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Biomolecular modification of implant surfaces

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In this review, surface modification of implant devices by immobilization of biological molecules is discussed. A brief introduction to the development of biomolecular surface science is presented, followed by a review of current activities in selected fields. Bone-contacting devices and some cardiovascular implant devices are reviewed as paradigmatic examples of research that is currently taking place. Advances in the basic fields of cell and tissue biology, in addition to concurrent developments in surface science tools, suggest that 'peri-implant biologics', or the control and direction of the host response at the implant–tissue interface by implant-surface-linked biomolecules, could be a major area of growth in the medical devices field in the next few years.

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Relevance of biomolecular modifications of implant device surfaces

Biomolecular surface modification involves processes aimed at the introduction of biological molecules on material and device surfaces. Through these approaches, researchers and medical device producers try to endow implant surfaces with the typical properties of biological molecules: biocompatibility (in a very broad sense); specific recognition and signaling; and bioactivity, that is, active involvement in and/or active stimulation of a given cell or tissue response. The ultimate goal is then the control and/or stimulation of specific mechanisms of response at the implant–host tissue interface.

Biomolecular modification of biomaterial surfaces has long been an intriguing field of activity in biomaterial surface science and a recognized tool in medical device technology. For example, enhanced resistance to blood coagulation through specific interactions at the blood–material interface can be obtained by heparin coatings on blood contacting devices. A number of heparin coatings have been on the market for several decades. Biomolecular approaches to surface modification are of special importance today owing to the explosion of knowledge at the basic level of cell and tissue biology; the enhanced capabilities of analytical and synthetic surface science; and the present market situation where innovation is crucial and often highly rewarding.

From a historical point of view, and without forgetting the contribution of significant pioneering work [1–3], the late 1980s and the first half of the 1990s can be considered the period when early biomolecular surface studies coagulated into a comprehensive and self-standing field of investigation.

The main events that promoted this transition can be tracked at the basic level of scientific studies and in the market, as follows:

- The recognition of the role played by biological molecules immobilized on materials and device surfaces in directing interactions at the host–tissue interface. Molecules involved ranged from polysaccharides (heparin and hyaluronan [HA]) [4–6], to proteins (collagen and, albumin) [7–9], to phospholipid components (phosphorhylcholine) [10];
- The development and commercialization of medical devices bearing advanced, surface-engineered heparin coatings [11,12].

Biomolecular modifications of biomaterial surfaces definitely came of age in the 1990s with studies by Massia and Hubbel on surface-linked

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cell-adhesive peptides [13]. It was found that synthetic peptides that contain the amino acids arginine-glycine-aspartic acid (RGD) can mimic cell attachment activity of the parental molecule [14,15]. Linking of peptides to material surfaces has since become a preferred way to enhance cell interactions with biomaterials. Two lively editorials (and much work) by Ratner contributed to the foundation of a burgeoning field of investigation that involves definitions, such as biomimetic surfaces, bioactive surfaces, biomolecular surfaces and biological surfaces [16,17]. Among the thousands of excellent activities, meetings and publications dealing with these topics it is worthy to recall the review paper by B Kasemo entitled 'Biological Surface Science' [18], the monographic issue of the American Chemical Society journal *Langmuir* on the biomolecular interface [19] and the institution of Gordon Research Conference on Biointerface Science [101].

The focus of this review is on biomolecular modification of implant device surfaces (BMIDS). TABLE 1 provides a summary of the primary examples of studies and applications. Owing to the size of the field, extending the discussion to nonimplantable devices and materials, such as plasticware for cell culture, diagnostic, external assist devices and circuitry would make the subject unmanageable. The examples presented in the next section demonstrate the increasing role that BMIDS are playing in the field of medical device technology. Moreover, they are well suited to set the stage for further discussion in the following sections.

Leading examples: titanium dental implants & bone-contacting devices

Titanium (Ti) is the material of choice for load-bearing, bone-contacting applications since the pioneering work of Branemark [20,21]. In the dental field, Ti implants are used widely for a variety of indications. Interfacial interactions at the bone–implant interface are recognized as the key to osseointegration. A vast amount of literature on Ti surfaces and interfaces exists [21–24].

The need to address difficult clinical settings, for example, an intended implant site showing low bone density, low vascularity or insufficient quantity of bone (in terms of the width of the alveolar ridge); the quest for early or immediate loading of the device; and the increasing needs of an aging population, are still spurring an intense activity of research on novel and better approaches to the surface modification of Ti dental implants. To date, most surface modification approaches have been aimed at the control of surface topography, trying to exploit surface roughness-dependent effects on osteogenic cells and on platelet activation [25,26]. FIGURE 1 is a typical example of a recent approach to modification of the topography of Ti surfaces to control cell behavior: the scanning electron microscope image shows an osteoblast-like $SaOS_2$ cell grown in culture on a nanoporous surface obtained by electrochemical treatment [23,27]. The cell philopodia are actively exploring the surface, the insets show some of them penetrating the hundreds of nanometer-wide holes on the surface. Dental implants bearing nano- and microporous surface topography are currently on the market.

The stimulation of cells by surface topography has been, and currently is, the main tool available for dental implant producers. However, BMIDS could initiate a major revolution in the field [28]. Hammerle wrote recently: "Another fascinating area of development is the possibility of binding bone-stimulating agents to implant surfaces. Increased understanding of the function of growth factors and extracellular matrix (ECM) proteins regarding the recruitment, attachment, proliferation and

Table 1. Examples of biomolecular modifications of implant device surfaces.

