Herbert P. Jennissen*

Implantomics: A Paradigm Shift in Implantology

Abstract: Implantomics is the science of the *implantome*. The implantome is a blend of the two terms *implant* and *proteome*. The proteome is defined as the protein complement of the genome. The term proteome also implies the *mass screening of proteins* for the determination of all proteins – and indirectly of all genes – involved in a certain tissue or organ response. In this sense the term proteome is employed here in a new way to specify the totality of proteins associated with a foreign body inserted into the human body. It will be addressed, why the determination of the implantome is important and which role the implantome may play in the bone-implant interface.

Keywords: implant proteome, bone-implant interface, biocompatibility, osseointegration, peri-implant bone healing,

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1 Introduction

The term "paradigm Shift" was coined by Kuhn in 1962 [1]. Three steps are involved: "*crisis*", "*reform*" and emergence of a "*new paradigm*". The *crisis* appears to be, that after ca. 35 years of research biocompatibility and osseointegration are still only poorly understood (see comprehensive reviews [2,3]). Published conclusions of protein adsorption being only of minor importance in implantology are up for revision. A radical reform of this thinking has been triggered by a new technology, i.e. LC-MS/MS, allowing the complete, simultaneous identification of all initial proteins layered on a human implant [4]. A new paradigm is suggested as the emergence of a *proteomic scale* new understanding of protein function in implantology. Biocompatibility is primarily a function of the implant or biomaterial surface. Corresponding research is multidisciplinary involving e.g. surface physics, surface chemistry and surface biochemistry, terms which could be condensed to one word, *epiphanostics* (from greek "epiphaneia" = surface). On the biochemical level protein

adsorption is the first fundamental interaction between the human body and the surface of a prosthesis on implantation. Every implant poses a non-specific molecular recognition surface in the body with high affinity for the spontaneous adsorption of human proteins. The adsorbed protein layer is thus a result of the protein composition in the environment (periimplant protein pool) and the respective surface with affinity for proteins. As will be shown the proteomic approach (for definition see [5]) necessitates a reevaluation of present models in implant science. In a sandwich model of the bone-implant interface the implant proteome can be viewed as the sandwich-spread in-between. It's understanding may be the key to master biocompatibility and implant integration.

2 Materials and Methods

All methods related to the hip implant proteome are described in ref. [4], together with the primary proteome data, available as *supplementary Table 1* for download [4] allowing third party evaluations. Briefly [4] the *in situ* femoral stems were retrieved 2 min after implantation, washed with saline, quick frozen in liquid nitrogen and stored at -80 °C. Proteins were by solubilized by SDS and reducing agents at room temperature and analyzed by tandem LC-MS/MS with the Proteome Discoverer software (Thermo Scientific) by Mosaique GmbH (D-30659 Hannover) [4]. Biocontact hip implants (Braun Aesculap, D-78532 Tuttlingen) with a rough plasmapore TPS surface and a smoother glass pearl blasted surface were employed.

Table 1
Suggested Nomenclature for implant proteome research*

Blended Terms	Connotation
Implantomics	Science that deals with the proteome on implants in general
Explantomics	Science that deals with the explant proteome
Implantome	Genome- and host-based <i>in situ</i> protein layer on implants during physiological peri-implant healing
Explantome	Genome- and host-based <i>in situ</i> protein layer on explanted implants, due to pathological peri-implant events.
Integratome	Genome- and host-based physiological steady-state protein layer on a tissue-integrated implant <i>in situ</i>

**implantome genesis is the dynamic product of a foreign-body reaction of the host. The blended terms are fully free for scientific use, but commercially trademark protected. Non-use of the symbols ® und ™ does not imply a waiver of trademark rights.*

*corresponding author:

Prof. Dr. H.P. Jennissen, Institut für Physiologische Chemie, Universität Duisburg-Essen, Universitätsklinikum Essen, Hufelandstr. 55, D-45122 Essen, Germany; Email: hp.jennissen@uni-due.de

3 Results and Discussion

3.1 Protein Adsorption

The basic mechanisms of protein adsorption were elucidated in *single protein adsorption systems*. From equilibrium and kinetic studies it has been concluded that homogenous pure proteins are adsorbed to non-biological surfaces with high affinity by multivalent [6], cooperative [7] non-covalent [8] interactions involving thermodynamically irreversible adsorption hysteresis [9,10] with binding affinities of $K'_A \sim 10^{12} \text{ M}^{-1}$ and higher (**Fig. 1**). In physically controlled kinetic studies the adsorption of proteins is an exponential function beginning in milliseconds and reaching equilibrium in less than one minute [11].

