Perspective

Received: 17 October 2020 Revised: 2 December 2020 Accepted article published: 3 December 2020 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/pi.6162

Polyetheretherketone implant surface functionalization technologies and the need for a transparent quality evaluation system

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Abstract

For bone implants, osseointegration resulting in a good and fast bone–implant contact is of primary importance to secure a proper implant function and to avoid implant loosening or inflammation resulting in necessary revision surgeries causing pain to the patients and immense costs. In particular, polyetheretherketone (PEEK) is a promising implant material due to the close mechanical properties to bone, but it is entirely bio-inert, hindering osseointegration and making surface functionalization necessary. Many different surface functionalization technologies have been reported of both physical and chemical nature. The same is true for the other prominent implant materials titanium and ceramics. Although they already have inherently better osseointegration than PEEK, they are much harder and stiffer than bone and brittle in the case of ceramics. Surface functionalization, which can be subdivided into surface coating and material modification, needs to be judged from a quality and safety viewpoint. However, a literature research resulted in the realization that no quality standard yet exists for implant surface functionalizations. This makes it difficult to near impossible to compare the safety and performance of different surface-functionalized bone implants, clearly showing the need to establish a transparent quality evaluation system for bone implants. This perspective article gives the state of the art and then develops a quality evaluation system based on six main categories as important benchmarks for the quality of surface-functionalized bone implant materials. A simple catalog of questions can be answered, and from the resulting scores the Safety and Performance Evidence Level (SPEL) representing the safety and quality of a given implant can be calculated as a percentage. This simple SPEL system allows an easy and transparent judgment and comparison of bone implants, ensuring the easy identification of safe and well-performing high-quality bone implants in the future.

Keywords: bone implant; polyetheretherketone (PEEK); surface functionalization; quality evaluation; safety and performance evidence level (SPEL)

INTRODUCTION

Surgeons and patients, as well as medical device manufacturers, are frequently confronted with postoperative implant failures due to implant loosening or inflammatory reactions. These complications are often the reason for pain after surgery and lead to revision surgeries resulting in increased healthcare costs. These failures are not limited to certain indications or surgical techniques: they occur in all applications where implants must be placed in the patient's body. Reasons for failed surgeries can be (i) the implant materials available do not have the best biological performance due to their material characteristics; (ii) the surrounding bone – where the surgeon has to place the implant – is not stable or dense enough due to the patient's age and/or osteoporotic skeletal bones; or (iii) the surgeon is not adequately trained or educated. The table below (Table 1) decribes the most important abbreviations, terms and definitions used in the publication.

Implant materials that are approved for their use in humans can be roughly divided into three material categories: metals,

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Table 1. Terms and definitions

Abbreviation	Definition
BIC	Bone (to) implant contact (BIC) is a term that refers to how much of the implant surface is touching bone on a microscopic level and is graded as a percentage
MDR	Medical Device Regulation (MDR) provides the legal framework (EU) and stipulates mandatory requirements on how to plan, develop, manufacture and market medical devices
SPEL	Safety and Performance Evidence Level (SPEL): a scoring system indicating the evidence level for evaluating the safety and performance requirements of SFTs in percentages.
SPR	Safety and performance requirements (SPR): requirements that every medical product has to fulfill, according to the scope they belong to. These essential requirements are described by the Medical Device Regulation (EU) 2017/745, Annex I
SFT	Surface functionalization technology (SFT): surface modifications or surface coating to enhance osseointegration
Single arm	Single-arm study. The simplest study design is a single-arm trial. In this design, all subjects with the targeted medical condition receive a single intervention and are then followed over time to observe their response. This kind of study design is often seen in animal models too[1]
Split mouth	The split-mouth design is a common design in oral health research. In the most common split-mouth study, each of two treatments is randomly assigned to either the right or left half of the dentition. It can easily be adapted to various kinds of animal models. The attractiveness of the design is that it removes a lot of inter-individual variability from the estimates of the treatment effect[2]

ceramics and polymers. The first attempts to use metals in implantology were related to the reconstruction of fractures of the long bones and their joints. The British surgeon Sir William Arbuthnot Lane (1856–1943) together with British Dame Agnes Gwendoline Hunt (1866–1948), the world's first orthopedic nurse, and the Belgian surgeon Albin Lambotte (1866–1955) designed a fracture plate made of stainless steel.[3] The development of implant materials continues with titanium in the 1940s and 1950s[4] through ceramics (1975)[5–9] to polymers in dentistry and in spinal applications (since the 1980s).[10,11]

The use of different materials often corresponds to a specific medical indication. Generally, metals are mainly used in the treatment of fractures and bone defects, ceramics – in addition to metals – are used in dental surgery, and polymers have gained a special position in applications such as spinal surgery.[12]

Even though all the materials mentioned above can be described as state-of-the-art implant materials in their field of application and their use is well documented, all the materials listed have one thing in common: none of them is an ideal candidate for orthopedic use or use in dental surgical care. Mechanical properties and functional surface characteristics are not ideally matched: while titanium surfaces can be described as positively osseointegrative, the mechanical properties of titanium or other metals are often too oversized in relation to the surrounding bone. Ceramics are very brittle, and polymers such as polyetheretherketone (PEEK) are considered bio-inert but nevertheless a promising implant material because of their excellent mechanical characteristics. The good mechanical characteristics are the reason why PEEK is a standard material in spinal applications.[13]

As surgeons are forced to deal with such poor biological conditions, medical device manufacturers must enhance the performance of implant surfaces to achieve more natural material characteristics. To overcome the discrepancy of good mechanical properties but limited biological implant integration is the reason why coating technologies have built an impressive catalog of success in many different applications. With a growing need for coating technologies to functionalize the surface of polymeric medical devices, the medical industry saw enormous growth in coating application onto medical devices. Various types of coating technologies, coating materials and substances are available to date: spanning from plasma spray coating technologies to dip-coating techniques, from titanium or hydroxyapatite (HA), all of which enhance cell attachment onto orthopedic implants. But there are also various risks associated with the materials and methods mentioned above: amongst others, delamination, wear debris, abrasion, particle migration, infection or corrosion.

This perspective aims to highlight new developments of surface functionalization technologies (SFTs) and analyze their general suitability for serial use as implantable medical device applications. In addition, we highlight the need for a transparent quality assurance system. Focus is laid on the functionalization of implants to be used in orthopedics and dentistry, their clinical benefit, and potential inherent risk for users, patients and surgeons.

