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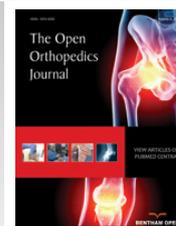
REVIEW ARTICLE

Nanoscale Surface Modifications of Orthopaedic Implants: State of the Art and Perspectives

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Abstract:

Background:

Orthopaedic implants such as the total hip or total knee replacement are examples of surgical interventions with postoperative success rates of over 90% at 10 years. Implant failure is associated with wear particles and pain that requires surgical revision. Improving the implant - bone surface interface is a key area for biomaterial research for future clinical applications. Current implants utilise mechanical, chemical or physical methods for surface modification.

Methods:

A review of all literature concerning the nanoscale surface modification of orthopaedic implant technology was conducted.

Results:

The techniques and fabrication methods of nanoscale surface modifications are discussed in detail, including benefits and potential pitfalls. Future directions for nanoscale surface technology are explored.

Conclusion:

Future understanding of the role of mechanical cues and protein adsorption will enable greater flexibility in surface control. The aim of this review is to investigate and summarise the current concepts and future directions for controlling the implant nanosurface to improve interactions.

Keywords: Chemical Modification, Implant Osseointegration, Nanosurface Modifications, Orthopaedic Implants, Physical Modification.

INTRODUCTION

Orthopaedic implants such as the total hip or total knee replacement are examples of efficacious surgical interventions with postoperative success rates of >90% at 10 years [1, 2]. Current statistics from the national joint registry identify that in 2014 over 144073 prostheses were inserted in NHS hospitals, with over 59269 implants to date in 2015 [3]. The failure rate of such implants in the United Kingdom is 6% at 5 years and 12% at 10 years implantation [3]. Implant failure results from osteolysis of wear particles that results in loosening of the prosthesis [4]. This is manifested in peri prosthetic pain for the patient and a reduction in mobility [5 - 7]. Prosthetic loosening results in a loss in bone volume due to a down regulation in bone stimulation and requires large implants at revision. The use of large implants further reduced bone volume and perpetuates the decline in patient function and risk of poor outcome.

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There are therefore serious healthcare costs associated both with the implant surgery and the subsequent care requirements and morbidity associated with poor outcomes from such surgery. They are therefore costly for the healthcare provides both in terms of resource allocation in surgeons time but also in post operative care of the patient. Therefore improving implant integration and reducing the need for revision surgery is paramount both for improving patient outcomes but in reducing associated healthcare and social costs.

In designing materials for critical sized bone defects an appreciation of the mechanisms that instigate reformation is necessary. Ultimately a synergy between osteoconduction, osteoinduction and osteogenesis must be sought [8]. Osteogenesis is the construction of new bone from osteocompetent cells. Osteoconduction is the formation of one along a preformed biologic or alloplastic scaffold with host osteocompetent cells. Osteoinduction is the establishment of new bone by stimulation and proliferation of mesenchymal cells by inductive proteins. Therefore to yield regeneration materials much contain or stimulate all of these key elements. Hence a route to reducing implant failure must seek to initiate and synergise these three elements. Namely using bulk and surface implant material properties to induce osteoconduction and osteogenesis.

A variety of materials have been investigated to manufacture biomedical implants, namely metals, ceramics, polymers and their composites [9 - 11]. Nevertheless metals remain the benchmark in implantology due to their superior mechanical properties, particularly higher resistance to corrosion and biocompatibility. In implantology the biocompatibility can be particularly defined as the materials ability to yield specific biological functions without stimulating adverse immune and cell reactions. Metals such as stainless steels, titanium or its alloys are particularly favoured in orthopaedics due to their proven record of stimulating osseointegration. Chromium cobalt metals have also been explored due to their low friction coefficient and greater wear resistance and have been adopted in knee and hip joints [12, 13]. Alternatively tantalum has been utilised in spinal surgery as a 3D porous scaffold to improve osseointegration [14].

One future direction is the transition to utilising biodegradable materials to synthesis parts of implants. Magnesium and iron based alloys are gaining increasing focus for musculokeletal applications, where they have been employed for non permanent devices namely wires or plates.

The focus in biotechnology from the micro level to the nanoscale has shed light on the significant control yielded by manipulating the nanosurface properties of materials [15 - 25]. Particular manipulation of nanoengineered surfaces allows control of the molecular and cellular events guiding protein adsorption, cell adhesion and proliferation. Therefore various nanotechnology techniques have been explored to construct nanoscale surfaces to materials to attempt to manipulate and improve integration.