Device	Biomolecule	Rationale
Vascular prostheses	Heparin albumin	Prevention of blood clotting
	Phosphorylcholine	Prevention of blood clotting and decrease of nonspecific adsorption
	Peptides and receptors for progenitor cells	Increased rate of endothelialization
Intraocular lenses	Heparin and hyaluronan	Fouling resistance and heparin decreases complement activation
	Phosphorylcholine	Fouling resistance
Soft-tissue prostheses	Collagen and hyaluronan	Decrease of fybrosis, prevention of postsurgery adhesion
	Peptides	Increased cell adhesion
Vascular stents	Heparin and phosphorylcholine	Prevention of blood clotting
	Hyaluronan and fibrin	Prevention of blood clotting and stimulation of healing
	Peptides and receptors for progenitor cells	Increased rate of endothelialization
Orthopedic devices	Peptides, collagen, other extracellular matrix proteins, growth factors and glycosaminoglycans	Stimulation of osteointegration

Reports work involving surface immobilization, it does not consider the local release of bioactive molecules. References to specific applications can be found in the text.

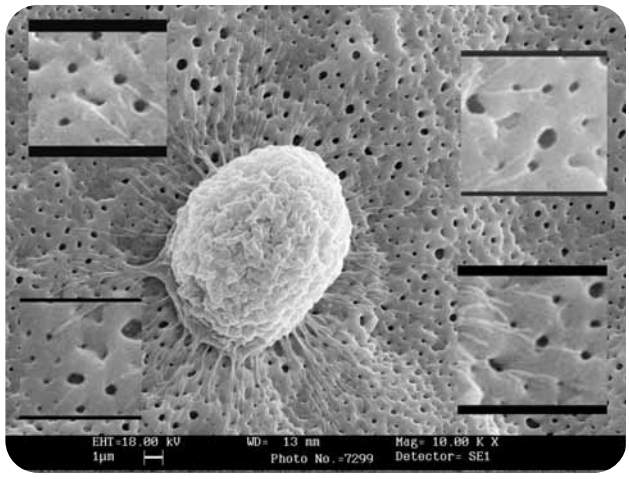

Figure 1. Example of currently adopted modifications of surface topography of titanium implants to control cell behavior. The nanoporous surface topography was obtained by electrochemical treatment. Note the pore diameter below 1 µm, and, in the insets, cell philopodia explorating the surface porosity.
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differentiation of osteoblasts has led to extensive research in this field … In summary, the topographic design of implant surfaces has reached a high degree of sophistication. Additional physical modifications, such as increasing the surface energy of the implant, can probably further improve osseointegration. Major progress, however, is expected to occur when surfaces with bone-stimulating agents become available" [29].

Studies in biomolecular modification of dental implants (and of bone-contacting Ti implant devices in general) have been aimed at surface linking of components of the ECM (it must be underlined that the delivery or local application of nonsurface-linked growth factors or bone morphogenetic proteins [BMPs], although investigated widely and undoubtedly interesting, do not fall within the realm of surface modification of devices). This has been achieved by the surface linking, either of short amino acid sequences (peptides), part of an ECM protein or of whole ECM proteins. The underlying strategy is to promote adhesion and differentiation of osteogenic cells on the implant surface to impart, to the bioinert Ti surface, either or both osteoconductive and osteoinductive properties. The scientific literature in this field has recently been reviewed [28]. Concerning adhesive peptides, this line of research stems from the already quoted fundamental findings by Pierschbacher and Ruoslahti [14,15], and Massia and Hubbell [13]. In essence, a heterodimeric cell membrane receptor family, known as integrins, is involved in cell adhesion to ECM proteins [30]. Integrins interact with short amino acid sequences within ECM molecules. In particular, the sequence RGD has been identified as mediating attachment of cells to several plasma and ECM proteins, including fibronectin, vitronectin, type I collagen, osteopontin and bone sialoprotein [31]. Cellular recognition of simple surface-linked peptides suggests their potential usefulness of conveying particular cell adhesion properties, thus, enhancing cell–material interactions. Cell-specific peptide sequences could, in principle, lead to cell-selective surfaces [32].

The second widely investigated approach involves coating of Ti surfaces by whole ECM proteins. Laminin, fibronectin [33–35], bone sialoprotein [36] and collagen [28] have been investigated in their relationship with adhesion and differentiation of cells involved in interfacial interactions with bone implants. Concerning the most widely investigated ECM protein (collagen), it plays an important role in osteoblast cell behavior [30], promoting not only cell adhesion but also osteoblastic differentiation of bone marrow cells and controlling a number of aspects of their progression along the osteogenic pathway [37–39]. Thus, the aim of BMIDS by collagen is to recruit more osteogenic cell precursors and/or provide a more favorable peri-implant environment for osteogenic cell differentiation following instructions from cell–matrix interactions and from diffusible molecules.

