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Original Article

Use of nano-sized clay crystallites to restore adhesion among tumor and aging stem cells - a molecular simulations approach

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Abstract: Adhesion of cells to the ECM is key to the regulation of cellular morphology, migration, proliferation, survival, and differentiation. The decrease in or loss of the cell's ability of mutual adhesiveness has been considered as one of the specific abnormalities in the surface properties of malignant cells. A change in the association of plasma membrane with cytoskeletal structures also seems to have a close relation with these abnormalities. Similar to the role of adhesions in tumor cells, stem cells' self-renewal is also tightly controlled by the concerted action of stem cell-intrinsic factors and signals within the niche. This study has demonstrated through molecular simulations the potential use of smectite (Na-montmorillonite) clay crystallites to create adhesions among tumor and stem cells. High electrostatic energies and cohesive energy densities measured in the simulations after the sorption of clay crystallites on cell-cell and cell-ECM complexes validate the concept of using these crystallites for the purposes. As results of this study are quite promising and clay crystallites could be considered as an option to restore adhesions in tumor and stem cells, other confirmatory tests and live cell culture studies are in process for the final validation.

Keywords: Stem cell adhesions, nano-sized clay, molecular simulations

Introduction

The integrity of the human tissues is promoted through the cell to cell and cell to extracellular matrix (ECM) adhesions. Cell to cell adhesions is mediated through adhesion molecules of cadherin family while cell to ECM adhesion is promoted through various receptors including syndecans, dystroglycan, and integrins [1]. Classical cadherin molecules mediate cell-cell adhesion via homophilic interactions between the extracellular domains of cadherins on adjacent cells and via interactions of cadherin intracellular domains with cytoskeleton-associated proteins [2]. Integrins are heterodimeric (consisting of α and β subunits) transmembrane molecules that mediate cell-ECM interactions [2]. The extracellular domains of integrins can bind directly to ECM proteins such as laminin, collagen, and fibronectin [3, 4]. In addition to ECM components, integrins can also bind to other cell-surface adhesion molecules such as intercellular adhesion molecule 1 (Icam1, also known as CD54) and vascular cell adhesion molecule 1 (Vcam1, also known as CD106). These cell adhesion molecules are known to be present in some stem cell niches [3].

In the review of the relationship of the turnover of the focal adhesions (FA) and the cancer cell migrations [5], studied the cell adhesion to the ECM and determined the turnover of FAs using cells cultured on an ECM-coated substratum. It was discovered that adhesion of cells to the ECM is key to the regulation of cellular morphology, migration, proliferation, survival, and differentiation [6, 7]. Numerous proteins are involved in integrin-mediated cell adhesion, and these proteins are collectively referred to as the adhesome [8]. Among the latter, Talin is a key regula-

