A current perspective on high-throughput polymer science

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Recent years (2000–2003) have witnessed an upsurge in the application of combinatorial and high-throughput experimental methods to polymers. This review highlights the most recent developments in high-throughput polymer science, in order to put into perspective the articles selected for this special section of *The Journal of Materials Science*. This article begins by defining the unique challenges in polymer (and materials) high-throughput screening (HTS), with respect to other applications of combinatorial and high-throughput methods in drug discovery and catalyst development. Advances in the preparation of HTS libraries are then discussed, with a distinction made between libraries for synthetic investigation and libraries for formulation and characterization. Then, the recent applications of HTS to polymer analytical methods (for chemical characterization of synthetic libraries) and physical characterization methods (for formulation libraries) are presented. © *2003 Kluwer Academic Publishers*

1. Introduction: unique challenges of polymer high-throughput screening

Polymer high-throughput screening and characterization differs significantly from the more well-established combinatorial screening practiced in drug discovery, genomics, proteomics, and catalyst research. For example, in polymer and materials science, the goal of experimentation is not usually to search for a yes/no answer, e.g., does the drug work as expected? or does the antibody bind the target protein? Rather, there are an extremely large number of variables that must be tuned in order to achieve a specific target property. Because polymers are used in so many industrial and commercial applications, there is a large collection and range of target properties of interest, and usually a combination of properties is desired. Often, optimization of one property, e.g., modulus, causes detrimental effects in other properties, such as strength, permeability or clarity. In addition, in polymer science, there is a major focus on formulating mixtures of polymers and additives (plasticizers, UV stabilizers, etc.), in addition to de novo synthesis. Often, polymers achieve desired target properties only after formulation steps—and the number of combinations of formulation variables (compositions and processing steps) is formidable! For these reasons, polymer HTS presents several distinct challenges:

(1) *no universal screen* for positive 'hits'

 many non-high-throughput tests exist for polymers

(2) polymer properties are *continuous* variables

very few yes/no assays for selecting optimal materials

(3) some properties are *size*-dependent

how can results from miniaturized libraries be scaled?

(4) *most* properties are *process history-dependent*

 annealing temperature, orientation, fatigue history

(5) *data mining and statistical techniques* for library design and analysis do not have a history of common use in polymer science

In this brief review, the goal is to present the most recent work that aims to overcome and address these challenges. First, however, the reader should be aware that several excellent reviews of polymer [1–6] and materials [7–10] combinatorial and high-throughput screening have preceded this article. Information contained in these previous reviews is not presented in detail here. In addition, there is a tremendous literature on the topic of high-throughput protein and DNA screening using parallel arrays [11, 12] or resin-beads [13, 14], which is not reviewed here as these techniques are used as utility tools in proteomics and genomics, but not as tools for pure and applied polymer science. Also, there is a vast literature on the subject of polymer-based resins for use as solid supports in combinatorial organic synthesis. Because the focus is screening non-polymers, this work is not covered here, except where combinatorial synthesis is used to generate a diverse library of polymeric supports themselves.

2. Library preparation for high-throughput polymer screening

Two approaches have been applied to developing polymer combinatorial libraries: **discrete** and **continuous**. As the name suggests, discrete libraries have many individual 'samples' that are separated into distinct reaction or mixing wells. Discrete sampling allows precise

COMBINATORIAL AND HIGH-THROUGHPUT POLYMER SCIENCE
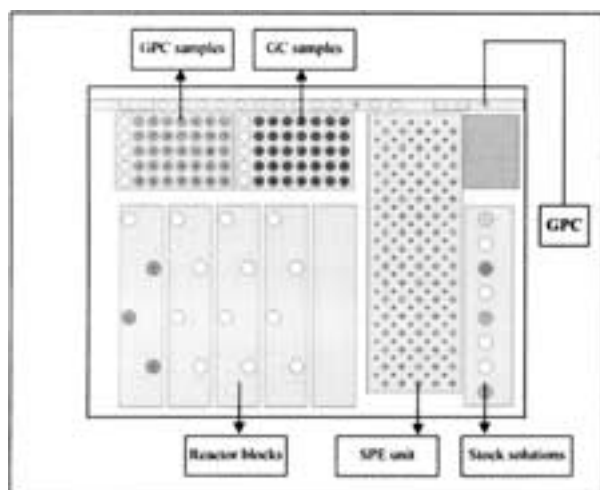
Figure 1 Schematic of an automated discrete library synthesis station: the Chemspeed ASW 2000. Reaction solutions are mixed using aliquots from the stock solutions and transferred to the reactor blocks using automated liquid handling equipment. Following synthesis in the heated and cooled reactor blocks, the products are sent to analytical instruments. A portion of each sample is sent, respectively, to either a gas chromatograph (GC) to monitor monomer conversion, or to a solid phase extraction (SPE) unit for purification prior to gel permeation chromatography (GPC) to measure molecular mass.

control over mixing by utilizing automated instruments and experimental design software already commercially available. Fig. 1 shows a schematic of a commercially available discrete library apparatus, the Chemspeed ASW 2000 automated synthesizer. In this computer-controlled instrument, five reactor blocks can be used in parallel and each block has 4 to 16 reaction vessels with volumes of 13 mL to 100 mL, respectively. The reaction vessels can be heated or cooled with a jacketed oil bath or reflux condenser. Discrete libraries offer the ability for true 'combinatorial' chemistry, by designing libraries that explore statistically relevant combinatorially designed selections of composition. For this reason, they are often the preferred library for combinatorial synthesis investigations, where the goal is to discover a new catalyst or product, or optimize a reaction. Despite these great strengths, the discrete technique does require careful selection of compositions via experimental design and is limited in its ability to mimic realistic processing and formulation conditions for polymers, e.g., shear conditions, temperature history, etc. In addition, if temperature or pressure are desired as combinatorial parameters, tedious and expensive addressing of pressure and temperature control to each sample well is required.