Fig. 1. Multiple metastable protein states in hysteric Model of protein adsorption [9,12,13]. The steps k_{+2} , k_{+3} , k_{+5} are slow and the steps k_{-6} , k_{-7} are fast. $t = \text{time}$, $P^{I-III} = \text{adsorbed protein in 3 conformational states of increasing affinity } (K_A)$

In early attempts for understanding more complex systems. Horbett et al. [14] and Cooper's group [15] analyzed pure *binary-protein adsorption systems*, demonstrating competition and heterologous protein-protein displacement on polymers. On defined alkyl-residue lattices protein-protein displacement was shown to be a form of negative cooperativity [16]. A novel observation in binary systems is "protein interference" [17]. Much earlier Vroman had studied more complex *multi-protein adsorption systems* of fibrinogen from blood plasma [18] (Vroman effect). Because of the immense analytical problems this work had been largely discontinued. This has now changed. Today the highest imaginable scale of complexity in the form of *proteomic-scale or myriad-protein adsorption* on surfaces has become a reality [4], allowing thousands of individual proteins to be monitored simultaneously during adsorption and desorption.

At the advent of the field of biomaterials in the 1970ies and 1980ties it was without question that the first proteins adsorbed on an implantable device originate from blood plasma [13,19,20] initiating periimplant endosseous healing

similar to fracture healing [21] and leading to implant integration [22]. Based exclusively on *in vitro* data this has been the doctrine to the present day [23,24]. In their paper from 1987 on the Big Twelve proteins Andrade and Hlady [13] demonstrated 150 electrophoretic bands from plasma and estimated that a cell contains ca. 5,000 proteins. Today's estimates with splice variants lie at ca. 70,000 human proteins. Current doctrine teaches that adsorption from blood plasma leads to preferential adsorbates such as albumin, fibrinogen and IgG [25], followed by blood coagulation, the formation of a fibrin network as guide rail for oncoming cells e.g. Macrophages [26,27] and stem cells.

Implant surface properties also play a decisive role [28]. Such surfaces are now available and classified either as ultra-/superhydrophilic displaying contact angles $< 10^\circ$ [29] or as hyperhydrophilic with complex and/or imaginary contact angles [30]. The rapid, spontaneous reversal from hydrophilicity to hydrophobicity can be prohibited for years in a dry state by an exsiccation layer of salt [31].

3.2 The Hip Implantome

3.2.1 The Origin of the Hip Implantome

As detailed above, the adsorbed protein layer is a result of the periimplant protein pool and the implant surface. The primary implantome originates not only from the blood plasma proteome [23,24], but from a mixture of blood plasma with the periimplant operational humor (Humor operationis or operationl protein fluid), the composition of which is still unknown. The operational humor depends on the operator and the operational and patient situation. Thus tissue injuries result, which release large amounts of intracellular proteins but are operationally mandatory. To a certain extent the situation cannot be planned. On the other hand in the future an optimal implantome might be rationally prefabricated.

3.2.2 The Primary Implantome and the Early Bone-Implant Interface

The bone-implant interface in general, and for titanium implants specifically, possesses biochemical, biological and mechanical aspects which must be experimentally assessed. In literature searches very little can be found on the early interface healing stages and biochemistry appears to be an implantomic and interfacial orphan.

The initial protein layer on an implant, i.e. the primary implantome, is the crucial host response and an integral part of the initial bone-implant interface during the earliest

healing stage. An early paper on the composition of the bone-metal interface was described nine days after implantation in a rat model by Donath et al. [26] and appears to be 7-15 μm wide. The earliest cells to appear after 3 days are histiocytes and multinucleate giant cells, probably related to M2 type macrophages in agreement with a macrophage model of osseointegration (see ref. [27,32,33]).

In the current paper by Jäger et al. [4] it was found that the primary human hip implantome, at a post-insertion time of 2 min, consisted of 2802 (#peptide number ≥ 2) unique

Table 2

Profile of Plasma Proteome in the hip Implantome^[1]

Nr	Name	Symbol	Abundance [ppm]	
			mean	SD
1	Serum albumin	ALB	48927	17300
2	Alpha-1-antichymotrypsin	SERPINA3	2342	1174
3	Titin	TTN	1961	98
4	Carbonic anhydrase 1	CA1	1888	21
5	Collagen alpha-1(V) chain	COL5A1	1820	1486
6	Catalase	CAT	1678	947
7	Keratin, type II cytoskeletal 1	KRT1	1540	2002
8	Fibrinogen beta chain	FGB	1518	529
9	SH3 domain-binding glutamic acid-rich-like protein 2	SH3BGL2	1411	1946
10	Keratin, type I cytoskeletal 10	KRT10	1359	811
11	Serotransferrin	TF	1312	256
12	Fibrinogen gamma chain	FGB	1161	248

^[1]Data from supplementary Table 1 ref. [4]. Hemoglobin, the most abundant protein, is not shown, because it is an intrinsically intracellular protein.

