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Research Paper

SIMULATED MOLECULAR LEVEL ANALYSIS OF RGD (ARG-GLY-ASP) INTERACTIONS ON TANTALUM AND TITANIUM BIOMATERIAL SURFACES

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Titanium and its alloys are being widely used as orthopaedic implants based on their desirable properties of relatively low modulus, good fatigue strength, formability, machinability, corrosion resistance, and biocompatibility. However, titanium and its alloys cannot meet all of the clinical requirements due to lack of level of osseointegration required for implant longevity. Porous tantalum metal is currently used in orthopaedics for manufacturing of structural components for primary and revision total hip revision surgery. Tantalum has shown a higher biocompatibility compared to titanium in various in vivo tests. RGD sequence has been identified in mediating the attachment of cells through specific ligand-receptor interactions. The biocompatibility of implant surfaces are directly related to the ease with which the RGD sequence interacts with the implant surface. A molecular level RGD adhesion characteristics have been compared for studying the biocompatibility of titanium and tantalum surfaces based on the molecular orbital theory. The results suggest the possibility of adapting the methods for predicting the biocompatibility of forthcoming implant materials at the development stage itself.

Keywords: Spartan 04, Molecular orbital theory, Biocompatibility, RGD

INTRODUCTION

Long term mechanical fixation of implants in vivo is based on the formation of bone around the implant (Puleo and Nanci, 1999; Bennettl *et al.*, 2001; Jason and Kristi, 2002; Meyer *et al.*, 2005; Xufeng *et al.*, 2005; Ganesan and Thomas, 2006; Kelly and Hideo, 2009; Edorta *et al.*, 2011; and Vandrovcova and Bacakova, 2011). Metallic implant materials have a significant impact in the biomaterials field, especially in applications requiring mechanical properties like mechanical strength and toughness. Among metallic materials, titanium and titanium alloys are considered the best

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choice for manufacturing permanent implants. The biocompatibility of titanium is due to the dense and well-adhered oxide layer (Mangal *et al.*, 2010). Commercially pure titanium and Ti-6Al-4V alloy are the two most common titanium-based implant biomaterials (Eisenbarth *et al.*, 2004; and Mangal *et al.*, 2010). Pure Titanium has poor osseointegration properties which can lead to aseptic loosening and premature failure of the uncemented implants in vivo (Eisenbarth *et al.*, 2004; and Mangal *et al.*, 2010). Porous tantalum metal is currently used in orthopaedics for manufacturing of structural components for primary and revision total hip revision surgery. Tantalum has shown a higher biocompatibility compared to titanium in various in vivo tests (Mangal *et al.*, 2010). The efficacy of bone regeneration is determined mainly by surface characteristics such as the chemical composition and physical properties of the implant that controls initial protein adsorption. These properties alter the adsorption of proteins which mediate the adhesion of desirable (osteoblast) and undesirable (fibroblast) cells (Ganesan and Thomas, 2006). It is now well recognized that osteoblasts preferentially adhere to specific amino acid sequences such as arginine-glycine-aspartic acid (RGD) and heparin-sulfate binding regions in adsorbed proteins (Puleo and Nanci, 1999; Bennettl *et al.*, 2001; Jason and Kristi, 2002; Xufeng *et al.*, 2005; Ganesan and Thomas, 2006; Kelly and Hideo, 2009; Edorta *et al.*, 2011; and Vandrovcova and Bacakova, 2011). Understanding cellular recognition to proteins initially adsorbed on biomaterial surfaces is important to a longer implant life. A molecular level analysis has been conducted to assess the interactions of RGD sequence with the surface oxide layers of Titanium and Tantalum implants.

Quantum Chemical Calculations

Quantum chemical calculations use quantum mechanics to study and predict the chemical properties and behaviour of molecules. Quantum mechanics describes molecules in terms of interactions among nuclei and electrons, and molecular geometry in terms of minimum energy arrangements of the nuclei. All quantum chemical calculations ultimately trace back to the time-independent Schrödinger wave equation (Equation (1)), which can be generalized as a multi nuclear, multi electron system (Hehre, 1998 and 2003; Bowden and Tabor, 2001; and Jayadas and Nair, 2006).

$$\hat{H}y = Ey \qquad ...(1)$$

Here E is the electronic energy in atomic units, y is a many electron wave function and $\hat{H}$ is the Hamiltonian operator, which in atomic units is given by Equation (2).