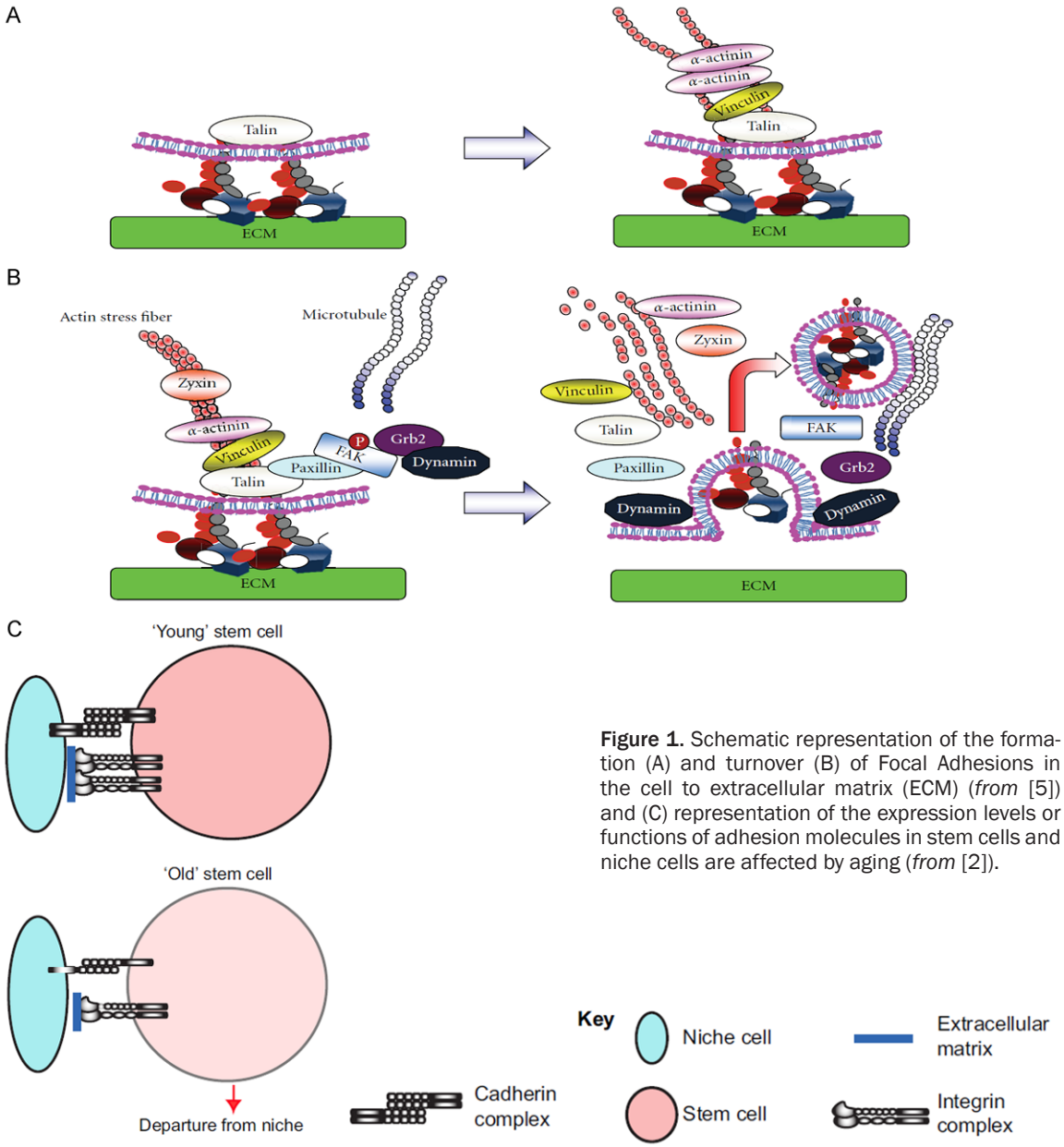

Figure 1. Schematic representation of the formation (A) and turnover (B) of Focal Adhesions in the cell to extracellular matrix (ECM) (*from* [5]) and (C) representation of the expression levels or functions of adhesion molecules in stem cells and niche cells are affected by aging (*from* [2]).

tor of the initial step of FA assembly. The binding of talin to integrin stabilizes the ligand-induced clustering of the latter at an initial stage of FA formation by mediating crosslinking of integrins with filamentous actin (F-actin) and F-actin-binding proteins such as vinculin and α-actinin (**Figure 1**).

Both α- and β-subunits of integrins are type I transmembrane proteins and contain both a large extracellular domain responsible for binding to ECM ligands and a cytoplasmic portion (CP) that recruits multiple intracellular proteins. Each integrin recognizes a distinct ECM ligand and the common integrin binding motif, Arg-Gly-Asp (RGD), is shared by several ECM proteins, including fibronectin, vitronectin, and fibrinogen. Integrin binding to laminins and collagens occurs at other recognition motifs [1].

The decrease in or loss of the cell's ability of mutual adhesiveness has been considered as one of the specific abnormalities in the surface properties of malignant cells. A change in the association of plasma membrane with cytoskeletal structures also seems to have a close relation with these abnormalities.

Similar to the role of adhesions in tumor cells, stem cells' self-renewal is also tightly controlled

by the concerted action of stem cell-intrinsic factors and signals within the niche [2]. Niche signals often function within a short range, allowing cells in the niche to self-renew while their daughters outside the niche differentiate. Thus, for stem cells to continuously self-renew, they are often anchored in the niche via adhesion molecules. In addition to niche anchoring, however, recent studies have revealed other important roles for adhesion molecules in the regulation of stem cell function, and it is clear that stem cell-niche adhesion is crucial for stem cell self-renewal and is dynamically regulated (**Figure 1**).

