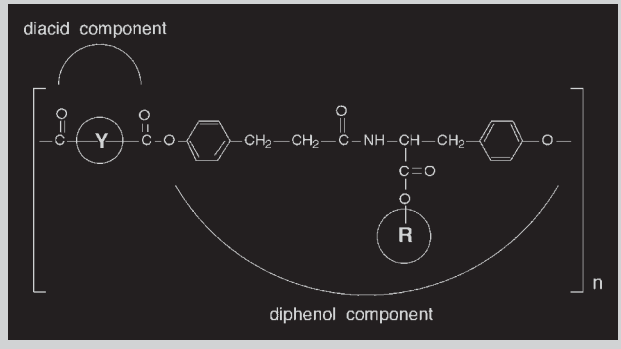
Summary: The advent of high-throughput combinatorial synthesis techniques in drug discovery has stimulated efforts to apply these techniques to the discovery of biomaterials. To be of practical utility, combinatorial approaches to biomaterials design require (i) the availability of parallel synthesis techniques to generate libraries of polymers, (ii) efficient assays for the rapid characterization of biorelevant material properties, and (iii) computational methods to efficiently model different biological responses in the presence of polymers. Here we report the integration of these methodologies and illustrate the potential of this approach to accelerate the development of new biomaterials. The parallel synthesis of a library of 112 biodegradable polyarylates has been reported previously. This library was used to develop efficient screening techniques to determine biorelevant polymer properties (fibrinogen adsorption, gene expression in macrophages, growth of fetal rat lung fibroblasts (RLFs)). A Surrogate (semiempirical) Model was developed (i) to determine molecular-scale polymer properties that correlate to various biological responses, and (ii) to predict fibrinogen adsorption and RLF growth on polymeric surfaces. For 38 out of 45 polymers, the model predicted the amount of fibrinogen adsorbed correctly within the error of the experimental measurements. The growth of rat lung fibroblasts was correctly predicted by the model for 41 out of 48 polymers. The correlation factor between the model's predicted values and the experimentally determined data was 0.54 ± 0.09 and 0.69 ± 0.12 for fibrinogen adsorption and RLF growth, respectively. The results presented here demonstrate the utility of combinatorial and computational approaches for the rational design of polymers for biomedical applications.



Design of the library of polyarylates, which are copolymers of a diacid and a diphenol. Chemical diversity was created by variations in the structure of the diacid (marked as "Y") and the pendent chain (marked as "R").

Integration of Combinatorial Synthesis, Rapid Screening, and Computational Modeling in Biomaterials Development

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Introduction

Combinatorial approaches have led to dramatic changes in the way lead compounds for the discovery of new drugs are identified.[1] The approach of creating millions of combinatorially synthesized moieties within a single reaction vessel, followed by the identification of potentially active compounds in a selective bioassay, is often referred to as combinatorial drug discovery ("CombiChem"). For this approach to be effective, rapid screening assays, as well as appropriate computational methods for data handling, need to be available. In spite of the conceptional simplicity of this approach, substantial technical hurdles had to be overcome before combinatorial techniques could be used with some success in drug discovery.[2]

One of the earliest material science applications of the combinatorial discovery approach was the search for catalytically active polymers by Menger et al.[3] They

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Jack R. Smith completed his undergraduate thesis in physics in the group of Stuart Field (University of Michigan) by modeling the dynamics of chaotic systems. Subsequently, he worked as an intern developing data acquisition algorithms for the Babar project in the group of Michael Levi (Lawrence Berkeley Laboratory, USA). His PhD work was performed under the supervision of Dawn Bonnell and Roger French (University of Pennsylvania, USA) and focused on using experiment and modeling to establish correlations between bonding, atomic-scale structure and optical properties in thin, amorphous films of CrOCN used in DUV phase-shift lithography. For this work he received the university's Stein Prize. In 2001, he was awarded the Chateaubriand Fellowship for postdoctoral study in France. In the group of Joel Chevrier at the European Synchrotron Radiation Facility (Grenoble), he studied the oxidation of AlPdMn quasicrystalline surfaces primarily using scanning probe techniques. Currently, he is a postdoctoral associate at the New Jersey Center for Biomaterials working closely with Drs. Knight and Kohn on the computational modeling of bioresponse to polymeric materials/surfaces.

Dr. Agnieszka Seyda is a graduate of McMaster University in Hamilton, Ontario (Canada) where she received her BS in Biochemistry in 1995. Her Ph.D. (2000) is from the University of Toronto, Department of Biochemistry. After graduating, Dr. Seyda was a postdoctoral fellow at Ontario Cancer Institute, Princess Margaret Hospital, Toronto, Canada. As a Research Assistant Professor with the New Jersey Center for Biomaterials at Rutgers University, Piscataway, New Jersey, her research focused on cell interactions with polymer surfaces at the level of gene expression. She is currently employed at Ethicon, a division of Johnson and Johnson, Inc.

Norbert Weber received his BS in Biology, MS in Cell Physiology and Ph.D. in Cell and Molecular Biology from the University of Tübingen, Germany where he also held his first postdoctoral position researching gene expression profiling in surface-activated leucocytes using cDNA-microarrays and Real-Time RT-PCR. He has also held a postdoc position at Rutgers, the State University of New Jersey at the New Jersey Center for Biomaterials. He is currently a Research Associate at Rutgers where his research focuses on fluorescence assays to detect surface-adsorbed proteins and attached cells, real-time measurements of protein adsorption and cell receptor binding to polymeric surfaces using a quartz crystal microbalance with dissipation monitoring (QCM-D).

Doyle Knight received his Ph.D. in Aeronautics at the California Institute of Technology in 1974. Following two years in the United States Air Force as an Aeronautical Engineer, and one year postdoctoral position at the California Institute of Technology, he joined the faculty of the Department of Mechanical and Aerospace Engineering at Rutgers – The State University of New Jersey. His research interests include Surrogate Modeling, design optimization, gas dynamics, turbulence simulation and high performance computing. He is an Associate Fellow of the American Institute of Aeronautics and Astronautics, and the author of more than 170 technical papers. He is the Director of the Rutgers University Center for Computational Design, established in 1996.

Dr. Sascha Abramson completed her undergraduate degree in English from the University of Pennsylvania in 1996. In 2002 she received her doctorate in Biomedical Engineering from Rutgers University for her work with tyrosine-derived polycarbonates. Dr. Abramson studied the physico-mechanical and biological properties of tyrosine-derived polycarbonates with a special emphasis on polymer degradation and the response of bone tissue to polymer implants. Dr. Abramson joined the New Jersey Center for Biomaterials and the Rutgers Center for Computational Design for a joint postdoctoral position in which she developed rapid screening protocols for degradable polymers. Dr. Abramson currently works at Celgene Cellular Therapeutics.