$$\hat{H} = -\frac{1}{2}\overset{electons}{\overset{o}{a}}\nabla_i^2 - \frac{1}{2}\overset{nuclei}{\overset{o}{a}}\frac{1}{M_A}\nabla_A^2$$

$$-\frac{1}{2}\overset{electrons}{\overset{o}{a}}\overset{nuclei}{\overset{o}{a}}\frac{Z_A}{r_{iA}} + \overset{o}{a}\overset{electons}{\underset{i<j}{a}}\frac{1}{r_{ij}}$$

$$+\overset{o}{a}\overset{nuclei}{\underset{A<B}{a}}\frac{Z_A Z_B}{R_{AB}} \qquad ...(2)$$

Z is the nuclear charge, M_A is the ratio of the mass of nucleus A to the mass of an electron. R_{AB} is the distance between the nuclei

A and B, r_{ij} is the distance between electrons i and j and r_{iA} is the distance between electrons i and nucleus A.

The many-electron Schrödinger equation cannot be solved exactly and hence approximations need to be introduced. One way to simplify Schrödinger equation is to assume that the nuclei do not move. This is called Born-Oppenheimer approximation, and leads to "electronic" Schrödinger equation (Equation (3)).

$$\hat{H}^{el} y^{el} = E^{el} y^{el} \quad ...(3)$$

Hamiltonian for the "electronic" wave equation is given by Equation (4).

$$\hat{H}^{el} = \frac{1}{2} \overset{electons}{\overset{o}{\underset{i}{a}}} \tilde{N}_i^2 - \overset{electrons}{\overset{o}{\underset{i}{a}}} \overset{nuclei}{\overset{o}{\underset{A}{a}}} \frac{Z_A}{r_{iA}} + \overset{o}{a} \overset{electons}{\overset{o}{\underset{i<j}{a}}} \frac{1}{r_{ij}} \quad ...(4)$$

The Hartree-Fock and semi empirical techniques are approximate methods to solve "electronic" wave equation. Equation (3) is a typical eigen value problem, the solution of which yields multi electron eigen functions (y^{el}) and the corresponding energy levels (E^{el}).

The Hartree-Fock method is also described as an *ab initio* ("from the beginning") method. In this method the many electron wave function (y^{el}) is expressed as a function of molecular orbitals (y_i). Molecular orbitals are expressed as linear combinations of a finite set (a basis set) of prescribed functions known as basis functions as shown in Equation (5).

$$y_i = \overset{o}{\underset{m}{a}} C_{mi} f_m \quad ...(5)$$

C_m are unknown coefficients determined iteratively in Hartree-Fock procedure.

The set of molecular orbitals leading to lowest energy are obtained by a process referred to as "Self-consistent-field" or SCF procedure (Jayadas and Nair, 2006). In the present study 6-31G, a basis set consisting of six Gaussian type basis functions, was used for calculations.

Semi empirical methods are simplified versions of Hartree-Fock theory using empirical (derived from experimental data) corrections in order to improve performance (Hehre, 2003). In the present study, the semi-empirical method has been used. The quantum chemical descriptors used in the study are

Qr: Net electrostatic charge-Atomic charges chosen to best match the electrostatic potential at points surrounding a molecule, subject to overall charge balance (Hehre, 2003; and Jayadas and Nair, 2006).

E_HOMO: Energy of the Highest Occupied Molecular Orbital. E_HOMO represents the energy of the least tightly held electrons in the molecule.

E_LUMO: Energy of the Lowest Unoccupied Molecular Orbital. LUMO describes the easiest route to the addition of more electrons to the system.

Frontier Molecular Orbital (FMO): Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) are the frontier orbitals.

MOLECULAR MODELLING

Different molecular modelling packages use different molecular data input which includes both textual type data input and graphical data

