Review

Application of low-pressure cell seeding system in tissue engineering

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Summary **Tissue engineering has been one of the most promising strategies for the regeneration of impaired tissue. Application of three-dimensional porous scaffolds has greatly improved the outcome of tissue engineering in many categories. Cell seeding is one of the key issues in tissue regeneration. It depends not only on the biocompatibility and affinity of the scaffold, but also on the seeding techniques. Current seeding techniques such as centrifugation and perfusion have enhanced better cell seeding, but still have their limitations. How to seed cells more efficiently and uniformly, especially in the inner parts of the scaffolds, and with no impairment to the cells, has been one of the major challenges in using porous scaffolds for tissue engineering. Low pressure seeding meets the above requirements and can easily be integrated into other seeding systems. Here we review, based on the literature, and discuss the feasibility and application of this low pressure system to promote tissue regeneration.**

Keywords: Cell seeding, low pressure system, porous scaffold, tissue engineering

1. Application of low pressure cell seeding system in tissue engineering

Three-dimensional (3D) porous scaffolds are widely used in tissue engineering. With the development of material science and engineering, these 3D scaffolds greatly improved the efficiency of tissue regeneration of bone, cartilage, nerve, skin, *etc.* (*1-4*), especially when they are made from biodegradable biomaterials such as β-tricalcium phosphate (β-TCP), collagen, gelatin, fibrin, poly(glycolic acid), and poly(lactic acid). The porosity of the scaffolds enhances conductivity for the cells and the culture medium containing growth factors, promotes the proliferation and distribution of cells through the connected pores of the scaffolds, and accelerates the degradation of biomaterials. The results of experiments using porous 3D scaffolds have shown great superiority over solid scaffolds, in which the cells could be cultured only on the surface. However, it has been found that there are not as many cells in the inner parts of the scaffolds as anticipated, and the regenerated tissue only presents in the superficial layer of the scaffolds. In addition, cell distribution throughout the scaffolds is far from uniform, and the center areas of the scaffolds are often found to contain few cells (*5,6*). How to improve cell seeding and how to make it efficient and uniform, especially in the inner parts of porous scaffolds, has been one of the key challenges in using porous scaffolds for tissue engineering.

Current 3D porous scaffold seeding techniques include static seeding, centrifugation seeding, perfusion seeding, rotary seeding and combinations of these techniques (*5-9*). Static seeding is most frequently used because of its simplicity and the low requirement for equipments other than a pipette. However, the efficiency of static seeding is always low even with an excellent biocompatible scaffold and big pores as is shown in Figure 1. Dai *et al*. (*10*) reported that when

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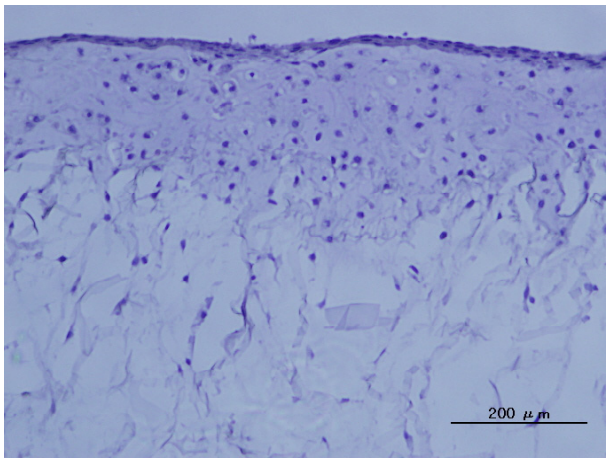

Figure 1. HE staining of bovine articular chondrocytes seeded on the porous collagen scaffold, cultured for 2 weeks. Cells grow only on the superficial layer of the scaffold.

combining human bone marrow mesenchymal stem cells with 75% porous β-TCP for bone regeneration, the cell distribution in the TCP center checked by scanning electron microscopy was very low, and that results were even worse when the size of the TCP blocks was over 5 mm. Figallo *et al*. (*11*) obtained similar results when seeding human fibroblasts onto micropatterned hyaluronic acid 3D scaffold. These findings have prompted the development of other new methods for cell seeding.

In centrifugation seeding, a moderate centrifugal force is applied during the seeding process. A result was reported by Dar *et al*. of a rather uniform distribution of cardiomyocytes throughout 3D alginate scaffolds during cardiac tissue engineering, with a volume cell density above 60% (*12*). Mironov *et al*. approached the maximum possible volume density (65.6%) based on theoretical sphere packing models using an *in situ* cross-linkable hyaluronan (HA)-based synthetic extracellular matrix (sECM) (*13*). These results were encouraging. Nevertheless, there was a very important issue to be resolved. That is, how do cells survive and function normally after centrifugation? Is centrifugation detrimental to cells and could this method be applied to other materials, including inflexible ones? During centrifugation, the orientation of the scaffold is always difficult to control especially because when the scaffold pieces are small they tend to stack together and overlap with each other. This does not allow a satisfactory distribution of homogeneous cells.