Professor Joachim Kohn is the Board of Governors Professor of Chemistry at Rutgers University and an Adjunct Associate Professor of Orthopedics at the New Jersey Medical School. He currently serves as the Director of the New Jersey Center for Biomaterials. His research interests focus on the development of new biomaterials for prostheses, implantable drug or gene delivery systems, and tissue regeneration scaffolds. He is mostly known for his pioneering work on "pseudo-poly(amino acid)s" – a new class of polymers that combine the nontoxicity of individual amino acids with the strength and processibility of high quality engineering plastics. He emphasizes the use of combinatorial approaches for the design of new polymers, and uses computational modeling and simulations for the elucidation of cell–polymer interactions. He has published 167 scientific manuscripts and reviews and holds 31 patents. Professor Kohn has extensive technology transfer experience. He serves on the scientific advisory boards of 4 companies. Professor Kohn is the recipient of numerous awards and honors, including the 2003 Clemson Award for Basic Science of the Society for Biomaterials.

created random patterns of pendent chains along a polymer backbone and identified a number of polymer mixtures that exhibited phosphatase-like activity. However, the simple CombiChem approach of generating many different polymers within one single reaction vessel is limited by the difficulty of separating the mixture into individual polymers. Indeed, Menger was unable to identify which specific polymer composition in each of his catalytically active mixtures was associated with the observed phosphatase-like activity.

The problems associated with the separation of mixtures of many different materials into discrete compositions were circumvented by the use of spatially resolved libraries. This approach was applied for the first time in a combinatorial search for photoluminescent inorganic phosphor compositions: Schultz et al. created different material compositions within a two-dimensional X-Y grid. A simple optical reading of the photoluminescence as function of the X and Y coordinates within the grid provided the necessary high-throughput assay and allowed the identification of lead compositions with potentially useful photoluminescence.[4] This concept of spatially resolved libraries was later adapted by Amis and his coworkers at the National Institute for Standards and Technology (NIST) to explore the properties of polymer blends within a two-dimensional grid in which all possible blend compositions were represented by a pair of X,Y coordinates.[5,6]

For most practical applications in materials science, a parallel synthesis approach seems to be more useful than the creation of many different materials within one single reaction vessel. Using parallel synthesis, a library of materials can be created in such a way that each individual material is obtained in pure form in a separate reaction vessel. This approach was used in 1997 in Kohn's laboratory to prepare the first combinatorially designed polymer library.[7,8] Their approach consisted of the synthesis of strictly alternating A–B copolymers. In this approach, first, structural templates for the A and B monomer are defined and a polymerization scheme is developed. Next, structural modifications are introduced in a systematic fashion into the A and B templates, resulting in the formation of families of related monomers (A1, A2, A3, etc. and B1, B2, B3, etc.). Finally, each of the A monomers is copolymerized with each of the B monomers in separate reaction vessels. Specifically, Kohn's group used eight different diacids (the A monomers) and 14 different diphenols (the B monomers) to create a library of $8 \times 14 = 112$ structurally related polyarylates (Figure 1).[8] The same approach was later used by Langer et al. to synthesize a library of copolymers that were screened for use as synthetic transfection vectors.[9]

The term ''biomaterial'' describes a material intended for use in a medical device or implant.[10] While commonly used biomaterials include metals, ceramics, and natural materials (such as collagen),[11] within the framework of this publication we will focus on the application of combinatorial and computational approaches to the identification of new, synthetic polymers as potential biomaterials. While the traditional application of polymers as engineering plastics is well established and supported by an extensive understanding of structure-performance correlations, a comprehensive understanding of the interactions between implanted polymers and the surrounding cells and tissues has not yet been established, making it impossible to apply a rigorous, rational design process to the identification of new biomaterials.[12,13] Therefore, a combinatorial approach to biomaterials design appears to be particularly promising, since this approach can be applied successfully when clear correlations between the basic design variables (e.g., biomaterial chemistry and structure) and the performance of the polymer (e.g., cell-biomaterial interactions) are not available. Furthermore, combinatorial methods can be the most effective when a large number of variables make it impossible to explore the entire range of experimental variables in an exhaustive fashion.

Computational techniques to build, screen, and mine libraries of compounds have evolved rapidly in recent years as an efficient strategy for molecular discovery and optimization.[14–19] To the best of our knowledge, however, these powerful computational strategies have not yet been applied to optimal biomaterial design. Likewise, the use of semiempirical models, such as Artificial Neural Network (ANN) models, has been limited. ANNs have been successfully applied to a wide range of modeling problems. For instance, Baluja[20] developed an ANN to control a passenger vehicle on a highway, Chandra and Sudhakar[21] developed an ANN for printed numeral recognition, and Yoon et al.[22] developed an ANN (denoted DESKNET) to diagnose papulosquamous skin diseases. This latter system is utilized in medical education, achieving a correct diagnosis for 70% of the skin diseases. In spite of the widespread use of ANNs and similar semiempirical modeling techniques in many fields, a detailed literature search revealed only two prior publications that are relevant to the prediction of a cellular response based on the measurement of some physical material properties. The first publication developed a multivariant model that was successful in establishing a correlation between the surface properties of common polymeric materials and the amount and retention of fibrinogen adsorbed from a complex mixture.[23] The second publication described a similar multivariant model to investigate the relation between endothelial cell growth and surface properties of plasma-deposited films.[24,25]

In order to be of practical utility, the previously reported parallel synthesis of polymer libraries needs to be integrated with efficient screening assays, as well as appropriate computational methods for data handling and modeling. While it would be premature to speak in terms of ''high throughput'' assays at this point, the development of efficient rapid-screening assays for biologically relevant material properties that can facilitate the extensive testing of dozens or

