Functional Tissue Engineering of Articular Cartilage

Cindy Xue, Chao Wan

Department of Biomedical Engineering, The Chinese University of Hong Kong, Hong Kong SAR

Introduction

Background

Articular cartilage has limited ability to self-repair due to its avascular nature, which makes the cartilage tissue diminished caused by trauma or degenerative pathology, subsequently resulting in Osteoarthritis (OA). OA is a very common degenerative joint disease for the elderly, which causes dysfunction of the joint and tremendous burden to the patient and the society. However, current therapies remain not satisfied. Cartilage is not only avascular, but also has a very complex structure, which consists of the superficial, middle, and deep zones which are also responsible for their own jobs in the cartilage, for example, the superficial zone contributes to the tensile modulus of the cartilage to resist shear stress at the joint surface, by producing collagen type II.

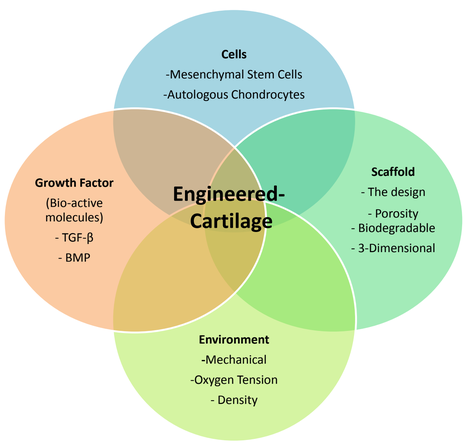


Fig.1 shows the need of the interaction or combinations among those factors to regenerate nature-like cartilage

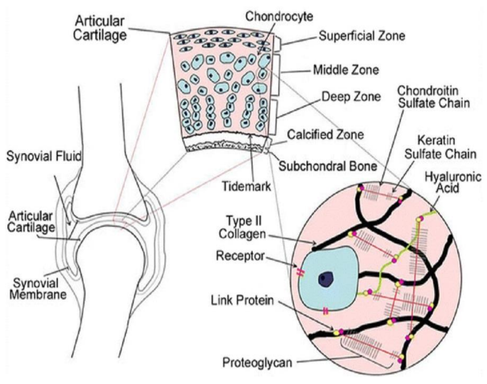


Fig.2 shows the complexity of the cartilage which we should take into considerations for cartilage regeneration[1]

In order to maintain the complex structure of articular cartilage, it is important to consider about these factors during tissue engineering of the cartilage, among which the keys are the type of the cells, the scaffolds, the environment and the growth factors.

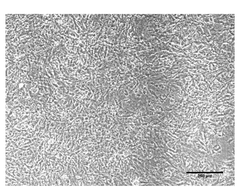
In this project, we encapsulated the chondrocytes in the alginate, a biocompatible and degradable material facilitate to resemble the cartilage matrix[2], and to redifferentiation of the dedifferentiated chondrocytes during the 3 dimensional expansion[3]. Hence, we are trying to find a better environment used for growing or maintaining the chodrocytes, so that it will not dedifferentiated.

Objective

To define the best combination of the materials used for maintaining the chondrocytes, or for preventing the dedifferentiation of the chondrocytes, and furthermore to promote the growth of the chondrocytes in a 3D environment.

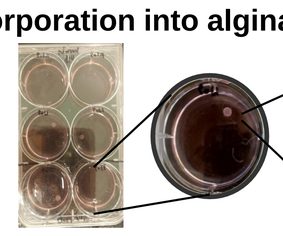
Methods

Cell Culture



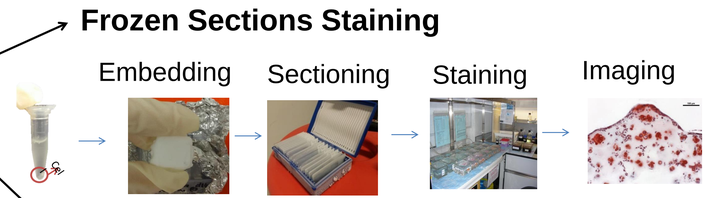
The chondrocytes cells were isolated and expanded for up to 3 weeks for 3D reconstruction.

Incorporation into alginate



The cells were incorporated into the alginate to form a 3D structure.

