Computer Simulated Screening of Dentin Bonding Primer Monomers Through Analysis of Their Chemical Functions and Their Spatial 3D Alignment

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Abstract: Binding interactions between dentin bonding primer monomers and dentinal collagen were studied by an analysis of their chemical functions and their s 3D alignment. A trial set of 12 monomers used as primers in dentin adhesives was characterized to assess them for binding to a complementary target. HipHop utility in the Catalyst software from Accelrys was used for the study. Ten hypotheses were generated by HipHop procedures involving (a) conformational generation using a poling technique to promote conformational variation, (b) extraction of functions to remodel ligands as function-based structures, and (c) identification of common patterns of functional alignment displayed by low energy conformations. The hypotheses, designated as pharmacaphores, were also scored and ranked. Analysis of pharmacaphore models through mapping of ligands revealed important differences between ligands. Top-ranked poses from direct docking simulations using type 1 collagen target were mapped in a rigid manner to the highest ranked pharmacophore model. The visual match observed in mapping and associated fit values suggest a strong correspondence between direct and indirect docking simulations. The results elegantly demonstrate that an indirect approach used to identify pharmacaphore models from adhesive ligands without a target may be a simple and viable approach to assess their intermolecular interactions with an intended target. Inexpensive indirect/direct virtual screening of hydrophilic monomer candidates may be a practical way to assess their initial promise for dentin primer use well before additional experimental evaluation of their priming/bonding efficacy. This is also of value in the search/design of new compounds for priming dentin. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2008

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INTRODUCTION

Hydrophilic primer monomers used in current dentin bonding procedures create a hybridized layer of collagen and resin by their permeation through the subsurface region of demineralized dentin in the conventional etch, prime, and bond technique followed by polymerization of the infiltrated monomer.[1] In the self-etch bonding procedure, the monomer penetrates through the porous dentin smear layer and subsurface region of the intact dentin to form the hybrid layer. In either case, the capability of a monomer to permeate into the tissue substrate and form a well-bonded hybrid layer depends on the priming efficacy of the primer. The ability to wet, permeate, and bond to collagen and/or mineral component of the tissue is therefore a critical requirement for primer monomers. Predictable interaction with type 1 collagen, a biological protein, is especially valuable in dentin bonding. Low-cost methods to evaluate the interaction of candidate monomers to dentinal collagen (type 1 collagen) are therefore necessary to screen candidate monomers before expensive *in vitro* or *in vivo* evaluation.

In recent years, computational methods have become important tools to virtually screen candidate molecules for biological activity at very low cost, and the compounds that successfully underwent the virtual screening scrutiny were targeted for the more important experimental and clinical evaluation. Such virtual screening has become possible because of the availability of reliable computer mod-

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eling methods to assess their potential for success. Two methods used in computer modeling screening are the direct (protein-based) and indirect (ligand-based) methods. In direct methods, flexible low energy conformations of a ligand that dock to a specific site or sites of a known target structure are identified, and the binding of the ligand assessed using interaction energy criteria. Such methods to characterize dentin bonding primer ligands have been reported in the past.[2] Prior knowledge of the target structure is essential in the direct docking methods. In the indirect methods, the target active site profile is deduced from the complementary features in the most likely spatial patterns of 3D ligand conformational poses, and hence the 3D structure of the target is not an essential input condition. Rather, sets of molecules known for their proven biological activity to the target molecule are used in the identification of the group of functional features and their 3D placement that facilitate their interaction with the target sites. In this study, the objective was to identify such a common template or 3D arrangement of functional groups (referred to as pharmacaphore) of a set of dentin bonding primer monomers that may be responsible for their adhesive interactions with dentinal collagen. An indirect method was used without using the structure of the intended target protein (type 1 collagen).

The term pharmacophore was introduced by Paul Ehrlich in the early 1900s to refer to the "molecular framework that carries (phoros) the essential features responsible for a drug's (pharmacon) biological activity."[3] Later, with improved understanding of intermolecular interactions between ligands and giant molecular targets with spatial and/or functional features capable of binding to appropriate ligands, it became abundantly clear that while the presence of specific functionalities in a ligand was a necessary chemical condition for the desired activity, their mere presence was not sufficient. The three-dimensional disposition of these functionalities is also critically important, that is, whether they can functionally and geometrically align optimally to the complementary features in the target molecule during bonding. The term pharmacaphore has acquired much broader meaning beyond drug–receptor interactions and cover any ligand–target interaction, where a specific 3D arrangement of functional groups in the ligand is required to exhibit a desired biological or other activity.[4] Pharmacaphore is best defined as a collection of chemical or functional features with the necessary and essential geometric 3D constraints on these features for favorable interaction with a target.[5–11] Expression of specific biological activity or binding of a ligand to an intended target is therefore considered to be associated with pharmacaphoric pattern of functional placement in the ligand 3D structure. The common alignment of functionalities in space for the model compounds is assumed to be responsible for the known bioactivity of these compounds to the intended target, and the pharmacaphore template encoding this 3D functional alignment is used as a search criterion for new bioactive compounds. Although such a method is the only computational method available to study ligand–target interactions in the absence of knowledge of the target structure, the pharmacaphoric pattern identification is also a useful part of a broader approach to study ligand–target interactions even when prior knowledge of target molecular or crystallographic structure is available. The pharmacaphore is essentially a ligand functional recognition configuration to bind to an intended target. The identification of a valid pharmacaphore model from previously known bioactive compounds can therefore be used in the search for new compounds of promising biological activity to that target. Such a search is typically focused on chemical compounds in known databases such as MDL-SC*, CSD†, CAP‡, Maybridge2004§, and others in which the structure and other relevant details of available compounds are already provided for low-cost virtual screening to identify new compounds.

In this study, the objective was to generate a pharmacaphoric model for a trial or training set of ligands. The ligands studied included those that are currently used as primers in dentin adhesive systems as well as some promising candidate molecules, which are not currently used in dentin bonding, but have potential for such use. The underlying hypotheses of the study are as follows:

1. Dentin bonding primer ligands show one or more common 3D frameworks of spatially aligned reactive chemical functions in their low energy conformations.
2. Each framework of alignment of functional features represents a pharmacaphore model (hypothesis) encoding a set of specific functionalities and their 3D placement constraints that promote expression of biological activity or binding to the intended target.
3. Top-ranked low energy ligand conformations in docked ligand–collagen complexes in direct docking simulations show 3D alignment of common functions in conformity with pharmacaphore concept.

