ELSEVIER

Advanced Drug Delivery Reviews 53 (2001) 123–130

Advanced
DRUG DELIVERY
Reviews

www.elsevier.com/locate/drugdeliv

Combinatorial chemistry and biomedical polymer development

S. Brocchini

Biomedical Polymers Group, Department of Pharmaceutics, School of Pharmacy, University of London, 29 Brunswick Square, London WC1N 1AX, UK

Abstract

Polymers are ubiquitous components of products manufactured for medical and pharmaceutical applications. Widely used commodity polymers were the first polymers to be utilised in biomedical applications. These polymers were not developed with biocompatibility established at the onset and many speciality polymers have been developed in recent years to begin to meet the multifaceted demands for medical development, the optimisation of structure–property correlations and ultimately, clinical use. In the broader area of materials research, combinatorial or high throughput strategies used for drug development are recognised to have potential for discovery and process development. Much of the application of combinatorial chemistry in drugs research has been dependent on the use of polymeric reagents, substrates and supports. The chemistry of the reactions on polymers in solid and liquid phases have also played a major role in combinatorial drugs research. There is considerable interest in combinatorial materials research and this review outlines how this research may be applied for biomedical polymer development.

Keywords: Combinatorial chemistry; Polymer synthesis; Biomedical polymers

Contents

1. Drugs and materials

Modification and redesign of some combinatorial strategies has occurred due to the differences between drug candidates and functional materials. Typically drugs are structurally defined molecules with, in principle, a similar set of criteria that must be met during development. Combinatorial chemistry for drugs research has focused on single compounds as targets. It has been possible to generate quantifiable descriptors resulting in the generation of increasingly rational diversity that can be used for discovery and optimisation. In contrast, the properties of functional materials (i.e. polymers, ceramics, metals; hybrid systems such as inorganic and solid state materials, alloys and composites)

E-mail address: steve.brocchini@uslop.ac.uk (S. Brocchini).

0169-409X/01/$ – see front matter
PII: S0169-409X(01)00224-1

derives from composition and morphology that span statistical distributions [1–4]. This results in complex interactions within the bulk and surface structures of materials where theory and predictive models are still far from complete.

Generally polymers are produced from monomers in a polymerisation reaction where all the bond forming reactions are conducted in one reaction. This leads to molecular weight variation and polymer molecular weights are expressed in terms of averages. Structural variation is also prevalent in functional polymers and it is generally not possible to know exactly the structure of each polymer molecule. Nonetheless, like any drug, biomedical polymers must meet strict regulatory requirements to gain approval for clinical use. Biological targets and applications are being identified where functional biomedical polymers are increasingly being developed so their broad profile of materials, physicochemical and biological properties together must display elements of specificity that is usually associated with drugs [5–7]. Many critical issues relating to toxicology (both acute and cumulative), metabolic fate and immune response, apply to both drugs and biomedical polymers research. At the preclinical stage, development of functional biomedical polymers can be thought as an activity similar to what medicinal chemists do to develop new drugs with the added burden that a wide range of polymer properties must be optimally matched to the medical application [8]. Development of degradable polymers has additional levels of complexity because structure–property correlations will change with time. Also, the range of drug delivery applications reliant on functional polymers requires that development of the ‘delivery system’ cannot be disassociated from the development of the therapeutic agent itself [9].

2. Elements of the combinatorial process

Interest in combinatorial strategies for materials development has been due to the economics of industrial research (i.e. reduced cycle times) [1] and issues of innovation (i.e. increased possibility for serendipitous discoveries) [3]. Candidate materials which have been examined include high temperature superconductors, catalysts and magnetic materials [10]. These materials have tended to be inorganic and solid state materials. Such materials are fundamentally different from biomedical polymers. However, the practise of combinatorial strategies from drugs research and materials research illustrates how some of these strategies can potentially be employed in biomedical polymer development.