Continuous libraries offer a quick and streamlined method to search large ranges of composition and processing parameters through the creation of gradients in these parameters. Because most polymer processing is semi-continuous, e.g., melt extrusion, fiber and film drawing, the continuous library approach offers a better opportunity to capture realistic process steps compared to the discrete library approach. For this reason, many applications of continuous libraries have been focused on investigating formulation effects, as opposed to reactions and synthesis. In fact, the very first attempts in combinatorial materials science utilized composition-

gradient libraries of metals and metal oxides [15] and this trend continues today for developing phase diagrams for inorganic materials [16]. The goal of experimental design here is to choose the appropriate parameters (those with the greatest influence) and their ranges (where properties are most sensitive). Parameters such as composition, temperature, thickness, and surface energy are continuously varied in a gradient polymer library using specialized coating and mixing equipment. Recent examples include the composition-gradient flow-coating technique [1, 17], continuous polymer surfaces for biomolecule arrays [18], formation of thin polymer layers with gradients in molecular weight [19] and grafting density [20], and gradient-compositions produced with extrusion [21, 22]. Fig. 2a shows a digital image of a typical composition gradient library film [1, 17], in this case using a blend of poly(D,L-lactide) (PDLA) and crystalline poly(ε-caprolactone) (PCL). One can see the graduated change in color and clarity as the PDLA (non-crystalline) composition is increased linearly across the gradient. Fig. 2b presents a typical thickness-gradient produced via flow-coating a film of isotactic polystyrene [23].

Exploration of processing variables, particularly temperature, is particularly convenient with continuous temperature gradient instruments that are simple and cost-effective to produce. In fact, temperature gradients are common in pharmaceutical crystallization research. One approach for polymers utilizes an aluminum heating stage with a heater on one end and cooler on the other [1, 17]. Fig. 2c shows typical temperature variations along a silicon substrate surface that is being annealed on one such temperature-gradient stage [23]. It is important to note the limitations of continuous libraries, relative to discrete. First of all, there are few stand-alone commercial instruments for the preparation of composition, temperature, or thickness gradients for polymers. Usually instruments must be assembled from individual components by the user, which offers an advantage in cost, but does require a time-investment. Gradients ultimately lead to diffusion, convective flow, and heat transfer; but, these can be kept to an acceptable minimum with careful control and design of the sampling regions. In addition, the continuous libraries can only be properly referred to as high-throughput techniques, since they do not allow the combinatorial exploration (in the strict mathematical sense) of discrete arrays.

Libraries, whether they are discrete or continuous, can be divided into two categories based upon the purpose of the experiment. **Formulation libraries** mix pre-synthesized polymers to explore the effects of blending polymers and additives as well as processing variables like temperature and shear rate. **Synthesis libraries** are used to perform reactions to synthesize polymers de novo from monomers or oligomers, as a function of reactant stoichiometry, catalysts, pressure, or temperature. This distinction is very important, as the goal of identifying a new synthetic product is very different from searching for an optimal formulation or process condition. By a large margin, most of the work in polymer HTS has been focused on synthesis libraries. One of the greatest needs in high-throughput polymer
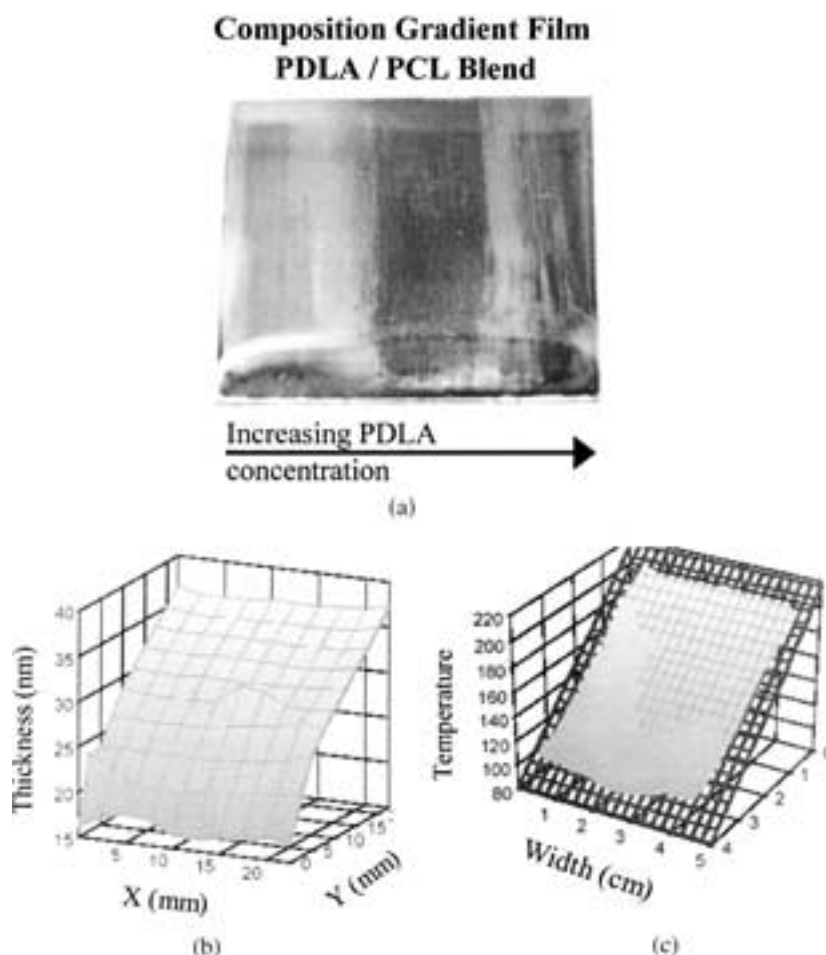
Figure 2 (a) Photograph of a continous composition gradient library of poly(D, L-lactide) and poly(ε-caprolactone), (b) a plot of a typical thickness-gradient library (isotactic polystyrene on a silicon wafer) (reproduced from [23], (c) a plot of a typical temperature-gradient produced with the aluminum gradient stage [23] showing both the measured temperatures on a silicon wafer placed on the stage (gray) and the expected linear profile based upon thermocouples embedded in the stage (black grid). (Reprinted with permission from [23]. Copyright (2003) American Chemical Society)

science is the development of new formulation library preparation and analysis techniques. Consider, for example, that most commercial polymers are mixtures of two or more (usually more) individual polymers or modifiers. Polymer properties are extremely dependent on the method of mixing, e.g., melt extrusion, milling, or solvent-based processing; the presence of mechanical stresses that might orient chains; and, the annealing temperature history.