MATERIALS AND METHODS

Computer Simulation Methods

Catalyst software (version 4.11)¶ was used in the study. Catalyst offers two methods to study pharmacaphore model generation: HypoGen and HipHop.[12–15] Hypotheses are generated in both methods. A hypothesis is a set of common characteristics that distinguish a set of molecules. HypoGen generates hypotheses based on activity characteristics of the molecules, and HipHop generates function or feature-based hypotheses. When specific data are available

* MDL-SC (MDL Screening compound Directory) at http://www.mdl.com/products/pdfs/scd_ds.pdf formerly also known as ACD (Available Chemicals Directory)-SC."

† CSD (Cambridge Structural Database), at http://www.ccdc.cam.ac.uk/products/csd/

‡ CAP (Chemicals available for purchase) Database, Accelrys, More information at http://www.accelrys.com/products/chem_databases/databases/CAP.html

§ Maybridge2004 Chemical Database from Accelrys Inc. More information at www.chem.ac.ru/Chemistry/Databases/MAYBRIDGE.en.html

¶ Accelrys, 9685 Scranton Road, San Diego, CA 92121.

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to represent the biological activity of model compounds, and the activity of these compounds covers a wide range (of 4–5 orders of magnitude), HypoGen offers a practical approach to model an appropriate pharmacaphore for ligand–target interactions.[12] Unfortunately, no valid data are available for the binding activity of dentin primer monomers to use the HypoGen program. When no activity data are available (as is the case for dentin primer monomers) or when the known activity data are closer together in a narrow range, the HipHop program is used to generate pharmacaphore models for a small set of ligands (typically <15). We used HipHop to identify low energy conformations and to extract 10 pharmacaphore hypotheses using the common patterns of functional alignment in the ligands.

Pharmacaphore Search Procedures in HipHop Utility

In the overall approach of pharmacaphore search, a number of general steps are used to arrive at the template or templates for consideration.[6] The steps include:

1. Proper selection of starting ligand molecular structures of a small, but diverse set of compounds to give valid and representative outcomes. Some important and well-established bioactive molecules of the training set are also typically specified as reference molecules with greater weight (than other members of the trial set) in arriving at the pharmacaphore hypothesis.
2. Conformational search to identify different low energy conformational states.
3. Extraction of features in the ligands. Features used in different algorithms include atoms, topological features (e.g., phenyl ring, carbonyl group etc.) or chemical functions. Hip-Hop uses chemical functions as features. Typical functionalities used are hydrogen bond acceptor, hydrogen bond donor, acid functionality (negative ionizable function at physiological pH 7), base (positive ionizable function at physiological pH 7), aromatic ring and hydrophobic group.[6]
4. Feature-based molecular model identification: The extracted features are combined to configure a respective function-based ligand structural model.
5. Pharmacaphore hypothesis generation: The features of all ligand molecules in the respective structural models are matched, and models of their common 3D placement are generated as pharmacaphore candidate hypotheses.
6. Scoring: The hypotheses are scored and a preselected number (typically up to 10) of highest ranked hypotheses used for analysis and further assessment.

HipHop Representation of Pharmacaphore

In the HipHop algorithm, a pharmacophore model or hypothesis is generated as a distinct three-dimensional configuration of chemical functions located at centers of tolerance spheres.[5,11,13] A tolerance sphere defines that area in space that should be occupied by a specific type of chemical functionality in the bound ligand structure. Hydrogen bond donors and acceptors also include a vector to incorporate the constraint of bond directionality. Misalignment of any functionality from its reference sphere of tolerance or directional constraint can be expected to weaken the interactions between the chosen functionality and its complementary functional site in the target. Therefore, the match of the 3D functional alignment of each individual ligand to the pharmacaphore model was used to screen candidate monomers for priming application.

Training Set of Ligands

The training set (Figure 1) was selected to represent a spectrum of common functional features present in dentin bonding primer monomers, including (a) acidic groups (e.g., phosphoric or carboxylic acid), (b) hydrogen bond donor/acceptor functions (e.g., —C=O/—NH groups), (c) spacer groups (e.g., —CH_2— chains), and (d) other hydrophobic sites (e.g., acrylic groups and aromatic rings). The ligand set included primer molecules based on oxygen, phosphorus, and nitrogen as hetero-atoms for potential binding interactions. Some molecules in the training set are currently used as dentin primer monomers, and others have been shown to be promising. The structures and short designations of all the ligands studied are listed in Table I. F1 T1

Methods and Default Parameters of Pharmacaphore Search

Pharmacophore modeling and analysis were carried out on a SGI workstation (Octane) running IRIX 6.5.28m operating system with a 400 MHz processor with CPU MIPS R12000. The 3D structures were built in Sybyl molecular modeling software (version 7.1**). All structures of primer molecules included in the study were imported into Catalyst to create a training set, and energy minimized to the closest local minimum using the generalized CHARMm[14] force field as implemented in Catalyst. Confirm module with *Best Conformer Generation* option was used to generate conformational models. Best conformer generation option uses a poling algorithm to generate unique but diverse conformations. The algorithm, originally described by Smellie et al.,[16] uses a poling function that prevents conformations of molecules from being too close together (i.e., promotes diversity to prevent missing of important "hits"), and allows the exploration of the conformational space of molecules within a user-defined energy window. The maximum number of conformations opted for consideration was 500, and the energy window of the conformational states considered was set to 20 kcal/mol above the minimum energy conformational state in the ensemble. Based on the diversity and experimental priming efficacy of the functionalities (as assessed by immunochemical binding studies[2,11,17]) in the molecules, GPDM, MMEM, NMGlu, and MPDP were used as reference ligands. Set