Combinatorial efforts tend to be based on the integration of library synthesis and high throughput screening, both activities being conducted on small scale and by automated processes [3]. Analogous to the computational methods being used in genomics, the analysis of data and the use of automation are important in combinatorial materials research [4]. Modelling and combinatorial mathematics [2] are being employed to sample combinations of parameters in a particular set of materials. Such activities may have relevance for property prediction [11].

Library synthesis by a variety of deposition technologies (e.g. vapour, electrochemical, sputtering, pulsed laser ablation) have been used to prepare libraries of these materials [1–4]. Photolithography and masking techniques are useful to prepare spatially distinct materials of known composition and processes. Another preparative technique is to use component gradients to give a continuous spread in composition by thin film approaches.

For biomedical polymers, synthesis is best accomplished in solution where individual polymers are made in parallel. Each polymer is prepared in a separate solution. This allows for discrete polymers to be synthesised [12]. Ink jet methodology can operate with nanoliter amounts of reagent solutions and may be potentially important for generating large numbers of polymers on small scale [4]. Novel approaches for library synthesis of polymers will continue to emerge [13,14].

One element for the advent of combinatorial strategies in drugs research was biological assay methodology during preclinical studies was steadily being developed to screen a greater number compounds than could be prepared by chemists in a serial manner. In materials research, conventional techniques needed for screening in a library format have required some modification to be useful. Non-destructive optical imaging techniques have been developed [4] for interrogating bulk/surface characteristics [15]. In the case of biomedical polymers,

relevant techniques for screening physicochemical properties include high throughput protein crystallisation [16], MALDI-TOF [17] and electrospray ionisation mass spectrometry [4], parallel calorimetry, rapid gel permeation chromatography and capillary electrophoresis [18].

Screening strategies can differ where both trends and spikes (i.e. the hits) may be delineated [1–4,19]. Initial screening of a material library screening typically provides information about trends. Iteration is necessary to optimise relevant correlations. Histogrammes that display a small number of active compounds or materials with the sought activity are a familiar aspect of combinatorial chemistry in drug research. In biomaterials research, trends are also critical because if a new polymer system emerges to meet an application endpoint, then it would be important to be able to use close compositional or processed analogues for other applications. A set of trends that may be important in biomedical research is to produce a library of candidate polymers that (A) share a set of common properties (e.g. degradation mechanism) while (B) displaying an incremental variation over a wide range for other properties (glass transition temperature). This combination of property variation may be important to optimise complex biological properties in addition to all the other necessary physicochemical and materials properties than require optimisation. This approach to combinatorial development may be a more accurate reflection of how in nature, structure and function are matched. For example all proteins share (A) many similar properties (e.g. they are polyamides, water soluble) while at the same time (B) other properties incrementally over a wide range (e.g. substrate selectivity, receptor binding, turnover) [12].

3. Summary of design criteria for biomedical polymers

Biocompatibility issues are of primary importance for biomedical polymer development. While many applications encompass the broad fields of drug delivery and tissue replacement [8], different design criteria and polymer properties are required that are dependent on whether a polymer is utilised as a (1) material, (2) excipient or (3) soluble molecule [9]. Applications for each of these categories include:

- *material* (e.g. site specific drug delivery; continuous and prolonged drug delivery; tissue/organ improvement, substitution or regeneration; orthopaedic fixation; dental restoration; ophthalmic devices; packaging)
- *excipient* (e.g. gels, binders, emulsifiers, viscous oils, coatings, diluents, stabilisers and carrier particles)
- *molecule* (e.g. physiologically soluble drug or protein–polymer conjugates; polyvalent ligands; sequestering agents; non-viral vectors; stimuli responsive agents; aggregating reagents for nano/macro particle fabrication)

The properties that require optimisation between ‘material’ and ‘molecule’ applications are more clearly differentiated. ‘Excipient’ and hybrid systems (e.g. gel networks, PEGylated liposomes and surface functionalised solids) require optimisation of properties that may not be as clearly defined. In the context of drug delivery, application dependent polymer properties are critical to favourably alter pharmacokinetics to optimise efficacy. For example, in polymeric devices, polymer–drug (e.g. protein) interactions during storage, upon hydration and during polymer degradation [20,21] will be critical for maintaining drug efficacy [7,8,22].