3. Synthesis and formulation of high-throughput and combinatorial libraries: recent progress

3.1. Discrete library approaches

One of the early uses of discrete libraries in polymer science was screening for new or optimized polymerization catalysts [7, 24]. An example of the excellent recent work along these lines is the discovery of single-site chromium catalysts for ethylene polymerization [25]. The recent past has seen the application of discrete parallel library polymerization to investigate and optimize a wide variety of polymerization reactions. For example, an automated synthesizer (Chemspeed ASW2000) with 16 parallel reactors that have individual temperature control was used to optimize the reac-

tion temperature for the ring-opening polymerization of 2-ethyl-2-oxazoline [26]. Products were characterized with gel permeation chromatography (molecular mass) and gas chromatography (monomer conversion). Fig. 3 shows the molecular mass versus reaction time at multiple temperatures, with several repeated temperatures as internal standards. Each profile leads to a reaction rate constant (k) and an Arrhenius plot ($\ln(k)$ vs. T^{-1})
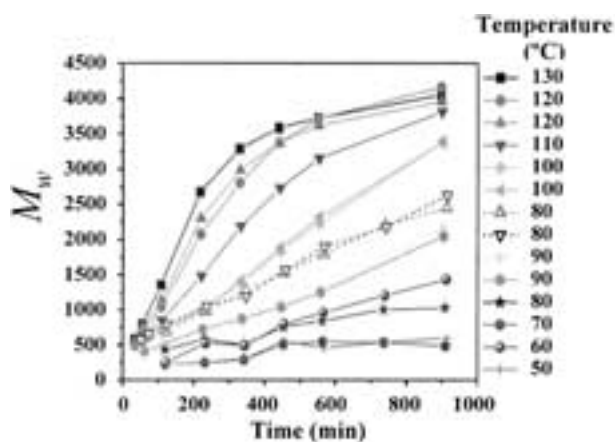

Figure 3 Mass-averaged molecular mass (M_w) versus time for polymerization of poly(2-ethyl-2-oxazoline) in dimethylacetamide (solid lines) and acetonitrile (dashed line). (Reproduced with permission from [26].)
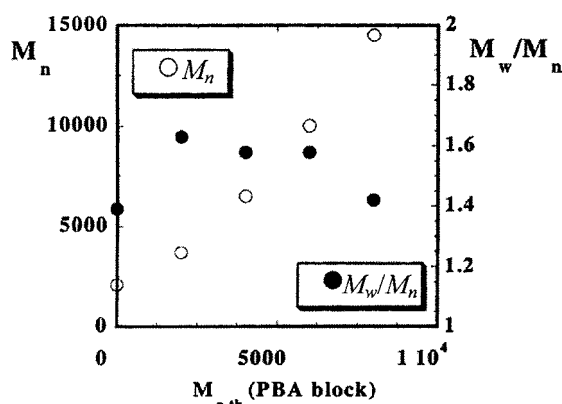
Figure 4 Number-averaged molecular mass (M_n) and polydispersity (M_w/M_n) versus theoretical (calculated) M_n of the butylacrylate block for the controlled radical copolymerization of poly(ethylacrylate-*b*-butylacrylate). (Reproduced with permission from [27].)

Figure 5 Dependence of M_n and polydispersity index (PDI) on monomer conversion in the ATRP of MMA in *p*-xylen at 90°C in a conventional set-up (■) and in the automated synthesizer (3 parallel reactions: ○, △, ◇). (Reproduced with permission from [28].)

leads to a value of the activation energy of 68.7 kJ/mol, similar to the values reported for 2-methyl-2-oxazoline.

Parallel discrete synthesis has been applied recently to controlled radical copolymerization of acrylates [27]. The method of macromolecular design via the interchange of xanthates was utilized to prepare block copolymers of ethyl- and butyl-acrylate. Excellent agreement between conventional reactions and parallel reactions was observed and Fig. 4 indicates the excellent control over number average molecular mass and the polydispersity index (PDI) as a function of the theoretical molecular mass of the butylacrylate block. The linear nature of the plot indicates the 'living' character of the controlled radical polymerization. Recently, the first detailed study of parallel synthesis using another controlled radical polymerization method, atom-transfer radical polymerization (ATRP), was demonstrated successfully [28]. High-throughput screening of ATRP reaction conditions is of great interest due to the many parameters that affect ATRP systems, making reaction optimization very difficult with conventional synthesis methods. The ATRP of methyl methacrylate, a well-characterized system, was carried out in *p*-xylene at 90°C with initiator (ethyl 2-bromoisobutyrate) and catalysts (CuBr + *N*-hexyl-2-pyridylmethanimine). Three parallel reactions were carried out in the automated synthesizer in order to determine the reproducibility with respect to conventional synthesis. As noted above, the scale-up from parallel to conventional synthesis is a unique challenge. The apparent rate constant of conventional synthesis was 4.5×10^{-5} s^{-1}, which compares favorably to those of the three parallel reactions carried out in the automated synthesizer: $4.7 \times 10^{-5}, 4.5 \times 10^{-5}$, and 5.0×10^{-5} s^{-1}. Fig. 5 shows that M_n values increased linearly with monomer conversion, characteristic of a controlled 'living' polymerization. In addition, Fig. 5 indicates that M_n and polydispersity (M_n/M_w) from parallel syntheses are close to those obtained in conventional experiments.

In the arena of complex polymeric materials and mixtures, Hawker and coworkers have utilized high-throughput synthesis to prepare novel functionalized star polymers with well-defined nanoscale dimensions
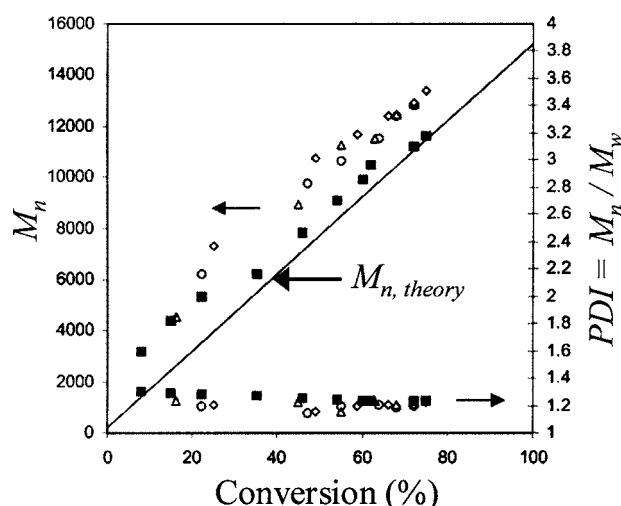
[29], using nitroxide-mediated controlled living radial polymerization. Poly(*p*-ethylphenol)/vanadium oxide (V_2O_5) nanocomposites have also been prepared in combinatorial libraries [30]. Rapid screening with Raman spectroscopy lead to the optimization of crystallized V_2O_5 production. Using conventional 96-well microtiter plates and liquid-handling equipment, Mukawa and coworkers have recently synthesized libraries of molecularly-imprinted polymers targeted to detect cinchona alkaloids [31]. The binding capacity of the imprint polymers was screened with a fluorescence plate reader, and the results were shown to reproduce imprints produced with conventional synthesis and characterization methods. Recently, a library of ligands based upon a chromium bidentate salicylaldimine ligand was used to determine structure-activity relationships and to discover a novel tridentate chromium catalyst. Another discrete library synthesis example utilized parallel suspension polymerization to produce libraries of resins (crosslinked polystyrene beads) for solid phase synthesis [32]. In another high-throughput investigation of resin polymers, parallel libraries of soluble poly(styrene-co-chloromethylstyrene), PEG stars, and substituted poly(norbornylenes) were developed as alternatives to insoluble resin supports [33].