** Tripos, St. Louis, MO.

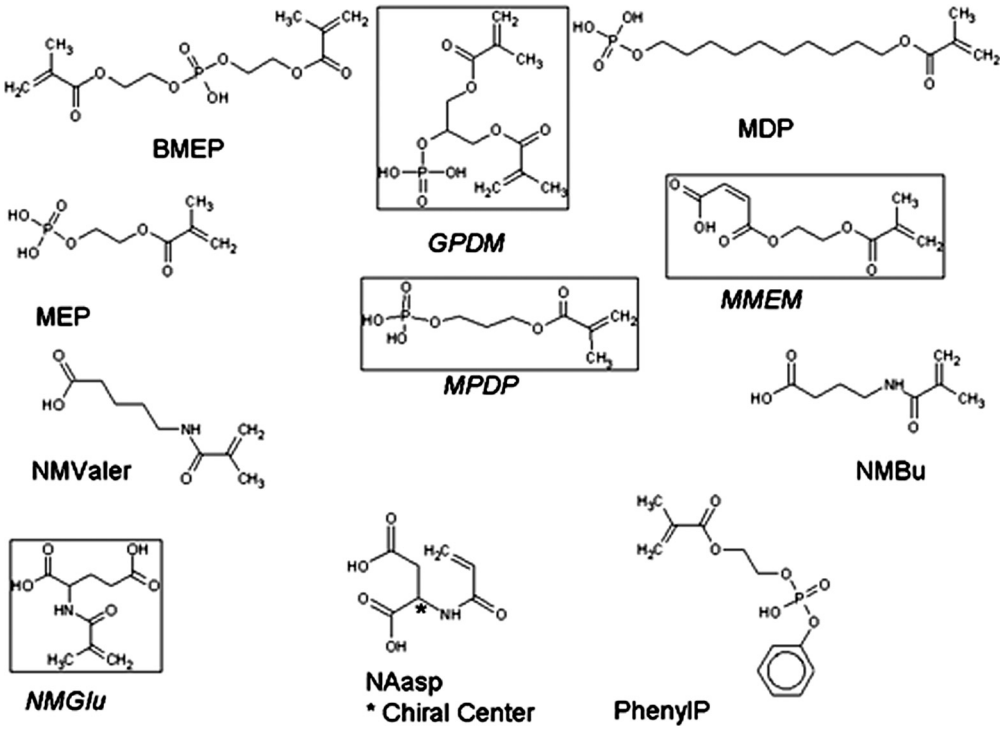


Figure 1. Chemical structures of primer monomers used in the HipHop calculation. The molecules shown in boxes were chosen as reference compounds with Principal = 2, MaxOmitFeat = 0 HipHop parameter settings. The remaining compounds are nonreference compounds with Principal = 1, MaxOmitFeat = 1. Note that MaxOmitFeat values 0 and 1 indicate whether all features are mapped or, all features but one are mapped into the hypothesis, respectively.

Stereochemistry tool in the *Toolbox* was used to assess and include the stereochemistry of the appropriate molecules (S-NAasp and R-NAasp). Default parameters were used for the HipHop calculations except for selected cases listed under footnote.[††]

Mapping of Direct Docking Conformations to Pharmacaphore Models

Conformations that formed low energy complexes with target (RCSB PDB[18] file 1QSU) in direct docking studies of selected ligands were also mapped to the pharmacaphore hypothesis generated in the present study using indirect methods with no target. The docked conformations were generated using AutoDock 3.05 by Lamarckian genetic method of docking used in our earlier studies described in previous publications.[2,11,17] Top-ranked docked ligand conformations (the conformation with the lowest docking energy for each ligand) from selected ligands were extracted and mapped to the pharmacaphore hypothesis-1. The mapping was done under conformational rigidity constraint (i.e., with no changes in dihedral angles).

In Vitro Binding Assay and SEM Evaluation

Top two primer monomers of the training set in the Pharmacaphore modeling was also evaluated by *in vitro* immunochemical binding assay. The assay was carried out using gold-labeled immunochemical-binding methods reported previously.[2,11] Human dentin sections demineralized with phosphoric acid (to expose collagen fibrils) were separated into control ($N = 3$) and experimental samples ($N = 3$). The samples in the experimental group were treated with primer ligand solution for a minimum of 1 hr to mediate binding interactions between the ligand and type 1 collagen. The control samples were treated with distilled water under the same conditions. All samples were then treated with anticol-

TABLE I. List of Primer Monomer Compounds Studied

Number	Short Designation	Compound Name
1	GPDM	Dimethacrylglycerol monophosphate
2	MPDP	3-Methacryloyloxypropyl phosphate
3	NMGLu	*N*-methacryloyl glutamic acid
4	NMValer	*N*-methacryloyl valeric acid
5	MEP	2-Methacryloyloxy ethyl phosphate
6	PhenylP	2-Methacryloyloxyphenyl phosphate
7	(R)NAAsp	(*R*)-*N*-acryloylaspartic acid
8	(S)NAAsp	(*S*)-*N*-acryloylaspartic acid
9	MMEM	Monomethacryloyloxyethyl maleate
10	NMBu	*N*-methacryloyl butyric acid
11	BMEP	Bis(2-methacryloyloxyethyl) phosphoric acid
12	MDP	10-methacryloyloxydecamethylene phosphoric acid

[††] Spacing: 250 pm (2.5 Å), catHypo.forceAbsoluteStereochemistry = 1, compare.considerMirror = 0, hipHop.extended.output = 1.

lagen type 1[‡‡] for antibody binding to collagen molecules. The extent of antibody binding to collagen was assessed by immunochemical binding assay. The immunochemical assay was done by treatment with secondary antibody (antimouse, antigoat IgG labeled with 10-nm size colloidal gold particles[‡‡]), followed by silver enhancement of gold labels and scanning electron microscopy in a Hitachi SEM S-2500. The silver enhancement is a routine technique used to increase the size of gold labels by silver deposition on the gold particles using a silver staining kit.[19,20]