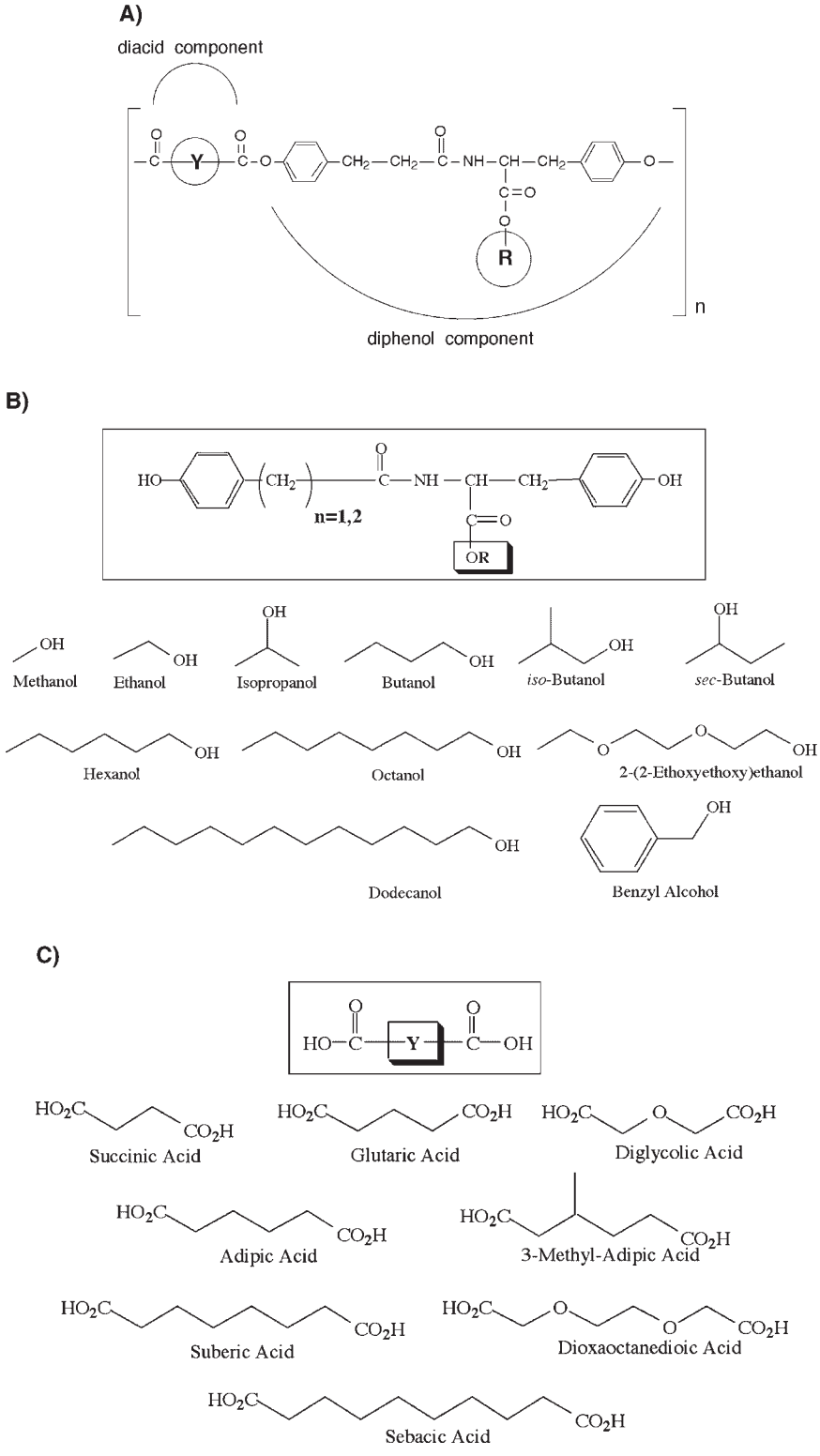

Figure 1. Design of the library of polyarylates. (A): Polyarylates are copolymers of a diacid and a diphenol. Chemical diversity was created by variations in the structure of the diacid (marked as "Y") and the pendent chain (marked as "R"). (B): Representative examples of the pendent chain structures used to modify the diphenol component. (C): Representative examples of the diacid structures used to modify the polymer backbone.

hundreds of polymers represents an important research challenge. A second challenge relates to the need to adapt commonly used computational techniques to appropriately account for the experimental uncertainty inherent in biological data sets. Finally, a third research challenge is to develop a better understanding of which molecular-scale polymer properties are the best predictors of biorelevant polymer performance, such as cell growth, toxicity, or biocompatibility. Some of these challenges are being addressed by the work presented here.

Materials and Methods

Polymer Synthesis and Characterization

Polymers from a combinatorial library of tyrosine-derived polyarylates were synthesized according to published procedures.[8] The polymers consisted of a series of commercially available diacids and tyrosine-based diphenols which were synthesized as previously described.[26] The 112 polymers of the library were subjected to a characterization protocol consisting of purity and chemical-structure analysis by proton and carbon NMR spectroscopy, High-pressure liquid chromatography (HPLC), and elemental analysis, followed by molecular-weight determination by gel-permeation chromatography, detailed thermal analysis by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), and air-water contact angle measurement by goniometry.[8]

Cell Proliferation Studies on Flat Surfaces

Cell proliferation studies were either performed on spin-coated glass cover slips or on compression-molded disks that were inserted into the bottom of wells in 96-well plates.[8,27] The number of cells present on the growth substrata was estimated using a commercially available MTS colormetric assay (Promega, Madison, WI).

Surface Protein Adsorption by Immunofluorescence Assay

384-Well microtiter polypropylene plates (Cat. No. 264576) were obtained from Nalge Nunc International (Rochester, NY, USA), while 96-well polypropylene plates were obtained from VWR (Bridgeport, NJ). The assay has been reported previously.[28,29] Briefly, polymers were dissolved in methylene chloride (5% (w/v)). Next, the polymer solutions were filtered through 0.45 μm PTFE filters (Whatman Inc., Clifton, NJ, USA). Then, individual microtiter wells on the plates were filled with test polymer solutions. To evaporate the solvent, the plates were kept at a temperature of 50 °C for 3 hours in a drying oven. This process generated thin, macroscopically smooth polymer films inside the wells. The wells were then exposed for 90 min to 25 μL of fibrinogen solution in phosphate buffer, followed by rinsing with PBS. After blocking of non-specific antibody binding sites by incubation with bovine serum albumin, the plates were rinsed with PBS and exposed to fluorescently labeled polyclonal anti-fibrinogen antibodies. After appropriate washing steps, the amount of surface-adsorbed fibrinogen was determined by measuring the fluorescence in each well in a fluorescence reader (Spectra Max Gemini, Molecular Devices, Sunnyvale, CA, USA). Human fibrinogen adsorption to non-coated polypropylene wells was used as an internal control to normalize the fluorescence signals against an internal standard.

Gene Expression by Real Time RT-PCR

In Vitro Rat Peritoneal Macrophage Culture

Monocyte-derived macrophages were harvested from the peritonea of adult Sprague–Dowling rats treated with 50 mL of PBS at 0 °C. Monocytes were plated on polypropylene plates in Dulbecco's Modified Eagle's Media (D-MEM) supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 U/mL penicillin G (Sigma, St. Louis, MO), 100 μg/mL streptomycin (Sigma, St. Louis, MO) and allowed to achieve a quiescent state by incubation for 48 hours. After this period, the macrophages were plated on different, solvent-cast polymer surfaces in 96-well polypropylene plates as described before.[28,29] Macrophages in either quiescent or lipopolysaccharide (LPS) activated state were assayed by real-time Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) for the expression of IL-1β and IL-6.

