukam F W, von der Mark K and Schmuki P 2009 TiO2 nanotube surfaces: 15 nm—an optimal length scale of surface topography for cell adhesion and differentiation *Small* **5** 666-71

[180] Park J, Bauer S, Schmuki P and von der Mark K 2009 Narrow window in nanoscale dependent activation of endothelial cell growth and differentiation on TiO2 nanotube surfaces *Nano letters* **9** 3157-64

[181] Kim D, Choi B, Song J, Kim S, Oh S, Jin E-H, Kang S-S and Jin E-J 2011 TiO2 nanotube stimulate chondrogenic differentiation of limb mesenchymal cells by modulating focal activity *Experimental and Molecular Medicine* **43** 455-61

[182] Peng Z, Ni J, Zheng K, Shen Y, Wang X, He G, Jin S and Tang T 2013 Dual effects and mechanism of TiO2 nanotube arrays in reducing bacterial colonization and enhancing C3H10T1/2 cell adhesion *International journal of nanomedicine* **8** 3093

[183] Zhang R, Wu H, Ni J, Zhao C, Chen Y, Zheng C and Zhang X 2015 Guided proliferation and bone-

forming functionality on highly ordered large diameter TiO 2 nanotube arrays *Materials Science and Engineering: C* **53** 272-9

[184] Yu W q, Jiang X q, Zhang F q and Xu L 2010 The effect of anatase TiO2 nanotube layers on MC3T3-E1 preosteoblast adhesion, proliferation, and differentiation *Journal of Biomedical Materials Research Part A* **94** 1012-22

[185] Zouani O F, Chanseau C, Brouillaud B, Bareille R, Deliane F, Foulc M-P, Mehdi A and Durrieu M-C 2012 Altered nanofeature size dictates stem cell differentiation *J Cell Sci* **125** 1217-24

[186] Anitha V, Lee J-H, Lee J, Banerjee A N, Joo S W and Min B K 2015 Biofilm formation on a TiO2 nanotube with controlled pore diameter and surface wettability *Nanotechnology* **26** 065102

[187] Puckett S D, Taylor E, Raimondo T and Webster T J 2010 The relationship between the nanostructure of titanium surfaces and bacterial attachment *Biomaterials* **31** 706-13

[188] Kummer K M, Taylor E N, Durmas N G, Tarquinio K M, Ercan B and Webster T J 2013 Effects of different sterilization techniques and varying anodized TiO2 nanotube dimensions on bacteria growth *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **101B** 677-88

[189] Ercan B, Kummer K M, Tarquinio K M and Webster T J 2011 Decreased Staphylococcus aureus biofilm growth on anodized nanotubular titanium and the effect of electrical stimulation *Acta biomaterialia* **7** 3003-12

[190] Xu Z, Lai Y, Wu D, Huang W, Huang S, Zhou L and Chen J 2015 Increased mesenchymal stem cell response and decreased staphylococcus aureus adhesion on titania nanotubes without pharmaceuticals *BioMed research international* **2015**

[191] Kumeria T, Mon H, Aw M S, Gulati K, Santos A, Griesser H J and Losic D 2015 Advanced biopolymer-coated drug-releasing titania nanotubes (TNTs) implants with simultaneously enhanced osteoblast adhesion and antibacterial properties *Colloids and Surfaces B: Biointerfaces* **130** 255-63

[192] Necula M G, Mazare A, Ion R N, Ozkan S, Park J, Schmuki P and Cimpean A 2019 Lateral Spacing of TiO2 Nanotubes Modulates Osteoblast Behavior *Materials* **12** 2956

[193] Bai Y, Park I S, Park H H, Lee M H, Bae T S, Duncan W and Swain M 2011 The effect of annealing temperatures on surface properties, hydroxyapatite growth and cell behaviors of TiO2 nanotubes *Surface and Interface Analysis* **43** 998-1005

[194] Khrunyk Y Y, Belikov S V, Tsurkan M V, Vyalykh I V, Markaryan A Y, Karabanalov M S, Popov A A and Wysokowski M J N 2020 Surface-dependent osteoblasts response to TiO2 nanotubes of different crystallinity **10** 320