LITERATURE EVALUATION

We wanted to take a closer look at the safety and performance aspects of different coatings. Therefore it was our search strategy to base on recent meta-analyses and (systematic) review articles. We wanted to detect, find relevant aspects of and find the regulatory and clinical status of surface coating technologies related to safety and performance endpoints of the respective technologies under evaluation and in comparison to all relevant alternative SFTs.

High-evidence studies comparing treatment methods should be in the scope of literature identification; thus focus is put on (systematic) review articles and meta-analyses. For this literature search, PubMed (MEDLINE) was chosen as the main data source for the following reasons.

- PubMed comprises over 26 million citations for biomedical literature from MEDLINE, life science journals and online books. PubMed citations and abstracts include the fields of biomedicine and health, covering portions of the life sciences, behavioral sciences, chemical sciences and bioengineering. PubMed also provides access to additional relevant websites and links to the other National Center for Biotechnology Information molecular biology resources.
- The majority of journals selected for MEDLINE are based on the recommendation of the Literature Selection Technical Review Committee, a National Institutes of Health (NIH) chartered advisory committee of external experts analogous to the committees that review NIH grant applications.

Table 2. Literature search details

Database searched	Keywords and search terms	Date	Results
MEDLINE/PubMed	(PEEK AND COATING) AND (Safety AND Performance) AND Osseointegration Filters: no filters applied	24 September 2020	0
	COATING AND (Animal Study) AND Osseointegration Filters: (systematic) review articles and meta-analyses, English language only	24 September 2020	54
	'Surface Functionalization' AND (test OR ASTM OR verification OR validation) Filters: (systematic) review articles and meta-analyses, English language only	24 September 2020	74
Web of Science	(PEEK AND Coating AND ASTM)	24 September 2020	7
	'PEEK coating' AND ISO	24 September 2020	7
	(PEEK coating) AND (clinical study)	24 September 2020	44

PEEK, polyetheretherketone.

The Web of Science database was additionally used for literature search. The literature search details are summarized in Table 2.

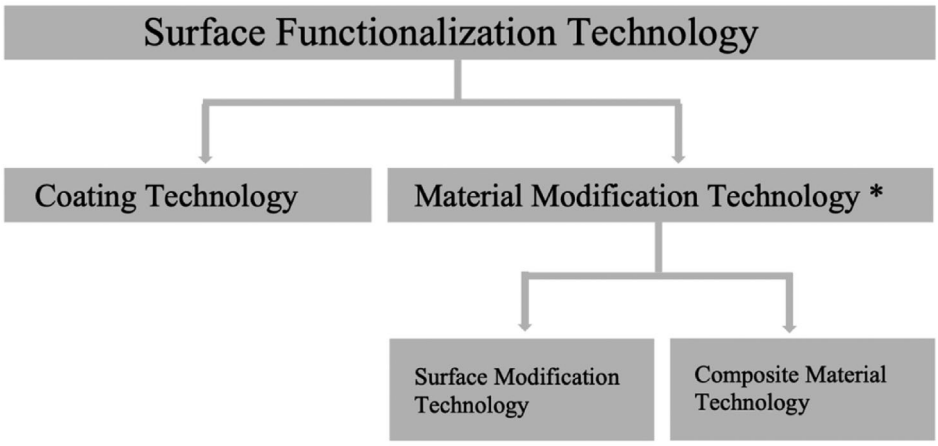
SAFETY AND PERFORMANCE EVALUATION OF PEEK COATINGS AND MODIFICATIONS

However, the initial literature research quickly set a limit to this ambitious undertaking. It became clear that in the field of safety and performance evaluation of coatings reported in the literature, pioneering work is still to be done because only 5% of the references found in the literature search were usable to evaluate safety and performance as required by the Medical Device Regulation (EU) 2017/745 (MDR)[14] aspects of the described materials. The main problem encountered is that different evaluation methods are used in the different studies so that a comparative evaluation of the available materials is impossible and results in comparing apples to oranges.

In the literature, the term coating is not clearly defined. The term is often used to describe different technologies and methods. However, in order to be able to investigate and evaluate the safety and performance characteristics of different functionalization technologies, it was essential to first clearly distinguish between the methods (i) modification and (ii) coating by a definition (see Fig. 1 and Table 3). Either one of the methods can apply to an implant surface, or both methods can be applied.

The more complex the operating principle and functionality of medical devices, the more precisely these products must be developed, documented and tested. This includes risk analysis and risk assessment to prove safety,[15] clinical evaluation[14–16] or testing to prove performance and efficacy.[14]

In order to evaluate relevant performance and security aspects in a transparent and standardized way, it is also essential to classify the different technologies into different risk classes. Since all technologies examined in this paper are related to their use in the patient's body, all SFTs must meet specific regulatory and



* While coatings in most cases do not influence the chemical composition of the substrate material, material modifications have a direct influence and impact on the composition of the modified material. Therefore, it must be proven by means of risk analysis and material investigations whether and to what extent the applied modification technology has influenced the regulatory status of the original material. It is possible that the material has lost its classification as implantable grade material after modification.

Figure 1. Surface technology classification flowchart.

Polymer International published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Table 3. Definition of different types of surface functionalization

Term/ method	Definition
Coating	Coating is a surface functionalization method using a covalently or non-covalently applied (additional) material layer to the implant's surface incorporating one or more substances to achieve the desired characteristics the coating is intended for. To be applied to medical devices and for use in medical technology, the coating method shall be verified, and the coating functionality must be validated
Modification	Surface modification is surface functionalization utilizing a material mixture (composite material) or by means of chemical or physical change of the implant's surface to achieve the desired characteristics the surface modification is intended for.
	All material modification methods shall be verified for use in medical technology, and their functionality must be validated

legal requirements. These requirements are described in MDR (EU) 2017/745[14] and apply at the legal level.

A medical device's safety refers to all its components, parts, development or manufacturing steps. If the completion of a medical device does not follow a controlled development, neither safety nor a consistently high-performance level can be assumed. If a development does not follow the procedures defined in the MDR and the applicable standards, a product cannot be brought to market.

Incomplete development documentation, missed verification and validation measures, or insufficient attention to regulatory requirements are often the reason why SFTs are frozen at an academic-experimental development stage and have no future in industrial application.