Based on the above premise, it is evident that loss of adhesions is the hallmark of both tumor and aging stem cells. In addition to several other consequences of loss of adhesions such as lack of communications, it results in the migration of the tumor cells to migrate to other parts of the body and create metastatic cancer. Most of the deaths in cancer patients occur due to the formation of the metastatic cancers. Restoration of tumor cell adhesions, therefore, may restore the cell communications and also control the tumor cells' migration resulting in the formation of metastatic cancers. Moreover, restoration of the stem to niche cell adhesions may also restore the differentiation ability of the divided aged stem cells resulting in the formation of new tissues.

One of the options of the restoration of adhesions among tumor or stem cells is the use of inorganic nano-sized clay crystallites. Among clay minerals, smectite is electrostatically charged particles. The charge deficiency in smectite clay, occurring due to isomorphous substitution in their molecular structure, is balanced by cations sorbed on their surfaces. Due to the charged structure, smectite clay particles have an affinity for other charged substances such as bacteria and the toxins. Due to this property, smectite clays have been used as alternative medicine for several ailments [9-15]. In addition to the medicinal use of smectite clays, and few studies to use clay nano-particles as medicine carrier [16, 17]. Recently, [18] and [19] have explored the possibility of use of clay nanoparticles as scaffolds aids during the regrowth of the bone structure. They used simulation-driven approach to demonstrate the use of nano-clays modified with amino acid to mineralize hydroxyapatite that mimics the biomineralization process. Although these studies [18] and [19] are a step towards the use of clay particles to enhance the biological processes, these and none of the other studies have ever considered clay nano-particles as adhesion restoring agents among cancer and stem cells.

In this study, we have demonstrated the possible use of the smectite clay minerals to restore adhesion among cancer and aging stem cells using molecular level simulation approach. The use of clay nanoparticles could lead to preventing cancer cell migration and possible promotion in the stem cell differentiation processes. This study encompasses the molecular level study of the interaction of the clay crystallites with cell-cell and cell-ECM environment using Monte Carlo (MC) and molecular dynamics (MD) simulation techniques.

Simulations study

The essential inputs for any molecular simulation scheme are the choice of the representative molecules/crystallites, formulation of the representative unit cells with periodic boundary conditions, and the application of a force field to run the appropriate ensemble. This study consisted of the creation of cell-cell and cell-ECM configurations in a molecular simulation software followed by the sorption and simulations of smectite clay crystallites with varying cation exchange capacity (CEC) on the formulated configurations. The molecular simulations were carried out using Monte Carlo (MC) and molecular dynamics (MD) techniques using Materials Studio software [20]. Due to the large volume of computations involved in the simulations, these calculations were carried out at the high-performance computing facilities (HPC) at KFUPM, KSA. Cohesive energy density (CED), considered as a measurement of the cohesiveness of the molecular system was determined for all the simulated configurations.

Selection and formulation of clay crystallites

Unit molecules used in the formulation are Na-montmorillonite crystallites of three different CECs. To study the relative effect of CEC on the simulation behavior, Na-montmorillonite molecules of three different CECs of 54, 90 and 144 meq/100 g were used. For the purpose of identification, these molecules were respec-

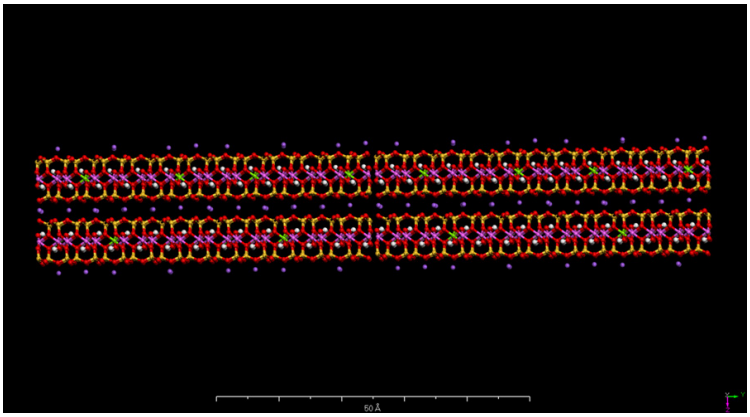

Figure 2. Unit crystallite of Na-montmorillonite (26×108×20 Å) with CEC of 90 meq/100 g and sodium as an exchangeable cation. Scale bar = 50 A.