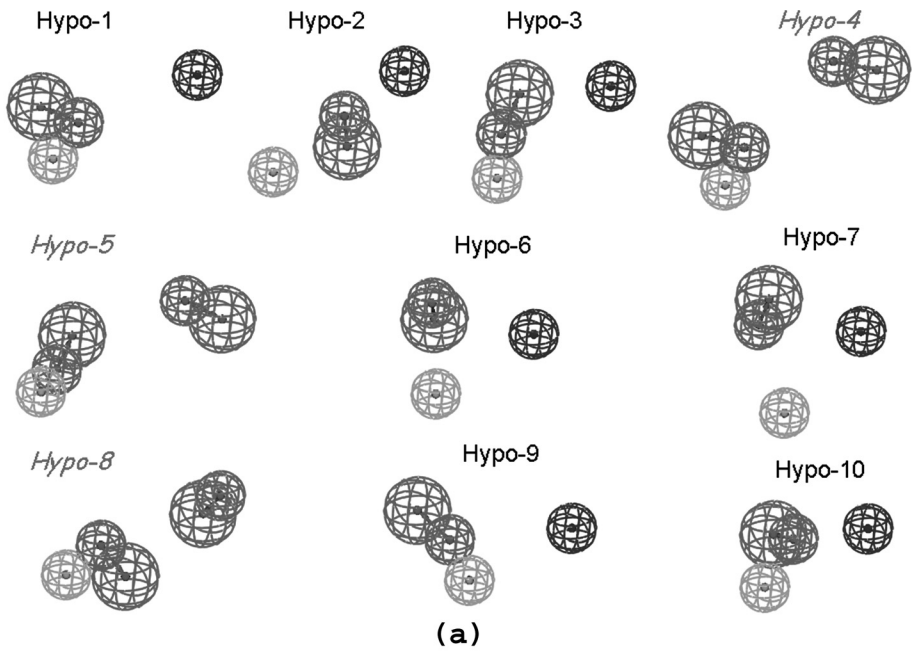
RESULTS

Figure 1 shows the structures of the primer molecules included in the training set. The top 10 potential HipHop pharmacophore models [hypotheses] derived from the training set of primer molecules are shown in Figure 2(a). Figure 2(b) illustrates hypothesis-1 with specific details of the 3D distance matrix between functions. Table II lists details describing each hypothesis, such as the functional groups extracted and explored, the rank score and the hit masks. Direct hit mask values indicate whether (1) or not (0) a training set molecule mapped all the functional features to the current hypothesis, and the partial hit values denote whether the molecule has missed all but one (1) feature or not (0) in the pharmacophore models. The pharmacaphore frameworks are common combinations or groups of chemical functions and their 3D constraints in space identified in the low energy conformations of all ligands in the training set. All models identified in the search were made up of three chemical functions with a common spatial alignment (within a well-defined spatial range of tolerance). The functions included one or more combinations of (a) a negative ionizable group (N), (b) a hydrophobic group (H), and (c) hydrogen bond acceptor (A). Clustering the top 10 hypotheses using Hierarchical Average Linkage method at the two-cluster level showed that hypotheses 4, 5, and 8 belong to a different cluster than the rest of the models (see Table II). These three hypotheses had the HAA functions, and the other seven hypotheses had NHA functions. Typically the models represent 10 of the highest ranked 3D placements of functional features in the hypotheses, to which the matching functional features of low energy conformers of all the ligands in the training set are most successfully mapped within the defined tolerance range of their spatial alignment. The adhesive behavior of individual ligands to the intended target may therefore be promoted by a favorable match of the 3D functional placements in the ligand to that in the pharmacaphore templates generated. The ranking scores of the 10 hypotheses ranged between 80.27 and 94.57. The higher the ranking score for a given hypothesis, the lesser is the probability that the successful mapping occurred by chance, and the top-ranked models may reasonably be considered to represent potentially valid functional placements promoting adhesive interactions to the assumed target. For this reason, we used hypothesis-1 with the highest ranking in the NHA groupings and hypothesis-4 from the HAA groupings to illustrate how well the individual ligands were mapped to the pharmacaphore models. Figure 3 illustrates the mapping of selected individual ligands of the trial set to the pharmacaphore hypothesis-1. The details of the outcomes of the mapping for each individual ligand are also listed in Table III. The details in order of each column are: the compound name, how the compound is used as principal (value of 1 indicates that the molecule must map into the hypothesis fully or partially to be considered active, value 2 designates reference compound with higher weight during the construction of pharmacaphore hypotheses), MaxOmitFeat (value 0 and 1 indicate whether all features of the compound are mapped or, all but one must be mapped into the hypothesis, respectively), the number of conformers used in generating the hypothesis, conformational energy of the top-ranked conformer and the corresponding best fit. Figure 3 and Table III show major differences in the mapping, and several parameters of the mapping used or computed by the program. For example, the mapping of individual ligands in Figure 3 illustrates important differences in the locations of the function centers relative to the pharmacaphore tolerance sphere centers representing the respective functions. Similarly, there are also differences in the alignment of the hydrogen bond directionality vector from that in the pharmacaphore. Significant differences in the number of conformations used in building the pharmacaphore are also seen. The conformational energy values of the best-fit conformers and the corresponding fit values [see Table III] show differences as a function of ligands.

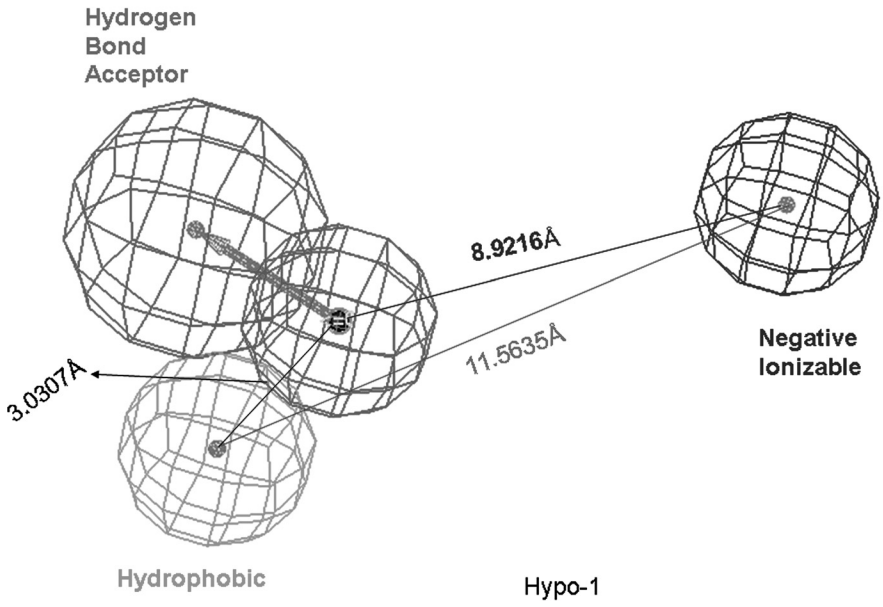
When direct docking is done on a target, the bound conformational states are generated at energetically favored target sites. Figure 4(a) is an example of selected conformations of ligands docked to the type 1 collagen molecule using AutoDock 3.05. The docked conformations typically exhibited spatial alignment of their functions to active sites in the collagen target. We examined whether these alignments matched the pharmacaphore pattern (hypothesis-1) determined by indirect simulations with no target. Figure 4(b–d) shows these docked ligand conformations mapped to the pharmacaphore hypothesis-1. It is seen that the conformations map the functions within the pharmacaphore tolerances.

