Expert Opinion

Combinatorial design of biomaterials for drug delivery: opportunities and challenges

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Background: The rational design of biodegradable polymeric devices for controlled drug delivery and tissue engineering is an important area of research for advancing new therapies for cancer, diabetes and immune-related disorders. In an era of escalating costs for discovery-based research, there is an urgent need to develop new and rapid methods to design drug delivery systems. *Objective/methods*: By merging this field of study with rapid and high throughput methods of design, optimization and development, researchers have been able to accelerate the discovery and design processes for these devices. Combinatorial research enables the rapid identification of key regions of interest. *Conclusion*: This review focuses on the opportunities and challenges in the area of combinatorial biomaterials design for drug delivery, as there has been a great deal of significant progress over the past decade to propel this approach for the rational design of biomaterials.

Keywords: biomaterials, combinatorial library, drug delivery, high throughput screening

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1. Introduction

Polymeric biomaterials are used in a wide range of biomedical applications from bio-absorbable prostheses [1] and drug delivery [2-5] to tissue scaffolding [6] and sutures. Their role in drug delivery has been a great success due to their biocompatibility and highly tunable behavior, which results in control over release kinetics [2,7], immune modulation [8], protein stability [7,9,10] and responsiveness to environmental changes such as pH [11]. The rational design of key polymer properties and controlled experimental conditions is essential so that their potential in these applications can be fulfilled. This is typically carried out through careful, time-consuming, conventional (i.e., one experiment at a time) design and development with multiple varying parameters, including polymer properties (e.g., chemistry, degradation mechanisms and kinetics, glass transition temperature, melting temperature), experimental conditions (e.g., temperature, buffers, pH, ionic strength) and polymer–cell interactions (e.g., cytotoxicity, proliferation, adhesion), with each variable differentially affecting the resulting experimental outcomes. Often this trial-and-error process, testing one variable at a time, can become an expensive and non-reproducible proposition, in which experiments take up a considerable amount of time. This is a multivariate problem and efficient methods enabling numerous variables to be tested simultaneously are required to advance the development of biomaterials for applications in drug delivery and tissue engineering [12].

The combinatorial approach to discovery was initially propelled into the spotlight by the pharmaceutical industry [1,13]. Difficulty in efficiently evaluating complex combinations of proteins, biomolecules and biological components

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directed the focus on combinatorial techniques, including biological assays and high-throughput automated sampling and data quantification (i.e., auto samplers and plate readers). The combinatorial approach has been applied to biomaterials and drug delivery development and has focused on the synthesis, characterization and optimization of the biomaterial properties best suited for specific applications in drug delivery. Drug delivery system design is a multivariate problem with interplay between the biomaterial, the drug, the processing conditions and the *in vivo* conditions into which the drug is delivered. Optimizing these variables to administer the drug at a specific rate to a specific organ or tissue is a daunting challenge that could benefit from the use of combinatorial methods. This methodology also plays a central role in the subsequent biological screening of cellular interactions with these biomaterials. The versatility of the combinatorial methodology makes it amenable to a number of applications and processes allowing for accelerated discovery, optimization and reduced experiment-to-experiment variability. It must be borne in mind that the combinatorial method is a screening tool, enabling the rapid identification of 'hot spots' or areas of interest in a large search space and it must be validated by conventional experimentation.

The various steps in the combinatorial process are shown in **Figure 1**, beginning with experimental design. The primary goal of this step is to design a process which focuses on a limited parameter space by employing previous knowledge, thus resulting in the most efficient method for collecting useful data. The experimental design is then implemented to rationally design and synthesize either a discrete or continuous combinatorial library, in which properties are varied systematically in one or more directions. Next, high throughput characterization techniques are utilized to investigate the structure–property relationships within the parameter space of the library, which minimizes experimental variability. The large data sets obtained through these analyses are then validated by informatics and statistical methods, which provide insight into the design and development for further experiments. The key findings and results discovered through the combinatorial approach are then validated by conventional techniques, allowing for an accelerated approach to the design and optimization of biomaterials for drug delivery. Throughout the process, there is feedback built into the system to enable optimization at every step.

