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Interaction of RGD peptide on Cobalt metal surface: A computational modeling study to design effective biocompatible surface for bone-tissue engineering

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Abstract: Interaction of organic and inorganic materials at the surface dictates the chemistry and energetics behind bone growth and regeneration process during bone-tissue engineering. Not all surfaces in the bone are bioactive but some can be used as biomedical implants. Researchers studied interaction of peptides with metallic surfaces to better understand the role of various inorganic surfaces like Ti and TiO_2 during bone regeneration process. Current study focusses on interaction of RGD peptide on more biocompatible Co-Cr alloy, and as a precursor to that study, most favorable RGD-Cobalt metal interaction is established in this paper, along with suitable forcefield. If we analyze the absolute adsorption energy values of RGD on Cobalt surface, we find that all the values are negative, ranging from -158 kCal/mol to -305 kCal/mole. The positive adsorption energy arises due to values of optimized Cobalt 4x4x4 surface, which is ~50-60 kCal/mol. The force field takes into account the multibody potential interaction between surface atoms, between surface atom and peptide atoms, and it is difficult for a force field to accurately model both type of interactions, since the cobalt surface will have a lot more coulombic interactions and electron exchange parameters compared to peptide -metal interactions. In future work, modeling studies will be done to incorporate more coulombic and multibody potential terms, that can more accurately define the energy values of metal surfaces. Also, in future studies, OPLS forcefield will be studies with other small peptides like YIGSR, another cell adhesion triggering peptide, on cobalt metal surface, whose dimension can be changed to see if that can make more thermodynamically favorable adsorption surface.

IndexTerms **– Bone-Tissue Engineering, Hydroxyapatite, RGD, Cobalt, Forcefield**

I. INTRODUCTION

Interaction of organic and inorganic materials at the surface dictates the chemistry and energetics behind bone growth and regeneration process during bone-tissue engineering. Cell proliferation and cell signaling process is the onset of this bone growth process which involves cell adhesion, and spreading. Organic -inorganic interactions at the biomaterial surface plays the key role for the above-mentioned processes. However, thermodynamically unfavorable biomolecule-inorganic material interaction at the surface can also induce negative behaviour during bone growth, which can lead to cell apoptosis and cell death. Not all surfaces in the bone are bioactive. Specific bioactive surfaces can induce interaction with extra-cellular proteins, or bioengineered motifs, which, on favorable interaction with the substrate bone-mineral, can enhance the bone regeneration process.

Bone-tissue engineering is the process of regeneration of damaged bone tissue and restoration of its normal biological activity. Extensive research has already been done on the regrowth process of the bone, both *in vivo* and *in vitro* on the experimental front **[I].** The main inorganic component of bone is hydroxyapatite, with specific substitution of -OH and $-CO_3^-$ groups in the lattice **[2,3]**. Not all the surfaces or miller planes of hydroxyapatites are favorable for interactions with extracellular proteins, and a considerable amount of resources are lost during the search for finding the most thermodynamically favorable hydroxyapatite surface for bone regeneration, experimentally. A similar challenge is also faced while inserting a biocompatible inorganic surface inside the body as biomedical implant which will enhance the bone tissue engineering process. Hydroxyapatite surface needs support from other compatible inorganic surface to grow back the damaged tissue. These additional inorganic surfaces, sometimes coupled with organic polymeric structures, act as biomedical implant, on which the bone regeneration takes place, in a similar way of peptide-hydroxyapatite interaction.

We know computational modeling study has been used by researchers for past few decades to tackle these challenges effectively, and reduce the time and cost for carrying out above mentioned experimental procedures involving peptide and inorganic surfaces/biomaterials. We have studies interaction of biomolecules on inorganic surfaces

In our previous work, two peptides RGD and YIGSR, and their interaction on various hydroxyapatite surfaces has been studies extensively **[4,5]**. The initial orientation and position of the peptide on hydroxyapatite surface dictates the stability and adsorption energies, and same pattern was observed during our study of RGD-TiO_2 interactions **[6,7]**. TiO_2 forms a very thin layer on Ti surfaces, which increase calcium ion interaction of the RGD peptide that starts the cell adhesion and tissue engineering process.

But TiO_2 surface develops various cell apoptosis and other intercellular disorder during the regeneration process, which prompted scientists to think for alternatives as biomedical implants. We have studies interaction of RGD peptides on pure Titanium surface, which gave us excellent thermodynamic stability, and showed better Osseo integrative properties. Titanium surfaces can be modified by

polythene glycol **[8,9]** like materials to give it more flexibility during the tissue growth process, but that creates additional complexity in the strengthening of bone.

One viable alternative which could keep the metal biomedical surface's strength and elasticity intact, is doping of Titanium surfaces with metals like Aluminium (Al), Chromium (Cr), Vanadium (V) etc. We have studied RGD on Ti-Cr surfaces and Ti-Cr-V surfaces, and observed that Ti-Cr surface gives better energetics compared to doping with Vanadium.

