Combining Mesenchymal Stem Cell from Stromal Vascular Fraction with Scaffold (in silico, Biocompatibility, and Attachment Study)

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Abstract

AIM: The aim of this study is to evaluate the in silico, biocompatibility, and attachment of mesenchymal stem cell (MSC) from stromal vascular fraction (SVF) combined with scaffolds.

METHODS: This research is true experimental study. In silico study using HEX version 8.0. Attachment of MSC from SVF to scaffolds was evaluated using electron microscope and measured. Biocompatibility was evaluated using viability and apoptosis of MSC from SVF after combined with MSC with scaffold.

RESULTS: From in silico study, the strongest bond to scaffold is between MSC from SVF to hydroxyapatite (HA) in both receptor which was integrin alpha V to HA which need a total energy of −89.24 (J/Mol) and integrin beta 2 to HA which need a total energy of −177.8 (J/Mol). MSC from SVF cells is capable to combine with three types of bone substitution material. At HA-calcium sulfate administration, SVF cells had apoptosis of 30.27%, and viable cells were 69.73%. At the administration of bovine bone cancellous, SVF cells had apoptosis of 22.20% and viable cells of 77.80%. From microscope electron study, the best result of attachment was obtained from MSC to HA-calcium phosphate with an average value of 12.66 cluster cells counting per 100 µm² scaffold material.

CONCLUSION: From in silico study showed that MSC from SVF could attach to scaffold with stronger binding between integrin alpha V and integrin beta 2 to HA scaffold. Bovine bone cancellous has the best biocompatibility and attachment among other scaffold with the highest viability and lowest cell apoptosis. From microscope electron study, we can prove that MSC can make a cluster cell in those scaffolds with HA-calcium sulfate having the biggest cluster cell counting.

Introduction

Tissue engineering (TE) is the intent to rejuvenate organ functionality and loss of tissue as an impact from aging, disease, or injury. Biomaterials, cells, and bioactive factors are often considered to be the core in the arrangement of 3D tissue-engineered structures for damaged tissue regeneration. Regeneration of tissues such as bone, tendon, cartilage, skin, and cardiac tissues has become the main target for TE to support and promote. It works at the injured site inducing tissue regeneration and acts as an artificial extracellular matrix. The scaffold supports cell attachment, proliferation, and differentiation. If the biocompatibility is good, the cells around it will infiltrate and proliferate. Scaffolds are implantable in the body as acellular scaffolds, or in communion with cells and/or cytokines, growth factors, and genes (bioengineered scaffolds). The latter has the benefit of better promotion of tissue regeneration, notably when the tissue does not have good self-regenerating capability, as in pathological conditions [1].

A structure which is capable of support and/or promotes tissue regeneration is called a scaffold. It should have a 3D and good micro-architecture and macro-architecture with an interconnected pore network. Scaffolds applied for TE should have great biocompatibility and no serious adverse immunological reaction. The frequency of research on biomaterials and its application in TE has greatly increasing in recent years. Various artificial bone substitute materials are available such as hydroxyapatite-calcium sulfate (HA-CaSO₄), synthetic HA, bovine HA, nanoparticulate HA paste, and morselized bovine xenograft. HA has been widely studied because of its similarity to bone in its composition and mineralogical structure. High biocompatibility and bioaffinity of HA make it slowly integrated and replaced by host bone [1].

In theory, a scaffold should undergo in vitro biocompatibility test before applied in animal or used in clinical trial but no data about biocompatibility have acquired yet. In vitro biocompatibility tests propose preliminary evaluations of newly developed materials,
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 are using the autograft 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 [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, scanning electron microscopes (SEM), and low energy electron microscopy. 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 scaffold [4].

In this paper, we want to make an in silico study, biocompatibility, and attachment of MSC from SVF to scaffolds.

## Materials and Methods

**In silico study:** Molecular docking with HEX version 8.0. Hex is an interactive protein docking and molecular superposition program, written by Dave Ritchie. Hex understands protein and DNA structures in Protein Data Bank (PDB) format, and it can also read small-molecule structure data files [5]. The sample are: Integrin alpha V (PDB ID 1JV2), integrin beta 2 (PDB ID 3K6S), nanoparticle HA (PUBCHEM ID 18986957), HA (PUBCHEM ID 14781), CaSO$_4$ (PUBCHEM ID 244497), and calcium phosphate (PUBCHEM ID 24456). Biocompatibility was measured by viability and apoptosis of SVF cells with markers Annexin V and propidium iodine through flow cytometry. Population density of adipocyte derivative stem cells measured the number of cluster cells seen in imaging SEM. To reduce measurement bias, the measurement was done 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]. This study was experimental in vitro with post-test control group design, and conducted at Biomedical Laboratory of Faculty of Medicine Universitas Brawijaya Malang. This research has been granted an ethical clearance by the medical research ethic committee of Saiful Anwar Hospital Malang with ethical clearance number 405/37/K.3/302/2018. The chairman of the ethical committee is dr. Mohammad Saifur Rohman, SpJP(K), PhD.