The quoted review presents a more in-depth discussion of BMIDS of Ti implant devices, including *in vitro* and surface chemistry aspects [28]. For the scope of the present review it is of interest to consider briefly some of the most relevant *in vivo* results: Ferris and colleagues presented the first *in vivo* study demonstrating evidence of increased bone formation by RGD-coated implants [40]. The study was designed to evaluate the quality and quantity of the new bone formed in response to Ti rods coated with the peptide sequence arginine-glycine-aspartic acid-cysteine (RGDC) in rat femurs. The peptide was immobilized to the surface using the chemistry commonly adopted for self-assembled monolayers: smooth Ti samples were gold coated and RGDC immobilized using gold–thiol chemistry in water–alcohol solutions. Histomorphometric analysis demonstrated a significantly thicker shell of new bone formed around RGD-modified versus plain implants at 2 and 4 weeks. Mechanical pull-out testing conducted at 4 weeks revealed that the average interfacial shear strength of peptide modified rods was greater than control rods, although this difference was not statistically significant.

New bone formation in a porous Ti fiber mesh implant, coated with a cyclic RGD peptide containing a phosphonate anchor was evaluated by Kroese-Deutamn and colleagues [41]. The Ti meshes were soaked in the coating solution and the peptide was allowed to immobilize overnight. The RGD–Ti implants were inserted into the cranium of a rabbit and were compared with porous Ti fiber mesh disks without RGD sequence and with an open control defect. Histologic and histomorphometric examinations were performed 2, 4 and 8 weeks postoperatively. A significant increase in bone formation, or bone ingrowth, was detected in the RGD–Ti group compared with the Ti group after 4 and 8 weeks.

In addition, studies by Elmengaard and colleagues, involving implants in the tibia of mongrel dogs, demonstrated significant improvement in terms of increased bone ingrowth for RGD-coated implants over uncoated controls [42,43].

Using a model more directly related to dental implants, Schliephake and colleagues evaluated peri-implant bone formation around a Ti screw implanted in dog mandibles [44]. Implants had either a turned machined Ti surface or they

were coated with collagen type I to which cyclic RGD was linked at high and low concentrations, as determined by the peptide concentration in the coating solution. Peri-implant bone regeneration was assessed histomorphometrically after 1 and 3 months in five dogs by measuring bone implant contact and the volume density of the newly formed peri-implant bone. After 1 month, bone implant contact was significantly enhanced in the group of implants coated with the higher concentration of RGD peptides compared with Ti surface; however, no significant difference was detected between the groups with organic coating (collagen and RGD low and high). Volume density of the newly formed peri-implant bone was significantly higher in all implants with organic coating, again no significant difference was found between the collagen coating and RGD coatings. After 3 months, all implants with organic coatings gave better results than uncoated implants without showing differences between the three different groups, that is, type I collagen (that *per se* contains RGD) performed without a statistically significant difference compared with RGD coatings.

Another interesting comparison between RGD and collagen coatings on Ti implants has been presented by Bernhardt and colleagues [45]. Ti implants coated with collagen type I, type III or RGD peptide were placed in the femur of goats, together with an uncoated reference. Collagen coatings were performed after allowing overnight fibrillar assembly. Bone contact and volume were determined 5 and 12 weeks postimplantation using histomorphometry and synchrotron radiation micro computed tomography. Both methods revealed similar tendencies in bone formation for the differently biofunctionalized implants: after 5 and 12 weeks, all three coatings demonstrated a significant increase in bone volume over the uncoated reference, with the highest results for the collagen coatings, while the RGD coating demonstrated only a slight improvement compared with the reference material.

Studies based on collagen are a good example to underline a peculiar aspect of this field, often overlooked in published papers. The first full paper providing *in vivo* evidence of enhanced osseointegration by surface modification of Ti implants by collagen was published in 2003 [46]. The paper presents surface characterization and *in vitro* and *in vivo* (4 weeks in rabbit femur) data concerning type I collagen covalently linked to a Ti surface-grafted polyacrylic acid layer. A number of *in vivo* studies on collagen-coated Ti implants have since been published [44,45,47–50], showing enhanced performances or no significant effect [50] (reviewed in [28], the same inconsistent results are reported in *in vitro* studies). It is important to stress here that, as for any other surface modification process involving immobilization of biomolecules, no 'general' collagen-coated surface exists. Rather, very relevant details concerning molecular behavior, such as surface density, stability in aqueous media, resistance to hydrolysis and degradation, conformational freedom, presentation of active epititopes are directly related to the way the biomolecule is linked to the surface, that is, to the specific surface-engineering process adopted. Detailed surface analysis, including a number of different and complementary surface sensitive tools, should be a mandatory component of every study on BMIDS [28,51].

Another point to highlight is that the rationale behind studies presented to date is the effect on cell adhesion and/or differentiation, that is, they are based on the cell-adhesion and growth paradigm [52]. As a consequence, the biomolecules involved are proteins or their components, in particular those that are involved in cell adhesion. Present research on biomaterial science underlines the role of the whole process of wound healing in implant integration [53]. In this respect, it is of interest to quote a couple of very recent studies that extend the breadth of biomolecules of interest to ECM glycosaminoglycans (GAGs). In particular, Rammelt and colleagues have recently demonstrated that a GAG, chondroitin sulphate (CS), coadsorbed with collagen, enhances the effects on bone remodeling at peri-implant interfaces, underlying the role that ECM GAGs can play in osseointegration [54,55]. In particular, Ti pins were coated with type I collagen, RGD peptide or type I collagen and CS, while uncoated pins (Ti) served as controls [55]. The pins were inserted as intramedullary nails into the tibia of male adult Wistar rats. In agreement with previous *in vitro* findings on the effect on bone cells [56], results indicate significantly increased bone formation with respect to total bone contact around Ti implants coated with the artificial ECM consisting of collagen and CS. Some interesting speculations are presented to account for the results observed, ranging from enhancement of interfacial interactions with growth factors and cytokines to surface charge effects.

