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In dental implant surfaces, NanoWar has begun… but NanoQuest is still at stake!

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Abstract

Dental implant surface characteristics are defined by a quartet of parameters, respectively at the macro-, micro-, nano- and chemical scale. Many companies are now claiming to use nanofeatures on their implant surface, while in fact only a minority of products really displays significant nanostructures. In this article, the exact terminology of nanostructures was described, and many technologies developed to produce nanostructures on titanium implants were reviewed. Practically, only a few techniques are applicable on dental implants. The most frequent forms of nanofeatures encountered in dental implant surfaces are the nanoroughness (eg Intra-Lock Ossean, AstraTech Osseospeed) and the nanoparticles in various crystalline forms (eg 3I NanoTite, Straumann SLActive). Very little is known about the real impact of these nanocharacteristics, as an element of the quartet of parameters that influence the osseointegration. The first step is to clarify the definitions to avoid the commercial confusion.

Keywords. Dental implant, nanostructure, osseointegration, titanium.

1. NanoWar has begun…

Among the few characteristics that define a dental implant system, the surface treatment and the implant macrodesign are the 2 main parameters that define the bone implant interface, from the early bone peri-implant healing to the long-term stability of the osseointegrated interface [1]. Logically, as a key characteristic of each commercially available system, the implant surface is both a very active research topic and a strong commercial argument for companies playing in a very aggressive competitive and growing market [2]. Many companies used the arguments of the microtexture of the implant surface topography (microroughness for SLA-Sand-blasted Acid-Etched surfaces, microporosity of anodized surfaces) and/or of the use of chemical modifications (such as Fluoride low impregnation or Calcium Phosphate CaP deposition) in the past, in order to claim better biological interactions and faster and better osseointegration of the implants [3].

In the last years, the use of the term « nano » became a real fashion, and many companies started to refer in their advertisements to the nanocharacteristics of their products, particularly of the dental implant surface. In this sense, on a very competitive

market, NanoWar has begun... The term « nano » started to be used in all circumstances, and it is clearly a source of abuse and confusions, playing on the meaning of this word. However, there is no doubt on the exact definition of nanocharacteristics, as described in engineering and traditional non-dental science, for example for the design of electronic chipsets [3].

In a recent article, a system of characterization of dental implant surface was suggested, and it proposed a clear terminology to characterize the surface topography and patterns at the micro- and nanoscale, following the well defined rules used since many years in general surface science [3]. Then, in a following article analyzing in details 14 significant implant systems available on the market [4], it was shown that only 4 of them had really characteristics that may be considered as nano **(Figures 1 and 2)**. Among these 4 systems, one surface is no more promoted due to mixed clinical results (Nanotite, Biomet 3I, Palm Beach Gardens, FL, USA)[5,6], 2 were never copied due to the secret of their production (Osseospeed, AstraTech, Mölndal, Sweden, and Ossean, Intra-Lock, Boca Raton, FL, USA)[7,8] and one was considered « nano » because it was covered with dry NaCl crystals (SLActive, ITI Straumann, Basel, Switzerland)[4]. Most other dental implant systems available worldwide are often copying the main surfaces analyzed in this first article, what means that in fact only a few percentages of implant systems can claim to have some real nanofeatures.

Are you nano? Between commercialism and misunderstandings, the commercial war has started, but the quest for nanotextured implant surfaces is still uncertain.

2. What is exactly a surface nanostructure?

A nanostructure is an object of intermediate size between molecular and microscopic (micrometer-sized) scales, and measuring between 1 Angström (0.1nm) and 100nm. This range of size is clearly found in all the definitions in the various fields of nanosciences [3].

Nanostructures must be described through their number of dimensions on the nanoscale.

- Structures with one nanometric dimension (a peak height or a layer thickness) are repeated to create a nanotextured surface. A repetitive and homogeneous texture with one dimension on the nanoscale was termed nanoroughness or nanorugosity.