The keystone of this process for biomaterial design begins with the creation of a single, multi-dimensional library [14]. This has been made possible by major advancements in lithography and robotics, enabling highly expedited deposition and synthesis of these libraries [15]. The libraries are amenable to high throughput characterization, employing methods such as Fourier transform infrared spectroscopy (FTIR), optical microscopy and gel permeation chromatography (GPC). This parallel characterization results in the rapid evaluation of properties such as phase behavior, contact angle and glass transition temperature, all of which play a critical role in the design of specific biomaterials as drug carriers.

The main goal of the combinatorial approach in drug delivery is to explore a vast array of biomaterials in a single, high throughput experiment which covers a large parameter space and allows for parallel screening. This reduces experiment-to-experiment inconsistency and provides a large database of information for employing informatics to identify hot spots [16]. This review provides a discussion of the various combinatorial methods that have been developed to synthesize and characterize biomaterials, the cell-based high throughput screening methods to characterize their interactions with cells and combinatorial drug delivery [17-19]. The article concludes with the authors' opinion on the challenges and opportunities provided by the combinatorial approach in the discovery and development of rapid and optimal drug delivery systems based on polymeric biomaterials.

2. Combinatorial biomaterial library fabrication and characterization

The combinatorial approach has been widely applied to synthesize libraries of polymeric biomaterials, as summarized in **Table 1**. Often, polymerization reaction conditions and duration cannot be varied, so making the process combinatorial involves synthesizing multiple polymers simultaneously while varying properties such as composition or molecular weight. Many polymeric biomaterials syntheses have been reported using a parallel approach [16]. Polymer libraries of poly(dimethylsiloxane) (PDMS) and poly(ε-caprolactone–block-dimethylsiloxane-block-ε-caprolactone) triblock copolymers were synthesized by Ekin and Webster with the use of combinatorial experimentation in which novel PDMS oligomers were synthesized by reacting ethylene carbonate with 3-aminopropyl terminated PDMS oligomers [20]. A secondary reaction of these oligomers with ε-caprolactone resulted in the triblock copolymers. Polyanhydrides based on 1 – 6 *bis*(*p*-carboxyphenoxy)hexane (CPH) and sebacic acid (SA) have been synthesized using a rapid microwave polymerization technique described by Vogel *et al.* [21]. A high throughput deposition and polymerization method of fabricating discrete polyanhydride libraries based on rapid prototyping and thiolene photopolymerization has been described by Vogel *et al.* [22]. In this method, thiolene-based multi-wells were used to robotically deposit anhydride monomers and the monomer library was then subjected to melt polycondensation under vacuum to result in a library of polyanhydrides. Langer and co-workers developed a method for synthesizing a library of poly(β-aminoesters) via the addition of bifunctional amines to bisacrylamides [23,24]. Brocchini and co-workers synthesized polyester libraries derived from serinol, producing a 16-member library by polymerizing four

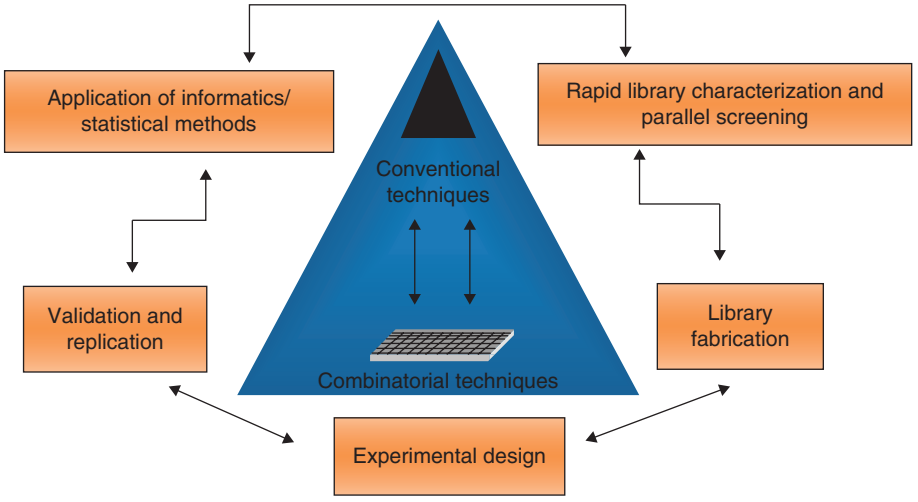

Figure 1. The combinatorial methodology.