To be able to describe the marketability of SFTs in detail, it is vital to know their influence on the risk classification of a medical device. The MDR, for example, stipulates that medical devices containing materials of animal origin have a higher risk and must therefore overcome higher approval hurdles than medical devices without materials of animal origin.[17] Knowing the influence of surface functionalization, it is possible to predict its impact on the approval of the medical device. Therefore, we have decided to develop a risk classification (Table 4, Fig. 2) and safety and performance requirements for coating technologies (Table 5) that are closely related to MDR (EU) 2017/745.

Table 4. Risk classification of surface functionalization methods and materials

Class 1	Low risk	Non-active, non-degradable surface coating based on materials of non-animal origin
Class 2a	Medium risk	Any surface modification or active and/or degradable surface coating based on materials of non-animal origin
Class 2b	Medium risk	Surface functionalization, incorporating materials of animal origin
Class 3	High risk	Surface functionalization to enable drug delivery

Besides the risk classification of SFTs, there are essential safety requirements, which must be met and which are formulated as 12 clauses, some of which find their direct reflection in an MDR clause (Table 5). Table 5 shows that SFTs have to meet multiple requirements to ensure their quality and riskless application.

OVERVIEW OF DIFFERENT COATING TECHNOLOGIES FOR POLYMER SURFACES

In analyzing relevant safety and performance characteristics of SFTs, we have limited ourselves to technologies for improved osseointegration of implants used in orthopedics or dental surgical care. Table 6 lists the results of our literature and market (benchmark) search analyzing the availability of SFTs and the description of their performance that have a 'kind of regulatory clearance' and are used in an industrial series standard (Table 6).

In Table 6, it becomes evident that a number of different coating technologies are applied, and these techniques are shown in more detail in Table 7.

ANALYZING SAFETY AND PERFORMANCE CHARACTERISTICS UNDER SIMULATED USE

Our literature analysis has shown that often specific verification and validation methods are chosen to produce outstanding results that can 'easily be published' because they give the impression that the technology and/or product tested has achieved a particular performance. However, only the analysis method was 'trimmed' to emphasize certain performance characteristics.

When analyzing the relevant safety and performance characteristics of SFTs and, directly related to them, the safety and performance characteristics of medical devices, one thing must be undisputed: the test strategy and setup must be selected to analyze and describe a possible worst-case behavior of the technology. Therefore, it is of absolute importance to define and stick to that test method and setup so that the technology analyzed is stressed and challenged in the best possible way during the verification and validation activities. This requirement applies to all mechanical, *in vitro* and *in vivo* test strategies.

Almost all commonly used coating materials and technologies suffer from debris, delimitation and abrasion because they only rely on physical forces of coating attachment to the implant surface (mainly weak van der Waals forces). In addition, particle-filled polymer composite materials suffer from the general incompatibility of the hydrophobic PEEK polymer (water contact angle 75°–95° [18–20]) and the hydrophilic HA mineral particles, which results in an incompatible interface between nanoparticles and polymer matrix, resulting in uneven particle dispersion in the materials. This results from the higher affinity of the nanoparticles to each other than to the polymer matrix. In addition, the bonding of the inorganic nanoparticles to the PEEK matrix is weak.[13]

Coating technologies incorporating titanium are also suspected to corrode, particularly in acidic environments, and cause inflammatory reactions. Table 8 summarizes the different disadvantages

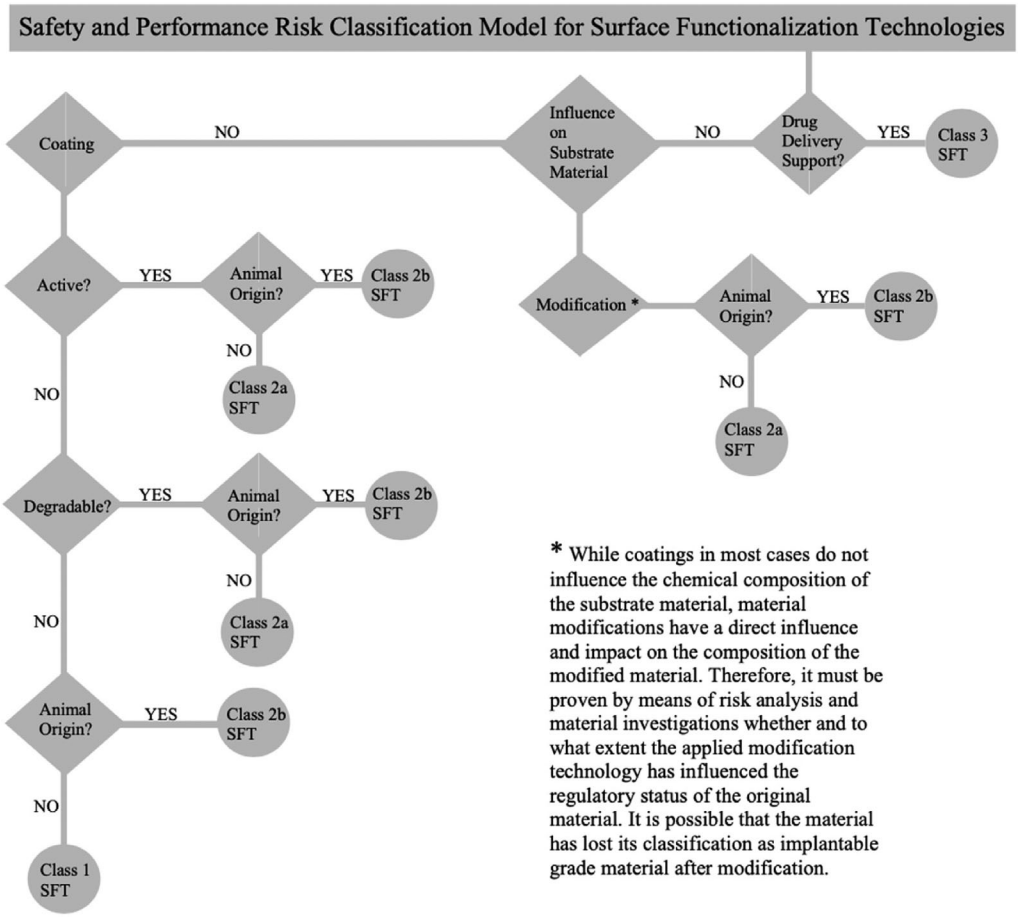


Figure 2. SFT risk classification decision tree.

and drawbacks of commonly known and regulatory cleared coating technologies as well as the applied test methods. The results presented here have been analyzed and evaluated by PubMed literature searches (see Table 2) and/or during simulated use tests performed by the authors and/or affiliated companies and institutes.