In this review we will focus on current methods utilised for structuring the nanosurfaces of implantable metals, focusing on those that offer clinical and commercial applicability in orthopaedics [15, 16]. We will then highlight the improved biological integration achieved with such nanosurface properties through *in vitro* results. Finally we will correlate this through to established *in vivo* models and clinical translation. We will conclude with future directions for such orthopaedic implants.

TECHNIQUES IN NANOSCALE SURFACE MODIFICATIONS

The needs of modern biomaterials have shifted the focus from 'top down' synthesis of the micro and nano scales of biomaterial structures to the 'bottom up' synthesis [26]. In this context various techniques can be adopted to manipulate the surface properties of implantable materials. Furthermore the advantage of requiring a prefabricated implant, rather than a material that generates *in situ*, is that it leaves a wide array of available techniques. The only hinderence to some of the available methods is the longevity in the surface modification and it's robustness in sterilisation of the implant for clinical use [27 - 29]. It is now recognised that host material immune tissue reactions are governed by nanometric cues for cell surface interaction [30]. Hence micron sized features are too indiscriminate for directing cellular activity and mechanical translation [31 - 33]. The unifying criteria for nanosurface modification techniques are threefold, namely those that are able to concurrently reach all surfaces in devices with complex topography. Secondly to modify nanoscale features on the commercial scale and finally to be industrially integrated. Henceforth the methods can be divided into the mechanical, chemical and physical modifications.

MECHANICAL TECHNIQUES

The mechanical surface modifications used to obtain superficial changes are through subtraction or attrition process

[34, 35]. These obtain specific surface topographies that improved adhesion in bonding and biomineralisation due to increased surface area [36]. The main limitation with mechanical techniques is that previously their modification was limited to the microscale. Recently a novel surface mechanical attrition treatment, SMAT, has been developed that allows surface roughening on the nanoscale [37 - 39]. This nanophase attrition process particle or grain sizes less than 100nm [40]. The higher percentage of particle boundaries yielded by having nanoscale roughening yields greater osteoblast cell adhesion [37 - 40]. Human bone consists of grain sizes from 20-80nm and 2-3nm in diameter for osteoid minerals [40]. Such roughening increases the adhesion energy which is reflected in the positive cellular response. The SMAT technique may be a commercially viable technique for modification of current orthopaedic implants, however it is limited on its flexibility in controlling the intracellular response beyond local adhesion energy.

CHEMICAL TECHNIQUES

One method to improve implantable materials is to graft bioactive peptides or proteins through covalent linkage onto the implant surface [41 - 43]. There are three major techniques for biochemical treatment of a metal surface, mainly physicochemical adsorption [44], covalent binding of a molecule and peptide inclusion into a carrier material [45 - 48]. Table **1** summarises these methods. One method in order to achieve this is the use of self assembled monolayers using a biofunctional molecule with a head group that interacts strongly with the metal. Protein grafting itself is limited due to the low chemical stability and solubility in the biological environment. However the use of beta transforming growth factors and bone morphogenetic proteins have been studied, along with the Arg-Gly-Asp amine sequence which is the minimal cell recognisable sequence in adhesive proteins [45, 46, 49]. Hence this technique grafts biologically active moieties onto the surface but yields control of the relative densities or arrangement that may itself have unique interplays with cell signalling.

Table 1. *In vitro* **chemical methods for nanosurface modification.**

In Vitro Studies				
Method	**Controlled Variables**	**Nanoscale surface features**	**Outcomes**	**References**
Anodization	Nanotube length, oxide layer thickness, nano crystallinity, pore size	Nanotubes with a diameter < 100nm	Enhanced adhesion, proliferation, matrix secretion and mineralisation in bone models. Promotion of human MSC growth and differentiation, increased assembly of focal adhesions. Increased chondrocyte adhesion and keratinocyte proliferation	[50, 52 - 55, 60, 63, 68, 91, 94-96, 104, 118-124]
Oxidative nanopatterning	Oxide deposition thickness, chemical moeities, Micro & nanotopograpy	Nanoporous diameters of 20-100nm	Increased osteoblast activity and limits to fibroblast growth. Increased Bone sialoprotein, osteoopontin, alkaline phosphatase, RunX2 expretion. Stimulation of Human umbilical cord stem cells	[29, 34, 65, 97, 99, 100]
Chemical Vapor Deposition & Sol-Gel processes	Nanosurface roughness. Nanolayer thickness and nano-crystallinity	Nanotopography	Increased osteoblast adhesion and proliferation	[64, 91, 110, 112, 125 - 132]
Biochemical functionalisation	Thickness of biochemical coatings. Control of the functional groups	RGD protein motifs, extrcellular proteins and amino acid segments. Nanorosette, antibiotics, non fouling and anticoagulant sequence	Increased osteoblast activity including adhesion, gene expresion and proliferation	[41 - 43, 50, 73, 133 - 136]
Acid/Alkali Treatment	Acid solution concentration, relative thickness of the oxide layer.Porosity, layer thickness, two layer structure Crystallinity	Nanosurface reactive groups.	Acid etching leadings to thin surface oxide layers, that grow slowly in air. Two step chemical reactions can be epmployed to improve bioactivity. Alkali and heat treatment improved pore size and corrosion resistance. Apatite formation increased with alkali treatment	[29, 137, 138] [43, 105, 112, 139, 140]