Table 1. Polymeric biomaterials and the various high throughput screening methods that have been developed to study these materials.

Polymeric biomaterial	High throughput screening	Ref.
Polyanhydrides	Cytotoxicity Blend phase behavior Immune activation Drug delivery	[1,22,23,25]
PEG and polyesters	Cell adhesion and growth	[25,51]
Aminoesters	Transfection	[23,24,47-49]
Poly(ethylene glycol)-4000 diacrylate (PEG4000DA) and acryloyl-poly(ethylene glycol)-RGDS (Acr-PEG-RGDS)	Cell adhesion	[33]
Polyarylates	Cell proliferation	[31,47,50]
PLGA and poly(ε-caprolactone) (PCL) PVME and PS	Blend phase behavior Cytotoxicity Cell adhesion, growth, aggregation and protein production Cell alkaline phosphatase expression	[27,28,34,52]
Plasma polymerized hexane (ppHex) and plasma polymerized allyl amine (ppAAm)	Cell adhesion and growth 3D polymer scaffold synthesis	[35,43]
Poly(dimethylsiloxane) (PDMS) and poly(ε-caprolactone–block-dimethylsiloxane-block-ε-caprolactone)	Synthesis Structural characterization (NMR, DSC, FTIR and GPC)	[14]
Poly(dichlorodimethylsilane)	Cell adhesion	[36]
Poly(2-hydroxyethyl methacrylate)	Cell adhesion	[37]
Poly(L-lactic acid) (PLLA) Poly(D,L-lactic acid) (PDLLA) Polylactic-co-glycolic acid (PLGA)	Cell proliferation 3D polymer scaffold synthesis Bone growth	[38,39,41,57]
Polyglycolide and poly(hydroxyacetic acid)	3D polymer scaffold synthesis	[40]
Collagen-glycosaminoglycan (CG)	3D polymer scaffold synthesis	[58]

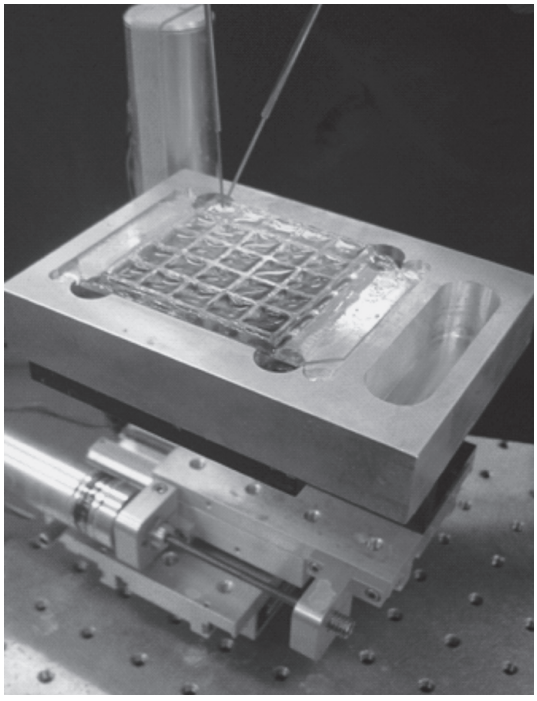

Figure 2. A discrete polyanhydride library polyanhydrides, increasing in SA composition from right to left and front to back (CPH is designated by the darker shade of grey and SA by the lighter shade of grey) [26].
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N-substituted serinol-diol monomers with four commercially available diacids [25].