Real-Time RT-PCR

The PCR reaction was performed on a Rotor-Gene™ Four-Channel Multiplexing System (Corbett Research, Mortlake, NSW, Australia). Data was analyzed using the Rotor-Gene software and the manufacturer's general protocol was followed with regard to RNA isolation, reverse transcription, and amplification. Specifically, total RNA was isolated from adherent cells six hours post seeding using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, 50 μL of TRIzol® Reagent was added to each well. Duplicate samples were combined to ensure sufficient amounts of RNA, yielding 100 μL of cell extract per surface per condition. The extract was allowed to stand for 10 min then mixed with 50 μL chloroform and inverted repeatedly for 15 s followed by 3 min incubation and centrifugation for 15 min at 10 000 rpm. The resulting aqueous layer was then combined with 100 μL of isopropanol and mixed thoroughly. RNA was then pelleted and resuspended in 30 μL of UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen, Carlsbad, CA) containing 0.5 μL of DNase I

(Ambion, Austin, TX) according to the manufacturer's specification. DNase I was inactivated by incubation at 70°C for 5 min. Messenger RNA was reversibly transcribed by combining 10 μL of the total RNA with 1 μL of oligo(dT)12–18 primer (Integrated DNA Technologies, Coralville, IA) and 1 μL of 0.01 M dNTP mix and incubating for 10 min at 65 °C. A mixture of 4 μL of 5x first-strand buffer, 2 μL of 0.1 M DTT, and 1 μL of RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/μl) (Invitrogen, Carlsbad, CA) was then added to the sample and incubated for 2 min at 42 °C. Reverse transcription was then initiated by the addition of 0.5 μL (100 units) of Superscript II® (Invitrogen, Carlsbad, CA) and the reaction was incubated at 42 °C. After 50 min, the reaction was inactivated by heating the mixture to 70 °C for 15 min. All samples were diluted to 100 μL total volume using UltraPure™ DNase/RNase-Free distilled water. The reversibly transcribed product was then stored at −20 °C until used for PCR. PCR was performed using a Platinum® *Taq* DNA Polymerase (5 U/μL) (Invitrogen, Carlsbad, CA). Five microliters of reversibly transcribed product was used for each PCR reaction. A mix of 5 μL of 10× PCR buffer, 2.5 μL of 0.05 M $MgCl_2$, 1 μL of 0.01 M dNTP mix, 0.5 μL of 10× SYBR® Green I nucleic acid gel stain (10 000X concentrate in DMSO) (Molecular Probes, Eugene, OR) and 0.25 μL of Platinum® *Taq* DNA Polymerase were used per reaction tube. UltraPure™ DNase/RNase-Free distilled water was then used to bring each reaction volume to 50 μL. Primer pairs (Table 1) were added to a final concentration of 1×10^{-6} M. The amplification was then performed for 35 cycles (94 °C for 20 s, 56 °C for 20 s and 72 °C for 20 s per cycle). After data analysis using the Rotor-Gene software, expression ratios were determined by dividing the signal intensity of the product of interest by that of the corresponding *β*-2-microtubulin.

in vitro Cell-Growth Measurements

Sample Preparation

Previously published procedures[8] to culture rat lung fibroblast (RLF) cells on flat surfaces of various test polymers were modified and adapted from a 24-well plate format to a more efficient 96-well plate format. In the 24-well plate format, the polymers were spin coated onto 15 mm glass. Once dry, the cover slips were loaded polymer side up into 24-well polystyrene plates. For the 96-well plate format, polymers were either compression molded into thin, round disks which could be fitted tightly into the bottom of each well, or polymers were directly coated onto the well surface by solvent casting. In this case, polypropylene plates were used[28,29] and 50 μL of a 7% (w/v) solution of test polymer in methylene chloride produced the best surface coverage. After extensive drying, incubation at 37 °C for three days did not dislodge any of the films from the polypropylene well surface. All three methods of sample preparation provided similar cell-growth results.

Cell Culture and Data Collection

Rat lung fibroblast (RFL-6) cells were grown on the test surfaces for seven days as described before[27,30] and metabolic activity was measured with a commercially available kit (CellTiter96®, Promega, Madison, WI). The measured value for the metabolic activity was then normalized to tissue culture polystyrene which served as internal standard. The Normalized Metabolic Activity (NMA) value was used in the computational models.

Computational Modeling

Overall Strategy

First, polymer descriptors were generated for each polymer. Then, the significance of each descriptor with respect to the set of experimental bioresponse data was ascertained. Finally, the three most significant descriptors in conjunction with the experimental data for polymers contained within the training set, were used as inputs into an Artificial Neural Network (ANN) to predict protein adsorption and cell growth for those polymers included in the validation set. The experimentally determined values were then compared with the predictions derived from the model.

Software Used

The Molecular Operating Environment platform (MOE Version 2003.02, software available from Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, Canada H3A 2R7 http://www.chemcomp.com)

Table 1. Forward and reverse primers for RT-PCR.

Gene	Primer	Sequence
IL-6	Forward primer	5′ ATT CTG TCT CGA GCC CAC CA 3′
	Reverse primer	5′ CTG AAG GGC AGA TGG AGT TGA 3′
IL-1*β*	Forward primer	5′ TGC AGG CTT CGA GAT GAA CA 3′
	Reverse primer	5′ ACA TGG GTC AGA CAG CAC GA 3′
β-2-microglobulin	Forward primer	5′ CTC ACA CTG AAT TCA CAC CC 3′
	Reverse primer	5′ AAG AAG ATG GTG TGC TCA TTG 3′

and Dragon software (http://www.disat.unimib.it/chm/Dragon.htm) were used to calculate 101 molecular descriptors for each polymer.

Identification of the Three Most Significant Descriptors for a Given Bioresponse

Together with two experimentally measured quantities, glass transition temperature (T_g) and air-water contact angle (θ), and an additional structural descriptor, the Total Flexibility Index (TFI), a total of 104 individual descriptors were available for each polymer. A C5 Decision Tree routine (ID3 Algorithm available from RuleQuest Research Pty Ltd, 30 Athena Avenue, St Ives NSW 2075, Australia. http://www.rulequest.com/see5-info.html) was used to calculate a measure of significance, the Information Gain (IG)[31] of each of the 104 descriptors for a given bioresponse. A Monte Carlo approach was used to account for experimental uncertainty. A sequence of 500 000 computer-based (pseudo) experiments was performed varying the value of bioresponse for each polymer randomly but within a normal distribution (defined by the experimental standard deviation) about the mean. Each pseudo-experiment yields a single ''most relevant'' descriptor – the descriptor with the highest Information Gain. The results for all 500 000 pseudo experiments were tallied in a histogram. The three descriptors with the highest counts in this histogram were then selected as the input variables for the ANN.

Design of the Artificial Neural Network (ANN) Model

The ANN was a two-layer perceptron. Its inputs are the three descriptors obtained from the Decision Tree analysis, and its output is a prediction of a specific bioresponse. ANN methodology is comprehensively described elsewhere.[32] Using half of the experimental data set (the training set), the ANN was trained by minimizing the mean-square difference between the ANN-predicted values and the experimental values. Training was accomplished using a Genetic Algorithm.[33] The accuracy of the ANN was assessed by comparison of the predicted values and experimental values for the remaining half of the experimental data set (the validation set). The effect of experimental uncertainty on ANN predictive capability was assessed via a Monte Carlo analysis. A sequence of 100 experimental data sets was generated wherein the mean value of the bioresponse for a given polymer was perturbed by a random number obtained from a normal distribution derived from the standard deviation of the experimental bioresponse values measured for that polymer. For each experimental data set, an ANN was built using half of the experimental data set selected at random to train the ANN. The accuracy of the ANN was determined by comparison of the predicted and experimental values for the remaining half of the experimental data (the validation data set).