Alternatively the metal surface can be modified through electrochemical process, most notably anodic oxidation [50, 51]. Anodic oxidation utilised electrode reactions with electric field guided oxygen and metal ion diffusion that creates an oxide film throughout the surface [52, 53]. It is already established to produce protective films on metal surfaces [54, 55]. Acids such as H_2SO_4, H_3PO_4 and acetic acid can be used as electrolytes that improve adhesion and bonding [56]. Such techniques have been hugely translated in the aerospace industry. One of the advantages of anodic

oxidation is the vast range in controls that can be modified, namely anode electrode potential, electrolyte, temperature and the current through the circuit [57]. Smooth titanium surfaces have been modified into nanotubular structures to improve orthopaedic integration and control the spacing and diameter between nanotubes. Alternatively multiwall carbon nanotubes [58], pillar-like structures or nanocrsystalline hydroxyapatite coatings [59] have been synthesised as surface modifications though anodic oxidation [50, 60 - 63].

Modification using acid or alkali solutions is also a popular method for achieving nanosurface properties on metal implants. Acid etching produces a thin surface oxide layer, less than 10nm, which also grows in air [34, 35, 64]. Furthermore hydrofluoric acid can be utilised to attach fluorine surface groups that improves antibacterial effects and bone formation [61]. The oxidation process has been shown to generate nanopits on metal alloys. Furthermore by manipulated the length of exposure to acid or alkali or the reaction temperature the surface properties can be altered [65]. In particular the roughness and wettability. The reaction of hydrogen peroxide with titanium yields a ti-peroxy gel that in simulated body fluid improves apatite formation [66, 67]. Furthermore heat treatment of this gel layer can modify the inherent pore size and calibrate the bioactivity.

Chemical vapor deposition utilised chemical reactions in the gas phase in the presence of a sample surface which results in the deposition of a non reactive compound on the substrate surface. It is beneficial as it is widely utilised in industry to produce organic and inorganic layers on metals or semiconductors. Continuing this industry to clinical translation; diamond films have been deposited on titanium in order to investigate the mechanical and biological improvements, representing a highly novel translation of industrial processes into the biochemical world [68, 69].

The sol-gel transition reaction is an alternative method for altering metal surface properties that grafts rather than etches the metal surface. However historically the sol-gel method deposits coatings of less than 10μm rather than on the nanoscale. Furthermore the reaction interface is not between the sample surface and the solution/gel but within the solution itself. In one example a sol-gel process was utilised to investigate the functionalization of TiO_2 nanoparticles, proving its use to potentially graft nanoparticles or biochemical moieties (RGD) on a metal surface.

PHYSICAL TECHNIQUES

Physical surface modification circumvents an *in situ* chemical reaction to instead utilise physical spraying of coatings or atomic rearrangements with ion implantation [70]. Table **2** highlights these techniques. Plasma surface modification uses plasma, considered to be the 4th state of matter, which is a highly excited atomic, molecular, ionic or radial species [71 - 73]. Under vacuum conditions the excited plasma gas is excited by radiofrequency, microwaves or electrons and undergoes many surface reactions. Alternatively physical vapour deposition uses vacuum condensation of a thin material to coat it onto a surface. A modification, electron beam physical vapour deposition, heats a the material to be deposited with electrons prior to transporting it in vapour form before condensation and coating onto the surface within a vacuum chamber [64]. These methods have been investigated to yield improved nanonodule or nanorough layers.