A study from our group has shown that another GAG, HA, covalently linked to the surface of Ti implants, induces a significant increase in peri-implant bone formation in a 4-week rabbit model of femur implants [57]. GAGs are very hydrophilic and water soluble, and, as discussed above, surface immobilization plays a key role in determining interfacial presentation of the biomolecule [51]. In the quoted study, HA was covalently linked to surface amino groups introduced on the Ti surface by glow-discharge plasma. Results demonstrate, in both major bone macroarchitectures (cortical and trabecular), that covalently linked HA stimulates bone regeneration at the implant interface, at least in the animal model tested. FIGURE 2 demonstrates histological sections of control (A) and HA-coated (B) implants in trabecular bone. A very significant increase of the percentage of interface covered by bone in HA-coated samples is observed. As reported in the quoted paper, improvements of histomorphometry parameters were significant ($p < 0.05$) in cortical and very significant ($p < 0.01$) in trabecular bone. As in the case of collagen–CS coatings [55], the complexity of GAG interactions with ECM components and with cytokines and growth factors expressed in wound healing does not permit firm conclusions on the underlying mechanisms to be drawn, but suggests some speculation based on the literature [57]. In particular, since the 1950s it has been established that considerable HA is synthesized in the early stages of callus formation during the repair of fractured long bones [58]. HA is a prominent ECM component during bone morphogenesis [59,60] and it is found

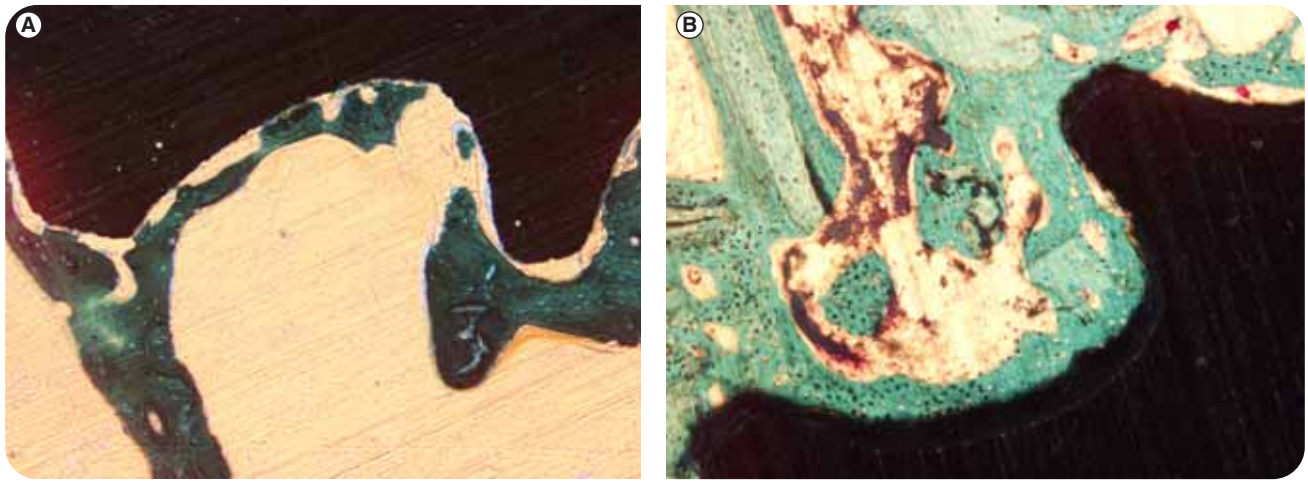

Figure 2. Examples of histological sections obtained from 4-week implants in rabbit femur trabecular bone (×10). (A) Control titanium implant; (B) hyaluronan-coated titanium implant. Note the significant increase of the interfacial interaction between the implant devices and new bone tissue (complete data are reported in [57]).

whenever there is a need for rapid cell proliferation, repair and regeneration. During an early stage of osteogenesis, when only undifferentiated mesenchymal cells are found, HA reaches peak levels [61]. According to Bernard, "In terms of its correlation with wound healing mechanisms and hard tissue development, HA can be thought as a 'primer' in cell regeneration" [62]. Moreover, Zou and colleagues reported that 800 kDa HA added to bone marrow stromal cells cultured *in vitro* accelerates cell proliferation, increases alkaline phosphatase activity and osteocalcin gene expression, and that HA interacts with BMP-2 to generate direct and specific cellular effects [63]. Ito and colleagues have shown that locally applied 900 kDa HA has a positive effect in bone ingrowth in Ti fiber mesh implant in rats [64]. According to Cho, HA shows a positive effect in early bone consolidation in distraction osteogenesis [65]. It is then possible to speculate that the interfacial HA-rich layer creates an environment conducive to interfacial cell migration, in line with the biological role of HA in bone morphogenesis [61,62].