With all these different materials and substrates used in coating applications and because of the various coating technologies to apply these materials onto the surface of implants, users can easily get lost in the jungle of different efforts undertaken to test the mechanical characteristics of the coating layer, for example the stability of the various coatings on the implant's surface, the biocompatibility of the modified implant surface and the clinical benefit of the different engineered implant surfaces.[26]

On the one hand, there are some standardized test methods to prove the coating layer's mechanical stability on the implant's surface. On the other hand, these methods cannot be used to characterize the mechanical behavior of the various technologies in the scope of this perspective paper as they are developed to test mainly metallic coatings with a coating layer on a three-digit micrometer scale. Different setups of *in vitro* cell test methods and animal models can be found in the literature for biocompatibility testing.

The literature review carried out to investigate verification and validation methods (mechanical tests, cell tests, animal studies) for surface functionalization revealed a very inhomogeneous picture concerning the setup and study protocols. In order to create transparency regarding safety and performance, and to compare the performance characteristics of different technologies, it would be desirable to perform verification and validation activities according to a standard protocol. While attempts have already been made to establish a standardized procedure for mechanical testing (ASTM standards), and several standards or guidelines (ISO) exist for *in vitro* biocompatibility testing, this is still inconsistent in the field of cell and animal testing, although different models seem to prevail. However, it is essential to know that different test setups can also influence the results.

IN VITRO TESTING (CELL TESTS) TO ANALYZE THE SAFETY AND PERFORMANCE OF SURFACE FUNCTIONALIZATION TECHNOLOGIES

All materials used must be evaluated for biocompatibility using *in vitro* assays to protect patient health and safety. The recommended approach and principle to investigate this starts with testing the biological behavior of cell cultures on these materials. The traditional concept of biocompatibility is regarded as a lack of adverse reactions between the host and the tested material, addressing the evaluation for general safety. A further definition

Table 5. Essential safety and performance requirements for surface functionalization technologies

Clause	Related MDR clause	Description
1		SFTs shall be planned and developed in a structured and documented way. All design and development steps must be reviewed, evaluated and approved
1a		Crucial design and development steps must be approved in a risk-based approach. Main design and development must be verified
1b		SFT must be validated
2	SPR 1	Surface functionalization technologies (SFTs) shall achieve the performance intended by their manufacturer and shall be designed and manufactured in such a way that, during normal conditions of use, they are suitable for their intended purpose. They shall be safe and effective and shall not compromise the clinical condition or the safety of patients or users' safety and health
2a		SFTs for enhanced osseointegration shall allow for early bone formation and an adherent and dense cell layer
2b		SFTs for enhanced osseointegration shall allow for high bone to implant contact with a balanced ratio of old 'parent' bone and new bone
3	SPR 10.1	SFTs shall be designed and manufactured in such a way as to ensure that the characteristics and performance requirements referred to in MDR, Annex 1, Chapter I are fulfilled. Particular attention shall be paid to:
3a		the choice of materials and substances used, particularly as regards toxicity and biocompatibility, metabolic reactivity
3b		the compatibility between the materials and substances used and biological tissues, cells and body fluids, taking account of the intended purpose of the SFT and, where relevant, absorption, distribution, metabolism and excretion
3c		the mechanical properties of the SFT on the implant, reflecting, where appropriate, wear resistance and abrasion
3d		surface properties such as homogeneity and (layer) thickness
3e		the confirmation that the SFT meets any defined chemical and/or biological specifications
4	SPR 10.2	SFT modified devices shall be designed, manufactured and packaged in such a way as to minimize the risk posed by contaminants and residues to patients, taking account of the intended purpose of the device, and to the persons involved in the transport, storage and use of the devices. They must be taken into account that packaging materials may react with the SFT
4a		Confirmation that packaging material does not interact with or react to the SFT
5	SPR 10.4.1	SFTs shall be designed and manufactured in such a way as to reduce as far as possible the risks posed by substances or particles, including wear debris, degradation (products) and processing residues, that may be released from the SFT
6	SPR 10.6	SFTs shall be designed and manufactured in such a way as to reduce as far as possible the risks linked to the size and the properties of particles, which are or can be released into the patient's or user's body unless they come into contact with intact skin only. Special attention shall be given to nanomaterials
7	SPR 11.1	SFTs and their manufacturing processes shall be designed in such a way as to eliminate or to reduce as far as possible the risk of infection to patients, users and, where applicable, other persons. The design shall:
7a		allow easy and safe handling
7b		as far as possible, avoid any microbial leakage from the device and/or microbial exposure during use
7c		prevent microbial contamination of the device or its content such as specimens or fluids
8	SPR 11.2	SFTs shall be designed to allow for safe cleaning, disinfection and/or sterilization
9	SPR 12.2	Devices that are composed of substances or of combinations of substances that are intended to be introduced into the human body and that are absorbed by or locally dispersed in the human body shall comply with the relevant requirements laid down in Annex I to Directive 2001/83/EC for the evaluation of absorption, distribution, metabolism, excretion, local tolerance, toxicity, interaction with other devices, medicinal products or other substances and potential for adverse reactions, as required by the applicable conformity assessment procedure under this regulation
10	SPR 13.2	For SFTs manufactured utilizing tissues or cells of animal origin, or their derivatives, which are non-viable or rendered non-viable the following shall apply:
10a		where feasible taking into account the animal species, tissues and cells of animal origin, or their derivatives, shall originate from animals that have been subjected to veterinary controls that are adapted to the intended use of the tissues. Information on the geographical origin of the animals shall be retained by manufacturers
10b		sourcing, processing, preservation, testing and handling of tissues, cells and substances of animal origin, or their derivatives, shall be carried out in a way so as to provide safety for patients, users and, where applicable, other persons. In particular, safety with regard to viruses and other transmissible agents shall be addressed by implementation of validated methods of elimination or viral inactivation in the course of the manufacturing process, except when the use of such methods would lead to unacceptable degradation compromising the clinical benefit of the device
10c		in the case of SFTs utilizing tissues or cells of animal origin, or their derivatives, as referred to in Regulation (EU) No 722/2012 the particular requirements laid down in that Regulation shall apply
11		SFTs shall meet labelling requirements clearly highlighting the methods used to verify and validate the safety and performance of the respective SFT
12		Where applicable, SFTs must meet all SPRs stipulated in MDR, Annex 1

MDR, Medical Device Regulation, SFT, surface functionalization technology; SPR, safety and performance requirements.