Table 2. *In vitro* **physical methods for nanosurface modification.**

In Vitro Studies				
Method	**Tunable surface properties**	**Nanoscale surface features**	**Outcomes**	**References**
Plasma deposition, vapor deposition	Nanoscale surface roughness and layer depth		Enhanced osteoblast activity and calcium deposition	[59, 64, 70, 88, 89, 101, 141, 142]
Ion implantation	Depth and surface concentration of implanted chemical groups	Grafted ions such as fluorine, sodium *etc*	Increased osteoconduction, proliferation and induction	[61, 95, 143]
Thermal Oxidation	Nanotopography	Titanium oxide nano layers	Improved oxteoblastcell spreading, surface adhesion, proliferation and cell line differentiation	[144 - 149]
Titanium discs subjected to laser irradiation	Rough nano to micro surfaces	Uncharacterised nano surfaced irradiation	Stimulated osteoblast differentation	[150]

Alternatively ion implantation allows the fine control of selected elemental ions on the material surface. Unlike aforementioned chemical methods, ion implantation allows precise control over the concentration and depth coating of the implanted elements. Thermal oxidation is able to alter the crystalline structure on the titanium oxide layer. Yet the temperatures involved during the annealing process can yield superficial stresses or modifications of previous nanosurface structures, potentially highlighting a lack of control in its application.

IN VITRO SIGNIFICANCE OF THE NANOSCALE

Our understanding of the propagation and magnification of molecular level signalling to micrometer scale events has improved over the last decade. Hence the utilisation of nanotechnolgy to engage and influence small molecular changes that propagate much larger responses on a tissue level has also reciprocally improved [74 - 79]. As living cells are constructed from structural and molecular components that are interconnected through a hierarchical system at different levels the molecular signalling is able to create seismic shifts on a larger structural level [75, 77, 78, 80, 81]. The static model of bone tissue must be replaced with a more fluid appreciation of the role of cellular tensegrity and mechanotransduction in maintain the homeostatic composition of bone. Accordingly multicellular cell substrate interactions at the molecular level can be controlled and propagated through adequate manipulation, potentially, of the substrate surface interaction.

It is therefore important to visualise the bone scaffold not as isolated solid structure in isolation but as part of a collective pendulum of tissue and cellular stress. As cells hang and interact to their extracellular matrix through focal adhesions and integrin molecules they sense the tension within the system. Integrins transmembrane proteins anchor cells and transmit mechanical forces in the size of 15-30nm [45]. In addition they communicate with key proteins such as the RGD sequence, which communicates to cells though its surface density. Ingber and colleagues have identified the importance of cytoskeletal reorganisation to simple extracellular nano mechanical cues [74 - 79]. Hence nanoscale surface features have been shown to be able to regulate focal adhesion kinase signalling in osteoblastic cell lines. Further nanoporous coatings can activate GTPase RHO which is crucial in intracellular signalling. Therefore the control of the nanostructure in implants is crucial in bridging the mechanical communication between the external environment and the internal bone structure.

As the surface and environmental contact is the key first event in dictating the water colonisation and adsorption is therefore central to laying the foundation for correct protein adsorption [82]. As this process takes place over the nanoseconds it can be often overlooked. The water shell controls the plasma and ECM protein adsorption, including the protein orientation, coverage and denaturation. The subsequent adlayer will ultimately control the rate of cell proliferation, spreading and adherence and potentially their overall phenotype [83 - 86]. It is likely that the process is an overall cascade where the surface to protein adlayer interaction ultimately guides the protein cellular interplay [87 - 90]. However further research will hopefully delineate this relationship.

Therefore the *in vitro* protein-substrate interplay is key in harnessing the eventual cellular integration of any implant.

Nanosurface modification is able to control protein adsorption and the biochemical construction of the protein layer [91]. Constructing 'bottom up' nanoscale features can ultimately direct the surface hydrophilicty, the oxide thickness or the distribution of functional groups [90, 92]. Surface topography as been highlighted to also direct protein orientation and denaturation, which downstream obviously controls cell surface proliferation. However not all nanoscale features are able to control cell outcome. It has been shown that only nano features up to 10nm, similar to proteins, are able to change cell morphology and activity. The role of nanorough surfaces has been studied with regards to key proteins such as fibrinogen, albumin and fibronectin. Particularly with fibronectin, a key adhesion molecule, the depth of the protrusions was key. This was ultimately central to controlling the size of focal adhesion constructed.

One of the drawbacks for integrating nanosurface modifications is [87] appreciating their short term and long term stimulation of cell interactions. Short term interactions imply the establishment of adhesion and proliferation, whilst long term effects are the stimulation of matrix, osteoid production and mechanical remodelling. Such dynamic processes and the implications from nanosurface modificaitons are difficult to quantify. Anodized titanium surfaces have had some success in co-ordinating both these effects [51]. In particular titanium surfaces have demonstrated the proliferation and differentiation of mesenchymal stromal cells, MSC, to an osteogenic lineage as well as concurrent cytoskeletal changes through focal adhesions. In the MSC work they highlighted that smaller nanosurface modifications, 15nm, compared to larger nanotubes, 70-100nm, were more effective in controlling cell adhesion, spreading and differentiation [30, 34, 35, 63, 93 - 96]. An additional factor that is key to reducing implant failure is the consideration of reducing bacterial load. Nanorough titanium surfaces have also been shown to be an effective antibacterial tool. A key area in prosthetic development is the use of bone anchors to improve prosthetic integration. Anodization has been shown to improve current implants particularly with their antibacterial features, reducing one of the limitations of using these anchors [94, 95].