3.2. Continuous library approaches

Continuous formulation libraries with variations in composition, temperature, thickness, and surface energy have been utilized in the past to investigate the effects of mixing and processing conditions on a wide range of polymer phenomena [1, 3, 17, 34–37]. However, significant questions have remained regarding the use of these gradient libraries for parallel synthesis. Recently, the synthesis of segmented polyurethaneurea block copolymers was demonstrated using high-throughput composition and temperature-gradient libraries [38]. Segmented polyurethaneurea libraries were synthesized by first preparing two solutions: (1) toluene diisocyanate(tetramethylene
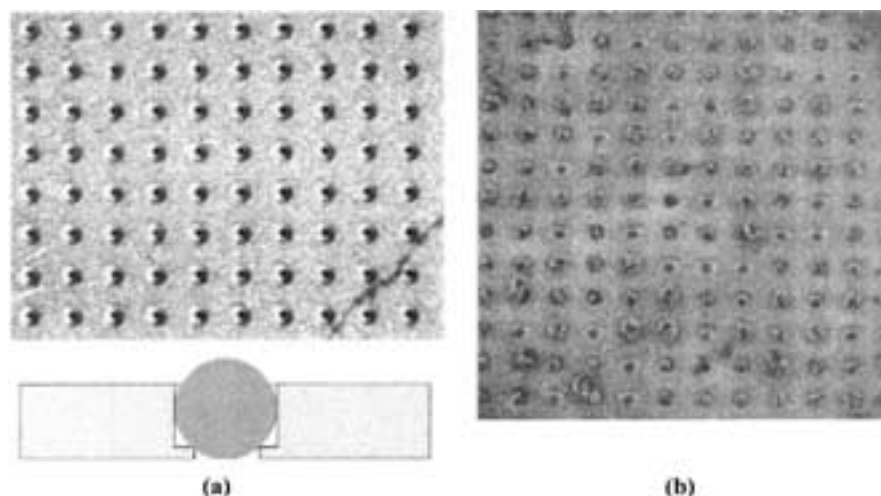
Figure 6 (a) 800 μm $\times$ 1000 μm field of view optical micrograph and the cross-section of the specially designed resin arraying substrate. (b) 1400 μm^2 field of view TOF-SIMS image of an array of stearic acid–Wang–PS resins. (Adapted with permission from [39].)

glycol) prepolymer (Air Products and Chemicals, AIRTHANE® PET-85A, $M_W = 2500$, NCO functionality $= 2.0$, mass% NCO $= 3.3\%$) and (2) trimethylene glycol di-p-aminobenzene chain extender (Air Products and Chemicals, VERSALINK® 740-M), to a total concentration of 30 mass% in tetrahydrofuran (VWR, ACS Grade). A composition-gradient film library, similar to that pictured in Fig. 2a, was prepared using these two solutions with the technique described in detail elsewhere [17] The library, after solvent removal, contained a gradient in chain extender (diamine) concentration from 60 to 160 mol% stoichiometry. The reaction begins immediately upon mixing at room temperature, but the composition gradient films were placed on a vacuum temperature-gradient heating stage, orthogonal to the composition gradient direction. As presented in the characterization section below, the spectral and mechanical properties of the libraries compared favorably with uniform samples prepared conventionally.

Recently, a microextruder was used to prepare libraries with composition-gradients in the extruded rods [21]. The apparatus was used to investigate the effect of pigments and UV-stabilizers on bisphenol-A polycarbonate weathering, a study that is highlighted in the High-Throughput Characterization section below.

4. Advances in development of high-throughput screens of polymer properties

4.1. Chemical characterization (usually synthesis libraries)

Following a parallel synthesis, characterization seeks to determine if the desired reaction occurs, if the products have the expected structure, reaction conversion, and the extent of polymerization (molecular mass). For these purposes, analytical chemistry techniques must be adapted to high-throughput screening and must be compatible with the libraries (discrete versus continuous). Time of flight secondary ion mass spectrometry (TOF-SIMS) was recently applied as a rapid screening tool for arrays of polystyrene resins functionalized with a wide variety of linker molecules and analytes [39]. These resins are used widely for SIMS detection of products in combinatorial chemistry of small molecules. A chemical assay of polymer resins was designed using substrates fabricated with holes of 50 μm resins and containing up to 10,000 resin beads/cm^2. As many as 400 beads can be assayed in one TOF-SIMS image. Fig. 6a shows the cross-section of an individual hole on the array, where the openings on both ends are for loading purposes. Fig. 6b is a TOF-SIMS image showing detection of a stearic acid-PS resin model system. The dark color denotes the presence of stearic acid.

Automated Raman spectroscopy has been shown to be a useful characterization technique for molecular structure of ethylene/1-hexene copolymers [40]. Raman spectroscopy is an ideal tool for high-throughput characterization since it does not require time-consuming sample preparation. In this study the molecular structure as well as macroscopic properties like density and mechanical properties (elastic modulus and yield stress) were estimated from Raman spectra. An adjustable x-y-table automatically positioned samples in a 48-well microtiter plate underneath the sample head of the spectrometer. In all, more than 100 ethylene/1-hexene copolymers from metallocene, Ziegler-Natta, and chromium catalysts were investigated. Raman peaks around 1000–1200 cm^{-1} (stretching modes of C—C bonds) contain information on the content of the comonomer 1-hexene. Using a principal components calibration, the density and methyl group content (CH$_3$/1000 C) of library compounds could be predicted to within 1% from the Raman spectra (with respect to known values determined conventionally).