input. Spartan 02, the molecular modelling package used in this study, has a powerful graphical user interface for model building (Jayadas and Nair, 2006). Protein molecules are built from *sp2* and *sp3* hybridized carbon and oxygen atoms and hydrogen atoms. The atoms are selected from the model kit available with the package and placed on the work area. Bonds can be formed by clicking on the appropriate free valences of the atoms which are already placed on the work area. The Titanium and Tantalum atoms are modelled by selecting atoms with appropriate free valences from the model kit. Calculation options are set up by selecting "Calculations" from the "Setup" menu. Calculations are performed for "Equilibrium Geometry" using the "Semi-Empirical" method using basis set "PM3" by making appropriate selections in the dialogue box. The Highest energy Occupied Molecular Orbital (HOMO) and the Lowest energy Unoccupied Molecular Orbital (LUMO) of the molecules are calculated. These orbitals of interacting molecules are the pair that lies closest in energy of any pair of orbitals in the two molecules, which allows them to interact most strongly. The orbital's like HOMO and LUMO, their energies, and atomic charges are obtained by checking the appropriate boxes in the dialogue box. Calculations are started by clicking the "Submit" button on the dialogue box and the Output obtained by selecting the appropriate display functions. Molecular modelling of RGD strand Ta_2O_5 and TiO_2 have been made and their HOMO, LUMO images and charge densities are calculated. The charge density spread of the various molecules are shown in Figures 1-6. The interactive molecular models of oxides of Titanium and Tantalum with the RGD strand are shown in Figures 7 and 8 respectively. Blue indicates net positive charge and red indicates net negative charge areas in the molecular model. The semi-empirical calculations for the charge densities and energy levels E_HOMO and E_LUMO are shown in Table 1.