Oxidative nanopattering exhibits a similar cellular response to that of anodization and it is likely that both these

modalities will have a key role in future implant engineering strategies. Grossly, oxidative nanopattering has demonstrated the ability to upregulate osteoblastic cell lines and inhibit fibroblastic lines [97, 98]. Despite discussion concerning the ability to dicate the protein adlayer, it appears oxidative nanopatterning appears to influence the rate of cell growth and differentiation rather than adhesion. This has been substantiated by gene and protein expression investigation [65, 99, 100], with early upregulation of bone sialoprotein (BSP) and osteopontin and fibronectin assembly. Surface roughness manipulation of 0.2nm by carbon nanofibers has also demonstrated relative 'selective adhesion' of cell lines by apparent downregulation in chondrocyte and smooth muscle cell activity compared to osteoblasts. Such relative control over adhesion and proliferation is key in the vital stages of tissue regeneration and healing. Titanium etched surfaces with H_2SO_4/H_2O_2 or NH_4OH/H_2O_2 also showed gene expression upregulation of integrin alpha – 5 and hyaluronin and fibronectin assembly; highlighting the control of cell adhesion and migration [65]. In mesenchymal stromal cell investigation nanopatterned titanium increased expression of alkaline phosphatase and runx2 [29].

Sol gel methods of implant surface modification may be advantageous for improving bioactivly over aofrementionned surface modification techinques. In particular the ability to control surface area, porosity, composition, dissolution rate and adsorption are attractive to implant technology [68, 69, 101]. The use of coatings such as titanium oxide, calcium phosphate and composites can improve mineral formation. Ti-OH based coatings have been shown to induce calcium phosphate formation *in vitro*. Li *et al*. demonstrated micrometer depth HA coatings up to 2 weeks in simulated body fluid [56, 102 - 105]. Alternatively Liu and collegues deposited titanium oxide nanoparticles on NiTi alloys that yielded 205nm thick films [27, 106 - 109]. The adoption of composite titania and hydroxyapaptite coatings bridges the gap from pure metal coatings that are traditionally poorly bioactive. Titanium dioxide, for example, strongly adheres to titanium but has limited bioactivity. Comparatively hydroxyapatite has good bioactivity but poor adhesion to the implant. In one example a homogenous rough porous titanium oxide hydroxyapatite coating was created, demonstrating optimal conditions for cell adhesion [103, 110 - 113].

Physical methods such as plasma surface modification techniques yield a more molecular surface modification approach than previously represented techinques. Micro arc oxidation [114, 115] or plasma electrolysis improved *in vitro* proliferation with a titanium oxide layer. Increasing the voltage resulted in a thicker and rougher oxide layer that produced a reciprocal rise in calcium and phosphate ions and cellular ALP activity [114, 116, 117]. Alternatively MAO has coated magnesium ions onto implant surfaces. This has created a more rapid bone integration.

IN VIVO SIGNIFICANCE AND CLINICAL EXPERIENCE

A key problem with *in vivo* characterisation of nano surface modification, as with the *in vitro* work, is the lack of standardisation between models. The use of different animal models, mechanical and anatomical conditions make it difficult to compare outcomes between investigators. The adoption of a benchmark *in vivo* model is difficult as the nature of the animal used is dependent on the size of the implant and modification. Further to this the cellular and mechanical properties largely differ between animal and human bones. Table **3** summarises the variety in animal models conducted for nano surface bone research. Canine bone structurally has a higher mineral density due to a secondary central osteonal bone surrounded by a set of plexiform bone. Although canine bone is apparently equal to human bone, it is able to withstand much higher strain resistance. Furthermore it also withstands a greater compressive stress, which will significantly impact the conclusions drawn from canine implant models for human use [151]. Sheep bone has been identified to have a larger amount of bone ingrowth compared to humans, therefore implant models may overestimate the ability of modifications to encourage osseointegration. Furthermore the mechanical moduli of such *in vivo* models are significantly crucial when investigating the micromechanical influences that contribute to long term failure or integration. Alternatively in investigating the effects of loading on osseointegration, as force distribution will differ significantly in bone with greater strain resistance [152, 153].