### Results

From the in silico 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 Figures 1 and 2 and Tables 1 and 2, 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 3).

![Figure 1: Interaction between integrin alpha V and nanoparticle](image1.png)

![Figure 2: Interaction between integrin beta 2 and nanoparticle](image2.png)
MSCs were cultured from SVF. After cells became confluent, they can be used for treatment.

Table 2: Interaction between integrin beta 2 and nanoparticle shown by color

| Ligand                | Color |
|-----------------------|-------|
| Calcium sulfate       |       |
| Calcium phosphate     |       |
| Nanoparticle hydroxyapatite |       |
| Hydroxyapatite        | Black |

This shows that MSCs from SVF cell cultures conducted by the study have MSC characteristics derived from fatty tissue, as shown in Figure 3. Then, from the examination of MSC phenotype characteristics of SVF cells gave CD34 and CD44 expression positive and CD45 expression negative, as shown in Figure 4.

Table 3: Total energy needed for the binding between ligand and macromolecule

| Receptor    | Ligand                | Total energy (J/Mol) |
|-------------|-----------------------|----------------------|
| Integrin alpha V | Calcium sulfate   | -79                  |
|              | Calcium phosphate     | -46.87               |
|              | Nanoparticle hydroxyapatite | -76.2               |
|              | Hydroxyapatite        | -89.24               |
| Integrin beta 2 | Calcium sulfate   | -167.68              |
|              | Calcium phosphate     | -127.94              |
|              | Nanoparticle hydroxyapatite | -137.99             |
|              | Hydroxyapatite        | -177.8               |

Figure 3: Stromal vascular fraction cell culture results have been confirmed.

Figure 4: (a and b) Results of CD34, CD44, and CD45 measurements as mesenchymal stem cell phenotype characteristics of stromal vascular fraction cells.

After that, mesenchymal stem cell samples were treated with scaffolds (HA calcium phosphate, calcium phosphate, and bovine bone cancellous), incubation for 24 h, then analyzed by flow cytometry with Annexin V markers and propidium iodine to assess the presentation of cells with late apoptosis and viable cells. The results are shown in Figure 4.

Figure 5: Average presentation of stromal vascular fraction cell apoptosis in each treatment

From the results of one-way ANOVA test, on the data of apoptosis and viability of SVF cells found a significant value of 0.000 where p < 0.05, this indicates that there were significant differences in the effect of three bovine bone cancellous, HA-calcium sulfate, and phosphate calcium phosphate substance to viability and apoptosis of stromal vascular cells, as shown in Figures 5 and 6. From Tukey HSD post hoc test, it was found that significant value of p < 0.05 between each treatment, especially control of HA-Ca sulfate, Ca phosphate, and bovine bone cancellous. This indicates that the provision of bone substitution material has a significant effect on viability and cell apoptosis of MSCs from SVF.

Figure 6: Average stromal vascular fraction cell viability percentage on each treatment

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

Table 4: Descriptive analysis of cluster cells counting per 100 µm² based on each scaffold

| Group              | n | Mean  | Std. deviation |
|--------------------|---|-------|---------------|
| Bovine bone cancellous | 6 | 8.008 | 0.085         |
| HA-calcium sulfate  | 6 | 12.660| 0.413         |
| Calcium phosphate   | 6 | 8.467 | 0.322         |
| Control             | 6 | 0.925 | 0.085         |
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:

1. Observations of bovine bone cancellous from six replications obtained an average value of 6.008 and a standard deviation of 0.085
2. Observations of HA-CaSO₄ (Perosal) from six replications obtained an average value of 12.66 and a standard deviation of 0.413
3. Observations of calcium phosphate (Kasios) from six replications obtained an average value of 8.467 and a standard deviation of 0.322
4. Observations of control from six replications obtained an average value of 0.925 and a standard deviation of 0.085.

Discussion

These days, various protein docking programs have been made available as web servers. These range from rapid PatchDock server to much more computationally intensive approaches incorporating models of flexibility such as RosettaDock and Haddock. Several fast Fourier transformation (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. However, despite all of that assumption, Cartesian grid-based FFT docking algorithm is inherently overpriced.