Table 6. Benchmark research/safety and performance claimed by SFT supplier

Manufacturer	Brand	Reported mechanical testing	Reported cell test	Reported animal model[1] Animal[2] Implant design[3] Surgical location	Type of surface functionalization
Promimic	HA^{nano} Surface®			Comparative animal model [1] Rabbit [2] Screw design [3] Tibia (left and right) hind leg	HA surface spray coating
Plasmatreat	Openair-Plasma®		NR		Titanium plasma spray coating
DOT	Plasma spray coating	ASTM F1854 ASTM F1147	DIN EN ISO 10993-1 DIN EN ISO 3274 DIN EN ISO 4287 DIN EN ISO 4288	Comparative animal model [1] Rat [2] Cylindrical design [3] Tibial metaphysis	Physical vapor deposition Titanium plasma spray
DOT	BONIT coating	ASTM F1185 ASTM F1609	DIN EN ISO 10993-1 DIN EN ISO 10993-5 DIN EN ISO 10993-10	Comparative animal model [1] Miniature pig [2] Pins [3] Maxilla	Electrochemical calcium phosphate coating + HA
Elos Medtech	HA NANO™ Surface	ASTM F1147	ISO 10993	Comparative animal model [1] Rat [2] Screw design [3] Tibia	HA dip-coating process
Eurocoating/ Lincotek Medical	Spondycoat®-T 371A	ASTM F1147 (PEEK)	ISO 10993-1	Comparative animal model [1] Sheep [2] Screw design [3] Ilia crest	Titanium coating with low roughness (approximate R_a values 4–10 µm) mainly indicated for thin layers (thickness 60–120 µm)
Eurocoating/ Lincotek Medical	Spondycoat®-T 107	ASTM F1147	ISO 10993-1	Comparative animal model [1] Sheep [2] Screw design [3] Ilia crest	Titanium coating with high roughness (approximate R_a values 20–40 µm) and a thickness of 125–250 µm
Eurocoating/ Lincotek Medical	Ti-Growth®	ASTM F1147	ISO 10993-1	NR	Porous titanium coating with high roughness (approximate R_a values 40–80 µm) and an approximate thickness of 300–500 µm
Eurocoating/ Lincotek Medical	Spondycoat®-HA	ASTM F1147)	ISO 10933-1	NR	HA coating with low roughness (approximate R_a values 4–8 µm) mainly indicated for thin layers (approximate thickness 45–85 µm)
IHI IonBond	Medthin™ 65 Ti	ASTM F1147	ISO 10993-1	NR	A thin film with tunable surface roughness which can be deposited at a thickness of up to 20 µm
Orthobion	TSC	NR	NR	Comparative animal model [1] Sheep [2] Cylindrical dowel [3] Femur and tibia	Micro-surface roughness Nano titanium particle coating
stimOS	MBTv				

Table 6. Continued

Manufacturer	Brand	Reported mechanical testing	Reported cell test	Reported animal model[1] Animal[2] Implant design[3] Surgical location	Type of surface functionalization
		Test strategy for nm-modification layer: drop test in combination with ASTM D3359 CY5 Staining	Comparative cell tests	Comparative animal model [1] Sheep [2] Screw design [3] Ilia crest	Covalently attached polysaccharide nm thin film mineralized with amorphous calcium phosphate
stimOS	MBTg	Test strategy for nm-modification layer: drop test in combination with ASTM D3359 CY5 Staining	Comparative cell tests	Comparative animal model [1] Sheep [2] Screw design [3] Ilia crest	Covalently attached gelatin nm thin film mineralized with amorphous calcium phosphate
Seaspine	NanoMetalene	CP Titanium Surface ASTM F67	NR	Comparative animal model [1] Sheep [2] Cylindrical dowel [3] Femur and tibia	Micro-surface roughness Nano titanium particle coating

HA, hydroxyapatite; MBT, mimicking bone technology; NR, not reported; PEEK, polyetheretherketone; SFT, surface functionalization technology.

might be the ability of a (bio-)material to induce an appropriate and advantageous host response during its intended clinical usage, addressing the performance of the material for its intended application. Although discrepancies regarding biocompatibility assessments produced by cell culture assays and the *in vivo* biocompatibility upon implantation into a living host exist, such screenings are imperative for a first and quick assessment of new materials. The general awareness of these difficulties has provided the incentive to standardize the methodology of *in vitro* assays and regulate their application at national and international levels. From these efforts, a large number of screening methods exist for measuring the biocompatibility, which vary in

Table 7. Overview of different regulatory cleared surface functionalization technologies for polymeric implant surfaces found in the literature

Coating technology	Description
Titanium plasma spray coating	Pure titanium coating applied by vacuum plasma spray process. The purity of the basic material corresponds to the ISO 5832-2 implant standard. With a thickness of 100 to 300 µm, titanium plasma spray coating contributes effectively to surface roughness, a good primary stability and improved osseointegration
Titanium sputter coating	Physical vapor deposition technique, resulting in a coating layer which promotes osseointegration with a thickness in the three-digit-nanometer range
Plasma-sprayed HA	Thermal spray technique to produce an HA layer with a thickness from 30 to 200 µm depending on the coating conditions. Due to its chemical identity with the mineral component of bone, hydroxyapatite ceramics ($Ca_5(PO_4)_3OH$) have proven to be an appropriate material for bone replacement
TiO_2-CaP dip coating	To achieve even thinner coating layers, TiO_2-CaP dip coating was introduced on the nanometer scale. The implant surface is masked by improved biocompatible titanium oxide, which has advantageous effects in many fields of medical applications. At the same time, incorporated calcium ions are released to accelerate faster bone ingrowth
HA enhanced PEEK	Material enhancement in spinal device technology. HA, as a well-known osteoconductive material, is integrated within a PEEK matrix, making it available on the surfaces of a device only after processing the implant material by milling
Biochemically covalently bonded surface modification	The material surface is modified by a covalently bonded surface coating. The chemical bond prevents detachment and delamination. Biological molecules, also in combination with HA or amorphous calcium phosphate, can be used for the coating to mimic the natural bone, thus providing superior osseointegration

HA, hydroxyapatite; PEEK, polyetheretherketone.

methodology, applied cell line and the assay setup. All components can influence the intended assay outcome.