Figure 5 shows SEM micrographs of demineralized dentin surfaces of control and experimental surfaces after their respective treatments. The control samples showed silver enhanced gold label depositions on the sample surface, but such deposition is very sparse in ligand-treated samples. Since the gold labeled secondary antibody is designed to bind to the primary antibody binding to type 1 collagen, the relative absence of gold deposition in MDP- and MMEM-treated sample surfaces (and their contrast with the gold deposition spots in the control specimen) indicates that the ligands bind to the exposed collagen fibrils preventing primary antibody access to bind to the target. Thus the ligands, which gave top "best fit" values in pharmacaphore modeling, show evidence of strong *in vitro* binding to dentinal collagen.

‡‡ Sigma-Aldrich Chemicals, St. Louis, MO.



(a)



(b)

Figure 2. (a) Top 10 pharmacophore models (hypotheses) from HipHop calculation. Details of functional features for each hypothesis are shown in Table II. Hypo-4,5, and 6 belong to a different cluster, and their respective captions indicated in red. (b) The top-ranked three-feature NHA pharmacophore model (hypothesis-1). [N: Negative Ionizable, H: Hydrophobic, and A: Hydrogen Bond Acceptor]. The hydrogen bond direction vector is also indicated from the acceptor to the hypothetic donor site corresponding to the center of the larger donor tolerance sphere. Distance matrix between functional groups is also shown. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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DISCUSSION OF RESULTS

Many hydrophilic monomers have been used successfully as primers in dentin bonding.[1] In this study, we computationally identified highly likely common frameworks of functional alignment of several dentin bonding primer monomers. This was done by identifying the chemical functions and the common 3D arrangement of these functions in low energy conformational states of these ligands. Low energy conformations, being thermodynamically most

TABLE II. Details of 10 Top-Ranked Hypotheses

Hypotheses	Features	Rank	Direct Hit	Partial Hit	Max Fit
1	NHA	94.5720	111111111111	000000000000	3
2	NHA	91.1065	111111111111	000000000000	3
3	NHA	87.1407	111111111111	000000000000	3
4	HAA	83.6057	111111111111	000000000000	3
5	HAA	83.6057	111111111111	000000000000	3
6	NHA	83.4696	111111111111	000000000000	3
7	NHA	82.4806	111111111111	000000000000	3
8	HAA	82.0086	111111111111	000000000000	3
9	NHA	81.0379	111111111111	000000000000	3
10	NHA	80.2707	111111111111	000000000000	3

N, NegIonizable Hydrophobic and A, Hydrogen Bond Acceptor.

stable, constitute the vast majority of actual conformational states present in the distribution of conformations in any assembly of molecules under ambient conditions, and they are assumed to have the highest weight in determining the binding properties of the primer monomers. The frameworks of functions and their 3D alignments of ligands encoded in their pharmacaphore hypotheses are therefore considered as valuable reference models for predicting potential ligand–target interactions associated with priming efficacy of the ligand.

The computational program output provides a number of parameters for consideration in assessing the promise of a given candidate primer monomer for dentin bonding. These include (a) the best fit among all conformations of a specific ligand mapped to the pharmacaphore, (b) the conformation energy of the best-fit conformation, (c) the number of conformations of a given ligand in the training set used in generating the pharmacaphore, and (d) whether all features of a specific ligand are mapped in the pharmacaphore model or not. These are useful parameters for analysis to ascertain the priming efficacy of the individual monomers used in the study. The results of the study indicate important differences in the alignment of functions of individual ligands with respect to the desired alignment, as projected in the pharmacaphore hypothesis. These results may be relevant in determining the differences in the adhesive proper-

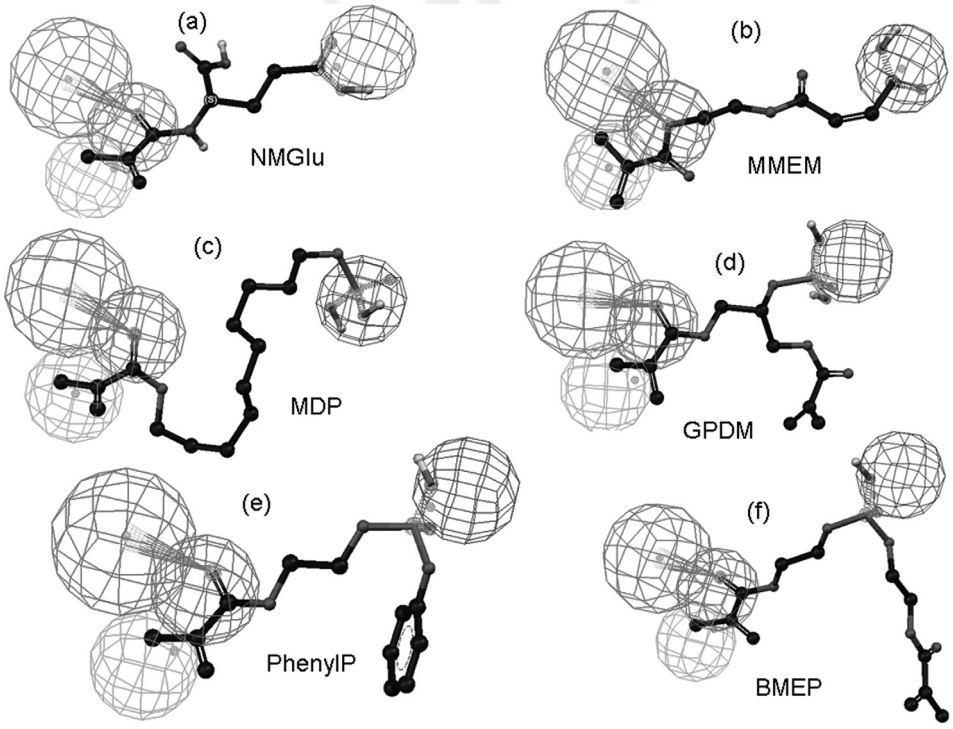

Figure 3. Mapping of selected primer monomers [NMGlu (a), MMEM (b), MDP (c), GPDM (d), PhenylP (e), and BMEP (f)] to the top-ranked pharmacophore model (hypothesis-1). Best Fit with MaxOmitFeat = 0 was used for the fit. Only polar hydrogen atoms are shown. Note the differences in the mapping of individual ligands with respect to the pharmacaphore. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE III. Summary of HipHop Run