In summary, studies on biomolecular modification of Ti bone-contacting and, in particular, dental implants, highlight many of the typical aspects of BMIDS: interfacial performances of a bioinert device can be upgraded, drawing guiding principles from the growing body of knowledge on ECM molecules and on wound-healing mechanisms. Results are sometime contradictory; improvements are found in some instances and not found in other works on the same molecule. Very different approaches to surface modification by the same molecule are presented, often proper surface characterization is lacking. Finally, explanations of a given behavior are often based on speculations owing to the intrinsic complexity of the field.

Biomolecular strategies in the current evolution of vascular implants

The previous discussion on bone-contacting devices shares a number of features with studies taking place in other fields of biomedical materials and devices. Researchers in cardiovascular implant materials, for instance, have long been involved with studies aimed at promoting endothelialization of artificial vascular grafts, as a preferred way to impart to synthetic graft materials the biocompatibility and function of natural vessels. Here the material of choice is polymeric, that is, expanded polytetrafluoroethylene (ePTFE). The problem related to the use of ePTFE is suboptimal resistance to clot formation and intimal hyperplasia (IH), that is, growth of cellular tissue that decreases lumen patency. IH formation is thought to reflect an accumulation of several separate entities, including inflammatory, coagulatory and hemodynamic factors. The lack of a functional endothelial monolayer on the prosthetic graft is critical both for coagulation and inflammation, because the endothelium constitutes the first-line homeostatic defense mechanism by exerting anticoagulatory and anti-inflammatory effects. Endothelial cell (EC) seeding at the luminal surface of prosthetic vascular grafts is a valuable strategy to improve graft patency, and biomolecular approaches to endothelialization are actively being investigated.

A number of common features can be found between the path of evolution of ePTFE grafts and the previously reported developments of Ti bone-contacting devices. Control of surface topography, in terms of porosity, has been investigated widely [66], followed by nonspecific surface treatments, such as glow-discharge treatments already used to promote cell adhesion to plastics [67]. Attempts to promote endothelialization, both in terms of cell adhesion and cell function, by specific biochemical cues linked to the bioinert ePTFE surface, have been pursued. A comprehensive review of early studies on endothelialization-promoting surface modifications of ePTFE grafts can be found [68]. Biomimetic approaches based on biomolecular modifications of synthetic graft materials are presently being actively investigated. As an example, Li and colleagues have presented an interesting *in vitro* and *in vivo* study on ePTFE vascular grafts treated with P15 peptide [69]. P15 peptide is the 15 amino acid sequence glycine-threonine-proline-glycine-proline-glutamine-glycine-isoleucine-alanine-glycine-glutamine-arginine-glycine-

valine-valine (GTPGPQGIAGQRGVV) identified as the cell-adhesion domain within type I collagen. P15 and MAP4 peptides, a four branched peptide where each arm bears a P15 sequence, were covalently bonded onto ePTFE grafts. MAP4 peptides are then much larger than the short amino acid sequences commonly investigated (RGD or similar) and, as discussed in the quoted papers, the large molecular size should help the peptide to provide suitable molecular conformation for approaching cells and the multiple cell-adhesion domains should improve cell attachment, adhesion and proliferation. Results of *in vitro* studies show a much higher adhesion and proliferation of ECs on peptide coated ePTFE compared with the uncoated control. This is not a particularly difficult task since hydrophobic ePTFE is a notoriously bad substrate for cell adhesion. More interestingly, *in vivo* studies in sheep models were conducted by placing four P15-treated grafts and two controls as arteriovenous grafts between the femoral artery and vein or the carotid artery and jugular vein in two sheep [70]. The amount of neointimal hyperplasia present at the arterial and venous sides of the anastomosis and the degree of endothelialization on the luminal surface of the grafts were assessed. Results demonstrated that the thickness of the neointimal hyperplasia of untreated grafts was three-times that of P15-treated grafts ($p < 0.05$) at the venous side of the anastomosis. P15-treated grafts also had a higher degree of endothelialization on the graft lumen.

While the previous study is a good example of biomolecular modifications of vascular implant devices by engineered peptides, another interesting area of study involves the capture of relevant circulating cells. The underlying strategy is to promote homing of cells onto the material surface via presentation of antibodies to specific cell surface receptors, mimicking approaches used already in affinity chromatography. Interest is focused on bone marrow-derived endothelial progenitor cells (EPCs) that have emerged as a promising source of autologous ECs. EPCs are a subset of $CD34^+$ cells with the potential to proliferate and differentiate into mature ECs [71]. EPCs have the capacity to home to sites of vascular injury, thus promoting the process of re-endothelialization. Animal studies have shown that *in vitro* seeding of prosthetic vascular grafts with $CD34^+$ cells markedly increases graft endothelialization in animal models [72–75]. Surface linking of specific antibodies can be obtained by typical techniques of surface engineering: surface functionalization or the introduction of specific chemical groups to the synthetic material surface; linking of a spacer or a molecule (often a hydrophilic polysaccharide that minimizes nonspecific adsorption of proteins); and surface coupling of antibodies at a given surface density.