The biocompatibility evaluation process generally begins with cytotoxicity assays and their evaluation, which measure cell death or cell influence caused directly by the material upon contact or upon contact with their extracts. A general guidance for *in vitro* cytotoxicity testing is presented in ISO 10993-5. For dental materials, DIN EN ISO 7405:2019-03 offers further guidance in the evaluation of new materials. Herein, different methods are presented for cytotoxicity detection. Cytotoxicity testing mostly uses fibroblasts within a short testing time, and then claims are made about the investigated influence: The manufacturer can select appropriate methods, based on the intended use of the material and the known and assumed toxicity profile of the material or its components and in preference to costs, experience or other reasons. The evaluation of data is frequently presented at single time points, the kinetics over an extended period are not considered, and often appropriate controls are not included. This neglects effects that influence later cellular stages or effects of lag time to proliferation.

Despite this attempt to standardize assays, evaluation discrepancies are still prevalent, notwithstanding seemingly similar experimental conditions. The application of different methods of cytotoxicity evaluations may even produce different assessments for the same materials, making it increasingly difficult to predict the *in vivo* performance or safety.

A test for the safety of a material is the minimum requirement of *in vitro* evaluation. No adequate standards exist to evaluate the performance of a new bone implant material. Many factors can be counted to be addressed to assess the *in vivo* performance: cellular adhesion, cellular proliferation, specific metabolic activity of the cells, and many more. All these parameters can vary significantly according to the applied method, setup and choice of the cellular system.

One of the reasons for the discrepancies may be due to the choice of the cell line for *in vitro* tests, which assesses the property of investigation with regard to safety or performance. The ISO 10993 guideline on the standardization of cell culture experiments is to advocate the use of permanent cell lines to achieve reproducibility testing between different laboratories in the

Table 8. Overview of test methods and results to analyze the safety and performance of surface modification technologies

Coating technology	Test method	Results
Titanium plasma spray coating on PEEK HA plasma spray coating on PEEK	In-house research: according to EN ISO 7438, appropriate tests were performed to evaluate adhesion and elongation of the coatings under bending tension. $N = 12$ (2×6)	Cracks and beginning delamination after EN ISO 7438 testing (Fig. 3) Films deposited by thermal spraying suffer from poor coating–substrate adherence and nonuniform crystallinity, which reduce the lifetime of such coated implants. Thermal spray coating requires a high sintering temperature, which may result in crack propagation on the surface of the coating[21]
Titanium nanocoatings on PEEK CaP nanocoatings on PEEK	Titanium and CaP nanocoated implants were tested in usability studies. All implant geometries were the same and made out of PEEK. The test implants, as the study group, were additionally surface coated, either with a CaP nanocoating or a Ti nanocoating, whereas a control group was uncoated. The experimental setup was designed to mimic cage impaction into the intervertebral disc space. The cage surface was inspected before and after the impaction.[22] $N = 12$ (2×6)	Abrasion of the tip of the ridges was detected in all three test groups. Additionally, in the case of the Ti nanocoated cage, some areas were detected where the coating had almost disappeared[23]
HA-filled PEEK	In-house research: *in vivo* sheep model – histological analysis after implant extraction. $N = 12$ (2×6)	The HA-filled PEEK compound in this study had an HA content of 20%. In the cortical bone area, in particular, a relatively wide range of BIC values was found in comparison with the other implant groups. This could be attributed to an uneven distribution of the HA particles within the matrix and the implant surfaces (Fig. 4) HA does not show a robust physical/chemical affinity to the PEEK matrix itself, due to the high chemical contrast between the two materials, resulting in weak binding of the HA particles to PEEK[13] Compounding the PEEK matrix with HA particles makes PEEK more brittle[22,24,25]
Biochemically covalently bonded mineralized coating (MBT)		Homogeneous BIC on MBT implants. Stable anchorage of the coating surface onto the polymeric implant surface (Fig. 5)

BIC, bone (to) implant contact; HA, hydroxyapatite; MBT, mimicking bone technology; PEEK, polyetheretherketone.

Table 9. Cell lines used in *in vitro* (cell test) testing of surface functionalization technologies

Cell line	Description
Osteoblast MC3T3-E1	Derived from *Mus musculus* (mouse) calvaria Cells have the capacity to differentiate into osteoblasts and osteocytes
Fibroblasts L929	Mouse fibroblast cell line

standard assays as part of biocompatibility screening. However, immortalized cell lines represent simple replicating systems, lacking the specific metabolic potential that cells have *in vivo*. Furthermore, their growth characteristics and sensitivity to certain toxins can vary greatly from culture to culture, making it increasingly difficult to predict the *in vivo* performance. Additionally, the material's apparent response can be significantly affected by the cell line or even a subclone of the same cell line selected for the test.

One example is the use of osteoblastic cell lines for bone or dental implant applications, for instance the MC3T3. Although described as 'one of the most convenient and physiologically relevant systems for study of transcriptional control in calvarial osteoblasts',[27] many researchers and manufacturers are not aware of the specifics with regard to the origin or performance of these cell lines. In 1999 Wang *et al*. isolated a series of 52 single-cell clones derived from the parent MC3T3-E1 cell line.[28] These cell lines were then further characterized regarding their different cellular activities, including their expression of several hallmark osteoblastic genes, metabolic activity and mineralized matrix deposition. The subclonal cell lines differed slightly from each other; specifically, they had high or low differentiation/mineralization potential after growth in the presence of ascorbic acid.[28,29] Several of these subclones are currently commercially available. Despite the availability of these subclones and knowledge of their resemblance or non-resemblance to osteoblastic function, only a meager number of published papers clearly specified which subclone of MC3T3-E1 was studied.

Further discrepancies in the performance evaluation might arise due to the choice of another cell line, again due to different cellular properties such as differentiation profiles. MG-63 osteoblast cells have been repeatedly reported to have significantly higher proliferative rates at growth day 2, whereas SaOs2 cells have a significantly higher initial level of alkaline phosphatase activity when directly compared to the corresponding primary cells.[30] If two investigating researchers tested the metabolic activity at day 2 of the same material but using different cell types, one group may recommend the material while the other users may rate the same material as ineffective.

