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RESEARCH ARTICLE

Insilico and Electron Microscope Study about Attachment of Mesenchymal Stem Cell Derived from Stromal Vascular Fraction to Scaffold (Bovine Bone Cancellous, Hydroxyapatite-Calcium Sulphate, Calcium Phosphate)

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Abstract

Objective: Insilico study using molecular docking with Hex had already surpassed all of the other previous approach in assessment of molecular binding to scaffold. The combination of insilico study to investigate the molecular binding to scaffold and electron microscope to assess the population density of adipocyte derivative stem cells by measuring the number of cluster cells could show the inter cell communication between adipocyte derived stem cell and scaffold. The aim of this study is to prove the attachment of mesenchymal stem cell (MSC) from stromal vascular fraction (SVF) to scaffold. Methods: The research is true experimental research using molecular docking with HEX version 8.0. Electron microscope used to measure the density and number of cluster cells of adipocyte derivative stem cells. Results: The strongest bound to scaffold is between MSC from SVF to hydroxyapatite in both receptor which was Integrin Alpha V to Hydroxyapatite which need a total energy of -89.24 (J/Mol) and Integrin Beta 2 to Hydroxyapatite which need a total energy of -177.8 (J/Mol). The highest impact obtained from hydroxyapatite-calcium phosphate with an average value of 12.66 cluster cells counting per 100 μm^2 scaffold material observed by electron microscope. Conclusion: This study showed that MSC from SVF could attach to scaffold with stronger binding between Integrin Alpha V and Integrin Beta 2 to hydroxyapatite. From microscope electron study we can prove that MSC can make a cluster cell in those scaffolds with hydroxyapatite-calcium sulphate having the biggest cluster cell counting.

Keywords: *Hydroxyapatite, Integrin Alpha V, Integrin Beta 2, Mesenchymal stem cell, Scanning electron microscope, Stromal vascular fraction.*

Introduction

Tissue Engineering (TE) point to improvement, restoration, and maintenance of damaged tissues caused by diverse factors such as injury, disease, or congenital disabilities. Tissue regeneration and healing in conventional method is using the auto graft method and mainly dependent on the presence of donor tissues, coupled with other unwanted effects such as pain and risks to patients such as morbidity of donor tissue and infectious diseases. Nowadays artificial scaffolds have been applied and used as a supporting structure for cell cultures and domination of cell growth in repair of defective tissues or organs. While the cell regenerated, the scaffold temporarily supports in cell regeneration and gradually biodegrades either in the course of the healing process or after, and a new tissue with a suitable shape and properties is produced [1]. Fundamentally TE paradigm composed of scaffolds, signals, and cells. Combination of these 3 elements or independent use of each could be used to attempt to generate tissues in an infinite number of arrangements.

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But with an increasing complexity of design, there are bigger challenges to translation. For example, receiving regulatory approval for an acellular scaffold requires substantially less time and fewer resources than does a drug-eluting scaffold that has been pre-seeded with stem cells. Scaffolds in TE are cytocompatible biomaterials that cells can adhere to and/or replace with extracellular matrix to generate native tissues. Whether a simple morselized autologous bone or complexes inject able, thermally responsive synthetic hydro gels capable of mineralizing in situ could be used as a scaffold.

Signals in the TE paradigm could be defined as internally or externally derived environmental factors that can affect the regeneration of tissues. Same as scaffold, these signals can be further deranged into subcategories including mechanical, biological, chemical and electrical cues. Cells, in order to create living tissues, as well as integrate living engineered tissue with native host tissues, must be present. Cells can be recruited into an implanted scaffold by methods such as chemokines release, cell ligands attachment to scaffold, or scaffolds containing cells which could be implanted into a defect.

Unlike other TE fields, there is less controversy in regard to stem cell type in orthopedic TE. Until this day, mesenchymal stem cell (MSC) is the most commonly utilized cell type [2].Stromal vascular fraction (SVF) is a heterogeneous, versatile, and clinically relevant cell system.

SVF is known to have MSC, fibroblasts, smooth muscle cells, mural cells, blood cells, macrophages, and a whole cadre of other stem cell phenotype. Interaction of all these cell types contributes to SVF's overall therapeutic potential [3].Electron microscope is subcategorized into transmission electron microscopes (TEM), scanning electron microscopes (SEM) and low energy electron microcopy (LEEM).

All of these serve in purpose of a significant demand exist for the development of novel techniques capable of imaging nanostructures, macromolecules, and surfaces to provide analytical capabilities with sub-nanometer resolution [4]. In this paper we want to make a study of insilico and electron microscope about attachment MSCderived from SVF to scaffold.