Results and Discussion

Synthesis

Traditional Materials Development versus Combinatorial Materials Design

Kohn's group has used tyrosine-derived monomers to prepare a wide range of polymers. Among those, tyrosine-derived polycarbonates[34] and tyrosine-derived polyarylates[8] are of particular relevance to highlight the difference between a traditional polymer development approach and a combinatorial approach. The polycarbonates were prepared entirely by conventional polymer synthesis techniques: each of the four different tyrosine-derived polycarbonates described in the literature[34] was prepared sequentially. Nine months were required to optimize the synthesis for each of the test polymers and to prepare sufficient quantities for testing.

The polymers had identical backbone structures but differed in the length of an alkyl ester pendent chain attached to the backbone of each repeat unit. A substantial amount of data was collected on the effect of increasing pendent chain-length on selected polymer properties, such as glass transition temperature, rate of chemical degradation, surface hydrophobicity, and mechanical properties. However, while each polymer was carefully characterized, it was impossible to distill data collected for individual polymer properties into generally applicable correlations that would permit extrapolations beyond the four specific polycarbonates tested. Further, while cell culture data were reported,[34,35] it was impossible to predict the biologically relevant performance characteristics of any one polymer based on its molecular structure and basic biophysical features.

In contrast to the ''random walk'' through polymer design space taken during the development of polycarbonates, one of the main goals of modern biomaterials research is to develop a new generation of functional biomaterials that are rationally designed to produce a biological response that is optimal for the intended clinical application. In view of this challenge, tyrosine-derived polyarylates were designed as a combinatorial library and all 112 currently available polymers were prepared within one week in a ''home-made'' parallel synthesis reactor.[8]

Design of the Library of Polyarylates

The basic structure of desaminotyrosyl-tyrosine alkyl esters (DTR, see Figure 2) consists of a unit of ''desaminotyrosine'' and a unit of L-tyrosine alkyl ester, linked together by a regular peptide bond. DTR is a derivative of naturally occurring tyrosine dipeptide with the important structural modification that the N-terminus of the peptide has been replaced by a hydrogen atom and the C-terminus of the peptide is protected by a pendent chain ''R'' of variable length and structure (Figure 3). This particular design gives rise to a versatile diphenolic monomer.

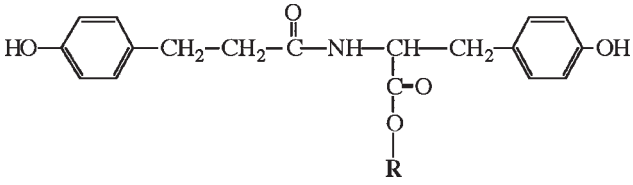

Figure 2. Chemical structure of DTR diphenols. Note that the monomers form an homologous series, differing only in the length of their respective pendent chain (R). Commonly used pendent chains are ethyl (E), butyl (B), hexyl (H), octyl (O), and dodecyl (D) esters.

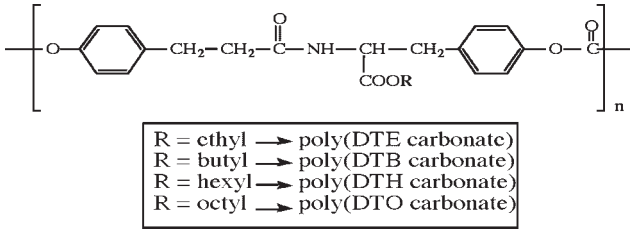

Figure 3. Chemical structure of tyrosine-derived polycarbonates. See Figure 2 for corresponding structures of monomers.

In polyarylates, a diphenol and a dicarboxylic acid are copolymerized, forming a strictly alternating A–B copolymer similar to the copolymerization of a dialcohol and a dicarboxylic acid (leading to a polyester) or the copolymerization of a diamine and a dicarboxylic acid (leading to polyamides). Thus, if the dicarboxylic acids are regarded as the A monomer, and the diphenols are the B monomer, a combinatorial design is obtained when a set of x structural variations of ''A'' and y structural variations of ''B'' are copolymerized in all possible combinations. Considering the ultimate applications of the polymers as medical-implant materials, the specific dicarboxylic acids used were selected from the constituents of the Krebs Cycle, various nutrients, and from the FDA's list of approved food additives (EAFUS Listing). Overall, eight dicarboxylic acids and 14 diphenols were used, resulting in 112 individual polymer compositions.[8,27] For the initial synthesis of the library, about 100 to 200 mg of pure polymer was obtained from a 25 mL reaction vessel.[8]

Polymer Characterization and Development of Data Sets

Increasing the pace of polymer synthesis is not useful without a corresponding acceleration in the pace of polymer characterization and evaluation. To analyze the more than 100 polymers contained in the library of polyarylates, Kohn et al. developed a series of new assays for the rapid characterization of large polymer sets.[8,27,36–40] With only 30 mg of polymer sample, glass transition temperature, melting temperature, decomposition temperature, the position of low-temperature transitions, an estimate of the flexural modulus, surface hydrophobicity, surface chemical composition (by XPS) and surface topography by scanning electron microscopy (SEM) and/or atomic force microscopy (AFM) were obtained. Using miniaturized compression-molded thin films, an additional 15 mg of polymer was sufficient to generate disks which were inserted in 96-well tissue culture plates and used to evaluate the ability of these polymers to support cell growth.[27]

In the course of the characterization of the 112 polymers, data sets with hundreds of individual data points were obtained. The most intuitive way to analyze such large data sets is by visual inspection of appropriately arranged graphic presentations (Figure 4). By defining a new structural polymer descriptor, the ''total flexibility index'', the graphic presentation of Figure 4 could be transformed into a correlation between chemical structure and the glass transition temperature (Figure 5).[27] As this correlation was shown to extend beyond the 112 polymers contained initially in the polyarylate library,[41] measurements performed on a representative subset of about 40 polymers made it possible to predict the glass transition temperature of every polyarylate that can theoretically be derived from the copolymerization of structural variants of the particular templates of ''A'' and ''B'' used here.

To facilitate data analysis, it is possible to define a library space.[41] If n different properties are being measured for each polymer, the corresponding data set can be mapped in ''n-dimensional space'' from which any number of two-dimensional projections can correlate two polymer properties of interest. For example, using the correlations for glass transition and air-water contact angle, the ''boundaries'' of the library space were determined (Figure 6). For all combinations of glass transition temperature and air-water contact angle that fall within the shaded area of the plane, a polymer structure can be identified that will exhibit the desired combination of properties.

