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Molecular Modeling of Bone Morphogenetic Protein for Tissue Engineering Applications

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Abstract

Bone morphogenetic proteins (BMPs) are an important type of growth factors directly involved in many developmental processes for tissue and organ restoration. BMP-2 is the primary growth factor that directs osteogenic (bone) tissue formation in stem-cell based tissue regeneration. However, its interaction with implant materials at the molecular level needs further exploration for clinical usage. In this paper, molecular dynamics (MD) simulations were used to study the dynamic behavior of BMP-2 on a flat gold substrate which is typically used in medical devices. Water and a saline media (sodium chloride 0.15M) under different ambient conditions were used as dissolution media for the solvation of the biomolecule to represent the cellular environment. Protein adsorption was analyzed for three different temperatures: room temperature (293K), *in vivo* conditions (310K), and boiling point of water (373K). Results obtained showed that BMP-2 was resistant to high temperatures at the nanoscale since no denaturation occurred. The presence of salt ions interfered with the protein adsorption forming a barrier layer between the protein and the substrate resulting in a weaker interaction. The investigation of BMP-2 protein interaction under different physiological conditions reveals the complex biochemical cues for tissue engineering applications.

Keywords
Tissue engineering, growth factors, bone morphogenetic proteins, molecular dynamics, nanoscale biochemical cues.

1. Introduction

In recent years, the number of orthopedic procedures performed in the United States has drastically increased due to the aging of the population, traffic accidents and sports injuries. Orthopedic surgeries face problems such as insufficient supply of donor tissue, immune rejection and disease transfer [1, 2]. Tissue engineering has emerged in the past few years as an alternative treatment for standard orthopedic procedures, by using artificial scaffolds to mimic a biological environment of cells, allowing the effective regeneration of diseased or damaged tissues and organs [3].

Understanding the cellular environment is essential for the performance of bioengineered systems. Although, engineering bioscience has significant contributions in various fields, including pharmaceutical engineering, molecular engineering, biomedical reaction engineering, and metabolic process control, tissue engineering still presents significant challenges. Some of these challenges include adequate reproduction of the environment for the development of the functions of the tissues, scale-up to obtain significant microenvironment to clinical scales, and automation of cellular devices and therapy systems [4].

The functional tissue restoration is achieved by combining together cells, scaffolds, and signals [5]. Cells used for regenerative medicine can be stem cells, progenitor cells or differentiated cells, which can also be used in combination such as primary cell with a partner cell. Scaffolds are needed for the fabrication of the tissue or used as a transportation system for the cells that are used in a therapy. Both synthetic biomaterial or natural extracellular matrix can be used as scaffolds [6]. Cellular signaling is responsible for the communication between cells and the body and is essential to the coordination of number, position and function of the cells [7]. The bio-chemical signals can include growth factors and chemotactic factors [6].

Recent advancements in tissue engineering are using biosensors to maintain 3D cell cultures and develop “organs-on-chips” model. These sensors can detect concentrations of biomolecules in real time for the understanding of cellular activities. Gold is been widely used as material for this purpose due to its optical, chemical, and magnetic properties[8]. Applications include nanofibers for engineered heart tissue [9], immunosensors [10], and acoustic sensors in biological systems [11].

Bone morphogenetic proteins (BMPs) are an important type of growth factors directly involved in many developmental processes for tissue and organ restoration. BMP-2 is primary growth factor that directs osteogenic (bone) tissue formation in stem-cell based tissue regeneration, allowing a reduction in the healing time and the risk of rejection. Due to its osteoinductive properties, this protein was approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) to be used in clinical trials for the treatment of tibia fracture, spinal fusions, and as dental graft [12]. The biocompatibility of implants and the efficiency of tissue scaffolds for the differentiation and proliferation of cellular tissues can be controlled by protein adsorption [13, 14].