Materials and Methods

Insilico study: Molecular Docking with HEX version 8.0

He x is an interactive protein docking and molecular superposition program, written by Dave Ritchie. Hex understands protein and DNA structures in PDB format, and it can also read small-molecule SDF files [5].

Sample:

Integrin Alpha V (PDB ID 1JV2)

Integrin Beta 2 (PDB ID 3K6S)

Nanoparticle hydroxyapatite (PUBCHEM ID 18986957)

Hydroxyapatite (PUBCHEM ID 14781)

Calcium Sulphate (PUBCHEM ID 24497)

Calcium Phosphate (PUBCHEM ID 24456)

Electron microscope: Assessment of population density of adipocyte derivative stem cells by measuring the number of cluster cells seen in imaging scanning electron microscope / SEM to reduce measurement bias, then measured the number of cluster cells by two observers, namely researchers and expert analysts in the field of stem cells. In addition, cells measured for density on SEM imaging were performed under a light microscope to prove that cells imaged in SEM were adipocyte derivative stem cells [6].

Results

From the insilico study we have obtained a result from the docking of MSC from SVF to scaffold. Integrin Alpha V and Integrin Beta 2 were chosen as a macromolecule because more than 80% of MSC's will express Integrin Beta 2 and 20-50% will express Integrin Alpha V. Both of these proteins will have a huge role on attachment and adhesion.

Docking has been done and shown in Figure 1-2 and Table 2-3, to locate the binding of nanoparticle ligand and to study for how much energy it needs. More negative value of Total E means the stronger the bond between the ligand and macromolecule (Table 1).

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Table 1: Total energy needed for the binding between ligand and macromolecule

Receptor	Ligand	Total Energy (J/Mol)
Integrin Alpha V	Calcium sulphate	-79
	Calcium phosphate	-46.87
	Nanoparticle Hydroxyapatite	-76.2
	Hydroxyapatite	-89.24
Integrin Beta 2	Calcium sulphate	-167.68
	Calcium phosphate	-127.94
	Nanoparticle Hydroxyapatite	-137.99
	Hydroxyapatite	-177.8

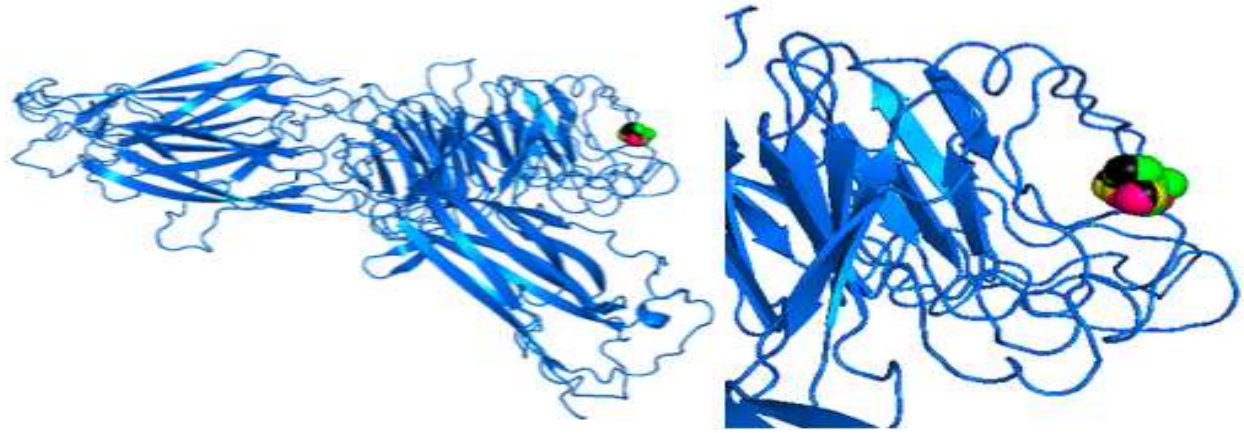

Figure 1: Interaction between Integrin Alpha V and Nanoparticle

Table 2: Interaction between Integrin Alpha V and Nanoparticle shown by color

Ligand	Color
Calcium sulphate	
Calcium phosphate	
Nanoparticle Hydroxyapatite	
Hydroxyapatite	black

