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ORIGINAL ARTICLE

Activation of Polymorphonuclear Leukocytes by Candidate Biomaterials for an Implantable Glucose Sensor

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Abstract

Background:

Continuous monitoring of glucose by implantable microfabricated devices offers key advantages over current transcutaneous glucose sensors that limit usability due to their obtrusive nature and risk of infection. A successful sensory implant should be biocompatible and retain long-lasting function. Polymorphonuclear leukocytes (PMN) play a key role in the inflammatory system by releasing enzymes, cytokines, and reactive oxygen species, typically as a response to complement activation. The aim of this study was to perform an *in vitro* analysis of PMN activation as a marker for biocompatibility of materials and to evaluate the role of complement in the activation of PMN.

Methods:

Fifteen candidate materials of an implantable glucose sensor were incubated in lepirudin-anticoagulated whole blood. The cluster of differentiation molecule 11b (CD11b) expression on PMN was analyzed with flow cytometry and the myeloperoxidase (MPO) concentration in plasma was analyzed with enzyme-linked immunosorbent assay. Complement activation was prevented by the C3 inhibitor compstatin or the C5 inhibitor eculizumab.

Results:

Three of the biomaterials (cellulose ester, polyamide reverse osmosis membrane, and polyamide thin film membrane), all belonging to the membrane group, induced a substantial and significant increase in CD11b expression and MPO release. The changes were virtually identical for these two markers. Inhibition of complement with compstatin or eculizumab reduced the CD11b expression and MPO release dose dependently and in most cases back to baseline. The other 12 materials did not induce significant PMN activation.

Conclusion:

Three of the 15 candidate materials triggered PMN activation in a complement-dependent manner and should therefore be avoided for implementation in implantable microsensors.

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Abbreviations: (A2020) araldite 2020, (AAO) aluminium oxide, (ANOVA) analysis of variance, (CD11b) cluster of differentiation molecule 11b, (CM) cellulose ester, (CT) ceramTec GC, (ConA) concanavalin A, (DP) DuPont 951, (ETek) epo-Tek 353ND, (ISF) interstitial fluid, (LTCC) low-temperature cofired ceramic, (Me) stainless steel, (MFI) mean fluorescence intensity, (MPO) myeloperoxidase, (MWCO) molecular weight cut off, (PAR) polyamide reverse osmosis membrane, (PATF) polyamide thin film membrane, (PBS) phosphate buffer saline, (PC) polycarbonate, (PDMS) polydimethylsiloxane, (PMN) polymorphonuclear leukocyte, (ROI) reactive oxygen intermediate, (S3140) silicone 3140, (S3145) silicone 3145, (Si) silicon, (SiO_2) silicon dioxide

Keywords: biocompatibility, biomaterials, complement, implantable device, polymorphonuclear leukocyte

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Introduction

Global prevalence of diabetes mellitus is increasing, with an estimated 250 million people now suffering from the condition according to the latest survey by the American Diabetes Association. Latest figures released in January 2011[1] confirm that the number of diagnosed cases in the United States have risen to 18.8 million people, whereas another 7 million may live with the condition undiagnosed. Therefore, the main goal for an effective treatment of diabetes is the maintenance of blood glucose values within what is considered normal values for a healthy subject. Such tight control of glucose can best be achieved by continuous monitoring of the glucose level either directly in blood or in the interstitial fluid (ISF) representing an ultrafiltrate of blood.[2]

Continuous glucose measurements will track the glucose level in real time and thereby predict upcoming hypo- or hyperglycemic events at an early stage. To realize this potential, implantable glucose sensors should be able to measure the concentration of glucose within tolerances that are as good as that of current state-of-the-art *ex vivo* sensors. They should exhibit as small a drift as possible, be unobtrusive, and exhibit biocompatible properties with the tissues at the implantation site.