In contrast to cell lines, primary cells are isolated directly from tissues or organs. They have a finite lifespan and limited

Table 10. Parameter used in *in vitro* (cell test) testing of surface functionalization technologies for safety

Screening parameter	Description
Direct toxicity	Evaluation of cell survival in direct contact with the material under investigation
Indirect toxicity through extracts or leachables	Evaluation of cell survival of possible extracts or leachable toxins from the material under investigation
Cellular viability/health in direct contact	Evaluation of impacts on metabolic activity or other adverse cellular inhibition
Cellular viability/health in the influence of possible extracts or leachables	Evaluation of impacts on metabolic activity or other adverse cellular inhibition through leachable toxins from the material under investigation

Table 11. Parameter used in *in vitro* (cell test) testing of surface functionalization technologies for performance

Screening parameter	Description
Cell adhesion	Evaluation of cellular adhesion on the surface of the material
Cell proliferation	Evaluation of cellular growth within specific time-frames
Mineral/matrix deposition	Evaluation of cellular matrix deposition and its mineralization
Differentiation status (osteoblasts)	Evaluation of markers of differentiation, e.g. alkaline phosphatase

Table 12. Test geometries used in *in vivo* (animal model) testing of surface functionalization technologies

Test implant design/ geometry	Description	Predictable impact on test results
Cylindrical test dowel		If a cylindrical test implant is used, the bone is usually prepared with a hole of the same diameter as the test implant, and the implant is then placed in this hole. It can be assumed that the implant surface will not be subjected to excessive stress during this procedure. Possible abrasion and/or possible delamination is prevented
Screw design		If a test implant is used in the screw design, the bone is prepared with a hole usually 0.5 mm smaller than the test implant. Since the test implant is also tapered and screwed in, the implant surface is subjected to an additional stress test, which provides information about possible unwanted abrasion or delamination

Table 13. Safety and performance evidence level (SP evidence level for SFTs)

				Grading system	Impact score	Degree of fulfillment
eI	Design and development (only one answer possible; maximum 3 points = 100%)					
	Design and development	Verified and validated and certified ISO 13485		YES	3	100%
				NO	0	
		Verified and validated and according to GLP standard		YES	2	66%
				NO	0	
		Verified and validated		YES	1	33%
				NO	0	
		No validation		YES	0	0%
				NO	0	
					Subtotal	100% (1/6)
eII	Manufacturing method (only one answer possible; maximum 3 points = 100%)					
	Manufacturing process	Manufacturing process (industrial scale) is verified and validated and ISO 13485 certified		YES	3	100%
				NO	0	
		Manufacturing process (industrial scale) is verified and validated and according to GLP standard		YES	2	66%
				NO	0	
		Upscaling verified and validated		YES	1	33%
				NO	0	
		Manufacturing on an industrial scale is not possible		YES	0	0%
				NO	0	
					Subtotal	100% (1/6)
eIII	Pre-clinical testing: abrasion and delamination (only one answer possible; maximum 3 points = 100%)					
	Mechanical testing	Rationale and verified and validated and performed by an accredited laboratory		YES	3	100%
				NO	0	
		Rationale and verified and validated		YES	2	66%
				NO	0	
		Rationale		YES	1	33%
				NO	0	
		Other		YES	0	0%
				NO	0	
		None			FAILED	
					Subtotal	100% (1/6)
eIV	Pre-clinical testing: cell test (only one answer possible; maximum 4 points = 100%)					
	Cell testing	Relevant cell line and comparative setup and statistically relevant and GLP conform		YES	4	100%
				NO	0	
		Relevant cell line and comparative setup and statistically relevant		YES	3	75%
				NO	0	
		Relevant cell line and comparative setup		YES	2	50%
				NO	0	
		Relevant cell line and setup (see Tables 9–11)		YES	1	25%
				NO	0	
		Other		YES	0	0%
				NO	0	
		None			FAILED	
					Subtotal	100% (1/6)
eV	Pre-clinical testing: animal model (multiple answers possible; scores are added. Maximum 10 points = 100%)					
	Animal model	Animal study performed		YES	2	20%
				NO	0	
	Study setup	Split mouth		YES	4	40%
				NO	0	
		Comparative		YES	3	30%
				NO	0	
		Single arm		YES	2	20%
				NO	0	
	Animal	Sheep		YES	1	10%
				NO	0	
	Surgical side	Dense bone		YES	1	10%

Table 13. Continued

			Grading system	Impact score	Degree of fulfillment
			NO	0	
	Implant geometry	Screw design	YES	1	10%
			NO	0	
	Standard	GLP	YES	1	10%
			NO	0	
				Subtotal	100% (1/6)
eVI	Applicability (only one answer possible; maximum 4 points = 100%)				
	Limitation of clinical applicability	Surgical technique, storage, packaging, cleaning and sterilization requirements of the implant incorporating SFT are not affected by SFT	YES	4	100%
			NO	0	
		Only storage conditions of the implant incorporating SFT must be adapted to the requirements of SFT	YES	3	75%
			NO	0	
		Storage, packaging, cleaning and sterilization conditions of the implant incorporating SFT must be adapted to the requirements of SFT	YES	2	50%
			NO	0	
		Surgical technique must be adapted to the requirements of SFT	YES	1	25%
			NO	0	
		SFT cannot be stored using standard storage conditions guaranteeing a shelf life of (≥) 5 years	YES	0	0%
			NO	0	
				Subtotal	100% (1/6)

GLP, good laboratory practice; SFT, surface functionalization technology.

expansion capacity, have normal cell morphology, and maintain many important markers and functions observed *in vivo*. Thus, they represent the *in vivo* situation better than immortalized cell lines. However, testing conditions are complicated and more costly since these cells have to be isolated reliably. Furthermore, in contrast to cell lines, primary cells are very sensitive and often require additional nutrients not included in classical media. To optimize survival and growth, primary cells perform best in media customized for each cell type. Furthermore, the use requires the availability of tissues and organs for each test to be applied.

Today's direction of standardization lies in an initial risk assessment by an expert. In this process, data already available about physical, chemical and biological characteristics are evaluated, and a decision is made regarding further studies or even if further studies are necessary. If a material that has already been applied in practice was only slightly modified, then its harmlessness (i.e. acceptable risk) can be certified, for example based on the chemical analysis of extracts.

It is clear that further standardization and clear protocols are required in this field. Generally, the material has to be tested for both safety and performance. Herein, the experimental design for the screening must be based on the material's unique properties under investigation and its intended use. The experimental setup has to be clearly defined. Furthermore, the experimental design must include quantitative assays, statistical analysis and sufficient controls. Additionally, the appropriate cell line for the intended application should be utilized for all the required biocompatibility evaluations. To avoid confusion and misinterpretation, clear definitions of the specific terminology should be defined, used and updated to the state-of-the-art knowledge. This includes the specific definitions and identification of cell types involved. Suggestions for applicable cell lines, valuable screening parameters and suitable test parameters for the performance of a material are given in Tables 9, 10 and 11.