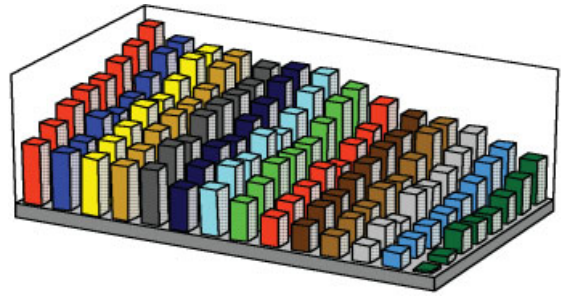

Figure 4. Bar graph presentation of the glass transition temperatures of 112 polymers in the polyarylate library. When appropriately sorted, the bar graphs clearly show the progression from low to high T_g, the upper and lower limits of possible T_g values, and intuitively illustrate that there may be a correlation between chemical structure and the glass transition temperature. Similar presentations were prepared for surface hydrophobicity, mechanical properties, and various cellular responses (not shown).

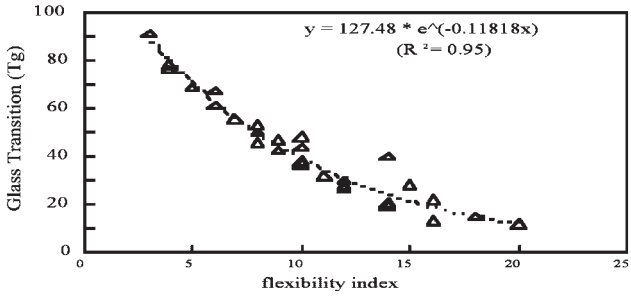

Figure 5. Exponential correlation between the glass transition temperature of individual polymers contained within the library of polyarylates and the "total flexibility index" (x), an empirically derived parameter that describes the chemical structure of the polymers. Using the equation $y = 127.48e(-0.11818x)$, the glass transition (y) of every one of the thousands of theoretical polymer structures contained within the library can be predicted.

Protein Adsorption Assay

Clean material surfaces that come in contact with serum-containing fluids in vitro or in vivo are rapidly covered by serum proteins. Numerous studies reveal that different synthetic, polymeric substrates vary in their ability to adsorb proteins and, therefore, vary in their ability to support cell attachment and growth.[42–45] In fact, the wide interest in protein surface adsorption in the biomedical community is motivated by the hypothesis that cellular responses to polymeric surfaces are mediated by the protein layer that adsorbs on the surface.[12,23,46,47] The type and conformation of the protein layer forming on a polymer surface may therefore be a major determinant of the suitability of a given polymer surface for biomedical applications. For example, adsorption of fibrinogen, one of the most prevalent proteins in blood, to a polymer surface reduces the blood compatibility of that surface.[23,48,49]

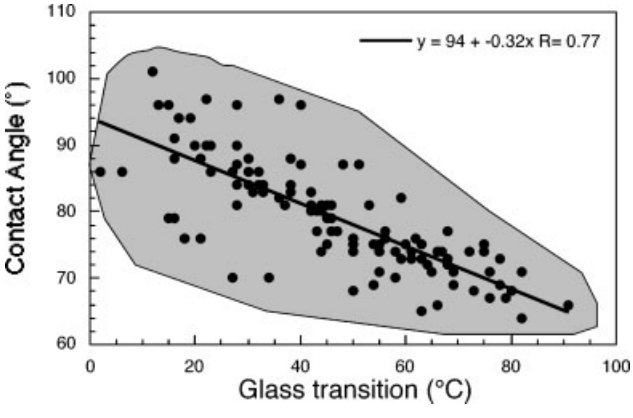

Figure 6. Illustration of the library space defined by a two-dimensional projection of the air-water contact angle (θ) and glass transition temperature (T_g). The dark points are the coordinates of polymers that were synthesized and analyzed. For all combinations of T_g and θ that fall within the shaded area, a polymer with corresponding properties can be found in the existing library of polyarylates.[39]

For the reasons mentioned above, a cost-efficient, rapid technique for the study of protein-polymer interactions was critically needed. Previously available assays for the detection of surface-adsorbed proteins, such as immunoblotting[50] or surface plasmon resonance,[51] are accurate but far too costly or too time consuming to be applied to the rapid screening of a large number of polymer samples. Therefore, we designed an immuno-fluorescence assay that could be conducted in commercially available, large capacity microplates using highly sensitive detection of surface-adsorbed fibrinogen with fluorochrome-labeled antibodies. Utilization of standard microplates allowed the use of conventional microplate processing and reading devices.

We screened a selected set of 45 polymers for polymer-adsorbed human fibrinogen using the immuno-fluorescence assay described above. The results show a polymer-dependent adsorption of fibrinogen (Figure 7). A group of polymers with low fibrinogen-binding capacity (left side of the chart) and a group with high fibrinogen-binding capacity (right side of the chart) could be identified. The statistical comparison of each of 10 low fibrinogen-binding polymers (nos. 1–10, Figure 7) with each of 10 high fibrinogen-binding polymers (nos. 36–45, Figure 7) showed significant differences in the amount of fibrinogen adsorption between the polymers ($p < 0.001$). This data set was used to train an Artificial Neural Network (ANN) model (see below).

Protein adsorption is strongly influenced by the hydrophobicity of the surface, as measured by the air-water contact angle (θ). Several studies therefore explored to what extent θ correlates with the adsorption of proteins when a material comes into contact with protein-containing solutions.[13] In turn, the layer of adsorbed proteins affects cell growth,[52] indicating that a simple measure of surface hydrophobicity (θ) may correlate with many cellular responses.[53] To test this hypothesis, we plotted the air-water contact angle (θ) versus fibrinogen adsorption for all 45 polymers (Figure 8). From the figure, it can easily be seen that the relationship between fibrinogen adsorption and θ is not a linear correlation ($R^2 = 0.43$). While linear curve fitting failed to shed light on the relevance of θ on fibrinogen adsorption, the ANN provided additional insights (see below).

Genotypic Expression of Proinflammatory Cytokines in Macrophages

Macrophages play a critical role in invoking inflammation and the foreign-body reaction to biomaterials. Upon initial contact of macrophages with foreign objects, a variety of cellular reactions are triggered, including the production of reactive oxygen species, and the release of pro-inflammatory (e.g., IL-1β and IL-6) and anti-inflammatory cytokines

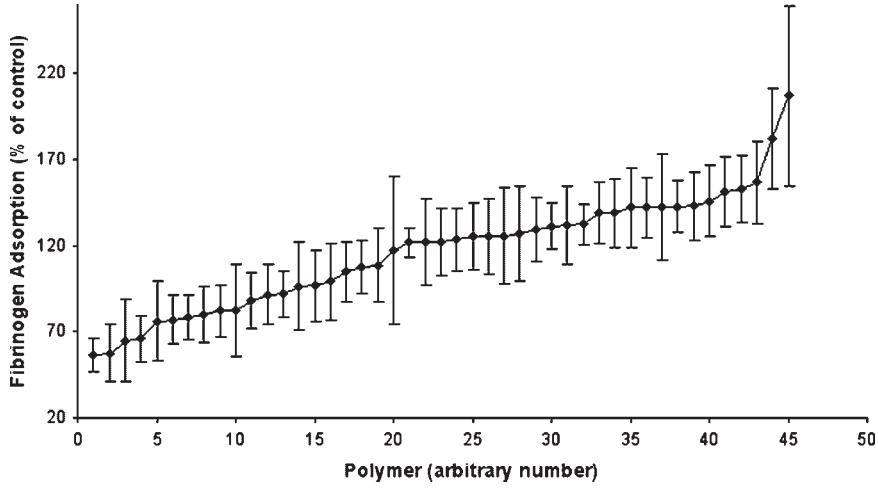

Figure 7. Immuno-fluorescence screening of adsorbed human fibrinogen on 45 polymers (mean ± SD; $n = 16$).