proteins of which numerically 77% were of intracellular origin and only 9% (i.e. 247 proteins) from blood plasma [4] (see **Table 2**). The intracellular proteins of the implantome originated from the bone, the bone marrow, the blood cell and the plasma proteomes respectively. Surprisingly the most abundant implant protein in the implantome was hemoglobin (10%) and not serum albumin (5%). Fibrinogen and IgG were absent from the most abundant first 36 proteins (see also [14]). Thus the *in vitro* evidence for the doctrine *plasma proteins* forming the initial protein layer [25], did not "translate" into the human *in vivo* proteome. Thus an old paradigm failed, at least in hip implantology. Nevertheless, the sequence of events strongly suggests, that the implantome *per se* mediates fundamental cell-biomaterial interactions.

Screening through the *supplementary Table 1*, deposited with the paper in ref. [4], some other additional details emerged. The only proteins related to bone healing were BMP-1, a protease, and inhibitors of BMP-2 i.e. Chordin, and Gremlins 1 & 2. The mediators BMP-2, VEGF, TGF- β and TNF- α were not present in the implant proteome at all. The largest single family of proteins contained in the hip

implantome [4] was the zinc-finger protein family with over 120 different protein entities containing the three high abundance proteins ZNF35, ZNF470 and ZNF 850.

3.2.3 Evolution from the Primary Implantome to the Integratome in the Bone-Implant Interface

A first step has now been made in the pilot determination of the primary implantome on a hip implant after 2 min *in situ* [4]. This protein layer is a principle component of the first "cell-containing bone-implant interface" What do we know about the last stage, the bone-implant interface of integration (i.e. integratome). Usually it is equated with the bone-implant contact, i.e. BIC. The residual interface gap in the BIC region is so small, that cells can no longer enter, which results in a "cell-free bone-implant interface". This by no means indicates that an implant proteome is absent from this interface! What is also often forgotten is, that the BIC generally only covers 50-60% of the integrated bone-implant interface [34]. What about the other 40-50% of the non-BIC regions in this area? Are they part of implant integration and the integratome? Recently a comprehensive review of the largely submicroscopic evidence obtained for the bone-implant-interface in the BIC region of integration has appeared [3]. It is concluded that the bone-implant interface zone at this stage is primarily fibrillar with an electron dense 20-50 nm thick layer of collagen fibrils and an additional finely fibrillar mineralized matrix of ca. 200 nm thickness, i.e. in sum 250 nm in width (i.e. enough room for an implantome). Generally only four proteins are repeatedly named in the bone-implant-interface of an integrated implant: collagen, bone sialoprotein, osteopontin and osteocalcin.

Three major biological phenomena thus appear notable in periimplant healing: (i) a large, dynamic cell containing bone-implant interface measuring 20-100 μm in width scales down 100-400-fold (= compaction) during integration to a (ii) long-term constant, cell-free bone-implant interface with a width of ~200-500 nm (BIC) [3], characterized probably by a steady-state turnover of implantome proteins and (iii) to non-BIC marrow-type (?) spaces. From this large interface compaction it can be speculated, that there may be a parallel reduction in the number of proteins but not to a complete disappearance of the implantome *per se*.

Interestingly the importance of proteins in biocompatibility, and with that in implantology as a whole, is strongly disputed: "clinically, protein adsorption is of minor importance in biocompatibility pathways" [2], and the "adsorption of macromolecules has only minimal effects in biocompatibility" [2]. In addition it is stated in the same paper that surface modification technologies in implantology – with the possible exception of nanostructures – also only

play a minor role [2]. This reasoning insinuates that proteins neither play a significant role in biocompatibility nor in periimplant healing and osseointegration. At present this cannot be disproven, but it is at odds with reason and many literature findings. One important point appears to have been overlooked, namely, that not only the fact alone of obtaining implant biocompatibility and/or integration, but also the time-frame i.e. the *rates* of healing and osseointegration are of utmost importance, especially in connection with hospital costs. That is where the implantome protein composition may very effectively come in.

Conclusions

It is the aim and mission of implantomics to gain fundamental insights into proteome function by clarifying new pathways of implantome maturation from the primordial implantome over intermediary implantomes to the final integratome. It is essential to elucidate the role of implant-specific implantomes in biocompatibility and osseointegration as well as to employ this information for future artificial implantome designs. A high priority should also be given to diagnostics, for an understanding of the mechanisms of premature implant loosening and failures by generalized, systematic explantome research and analyses.

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Abbreviations:

BIC, bone implant contact; BMP-2, bone morphogenetic protein 2; LC-MS/MS, LC (Liquid Chromatography), MS (Mass Spectrometry), MS/MS tandem mass spectrometry; VEGF, vascular endothelial growth factor; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TPS, titanium plasma sprayed.

Author Statement

The Author states no conflict of interest.

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