The fabrication of both continuous and discrete polymer libraries has been reported by Narasimhan and colleagues with polyanhydrides [1,26] and by Meredith with polystyrene (PS) and poly(vinyl methyl ether) (PVME) [27]. **Figure 2** shows an example of a robotically deposited discrete polyanhydride library with a composition gradient. Such libraries have been used to study polymer blend phase behavior annealed over a temperature gradient stage by optical microscopy [1,28]. The polyanhydride phase behavior from the combinatorial experiments exhibited upper critical solution temperature behavior [1], which is consistent with the conventionally obtained phase diagram by Kipper *et al.* [29]. Meredith and co-workers reported a PS/PVME phase diagram with lower critical solution temperature behavior [27]. The polymer phase behavior plays a significant role in drug release as it controls the drug distribution within the system, which influences the rate of drug release [2,30]. Such rapid analysis of phase behavior will provide a basis for the rational optimization and design of porous three-dimensional polymer scaffolds for tissue engineering and drug delivery.

Other polymer properties have been measured at high throughput by Kohn and co-workers [31]. Polyarylate libraries were characterized by GPC, differential scanning calorimetry (DSC) and contact angle measurements. The glass transition temperature (T_g) determined from DSC was found to decrease in correlation with the presence of oxygen moieties in the polymer backbone, while the water contact angle was found to decrease with increasing polymer chain length [31]. Knowledge of such properties and their trends within the polymer library are important for the fabrication of drug delivery devices such as tablets and micro- and nano-spheres.

High throughput characterization techniques such as FTIR, GPC and optical microscopy allow for the rapid screening of polymer libraries. GPC and FTIR employ automated sampling, which enables parallel and rapid validation of molecular weight and polydispersity (GPC), and chemical composition, drug interactions and molecular weight (all with FTIR). FTIR microscopy has been used as a high throughput technique to characterize the linear variation of composition in polymer libraries, as shown in **Figure 3** [26]. Optical microscopy has been utilized to observe phase behavior when such linearly varying libraries are annealed along a temperature gradient in a direction that is orthogonal to the composition gradient [28,32]. In addition to the methods described above, there are a number of studies in the literature on combinatorial synthesis and screening of biomaterials [33-38], including 3D gradients of polymer tissue scaffolds [39-43]. These accelerated methods are amenable to numerous other applications involving property determination and characterization of polymer systems.

Understanding the relationship between important biomaterial properties (e.g., chemistry, T_g, T_m, phase behavior and drug–polymer interactions) and drug release is paramount for the design and optimization of drug delivery vehicles. For example, Kipper *et al.* have shown that the phase behavior of biodegradable polymer blends, specifically the length scale of the microphase separation of polyanhydride copolymers, significantly affects the thermodynamic partitioning of drugs in these systems, thus affecting their release kinetics [44]. When the solubility of the drug or protein within a copolymer phase is exceeded, the drug or protein is forced to disperse into less favorable regions, thus resulting in a rapid initial release or burst of the protein [44]. This is also important for the design of multi-drug releasing polymer systems and degradable polymer systems for protein stabilization [9,10]. Properties such as the T_g and T_m are important because they dictate the processability of the materials. For example, the low T_g of certain polyanhydride copolymers controls the conditions under which microspheres of these copolymers are fabricated [45,46]. Likewise the crystallinity of the biomaterial also affects the processability and degradation kinetics and hence the release rates of drugs from these materials [44].

3. High throughput cell-based screening

Combinatorial approaches have led to the development of biological assays and high-throughput plate readers, allowing