Co-Cr alloys are most popular biomedical implant materials because of their extensive resistance to both corrosion and wear against in the long run. Structural features of Co-Cr alloys are studied extensively by scientists in recent years **[10]**, though study of interaction of Co-Cr, or individual metals with cell adhesion peptides like RGD, YIGSR are seriously lacking. Our present study focusses on interaction of RGD peptide with Co- metallic surfaces under various computational modeling conditions, which will pave the way for future studies with peptides-Cr-Co alloy interactions. Results of these studies with help the scientists to better understand the role of Co-Cr alloys during bone regeneration process, and it can be used effectively as biomedical implants.

II. MATERIALS, METHODS AND RESULTS

The current computational modeling study is done using Abalone II computational modeling software. It is an excellent molecular graphics software that allows the researchers to study various customized interactions between the biomolecules and inorganic surfaces. Abalone II allows chain building, geometry editor, bonds detection by distance, overlay, clash elimination etc., during the molecular model building process. The software also has inbuilt AMBER family of force-fields up to AMBER 03, and force fields like OPLS, OPLS-H, DREIDING Force Field for arbitrary molecules, fSPC and implicit water models. It has also several potential model controls for molecular dynamics study, which will be discussed as necessary in the paper. Though not exercised in the current study, Abalone II allows performing hybrid Monte Carlo, ORCA, NWChem.

RGD peptide was developed in the software using chain builder, and optimized using Hybrid Luis Storey-Conjugate Decent optimizer using AMBER 03 force field. Then molecular dynamics study was performed on the optimized RGD structure, after charge equilibration was done. The MD study of RGD peptide involved thermostat setting at 298.15 K, with time duration of each step of simulation fixed at 2 femtoseconds. AMBER 03 forcefield was used, where dielectric permittivity of the medium assumed as 1. Rm value is 1x108, whereas during the simulation, sigma is kept arithmetical and epsilon is kept as geometric. The final energetics of Molecular Dynamics simulated RGD is -234.72 kCal/mol. The contribution from other energy and potential components towards the final energy of RGD are as follows:

angle 21.3664962081947
torsion 12.7273163172009
improper 3.3847676676558
Van der Waals 9.61479620734057
electrostatic -301.672379216207

Next, the cobalt surface on which the RGD interaction to be studied, is chosen carefully. The cobalt surface has to have sufficient surface area so that all possible interactions with RGD can be taken care of. We have to keep in mind that the 2-body, 3-body and 4-body potential interaction terms with atoms present in RGD with Cobalt atom, is unknown in literature, and has not been studied before. Therefore, while choosing the forcefield, AMBER03 cannot be the best option for this simulation.

But, first a 4x4x4 Cobalt metal surface is built using crystal builder in the abalone software, putting "box" periodic boundary condition with parameters a=b=c=15 Å, and α=β=γ=900. After initial optimization of the structure, MD simulation was run with hybrid LS-CD command, at 298.15 0K temperature, with AMBER03 forcefield. Energy of this Cobalt 4x4x4 surface is 55.490 kCal/mol. We have used implicit water model, since it resembles overall aqueous environment around the cobalt metal surface, rather than single point charge interactions in three site fSPC water model. Since we have not used any point charge on Cobalt, keeping the overall surface neutral, all the contribution to total energy comes from van der Waals interactions.

Now the final interaction that is studied is interaction of RGD as cell adhesion peptide on Cobalt surface. The energetics of this interaction will give us clear ideas about what proportion of Cobalt surface can be used, for making Co-Cr biomedical implants, and also how Cobalt in general can interact with RGD to trigger cellular response during bone-tissue engineering process.

Here we have to use the earlier optimized and Molecular dynamics simulated RGD structure as starting point, and place it on the periodic boundary condition applied structure of Cobalt surface **(Fig. 1**), which has been already optimized and simulated using AMBER03 forcefield (55.49 kCal/mol) as mentioned earlier, with implicit water model. The main issue before starting the Molecular dynamics simulation run of RGD on Cobalt Surface was choice of appropriate forcefield. Earlier it was separately RGD, and separate Cobalt metal surface who MD has been studies, so there was no question of intermolecular interaction of heterogeneous surface and species (biomolecules and inorganic surface), so AMBER03 would have been a good choice. As mentioned earlier, whenever there is interfacial interaction between two heterogeneous surfaces like biomolecule (RGD) and inorganic surface (Cobalt surface), many body potential terms are needed to be incorporated inside the force field to get the best result. At the starting position, the periodic box size is increased from a=b=c=15Å to 20.056 Å keeping the other conditions same. Nose-Hoover thermostat is used, with 1 barometric pressure. Rrespa5 INTEGRATOR is used, with AMBER03 forcefield for the simulation. The adsorption energy of RGD on Cobalt surface under implicit water model is found as -158.727 kCal/mol.

When I used OPLS (Optimised Potential for Liquid Simulations) forcefield to study the interactions between RGD and Co-metal surface, the energetics are much more thermodynamically stable (Adsorption energy of RGD on Cobalt surface is -207.987 kCal/mol).