Figure 1: Charge Density Spread of Adenine Molecular Model

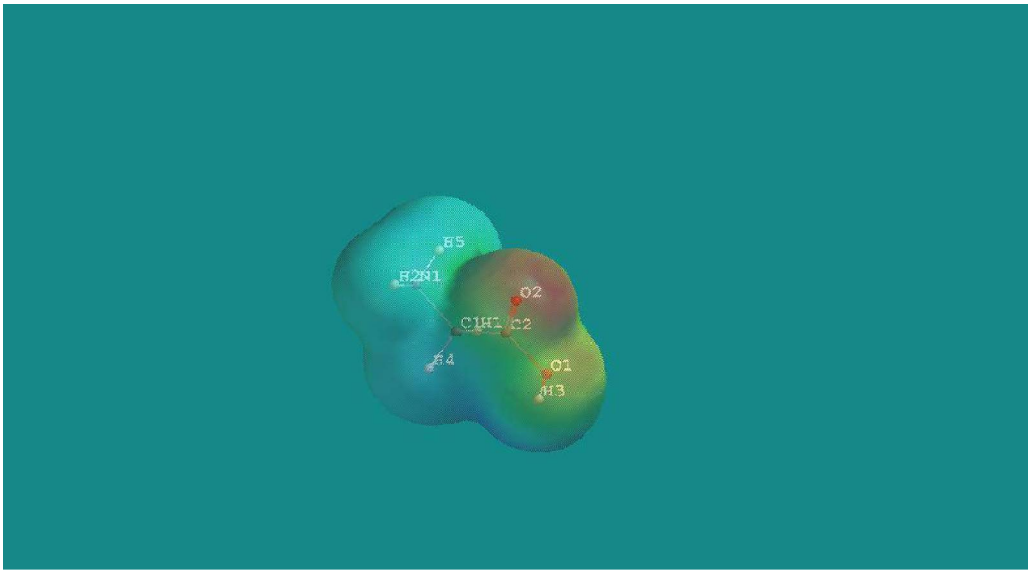

Figure 2: Charge Density Spread of Glycine Molecular Model

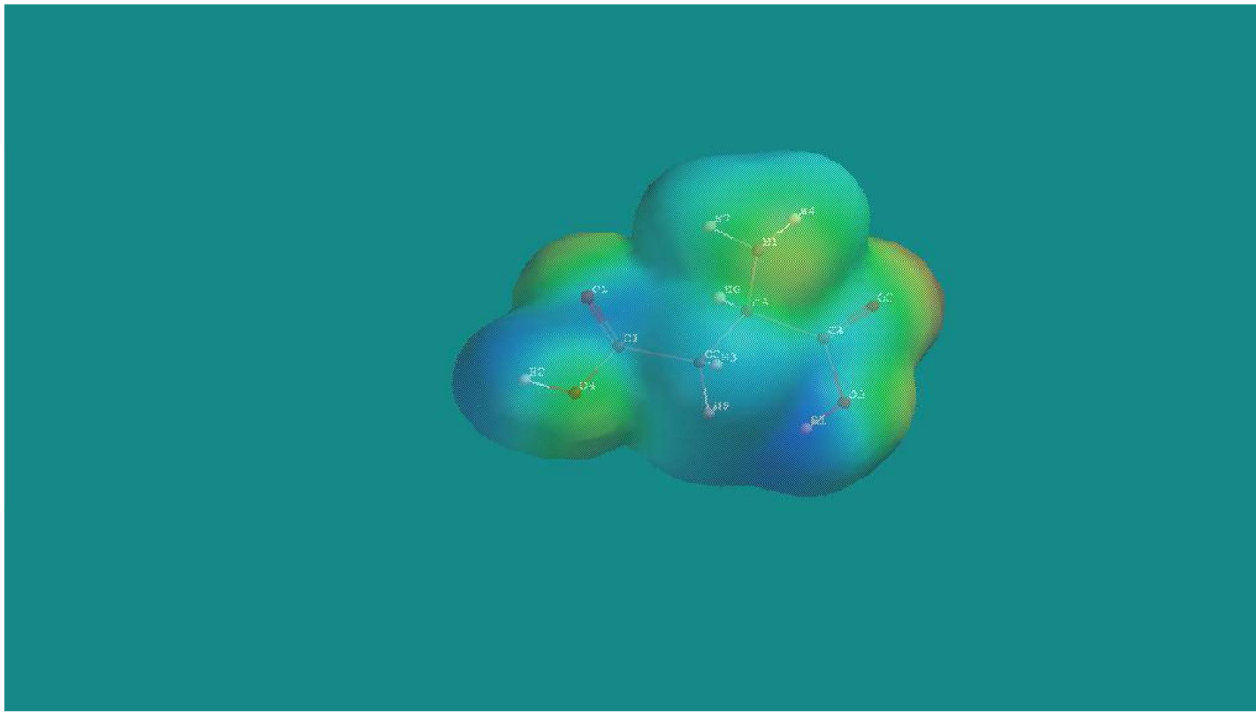