As discussed elsewhere there is also a lack of consensus on implant topography. One criticism is the nature of defining a surface by its modification process that than the topographical measurement or feature. In moderning the *in vivo* analysis of nanosurface modifications we need to transition from a view of segregating the macroscopic mechanical physical implant and the surface modifications to yield improved integration. Instead we should focus on a more dynamic view that sees the nanosurface modifications as an extension of an implant that is able to react to the external tissue mechanical cues. Based on previous *in vivo* results and future investigations such modified implants will undoubtedly play a large role in shifting implant technology.

Table 3. *In vivo* animal models for nanosurface modifications.

	Method	Nanoscale Features	Controlled Variables	Animal Model	Outcome	Year
Wilmowsky *et al.* [52]	Anodization of titanium nanotubes	Titanium nanotubes 20nm in diameter	Nanotube diameter	Pig Frontal Skull	Stimulation of Collagen I expression	2009
Yang *et al.* [171]	Electrochemical deposition of nano-HA particles	Nano-HA particles	NA	Rabbit Proximal Tibia	Improved bone-implant SA and contact with increased bone matrix	2009
Tavares *et al.* [137]	Oxidative nanopatterning of implant with H2SO4/Hydrogen peroxide	Nanopores of 20-25nm	Nanopores, surface depth and porosity	Dog Mandible	Improves bone to implant surface area and contact with matrix	2007
Abrahamsson *et al.* [172]	Titanium blasting with HF acid	Uncharacterised nanofeatures 50-200nm range	NA	Rabbit Femur	Stimulation of osteoblast gene upregulation, matrix formation and bone-implant surface interaction. Good osseointegration at 1 year	2008
Salou *et al.* [138]	Nanometer nanotubule surface modified implants	Nanosurface nanotubes37nm - 160nm diameter tubes	Tube dize	Rabbit Femoral Condyle	Bone to implant contact and bone growth values higher in Nanosurface modified implants compared to microsurface implants	2015
Schliephake [42]	Imbolised VEGF on oligonucleotides anchor strands using sandblasted etched implants	NA	NA	Rat Tibia	Significant improved bone implant contact	2015
Coelho *et al.* [141]	Plasma sprayed hydroxyapatite dental implants	NA	20-50nm tichkness bioceramic treated implants features	Dog tibia	The treated implants with thick coatings did no improve early bone to implant integration	2009
Kon *et al.* [173]	Osteochondral scaffold with magnesium hydroxyapatite during self assembly	Chemical surface modification with acetic acid with Mg-HA nanoparticles	Particle layer composition	Sheep Femoral Condyle	improved osseointegration with hydroxyapatite nanoparticles biomimetic scaffold	2010
Xue *et al.* [174]	PLGA Nanohydroxyapatite through thermally induced phase seperation	PLGA Nanohydroxyapatite scaffold	scaffold porosity, nanohydroxyapatite particles.	Rat knee	smooth and hyaline like cartilage with abundant glycosaminoglycan and collagen type II deposite	2010
Kuba *et al.* [175]	Micropit and nanonodule hybrid topography titanium oxide	Micropits & Nanonodules	Nodules in micropits and nanonodules addition	Rat Femur Model	Improved osteoconductivity	2009
Omori *et al.* [176]	Atmospheric plasma treamtent and stem cell immobilisation	Uniform round shaped deposits, dimaeter 350nm		Dog Femur	Continuous bone formation compared to controls	2015
Shouten *et al.* [177]	Electrosprayed calcium phosphate nanoparticles onto implant surfaces	Calcium phosphate nanoparticles	Nanoparticle size, particle spray	Iliac Crest Goats	Bone healing and fixation equal to grit blasted acid etched implants	2010
Bjursten *et al.* [178]	Titanium oxide nanotubes *vs.* titanium oxide gritblasted implant surfaces	Titanium oxide nanotubes	Nanotube size	Rabbit Tibia	Greater bone-implant surface area, calcium and phosphate deposition and bone matrix deposition in nanotube surfaces over grit blasted surfaces	2009
Meirelles *et al.* [179, 180]	Nano - Hydroxyapatite modified titanium implant	Hydroxyapatite nanoparticles & nanorough surfaces	Nanoparticle size, surface pores densitiy, depth and concentration	Rabbit Tibia	Rabbit Tibia gap model showed that there was similar bone healing in Nano HA implants to standard implants	2008

Evidence of the conflicting nature of *in vivo* protocols for nanosurface implant modifications can be seen in anodization models. In the anodization of titanium implants with TiO$_2$ nanotubes different clinical outcomes could be found *in vivo*. In one model utilising smaller nanotubes in frontal bone of pigs although there was an increase in collagen type 1 production, there was no overall improvement in osseointegration. Conversely in an alternative nanotube model utilising more than double the overall diameter the findings showed improves osseintegration yet reduced collagen proliferation. The lack of standardisation in surface topography and the subsequent alteration in overlayer crystalinity it is difficult to draw precise conclusions *in vivo*. Sol gel and anodic coating methods have been

utilised *in vivo* to quantify the positive effects of a nano-HA implant. Bone to implant contact was significantly improved with nano-HA coatings.