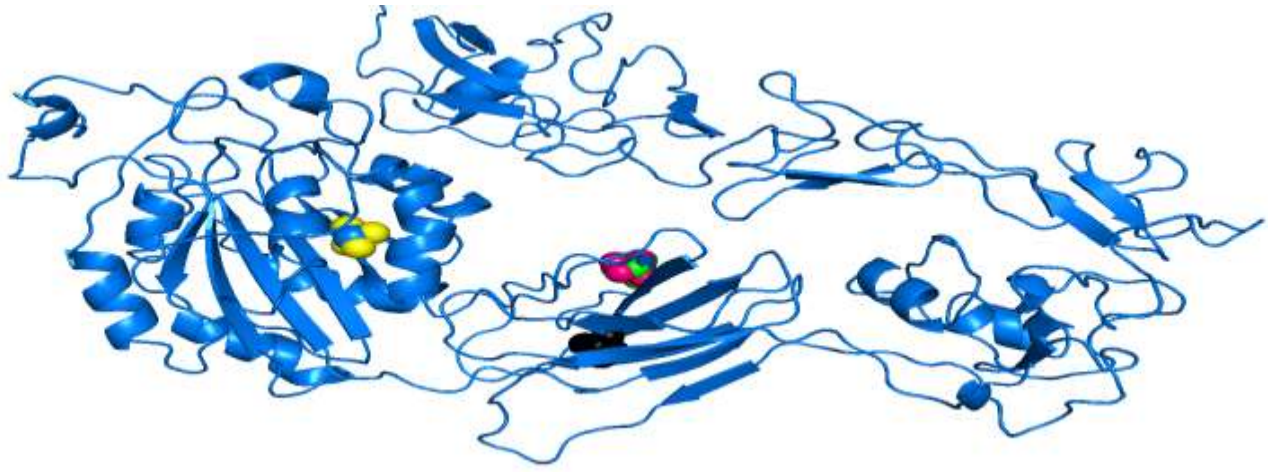

Figure 2: Interaction between Integrin Beta 2 and Nanoparticle

Table 3: Interaction between Integrin Beta 2 and Nanoparticle shown by color

Ligand	Color
Calcium sulphate	
Calcium phosphate	
Nanoparticle Hydroxyapatite	
Hydroxyapatite	black

From electron microscope study it had been proved that MSC from SVF could attach to scaffold and develop a cluster cells. The cluster cells were counted and the result was showed in Table 4.

Table 4: Descriptive analysis of Cluster cells counting per 100 µm²based on each scaffold

Group	N	Mean	Std. Deviation
Bovine bone cancellous	6	6.008	0.085
HA-Calcium Sulphate	6	12.660	0.413
Calcium Phosphate	6	8.467	0.322
Control	6	0.925	0.085

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Based on the results of the descriptive analysis in Table 4 it can be seen that the number of cluster cells per 100 µm2 is described as follows:

- Observations of Bovine bone cancellous from 6 replications obtained an average value of 6.008 and a standard deviation of 0.085.
- Observations of HA-Calcium sulphate (Perosal) from 6 replications obtained an average value of 12.66 and a standard deviation of 0.413.
- Observations of Calcium phosphate (Kasios) from 6 replications obtained an average value of 8,467 and a standard deviation of 0,322.
- Observations of control from 6 replications obtained an average value of 0.925 and a standard deviation of 0.085.

Through the results above, it can be said that the administration of Scaffold material in ADSC cells has a significant effect, and the administration of HA-Calcium sulphate (Perosal) material is higher than other groups. Figure 3 until 5 each shows the imaging of the scanning electron microscope and the descriptive analysis graph of cell cluster counting.