Name	Principal	MaxOmitFeat	Confs	Conf Energy	Best Fit
GPDM	2	0	287	19.6851	1.836
MPDP	2	0	259	15.1521	2.602
NMGlu	2	0	158	12.5193	2.080
NMValer	1	1	151	19.9766	2.907
MEP	1	1	172	15.8611	1.809
PhenylP	1	1	182	13.5300	2.007
(*R*)-NAAsp	1	1	59	7.0404	0.848
(*S*)-NAAsp	1	1	47	11.6455	1.155
MMEM	2	0	192	15.3080	3.000
NMBu	1	1	127	19.2939	2.119
BMEP	1	1	308	9.0992	1.926
MDP	1	1	394	10.7564	2.997

ties of the respective ligands. For example, the monomer MDP (evaluated under hypothesis-1) has a fit value of 2.997 (relative to a perfect fit of 3.0 for the ideal monomer conformation), a high number of different conformational states used in generating the pharmacaphore (specifically 394 conformations out of a maximum 500 conformations allowed for consideration), a relatively moderate conformational energy for the best conformation (10.7564 kcal/mol for MDP vs. 19.9766 kcal/mol for NMValer), and all three functions N, H, and A in the monomer were matched to the pharmacaphore. Thus the hypothesis-1 model predicts MDP to be a potentially excellent compound as dentin self-etch primer in keeping with the well-recognized self-etching and priming efficacy of the MDP in the Self-Bond priming system from Kuraray. Some of the ligands such as GPDM (known to be a popular primer) improved their fit and other parameters under hypothesis-4. It is distinctly possible that different pharmacaphore models may characterize binding of individual ligands to a type 1 collagen molecule with multiple binding sites along the cavity tracks. In contrast to MDP, The NAasp ligand was found to have low fit and a lower number of conformations mapped to pharmacaphore in both models. It is therefore predicted to be of low to moderate promise.

Direct docking simulation studies between flexible ligands and rigid collagen target give additional support to the indirect docking procedure. As reported previously,[2,11,17] docking simulations indicate binding to specific functional sites of the target, where low energy conformational states of the ligand energetically dock to form collagen–ligand complexes. In this study, it was also found that the bound conformational states of ligands identified in direct docking simulations using a target mapped closely to the tolerance range of the pharmacaphore model obtained only from the dentin primer monomers (with no consideration of the target). This indicates that common alignment patterns of functionalities (determined with no target) express the ability of the ligands to bind to the intended target, and is a very neat demonstration of the validity of the indirect modeling approach. The *in vitro* binding assay

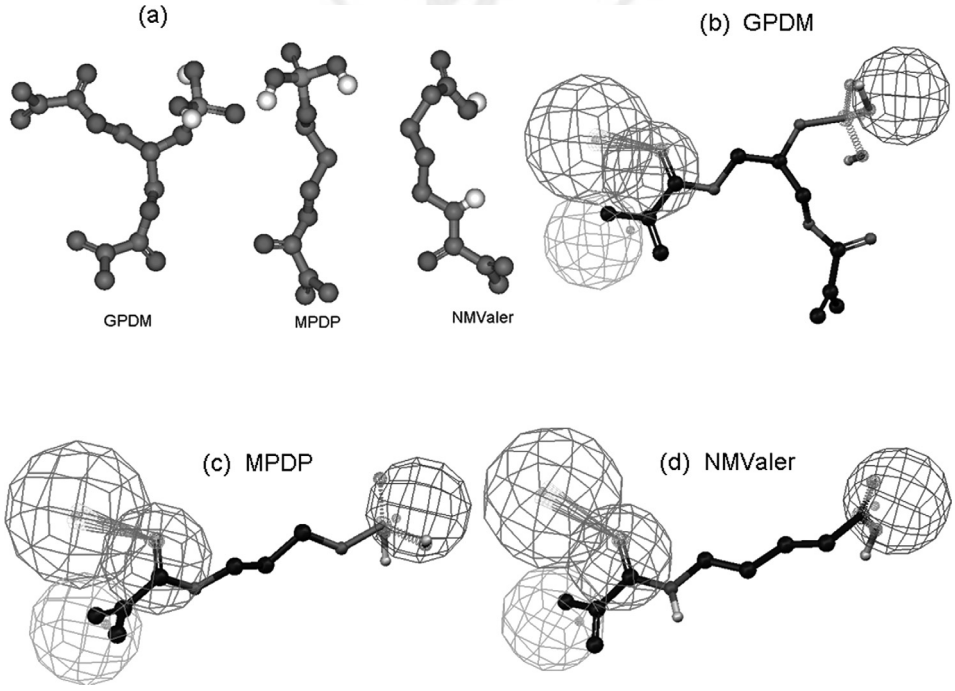

Figure 4. Autodock predicted top-ranked conformations were mapped (Best Map option) to hypothesis-1. (a) Top-ranked conformations of GPDM, MPDP, and NMValer extracted from AutoDock docking simulations, (b) Mapped GPDM conformation, (c) Mapped MPDP conformation, (d) Mapped NMValer conformation. Note that during mapping, AutoDock identified conformations were treated as rigid molecules. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