(e.g., IL-10 and TGF-β). The interest in studying macrophage interactions with material surfaces is motivated by the need to better understand the mechanism of this interaction, and by the need to design biomaterial surfaces that lead to controllable macrophage-surface interactions. We are currently addressing these objectives by developing efficient assays to explore the up- or down-regulation of gene expression of pro-inflammatory cytokines in macrophages that are in contact with different polymer surfaces. Here, we report preliminary results from the evaluation of 72 polyarylates (Figure 9). This is currently the largest set of polymers for which gene-expression data have been obtained within one single experiment. Since the 72 test polymers were carefully characterized in terms of polymer structure and material properties, the changes observed in the gene expression of IL-1β and IL-6 can potentially be correlated with the wide range of material properties, protein adsorption data, and cell-growth data obtained in the course of our studies.

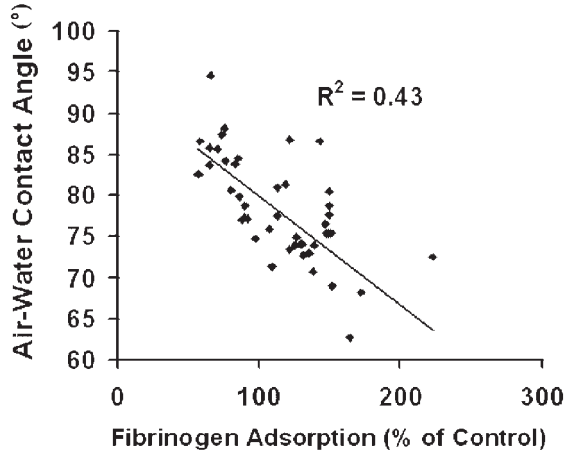

Figure 8. No correlation is evident between air-water contact angle θ and human fibrinogen adsorption. Amount of adsorbed fibrinogen ($n = 16$) is given in percent relative to a polypropylene surface which served as internal control.

Computational Modeling of Protein Adsorption and Cell Growth

Computational modeling is a necessity if large and complex data sets like those presented in the previous sections are to be useful in biomaterials development. In similar cases, semiempirical, or ''surrogate,'' modeling has been shown to be an efficient approach.[13] We present the results of our semiempirical Surrogate Model methodology to predict fibrinogen adsorption and fetal rat lung fibroblast growth.

Fibrinogen Adsorption

As outlined in Materials and Methods, a large number of structure-based polymer descriptors were calculated. Next a Decision Tree routine was used to identify those descriptors that were most relevant to fibrinogen adsorption. The three most significant descriptors obtained from the Decision Tree analysis are compiled in Table 2. These descriptors were used as inputs in the ANN, which was then trained on half of the available data set. The ANN predictions of fibrinogen adsorption on polyarylate surfaces are given in Figure 10. Overall, the ANN made correct predictions for 38 of the 45 test polymers (Figure 10). The average percentage root mean square (rms) error in prediction of the validation set was 35%, which compares favorably with the average percent relative standard deviation of the experimental measurements (i.e., 18%). The Pearson correlation coefficient for the validation data sets was 0.54 ± 0.12, also indicating a high degree of predictive capability.

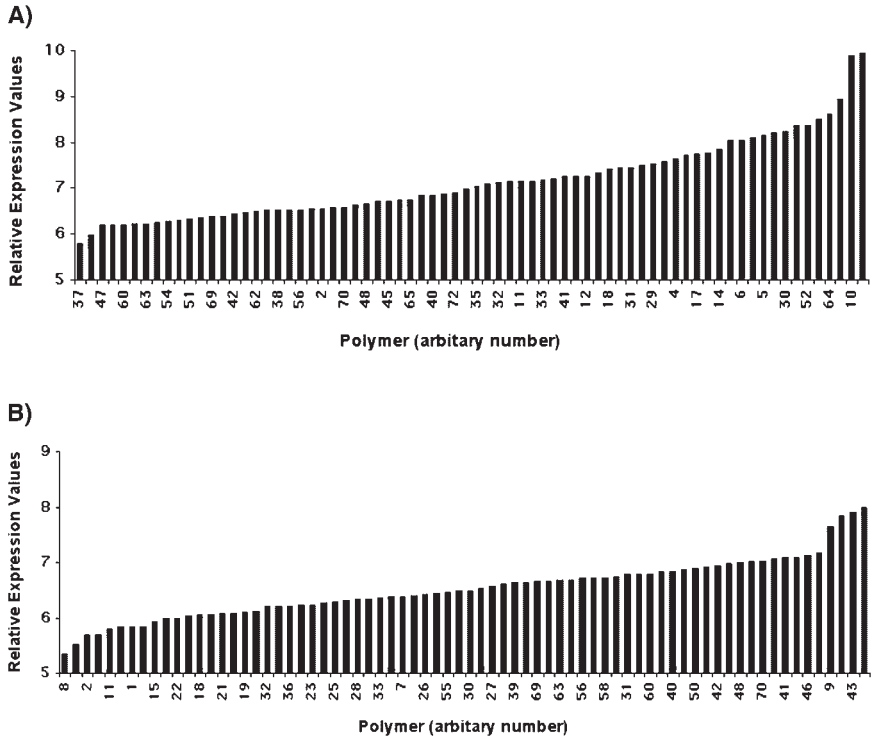

Figure 9. Gene-expression levels of pro-inflammatory cytokines by rat peritoneal macrophages grown on 72 different polymer films. Bars indicate relative expression values. Polymers were given a unique identification number (X-axis) and arranged in order of increasing expression values. The expression values shown represent the average of two separate experiments. (A): Gene-expression levels for IL-1b. (B): Gene-expression levels for IL-6.

Rat Lung Fibroblast (RLF) Proliferation

The results of the Decision Tree analysis relating to RLF proliferation are compiled in Table 3. Among the three most relevant descriptors was log *P*, an empirical estimate of the octanol/water partition coefficient of individual polymer chains. This is a very interesting result as several studies report an empirical correlation between surface hydrophobicity and cell growth. The ANN predictions of RLF NMA are given in Figure 11.