Biocompatibility, by definition, is the ability of a material to perform with an appropriate host response in a specific application,[3] and which in clinical medicine have been extended to a quality of being mutually tolerant with life.[4] Since the complement system plays a critical role in the inflammation response to implantable devices, it is reasonable to refer to complement compatibility as an initial important factor in predicting biocompatibility of a material.[5–7]

The complement system is an important part of innate immunity, and can be activated through the classical, the lectin, or the alternative pathway. These complement pathways converge at the C3-level, which results in formation of C3b. This is a key molecule that governs further activation of C5 to C5a and C5b, as well as triggering an amplification loop for the alternative pathway. C5a is a potent anaphylatoxin, whereas C5b participate in the formation of the terminal complement complex, which is the end product of complement activation.[8,9]

Transcutaneous glucose sensors approved for continuous glucose monitoring are based on enzyme technology, and the most long-lived sensors are qualified for use up to 7 days.[10] We propose that application of a microfabricated osmotic sensor technology in combination with selective biomaterials can extend the lifetime operation of the glucose sensor *in vivo*. The sensor makes use of an affinity assay, which does not consume any reagents or generate any toxic by-products that are detrimental for sensor operation with time.[11] Because it will be essential to avoid any biological responses that may interfere with detection of glucose, the sensor should not elicit any significant inflammatory response following implantation *in vivo*.[12–15] Hence, the purpose of these *in vitro* studies was not to accurately predict sensor performance *in vivo*,[16] but to enable a preliminary screen of biocompatibility in order to remove materials that would otherwise elicit a detrimental immunological response inside the body.[5]

Polymorphonuclear leukocytes (PMN) can be activated by invading pathogens as a favorable event to combat infection as well as by artificial materials, then often with unfavorable effects damaging the surrounding tissue with impairment of the device function. They play a key role in the inflammatory system through the release of enzymes and reactive oxygen species as well as producing proinflammatory cytokines, in particular, chemokines generating chemotactic signals attracting other leukocytes. Polymorphonuclear leukocytes are the predominant cells causing local inflammation in response to biomaterials during the first days after implantation.[17] Activation of PMN involves alterations of membrane receptors such as cluster of differentiation molecule 11b (CD11b) and L-selectin (CD62) that lead to an increased adhesiveness to artificial surfaces and initiation of degranulation. Polymorphonuclear leukocytes do also produce highly reactive oxygen intermediates (ROI) through a process known as oxidative burst. This is another potent mechanism of defense, but overproduction of these substances could also damage surrounding tissues as well as the implanted biomaterials.[18,19] The action of ROI is further augmented by myeloperoxidase (MPO) released from azurophilic granules during PMN activation.[18,20] All these processes can significantly alter the function of implantable devices and lead to sensor dysfunction. Thus, it is important to determine the PMN-activating properties of implantable materials.[21]

An important part of PMN activation is governed by the complement system of the innate immunity.[22,23] We examined the biomaterials intended for use in an

osmotic glucose sensor with respect to their complement activation potency, and found that the membrane materials activated complement through the alternative pathway.[24] Such activation leads to generation of anaphylatoxins C3a and C5a. These are known to stimulate PMN with subsequent degranulation and to generate production of ROI and up-regulation of membrane receptors.[22,23,25]

The aim of this study was therefore to perform an *in vitro* analysis of PMN activation following exposure to selected candidate materials for an osmotic glucose sensor and to evaluate how the activation of complement contributes to activation of PMN in response to such materials.

Materials and Methods

Material Preparation and Incubation

This study emphasizes candidate materials from a sensor produced by microfabrication, and consequently takes reference in the materials available for this sensor technology. Fifteen potential candidate materials were selected in which the biocompatibility's relation to activation of PMN has yet to be assessed (**Table 1**). The materials were divided into four groups depending on which part of the sensor they would be implemented (capsule, membrane, carrier, or sealing).