IN VIVO TESTING (ANIMAL MODEL) TO ANALYZE THE CLINICAL BENEFIT OF SURFACE FUNCTIONALIZATION TECHNOLOGIES

In the field of *in vivo* testing of SFTs, various setups and study protocols, different test geometries and different animal models are reported to be used in testing activities: this ranges from cylindrical test implants to screw designs. The implants are inserted partly in long bone (femur and/or tibia) and partly in the dense bone material of the iliac crest. The test implants are partly tested in dogs, rabbits, rats or sheep. Comparative test setups are reported as well as split-mouth design or single-arm models. Al-Otaibi *et al.* have conducted a comprehensive literature search and presented the results in tabular form.[31]

To study the pathophysiology of delayed fracture healing and non-union formation, appropriate animal models are needed. These animal models should be well standardized and, most importantly, should approximate the clinical situation in humans. Only studies using appropriate models will contribute to a better understanding of the mechanisms of the disease and will assist in the development of novel therapeutic strategies. Accordingly, not every animal model with a fracture that does not adequately heal may be suitable to study non-union formation.[32] A number of animal test models, such as rat/mouse, rabbit, sheep, goat and pig, have been developed to simulate the human *in vivo* environment and physical conditions to test the availability and comparability of bone substitute biomaterials. In order to mimic various orthopedic situations, many defect sites have been explored, such as calvaria, femora, ulna or pelvic bone (iliac crest). Several models have been developed in sheep. Utilizing drill hole defects to test bone graft substitutes, some are placing the drill holes in the long bones of the extremities, others are using a combination of femur drill holes and a slot defect in the tibia[23] or the pelvic bone. No large-animal model of bone regeneration has been accepted as

Figure 3. EN ISO 7438 bending tests of six plasma-spray-coated implant surfaces clearly show cracks in the coating layer. A further consequence may be delamination/abrasion. In-house research.

a standard testing model so far. But standardization may be the key to moving systematically towards better bone regeneration.[33]

Sheep are a convenient large-animal model for biomedical research because of availability, ease of handling and housing as well as animal cost. In orthopedic research, sheep are a well-accepted model for *in vivo* studies: sheep are useful to address the biomechanical, biochemical and histological processes of bone biology, due to similarities with humans in weight, size, bone and joint structure and the bone remodeling process.[34]

To study osseointegration and the osseointegrative characteristics of different implant surfaces or implant SFTs, we suggest a comparative split-mouth study setup using the pelvic bone (iliac crest) of sheep. A summary of suggested test implant designs is shown in Table 12.

CONCLUSION

To display verification and validation results in a transparent and comparable approach, we have defined a scoring system: the Safety and Performance Evidence Level (SP Evidence Level for SFTs) Scoring System (SPEL scoring).

The scoring system (Table 13) defined in this publication does not assess the values and results of the verification and validation activities performed but starts at a fundamental level: the scoring system does not assess the individual test results, but rather the 'evidence level' of the underlying verification and validation strategy. Thus, it is possible to relate each result to the verification and validation strategy and better assess the overall evidence level.

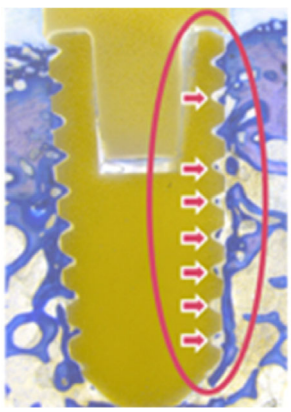


Figure 4. Histological analyses of HA enhanced PEEK implants: uneven distribution of HA results in an inhomogeneous BIC. In-house research.

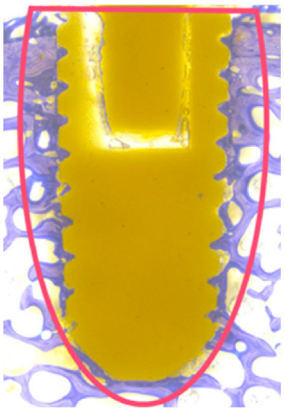


Figure 5. Homogeneous BIC on MBT implants. In-house research.

With this scoring system, data of SFTs are transparently displayed and test methods are aligned to such an extent that the potential user – surgeon or patient – can compare different technologies with each other.

A quality seal – issued by a neutral authority, such as a certification authority or notified body, accredited for the evaluation of medical devices – could provide the necessary transparency. The quality seal will indicate in combination (i) the surface functionalization method according to Table 3, (ii) the risk profile/risk classification of the SFT according to Table 4, (iii) the material incorporated and (iv) the SPEL and its degree of fulfillment for the respective SFT according to Table 13.

This scoring system is the first attempt to establish a standardized and transparent test system for the quality of bone implant materials, which already reveals the strengths and weaknesses of the many existing materials at first sight.

The scoring system is divided into six subsections: design and development, manufacturing, mechanical testing, biocompatibility testing, animal study, clinical applicability. In each subsection a maximum evidence level of 100% can be reached. Note that every subsection (sections I–VI) only represents 1/6% of the overall evidence level (degree of fulfillment). The overall evidence level can reach a maximum of 100% and is calculated as follows:

[e1(1/6)*(*XX*%) + e2(1/6)*(*XX*%) + e3(1/6)*(XX%) + e4(1/6)*(*XX*%) + e5(1/6)*(*XX*%) + e6(1/6)*(*XX*%)] = *XX*%

With this scoring system, the significance and applicability of verification and validation procedures that have been carried out to evaluate the marketability of an SFT can be determined. Thus an evaluation platform has been created which can be used system-independently of country-specific regulations (EU, MDR; USA, FDA; China, cFDA; Brazil, ANVISA etc.), in order to make comparable and transparent statements regarding the significance of test results.

Of course, the future will show the applicability of the scoring system and adaptations might become necessary but what is essential is that the system suggested here is the first step towards a transparent evaluation of bone implants. Our current focus lies on polymer implant materials, but the principle can easily be adapted to metal or ceramic implant materials.

CONFLICT OF INTEREST

The authors acknowledge their potential conflict of interest with the contents of this paper. stimOS GmbH and Solvay commercialize part of the presented technology/products and materials described in this paper. D.S., J.K and G.P. are affiliated with stimOS

Polymer International published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

GmbH, H.C. is co-inventor of a national patent owned by stimOS GmbH and C.K. is affiliated with Solvay.

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