However, to obtain a suitable delivery system for the BMP-2 to promote bone and cartilage formation, it is necessary to avoid protein degradation while maintaining its bioactivity. Therefore, the release rate of the protein needs to be proportional with tissue regeneration to obtain a convenient delivery system [15, 16]. Furthermore, the interaction of BMP-2 with implant materials at the molecular level needs to be investigated to avoid too strong adsorption of protein onto the substrate surface, causing protein denaturation and, consequently preventing tissue regeneration.

The objective of this research was to evaluate the influence of different temperatures on BPM-2 adsorption over medically relevant substrate (gold) using molecular dynamics (MD) simulations. These include room temperature (293K), *in vivo* conditions (310K), and boiling point of water (373K), The outcomes of this research will help understand the influence of temperature in the interaction between BMP-2 and gold substrates at the nanoscale.

2. Methods

In this research, molecular dynamics simulations were performed using Nanoscale Molecular Dynamics (NAMD) open source code version 2.11 [17]. NAMD is well-known for its robustness for parallel computing and compatibility with most force fields for the available CHARMM [18] and AMBER [19] packages. Virtual Molecular Dynamics (VMD) platform was used to model the simulations and analyze the results [20]. The simulations were performed on a 64-bit Linux platform (Fedora 21) using two graphical processing units (GPUs) from NVIDIA® Corporation (K40 and K20, with 2880 and 2496 cores, respectively).

A BMP-2 protein representation consisting of 1,641 atoms, with 1,664 bonds, at 2.7 Å resolution was obtained from the RCSB Protein Data Bank [21]. The protein was solvated in a minimum water sphere with an explicit TIP3P water model using VMD plugin. A second model was created by adding sodium chloride ions at a concentration of 0.15M. Both solvated proteins were placed on a flat gold substrate with dimensions of 200x200x20 Å. Figure 1 presents the initial model with the dissolution media excluded from the representation to better visualize the model. Protein adsorption was analyzed for three different temperatures: room temperature (293K), *in vivo* conditions (310K), and boiling point of water (373K), and two dissolution media: water and 0.15M sodium chloride solution.

All simulations were minimized for 0.2ns and executed for 10ns with a 2fs integration time step. Data were recorded every 0.1ns. The cutoff distance for Van der Waals interactions was 12 Å. Periodic boundary conditions were implemented in x- and y- directions. Constant temperature was assured using Langevin temperature control. All the atoms in the substrate were fixed while BMP-2 molecules were solvated in aqueous media to observe adsorption dynamics.

The bonded and non-bonded parameters for water molecules and protein were obtained from the CHARMM force fields [22]. The non-bonded force field parameters used in the simulations for gold were $\sigma_{ij} = 3.694$ Å and $\epsilon_{ij} = -0.039$ kcal/mol [23]. In a CPU+GPU configuration, the GPUs compute the non-bonded force evaluation while the CPUs compute the energy evaluations for molecular dynamics simulations [24, 25].