The sensor microimplant is built around a carrier of low-temperature cofired ceramic (LTCC), which is a multilayer ceramic platform that permits integration of microelectronic circuit components in three dimensions. This enables an ultracompact and thermally stable system architecture that is used in the artificial pacemaker. Consequently, a choice of two LTCC materials that differ slightly in their processing parameters are included in this study, CeramTec GC (CT) and DuPont 951 (DP). The membrane and pressure transducer are attached to the LTCC using a two-component epoxy resin, Epo-

Table 1.
Candidate Materials for Implementation in the Glucose Sensor

	Material	Abbreviation	Specification	Manufacturer
Encapsulation materials	Sylgard 184	PDMS	polydimethylsiloxane	Dow Corning Corp., Midland, MI
	Araldite 2020	A2020	Epoxy resin	Huntsman Ltd., Duxford, UK
	Stainless steel	Me	corrosion resistant, Type 316L	Fosstech Engineering, Stokke, Norway
Membrane materials	Silicon	Si	Silicon with native 2–3 nm oxide surface	Vestfold University College, Horten, Norway
	Silicon dioxide	SiO_2	Silicon with a 500 nm thick thermal oxidized surface	Vestfold University College, Horten, Norway
	Cellulose ester	CM	Ultrafiltration membrane (MWCO 5000 Da, ~2.5 nm)[a]	Spectrum Labs Europe B.V., Breda, Netherlands
	Polyamide	PAR	Reverse osmosis membrane (MWCO 0 Da, <1 nm)	Sterlitech Corporation, Kent, WA
	Polyamide	PATF	Thin Film membrane (MWCO 0 Da, <1 nm)	Sterlitech Corporation, Kent, WA
	Polycarbonate	PC	Track-etched membrane (MWCO 500 kDa, ~15 nm)	Whatman plc, Kent, UK
	Aluminum oxide	AAO	Anodic aluminum oxide (MWCO 50 kDa, ~5 nm)	Synkera Technologies Inc., Longmont, CO
Sensor carrier materials	CeramTec GC	CT	Low-temperature cofired ceramic	Ceramtec AG, Plochingen, Germany
	DuPont 951	DP	Low-temperature cofired ceramic	Dupont, Wilmington, DE
Sealing materials	Silicone 3140 coating	S3140	Silicone-based polymer	Dow Corning Corp., Midland, MI
	Silicone 3145 adhesive	S3145	Silicone-based polymer	Dow Corning Corp., Midland, MI
	Epo-Tek 353ND	ETek	Epoxy resin	Epoxy Technol. Inc., Billerica, MA

[a] Molecular weight cut-off

Tek 353ND (ETek), which cures with temperature. This adhesive also acts as a sealing material that protects the electrical connections from moisture. However, small distances between the electrical connections and the aqueous environment in the sensor cavity prompted two additional sealant materials to be considered: silicone 3140 (S3140) and 3145 (S3145), because of their good water-repellent properties. These will also act as a buffer between sensor components and the external capsule. The membrane and control chip are made from silicon (Si) and its glass-derivative silicon dioxide (SiO_2). Additional membrane materials include anodic aluminum oxide (AAO) and the polymers cellulose ester (CM), polyamide (PAR, PATF), and polycarbonate (PC). These were selected for their nanoporous nature and chemical property as well as the potential for integration on a miniaturized silicon membrane frame. The whole sensor is enclosed by an external capsule that protects internal components from the external environment. Sylgard 184 is a poly-dimethylsiloxane (PDMS) that is commonly used in microfluidics and lab-on-a-chip devices, whereas araldite 2020 (A2020) is a two-component epoxy resin that offers an additional degree of mechanical support. Both of these (alone or in combination) will be molded around the sensor assembly and cured at room temperature. Type 316L stainless steel (Me) used to encapsulate prototype devices was included in these studies for comparison.

The sensor (**Figure 1**) rests in the ISF and detects osmotic pressure generated in the sensor cavity (1) enclosed by the nanoporous membrane (2) and silicon pressure transducer (3). The membrane and transducers are attached by the adhesive (4) to the sensor carrier (5) and sealed with sealant materials (6), forming a flexible buffer against the external capsule (7). The sensor cavity maintains an active solution (8) based on the concanavalin A-dextran affinity assay,[11,26] which is protected against antibodies by the nanoporous membrane. Thus, the membrane, sealant, and encapsulant will be in direct contact with the ISF through the access channel (9); whereas the carrier, adhesive, and transducer will be in indirect contact through the membrane.