- Structures with 2 nanometric dimensions (nanometric diameter of a repetitive pattern) are repeated to create a nanopatterned surface. Good examples are nanotubes produced by anodization or chemically produced nanopatterned surfaces, where a 2-dimension geometric form is repeated infinitely.

- Structures with 3 nanometric dimensions are nanoparticles, i.e. the particle is nanometric in each spatial dimension. A surface covered with particles of these dimensions is nanoparticled. Although the size of most molecules is within the range of a nanoparticle, individual molecules are not referred as nanoparticles.

The same range of size (between 1 Angström (0.1nm) and 100nm) can be applied to all forms of nanostructures encountered in chemical and material sciences: nanoclusters, nanocrystals, etc. When nanostructures are associated together, they can then create the various forms of nanosurfaces cited above, but also nanopowders and nanomaterials (mostly by association of nanoparticles).

A last form of nanostructure is the nanothickness coating of the surface, for example with a Calcium Phosphate CaP layer. In this situation, only a layer between 0.1nm and 100nm can be considered as really nano.

These definitions are simple, neutral and based on the terminology used in nanosciences. All surfaces have per definition a topography at the microscale and at the nanoscale, respectively termed microtopography and nanotopography [4]. However, this nanotopography may be relatively smooth and may not present clearly identifiable, homogeneous and repetitive nanostructures. Without this kind of nanostructures, a surface can be considered to have no specific nanofeatures or nanocharacteristics, and therefore to not be « nano ». The term « nanosmooth » was advocated recently to describe the surfaces without significant nanofeatures [3,4].
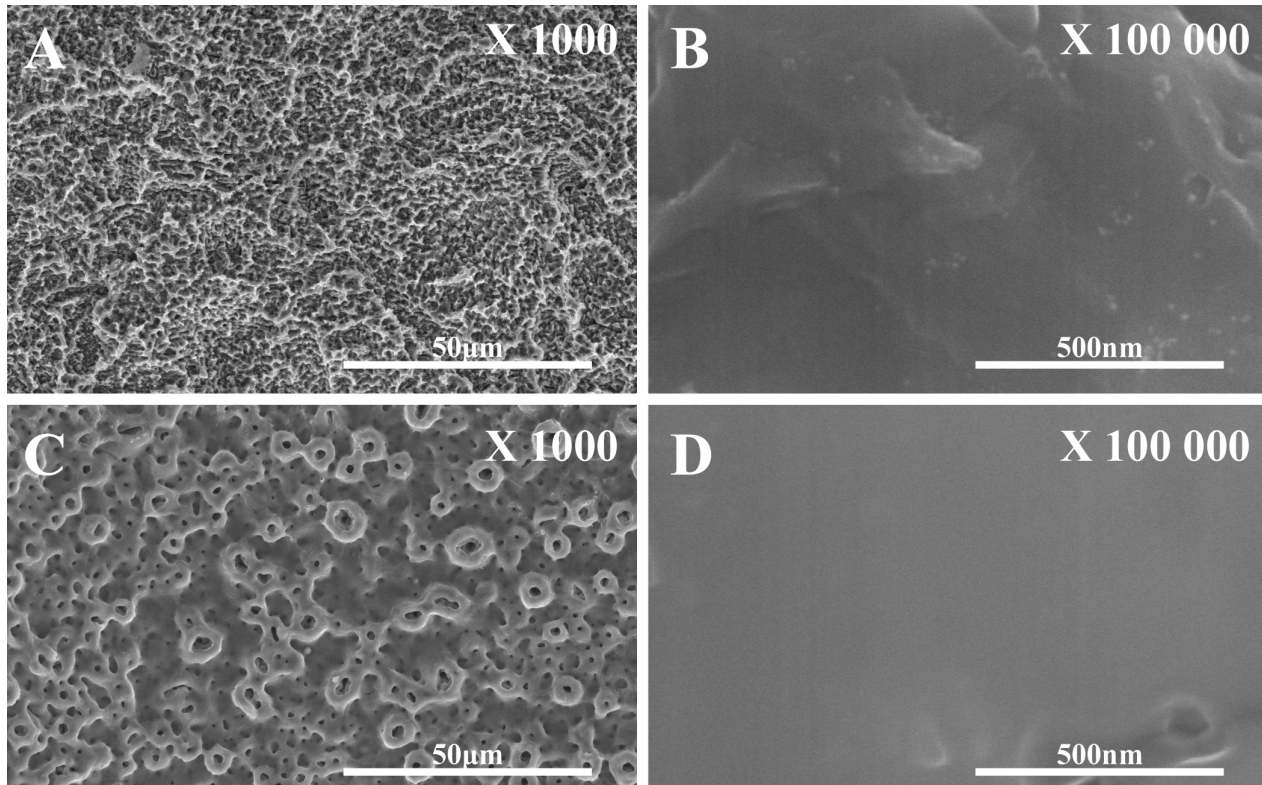