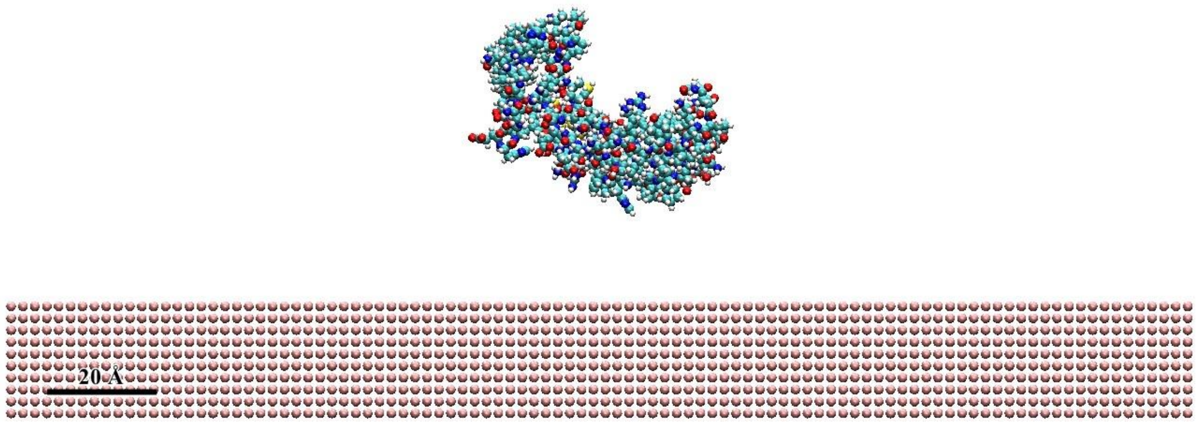

Figure 1: MD model of BMP-2 over a flat gold substrate

To maintain bioavailability of the BMP-2 growth factor, it is necessary that no significant folding and/or unfolding occurs to the protein, causing denaturation. To investigate the level of spreading of the protein during the simulations, the radius of gyration of the protein was calculated using Equation 1 [26].

$$R_g = \sqrt{\frac{\sum_i |r_i - r_{com}|^2 m_i}{\sum_i m_i}} \tag{1}$$

where, $|r_i - r_{com}|$ is the distance of the atom i with mass m_i to the center of mass of the protein.

3. Results and Discussion

Root mean square deviation (RMSD) was calculated over time for all simulations to determine if equilibration was reached. In this phase, there are no more variation of potential energy in the system and the RMSD reaches a steady state value. As it can be seen from
Figure 2, most of the simulations presented similar values of RMSD over time and reached equilibration. However, the system modeled at 373K using only water as the dissolution media is still searching for a lower energy state and did not reach equilibration.

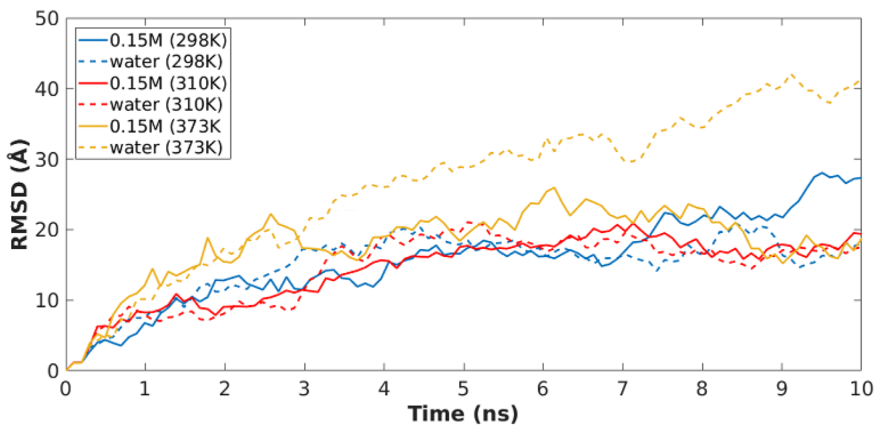

Figure 2: Root mean square deviation over time

Protein adsorption was measured using adsorption energy, which corresponds to the non-bonded energy (summation of the Van der Waals and electrostatic energy), of the interaction between the protein and the gold substrate. In all systems using gold as the substrate, the electrostatic energy is zero and the adsorption energy corresponds only to the Van der Waals energy.

Figure 3 presents the adsorption energy plot over time for different saline concentrations and temperatures. No protein adsorption occurred for the simulations performed at 298K using only water as dissolution media and at 373K using 0.15M saline solution. At 310K, both aqueous and saline (0.15M) models showed adsorption around 5ns. For the other simulations (0.15M saline solution at 298K and water at 310K), the protein took a longer time to get close to the substrate surface and only weak adsorption occurred.