Recent work at General Electric has utilized fluorescence spectroscopy and multivariate spectral descriptor analysis for high-throughput optimization of bisphenol A polycarbonate reaction conditions in a 96-well microtiter plate [41]. Reaction parameters that were varied included feed ratios, catalyst loading, time, temperature, and inert gas flow rate. Fluorescence measurements of library polymers were performed through the
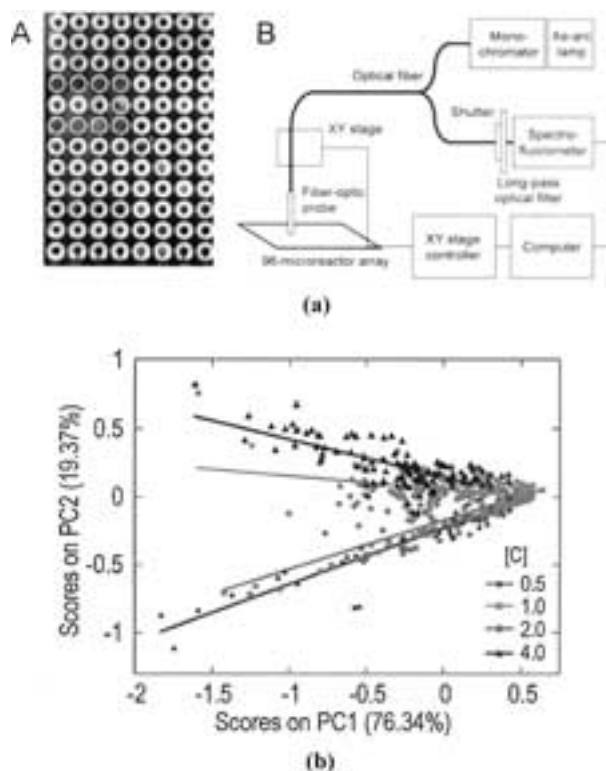
Figure 7 Optimization of multiple process parameters in 96-microreactor arrays: (A) reflected-light image of a 96-microreactor array with solid polymeric materials after a parallel melt-polymerization experiment and (B) setup for an automated acquisition of fluorescence spectra from the polymers in the microreactor arrays. (C) Results of the principal components analysis of the spectra of the polymeric materials made in the 96-microreactor arrays under all experimental conditions as a function of concentration of catalyst, C. (Reprinted with permission from [41]. Copyright (2003) American Chemical Society)

glass bottom of the microreactors using a white light source, a monochromator, a portable spectrofluorometer, and a translation stage, illustrated in Fig. 7a. Principal components analysis (PCA) was further used to extract spectral descriptors from the spectra, in order to provide a pattern recognition model that correlated the features of fluorescence spectra with chemical properties (polymer molecular weight and the concentration of side products). Fig. 7b demonstrates the correlations between two of the principal components (PC1 and PC2) observed at four different catalyst loadings, and is indicative of the sensitivity of the fluorescence spectral descriptor analysis to changes in polymer chemistry and structure.

In addition to spectroscopic methods, chromatography is an important chemical analysis tool following synthesis of polymer libraries. Size exclusion chromatography (SEC) is the primary method for the analysis of molecular weight distributions. Interaction chromatography (e.g., HPLC or LC-CC), on the other hand, is used to detect chemical heterogeneity and functionality distribution. Unfortunately, these liquid chromatographic techniques are slow, with conventional analysis times in the range of 30 min per sample. Pasch and Kilz have recently demonstrated the use of "fast" liquid chromatography methods for high-throughput polymer screening [42]. The SEC analysis time was reduced to 2 min per sample by using high-throughput SEC columns with packing materials and aspect ra-

tios optimized for high fluid flow (column material obtained from PSS GmbH, Mainz, Germany). HPLC analysis time was reduced to levels consistent with high-throughput screening by using shorter columns with smaller particle sizes for improved separation efficiency. For example polyethyleneoxides with different terminal end groups were separated with regard to functionality within 6 min and epoxy resins were separated in less than 2 min.

4.2. Physical characterization (usually formulation libraries)

Using one-dimensional composition-gradient libraries of bisphenol-A polycarbonate plus a UV stabilizer prepared using microextrusion, Potyrailo and coworkers have demonstrated high-throughput screening of the effects of environmental light radiation exposure on polymers [21]. To demonstrate the applicability of the weathering screening strategy, an aromatic polymer, bisphenol-A polycarbonate (PC, GE Plastics) was used as a base polymer in combination with a rutile TiO_2 pigment (1 wt%, provided by GE Plastics) and different levels of UV absorber (Tinuvin 234, T234, Ciba-Geigy, Ardsley, NY). By changing the feed ratios of the feeders, TiO_2-pigmented PC compositions containing 0, 0.6, 1.0, 1.4, and 2 wt% UV absorber were extruded as a 1 mm diameter strand. An inline spectrofluorometer coupled to a CCD camera was utilized to image the blends as they exited the extruder. Weathered blends were imaged fluorescently using a similar spectrofluorometer and automated translation stages. The high-throughput fluorescence screen produced results in agreement with published trends, at a 20 times faster rate.

A systematic investigation into the surface properties of siloxane rubber/carbon black (CB) nanocomposites has been performed, using an automated scanning probe microscope [43]. The automated Solver P7LS large sample SPM (prototype, NT-MDT Ltd., Moscow, Russia), offers several features not common for conventional AFMs: (i) large sample holder and moving system for exact positioning of samples having diameters up to 20 cm, (ii) high-resolution optical microscope and integrated frame grabber for optical imaging of the sample, (iii) advanced control software allowing automated sample positioning and sample measurement using up to 16 different interaction/contrast modes simultaneously, and (iv) advanced data acquisition and database software allowing almost unlimited storage and data handling ability. The AFM is thus capable of measuring an infinite number of sample positions, limited only by the amount of data-storage capacity. The influence of CB concentration and silicone curing rate on roughness and conductivity was studied. The study found that decreased curing rate resulted in decreased roughness and surface conductivity.