A recent study evaluated the feasibility of capturing EPCs *in vivo* using immobilized anti-CD34 antibodies on ePTFE grafts [76]. The underlying hypothesis is that *in vivo* 'autoseeding' with immobilized anti-CD34 antibodies establishes a confluent, mature EC monolayer that may attenuate IH in the outflow tract of AV ePTFE grafts. Anti-CD34-coated ePTFE grafts were implanted between the carotid artery and internal jugular vein of 11 pigs. Bare ePTFE grafts were implanted at the contralateral side. Animals were sacrificed at selected time intervals and grafts were processed for histology and scanning electron microscopy analysis. At 3 and 28 days after implantation, 95 and 85% of the coated graft surface was covered by ECs. By contrast, no cell coverage was observed in the bare graft at 3 days, whereas at 28 days, bare grafts were partly covered with ECs (32%). These results indicate a significant increase of endothelialization on CD34-coated grafts. However, it must be noted that, speaking strictly from a physicochemical point of view, it is not possible to conclude whether the observed effect is indeed due to specific receptor–antibody interactions or to nonspecific improvement of cell adhesion to the ePTFE-treated (to link CD34 antibodies) surfaces.

It is a common finding of early biomaterial surface science that cell adhesion is unspecifically increased on hydrophilic compared with hydrophobic surfaces [77]. Another significant and noteworthy result of this study is that, despite improved endothelialization, IH at the venous anastomosis was strongly increased in anti-CD34-coated grafts compared with bare grafts. This increase in IH coincided with enhanced cellular proliferation at the venous anastomosis. A number of hypotheses are suggested to account for this behavior; it is of interest to quote them because they underline many challenges in this field: first, vascular smooth muscle cells (VSMCs), that is, cells involved in IH, are $CD34^+$ and they can originate from bone marrow-derived $CD34^+$ progenitor cells, implying that differentiation of captured cells into VSMCs can contribute to increased IH. Second, EPCs have the capacity to release potent proangiogenic growth factors, such as vascular endothelial growth factor, and hepatocyte growth factor that can also stimulate VSMC migration. Additionall, and very important from the point of view of surface engineering, interaction of ECs with subendothelial matrix components has been shown to be of pivotal importance for the protective function of ECs; the absence of a physiological microenvironment may also contribute to the apparent lack of protective effects. Furthermore, the turbulent flow pattern in AV grafts may have deteriorated differentiation into ECs, especially in the anastomotic region. Finally, adequate maturation of captured $CD34^+$ cells may have been hampered by the binding of the immobilized antibody to the CD34 epitope, that is, captured cells were unable to regain their functional capabilities. The reading of the comments and author's reply to the quoted paper can add further clues [78].

The topic of BMIDS to promote capture through CD34 antibodies of EPCs brings us naturally to a very interesting and active field of investigation: coronary stents in general and, in particular, drug-eluting stents (DES). DES were introduced on the market in the late 1990s and demonstrated significant improvement in the control of IH in a number of well-documented studies. It is of interest here to recall that DES, and the need for drug delivery, can be considered as evidence of failure of surface modification strategies to control cell and tissue response, at least for this specific application. Williams, an advocate of bioinert devices and surfaces, considers DES the paradigmatic example of adverse effects due to the lack of noninert implant devices [79].

Polymeric carriers are used commonly in commercially available DES but, according to several research reports, they promote severe inflammation in the vessel wall, inducing significant side effects and hypersensitivity [80]. This often requires an increase of drug dosage to overcome carrier-related inflammation, leading, however, to unwanted drug-related side effects. FIGURE 3A shows a typical example of side effects due to the exaggerated release of a cytotoxic drug from a DES. In this 28-day pig coronary model experiment, hemorrages due to impaired endothelialization of the lumen side of stent struts can be seen. They are the result of indiscriminate killing, by the drug, of peri-implant cells and of the ensuing impossibility of active healing; inflammation is also present. By contrast, FIGURE 3B shows a nicely endothelialized stent, in the same animal model. After initial studies that were primarily concerned with DES efficacy, today's research is strongly aimed towards reducing DES-related risks and at decreasing the amount of carrier and drug. In addition, the debate on DES safety is ongoing [81,82].

DES are complex implant devices that involve a number of disciplines and expertises, as reported in FIGURE 4. In general, the sustained release of a drug from a DES requires tight control of the polymeric carrier chemistry and properties. Bulk modification of polymeric carriers to improve biocompatibility would alter the kinetics of release. BMIDS, in turn, can increase the biocompatibility of drug carrier surfaces by modifications involving just the first few nanometers – without affecting the bulk properties of the carrier, and hence the drug release kinetics. Immobilization of naturally occurring biological molecules to drug carrier surfaces can improve the biological acceptance of synthetic polymers via the presentation of a more natural interface to the surrounding tissue. BMDIS can, thus, lead to multifunctional drug carriers that control drug release kinetics and also take care of interactions with interfacing tissue. As an example, the already mentioned HA, has been suggested as a natural aid against restenosis since it exerts a selective cytostatic action on VSMC by saturation/inhibition of membrane-specific migration (receptor for HA-mediated mobility) and proliferative receptors (CD44) [83,84]. HA-modified stents have been investigated *in vitro* and *in vivo* [85–87]. A recent report evaluated the surface modification of the synthetic carrier of a DES by covalent linking of HA in a 28-day model of pig coronary implants, demonstrating improvements in several inflammation-related parameters compared with controls [87].

Another very interesting example of biomolecular modifications of vascular stents is provided by the Genous™ R Stent developed by Orbus Medical Technologies [102]. This device exploits the same anti-CD34 coating technology described previously to capture EPCs from the blood flow and to promote enhanced endothelialization. The Genous R stent underwent extensive *in vitro* and *in vivo* characterization in animal models and it is used currently in clinical trials.