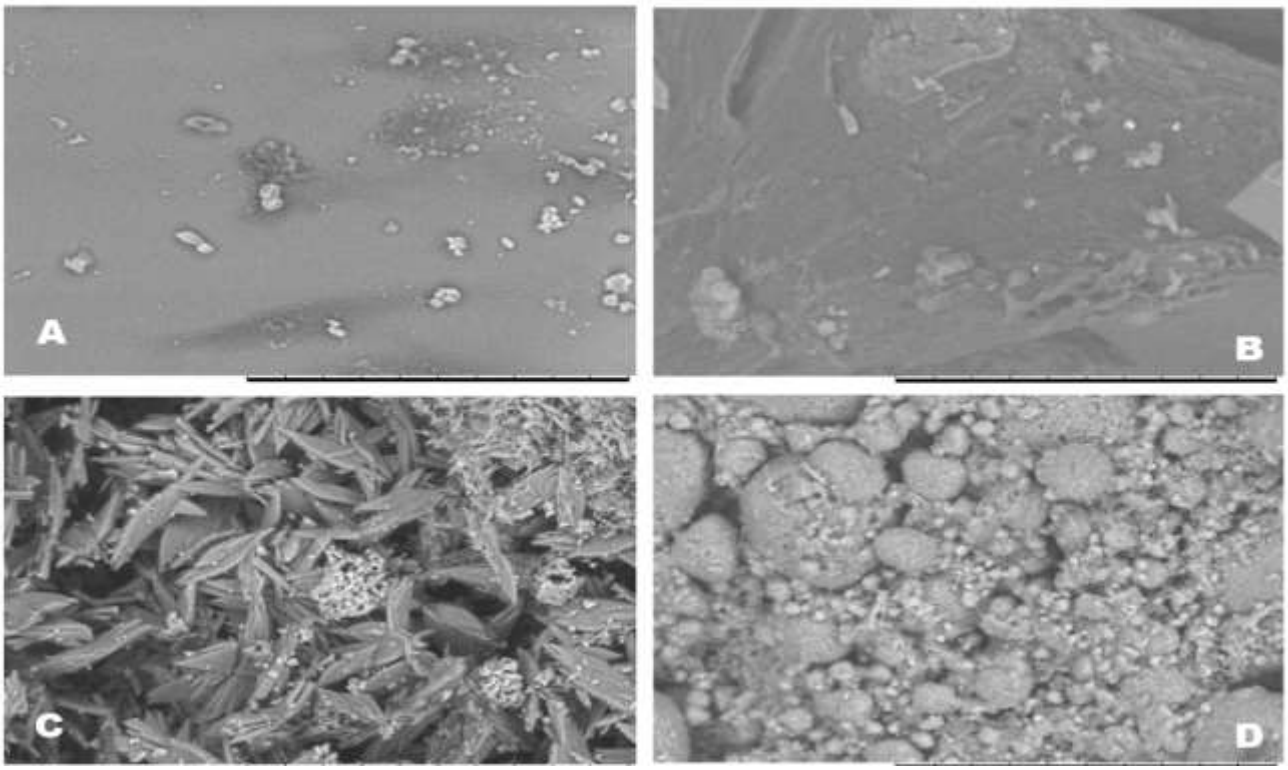

Figure 3: Imaging *Scanning electron microscope* of adipose derived stem cell in bonegraft medium (A) control, (B) *adipose derived stem cell* in *bovine cancellous graft* medium, (C) *adipose derived stem cell* in calcium phosphate medium, and (C) *adipose derived stem cell* in hydroxapatite-calcium sulfate. Magnification 1000x

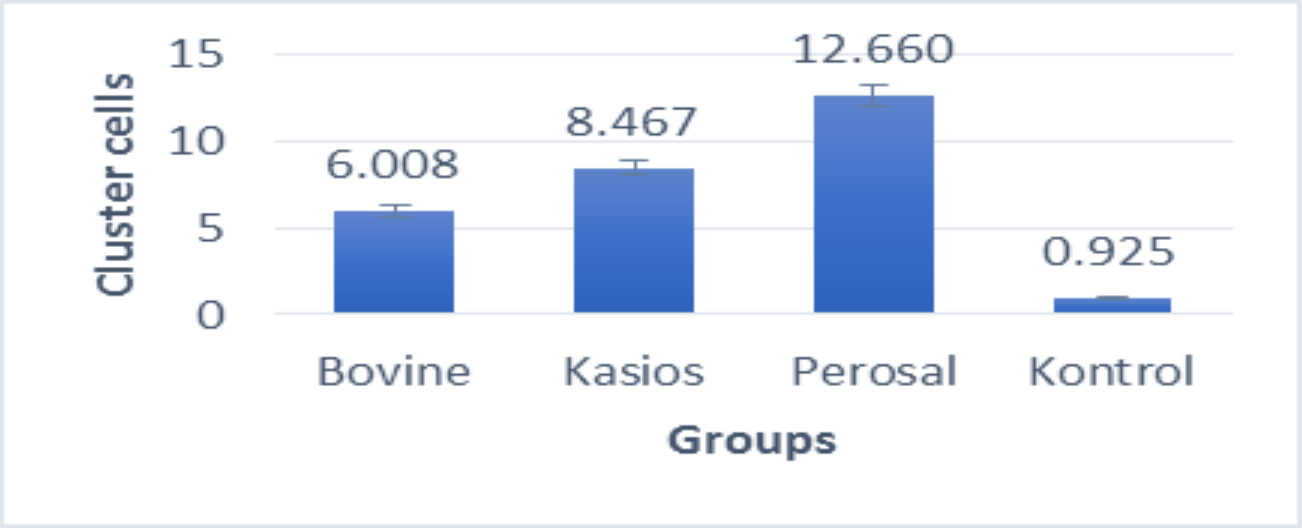

Figure 4: Descriptive Analysis Graph of Cluster cell counting per 100 µm² based on each Scaffold