Chemical structure and molecular weight characteristics of the polymer will influence structure–property correlations for all these applications. In ‘material’ applications, both processing and implant history will undoubtedly influence polymer properties that define performance. A biomedical polymeric material must be prepared, blended (or formulated) and fabricated into a device that is matched to a specific application. Optimised biological, thermal, mechanical, and physicochemical properties are required. To avoid long term polymer accumulation and device removal after the therapeutic requirement has been met, degradable polymers continue to be developed. Optimisation of structure–property correlations in respect to the biological profile and performance that are expected to change with time due to degradation processes is a crucial area of research. A degradable polymer must be processible,

survive sterilisation procedures and exhibit a satisfactory shelf life. Hydrolytic degradation of biomedical polymers in vivo is either (1) a bulk process or (2) a surface erosion process, and possibly a combination of the two processes. The rates and mechanisms of hydrolytic mechanisms in vivo that lead to polymer degradation are a function of (i) the chemical structure of the polymer, (ii) processing history, (iii) thermal properties, (iv) final fabricated device, (v) ultimate biological application, (vi) encapsulated excipients or loaded drugs, (vii) crystalline regions which will typically degrade more slowly potentially producing insoluble particles that can have harmful long term toxic effects and (viii) autocatalytic processes. Although a major challenge is to develop materials and their associated devices to elicit specific cellular responses, the interfacial surface properties of the polymer are also important for establishing biocompatibility and minimising the foreign body response. Surface texture, chemical functional spatial relationships and final processed geometry (e.g. dimensions and resolution of surface patterns) are critical considerations for biomedical device development. There has been intense interest to address the complex challenges in biomedical polymers research (for example Refs. [6–8,23–26]).

Utilisation of soluble polymers to exploit their properties as 'molecules' in medical applications that are dependent on parental administration will increasingly require uniform structure in respect to molecular weight distribution and structural homogeneity. This is analogous to proteins which are a class of biopolymers with defined structural characteristics. They have a distinct number and order of amino acids that make up the protein backbone that define solution structure and biological function. Optimisation of the desired biological interactions of soluble biomedical polymers with tissues, cells and viruses may depend on optimal structure–property correlations and require defined polyvalent interactions [27]. Circulation times and cellular uptake phenomena will depend on the solution structure and hydrodynamic radius of soluble conjugates [28–32]. Applications for soluble or semi-soluble polymers that are based on oral administration may be more tolerant of structure [33]. Nonetheless, the more structurally defined a soluble polymer and its constructs, the more able it will be to maximise efficacy.

4. Combinatorial biomedical polymer research

Biomedical polymers that possess inherent biological activity have long been investigated (for example Refs. [34,35] and references cited in Ref. [36]). Polyvalent interactions between multiple proteins and ligands are prevalent in biological systems (e.g. adhesion of influenza virus) and can involve interactions that occur at cell surfaces (e.g. clustered membrane bound receptors) [27,37,38]. Multiple simultaneous interactions of a macromolecular construct may potentially have unique collective properties that differ from properties displayed by the separate individual components of the construct interacting monovalently [39]. Several examples exist where synthetic polymers or polysaccharides have been used to present a polyvalent substrate designed to interact with more than one protein target (note references in Ref. [40]) and combinatorial approaches have been examined [41–45]. Polymers with active ester groups at each repeat unit have been used as precursors to generate libraries of candidate copolyacrylamides with conjugated asialoside pendent groups and that were screened for inhibition of agglutination of erythrocytes induced by influenza virus [46,47]. Other combinatorial approaches using polymeric precursors were focused on the development of enzyme [48,49] and receptor [50,51] mimics [10,52]. While these combinatorial methods involve the reactions of small molecules with polymers to generate a complex, although probably not random [48,49], mixture of polymeric molecules of varied molecular weight, enhanced activities were observed for polymeric products that were derived from distinct stoichiometries of precursor small molecules. In an effort to minimise structural heterogeneity in respect to molecular weight variation, narrow molecular weight polymeric precursors have been prepared [53]. Some of these evolving approaches have also involved the development of novel drugs that are dimeric–multimeric rather than polymeric in nature to minimise structural heterogeneity (for example [54–59]). Use of polyacrylamide and agarose gels as a means to present bioactive molecules to study cellular interactions may have potential for combinatorial evaluation [8]. And combinatorial approaches have also been used to examine molecularly imprinted polymers [60,61],