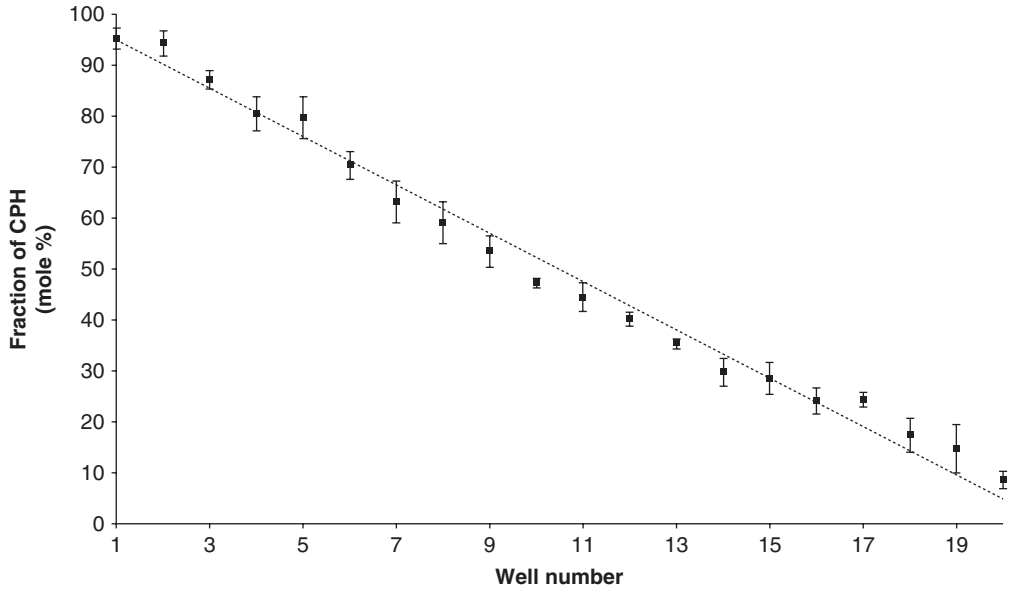

Figure 3. Mole percent of CPH varying along a discrete composition gradient library as determined in high throughput by FTIR microscopy [26].

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for rapid and parallel data acquisition and quantification [13]. When these high throughput methods are integrated with multi-dimensional polymer libraries, interactions of the polymeric carriers with drugs or cells can be assessed at high throughput. These include, but are not limited to, polymer chemistry effects on cell proliferation, adhesion, transfection, differentiation, cytotoxicity and immune modulation. Understanding and optimizing these processes can enable the development and optimization of protein therapies, tissue engineering scaffolds and vaccine delivery systems for localized treatments that target specific organs or tissues.

Cytotoxicity is an important factor to consider when designing polymeric biomaterials for *in vivo* applications. The concentration and composition of polymeric biomaterials often regulate cellular functionality and viability. High throughput methods have been developed to ascertain the cytotoxic effect of biocompatible polyanhydrides based on CPH, SA and 1,8-bis(*p*-carboxy phenoxy)3,6-dioxaoctane (CPTEG) on different cell lines [26]. The initial libraries studied were based on a gradient of 25 linearly varying polymer concentrations, which were fabricated using the multi-well system previously described [26]. These libraries were screened at high throughput using an *in vitro* cytotoxicity assay (i.e., the MTT assay) and demonstrated that concentrations of the CPH:SA copolymer system less than 14 mg/ml and of the CPTEG:CPH system less than 16 mg/ml resulted in total viability in Sp2/O myeloma cells when compared to the control groups. The average *in vivo* concentration for human applications is 0.5 mg/ml [8], thus demonstrating the viability of these copolymers as drug carriers. Further, 25-member polyanhydride libraries with a compositional gradient were synthesized and screened for cytotoxicity. It was observed that there were no resulting compositions of either copolymer systems found to be toxic to any of the cell lines [26].

Other role-specific biomaterials include transfection vectors, a group of specialized polymers studied by Langer and co-workers that collapse on DNA and transfect it into the cell [23,24]. This is a key process for controlling many intracellular functions by targeting the cell nucleus and has potential for use in cancer therapies. The commercially available transfection vector, polyethylene amine, has been problematic due to its inability to target and kill specific tumor cells. It was toxic to healthy cells, resulting in tissue damage and wounding. Langer and colleagues used high throughput techniques based on a library of poly(β-aminoesters) to address the cytotoxicity issue, which revealed a few specific cationic polymers that were not cytotoxic. These polymers were then further studied *in vivo* and found to eliminate the toxic effect on healthy tissue while reducing the tumor size by 40% [47].