Another drawback in the *in vivo* literature is the lack of characterisation of nano surface features. In one study using blasting and hydrofluoric acid treatment a range of uncharacterised nano features were created. Despite positive results to indicate improved osseointegration and bone implant fixation it is difficult to extrapolate the precise mechanism for this improvement. Such generalisation of the effects of nanotopography is misleading in presenting positive *in vivo* studies.

An alternative dimension to the implant cell interface is the role of bacterial colonisation. Competition between host and bacterial cells ultimately determines implant integration, and adequate primary host cell adherence will preclude bacterial colonisation. The prevention of a implant biofilm and control of the immune response are therefore crucial in designing nanosurface modifications [154, 155]. In the design of external fixation pins genatminicin loaded poly(d,l-lactide) PDLLA) coatings prevented implant osteomyltitis in titanium based pins [156, 157].

Hydrogen, chlorine, iodine and oxygen group surface modification are useful for their counter infective properties, their mechanical brittleness preclude them from use in orthopaedic devices. On the other hand selenium generates local superoxide radicals which has shown to reduced staphlacoccus aureus and staph [158] Epidermidis infection. Selenium presents itself an exciting alternative. In addition selenium also does not adversely affect osteoblast viability. Furthermore selenium nanoparticles have been identified to inhibit bacterial replication.

The use of more organic surface modifications, such as RNA II inhibitory peptide (RIP) and dihryoprrolone surface treatment are alternative surface modification that may aid orthopaedic implantation [159]. Macrophages have been shown to improve their antibacterial function when in contact with Chitosan modified surfaces, a shellfish derivative.

In cardiovascular material engineering drug eluting stents, DES, have delivered some degree of compensation to prevent bacterial colonisation or fibrous implantation [160]. Polymeric coatings in DES, along with nanoporous surface coatings of al203, have shown promising drug eluting capacities. Oxidative nanopatterning to titanium produced internetwork nanopores showed that thin films on materials not readily amenable to oxidation could allow anti adhesive properties. In this vein the use of nanopatterning and surface treatments such as altering nanotopography or addition of drugs such as vancomyocin could be used to reduce the bacterial colonisation of implants.

The utilisation of silver cations which disrupt bacterial cells walls, inactivate protein synthesis and DNA condensation as well as producing reactive oxygen species is a popular area of exploitation. Layer by layer deposition of silver nanoparticles integrated with titania showed good long term antibacterial properties and tissue integration [128].

The use of self assembled monolayers, SAM, are also an emerging surface modification that allows maintain dual surface function. The use of a nickel nanostamper with an alkanethiol SAM was investigated to create an anti adhesive layer [161, 162], which presents one strategy to reducing biofilm construction. Finally and more recently, the development of functional polymer brush coatings develops the concept of dual function surface modification. Such coatings contain an anti adhesive coating that repels bacterial invasion and an arginicine-glycine-aspartate functional group that enhances tissue integration.

One final consideration that cannot be avoided and often forms the centre for implant surface design is the implant micromotion [163, 164]. Many consider this to be the decisive factor in controlling interfacial healing. Beyond the focus on the nanosurface modification it is the motion associated interfacial principal strain magnitude, as well as its distribution, cycle of application, amplitude an waveform that influences the progression of osseointegration. Previous theories concentrated on the immediate and delayed loading of implants as initially it was believe that the timing of loading was crucial. However increasing understanding of the effects of mechanobiology in cell control highlights that it is the cells ability to understand changes in micromotion that guide bone remodelling and regeneration [164, 165]. Hence reciprocally an implants ability ot withstand varying micromotion is crucial. Attempts have been made to postulate a single value or range for the optimal stress and strain for interfacial bone healing. In one mouse tibial model a ~30% principal strain magnitude was perceived the upper limit for interfacial bone healing. Such theories avoided dealing with the central issue of micromotion and its effect on implant integration [166, 167]. Namely that the implant and bone must be in a relative mechanically fluid state that responds to a wide degree of change in the mechanical loading of the bone. Such a mechanical interface could be mathematically modelled to deliver a range of values particular for different bone types and animals. Such mathematical equations will then allow considerable design of the

nanosurface modifications necessary to deliver the appropriate physical cues for mechanobiology. In this instance self assembly or surface reassembly systems may be the most appropriate, that are able to alter surface modifications in response to surface mechanical cues. Therefore responding to micromotion to improve interfacial bone healing.