The material samples with liquid properties (PDMS, A2020, S3140, S3145, ETek) were prepared by administering 0.5 ml of uncured monomer in respective wells of a 24-well polystyrene plate and subsequently polymerized at room temperature (60 °C for ETek) for 24 hours in accordance with the recommendations of the manufacturer. Material samples with solid structure (Si, SiO_2, CT, DP) were cut into pieces of 1 cm^2. The nanoporous membranes and metal parts were cut into circular structures with

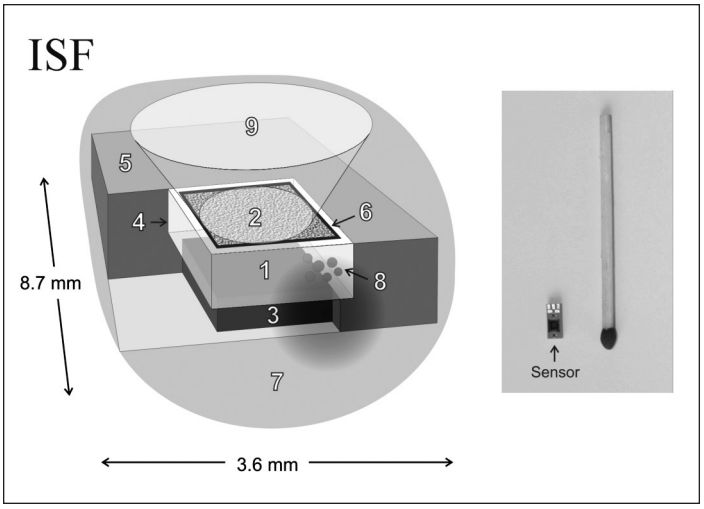

Figure 1. Conceptual illustration (left) of the microfabricated implantable osmotic glucose sensor, and the capsule less prototype (right). The whole packaged device measures 3.6 × 8.7 mm and resides in the ISF of subcutaneous adipose tissue. The unit samples glucose as an ultrafiltrate from blood through the open access channel of the capsule that provides direct access to the integrated 2 × 2 mm^2 osmotic pressure sensor. The labels are related to (1) chamber, (2) membrane, (3) pressure transducer, (4) adhesive, (5) carrier, (6) sealant, (7) capsule, (8) bioparticles, and (9) access channel. See text for details.

a surface area of 1 cm^2. Empty polystyrene wells were used as the negative control.

All materials, except the nanoporous membranes, were washed three times with ethanol and then rinsed with distilled water. Solid materials were additionally treated with an ultrasonic bath to remove residual microparticles that may persist on the material surface as a result of the manufacturing process. Nanoporous membranes were incubated in distilled water overnight in accordance with the manufacturer's instructions, and stored in phosphate buffer saline (PBS). All materials used in this study were rinsed with PBS solution prior to the experiment.

Whole blood from five healthy volunteers was used in the experiments. Informed consent was obtained before blood donation, and the study was approved by the local ethical committee. The blood was anticoagulated with the thrombin inhibitor lepirudin (Refludan®, Pharmion Germany GmbH, Hamburg, Germany) in a final concentration of 50 μg/ml.[23,27]

The experiments were performed by first administering 0.5 ml of blood in each well containing the respective candidate materials, prior to incubation at 37 °C in a climate room with the plate placed on a shaker platform. After 20 min, 45 μl of blood was withdrawn from the wells and used in flow cytometry in accordance to the

research protocol. The rest of the blood was incubated for 60 min, before the incubation was stopped by adding ethylenediaminetetraacetic acid to a final concentration of 20 mM. The samples were immediately transferred on ice and then centrifuged at 1400*g* for 15 min at 4 °C. The collected plasma was then centrifuged a second time at 1400*g* for 15 min at 4 °C, and the plasma was finally aliquoted and frozen at -70 °C. Whole blood incubated in empty polystyrene wells was used as the negative control.