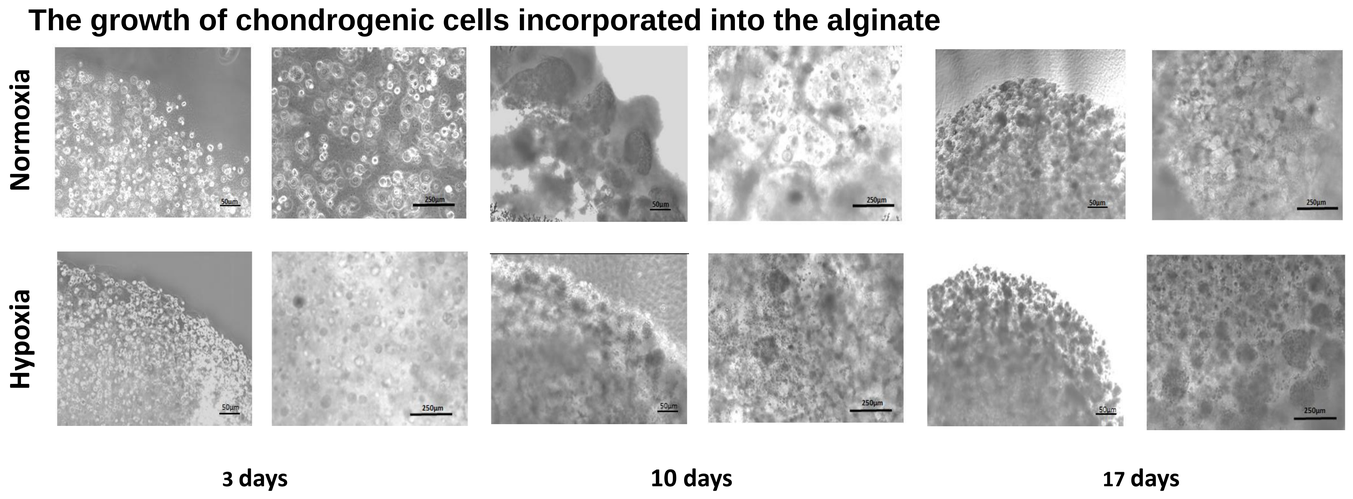
Frozen Sections Staining



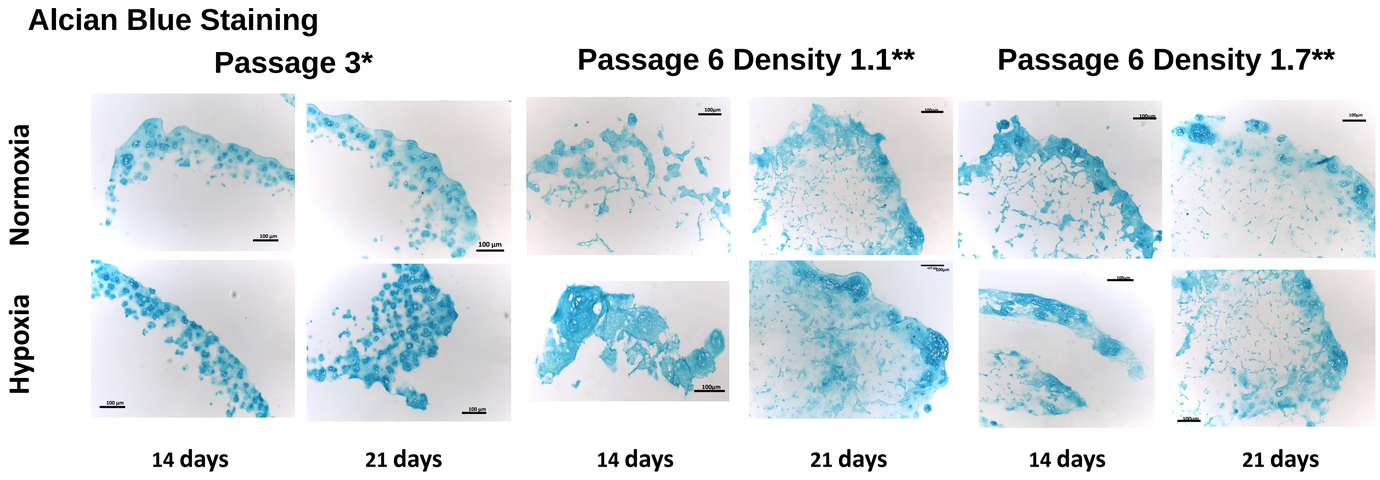
Gene expression Analysis

Results

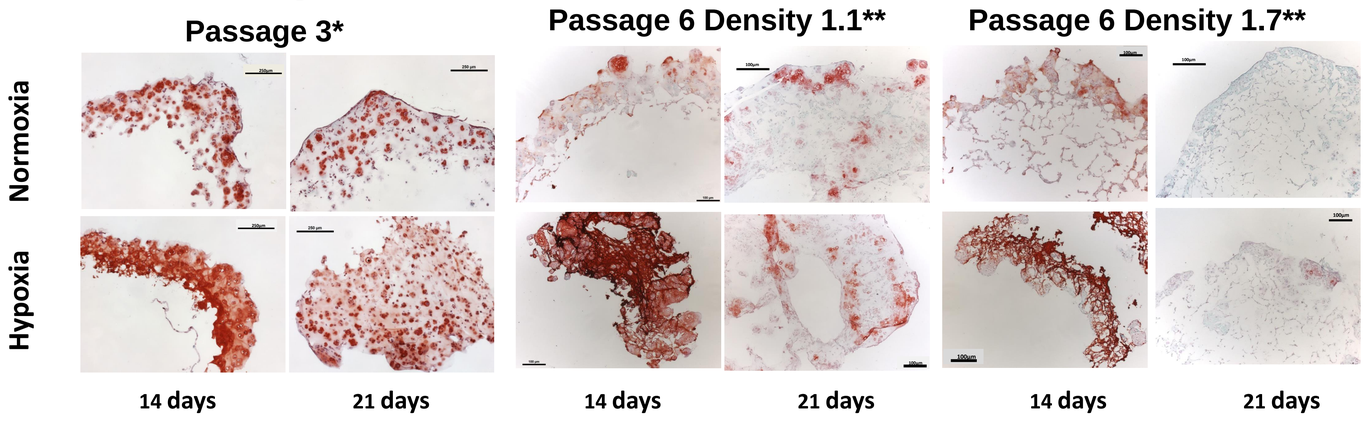
The growth of chondrogenic cells incorporated into the alginate



Alcian Blue Staining



Safranin O Staining



* Cells at passage 3 were used with density 1 x 10^6 cells in the 20µl alginate solution in a bead.

** Cell density as indicated in million cells per 20µl alginate solution in a bead.

Discussion and Conclusion

This experiment was conducted in three different settings, where the first one used the chondrocytes with passage 3 with cell density 1 x 10^6 cells in the 20µl alginate solution for a gel, while the second used the chondrocytes with passage no. 6 and cell density 1.1 x 10^6 cells in the 20µl alginate solution, and the last one used the chondrocytes with passage no.6 and cell density 1.7 x 10^6 cells in the 20µl alginate solution for a bead. All of these settings were cultured in both hypoxia condition (2% Oxygen), and normoxic condition (20% Oxygen).

By using the microscope to daily check the growth of the cells, we observed that in the hypoxia group, the cells grow faster and much denser. We could also observe that there are more mass cells compared to the group which cultured in the normoxic condition. This suggests that hypoxic condition enhances the growth of the cells.

In addition, from the staining results, they also suggests that hypoxic condition tend to show denser population of the chondrocytes compared to the normoxic group, both in the Alcian blue staining and the safranin O staining. This suggests that hypoxic condition serves as a better environment or better in mimicking the native environment for the chondrocytes to grow or be maintained, or be redifferentiated. While in the density factor, it shows that in the first 14 days, the cells are denser in the gel with the cell density 1.7, however, after some days later (21 days), it shows a better cell proliferation in the gel with cell density 1.1. This might be caused by the optimal concentration of the alginate used to culture the cells, as the structure, or morphology of