Overall (using training set and validation set data) the ANN predicted cell growth correctly for 41 out of 48 polymers to within the average percent experimental error of 23. Using a more stringent test, and considering the ANN predictions for the validation set only, the ANN predictions were within the average percent experimental error for 19 out of 24 polymers. This represents a 79% success rate of making correct predictions of cell growth on a wide range of polymer surfaces. The average percentage rms error of the validation set was 28% (correlation coefficient: 0.54 ± 0.09). The ANN was less successful in making correct predictions at both the low and high extremes of cell growth. Work is currently underway to understand the limitations of the model and to improve it.

Table 2. The three most significant polymer descriptors for the prediction of fibrinogen adsorption.

Descriptor Name	Definition	Significance Gain[a)]
T_g	Experimentally measured glass transition temperature	823
a_nH	Number of hydrogen atoms in the molecule	509
log *P*(o/w)	Log of the octanol/water partition coefficient (including implicit hydrogens) calculated from a linear atom type model	489

[a)] Decision Tree Monte Carlo descriptor results for fibrinogen adsorption. ''Significance Gain'' indicates the ratio between the number of pseudo experiments in which the given descriptor was found to have the highest IG to the number of experiments in which a random parameter was found to have the highest IG.

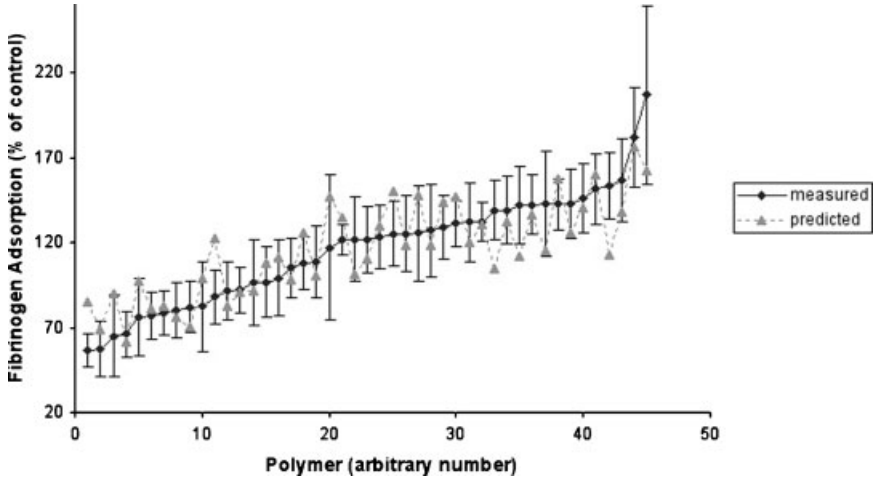

Figure 10. Comparison between experimental values for fibrinogen adsorption and the values predicted by the ANN. The experimentally determined amounts of fibrinogen adsorbed to different polymer surfaces are shown as diamonds. The error bars relate to the rms error of the experimental measurements. The values predicted by the ANN are shown as triangles. The ANN used the three descriptors given in Table 2 as inputs. For 38 out of 45 polymers, the ANN correctly predicted the experimental value within the error range of the measurement. This accuracy of prediction holds for both the training and validation sets.

Table 3. The three most significant polymer descriptors for prediction of rat lung fibroblast (RLF) growth.

Descriptor Name	Definition	Significance Gain[a)]
Slog *P*_ VSA9	The sum of the VdW surface area of atoms in the molecule for which the value of the log *P*(o/w) descriptor is greater than 0.4.	483
Hydrophilic factor	An empirical index obtained as a count of the number of hydrophilic groups.	331
Slog *P*_ VSA5	The sum of the VdW surface area of atoms in the molecule for which the value of the log *P*(o/w) descriptor is greater between 0.15 and 0.20.	316

[a)] Decision Tree Monte Carlo descriptor results for RLF cell growth on different polymer surfaces. ''Significance Gain'' indicates the ratio between the number of pseudo experiments in which the given descriptor was found to have the highest IG to the number of experiments in which a random parameter was found to have the highest IG.

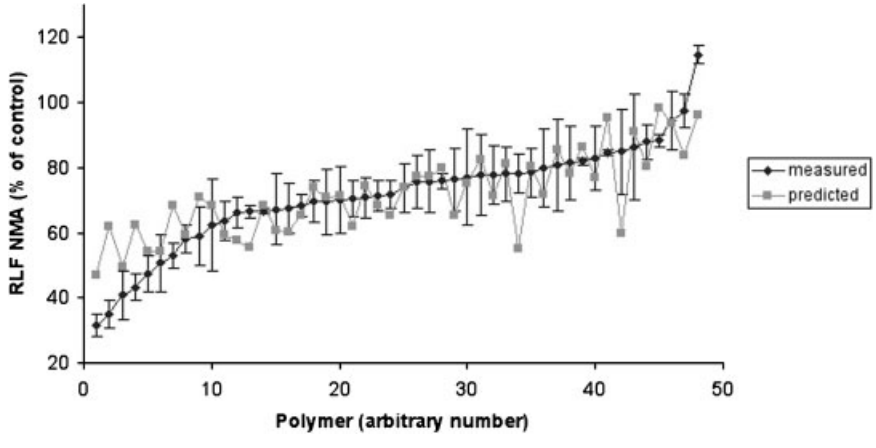

Figure 11. Comparison between experimental values for the normalized metabolic activity (NMA) of rat lung fibroblasts (RLF) and the values predicted by the ANN. NMA values are a measure of the number of cells growing on a polymer surface. The experimentally determined NMA values for different polymer surfaces are shown as diamonds. The error bars relate to the rms error of the experimental measurements. The values predicted by the ANN are shown as triangles. For 41 out of 48 polymers, the ANN prediction was within the average error range of the measurement. The ANN used the three descriptors given in Table 3 as inputs.

Conclusion

New approaches to biomaterials development are driven by three major, recent technological developments: (i) efficient parallel synthesis of polymers for biomaterials applications, (ii) rapid screening assays for various cellular responses (bioresponses) in the presence of polymers, and (iii) semiempirical modeling using techniques of machine learning. While all of these techniques are well known the field of drug discovery, they have so far not been applied to the challenge of biomaterials discovery in an integrated fashion.

Combinatorial libraries of more than one hundred biomaterials have now been prepared in sufficient quantities for in vitro assays. Rapid screening assays for biologically relevant material properties such as fibrinogen adsorption, cellular proliferation and macrophage genotypic expression have been developed and can be performed at substantially lower cost and in shorter time than previously possible. Surrogate (semiempirical) modeling techniques based upon machine learning technology can be effectively used to screen polymer libraries for identification of high-performance polymers. The successful prediction of protein adsorption and cell growth on different polymeric surfaces by a computational model presents a significant breakthrough and illustrates that the integration of combinatorial and computational techniques into a consistent design approach can reduce the time and cost required to identify biomaterials with optimum performance characteristics for specific clinical applications.

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