[195] Damodaran V B, Bhatnagar D, Leszczak V and Popat K C 2015 Titania nanostructures: a biomedical perspective *Rsc Advances* **5** 37149-71

[196] Oh S, Daraio C, Chen L H, Pisanic T R, Finones R R and Jin S 2006 Significantly accelerated osteoblast cell growth on aligned TiO2 nanotubes *Journal of Biomedical Materials Research Part A* **78** 97-103

[197] Liu G, Du K and Wang K 2016 Surface wettability of TiO 2 nanotube arrays prepared by electrochemical anodization *Applied Surface Science* **388** 313-20

[198] Papenburg B J, Rodrigues E D, Wessling M and Stamatialis D 2010 Insights into the role of material surface topography and wettability on cell-material interactions *Soft Matter* **6** 4377-88

[199] Indira K, Mudali U K and Rajendran N 2013 Corrosion behavior of electrochemically assembled nanoporous titania for biomedical applications *Ceramics International* **39** 959-67

[200] Liu C, Wang M, Wang Y, Huang W and Chu P K 2011 Electrochemical stability of TiO 2 nanotubes with different diameters in artificial saliva *Surface & Coatings Technology* **206** 63-7

[201] Hang R, Liu Y, Liu S, Bai L, Gao A, Zhang X, Huang X, Tang B and Chu P K 2016 Size-dependent corrosion behavior and cytocompatibility of Ni–Ti–O nanotubes prepared by anodization of biomedical NiTi alloy *Corrosion Science* **103** 173-80

[202] Gong D, Grimes C A, Varghese O K, Hu W, Singh R, Chen Z and Dickey E C 2001 Titanium oxide nanotube arrays prepared by anodic oxidation *Journal of Materials Research* **16** 3331-4

[203] Ahmadi S, Mohammadi I and Sadrnezhaad S 2016 Hydroxyapatite based and anodic Titania nanotube biocomposite coatings: Fabrication, characterization and electrochemical behavior *Surface and Coatings Technology* **287** 67-75

[204] Hilario F, Roche V, Nogueira R P and Junior A M J 2017 Influence of morphology and crystalline structure of TiO2 nanotubes on their electrochemical properties and apatite-forming ability *Electrochimica Acta* **245** 337-49

[205] Dang T-D and Thi Thanh Huyen B 2017 The Electrowetting and Corrosion Characterizations of Anodized As-Prepared Titanium Dioxide Nanotube *Journal of Electronic Materials* **46** 3279

[206] Pan J, Leygraf C, Thierry D and Ektessabi A 1997 Corrosion resistance for biomaterial applications of TiO2 films deposited on titanium and stainless steel by ion- beam- assisted sputtering *Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials and The Japanese Society for Biomaterials* **35** 309-18

[207] Mazăre A, Voicu G, Truscă R and Ioniţă D 2011 Heat treatment of TiO2 nanotubes, a way to significantly change their behaviour

[208] Yu W-q, Qiu J, Xu L and Zhang F-q 2009 Corrosion behaviors of TiO2 nanotube layers on titanium in Hank's solution *Biomedical Materials* **4** 065012

[209] Rafieerad A, Bushroa A, Zalnezhad E, Sarraf M, Basirun W, Baradaran S and Nasiri-Tabrizi B 2015 Microstructural development and corrosion behavior of self-organized TiO 2 nanotubes coated on Ti–6Al–7Nb *Ceramics International* **41** 10844-55

[210] Mazare A, Totea G, Burnei C, Schmuki P, Demetrescu I and Ionita D 2016 Corrosion, antibacterial activity and haemocompatibility of TiO 2 nanotubes as a function of their annealing temperature *Corrosion Science* **103** 215-22

[211] Zhao J, Wang X, Sun T and Li L 2007 Crystal phase transition and properties of titanium oxide nanotube arrays prepared by anodization *Journal of alloys and compounds* **434** 792-5