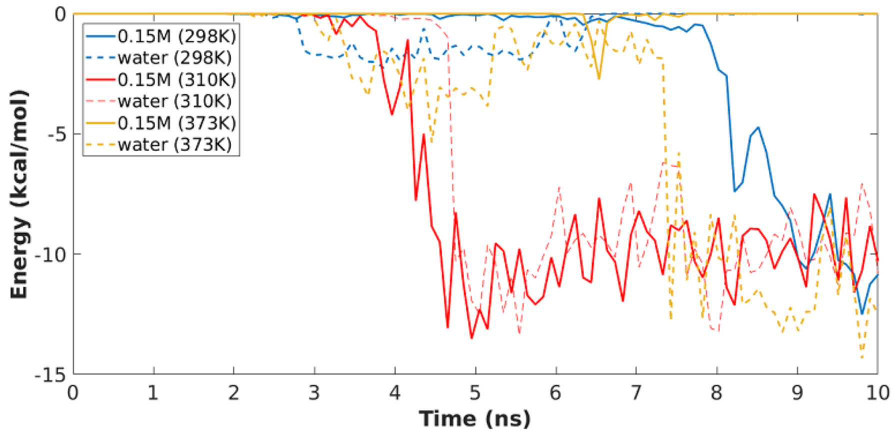

Figure 3: Adsorption energy of protein with substrate for different media and temperatures

A steady adsorption of the protein onto the surface occurred at 310K. Adsorption occurred in the same orientation towards the surface, exhibiting similar interactions with the surface. Different dissolution media seems to have no significant effects on the adsorption behavior of the protein at this temperature. However, at 373K, the presence of salt ions interfered with the protein adsorption forming a barrier layer between the protein and the substrate resulting in weaker interaction. Figure 4 presents the evolution of the simulations performed at 373K after 5ns and 10ns.

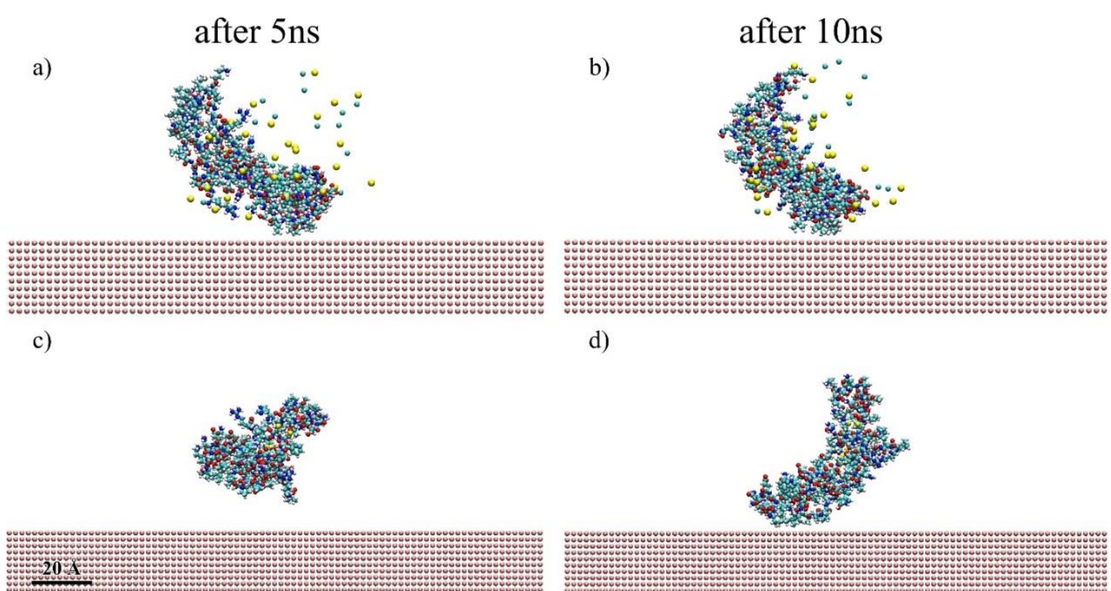

Figure 4: Side view of simulations performed at 373K, a) and b) 0.15M sodium chloride and c) and d) water as dissolution media. Water molecules were removed from this representation to better visualize the model

Based on the analysis of the radius of gyration no significant folding or unfolding occurred during the simulations, which indicates that no denaturation occurred to the BMP-2 structure. Thus, maintaining its properties even at the boiling temperature of water (see Figure 5). The protein did not unfold and spread onto the substrate surface as compared to the initial structure. This behavior is preferable so that the protein can adsorb to the surface while preserving its bioactivity [27].