With the Hex we can prove the attachment between MSC from SVF to scaffold and showed that the strongest bond is between MSC from SVF to HA in both receptor which was integrin alpha V to HA which need a total energy of −89.24 (J/Mol) and integrin beta 2 to HA which need a total energy of −177.8 (J/Mol) [6].

Our study use SVF cells that were produced from femur subcutaneous tissue with subcutaneous fat tissue resection method. After the confusion, the SVF cell sample assessed the phenotype characteristic of MSC first. In this study, the characteristic of the phenotype surface marker CD34 and CD44 was positive with negative CD45. In the result of flow cytometry, Ca phosphate administration induced SVF cell apoptosis compared with HA-Ca sulfate and bovine bone cancellous, and SVF cells were still viable with bovine bone cancellous. Based on the ANOVA one-way statistical test, there was a significant difference in the treatment and in the test Tukey HSD got the difference between each treatment [7].

According to Liu et al., extracellular research in MSCs in vitro by Liu et al. showed that proliferation and differentiation of MSCs derived from bone marrow would significantly decrease phosphate administration at both high concentrations and low concentrations. Phosphate significantly induces cell apoptosis. Increased calcium concentration does not alter cell proliferation but significantly inhibits the differentiation of MSCs. At the proper concentration of calcium it is able to increase the mineralization of cell [8].

Based on the research, optimum calcium and phosphate concentration for MSC cells can grow and differentiate, i.e., 1.8 mM and 0.09 mM. Increased or decreased concentrations of calcium and phosphate would lower the proliferation and differentiation ability of the MSC.13 In this study, the calcium phosphate supplied
in SVF cell samples did not see the concentration of calcium and phosphate, this resulted in SVF-induced apoptosis cells due to ineffective concentration. The influence of calcium and phosphate concentrations on cellular is a concern in the combination of calcium phosphate with SVF cells.

At HA-CaSO₄ administration, SVF cells had apoptosis of 30.27%, and viable cells were 69.73%. This condition is possible HA-CaSO₄ increases the acidity of SVF cell culture medium. Ion released by CaSO₄ material may affect the hyperosmolarity of the MSC culture medium. According to Kholine's research, 2012 HA-Ca sulfate can be used as MSC delivery material and does not affect the survival of MSCs. HA-Ca sulfate has biocompatibility in stimulating the adhesion of cells in biomaterial [9], [10].

At the administration of bovine bone cancellous, SVF cells had apoptosis of 22.20%, and viable cells of 77.80%. In research by Zambuzi et al., 2006, showed that bovine bone cancellous has an anorganic porous bone surface that can improve adhesion, proliferation and maturation of osteoblast cell culture. The inorganic matrix of bovine bone is a natural, porous, and has a xenogenic HA structure, which increases the ability of bone membrane deficiency and maturation. Bovine bone also has low macrophage activator ability and low osteoclast so it can be used as bone substitution material in situations requiring time in material implantation [11], [12].

Shahabipour et al. showed that there is an integration, adhesion, and maintaining MSCs in bovine bone cancellous during in vitro culture. Bovine bone cancellous has a three-dimensional structure to support and maintain MSC growth histologically and SEM [16]. In this study, SVF cells have high viability of 77.80% on bovine bone cancellous.

Based on the provisions of the International Federation for Adopted Therapeutics and Science together with the International Society for Cellular Therapy, to be used as an active biological material, SVF cells should have a cell viability of >70% cancellous can be a combination of SVF cells because they have SVF cell viability of 77.80% compared to HA-Ca sulfate 69.73% and Ca phosphate 47.46%.

The SEM study made it possible to control porosity percentage and the pores size. Study conducted by Khuder et al., 2017, shows 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 [13].

In this study, SEM imaging was also carried out in each treatment with a magnification of ×7000. Although not yet analyzed qualitatively, the imaging results suggest that 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. Hence, it is estimated that the relationship/inter-cell communication from ADSC increases after being combined with scaffold [14]. In our study, the best attachment of MSC was to HA CaSO₄ scaffold. It proved by the largest cluster cell number.

Conclusion

From in silico study showed that MSC from SVF could attach to scaffold with stronger binding between integrin alpha V and integrin beta 2 to HA scaffold.

Bovine bone cancellous has the best biocompatibility and attachment among other scaffold with the highest viability and lowest cell apoptosis. From microscope electron study, we can prove that MSC can make a cluster cell in those scaffolds with HA - CaSO₄ having the biggest cluster cell counting.

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