sensors [62] and patterned or functionalised surfaces [6,63–65] where polyvalent interactions may be critical for optimising performance.

Pseudo-poly(amino acids) [66,67] are being developed as degradable polymeric materials for drug delivery and orthopaedic applications. These strictly alternating polymers have hydrolytic bonds (e.g. ester, carbonate, iminocarbonate) in the polymer mainchain leading to polymer degradation in vivo. A combinatorial approach to study a class of tyrosine derived pseudo-poly(amino acid) was based on using two monomer sets for a total of 22 monomers to gave a library 112 of A–B strictly alternating polymers obtained by parallel synthesis [12,19]. Each set of monomers were prepared with defined but different, structure variations resulting in the polymer library possessing similar structural features (orthogonal homologation) that were not simply additive. A multitude of structure–property correlations and trends were established [19,68,69]. Several important properties (e.g. degradation mechanism, processibility, biocompatibility) were shared by the library polymers while a set of other properties incrementally varied over a wide range (glass transition temperature, air–water contact angle, relaxation properties, cellular response and mechanical properties). This work demonstrated that biomedical polymer libraries can be used (a) to increase in unique relationships the number of candidate polymers for medical applications, (b) to incrementally modulate specific polymer properties over a wide range while keeping other defined properties unchanged (this makes it possible to use similar polymers over a wide application range) and (c) the potential to systematise the study of structure–property correlations. This juxtaposition of trends allows for novel selection criteria. For example several polymers in the library had a glass transition temperature in the range of 35–37°C while displaying a wide range of air–water contact angles. These trends may also allow for a library to be designed and characterised with a relatively small number of polymers as a basis to predict the properties of many other related polymers that have not been prepared [11]. Although miniaturisation, automation and database advances are important for implementing combinatorial strategies, it was essential in this case that a considerable amount of research defining the chemistry and establishing biocompatibility had been conducted with these polymers before a combinatorial library was produced [24,70,71]. To find application relevant hits within these trends it is necessary to screen for specific biological profiles if the material is to be used in tissue engineering. If a drug delivery device is being developed, formulations that are then processed into the desired device must then be evaluated.

Research to systematise formulation technology with copolymeric excipients [72–75] and nanoscopic particles [76] have been examined by combinatorial approaches. Using computational methods many excipients that are generally recognised as safe have been evaluated to determine a multitude of trends that can be matched to the physicochemical properties of the pharmacologically active compounds. A doxorubicin formulation using a combination of two pluronics has shown this formulation may have broader efficacy than current clinical formulations of doxorubicin [77].

5. Conclusion

Combinatorial strategies have been an intensive area of research since the early 1990's. While early examples demonstrated many elements that parallel synthesis can provide libraries of compounds or materials, recent work demonstrates that the integration of synthesis, characterisation, and computational methodology is necessary to generate information that can be used iteratively to optimise correlates. Combinatorial strategies do not replace the necessity for the intellectual insight of the scientist that is require to apply chemical, biological and physical processes to prepare and characterise libraries. Nor can combinatorial strategies replace the imagination of the scientist to generate new hypotheses leading to the development of biomedical polymers to meet future medical needs.