CONSIDERING THE BIOCOMPATIBILITY AND CYTOTOXICITY OF IMPLANTABLE METALS

An inevitable by product of constructing bottom up strategies for cellular integration on the nanoscale is the implications and toxicity of such surface modifications. Implanted metals and their surface structure will have some undesirable *in situ* effects. Investigating such effects is therefore a necessity. As previously discussed; wear debris commence a cascade of events that result in implant loosening and rejection. Therefore ions or nanoparticle debris will stimulate an adverse immune reaction that may potentiate implant failure. Although titanium and its alloys are considered largely biocompatibility it is difficult to absolutely quantify the degree of cytotoxitiy from novel titanium alloys, or those of associated metals. Chromium, nickel and cobalt all have been shown to have some level of carcinogenicity [168 - 170]. However the adverse effects of *in situ* metals is relatively well studied compared to those of self assembly systems or bioactive moieties. Such complex systems may instil more widespread effects such as cellular DNA damage [170].

OVERVIEW OF STRATEGIES

The aim of this paper was to overview the current chemical, physical and mechanical surface modifications to improve nanosurface functions of potential orthopaedic implants. In reviewing the outcomes of potential *in vitro* models and correlating these to *in vivo* research we charter the potential evolution and feasibility of future techniques. Despite the overwhelming evidence of the nanoscale to influence the landscape of cell proliferation few of these attempts at nanoscale modification have transitioned to the operating table.

Previous *in vitro* work had concentrated on a range of materials that were not medically relevant and synthesised used techniques such as electron or colloidal beam lithography or phase separation. However a directional change over the last 5 years has seen a focus on titanium based nanosurface modifictions particularly with techniques such as anodic oxidation or with self assembly monolayers. The next phase is to focus the experimentation from the larger nanoscale (greater than 100nm) to the much smaller nanometre scales (10-20nm) to enable direction of the protein adsorption as mentioned.

Such a calibration of efforts in surface modification anaylsis will enable a greater understanding of the potential for infleuencing the protein surface adsorption and the vromen effect. Current *in vitro* studies into manipulating protein adsorption are limited due to the lack of understanding of the likely co factors that may be involved in a multicellular *in vivo* environment. The role of protein adsorption of material surfaces and their control of cellular adhesion and intracellular signalling is now well chronicled. Nevertheless the interplay between protein adsorption and the material interface on the mechanical tension throughout the tissue needs increased focused. Such interplay is increasingly featuring in soft tissue material research. Although as discussed earlier in this paper, the transition from understanding these interactions in unicellular *in vitro* models to multicellular *in vivo* or 3D cultures is significantly different and necessitates caution.

Several critisms have previously been levelled at the systems for investigating the impact of surface modifications of orthopaedic implants. Firstly surface analysis lacks standardisation that enables cross comparison between experimental work. One example of such potential standardisation is the use of contact angle for analysis of wettability of potential surfaces. However the lack of homogeneity in experimental conditions, such as drop size or symmetry and the potential differences in substrate properties make for wide variations in the presented data. Furthermore many implants presented as containing nanosurface properties are infact uncharacterisaed 'nanorough' randomly orientated surfaces that lack reproducibility. In this context even apparent smooth surfaces should be considered nanosurface modified. The lack of standardisation continues into the *in vivo* models where a significant disparity exists between the animal and human bone. Furthermore a significant proportion of evidence in from maxillofacial implant models which are often non load bearing and cannot be extrapolated for implants that are for weight bearing long bones.

Neverthless the potential benefits from nanosurface modifications are twofold, firstly to increase surface area which secondarily increases the potential reactive sites for tissue implant interaction. The introduction of nanoporous coatings therefore has the potential to instil a three dimensal nano environment, beyond a nano modified 2D surface, that can effect physiochemical effects such as fluid mobility. Nanoporous coatings can increase the interaction with bioactive moieties and act as an intermediary layer to transmit changes in mechanical forces. The most exciting avenue for

exploration in these material modifications is the use of self assembly systems. These complex multifunctional arrays allow for an equilibrium between mechanical transduction and reducing bacterial colonisation.

Such systems and their potential as effectors on the complex cellular environment within the bone mean that future generations should be capable of 'reassembly' to reflect external mechanical changes. Such intelligent surface modifications will be capable of reducing the problems with micromotion and improve osseointegration at the implant interface.

To conclude, the potential of nanosurface modifications on orthopaedic implants is exciting and vast. Combining modern chemical and physical surface modification techniques with an understanding of the mechanical cues that govern cell phenotype and local tensigrity will enable a much more dynamic implant interface.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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