Combinatorial cell-based transfection screening has also been used for the design of polymer libraries by synthesizing diacrylates with primary or secondary amines. The findings of the cell uptake studies emphasize the importance of the size of the polymer/DNA transfection complex and the polymer nitrogen to DNA phosphorus (N/P) ratio. Hydrophobicity was also shown to play a significant role in the transfection process. The histidine components of the polymer allowed for up to 4 – 8 times better transfection efficiencies than commercially available products. In further studies, this library synthesis process was automated, permitting the study of 1000 reactions

per day, creating a 2350 multi-component library [47,48]. This library was used to show that transfection was controlled by polymer molecular mass and endgroups [47-49].

Combinatorial methods provide an ideal platform to investigate cell–biomaterial surface interactions. This enables a rapid means for measuring and screening cell behavior, including proliferation, migration and cell attachment. Kohn and co-workers used a library of biodegradable carbonates to study the relationship between cell proliferation and polymer properties, including hydrophobicity and molecular weight [31,50]. It was demonstrated that the cells grew as a function of oxygen incorporation in the polymer backbone [31]. Further studies exploring the relationship between the hydrophobicity of polyarylates and cell proliferation found a significant trend relating the cell growth to decreasing hydrophobicity of the non-oxygen containing polymers [50]. Similar results were reported correlating cell proliferation with the oxygen content of the polymer [16].

Combinatorial methods can also be used to explore alternate polymer systems for improved cell attachment. Brocchini *et al.* synthesized a library of polyesters in the presence of N-substituted serinols, which added another dimension to the library by allowing for the addition of functional groups to the side chains of the polymers [25]. It was shown that hexyl side chains on the polyesters inhibited cell attachment. Similar studies with poly(ethylene glycol) (PEG) libraries were carried out by Langer and co-workers in which a polymer blend array of 3456 spots was screened for cell attachment and growth [51]. The screening results indicate that very little cell adhesion occurred until the blend approached 30 wt% poly(L-lactide-co-glycolide) (PLGA). This is attributed to phase separation in the copolymers, preventing cellular adhesion in the PEG-rich sections, which have minimal attachment area [25].

Cell adhesion and proliferation are important aspects in the design of tissue engineered scaffolds. Meredith *et al.* have studied the relationships between cell behavior, adhesion and proliferation, polymer library microstructure/roughness and composition [52]. They created a continuous combinatorial library varying between different compositions of PLGA and poly(ε-caprolactone) (PCL) which was annealed over a temperature gradient to vary the microstructure and roughness. There were significant correlations between cell adhesion and the average surface roughness of the amorphous PLGA and the crystalline PCL under normal cell conditions at 37°C. The authors reported that the cell adhesion was at its highest at the high temperature annealed polymer regions and for the mid–high PCL regions on the polymer library. They also investigated the effect of protein adsorption on the polymer library [52]. Increasing hydrophobicity of the carbon-rich regions of the library was found to increase protein adsorption. Although protein adsorption was found to be independent of cell adhesion early on in the experiment, prolonged studies demonstrated that the highest amount of protein adsorption correlated to the regions of high cell attachment (i.e., mid–high PCL regions) [52].

Combinatorial techniques have been developed to identify the effect of stem cell differentiation on libraries containing di and triacrylates mixed with a photoinitiator on a poly(hydroxyethyl methacrylate)-based coating [49]. The authors reported that nearly all compositions provided the necessary framework for cell adhesion and growth, but when retinoic acid was added to the cell growth medium, the monomer composition appeared to differentially regulate these cellular functions. This demonstrates the ability to control cellular behavior, thus leading to better optimization of tissue scaffolds in an efficient high throughput approach.