References

[1] J. Hewes, High throughput methodologies: a new paradigm for chemicals and materials research, Chim. Oggi 18 (2000) 20–24.

[2] J. Cawse, Experimental strategies for combinatorial and high-throughput materials development, Acc. Chem. Res. 34 (2001) 213–221.
[3] E. McFarland, W. Weinberg, Combinatorial approaches to materials discovery, TIB Tech. 17 (1999) 115–116.
[4] P. Schultz, X. Xiang, Combinatorial approaches to materials science, Curr. Opin. Solid State Mat. Sci. 3 (1998) 153–158.
[5] D. Hem, J. Hubbell, Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing, J. Biomed. Mater. Res. 39 (1998) 266–276.
[6] J. Hubbell, Synthetic biodegradable polymers for tissue engineering and drug delivery, Curr. Opin. Solid State Mat. Sci. 3 (1998) 246–251.
[7] R. Langer, Drug delivery and targeting. Nature, 392(6679(Suppl.)) (1998) 5–10.
[8] L. Griffith, Polymeric biomaterials, Acta Mater. 48 (2000) 263–277.
[9] S. Brocchini, Synthetic Polymers in Drug Delivery, Pharmaventure – Drug Delivery Companies Report (2000) 66–72.
[10] B. Jandeleit, D. Schaefer, T. Powers, H. Turner, W. Weinberg, Combinatorial materials science and catalysis, Angew. Chem. Int. Ed. 38 (1999) 2494–2532.
[11] C. Reynolds, Designing diverse and focused combinatorial libraries of synthetic polymers, J. Comb. Chem. 1 (1999) 297–306.
[12] S. Brocchini, K. James, V. Tangpasuthadol, J. Kohn, A combinatorial approach for polymer design, J. Am. Chem. Soc. 119 (19) (1997) 4553–4554.
[13] P. Hodge, Some applications of reactions which interconvert monomers, polymers and/or macrocycles, React. Func. Polymers 48 (2001) 15–23.
[14] T. Chen, R. Vazquez-Duhalt, C. Wu, W. Bentley, G. Payne, Combinatorial screening for enzyme-mediated coupling. Tyrosinase-catalyzed coupling to create protein–chitosan conjugates, Biomacromolecules 2 (2) (2001) 456–462.
[15] J. Thao, A combinatorial approach for efficient mapping of phase diagrams and properties, J. Mater. Res. (2001) 1565–1578.
[16] R. Stevens, High-throughput protein crystallization, Curr. Opin. Struct. Biol. 10 (2000) 558–563.
[17] H. Rader, W. Schrepp, MALDI TOF mass spectrometry in the analysis of synthetic polymers, Acta Polym. 49 (1998) 272–293.
[18] L. Avila, Y. Chu, E. Blossey, G. Whitesides, Use of affinity capillary electrophoresis to determine kinetic and equilibrium constants for binding of arylsulfonamides to bovine carbonic anhydrase, J. Med. Chem. 36 (1993) 126–133.
[19] S. Brocchini, K. James, V. Tangpasuthadol, J. Kohn, Structure–property correlations in a combinatorial library of degradable biomaterials, J. Biomed. Mater. Res 42 (1) (1998) 66–75.
[20] J. Kohn, D. Schachter, Pharmaceutical formulation composed of a polymer blend and an active compound for time-controlled release, PCT-WO 01/49311A1, (2001) 46 pages.
[21] J. Kohn, D. Schachter, Pharmaceutical formulations for regulating the timed release of biologically active compounds based on a polymer matrix, PCT-WO 01/49249A1, (2001) 41 pages.