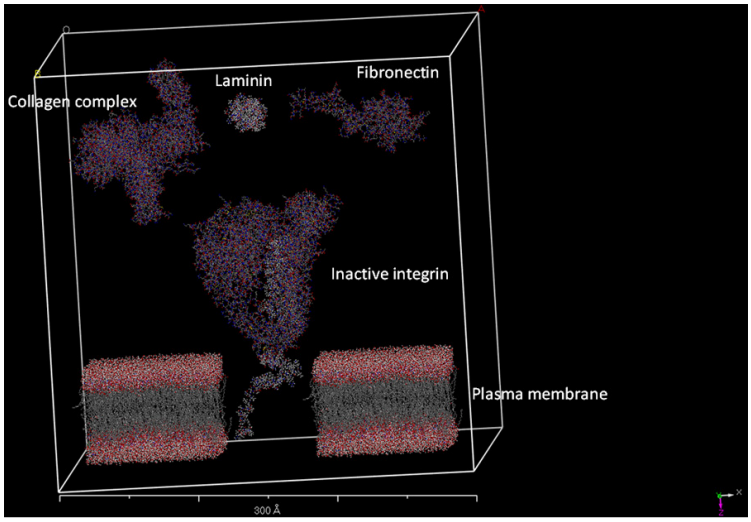

Figure 3. A cell-ECM complex with an integrin, plasma membrane and ECM proteins created in Materials Studio software. Scale bar = 300 A.

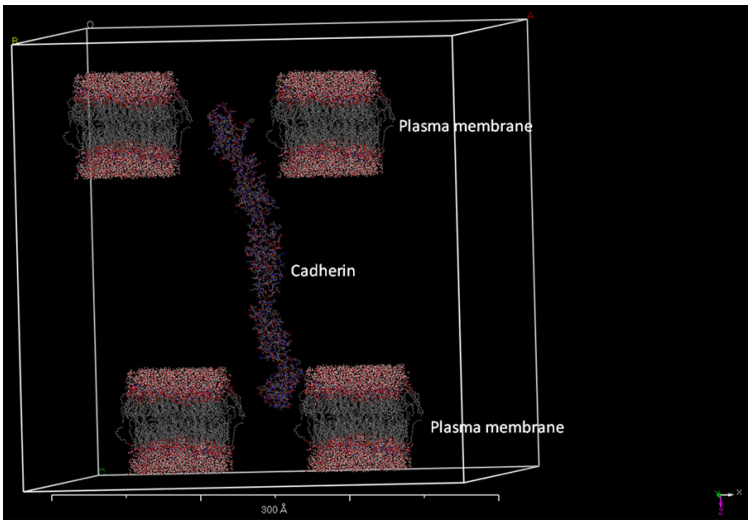

Figure 4. A cell-cell complex with a cadherin connecting plasma membrane created in Materials Studio software. Scale bar = 300 A.

tively named as Low CEC (LCEC), Medium CEC (MCEC), and High CEC (HCEC). Their charges were verified using the charge equilibration method QEq of the software.

For the clay minerals, all molecular level processes and interactions take place among the smallest particle size termed as fundamental crystal size or crystallite. The selection of dimensions of smallest or fundamental crystal/crystallite size is an essential step in the molecular level modeling studies. The literature contains several studies about the determination of the fundamental crystal size of the clay minerals [21-25]. Most of these studies have used XRD data and the physical imaging techniques such as Scanning and Transmission Electron Microscopy (SEM & TEM) for the purpose. In the current study, smallest molecular/crystallite size is selected from the authors' studies [26, 27]. In these studies, the smallest crystallite size was determined by the analysis of the XRD data using the Scherrer method [28] and the relevant literature [24]. Use of Scherrer method [28] resulted in an approximate range of crystallites from 29 to 58 Å. Simic and Uhlik [24] determined crystallite size of the smectite of sedimentary origin using Bertaut-Warren-Averbach (BWA) technique. The crystallite size of smectite varied from about 2.0 to 10.0 nm with the maximum occurrence of about 2.41 nm and the mean values ranging from 5.21 to 5.79 nm. Similarly, for the sedimentary environments clay minerals precipitate as small particles (<10 nm) and grow in diameter over time as water provides a continuous supply of clay crystallites or sometimes referred to as 'building blocks' of the structure [29].