[212] Hamlekhan A, Butt A, Patel S, Royhman D, Takoudis C, Sukotjo C, Yuan J, Jursich G, Mathew M T, Hendrickson W, Virdi A and Shokuhfar T 2014 Fabrication of anti-aging TiO2 nanotubes on biomedical Ti alloys *PloS one* **9** e96213

[213] Zalnezhad E, Maleki E, Banihashemian S M, Park J W, Kim Y-B, Sarraf M, Sarhan A and Ramesh S 2016 Wettability, structural and optical properties investigation of TiO2 nanotubular arrays *Materials Research Bulletin* **78** 179-85

[214] Wang Y, Wen C, Hodgson P and Li Y 2014 Biocompatibility of TiO2 nanotubes with different topographies *Journal of Biomedical Materials Research Part A* **102** 743-51

[215] Zhao L, Mei S, Chu P K, Zhang Y and Wu Z 2010 The influence of hierarchical hybrid micro/nano-textured titanium surface with titania nanotubes on osteoblast functions *Biomaterials* **31** 5072-82

[216] Fu Q, Bellare A, Cui Y, Cheng B, Xu S and Kong L 2017 The Effect of Hierarchical Micro/Nanotextured Titanium Implants on Osseointegration Immediately After Tooth Extraction in Beagle Dogs *Clinical implant dentistry and related research* **19** 486-95

[217] Gulati K, Li T and Ivanovski S 2018 Consume or Conserve: Micro-Roughness of Titanium Implants towards Fabrication of Dual Micro-Nano Topography *ACS Biomaterials Science & Engineering*

[218] Kontos A, Kontos A, Tsoukleris D, Likodimos V, Kunze J, Schmuki P and Falaras P 2008 Photo-induced effects on self-organized TiO2 nanotube arrays: the influence of surface morphology *Nanotechnology* **20** 045603

[219] Çırak B B, Karadeniz S M, Kılınç T, Caglar B, Ekinci A E, Yelgin H, Kürekçi M and Çırak Ç 2017 Synthesis, surface properties, crystal structure and dye sensitized solar cell performance of TiO2 nanotube arrays anodized under different voltages *Vacuum* **144** 183-9

[220] Anderson O, Ottermann C R, Kuschnereit R, Hess P and Bange K 1997 Density and Young's modulus of thin TiO2 films *Fresenius' Journal of Analytical Chemistry* **358** 315-8

[221] Munirathinam B and Neelakantan L 2016 Role of crystallinity on the nanomechanical and electrochemical properties of TiO2 nanotubes *Journal of Electroanalytical Chemistry* **770** 73-83

[222] Oh K, Lee K and Choi J 2017 Influence of geometry and crystal structures of TiO 2 nanotubes on micro Vickers hardness *Materials Letters* **192** 137-41

[223] Sarraf M, Zalnezhad E, Bushroa A, Hamouda A, Rafieerad A and Nasiri-Tabrizi B 2015 Effect of microstructural evolution on wettability and tribological behavior of TiO 2 nanotubular arrays coated on Ti–6Al–4V *Ceramics International* **41** 7952-62

[224] Xu Y, Liu M, Wang M, Oloyede A, Bell J and Yan C 2015 Nanoindentation study of the mechanical behavior of TiO2 nanotube arrays *Journal of Applied Physics* **118** 145301

[225] Shokuhfar T, Arumugam G K, Heiden P A, Yassar R S and Friedrich C 2009 Direct compressive measurements of individual titanium dioxide nanotubes *ACS nano* **3** 3098-102

[226] Kang S-H, Fang T-H, Chen T-H, Hsiao Y-J, Hong Z-H, Chuang C-H and Riccobono L 2014 Size effect on mechanical properties of TiO2 capped nanotubes investigated using in situ transmission electron microscopy *Microsystem technologies* **20** 515-20

[227] Vera-Jiménez A, Melgoza-Alemán R, Valladares-Cisneros M and Cuevas-Arteaga C 2015 Synthesis and mechanical/electrochemical characterization of TiO 2 nanotubular structures obtained at high voltage *Journal of Nanomaterials* **16** 379