Figure 3: Charge Density Spread of Aspartic Acid Molecular Model

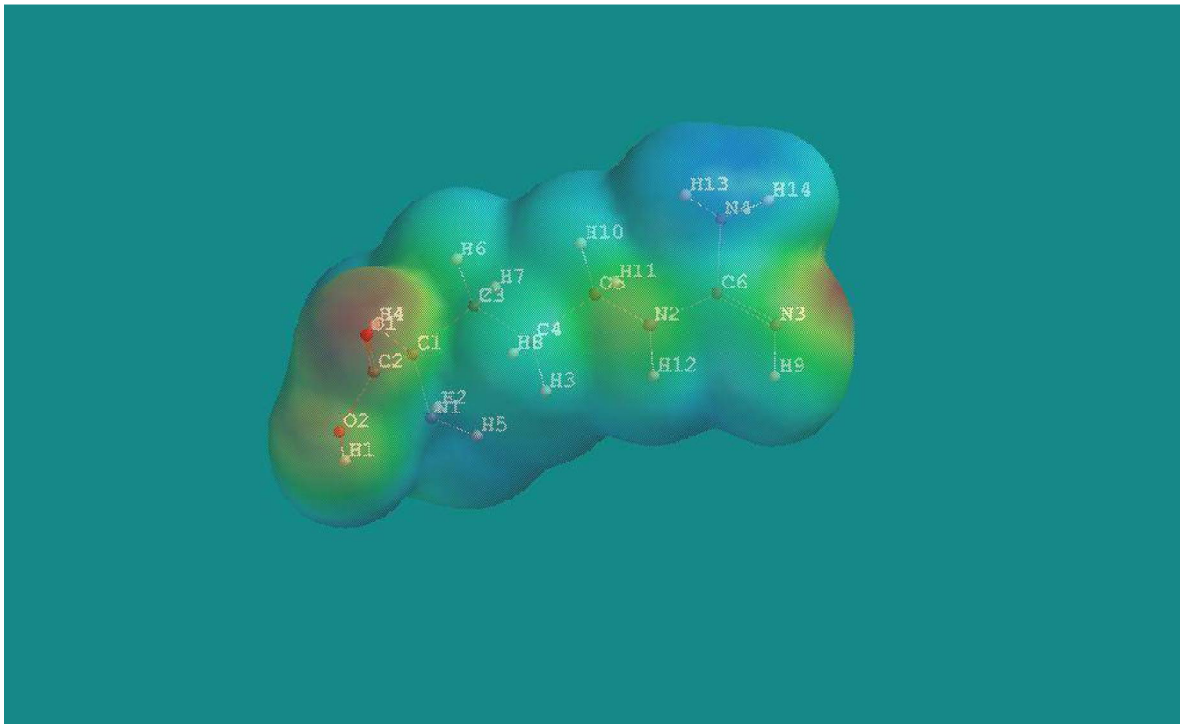

Figure 4: Charge Density Spread and HOMO and LUMO Orbitals of RGD Molecular Model