[22] K. Fu, K. Griebenow, L. Hsieh, A. Klibanov, R. Langer, FTIR characterization of the secondary structure of proteins encapsulated within PLGA microspheres, J. Control. Rel. 58 (3) (1999) 357–365.
[23] J. Hubbell, Biomaterials in tissue engineering, Biotechnology 13 (1995) 565–576.
[24] A. Nathan, J. Kohn, in: S. Shalaby (Ed.), Biomedical Polymers, Amino Acid Derived Polymers, Hanser, New York, 1994, pp. 117–151.
[25] A. Domb, J. Kost, D. Wiseman, Handbook of Biodegradable Polymers, in: A. Florence, G. Gregoriadis (Eds.), Drug Targeting and Delivery, Harwood Academic, Amsterdam, 1997.
[26] B.D. Ratner, New ideas in biomaterials science – a path to engineered biomaterials, J. Biomed. Mater. Res. 27 (1993) 837–850.
[27] M. Mammen, S. Choi, G. Whitesides, Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors, Angew. Chem. Int. Ed. 37 (1998) 2754–2794.
[28] R. Duncan, S. Dimitrijevic, E. Evagorou, The role of polymer conjugates in the diagnosis and treatment of cancer, STP Pharma 6 (1996) 237–263.
[29] R. Duncan, Drug–polymer conjugates: potential for improved chemotherapy, Anti-Cancer Drugs 3 (1992) 175–210.
[30] K. Ulbrich, C. Konak, Z. Tuzar, J. Kopecek, Solution properties of drug carriers based on poly[*N*-(2hydroxypropyl)methacrylamide] containing biodegradable bonds, Makromol. Chem. 188 (1987) 1261–1272.
[31] V. Chytry, M. Netopilik, M. Bohdanecky, K. Ulbrich, Phase transition parameters of potential thermosensitive drug release systems based on polymers of *N*-alkyhnethacrylamides, J. Biomater. Sci. Polym. Ed. 8 (11) (1997) 817–824.
[32] F. Uchegbu, H. Ringsdorf, R. Duncan: The Lower Critical Solution Temperature of Doxorubicin Polymer Conjugates, Proceed. Intern. Symp. Control. Rel. Bioact. Mater., 1996.
[33] H. Mandeville, C. Arbeeny, Bile acid sequestrants: their use in combination with other lipid-lowering agents, Curr. Opin. Cardiovasc., Pulm. Renal Invest. Drugs 1 (2) (1999) 241–246.
[34] W. Regelson, The Biologic Activity of Water-Soluble Polymers, in: N. Bikales (Ed.), Water Soluble Polymers II, Plenum Press, New York, 1973, pp. 161–177.
[35] A. Kaplan, in: L. Donaruma, R. Ottenbrite, O. Vogl (Eds.), Anionic Polymeric Drugs, Antitumor Activity of Synthetic Polyanions, Wiley, New York, 1980, pp. 227–254.
[36] R. Duncan, J. Kopecek, Soluble synthetic polymers as potential drug carriers, Adv. Polym. Sci. 57 (1984) 51–101.
[37] E. Toone, N. Burkhalter, S. Dimick, in: B. Erust, G. Hart, P. Sinay (Eds.), Carbohydr. Chem. Biol, Protein–Carbohydrate Interaction: Fundamental Considerations, Vol. 2, Wiley–VCH Verlag, Weinheim, 2000, pp. 863–914.
[38] J. Lundquist, S. Debenham, B. Toone, Multivalency effects in protein–carbohydrate interaction: the binding of the shiga-like toxin 1 binding subunit to multivalent C-linked glycopeptides, J. Org. Chem. 65 (24) (2000) 8245–8250.