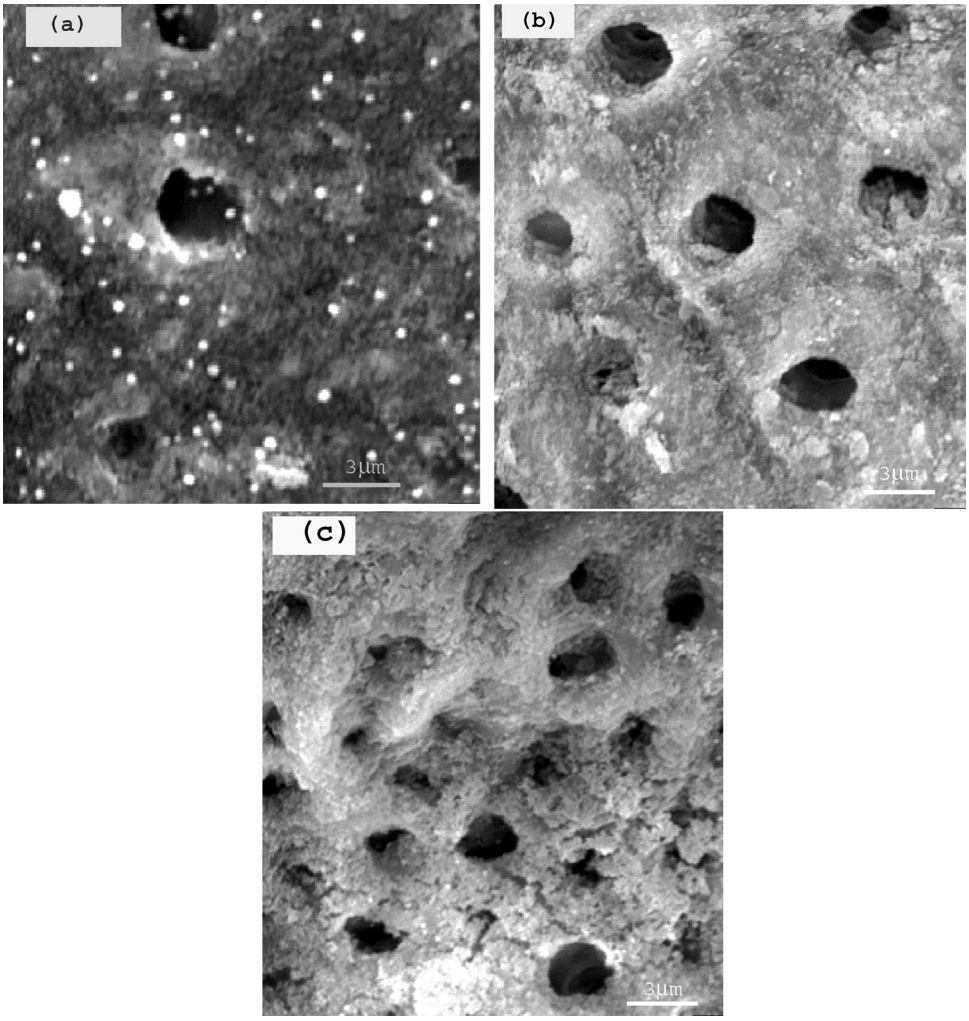

Figure 5. Typical SEM micrographs observed after immunochemical binding assay. (a) Control group, (b) MMEM-treated experimental group, (c) MDP-treated experimental group. Note how the ligand treatment precludes antibody binding to the demineralized dentin.

results also provided experimental evidence of ligand binding to dentinal collagen. Thus our results strongly support the conclusion that pharmacaphore modeling may identify chemical compounds with potential for binding to dentinal collagen.

The direct and indirect studies reported here and elsewhere by us are the first reports of virtual screening methods used in evaluating dentin-bonding primers. There are clearly benefits and limitations in the approach that need to be recognized. Thus, the virtual screening methods use the 3D functional make-up of the target structure directly or indirectly to develop useful criteria and parameters to assess ligand–target interactions and in searching for new compounds for the intended applications. Although the 3D constraints of the functional placements are critically important in the ligand–target interactions, the dissociation state of the ligands and the target structure are not currently included in the computational assessment methods we have used. These methods become important when pH values deviate from physiological conditions assumed in the studies. Future studies are being targeted to address these issues, since more recent programs under development include methods to incorporate ionization issues in the computations. In addition, there is a need to match these results to well-designed binding assays to provide activity data. Our immunochemical binding assays give useful qualitative and semiquantitative comparisons of predicted and observed priming efficacy, but much more additional work is needed to quantitatively correlate experimental and computational results. Thus, although more detailed work involving activity profiles, bond strength measurements, pharmacaphore models, and docking profiles is necessary for additional confirmation, our preliminary results strongly support the likelihood that promising collagen-binding ligands may conform to one or more pharmacaphoric hypotheses in their bound conformational states.

One of the advantages of the identification of pharmacaphore models is that these models can be used as reference templates to assess other candidate molecules for dentin bonding. Since the models are derived from the dentin ad-

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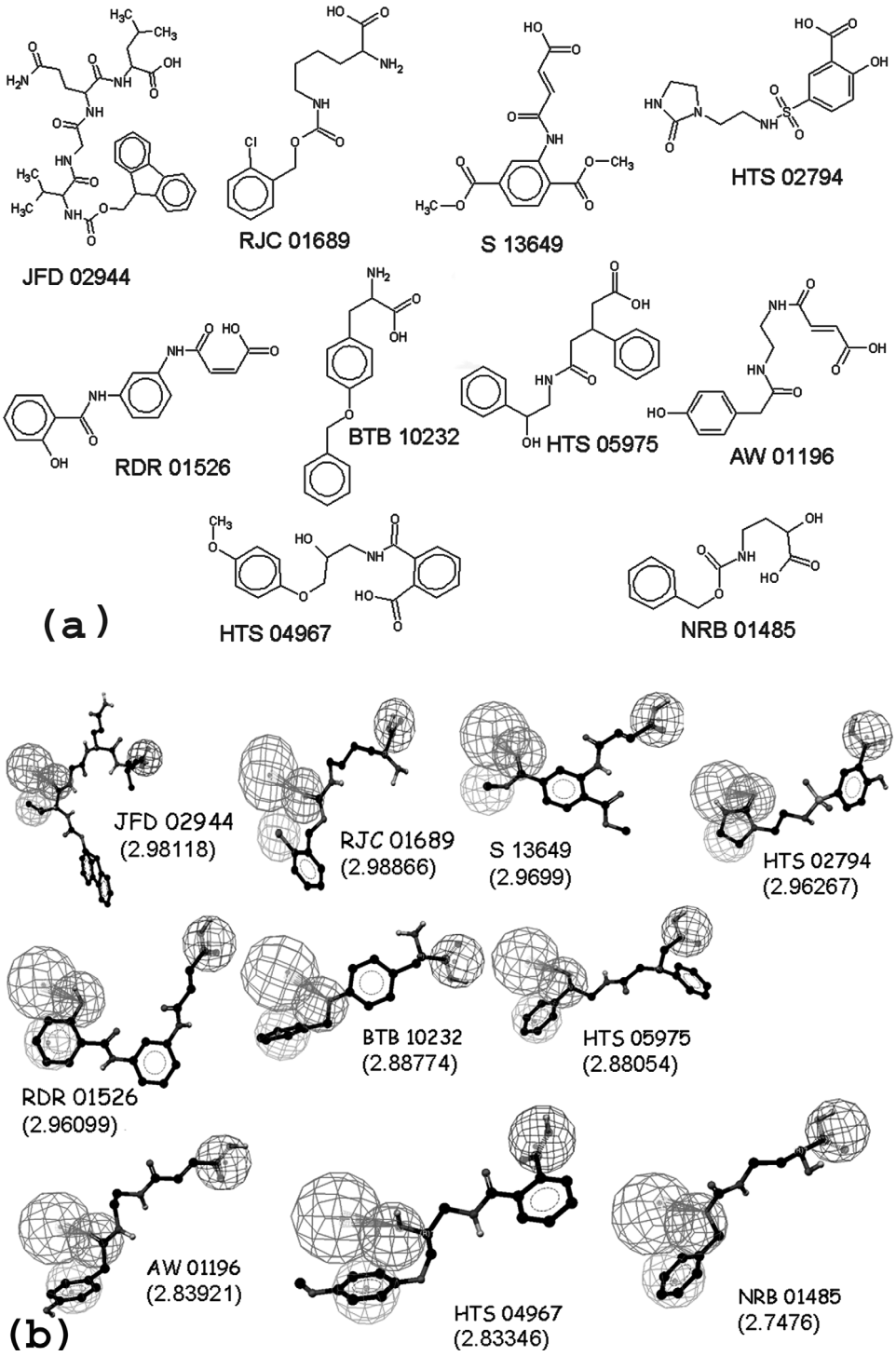