Recently, the thickness and temperature-gradient approach was applied to investigate crystallization of isotactic polystyrene over a range of film thickness of 13 nm to 120 nm and crystallization temperatures from $131^{\circ}C$ to $193^{\circ}C$ [23]. By using real-time optical microscopy on the temperature-thickness library,
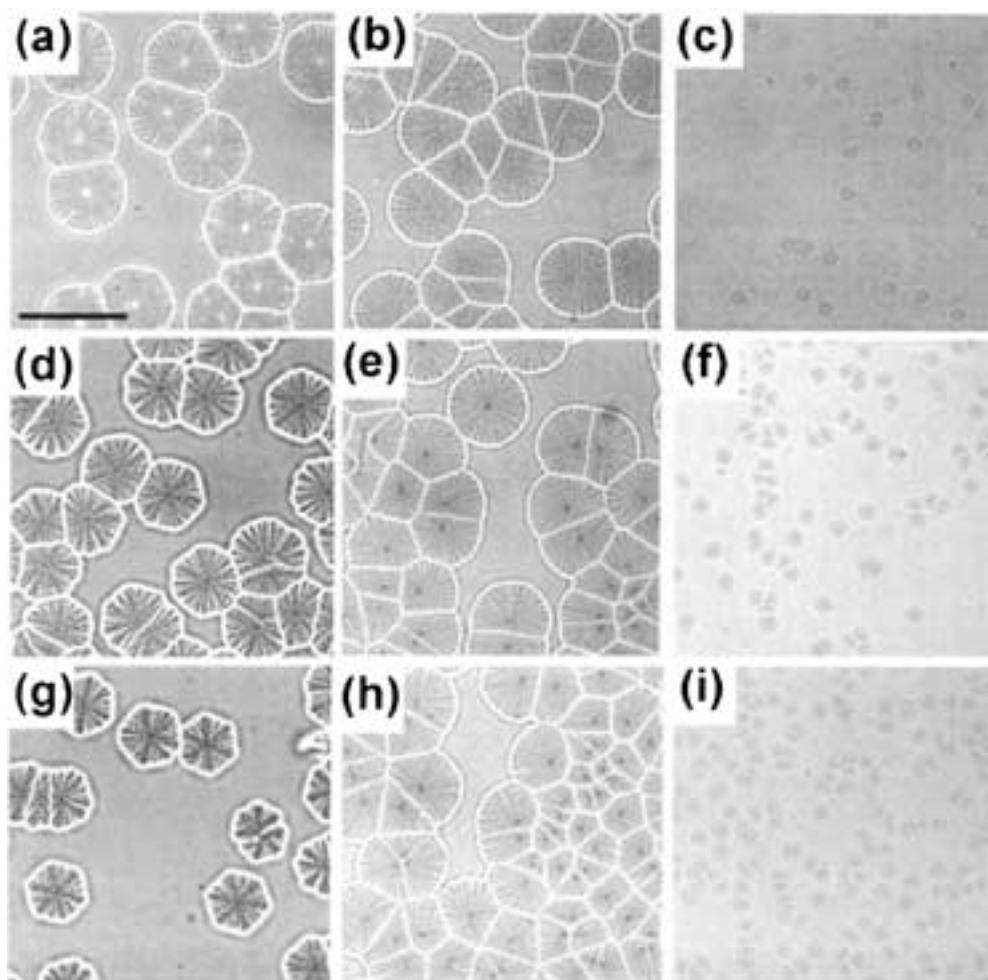
Figure 8 Selected images from a crystallizing temperature-thickness library of isotactic polystyrene. The images were taken from an array captured at time $t = 65$ min and correspond to the following temperatures and film thicknesses, (T_x, h): (a) (193°C, 75 nm), (b) (178°C, 76 nm), (c) (140°C, 81 nm), (d) (193°C, 61 nm), (e) (178°C, 65 nm), (f) (140°C, 60 nm), (g) (193°C, 55 nm), (h) (178°C, 59 nm), and (i) (140°C, 52 nm). The scale bar is 50 μm. (Reprinted with permission from [23]. Copyright (2003) American Chemical Society)

spherulite growth rates were measured as a function of film thickness and temperature. Fig. 8 shows selected images of growing spherulites from a typical library. The growth rate maximum occurred near 180°C and varied inversely to film thickness, in agreement with conventional measurements. In addition, the investigators found both hexagonal and disordered branched crystalline morphologies in ultrathin regions where thickness is less than the radius of gyration.

Of all polymer measurements, some of the most important and commonly reported are the mechanical properties. Mechanical characteristics such as modulus, tensile strength, elongation at break, and impact strength are used ubiquitously as tests in synthetic studies, formulation and blending investigations, and in product development or quality control. However, mechanical tests, including the tensile and Charpy or Izod impact measurements are time-consuming and require large volumes of sample. Hence, a miniaturized, high-throughput method for screening mechanical properties on combinatorial libraries would offer tremendous advantages over conventional 1-sample-1-measurement techniques. For discrete libraries synthesized in 96-well plates, Symyx Corporation has introduced a 96-pin tensile deformation apparatus that allows parallel measurement of tensile properties under both oscil-

latory and non-oscillatory conditions [44]. For continuous or discrete libraries, Meredith and coworkers have developed a high-throughput mechanical characterization (HTMECH) instrument, with a schematic given in Fig. 9a [38, 45]. This instrument mounts a one-, or two-dimensional gradient library film between two steel plates each with an identical grid of 100 holes. The circular holes isolate areas of the gradient library for mechanical analysis. The sample mounting grid is then driven at a controlled rate (between 0.001 and 1 m/s) onto a steel contact pin that measures the force versus time up until failure. The instrument has been used to investigate effects of curing temperature on the tensile and impact strengths of poly(urethaneurea) libraries [38], and recently has been applied to mechanical measurements on composition gradient libraries that vary chain extender concentration in poly(urethaneureas), as indicated in Fig. 9b. These measurements have indicated that tensile modulus measured with the radial-deformation HTMECH apparatus are identical to the tensile modulus obtained from conventional uniaxial tests (INSTRON). In addition, workers at the NIST Polymers Division have developed a novel method for measuring the modulus of polymer films, based upon the buckling instability induced by a linear deformation parallel to the film surface [46]. The frequency of
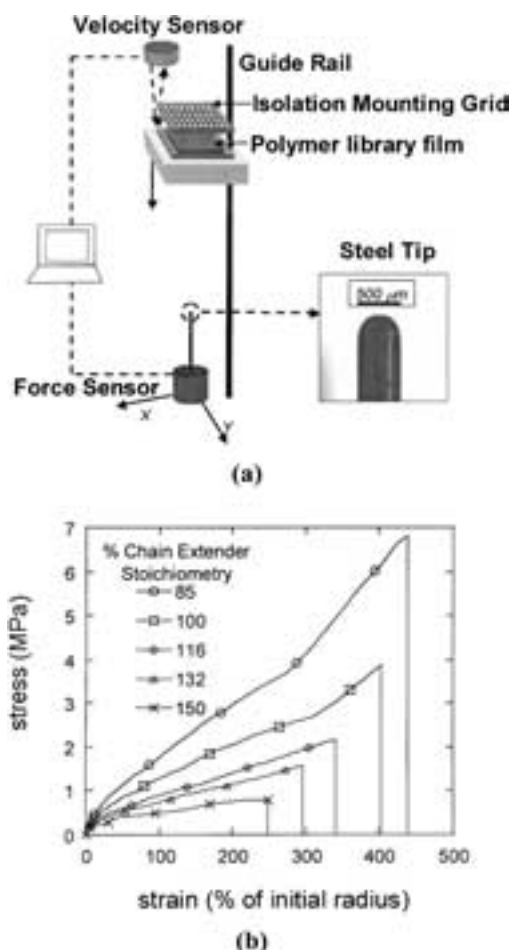
(a)

(b)

Figure 9 (a) Schematic of the High-Throughput Mechanical Characterization (HTMECH) apparatus, indicating the isolation grid for gradient libraries, contact tip, force and velocity sensors. (b) Stress versus strain data from a composition-gradient library of a poly(urethaneurea) cured at 90°C. The composition of the chain extender varied from 60% to 160% of the necessary stoichiometry.

oscillations in the film surface following buckling, determined from microscopy or light scattering, can be related to the film modulus. This simple technique offers future promise as an adaptation into high-throughput library screening.