Polymer chemistry is hypothesized to play a significant role in immune modulation, which is a key factor for the design and optimization of polymers as adjuvants. A high throughput technique based on cytokine analysis has been developed to study the effect of polymer chemistry on the immune response [26]. Using a CPH:SA polyanhydride library and a macrophage cell line, a correlation was identified between adjuvant hydrophobicity (i.e., increasing CPH content) and TNF-α production (**Figure 4**), and between adjuvant hydrophilicity (i.e., increasing SA content) and IL-6 production. The production of TNF-α typically leads to inflammation and endothelial activation, whereas IL-6 can induce fever, T and B cell growth and differentiation, and production of acute phase proteins [53]. Based upon the cytokines released from antigen presenting cells such as macrophages or dendritic cells, the adaptive immune response can be modulated between a T helper 1 (TH1) cell-mediated response and a T helper 2 (TH2) humoral response. The TH1 pathway activates macrophages and cytotoxic T cells, which are directed to eliminate the invading foreign body and are effective at neutralizing intracellular pathogens; the TH2 pathway activates B cells leading to antibody production and long-term cellular memory, and is effective at dealing with extra-cellular pathogens. Tuning the immune response is important for the optimal design of vaccine delivery systems and their ability to target and activate the necessary intracellular processes for successful prophylactic treatment. With the numerous applications for polymers as adjuvants in vaccine delivery devices, knowledge of how the polymer adjuvant modulates immune response pathways (i.e., cellular vs humoral) is vital for the rational design and optimization of these devices.

4. Combinatorial drug release

Vogel *et al.* designed a high throughput method to study drug release from a linearly varying polyanhydride library based on CPH and SA [22]. The previously discussed rapid prototyping technique for multi-compositional library synthesis and rapid characterization of polymer properties was modified to study drug release. A non-reactive ultraviolet dye, ethidium bromide bisacrylamide, was encapsulated in

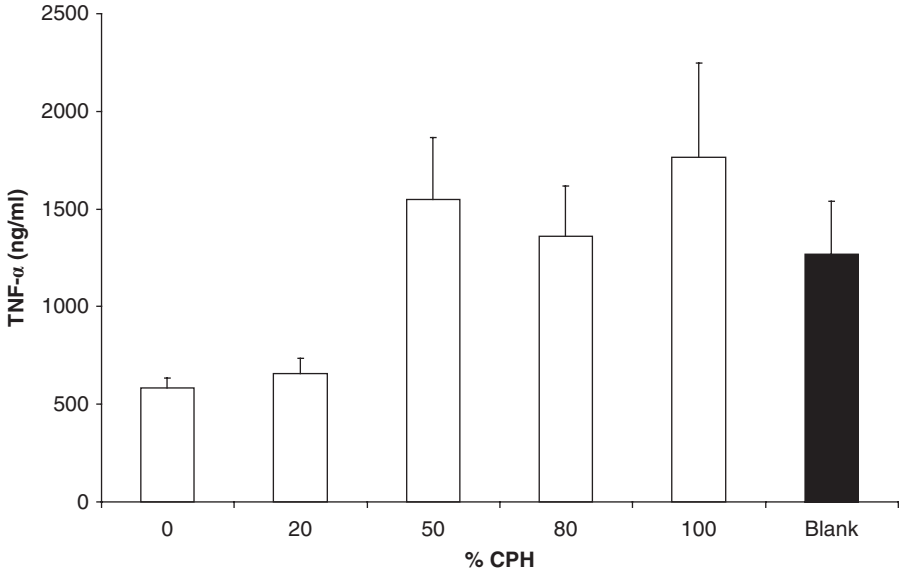

Figure 4. Correlation between mole percent of CPH and TNF-α production [26].
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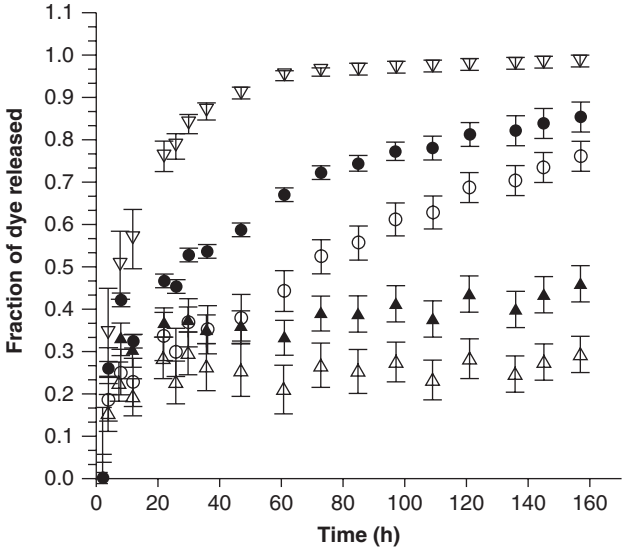