Figure 1. FE-SEM evaluation of some nanosmooth implants available on the market. (A) The Camlog surface (Camlog, Basel, Switzerland) is produced through a classical SLA (sand-blasting acid-etching) protocol. A significant microrough topography is produced. **(B)** At the nanoscale, the Camlog surface is nanosmooth. **(C)** The TiUnite surface (Nobel Biocare, Gothenburg, Sweden) is produced through anodization of titanium. During the process, a high impregnation with Phosphorus is obtained and large microporosities are produced, creating a specific micropatterning and extended cracks on the surface. **(D)** At the nanoscale, the TiUnite surface is completely nanosmooth.

Even with these clear definitions, it remains difficult to observe and characterize properly the nanostructures on dental implant surfaces. There is in fact no real accurate quantitative technique to evaluate the nanotopography on a microtextured surface, due to the interferences between the micro- and nano-architectures [3]. Repetitiveness and homogeneity are key parameters to define nanostructures as real nanofeatures, but are difficult to quantify and may be considered as qualitative morphological parameters.

The only instrument that allows to make a careful examination of these surfaces at the nanoscale is the FE-SEM (Field Emission-Scanning Electron Microscope)[3]. It offers a

much higher resolution than a classical SEM with tungsten source **[4,9]**, and allows to get accurate pictures at the nanoscale **(Figures 1 and 2),** even on surfaces with chemical modifications provoking significant charging effects (for example surfaces with Calcium Phosphate impregnation or coating are associated with charging effects and artifacts during the surface mapping using a focused electron beam reflecting across the surface)**[1]**.

There is unfortunately a very common approximation observed in the literature concerning the method of analysis of nanostructures, as the basic Scanning Electron Microscope (SEM) is often used as the gold standard for morphology characterization at the micrometer level **[9]**, but also used to investigate the nanoscale. While in fact, a FE-SEM is required to observe and characterize the nanotopography and associated nanostructures. Finally, coupled with a metrology software, FE-SEM pictures with 3° tilting can be combined and computed to get a three-dimensional reconstruction of the nanostructures for a better evaluation, sometimes advocated as a (semi-)quantitative morphology analysis **[1]**.

Other techniques such as Atomic Force Microscope (AFM) or light interferometer (IFM) are not suitable for this evaluation of the nanostructures on dental implant surfaces. AFM is using a piezoelectric probe that cannot follow the nanotopography within the complex geometry of the implant microtopography. IFM has a similar problem of physical limit related to the wavelengths of light that do not allow to map the nanostructures completely and properly, particularly when it is hidden in the shadow of the microtextures used on dental implant surfaces **[3]**. About morphology, FE-SEM remains the key instrument to perform morphology characterization at the micro- and nanoscale, and topography quantification when using the adequate metrology software **[4]**.