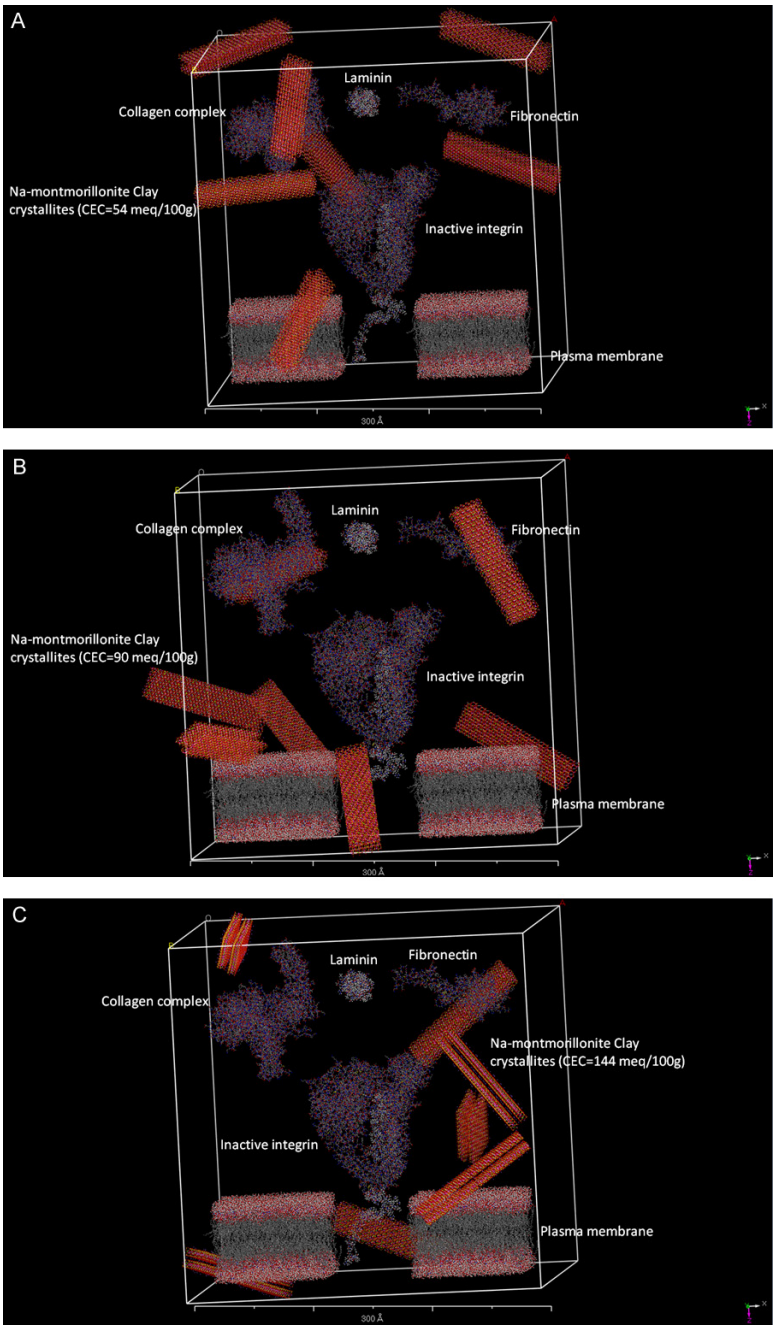

Figure 5. A. An integrin to plasma membrane and ECM proteins complex with sorbed LCEC (54 meq/100 g) crystallites. B. An integrin to plasma membrane and ECM proteins complex with sorbed MCEC (90 meq/100 g) crystallites. C. An integrin to plasma membrane and ECM proteins complex with sorbed HCEC (144 meq/100 g) crystallites. Scale bars = 300 A.

It was also demonstrated using TEM data that the <0.1 pm fractions of montmorillonite and regularly interstratified I-S consist of elementary particles 10 Å and 20 Å thick, respectively [30].

Based on already-published values and the XRD findings obtained in authors' research [26, 27], the fundamental crystallite size of 26×108×20 Å was chosen for the simulation as the fundamental particle/crystallite for Na-montmorillonite. A typical Na-montmorillonite model with a CEC of 90 meq/100 g and Na as the interlayer cation are shown in **Figure 2**.