Figure 5. Fraction of dye released from a compositionally varying polyanhydride library of CPH:SA copolymers. The SA content increases incrementally from the bottom (50%) to top curve (100%) [22].
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the polymer libraries upon combinatorial polymer synthesis and released from the libraries as they were incubated in buffer. The results produced clear trends between the rate of dye released and the degradation characteristics of the polymer carriers. The higher the percentage of the faster degrading component, SA, the more rapid was the dye release (**Figure 5**) [22]. These results were consistent with conventionally obtained dye release profiles from this polymer system [2].

5. Expert opinion

The above examples clearly demonstrate that combinatorial methods have been successfully applied to screen biomaterials for applications in drug delivery and tissue engineering. However, many challenges and obstacles remain in the path to further the design of biomaterials for drug delivery and tissue engineering. The literature in this area mainly focuses on discrete and continuous polymer libraries, which are typically films and their correlations to cellular behavior, which are applicable to areas such as tissue engineering. However, the majority of drug delivery systems employ injectable devices. This emphasizes the need for the development of new combinatorial techniques to fabricate micro- and nanoparticles for drug delivery. The development of such a technique would significantly accelerate discovery by fabricating particles of multiple chemistries simultaneously, as well as decreasing batch-to-batch variability commonly associated within the conventionally synthesized polymer particles, which employ techniques such as double emulsion and spray drying [46,54-56].

The challenges in library fabrication add to the difficulties encountered with screening of intra-cellular processes such as trafficking and transfection. The intracellular processes are often difficult to track due to lack of space and resolution in a typical combinatorial library, which only allows for a restricted population of cells. The behavior of cells can be affected by numerous external variables that can hinder the objective of the experiment itself. Screening of these

processes can be expensive and difficult to apply to combinatorial libraries, hampering their ability to provide a large library of data. Thus, new methods are required in which large populations of cells can be screened at high throughput for intra-cellular information and processes. Additionally, there are limitations on developing combinatorial methods for drug release since these methods rely upon following a tag (e.g., fluorescent) that needs to be incorporated into the drug of interest.

The implementation of high throughput techniques will lead to the generation of large libraries of data. Due to major changes in information generation and composition, there is a need for highly developed statistical analysis techniques to address the multi-dimensional error analysis and refined informatics tools to process the enormous libraries of data. These statistical and informatics methods will become an invaluable resource to efficiently mine and analyze large data sets, better focusing the library parameter space on key areas of interest. The development of such methods will require close collaborations between researchers in the areas of computer science, biomaterials and bioinformatics.

In combining the multiple aspects of combinatorial discovery for biomaterials design, it is clear that there is a lack of formalized educational programs that meld analytical methods, molecular biology, biomaterials, chemistry, computer programming, etc. The multi-faceted disciplines related to this field present many hurdles for newcomers to the area, requiring a significant amount of time and effort to master the cross-cutting ideas in combinatorial science. So there is an urgent need to develop novel educational programs that provide both a strong scientific grounding and a balanced exposure to the various techniques in this area.

6. Conclusion

Combinatorial and high throughput approaches to design and optimize biomaterials for drug delivery will become increasingly important as molecular structures become more complex, more variables are thrust into system design and processes become more expensive [16]. In this brief review, we have discussed initial efforts that demonstrate the viability and effectiveness of this approach and its ability to accelerate discovery, provide new insights and reduce time and cost. We have discussed several applications covering the vast array of possibilities for this approach to the rapid discovery and design of biomaterials. High throughput techniques have been employed for processes ranging from biomaterials synthesis and characterization to the downstream development of parallel methods for drug release and cell screening. With the large array of parameters affecting many design problems in drug delivery and tissue engineering, this technique can be an invaluable resource for rapid design and optimization of biomaterials. However, this method is fraught with numerous obstacles and eliminating these will have to be a primary goal in the next decade if combinatorial methods are to be used routinely for biomaterials discovery and design.

Declaration of interest

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