3. What are the effects of the nanostructures?

It is commonly considered that 4 levels of interactions exist during the osseointegration of an implant in the bone: the macrostructures, the microtopography, the nanotopography and the chemical level **[3,10]**. Each level has a different form of interaction with the bone tissue and a different effect for the integration of the implant **[11]**.

The macrodesign and the surface microtopography have first of all a function in the biomechanical interlocking of the implant in bone **[3]**. The macro- and microscale architecture also defines the space available for bone cells to interact with the surface and organize the bone apposition and remodelling: some implants use macrodesigns with larger healing chambers between the implant threads to promote a stronger bone apposition **[12]**. The microtopography was also advocated to influence directly the cell behavior, depending on the kind of microstructures (microroughness, micropores, microparticles) and the spaces between peaks and valleys of the topography **[3]**.

The material chemistry is considered to be responsible of the biochemical interlocking of the implant in bone **[3,13,14]**. If most dental implants use titanium as a core material to promote an ankylosis of the implant, many surface chemical modifications were proposed to increase the bone apposition and remodelling process through cell stimulation and mineral chelation (for example Fluoride or Calcium Phosphate CaP low impregnation, or Calcium high impregnation)**[7,8,13]**. Some implants were even coated with CaP layers, Hydroxyapatite layers or CaP nanocrystals to promote this chemical bone bonding between the implant and the bone **[15]**.

What remains for the nanomodifications? The osseointegration performance of a surface is influenced by its topography at the nanoscale following different biological mechanisms than on the microscale **[16]**. The nanotopography is supposed to influence the

surface energy and therefore the surface/protein interactions **[17]**. A significant surface energy allows to improve the surface wettability to blood and the adhesion and spreading of fibrin fibers and matrix proteins on the surface, and therefore to improve cell attachment and tissue healing, particularly during the early healing phases on the implant interface **[16]**. Many publications also suggested that a specific nanopatterning of surfaces even promotes cell proliferation and differentiation, through the direct modulation of cell behavior **[18-20]**. Among the many forms of nanostructures (nanoroughness, nanopatterning, nanotubes, nanoparticles)**[4]**, it remains however difficult to determine which nanofeature is the most efficient for the bone implant interface and is practically usable with adequate clinical results in dental implant surfaces, even if some excellent results were already reported with nanorough surfaces available on the market **[6,8]**.

One other specific aspect of nanofeatures is their potential impact in the peri-implant cervical area and the soft tissue attachment and peri-implant gingival sealing **[21]**, and more generally the effects of nanostructures to promote cell growth and reduce bacterial contamination in this competitive oral tissue area **[22,23]**. The implant surface is indeed a key factor of the peri-implantitis risk, peri-implantitis being often considered as a pathology of osseointegration **[24]**. Some recent results showed that nanoroughness applied in the implant cervical region is influencing positively bone maintenance **[21]**. It is interesting to notice that this implant system is commercially available, and using also this nanoroughness, combined with microroughness and chemical modification, on its bone/implant interface **[4,8,11]**. This result may be connected to several experimental results advocating that nanofeatures have a negative effect on bacterial growth and are a method to control bacterial contamination in many medical devices **[22,23]**.

4. Experimental and clinical results of nanofeatures on implant surfaces

There are in the scientific literature many articles describing various forms of nanofeatures prepared on implants and most of them were described as promoting positive effects *in vitro* and *in vivo* **[16]**. However, most of these works remain very experimental, and only few of them have concrete practical applications in products in current daily clinical use.

Among the many techniques that were described, we can for example cite the creations of nanotubes through anodization **[23,25,26]**. The process is quite similar to the anodization used to produce a specific microporous surface (for example the TiUnite system, Nobel Biocare, Gothenburg, Sweden)**[4,13]**, but using different parameters, the surface can be covered with lines of nanoporous structures only. One idea was even to dope the surface by filling these nanotubes with some drugs or minerals to boost chemically the osseointegration process through a slow release of a pharmaceutical preparation **[26]**. The system was considered with interest but finally abandoned for dental implants, as the lines of nanotubes are often considered too fragile to be used on oral implants enduring significant biomechanical constraints during the daily dental function. However, the cell modulation promoted by this kind of surface was described as very interesting to improve cell adhesion **[26]** and to reduce bacterial adhesion and proliferation **[23]** on titanium implantable devices and is still under evaluation in non dental applications like stents **[25]**, where no significant mechanical constraints are applied on the implant, reducing the risk of surface delamination.