Formulation of cell-ECM and cell-cell configurations

For the construction of the theoretical molecular level cell-ECM and cell-cell configurations, molecules of integrin, cadherin, and other associated ECM proteins such as laminins, collagen, and fibronectin were mainly acquired from protein data bank websites RCSB [31] and PDB-101 [32]. Also, plasma membrane files in protein data bank (PDB) format were acquired from University of Calgary website [33].

The integrin was created using three different parts formulating an inactive integrin. The top structure of the integrin that makes the extracellular portion extending outward from the cell surface (PDB entry 1jv2) was connected through the membrane by a short transmembrane section (PDB entry 2k9j) and the two short cytoplasmic tails extend into the cell (PDB entry 1m8o). Similarly, cadherin, in the form of large proteins that extend from the surface of the cell, were obtained from PDB entry 1l3w.

For the formulation of cell-ECM configuration (**Figure 3**), the tail of the inactive integrin was sandwiched between two parts of the plasma membrane and the rest was kept protruding out towards ECM proteins. In this configuration, ECM was mimicked using three main proteins, i.e., collagen, laminins, and fibronectin. Similarly, cell-cell adhesion configuration was cre-

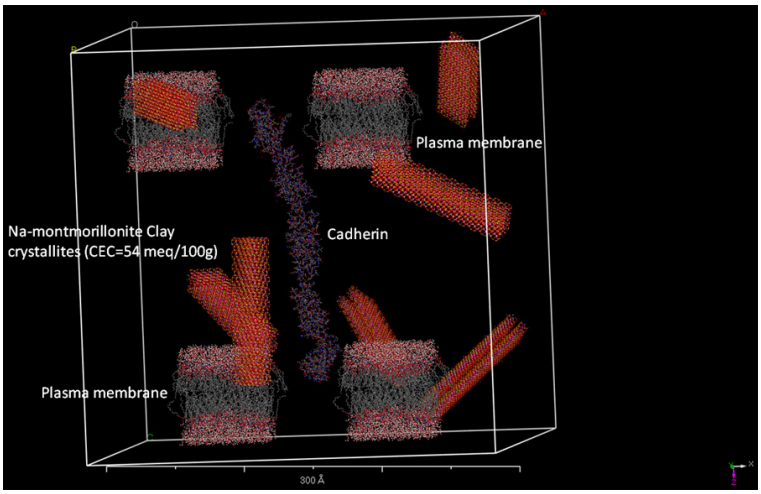

Figure 6. A cadherin to plasma membrane complex with sorbed LCEC (54 meq/100 g) crystallites. Scale bar = 300 A.

ated using the ends of cadherin sandwiched between two parts of plasma membranes on both sides and the software generated unit cell is shown in **Figure 4**. After placing the cell-cell and cell-ECM components within their relative positions and distances, the entire geometry was optimized by lowering the energy using geometry optimization option of Forcite module of the software.

Na-montmorillonite crystallites interaction with cell-ECM and cell-cell configuration

To simulate the interaction of Na-montmorillonite crystallites with cell-cell and cell-ECM complexes, seven (7) crystallites were sorbed on each configuration using Sorption module of the software. For the simulation purpose, Metropolis Monte Carlo method has been selected in the Sorption module of the software. In each sorption step, clay crystallites occupied spaces in the unit cell to lower the overall energy of the complex. Seven (7) crystallites were sorbed in each 2500 steps and then the energy of the system was minimized using Forcite module of the software based on Molecular Dynamics technique. The Forcite module of the Materials Studio software with the NPT (constant number of particles, pressure, and temperature) ensemble was used, and simulations were performed using a modified universal force field for 5 to 30 ps in 0.5-fs intervals or until a constant volume was reached. A Berendsen thermostat with a decay constant of 0.1 ps was used to control the temperature during the simulation. During the molecular-dynamics simulation, the assumed temperature was kept constant at 310°K (37°C). Simulations were carried out assuming atmospheric pressure (100 kPa) and a Berendsen barostat with a decay constant of 0.1 ps was used to control the pressure of the system. The final configurations after sorbing LCEC, MCEC, and HCEC crystallites on the cell-ECM complex are shown in **Figure 5A-C** respectively, while sorption of LCEC on cell-cell configuration is shown in **Figure 6**.