Figure 6. (a) Top hits from Maybridge2004 search. The best-fit values of each ligand studied in pharmacaphore match are listed in paranthesis under each structure. (b) Top hits from Maybridge2004 search mapped to pharmacaphore hypothesis-1. Note the close match of the compound functionalities to those of the hypothesis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

hesive molecules with known and/or promising ability to efficiently prime type 1 collagen of dentin, other molecules can be searched or designed to possess functions and alignment with closer match to the above pharmacaphore model or models. In addition, high throughput screening methods can also be used in future to identify lead compounds using pharmacaphore searching of a proper database. Current databases for high throughput search are, however, chemical compounds for drugs (that does not include a methacrylate functionality essential for primer monomers) in

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medical applications. Despite this limitation, we searched the Maybridge2004 Data base using the pharmacaphore hypothesis-1 developed in our study. The search yielded 1925 hits, and some of the top hits from the search are given in Figure 6(a). These compounds were also mapped to the pharmacaphore with hypothesis-1 in [Figure 6(b)], and the results indicate that these compounds may bind to collagen. The highest best-fit values of conformations of these molecules ranged between 2.748 and 2.998. By incorporating methacrylate functionality, these hits can be converted to dentin primer monomers. This aspect will be investigated in our future studies.

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CONCLUSIONS

Low energy dentin primer monomer conformations display common frameworks of functions and their 3D alignments. These frameworks potentially encode expression of their biological activity including binding patterns to type 1 collagen molecules in dentinal adhesion. These frameworks may be valuable to identify new dentin adhesives through virtual screening of compounds included in databases of suitable chemicals.

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REFERENCES

1. Nakabayashi N, Pashley DH. Hybridization of Dental Hard Tissues. Chicago: Quintessence Publishing Co.; 1998. p 37–83.
2. Vaidyanathan J, Kasinathan C, Vaidyanathan TK. Biomimetic recognition and immunochemical binding assay of collagen-ligand binding. J Adhes Dent 2003;5:7–17.
3. Ehrlich P. Ueber den jetztigen Stand der Chemotherapie. Berichte der Deutschen chemischen Gesellschaft (Reports of the German Chemical Society) 1909;42:17–47.
4. Gund P. Three-dimensional pharmacaphore pattern searching. Prog Mol Subcell Biol 1977;5:117–143.
5. Milne GWA. Pharmacophore and drug discovery. In: Schleyer PvR, Allinger NL, Clark T, Gasteiger J, Kollman PA, Schaefer HF III, Schreiner PR, editors. Encyclopedia of Computational Chemistry, Vol. 3. Chichester (UK): Wiley; 1998. p 2046–2056.
6. Dror O, Shulman-Peleg A, Nussinov R, Wolfson HJ. Predicting molecular interactions in silico. I. A guide to pharmacophore identification and its applications to drug design. Curr Med Chem 2004;11:71–90.
7. Miller MD. Molecular superposition. In: Schleyer PvR, Allinger NL, Clark T, Gasteiger J, Kollman PA, Schaefer HF III, Schreiner PR, editors. Encyclopedia of Computational Chemistry, Vol. 3. Chichester (UK): Wiley; 1998. pp 1693–1697.
8. Marshall G, Barry C, Bosshard H, Dammkoehler R, Dunn D. The conformational parameter in drug design: The active analog approach. In: Olson E, Christoffersen R, editors. Computer-Assisted Drug Design. Washington DC: American Chemical Society; 1979. p 205–226.
9. Beusen DD, Marshall GR. Pharmacophore definition using the active analog approach. In: Guner OF, editor. Pharmacophore Perception, Development, and Use in Drug Design. La Jolla: International University Line; 2000. p 21–45.
10. Güner OF. History and evolution of the pharmacophore concept in computer-aided drug design. Curr Top Med Chem 2002;2:1321–1332.
11. Vaidyanathan J, Vaidyanathan TK, Ramasubbu N, Ravichandran S. A computational and experimental analysis of ligand binding to type 1 collagen. Curr Comput Aided Drug Des 2005;1:397–422.
12. Clement O, Mehl AT. HipHop: Pharmacophores based on multiple common-feature alignments. In: Guner OF, editor. Pharmacophore Perception, Development, and Use in Drug Design. La Jolla: International University Line; 2000. p 71–84.
13. Li H, Sutter J, Hoffmann R. HypoGen: An automated system for generating 3D predictive pharmacophore models. In: Guner OF, editor. Pharmacophore Perception, Development, and Use in Drug Design. La Jolla: International University Line; 2000. p 171–189.
14. Sutter J, Güner OF, Hoffmann R, Li H, Waldman M. Effect of variable weights and tolerances on predictive model generation. In: Guner OF, editor. Pharmacophore Perception, Development, and Use in Drug Design. La Jolla: International University Line; 2000. p 499–511.
15. Brooks BR, Bruccoleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M. CHARMM: A program for macromolecular energy minimization and dynamics calculations. J Comput Chem 1983;4:187–217.
16. Smellie A, Teig SL, Towbin P. Poling: Promoting conformational variation. J Comput Chem 1995;16:171–187.
17. Vaidyanathan J, Vaidyanathan TK, Kerrigan J. Evaluation of intermolecular interactions of self-etch dentin adhesive primer molecules with type 1 collagen: Computer modeling and *in vitro* binding analysis. Acta Biomaterialia 2007;3:705–714.
18. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The protein data bank. Nucleic Acids Res 2000;28:235–242.
19. Namork E, Heier HE. Silver enhancement of gold probes (5–40 nm): Single and double labeling of antigenic sites on cell surfaces imaged with backscattered electrons. J Electron Microsc Tech 1988;11:102–108.
20. Hacker GW, Gu J. Gold and Silver Staining: Techniques in Molecular Staining Morphology. Advances in Pathology Microscopy and Molecular Morphology Series. Boca Raton: CRC Press; 2002.

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