Another form of nanomodification was the discrete calcium phosphate nanocrystalline deposition on a titanium dental implant surface, to enhance the contact osteoconduction on these surfaces [27], following the concept of chemical interlocking described as "bone bonding" [28]. Primary results were described as very promising and some famous implant system even used this technology to promote this concept of « bone bonding » (Nanotite, Biomet 3I, Palm Beach Gardens, FL, USA)[4]. However, the development of this technology was finally limited due to results inferior to other traditional non nanofeatured surfaces [5,6]. This result was never really explained scientifically, but was probably not related to the nanotexturing itself, but it was more probably due to the chemical composition of the nanoparticles (Calcium Phosphate percentage was more than 20% of the surface chemical composition)[4]. The same use of calcium phosphates or hydroxyapatites coating (for example nanopolymorphic crystalline hydroxyapatite coating)[29] was advocated by other authors, but the general fashion with these coatings is slowly declining nowadays, due to the lack of advantages (and sometimes the failures) observed in practical daily clinical results.

One very commonly tested method to create nanostructures is the chemical nanopatterning (for example oxidative patterning)[19,30]. In this domain, many *in vitro* results showed that repetitive nanopatterns created through the action of acidic-basic components are able to modulate positively the cell behavior [30]. Many works tried to analyze the cell gene expression in contact with various forms of nanopatterns, following the general concept of tissue engineering through nanoprogramming of the materials [31]. Some authors even tried to constitute theoretical libraries of potential nanopatterns and their *in vitro* cell effects [32]. The same kind of works can be found with other techniques of nanopatterning such as colloidal lithography [33]. These works are all very interesting, but remain quite far from the practical industrial applications, as dental implant surfaces are a 4-level system (macro, micro, nano, chemical) where all levels are interconnected and each level is influencing the effects of the other levels [11].

Another experimental method of nanomodification is to use the crystal phases of titanium (rutile, anatase and amorphous titanium) to create a titanium film with a roughness of about 8-10 nm synthesized by magnetron sputtering [34]. First results highlighted that the nanoscale topography created with the anatase phase of titanium promoted the best *in vitro* effects in terms of cell adhesion, proliferation and differentiation [34]. This experimental result is difficult to apply on microrough dental implant technologies, but may give some explanation on the good results offered by some forms of nanotexturization [8,11], as all surface treatments are also impacting the external crystal phase of the implant titanium core material [3].