The Berendsen methodology was selected as the most suitable for the single crystallites after several trials involving other thermostats and barostats available in the software. In Monte Carlo method, parameters chosen for ratios of exchange, conformer, rotate, translate, and regrow have been selected as 0.39, 0.2, 0.2, 0.2, 0.2 respectively, while the corresponding probabilities are 0.39, 0.2, 0.2, 0.2, and 0.2. Amplitudes adapted for rotation and translation are 5° and 1 Å respectively.

Electrostatic energy and cohesive energy density measurement

In this study, the effectiveness of clay crystallites to restore adhesions among cell-cell and cell-ECM complexes was evaluated through the changes in electrostatic attraction energies and cohesive energy densities. After the sorption of clay crystallites and the subsequent molecular dynamics of each of the configurations, energies were determined using Energy option of the Forcite module of the software. The Energy module provided total bond and non-bond energies of the configuration before and after the sorption of clay crystallites. Similarly, in this study cohesive energy density (CED) concept very closely explained the various molecular-level processes and interactions and mimicked the extent of adhesion created among the simulated complexes. Quantitatively, CED is the amount of energy needed for the transition of 1 mol of material from the liquid to the gaseous phase and is considered as a measure of the mutual attractiveness of molecules. The CEDs of the simulated complexes were

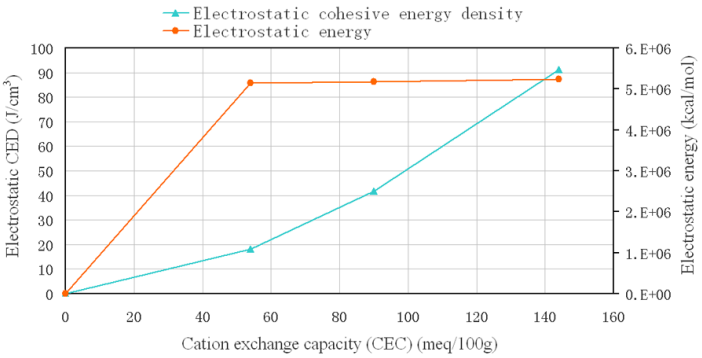

Figure 7. Variation of electrostatic non-bond energy and an electrostatic component of cohesive energy density (CED) with CEC of the clay crystallites (Note: zero on CEC axis represents the absence of clay).

also determined using the Forcite module of the software. For comparison purposes, electrostatic energy and electrostatic CED were plotted against the CEC of the crystallites in **Figure 7**.

Results and analysis of the molecular simulations

The sorbed Na-montmorillonite crystallites on cell-ECM and cell-cell configurations in **Figures 5** and **6** show that the crystallites, due to their charged nature, generally orient themselves with these configurations to provide an inter-connected network. This inter-connected network of crystallites results in the creation of an overall adhesiveness in the system. High electrostatic energies of an order of 5×10^{-6} kcal/mol and electrostatic CEDs of up to 50-100 J/cm^3, shown in **Figure 7**, are the evidence of the creation of adhesiveness in the complexes. It could also be noted from **Figure 7** that higher CEC clay crystallites have resulted in high level of adhesiveness. Therefore, for practical purposes, higher CEC clay would be preferred for the restoration of maximum adhesion among tumor or aging stem cells.

Although results of this study are quite encouraging, and clay crystallites could be considered as an option to restore adhesions in tumor and stem cells, other confirmatory tests and cell culture studies would be required for the confirmation of the findings of this study. Adhesion energies can be measured using bioforce probe (BFP), atomic force microscopy (AFM), and dual pipette assay (DPA) techniques. For the visualization of the interactions between clay crystallites and the cells, X-ray diffraction (XRD), environmental scanning electron microscopy (ESEM), and transmission electron microscopy (TEM) should be used. In addition to the use of these lab techniques, live cell cultures would be required to validate the findings of this study.

Conclusions and recommendations

This study has demonstrated through molecular simulations the potential use of smectite (Na-montmorillonite) clay crystallites to create adhesions among tumor and stem cells. High electrostatic energies and cohesive energy densities measured in the simulations after the sorption of clay crystallites on cell-cell and cell-ECM complexes validate the concept of using these crystallites for the purposes.

As results of this study are quite promising, and clay crystallites could be considered as an option to restore adhesions in tumor and stem cells, other confirmatory tests and cultures are in process. In this regard, bioforce probe (BFP), atomic force microscopy (AFM), and dual pipette assay (DPA) and live cell cultures are being planned/in the process for the validation purpose.

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Disclosure of conflict of interest

None.

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