Complement Inhibition

Materials that triggered an increased level of CD11b expression and MPO production were selected for further experiments using complement inhibitors. Compstatin analog Ac-I[CV(1MeW)QDWGAHRC]T, which binds to and inhibits cleavage of C3, and a control peptide were produced as described.[28] These were used in the experiments to a final concentration of: 25, 12.5, 6.25, and 3.125 mM. Eculizumab, (Soliris®, Alexion Pharmaceuticals, Cheshire, CT), a monoclonal antibody that specifically binds to the complement protein C5, was used in final concentrations of: 50, 25, 12.5, and 6.25 μg/ml. Whole blood was preincubated with these complement inhibitors for 5 min before the candidate materials were placed in the wells.

CD11b Expression

The CD11b expression on PMN was determined by flow cytometry. Blood was withdrawn from the wells containing the material candidate after a 20 min incubation period, prior to fixation using a 0.5% paraformaldehyde solution for 4 min at 37 °C. The cells were then stained for 15 min with anti-CD11b-PE antibodies (cat. no. 333142) or an isotype control IgG2a-PE antibodies (cat. no 349053), both obtained from Becton, Dickinson and Company (Franklin Lakes, NJ). Red blood cells were lyzed in accordance to protocol, washed twice, and cells were then resuspended in PBS containing 0.1% albumin and placed on ice in the dark until data acquisition was performed by the flow cytometer (FACScan, Becton, Dickinson and Company, Franklin Lakes, NJ). The PMNs were gated in a forward-scatter/side-scatter dotplot, and mean fluorescence intensity (MFI) values for CD11b were calculated.

Myeloperoxidase

Myeloperoxidase concentration in plasma was measured with a commercial enzyme-linked immunosorbent assay kit (cat. no HK324, Hycult Biotech, Uden, The Netherlands) in accordance to the manufacturer's instructions.

Statistics

Results from the CD11b and MPO experiments were statistically compared by one-way analysis of variance (ANOVA) between groups with Bonferroni post-test analysis. Data from the experiments with complement inhibitors were compared by one-way ANOVA in order to analyze the dose-dependent decrease of activation. All statistical data were collected and calculated with GraphPad Prism version 5.01 (GraphPad Software, San Diego CA). A p value <0.05 was considered as statistically significant.

Results

Activation of PMN

Expression of CD11b

The expression level of CD11b on the surface of PMN was measured with flow cytometry (**Figure 2**). The expression level of CD11b after incubation with three of the membrane candidates (CM, PAR, PATF) was significantly higher compared to the negative control (MFI 431, 511, and 462 vs MFI 240, $p < 0.05$). Material candidates used for the encapsulation, carrier, sealing, and the remaining four membrane candidates did not induce an expression of CD11b compared to the negative control.

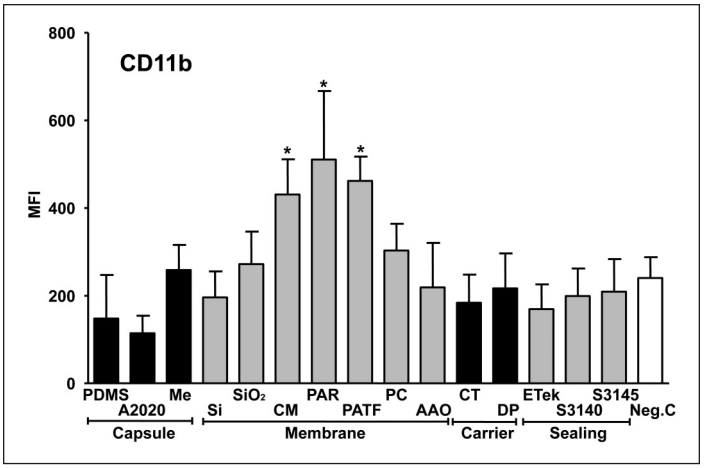

Figure 2. Expression of CD11b on PMN surface. The expression level of CD11b on PMN is shown with bars representing mean values with 95% confidence interval ($n = 12$). *$p < 0.05$ compared with the negative control. Neg.C, negative control.