Another example is the coating of dental implant surfaces with various forms of hydroxapatites (HA). If most HA layers are micrometric, some recent developments proposed to cover the implant surface with layers of Calcium Phosphate ranging from 30-50 nm to 300-500 nm, through the use of IBAD (Ion Beam Assisted Deposition)[15,35,36]. If it is possible to call « nanocoating » a 30-50 nm layer, the 300-500 nm layer is clearly a micrometric modification [15]. Experimental results with these 2 surfaces were reported as different, while in theory they have the same external chemistry. This example demonstrated that the concept of scale of interaction nano/micro is very real when considering the behavior of the cells that will be in contact with the external layer. This kind of surfaces was marketed as a NanoTite or Integra-CP (Bicon, Boston, MA, USA), while in fact the technology is using a layer superior to 100nm thickness, which is therefore not nano [4].
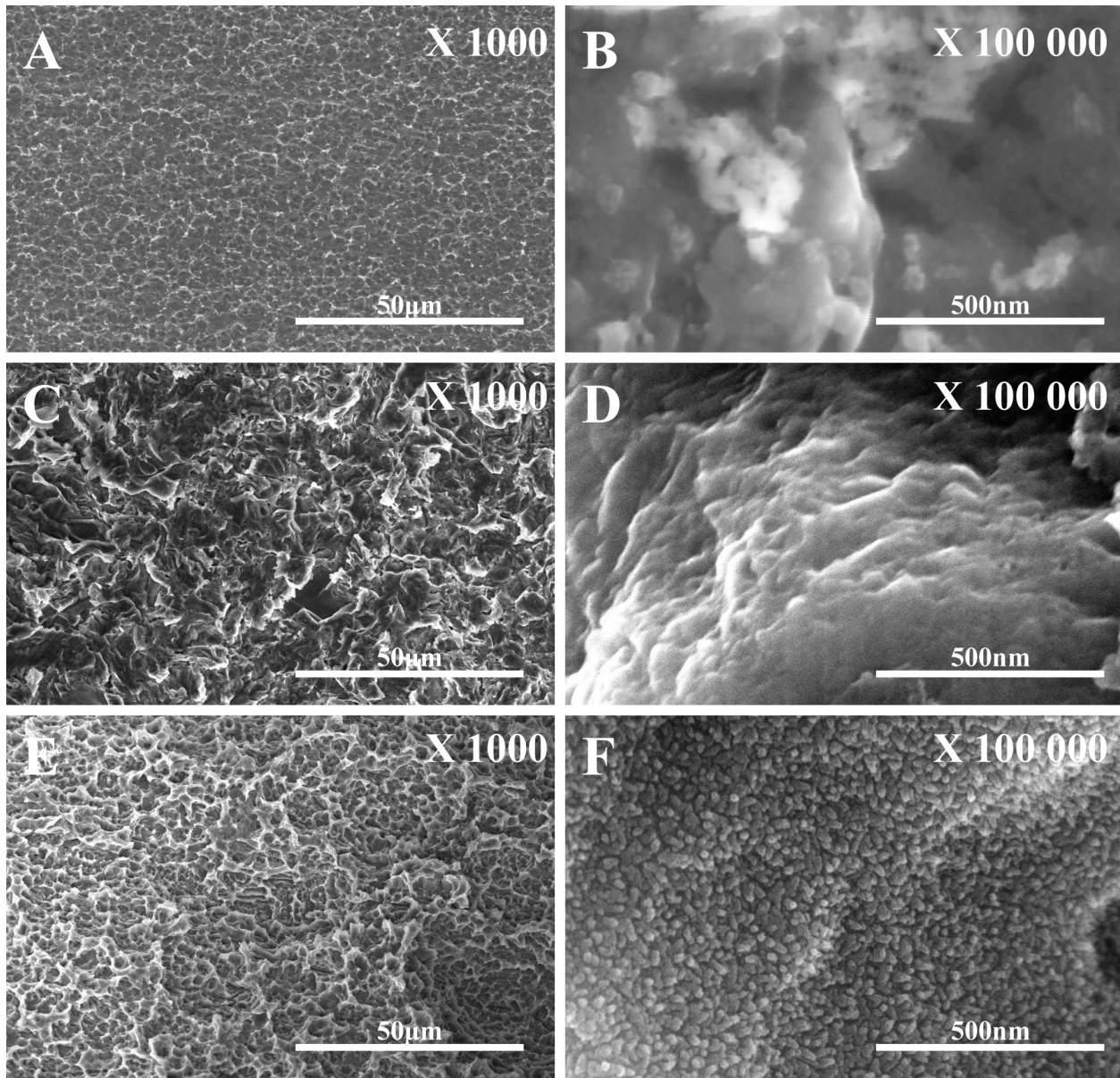
Figure 2. FE-SEM evaluation of some nanotextured implants available on the market.
(A) The 3I Nanotite surface (Biomet 3I, Palm Beach Gardens, FL, USA) is produced through discrete calcium phosphate nanocrystalline deposition on a titanium implant surface after acid etching. The surface microtopography is quite smooth, and covered by microcrystals of CaP. **(B)** At the nanoscale, the CaP nanocrystals of NanoTite are very visible and covering a significant portion of the surface. **(C)** The Ossean surface (Intra-Lock, Boca Raton, FL, USA) is a microrough nanorough CaP low impregnated surface, obtained through an initial resorbable blasting media treatment followed by specific post-processing. The microtopography presents a moderately rough aspect. **(D)** At the nanoscale, the Ossean surface presents a very significant nanoroughness. **(E)** The SLActive surface (ITI Straumann, Basel, Switzerland) is produced through a classical SLA (sand-blasting acid-etching) protocol, followed by an immersion in a specific physiological solution. A significant microrough topography is produced. **(F)** At the nanoscale, the SLActive surface is covered by an instable soluble layer of crystals of NaCl (Sodium Chloride) deposited by the physiological solution during the drying of the sample, thus giving a nanotextured aspect.