[39] P. Reece, K. Watson, W. Wu, B. Jin, G. Krippner, Preparation of poly(neuraminic acids) as influenza virus neuraminidase inhibitors, PCT-WO 98/21243, (1998) 56 pages.
[40] M. Sparks, K. Williams, G. Whitesides, Neuraminidase-resistant hemag glutination inhibitors: acrylamide copolymers containing a C-glycoside of *N*-acetylneuraminic acid, J. Med. Chem. 36 (1993) 778–783.
[41] G. Whitesides, J. Tananbaum, J. Griffin, M. Mammen, Molecules presenting a multitude of active moieties, PCT-WO 98/46270, (1998) 173 pages.
[42] S. Choi, M. Mammen, G. Whitesides, J. Griffin, Polyvalent presenter combinatorial libraries and their uses, PCT-WO 98/47002, (1998) 132 pages.
[43] D. Marquess, M. Mammen, J. Griffin, Methods for indentifying novel multimeric agents that modulate receptors, PCT-WO 99/66944, (1999) 359 pages.
[44] D. Joseph-McCarthy, L. Isaacs, G. Whitesides, M. Karplus, J. Hogle, J. Cheh, Anti-picomaviral ligands via a combinatorial computational and synthetic approach, PCT-WO 99/29280A2, (1999) 44 pages.
[45] L.E. Strong, L.L. Kiessling, A general synthetic route to defined, biologically active multivalent arrays, J. Am. Chem. Soc. 121 (1999) 6193–6196.
[46] M. Mammen, G. Dahmann, G. Whitesides, Effective inhibitors of hemagglutination by influenza virus synthesised from polymers having active ester groups. Insight in mechanism of inhibition, J. Med. Chem. 38 (1995) 4179–4190.
[47] S. Choi, M. Mammen, G. Whitesides, Generation and in situ evaluation of libraries of poly(acrylic acid) presenting sialosides as side chains as polyvalent inhibitors of influenza-mediated hemagglutination, J. Am. Chem. Soc. 119 (1997) 4103–4111.
[48] F. Menger, A. Eliseev, V. Migulin, Phosphatase catalysis developed via combinatorial organic chemistry, J. Org. Chem. 60 (1995) 6666–6667.
[49] F. Menger, J. Ding, V. Barragan, Combinatorial catalysis of an elimination reaction, J. Org. Chem. 63 (1998) 7578–7579.
[50] S. Patterson, B. Smith, R. Taylor, Tuning the affinity of a synthetic sialic acid receptor using combinatorial chemistry, Tetrahedron Lett. 39 (1998) 3111–3114.
[51] B. Linton, A. Hamilton, Host–guest chemistry: combinatorial receptors, Curr. Opin. Chem. Biol. (1999) 307–3 12.
[52] W. Motherwell, M. Bingham, Y. Six, Recent progress in the design and synthesis of artificial enzymes, Tetrahedron 57 (2001) 4663–4686.
[53] A. Godwin, M. Hartenstein, A. Müller, S. Brocchini, Narrow molecular weight distribution precursors for polymer–drug conjugates, Angew. Chem. Int. Ed. Engl. 40 (2001) 614–617.
[54] S. Schreiber, G. Crabtree, D. Holt, M. Zoller, New method for identifying and evaluating biologically active molecules, PCT-WO 96/13613, (1996) 30 pages.
[55] F. Menger, M. Rourk, Synthesis and reactivity of 5-fluoruracil/cytrarabine mutual prodrugs, J. Org. Chem. 62 (1997) 9083–9088.
[56] B. Stockwell, S. Schreiber, Probing the role of homomeric and heteromeric receptor interactions in TGF-beta. Signaling using small molecule dimerizers, Curr. Biol. 8 (13) (1998) 761–770.
[57] G. Crabtree, S. Schreiber, D. Spencer, T. Wandless, P. Belshaw, Genes, vectors and cells encoding ligand-binding chimeric proteins which may be oligomerized with multimeric synthetic ligands to induce a biochemical activity, US Patent 5869337, (1999) 96 pages.
