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Computational modeling of in vitro biological responses on polymethacrylate surfaces

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ABSTRACT

The objective of this research was to examine the capabilities of QSPR (Quantitative Structure Property Relationship) modeling to predict specific biological responses (fibrinogen adsorption, cell attachment and cell proliferation index) on thin films of different polymethacrylates. Using 33 commercially available monomers it is theoretically possible to construct a library of over 40,000 distinct polymer compositions. A subset of these polymers were synthesized and solvent cast surfaces were prepared in 96 well plates for the measurement of fibrinogen adsorption. NIH 3T3 cell attachment and proliferation indices were measured on spin coated thin films of these polymers. Based on the experimental results of these polymers, separate models were built for homo-, co-, and terpolymers in the library with good correlation between experiment and predicted values. The ability to predict biological responses by simple QSPR models for large numbers of polymers has important implications in designing biomaterials for specific biological or medical applications.

1. Introduction

Following implantation of a biomaterial in the body, protein adsorption occurs within seconds around the new implanted materials [1]. Cells thus interact with these adsorbed proteins rather than the biomaterial itself [2,3]. This initial protein adsorption plays an important role in determining the biocompatibility of the implant [4,5]. For example, within the context of a blood-contacting implant, the level of fibrinogen adsorption is a predictor of the implant's tendency to cause thrombosis: When fibrinogen is adsorbed strongly to an implant surface, the implant has a greater tendency to lead to thrombosis (blood clotting) than when an implant surface is designed to resist fibrinogen adsorption. Cells may then attach and grow further on implanted biomaterials. Some implant applications (for example contact lenses) require "non-fouling surfaces", e.g., surfaces that resist protein adsorption and subsequent attachment and growth of cells. Other applications (for example tissue engineering scaffolds) require that the implant surfaces support the attachment and subsequent proliferation of cells. Therefore, the levels of protein adsorption, cellular attachment, and proliferation on implant surfaces are important design parameters in the development of new biomaterials for any type of medical implant.

The recent advances in polymer combinatorial chemistry [6,7] have the potential to transform biomaterial development and translational use. Beginning with a small number of monomers, combinatorial parallel synthesis can generate thousands of polymers by varying the monomers and proportions of the monomers synthesized into homo-, co-, or terpolymers. These polymer libraries can now be synthesized in high throughput fashion [8] in sufficient quantity and purity that enables biological and physico-mechanical testing. Such tests could conceivably screen biomaterials with specific properties tailored for individual medical applications. However, given the large size of the polymer libraries such a screening process would be tedious, prone to experimental error, and require tremendous expense. Consequently, the capability to synthesize libraries of new polymers has now outpaced the ability to test the properties of the individual polymers for potential applications. Computational modeling may mitigate such issues by funneling the vast polymer libraries into a testable subset most likely to fit the specifications for a desired application.

The Combinatorial Computational Method (CCM) takes advantage of combinatorial synthesis, rapid screening and computational modeling as a biomaterial invention tool [9]. In this integrated

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approach a virtual library is formulated with a number of related monomer repeat units comprising all possible homo-, co-, and terpolymer combinations. In the place of comprehensive synthesis and testing of biological or material properties of the entire library, the semiempirical, quantitative structure property relationship (QSPR) method [10] may be able to predict particular polymer properties. This method is widely applied in the pharmaceutical industry to develop predictive models for a property of interest and ligand based design of compound libraries for virtual screening. To extend this technique to biomaterials, experimental values are obtained from representative "reference compounds" and QSPR is used to develop a predictive model that is extended to the larger library of polymers. Subsequently these predicted values are experimentally validated [11–17].

To test the validity of this method, the methacrylate family of polymers was selected as a model biomaterial system [18]. Polymethacrylates are extensively used in medicinal and industrial applications, and numerous methacrylate monomers are commercially available. For our analysis, 33 methacrylate monomers were selected as the building blocks of the polymer library (Fig. 1). In addition to homopolymers synthesized from the individual monomers, numerous combinations for co- and terpolymers are possible by varying the proportions of the different monomers. For this work, copolymers of all 33 monomers were selected in the defined ratios of 50:50, 25:75, and 75:25, leading to more than three thousand possible copolymers. Terpolymer blends of 33:33:33 were also included, leading to more than forty thousand polymer combinations in the virtual polymer library.