I have studied the RGD and Co-surface interaction using AMBER-GS forcefield, keeping all other conditions same as mentioned with above calculations (OPLS-H, AMBER03), and energy obtained is -184.048 kCal/mol.

Adsorption energy of RGD peptide on Cobalt 4x4x4 surface has been studied with various other forcefields to find the best one, and the result is tabulated below, for different forcefield calculations.

Effective Adsorption Energy is calculated as per the following equation:

E (effective adsorption energy) = E (Adsorption energy RGD on Cobalt 4x4x4 surface) – [E (Optimized RGD) + E (Optimized Cobalt 4x4x4 surface)]

Table 1: Absolute Adsorption energy of RGD on Cobalt 4x4x4 surface using different forcefield

Name of the force field used in Abalone II to study the adsorption energy of Optimized RGD peptide on 4x4x4 Cobalt surface	Adsorption Energy in kCal/Mol
AMBER 03	-85.221
AMBER-GS	-184.08
Amber i	-202.095
Amber ii	3.5×10^{11}
AMBER 99 SB	-205.684
OPLS	-304.431
OPLS - H	-207.987

Table 2: Effective adsorption energies of RGD on 4x4x4 Cobalt surface using various forcefield in implicit water model

Adsorption energy of RGD on Cobalt 4x4x4 surface	Energy of optimized RGD	Energy of Optimized Cobalt 4x4x4 surface	Effective Adsorption energy	Force Field
-158.727	-234.72	55.49	20.503	Amber 03
-184.08	-309.203	49.868	75.255	Amber GS
-202.095	-248.971	59.439	-12.563	Amber i
-205.684	-275.753	54.4427	15.6263	Amber 99 SB
-304.431	-292.711	50.6865	-62.4065	OPLS
-207.987	-293.576	52.613	32.976	OPLS-H

III. DISCUSSIONS

The current modeling work exercises the option of possibility of adsorption of RGD, which is a main peptide in triggering bone tissue engineering procedure during regeneration process, on cobalt bulk surface, in aqueous environment. The bulk Cobalt is taken as 4x4x4 surface with 20A0 as cell boundary length, which allows enough space for movement of RGD on metal surface regarding effective adsorption. Implicit water model provides aqueous environment, which takes into account for both RGD-Water interaction, metal surface-water interaction and RGD adsorption on metal surface. The reason behind studying the adsorption energy with various forcefield is that it will help us determine the best possible environment under which peptide and metal interaction involving pure metal can be studies. The previous work which was done with titanium, had separate nature of peptide-metal interaction than the current one. Also, it is to be noted that, in future work, the cobalt metal will be doped with Chromium, where percentage of doping will be of wide range, and we will have to find a suitable percentage of chromium doped cobalt where adsorption of RGD and other small scale tissue engineering peptides are most thermodynamically favourable.

The current set of data in Table 2 clearly indicates that most thermodynamically stable system is obtained OPLS system, where the absolute adsorption energy of RGD on Cobalt surface is -304.431 kCal/mol, and effective adsorption energy of RGD on Cobalt surface is -62.4065 kCal/mol. The effective adsorption energy value for AMBER-i fore field is obtained as -12.563 kCal/more. Other than these two values, all other effective adsorption values are positive values, which indicates that they are not thermodynamically favourable. But if we analyse the absolute adsorption energy values of RGD on Cobalt surface, we find that all the values are negative, ranging from -158 kCal/mol to -305 kCal/mole. The positive adsorption energy arises due to values of optimized Cobalt 4x4x4 surface, which is ~50-60 kCal/mol. The force field takes into account the multibody potential interaction between surface atoms, between surface atom and peptide atoms, and it is difficult for a force field to accurately model both type of interactions, since the cobalt surface will have a lot more coulombic interactions and electron exchange parameters compared to peptide -metal interactions.

In future work, modeling studies will be done to incorporate more coulombic and multibody potential terms, that can more accurately define the energy values of metal surfaces. Also, in future studies, OPLS forcefield will be studies with other small peptides like YIGSR, another cell adhesion triggering peptide, on cobalt metal surface, whose dimension can be changed to see if that can make more thermodynamically favourable adsorption surface.

Figures

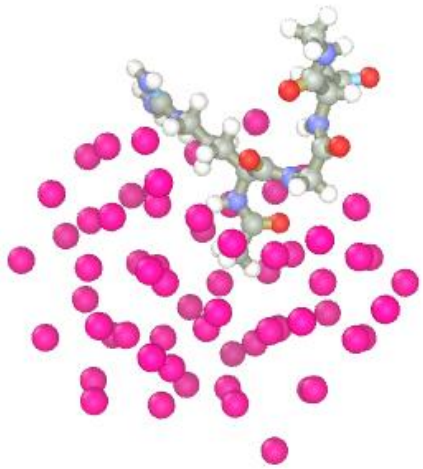

Figure 1: RGD adsorption on Cobalt 4x4x4 surface

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