Previously, thickness gradients have been used to explore ordering in diblock copolymer thin films [36, 37]. Recently, the thickness gradient library has been combined with a novel technique for preparing a continuous gradient in surface energy [47]. Briefly, a silicon wafer was coated with a self-assembled monolayer of octylchlorosilane, which was subsequently oxidized using a gradient of UV/ozone (UVO) radiation. The radiation gradient resulted in a continuous variation in the oxidation of the SAM film to more hydrophilic end-groups, leading to a variation in surface energy from 30 to 50 mJ/m^2. The two-dimensional library was used to map the morphology dependence of symmetric polystyrene-*block*-poly(methyl methacrylate) (PS-PMMA) thin films on film thickness and substrate surface energy. Fig. 10 shows a digital photograph of a library following 96 h of annealing at 170°C. The *h* increases from top to bottom and varies between 60 nm to 95 nm, while surface energy increases from left to right and varies between 35.5 mJ/m^2 to 44.5 mJ/m^2. Bright regions correspond to areas where diblock seg-
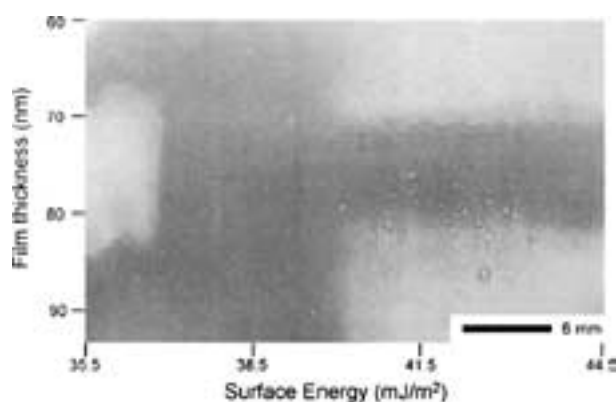

Figure 10 Optical photograph of a combinatorial map of a thin PS-PMMA block-copolymer film as a function of film thickness and surface energy gradients. Segregated islands and holes on the surface scatter light causing the film to appear lighter in color in the areas where they exist. The darker areas do not have ordered surface features. (Reproduced with permission from [47].)

regation (ordering) occurs, as a consequence of light scattering from the heterogeneous film surface. An inversion from symmetric to anti-symmetric dependence of lamellar morphology on film thickness was observed with a progressive change in surface energy. An intermediate neutral region was found between these limiting types of ordering.

5. Using polymer libraries to screen interactions with the external environment

A number of applications of polymer combinatorial and high-throughput libraries have focused not solely on the properties of the polymers themselves, but rather on the interaction between the polymer and some external environment. Most of the examples of this type of work fall into the areas of biomaterials or environmental engineering. Three pertinent examples of the use of libraries to characterize polymer-biological or polymer-environmental interactions are reviewed in this section.