Myeloperoxidase Release

Similar to CD11b, the concentration of MPO (**Figure 3**) in plasma after incubation with three of the membrane candidates (CM, PAR, PATF) was significantly higher than the negative control (500, 421, and 268.3 μg/ml vs 83.7 μg/ml, $p < 0.05$). Material candidates used for the encapsulation, carrier, sealing, and the remaining

four membrane candidates did not induce MPO release compared to the negative control.

Effect of Complement Inhibition on the Polymorphonuclear Leukocyte Activation

The effect of complement inhibition on the activation of PMN was evaluated by measuring the CD11b expression and the MPO concentration in lepirudin-anticoagulated whole blood incubated with materials after addition of compstatin and eculizumab. Based on the results from previous experiments examining CD11b expression on PMN and MPO concentration, the three membrane candidates that activated PMN were chosen to investigate the effect of complement inhibition.

CD11b Expression in Whole Blood with Complement Inhibitors

Complement inhibition by the C3-inhibitior compstatin led to a significant and dose-dependent decrease in the level of CD11b expression induced by all three materials (**Figure 4**). Maximum effect was obtained at 25 mM compstatin, where the expression of CD11b was reduced to the negative control value for all three materials.

Similarly, complement inhibition by the C5-inhibitor eculizumab significantly decreased the CD11b expression on PMN and dose dependently (**Figure 4**). The maximum was obtained at 50 µg/ml eculizumab, where the expression of CD11b was reduced to the negative control value for all three materials.

Unspecific binding of antibodies to the surface of PMN was measured in blood incubated with the isotype IgG2a control antibody. No unspecific bind was observed (**Figure 4**).

MPO Concentration in Whole Blood with Complement Inhibitors

Complement inhibition by compstatin led to a significant and dose-dependent decrease in the level of MPO secretion induced by all three materials (**Figure 5**). Maximum effect was obtained at 25 mM compstatin, where MPO concentrations for two of the three materials were reduced to negative control values.

Complement inhibition by eculizumab led to a dose-dependent decrease in the level of MPO secretion induced by all three materials (**Figure 5**). Maximum effect was obtained at 50 µg/ml eculizumab, where MPO concentrations for two of the materials were reduced to negative control values (**Figure 5**).

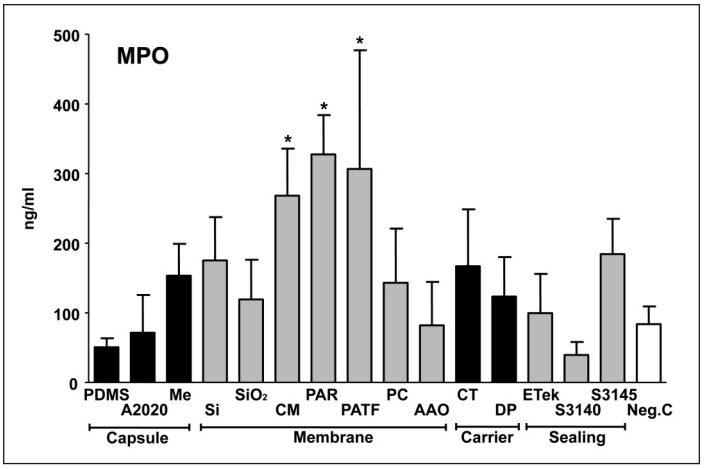

Figure 3. MPO concentration in plasma. The concentration of MPO in plasma after incubation with the 15 candidate materials. The bars represent mean values with 95% confidence interval ($n = 8$). *$p < 0.05$ compared with the negative control. Neg.C, negative control.