A subset of 130 homo-, co-, and terpolymers were chosen for synthesis and evaluation of protein adsorption and cell-material interactions. Experimental data within a certain range of cutoff value for standard deviation were considered "usable" for modeling. To build the model for the virtual polymer library an Artificial Neural Network (ANN) is constructed based on usable data collected for the 130 methacrylate polymers together with computational data of physicochemical polymer properties. The novelty in this approach is that the method makes the calculation of descriptors for any composition of co- and terpolymers easier, as the descriptors are calculated from linear combinations of the homopolymer descriptors. This process avoids the need to recalculate descriptors for each possible composition of co and terpolymers and provides enormous flexibility and extendibility of the model.

The end goal is to develop an integrated flexible computational model capable of predicting complex interactions of proteins and cells with polymer surfaces. This is accomplished by rank ordering the polymers based on their computationally calculated properties, aiming to predict values and identify trends as close as possible to the measured values obtained in validation studies. Rank ordering is achieved by dividing the experimental data for the reference polymers into different bins or classes (e.g. low, medium, high). We applied computational models to predict the properties of the same set of reference polymers. These predicted properties are then grouped into similar bins and comparisons are made whether a particular polymer is in the same bin as found from experiment or not. Rank ordering of biomaterials with respect to certain properties is important as it reduces the time and effort needed to complete costly cell and protein studies.

2. Methods

2.1. Experimental

The reference polymers (homo-, co-, and terpolymers) were synthesized using an automated parallel synthesizer (SLT 100 Accelerator, Chemspeed, Basel, Switzerland) utilizing previously published methods [8,18]. Briefly, reactors equipped with septa and reflux condensers were inertized, cooled to RT, and degassed reagents (purified monomers, chain transfer reagent, and solvent) were charged by syringe transfer using a 4-needle tool while being purged with argon. The reactions were vortexed at 600 rpm at 70 °C for 20 h under argon. The reactions were then cooled to 20 °C and precipitated manually. The polymers were dried under vacuum for $\geq$24 h at 60 °C. Using this robotic instrument it was possible to produce sufficient quantities of structurally related polymers with diverse pendant ester groups. Once synthesized, the polymer compositions were confirmed with proton NMR spectroscopy (Varian 500 or 400 MHz). Polymers were characterized for molecular weight and polydispersity using previously published methods [8] and had molecular weights of between 100 kDa and 200 kDa and polydispersity index of less than 1.6 (measured in either *N,N*-dimethylformamide or tetrahydrofuran and calculated

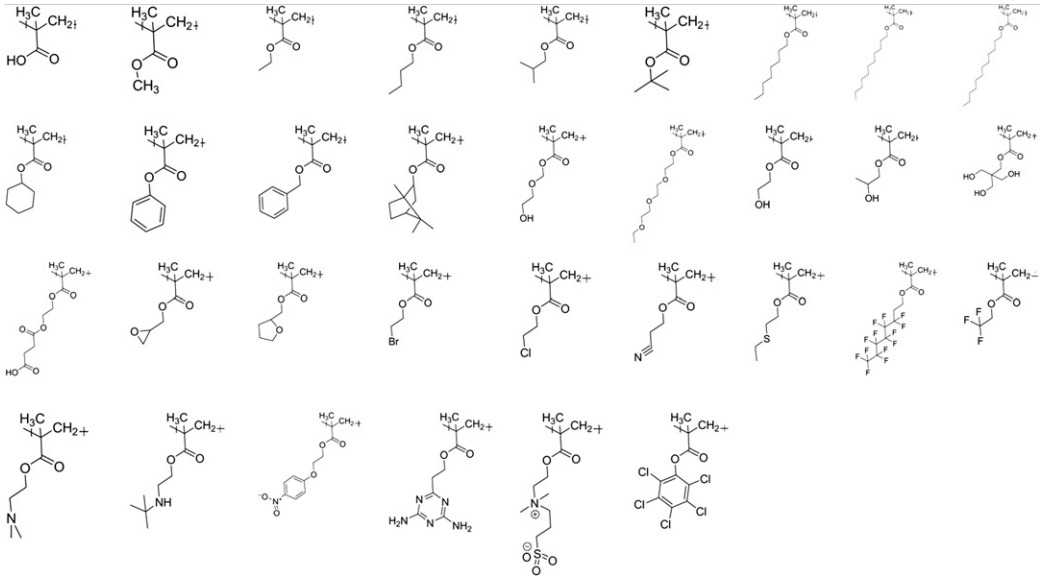

Fig. 1. Methacrylate polymers used in the library (in homopolymer form).