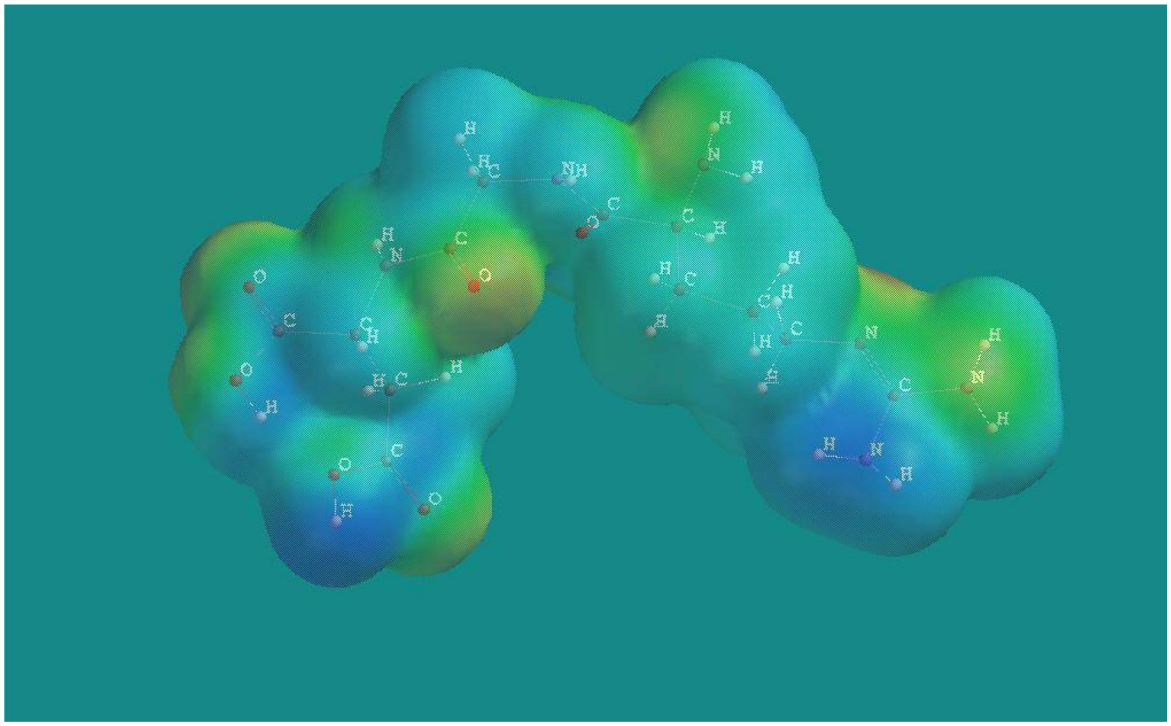

Figure 5: Charge Density Spread of Orbitals of Titanium Dioxide Molecular Model

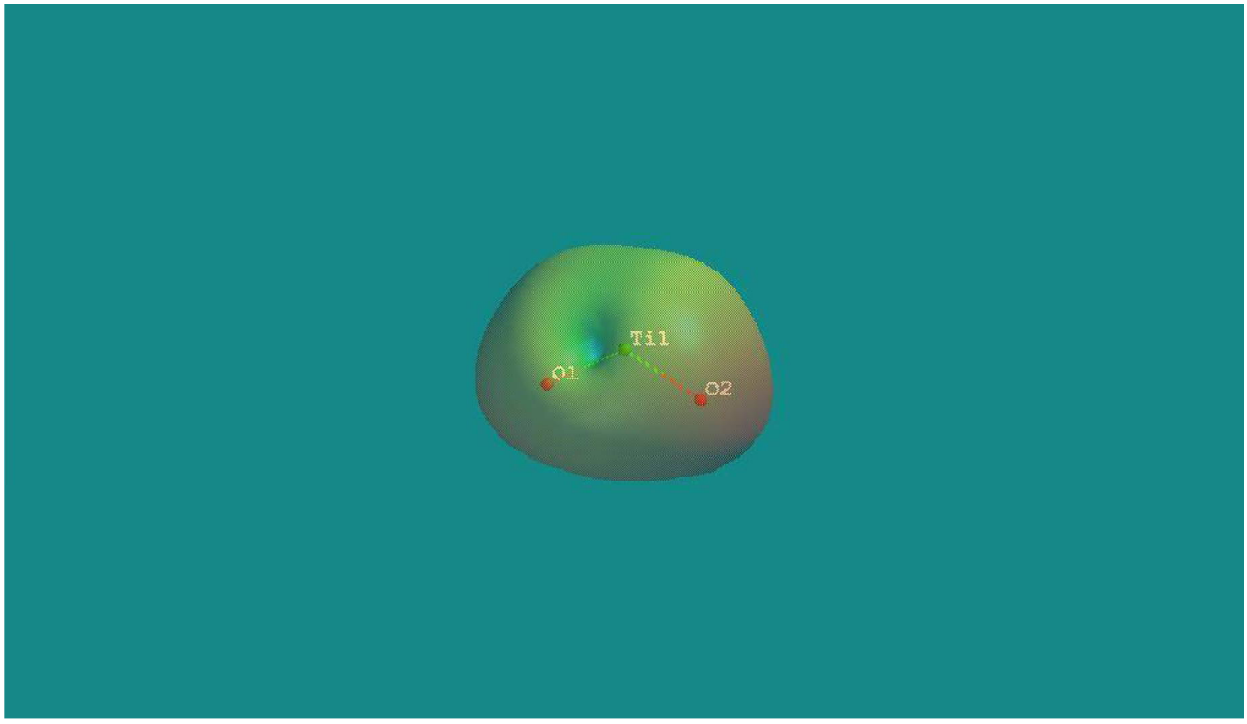

Figure 6: Charge Density Spread of Orbitals of Tantalum Pentoxide Molecular Model