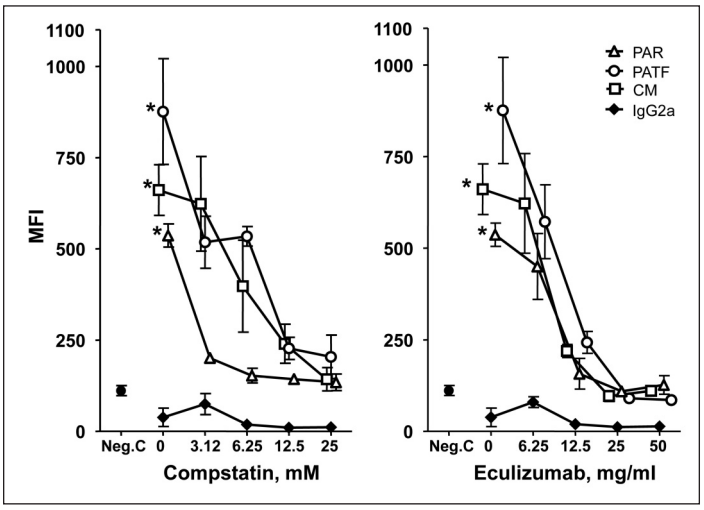

Figure 4. Expression of CD11b on PMN in whole blood with and without complement inhibitors. CD11b expression level for three material candidates is shown for different concentrations of compstatin and eculizumab. The bars represent standard error mean values ($n = 4$). *$p < 0.05$ compared by ANOVA for identifying the significance of the dose-dependent decrease. Neg.C, negative control; IgG2a, isotype control antibody

Discussion

This study documented a substantial variation in the PMN-activating properties of different candidate biomaterials intended for use in an implantable glucose sensor.

Assays used in this study were selected on the basis of international standard ISO 10993-4, which recommends the use of both complement assays and flow cytometry to detect increased leukocyte markers such as CD11b as a testing method to evaluate implantable devices.[29]

The 12 material candidates that did not show any significant increase in the CD11b expression and the MPO release were considered as biocompatible, and hence deemed possible candidates for use in other *in vivo* studies. The glucose-sensitive solution enclosed in the sensor chamber (concanavalin A and dextran) was not investigated in this study. Although concanavalin A is reported to exhibit a toxic response, the amount present in tiny biosensor implants is so small that in the case of membrane rupture this would not possess any overall toxic effect for the organism.[30] Further, the liquid properties of the affinity assay will permit it to dilute away from the sensor implant in contrast to the remaining sensor materials that may persist in generating a potential inflammatory response.

Three membrane candidates (CM, PAR, PATF) caused a virtually identical pattern of increase in the CD11b expression and the MPO release from the PMN. The results obtained for the cellulose membrane in our study are well correlated with literature. Although cellulose is a well-known material that has been used in dialysis membranes, the literature has suggested that this material is not biocompatible.[31–33] Different polyamide membranes have been widely used in implantable devices, but the reported studies concerning the biocompatibility of this material is controversial. Some clinical and *in vitro* studies (cell cultures) show a good biocompatibility of polyamide,[34–36] whereas other studies have claimed this material to be only moderately biocompatible.[37] Our studies showed that the polyamide membranes (PAR and PATF) activated PMN. These discrepancies can be explained by the different assays used to examine biocompatibility as well as the different types of polyamide used.