Expert commentary

BMIDS is a very active field of research. Since the quoted pioneering work, and its actual foundation almost 30 years ago, the area matured in a very rich multidisciplinary scientific effort. More and more convincing evidence exists, as reported in the literature, on the potential of stimulating peri-implant healing mechanisms by surface-linked biomolecules. While the scenario of the probable evolution of this field will be tackled in the next section, it is noteworthy here to underline a few points.

In the beginning, studies were mostly concerned with promoting cell adhesion to biomaterial surfaces but the target is now expanding. Peptides and proteins are still by far the most investigated class of biomolecules but many studies target selectivity and differentiation of (stem and mesenchymal) cells. Moreover, hydrophilic GAGs that, *per se*, do not support cell

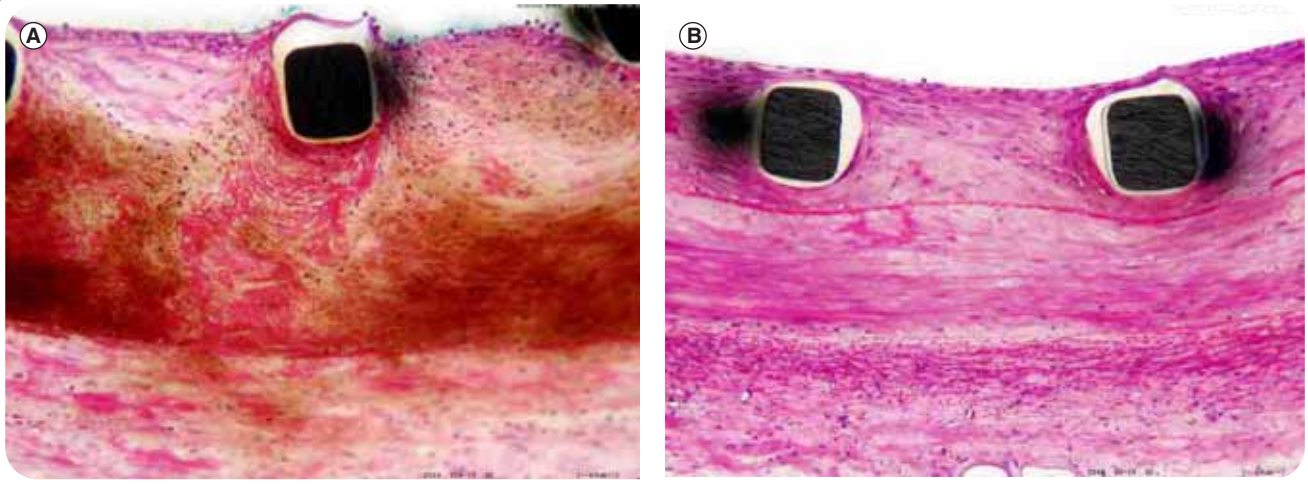

Figure 3. Examples of histological sections obtained from 28-day drug-eluting stent implants in a coronary pig model. The DES releases a cytotoxic antiproliferative drug. (A) Evidence of adverse effects due to drug overdosage: hemorrage and impaired endothelialization. (B) Correct drug dosage, stent struts are completely endothelialized, no major evidence of neointima proliferation is detected.

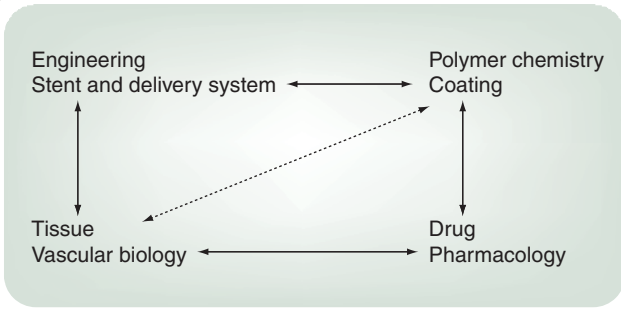

Figure 4. Relationships among the different disciplines involved in drug-eluting stent development. As discussed in the text, fine tuning of the drug carrier surface properties by biomolecular modifications can optimize interfacial interactions with the host tissue (dotted arrow) without affecting the kinetics of drug release that is controlled by the carrier bulk properties. Adapted from Hossainy, Drug eluting stent summit III, TCT 2004.

adhesion when covalently linked to biomaterial surfaces [88], have been successfully tested on bone-contacting devices [54–57]. A recent paper by Giachelli is a very good example of a more sophisticated approach to cell signaling by biomolecular modification of biomaterial surfaces [89].

From the practical viewpoint of actual exploitation of BMIDS, concerns exist on the effect of sterilization on molecular structure and function. As already reported, an increasing number of papers describe the method used for sample sterilization and concerns are now partially relieved [28]. On the other hand, very few, if any, data exist on another major point related to market exploitation, that is, shelf life and storage conditions of devices bearing engineered surfaces. This topic is not typical of scientific papers; more clues will probably arise from technical files of devices submitted for regulatory approval.