[58] J. Griffin, Y. Ji, M. Maimnen, D. Marquess, EJ Moran, J. Wray, Multibinding protein kinase inhibitors, PCT-WO 01/42243, (2001) 252 pages.
[59] Y. Ji, M. Natarajan, J. Griffin, Multibinding, multimeric ligands comprising calcium channel blockers, PCT-WO 99/63992, (1999) 166 pages.
[60] O. Ramstrom, L. Ye, M. Krook, K. Mosbach, Screening of a combinatorial steroid library using molecularly imprinted polymers, Anal. Commun. 35 (1998) 9–11.
[61] L. Sabourin, R. Ansell, K. Mosbach, I. Nicholls, Molecularly imprinted polymer combinatorial libraries for multiple simultaneous chiral separations, Anal. Commun. 35 (1998) 285–287.
[62] T. Dickinson, D. Walt, J. White, I. Kauer, Generating sensor diversity through combinatorial polymer synthesis, Anal. Chem. 69 (1997) 3413–3418.
[63] E. Ostuni, C. Chen, D. Ingber, G. Whitesides, Selective deposition of proteins and cells in arrays of microwells, Langmuir 1 (9) (2001) 2828–2834.
[64] C. Lowe, Combinatorial approaches to affinity chromatography, Curr. Opin. Chem. Biol. 5 (3) (2001) 248–256.
[65] N. Patel, R. Bhandari, K. Shakesfeff, S. Cannizzaro, M. Davies, R. Langer, C. Roberts, S. Tendler, P. Williams, Printing patterns of biospecifically-adsorbed protein, J. Miomater. Sci. Polymer Edn. 11 (3) (2000) 319–331.
[66] J. Kohn, R. Langer, Poly(iminocarbonates) as potential biomaterials, Biomaterials 7 (1986) 176–181.
[67] K. James, J. Kohn, in: K. Park (Ed.), Controlled Drug Delivery: Challenges and Strategies, Pseudo-Poly(Amino Acid)s: Examples for Synthetic Materials Derived from Natural Metabolites, American Chemical Society, Washington, DC, 1997, pp. 389–403.
[68] N. Suarez, S. Brocchini, B. Laredo, A. Bello, J. Kohn, The study of water uptake in new biomaterials by thermally stimulated depolarization currents, Biomaterials 19 (1998) 2347.
[69] A. Belu, S. Brocchini, J. Kohn, B. Ratner, Characterization of combinatorially designed polyarylates by time-of-flight secondary ion mass spectrometry, Rapid Commun. Mass Spectrom. 14 (2000) 564–571.
[70] J. Kohn, R. Langer, Polymerization reactions involving the side chains of α-L-amino acids, J. Am. Chem. Soc. 109 (1987) 817–820.
[71] J. Kohn, S. Brocchini, in: J.C. Salamone (Ed.), Polymeric Materials Encyclopedia, Pseudo-Poly(Amino Acid)s, Vol. 9, CRC Press, Boca Raton, FL, 1996, pp. 7279–7290.
[72] A. Kabanov, V. Alakov, B. Batrakova, Copolymer compositions for oral delivery, PCT-WO 99/39731, (1999) 80 pages.

[73] N. Galakatos, R. Langer, D. Putnam, Formulation arrays for screening, PCT-W0059627, (2000) 46 pages.
[74] V. Alakhov, E. Klinski, S. Li, G. Piertrzynski, A. Venne, E. Batrakova, T. Bronitch, A. Kabanov, Block copolymer-based formulation of doxorubicin. From cell screen to clinical trails, Coll. Surf. B 16 (4) (1999) 114–134.
[75] P. Lemieux, N. Guerin, G. Paradis, R. Proulz, L. Chistyakova, A. Kabanov, V. Alakhov, A combination of poloxamers increases gene expression of plasmid DNA in skeletal muscle, Gene Ther. 7 (11) (2000) 986–991.
[76] A. Boal, F. Lilian, J. DeRouchey, T. Thum-Albrecht, T. Russell, V. Rotello, Self-assembly of nanoparticles into structured spherical and network aggregates, Nature 404 (2000) 746–748.
[77] V. Alakhov, A. Kabanov, Block copolymeric biotransport carriers as versatile vehicles for drug delivery, Exp. Opin. Invest. Drugs 7 (9) (1998) 1453–1473.