Cell coating on silk fibroin mesh based on different motions

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Long Abstract

Introduction

The aim of this study is to coat silk fibroin meshes with autologous tissue, for use in a one-step surgical procedure for soft tissue repair. The experiments test the transport phenomena of coating the silk fibroin. The coating with SVF (stromal vascular fraction) is of particular importance because the heterogeneous mesodermal cell population combined with silk mesh might greatly improve hernia healing.

To establish a suitable mesh, biomaterial silk was knitted to a mesh and degummed. Prior to application, the silk from Bombyx mori is degummed to remove the sericin, which has been identified as an inflammation-inducing component. The remaining silk fibroin mesh was placed over CellcrownTM for the in vitro experiments applying different types of motion. The silk fibroin was grafted with lectin WGA (wheat germ agglutinin), which enhanced cell adhesion due to a higher binding affinity for the cell membrane. The generated silk meshes were then tested on three types of cells with different seeding strategies and a variation of adhesion time. The aim was to find a coating of the silk mesh with the maximum possible cell adhesion.

1. Methods and Experiments

The different seeding strategies were applied using different adhesion times. The variation of adhesion time was between minimum 15 minutes and maximum 90 minutes. As seeding strategy a rotational movement was applied during the adhesion time and compared to meshes seeded without moving the cell suspension during incubation. The silk fibroin fibres were coated with fibroblasts NIH3T3, hASC (human adipose- derived mesenchymal stem cells) and SVF. For NIH3T3 the best seeding strategy was the use of silk lectin WGA meshes for 15 minutes under rotational movement. For the hASC, the best adhesion was achieved after 90 minutes and the lectin WGA grafing on silk meshes raised cell adhesion most effectively after incubate for 30 minutes. Therefore, for hASCs, lectin WGA grafting can increase the rate of successful attachment to the mesh. Using the rotational movement after 30 minutes, the highest increase due to the lectin WGA grafting could be seen. For further studies, a seeding protocol under rotational movement with a limit of 30-minute adhesion time was applied.

As further application the SVF were applied on silk meshes. It was found that SVF coating to the silk fibroin meshes did not improve with lectin WGA coating. When the suspension of SVF was dripped onto the silk fibroin mesh, the SVF adhered in clusters, surrounded by an extracellular matrix; this restricts the binding affinity to the membrane and surrounds the cells. The SVF thus formed a layer that surrounded the silk fibroin and covered the silk mesh with autologous cells. The seeding strategy was to drip the suspension with SVF onto the silk fibroin mesh and to attach the SVF by gravitational flow. This was more effectively than the rotational movement during the adhesion time. The SVF spreads like a matrix over the silk surfaces independently from the presence of lectin WGA. As visualized in the SEM pictures 1 the proliferation is still equal on meshes with and without lectin WGA grafting after 14 days.

After adapting the seeding of the cell suspension to dripping on the mesh, an adaptation of the harvesting of the SVF was made. The harvesting, in which SVF is digested with collagenase, includes

a straining step at the end. The straining needs to be done within this step so that the SVF can pass through the pipette system. As seen in figure 2 the comparison of applying a cell strainer with 100 μm or 1 mm leads to a difference in DNA amount on the mesh. The smoother strained cell suspension needs five times more suspension flowing through than the rougher strained cells, which close the pores after transporting 1 ml of SVF suspension by gravitational force through the mesh. A cell strainer of 1 mm is recommended to fill the knitted pores of the silk mesh. By applying a large pore strainer and using a Perfusor® system to add the SVF suspension onto the mesh, a coating layer of SVF on the silk mesh was produced within 1 ml, after 1 minute. This application of the silk mesh coated with SVF can be used in future experiments.

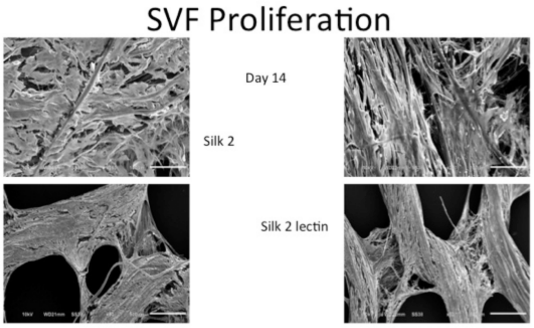

Figure 1. SVF Proliferation for silk 2 and silk 2 lectin within 14 days

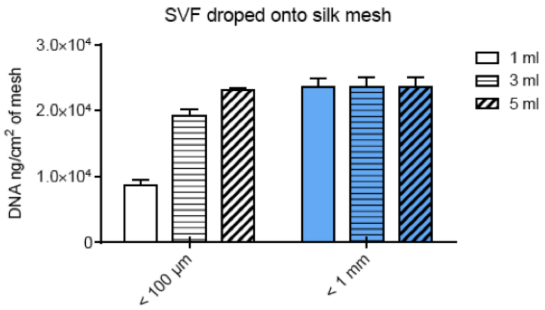

Figure 2. SVF quantity on the silk mesh

2. Conclusion

Based on the measurements performed, the following results should be highlighted. The first conclusion pertains to the lectin WGA grafting of the silk meshes. The lectin WGA enhanced cell adhesion due to a higher binding affinity for the cell membrane. Therefore, for hASCs, lectin WGA grafting can increase the rate of successful attachment to the mesh. Using the Adhesion Optimization after 30 minutes, the highest increase due to the lectin WGA grafting could be seen. For further studies, a seeding protocol under dynamic conditions with a 30-minute adhesion time is suggested.

The second conclusion is that lectin WGA grafting did not improve SVF adhesion on the mesh. While the lectin WGA improves cell membrane adhesion, which is effective for NIH3T3 and hASC, the SVF aggregates in clusters, surrounded by an extracellular matrix; this restricts the binding affinity to the membrane and surrounds the cells. The SVF behaves like a matrix that spreads out over the silk surfaces independently from the presence of lectin WGA, as visualized in the SEM pictures.

The third conclusion regarding SVF is that a knitted silk mesh, with its natural nanofibres, is suitable for catching SVF. A dynamic flow based on gravity and forcing itself through the mesh, is the preferable technique for adhering the SVF onto the silk mesh. The larger clumps of SVF have a great potential for coating the silk mesh with this technique. A properly coated silk mesh could favourably influence integration, quality of scar tissue, and potential local inflammatory response.

In conclusion, the silk can be knitted to a mesh to which cells adhere. The silk fibroin mesh can be coated with SVF, which enable a covering of the mesh with an autologous matrix within minutes, and the SVF clusters form a surrounding layer on the silk fibroin mesh.