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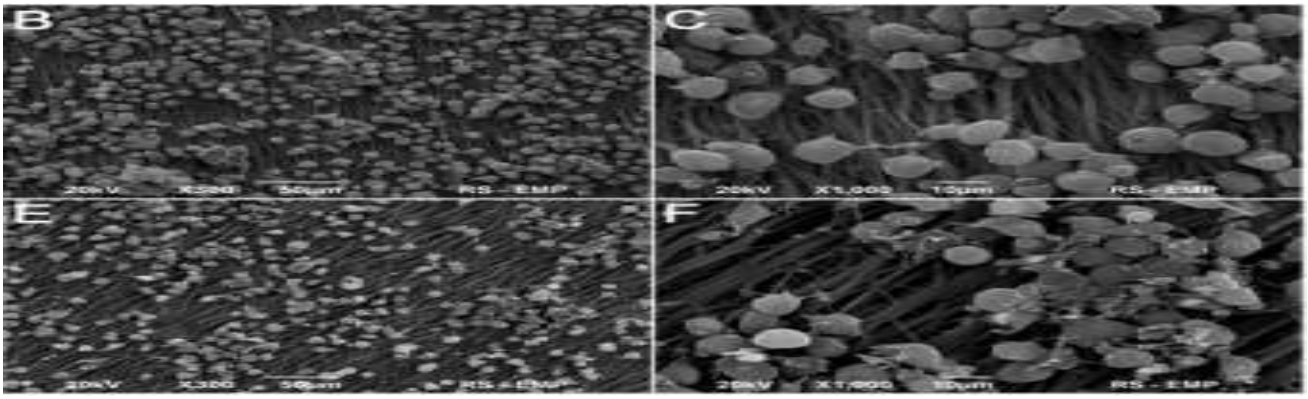

Figure 5: Imaging Scanning electron microscope of adipose derived stem cell

Discussion

These days, various protein docking programs have been made available as web servers. These range from rapid Patch Dock server to much more computationally intensive approaches incorporating models of flexibility such as Rosetta Dock and Haddock. Several FFT-based docking programs have also been made available as web servers. Similar as the geometric hashing approach, the FFT-based approaches assume that the proteins to be docked are rigid, but they sample densely all possible rigid-body orientations in the 6D search space.

But despite all of that assumption, Cartesian grid-based FFT docking algorithms are inherently overpriced. To counter those limitations of the Cartesian FFT approaches, the 'Hex' spherical polar Fourier (SPF) was developed using rotational correlations which significantly reduces execution times. Thus, Hex algorithm obtained an enormous speed-up by exploiting the huge computational power of modern graphics processor units (GPU) using the CUDA (Common Unified Device Architecture) development tools. A typical Hex docking calculation is up to two orders of magnitude faster than conventional Cartesian grid-based FFT docking approaches. The Hex SPF algorithm has been validated in CAPRI (Critical Assessment of Predicted Interactions) blind docking experiment and Hex prediction has been found commonly within the top 100 orientations in recent CAPRI scoring sections.

Therefore, Hex Server set a very fast and simple way to generate a high quality docking predictions for subsequent refinement With the Hex we can prove the attachment between MSC from SVF to scaffold, and showed that the strongest bound is between MSC from SVF to hydroxyapatite in both receptor which was Integrin Alpha V to Hydroxyapatite which need a total energy of -89.24 (J/Mol) and Integrin Beta 2 to Hydroxyapatite which need a total energy of -177.8 (J/Mol) [7].The scanning electron microscope (SEM) study made it possible to control porosity percentage and the pores size. Study conducted by Mahmood et al, 2017 show that SEM could show the superficial and internal structure of scaffolds. With this method it was possible to control the porosity percentage and the pores size. SEM analysis showed excellent micro pores structures with sizes varying from 9 to 526 μm [8].

In this study SEM imaging was also carried out in each treatment with a magnification of 7000x. Although not yet analyzed qualitatively, the imaging results suggest that adipocyte derived stem cell (ADSC) cell populations are denser in all three scaffolds than controls, cell density has been shown to affect cell-cell interactions and as an important factor, controlling subsequent cell proliferation and gene expression profiles. In addition, Cell proliferation is strongly influenced by the surface area of cell attachment and inhibition of contact between adjacent cells. So it is estimated that the relationship / inter-cell communication from ADSC increases after being combined with scaffold [6].

Conclusion

In conclusion, from insilico study we can prove that MSC from SVF could attach to scaffold with stronger binding between Integrin Alpha V and Integrin Beta 2 to hydroxyapatite, and from microscope electron study we can prove that MSC can make a cluster cell in those scaffolds with hydroxyapatite-calcium sulphate having the biggest cluster cell counting.

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