Langer *et al.* recently reported the parallel synthesis of 140 degradable poly(β-amino esters) via the addition of 20 amine monomers to seven diacrylate monomers [48]. To develop structure-function relationships they used high-throughput assays to investigate the ability of each DNA-complexing polymer to overcome cellular barriers to gene transfer. Diacrylate and amine monomers were selected (on the basis of degree of hydrophobicity and either structural or functional character) for the synthesis of the 140-member polymer library. Gel permeation chromatography indicated molecular masses ranging from 1000 to 50000, relative to polystyrene standards. Fifty-six polymers from the 140-member library were screened (luminescence screen in 96-well plate) for their ability to transfect COS-7 cells with DNA (Fig. 11). These 56 polymers were chosen based upon their ability to bind DNA and their water solubility. The majority of polymers in Fig. 11 showed little transfection, but complexes formed from two polymers displayed high levels of transfection. These two 'hits' later showed the highest levels of internalization in NIH-3T3 cells relative to "naked" DNA ($18\times$ and $32\times$, respectively).
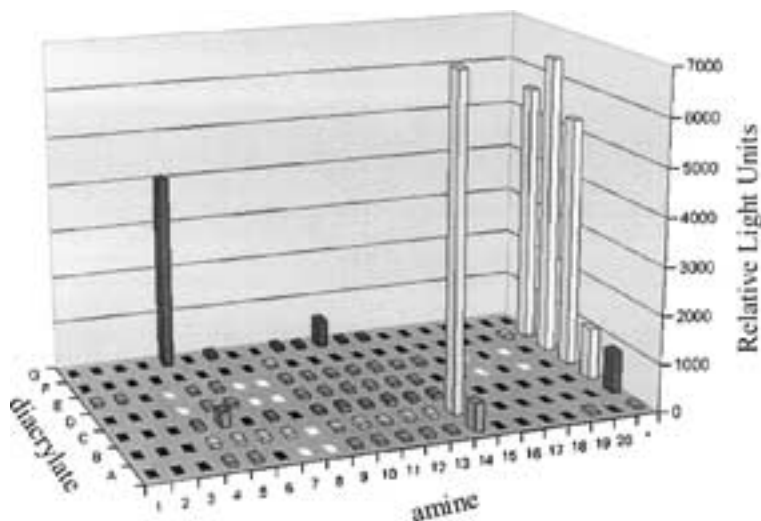
Figure 11 Transfection data as a function of comonomers for an assay employing pCMV-Luc dye (600 ng/well, DNA/polymer = 1:20). Light units are arbitrary and not normalized to total cell protein and experiments were performed in triplicate. Black squares represent water-insoluble polymers; white squares represent water-soluble polymers that did not complex DNA. The "*" column displays values for positive and negative controls. (Reprinted with permission from [48]. Copyright (2003) American Chemical Society)
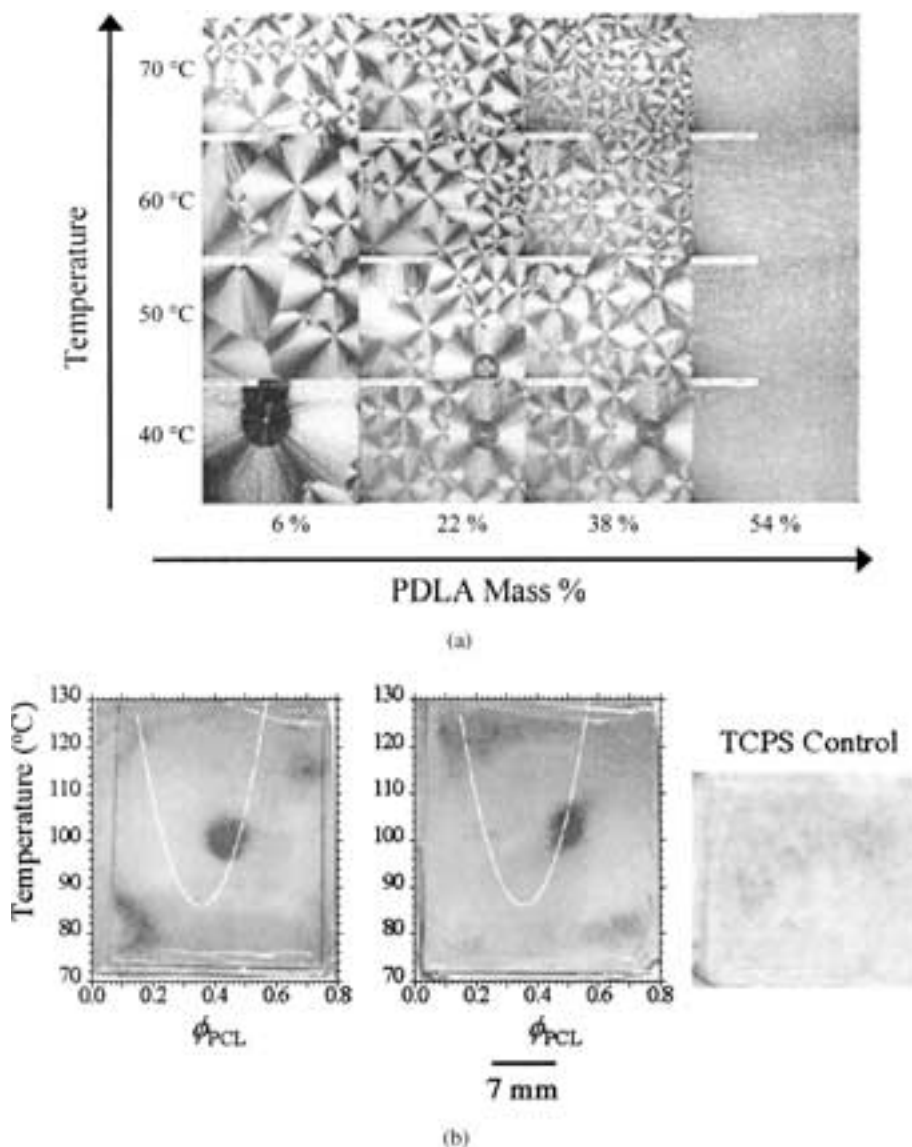

Figure 12 (a) Polarized optical micrographs taken at 5 mm spacing (horizontal and vertical) from a PDLA-PEO composition-temperature gradient library that explores changes in the size, crystallinity, and crystalline morphology of the PEO phase, which in turn generates surface structure and chemical diversity. Each image panel is 2 mm wide. (b) Photograph of a PDLA-PCL composition-temperature gradient library after seeding and culturing MC3T3 osteoblasts for 5d and staining for expression of alkaline phosphatase. White curve represents the LCST cloud point curve for the PDLA-PCL blend and a uniform TCPS substrate is shown as a control. (Reproduced with permission from [35].)

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Effective diameter and zeta potential measurements indicated that, in general, small particle size and positive surface charge led to the higher internalization rates.

One of the great challenges in biomaterials for tissue engineering is designing surfaces with the chemistry, microstructure, and roughness combination that leads to optimal cell adhesion, proliferation, and function. Because there are many ways to generate surface features and alter surface chemistry of polymers, the parameter space to be searched is vast. Composition-temperature gradient libraries of biomedical polymer blends have been used to generate surface libraries with significant diversity in surface chemistry and microstructure [35]. For example, Fig. 12a shows polarized optical micrographs from a library of PDLA blended with poly(ethylene oxide) (PEO), where composition- and temperature-gradients vary the nucleation and growth rate of crystalline PEO spherulites. Blends of PDLA with PEO are being investigated as tissue-engineering surfaces since PEO allows a neutral background (proteins and cells do not adsorb) for the protein and cell-adhesive PDLA patterns. Libraries like that in Fig. 12a are being combined with cell culture techniques to "discover" the dependence of cell adhesion and function on the active site patterns. As an example of this polymer-cell screening, Fig. 12b shows another composition-temperature library, PDLA-PCL (as in Fig. 2a) after 5d of culturing with MC3T3-E1 osteoblast cells. Temperature and composition vary the degree of liquid-liquid phase separation via the lower critical solution temperature (LCST) of this blend. This phase separation, as well as the crystallization of PCL, leads to diverse surface chemistries and roughnesses. The library was fixed and stained for alkaline phosphatase, a key indicator of osteoblast activity. Clearly, the libraries exhibit enhanced activity of the osteoblasts in an elliptical region near the high-PCL concentration side of the cloud-point curve for this blend.

In another example, a library of molecularly imprinted polymers was synthesized using β-lactam antibiotic pencillin G as a template. The 52-member library varied various functionalized monomers (acrylates) and crosslinkers as well as the ratio of components in the reaction mixture, to achieve a diverse population. A high-throughput radioligand binding assay was used to screen for selectivity to penicillin G, and a polymer prepared from methacrylic acid and trimethylolpropane trimethacrylate had the highest selectivity [49].

6. Conclusions
In the past three years (2000–2003), combinatorial and high-throughput methods have continued to grow and expand into new areas of synthesis and formulation in polymer science. In synthesis, parallel synthesis and high-throughput screening is being applied to new types of reactions, in particular controlled free radical polymerizations. Evidence from these studies shows that the combinatorial techniques can reproduce closely the properties of polymers synthesized in conventional laboratory reaction equipment. Perhaps even more dramatic is the progress that is being made in the inves-

tigation of formulation effects and physical properties. Studies of crystallization, mechanical properties, and others, as a function of formulation parameters such as thickness, composition, and annealing temperature, are yielding results that are in agreement with conventional methods, yet tens or hundreds of times faster.

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