the gels, which might affect its functionality is highly depending on the alginate concentration. While from the perspective of the passage numbers, they do show that the lower passage number would yield to a better result, as shown. Hence, it is also proven that cell passaging should be avoided to have a better result. The shape of the stained cells shows that the cells are more likely to grow at the edge of the structure. This might be caused by the process of the nutrition intake from the environment to the cells. The cells therefore move to the edge of the gels to get some nutrition or during 3D reconstruction the cells are easily located at the outer portion of the materials.

In summary, we have shown that cell passaging should be avoided as the lower the passage number, the better maintenance of chondrocyte phenotype, as passaging could weaken the cells or dedifferentiate the cells. In addition, we also show that hypoxic environment is better in mimicking the native environment of the chondrocytes in the body. There are also a lot of the other factors could affect the cultured cells, such as the cell density and the concentration of the alginate.

Acknowledgements

I would like to express my deepest gratitude towards Prof. Wan Chao who is the supervisor of this project and has guided me through out this project, and as well as his laboratory member and colleagues in SBS core laboratory who has helped me for the completion of this project.

References

[1] H. S, "Articular Cartilage Changes in Maturing Athletes," *Sage: Sports Health: a Multidisplinary Approach*, Apr., 2013. [Online]. Available: http://sph.sagepub.com/content/6/1/18/F4.expansion.html. [Accessed Dec. 1, 2014].

[2] Hauselmann, H. J., Masuda, K., Hunziker, E. B., et al. (1996) Adult human chondrocytes cultured in alginate form a matrix similar to native human articular cartilage. Am. J. Physiol. 271, C742–C752.

[3] Lemare, F., Steimberg, N., Le Griel, C., Demignot, S., and Adolphe, M. (1998) Dedifferentiated chondrocytes cultured in alginate beads: restoration of the differentiated phenotype and of the metabolic responses to interleukin-1β. J. Cell. Physiol. 176, 303–313.