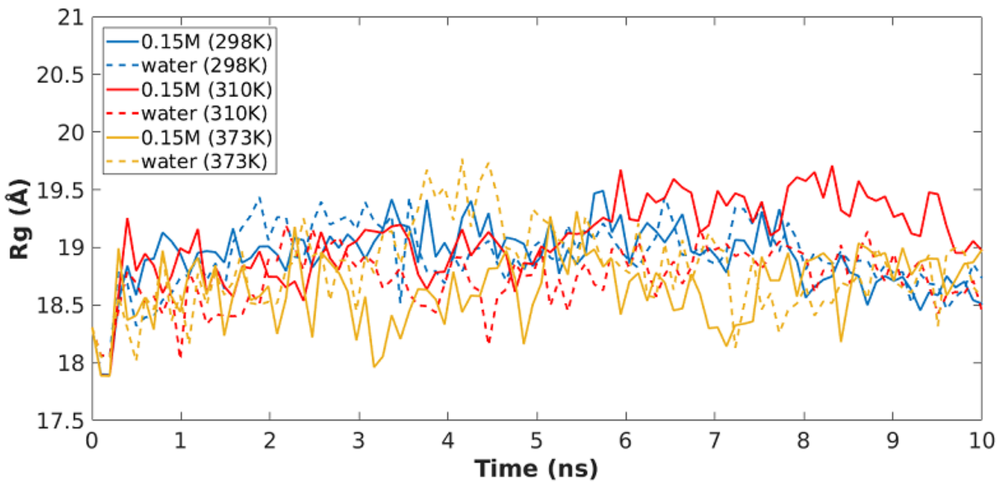

Figure 5: Evolution of the radius of gyration of the protein

In addition, we analyzed the secondary structure of the protein during simulations to confirm that no significant change occurred in its secondary structure (see Table 1). The β-sheet content of BMP-2 protein show a minor reduction. Similarly, minor variations of the α-helix occurred. This analysis confirms that no denaturation of the protein occurred even at high temperature (373K).

Table 1: Secondary structure of BMP-2 before and after 10ns simulations

	Initial structure	After 10ns					
Secondary structure		298K		310K		373K	
		0.15M	Water	0.15M	Water	0.15M	Water
α-helix	10.38%	11.32%	11.32%	11.32%	9.43%	11.32%	8.49%
3_{10}-helix	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.83%
β-sheet	68.87%	66.98%	66.98%	64.15%	61.32%	64.15%	61.32%

In summary, based on the results of this study, temperature has a significant impact on BMP-2 adsorption behavior onto gold flat substrate. Body temperature was observed to be preferable for stronger and stable protein adsorption. However, at higher temperature protein adsorption can occur without denaturation. These findings suggest that the BMP-2 protein could be used for experimental trials in high temperature processes, including autoclaves that are used in medical applications to sterilize implant materials.

4. Conclusion

This paper evaluates the effect of different temperatures on BMP-2 adsorption onto gold substrate at the nanoscale. Molecular dynamics MD simulations were performed to understand protein-substrate interactions at room temperature (293K), *in vivo* conditions (310K), and boiling point of water (373K). Water and a saline media (sodium chloride 0.15M) under different ambient conditions were used as dissolution media for the solvation of the biomolecule to represent the cellular environment. The results indicate that BMP-2 is resistant to high temperatures (373K) at the nanoscale since no denaturation occurred for both models (water and 0.15M saline solution). However, only weak adsorption occurred at this temperature. Stronger protein adsorption occurred at 310K using both dissolution media, which indicates that BMP-2 protein is suitable to be used for *in vivo* conditions. No significant variations of the secondary structure of the protein occurred, confirming that the protein can retain its bioactivity even at high temperatures.

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