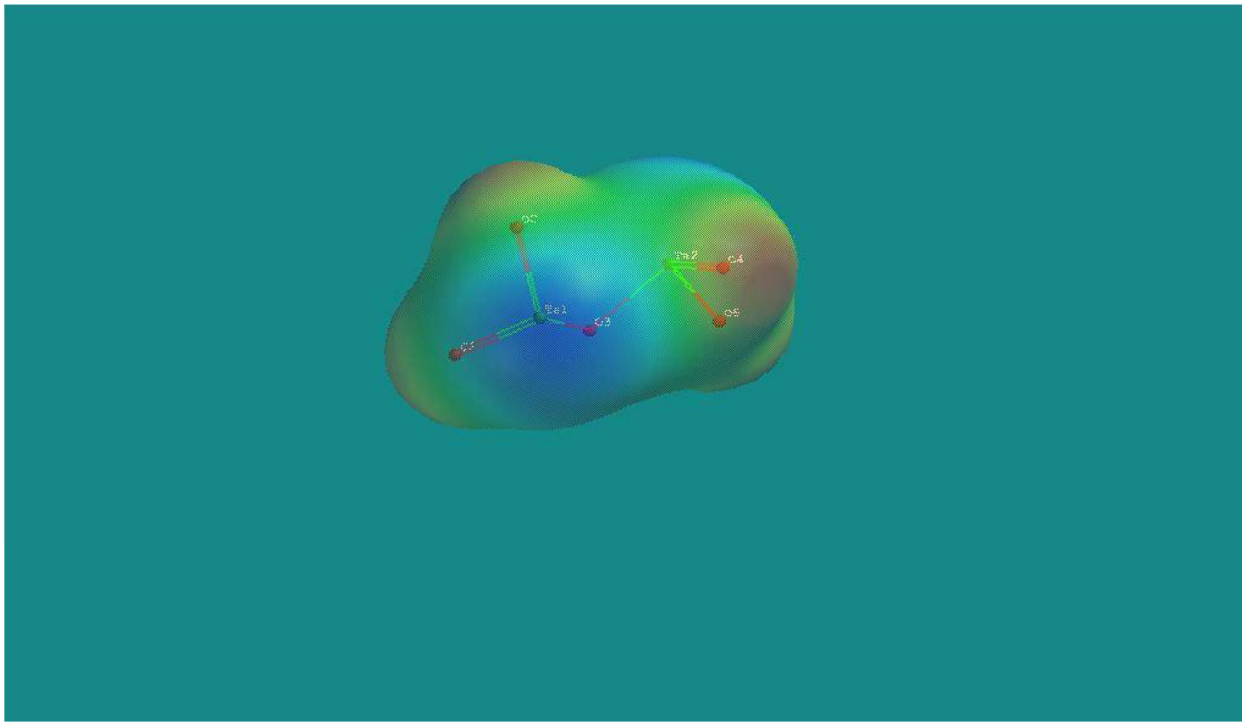

Figure 7: Charge Density Spread of Titanium-RGD Interactive Molecule

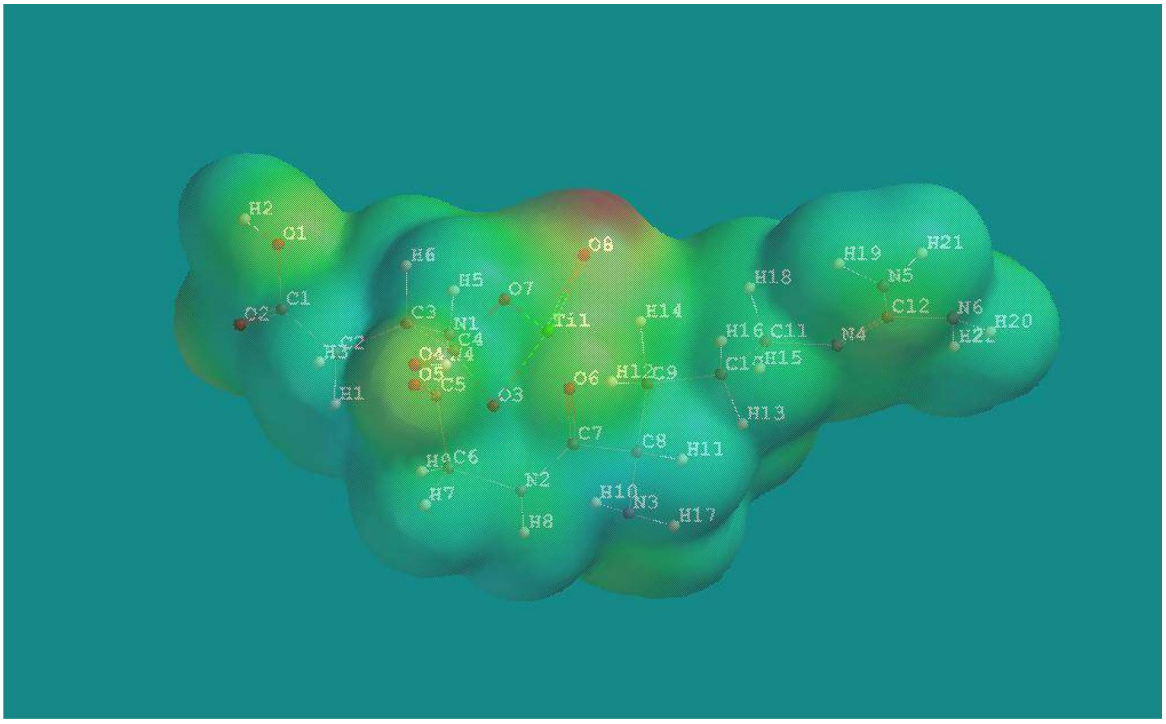

Figure 8: Charge Density Spread of Tantalum RGD Interactive Molecule

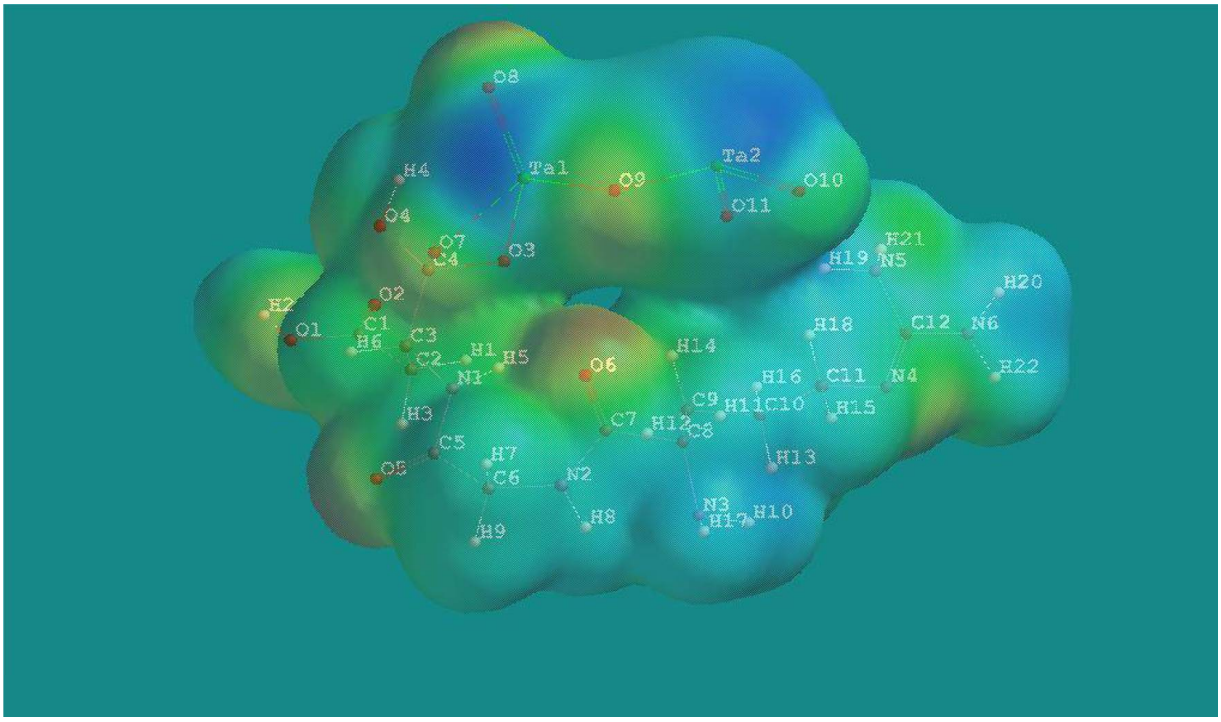

Table 1: Charge Density and E_HOMO and E_LUMO Values of the Molecular Models

Description	Charge Density of Oxygen Atoms	Charge Density of Metal Atoms	E_HOMO	E_LUMO
Ta_2O_5	–84	63(Ta)	–10.23	–3.46
TiO_2	–73	60 (Ti)	–11.25	–4.62
Arginine	–62	N.A	–9.90	–1.08
Glycine	–103	N.A	–3.08	–3.80
Aspartic Acid	–65	N.A	–9.33	–1.84
RGD	–83	N.A	–9.30	–3.30

RESULTS AND DISCUSSION

The charge density spread of the molecules indicates a net negative charge near the oxygen atoms and a net positive charge near the metal atoms. The E_HOMO and E_LUMO values of tantalum oxide and RGD are –10.23 and 3.3 respectively and those of Titanium dioxide and RGD are –11.25 and –3.3 respectively. As per molecular orbital theory a more favourable bonding characteristics exists for bonding between Oxygen atoms of Tantalum oxide with Carbon atoms of RGD strand than with Titanium oxide. However E_LUMO and E_HOMO values of Tantalum and RGD are –3.46 and –9.3 respectively which is less favourably aligned for bonding compared to that of Titanium and RGD with values of –4.62 and –9.3 thereby indicating more chances for bonding between Titanium and oxygen atoms of RGD strand than

Tantalum and oxygen atoms of RGD strand. However the charge density of oxygen atoms in Tantalum pentoxide is more comparable to that of charge density of Oxygen atoms in the RGD strand than that of Titanium dioxide. Moreover RGD approaches the implant surface from the extra cellular matrix and adsorbs onto the implant surface thereby the chances of bonding with the metal surface with sharing of the oxygen