Since a study demonstrated that the same three membrane materials did activate complement through the alternative pathway,[24] we hypothesized that the current activation of PMN was also mediated by complement. Consequently, by blocking complement activation with either the C3 inhibitor compstatin or the C5 inhibitor eculizumab before incubation of the materials in whole blood, a significant decreased CD11b expression and release of MPO by PMN was found. The results were virtually identical for the two inhibitors, indicating that the effects were mediated by C5 activation, since compstatin blocks C3 and subsequent C5 activation, whereas eculizumab is highly specific for C5. This is consistent with the well-known effect of C5a on PMN activation.[27] This finding underscores the role of the complement system as a primary inducer for leukocyte activation, since the latter was markedly attenuated by inhibition

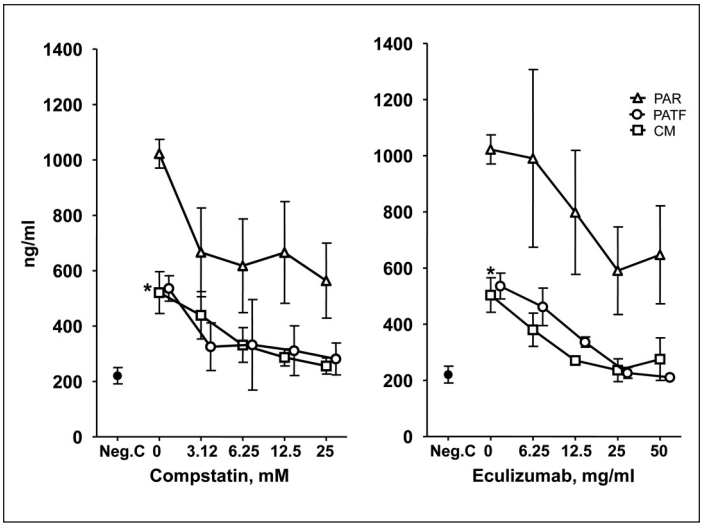

Figure 5. MPO concentration in plasma after incubation of the materials in whole blood with and without complement inhibitors. The concentration of MPO in plasma is shown for different concentrations of compstatin and eculizumab. The bars represent standard error mean values ($n = 4$). *$p < 0.05$ compared by ANOVA for identifying the significance of the dose-dependent decrease. Neg.C, negative control.

of complement, irrespective of the material in question. This novel approach in biocompatibility testing does not only correlate the activation of different systems but also shows the causal relationship between them.

The role of complement as a primary inducer of the expression of CD11b was confirmed, since complement inhibition completely abolished the CD11b expression for all the three materials. However, a complete MPO inhibition was obtained for only two of these (PATF and CM). Since some MPO release still occurred for PAR despite an optimal inhibition of complement, a direct activation of the PMN by the surface of the material[25] or by activated platelets[21] might also contribute to PMN degranulation. The membrane materials that were examined have various pore sizes, as indicated by their molecular weight cut-off (MWCO), from zero for PAR and PATF to 5000 Da for CM). The comparable magnitude of PMN activation triggered by these three materials suggests that the chemical structure and the degree of protein absorption on the surface of the materials may have a major contribution to their potential to activate complement and PMN.[38]

Collectively, however, the present data indicate that complement activation is the main inducer of PMN activation by the biomaterials investigated in this study. The abolished PMN activation for CM and PATF by complement inhibition shows the insignificant role of direct surface activation of the PMNs for these two

tested material candidates. Irrespective of the mechanism leading to complement activation by these surfaces, a main goal to improve biocompatibility would be to reduce the primary complement activation in order to avoid the secondary activation of PMN.

Conclusion

Examination of 15 material candidates for an implantable microfabricated glucose sensor identified 3 out of 7 membrane candidates as potent complement-dependent PMN activators that make them undesirable candidates for use in an implantable device. The remaining materials that were examined were rendered biocompatible in terms of PMN activation and suggested to be good candidates for use in an implantable glucose sensor. The results underscore the important role of complement in mediating immune system-triggered responses to a foreign material and should be included as an essential component in the future portfolio of immune system markers when studying the mechanisms involved in the biocompatible nature of a material. Because the possibility cannot be excluded that materials that do not activate complement and PMNs *in vitro* might turn out to be incompatible *in vivo,* future studies will seek to correlate the results from *in vitro* assays with *in vivo* studies.

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Disclosures:

Erik Johannessen is a former project manager employed by Lifecare. Dr. Johannessen is the inventor of a patent held by Lifecare (WO 2009025563).

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