Finally, a most relevant point in BMIDS is the very strict relationship between advancements in basic cell and tissue biology, interfacial biochemistry and surface science. The understanding of healing mechanisms and the understanding of the biological behavior of surface linked biomolecules are central to the development of BMIDS. To quote an example, the previously quoted HA shows molecular weight-dependent biological effects, oligomers often having completely different properties compared with high-molecular-weight chains [84]. It is not yet clear how the process of surface immobilization, and the concurrent decrease of the effective molecular weight of conformationally free sequences of repeating units, will affect the interfacial properties of surface-linked HA [51]. The clarification of this and a number of other topics related to the behavior of biomolecules at interfaces are actively pursued as reported in the introductory section [19,20], and significant fall-out effects are expected in the field of BMIDS.

Five-year view

Considerations on the evolution, in the next 5 years, of the field of BMIDS, must take into account the huge basic and applied research effort in this area: today, and since a few years ago, every regional, national or continental program of support to research contains a section on biomimetic or bioactive surfaces. In consequence, data and knowledge are constantly growing, and many researchers are working on BMIDS. It is highly probable that from this body of knowledge a new generation of biomolecular surface-engineered medical devices will arise shortly, at least in selected fields. As reviewed above, vascular stents able to capture EPCs are already in advanced clinical trials. Polymeric drug carriers for coronary and peripheral stents can now couple the control of the kinetics of release to the presentation of biomolecular cues to the tissue interface. Concerns related to the long-term safety of current DES are indeed reopening a market space for tissue-friendly stents with engineered surfaces.

Bone-contacting, dental and orthopedic implants are especially expected to undergo major changes in the next few years: programs involving CE marking of dental implants having biomolecular surfaces are underway. Extended clinical trials will give a full understanding of the benefits and potential, and could trigger a cascade effect in the related (and much bigger) market of orthopedic and spine devices. It is not difficult here to detect significant analogy with the field of orthopedic devices at large: more and more companies are currently involved with orthobiologics, or the exploitation of biological molecules in orthopedic procedures [90]. To quote a couple of examples, Infuse® is a collagen sponge that, loaded with recombinant BMP-2, substitutes autologous bone as filling material in Ti cages used for spinal fusion (US FDA approved in July 2002 and launched in Europe in 2003 as InductOs™). Ossigel is an injectable product used to accelerate the healing of fractures, which exploits the beneficial effects of HA on bone regeneration and basic fibroblast growth factor, which stimulates the proliferation of cells necessary for vascularization and bone growth [90]. The studies reviewed in the section on bone-contacting devices clearly indicate peri-implant orthobiologics, where BMIDS-enhanced bone formation at the implant–tissue interface can be the key to solve some of the remaining problems of dental and orthopedic implants, and possibly open new areas of indications. Furthermore, following previous considerations, 'peri-implant biologics', based on the control and guidance of tissue healing by BMIDS, could be a very significant area of growth of medical devices in the next few years.

Another major development will involve the nature and source of surface-linked bioactive molecules. As recently reviewed, a number of complex plant polysaccharides exist, which display, for example, anti-inflammatory or antitumor activity, or specific effects on cultured mammalian cells [91]. Advancements in separation and purification of complex plant polysaccharides, such as pectins are paving the way to a conscious exploitation of some of these properties. A multidisciplinary project founded by the European Commission is already underway, involving experts ranging from plant biology to purification and manufacturing of tailored polysaccharides, to surface modification and characterization of biomaterials, to

in vitro and *in vivo* biocompatibility assessment, to medical device manufacturing [103]. Reports on cell response to surface-linked pectin components have recently been published [91–93]. Cell–cell interactions rely on displayed carbohydrate moieties and on sugar–epitope-specific binding proteins. Specific sugar epitopes are thus the connecting link between the world of plant carbohydrate and mammalian cells. Understanding the sugar language and transferring it on top of medical devices is the goal of researches in this area. It is noteworthy to recall that, once the sugar language is properly understood, carbohydrate engineering in plants could offer unprecedented opportunities [103].

Finally, it is true that traditional permanent devices will be increasingly replaced by biodegradable materials. Even in the latter case, however, stimulation of natural healing mechanisms by biomolecules linked to the surface is an important asset [94].

Conflict of interest

The author is the cofounder, director and owns shares in Nobil Bio Ricerche srl (www.nobilbio.it), a company involved in the surface modification of medical devices. He is currently involved in programs on the biomolecular modification of bone-contacting and cardiovascular device surfaces.

Key issues

- Advancements in cell and tissue biology, coupled with advancements in synthetic and analytical surface science, are opening the way to the conscious exploitation of surface-linked biomolecules at the implanted device–host tissue interface.
- The field of biomolecular modifications of implant device surfaces, encompassing definitions such as biomimetic surfaces, biological surfaces, bioactive surfaces and biomolecular surfaces is currently among the most actively investigated areas of biomedical devices science and technology. A critical mass of knowledge is rapidly accumulating.
- Beside historical examples of heparin-coated surfaces, new biomolecular surface-engineered implants have reached or are reaching clinical studies or the market stage. Vascular devices surface-engineered to capture endothelial progenitor cells and stimulate endothelialization are tested in animal models and clinical studies; advanced programs are underway towards second-generation drug-eluting stent carriers, coupling drug release with the presentation of biological molecules to the host-tissue interface. Studies on dental and orthopedic implants are pointing towards peri-implant orthobiologics as the most rapidly evolving area of the more general field of peri-implant biologics.
- Limited published data exist on relevant issues, such as resistance to sterilization, and, most of all, the shelf life of biomolecular surface-engineered implant devices.

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