[228] Zou J-p and Wang R-z 2012 Crack initiation, propagation and saturation of TiO2 nanotube film [J] *Transactions of Nonferrous Metals Society of China* **22** 627-33

[229] Zou J-p and Wang R-z 2012 Debonding phenomenon of TiO2 nanotube film *Transactions of Nonferrous Metals Society of China* **22** 2691-9

[230] Bortolan C C, Campanelli L C, Bolfarini C and Oliveira N T C 2016 Fatigue strength of Ti-6Al-4V alloy with surface modified by TiO2 nanotubes formation *Materials Letters* **177** 46-9

[231] Campanelli L C, Silva P S C P d, Oliveira N T C and Bolfarini C 2017 Effect of the modification by titanium dioxide nanotubes with different structures on the fatigue response of Ti grade 2 *Materials Research* 0-

[232] Yu D, Zhu X, Xu Z, Zhong X, Gui Q, Song Y, Zhang S, Chen X and Li D 2014 Facile method to enhance the adhesion of TiO2 nanotube arrays to Ti

substrate *ACS applied materials & interfaces* **6** 8001-5

[233] Zhang G, Huang H, Liu Y and Zhou L 2009 Fabrication of crack-free anodic nanoporous titania and its enhanced photoelectrochemical response *Applied Catalysis B: Environmental* **90** 262-7

[234] Bjursten L M, Rasmusson L, Oh S, Smith G C, Brammer K S and Jin S 2010 Titanium dioxide nanotubes enhance bone bonding in vivo *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials* **92** 1218-24

Author statement

CRediT roles:

Daniel Martinez-Marquez, Karan Gulati, Christopher P. Carty, Rodney A. Stewart, and Sašo Ivanovski: Conceptualization; Daniel Martinez-Marquez and Karan Gulati: Data curation; Daniel Martinez-Marquez and Karan Gulati: Formal analysis; Daniel Martinez-Marquez and Christopher P. Carty: Funding acquisition; Daniel Martinez-Marquez: Investigation; Daniel Martinez-Marquez: Methodology; Karan Gulati, Christopher P. Carty, Rodney A. Stewart, and Sašo Ivanovski: Project administration; **Rodney A. Stewart, and Sašo Ivanovski:** Resources; Daniel Martinez-Marquez: Software; Christopher P. Carty, Rodney A. Stewart, and Sašo Ivanovski: Supervision; Daniel Martinez-Marquez, Karan Gulati, Rodney A. Stewart, and Sašo Ivanovski: Validation; Daniel Martinez-Marquez: Visualization; Daniel Martinez-Marquez: Roles/Writing - original draft; Daniel Martinez-Marquez, Karan Gulati, Christopher P. Carty, Rodney A. Stewart, and Sašo Ivanovski: Writing - review & editing.

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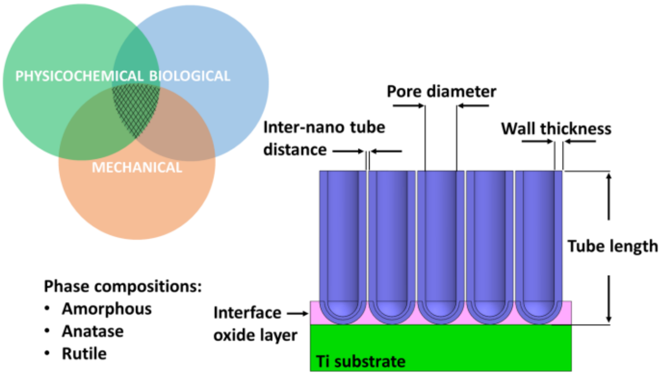
We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from k.gulati@uq.edu.au.



Graphical abstract

Highlights

1. Systematic literature search identifying titania nanotube (TNTs) characteristics that influence implant performance.
2. Web-based survey of experts to enable facilitation of a quality by design (QbD) study.
3. Determining the perceived contribution of TNTs characteristics on implants' biological, physiological and mechanical performances.
4. Highlights the most influential TNTs' characteristics to achieve desirable implant functions.