atoms are more prominent than vice versa. This suggests a higher compatibility in displacement of Oxygen from the RGD strand by Tantalum than Titanium. The comparative values of the orbital energies and the charge densities clearly indicate that Tantalum oxide will return a more favourable bonding characteristics compared to Titanium oxide surface. The results are in line with the various in vivo studies conducted previously (Bünger *et al.*, 2006; and Vamsi *et al.*, 2010a and 2010b). This suggests the scope of correctly predicting the biocompatibility of metal oxide surfaces with the molecular level bonding characteristics of their oxygen atoms with atoms of the RGD strand. The study has not taken into account the surface topography of the implant material which normally plays a significant role in cell adhesion. Further study is also required to account for Silanization (Rainer *et al.*, 2005), which has long been considered as the benchmark method for attaching organics to the native oxide coatings.

CONCLUSION

Comparison of bonding characteristics of RGD with Tantalum and Titanium surfaces have been made by molecular modelling and simulation of RGD strand and surface oxides of Tantalum and Titanium. The molecular orbital approach suggests a more conducive bonding environment between RGD and Tantalum Oxide compared to RGD and Titanium oxide. This is in line with the various in vivo test results obtained previously. The results suggest that the molecular Orbital approach can be employed to assess the biocompatibility of the newer implant materials which are being developed before biological tests are actually conducted. Further work is required to account for Silanization and orientation of the RGD strand as it approaches the implant periphery.

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