Many other experimental techniques also reported the production of nanostructures (for example TiO₂ nanoparticles coating to increase surface reactivity and early nucleation of apatite)**[37]**, but most works remain very experimental. Clinical results may be very different from the first published results, and many techniques do not have clear practical and industrial applications for mass-production dental implants.

5. NanoQuest is still at stake

Most of the commercially available implants do not display nanostructures **(Figure 1)[4]**. The first reasons is that most of the experimental methods described in the previous paragraphs were either inadequate for dental implants (fragile nanotubes) or difficult to apply properly and homogeneously on microtextured surfaces because of the interferences with the microtopography (oxidative nanopatterning, colloidal lithography, magnetron sputtering).

The second reason is that some of the technologies used to produce nanostructures did not allow to get the expected good clinical results. This is for example the case of the discrete calcium phosphate nanocrystalline deposition **(Figures 2A and 2B)**, which was finally no more promoted and replaced by a simple acid-etched surface without any nanostructures **[5]**. This experience may have discouraged some companies to move too quickly and for real in the nanotechnologies. Moreover, the use of nanocoating (thickness less than 100nm) remains rare, and much thicker micro-coatings are used by a very limited number of companies using a very specific implant design (Bicon, Boston, MA, USA)**[15]**. As a consequence, most surfaces available on classical screw implants - and giving excellent clinical results - are in fact terribly nanosmooth **(Figure 1)[4]**.

A few rare companies are using real nanoroughness as previously defined (Osseospeed, AstraTech, Mölndal, Sweden and Ossean, Intra-Lock, Boca Raton, FL, USA, particularly) through the development of some specific and secret production processing **(Figures 2C and 2D)[8]**. Several other companies are proposing an apposition of saline soluble nanocrystals as nanomodification (SLActive, ITI Straumann, Basel, Switzerland, and all its copies)**(Figures 2E and 2F)[4,38]**. These 3 systems offer excellent reported results, but their success is related to their combination of macrodesign, microtopography, nanotopography and chemistry – not to their nanofeatures alone. It is probable that a few other systems may present some real nanofeatures, but they still have to be well identified in the future **[39]**. Little is known on what would be the ideal nanostructures to use, and the ideal combination of features at the macro-, micro-, nano- and chemical scale.

Paradoxically, we have never so much heard about « nano » everywhere, while nanostructures are quite absent from the products available on the market, even if many companies are playing on the ambiguity to confuse users. While also we are still very far to really understand how the nanofeatures are influencing healing around dental implants and how they should be combined with other parameters. Yes, the NanoWar is raging, but the Quest for a better understanding and use of these features is just beginning. We can only hope that the commercial interests will not interfere too much with this Quest and to undermine the credibility of this field. In this article, we tried to give a clear vision of the current situation and recall, again, the key definitions of the topic. The non-commercial accurate definition of nanocharacteristics remains the best approach to avoid confusions.

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