In perfusion seeding, a continuous cell suspension perfusion is applied through 3D scaffold pores using bioreactors to assist in cell infiltration and to aid in nutrition. Although significant improvements have been achieved in seeding efficiency, uniformity, and viability (*14,15*), the use of a bioreactor always involves cumbersome equipment and the scaffolds usually need a specific design to match the bioreactor, which is troublesome in most cases.

What hinders cell penetration into scaffolds? Factors vary in different studies such as pore size and interconnectivity of the scaffold, cell density of the suspension, and biocompatibility between cell and scaffold. However, when 3D porous biomaterials are used, one very important thing that prevents cell penetration is the presence of air in the pores of the scaffold, which can explain the reason why static seeding cannot yield satisfactory results no matter what scaffold or cell line is used. In addition, the surface tension produced at the air/culture medium interface also keeps the cells from easily infiltrating into the inner parts. In centrifugation or bioreactor perfusion seeding, most of the air in the pores is drawn out and enables entry of the cell suspension; any possible surface tension eliminated at the same time also contributes to the promotion of seeding efficiency. Therefore, the most important issue is to discover how we can manage to do this without requiring the use of complex equipment, and how we can apply the method to most scaffolds and cell lines. Presumably a low-pressure method will help to achieve this goal.

Low-pressure has been frequently used in tissue engineering, usually for the degassing of scaffolds before further treating such as with bio-coatings. This method had also started to be used in cell seeding. As was described by several researchers (*16,19*), a hypothetical low-pressure cell seeding system could simply consist of a vacuum pump with a controller and a vacuum desiccator. The 3D porous scaffolds and the cell suspension are mixed into dishes, and the dishes are put into sterilized vacuum desiccators immediately. Then low pressure is produced by the pump to draw the air in the materials out by pressure difference, and to eliminate any surface tension produced by the air/culture medium interface, as is illustrated in Figure 2. This method enhanced cell seeding and infiltration in our research using bovine articular chondrocytes and a porous PLGA/collagen hybrid scaffold for cartilage

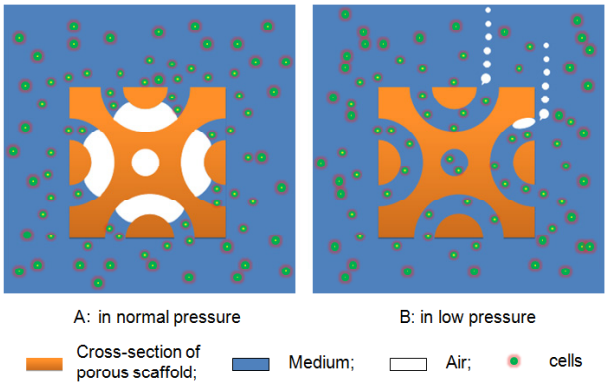

Figure 2. Schematic diagram of cell-seeding on 3D porous scaffolds in normal and low pressure conditions.

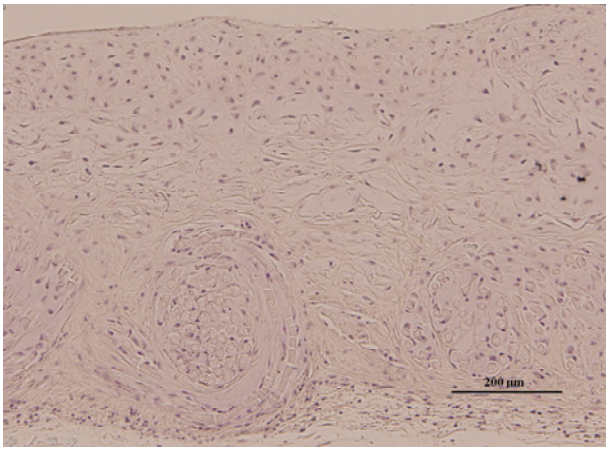

Figure 3. HE staining of bovine articular chondrocytes seeded on the porous PLGA/collagen hybrid scaffold, cultured for 2 weeks. Homogenous cells distribution was achieved throughout the scaffold.

tissue engineering, as is shown in Figure 3. The overall processing time is quite short, and the cell/scaffold composites could be moved out for further culture or treatment. This method is simple, convenient, and possibly universal for most tissue engineering research.

In 2001, Dong *et al*. (*16*) developed this low pressure seeding system, examined the relationship between pressure and cell seeding efficiency, and revealed that maximum cell seeding was achieved under a pressure of 100 mmHg. The long term *in vivo* effect of this method was also observed. MSCs/porous HA composites built with a pressure of 100 mmHg were transplanted into subcutaneous sites of rats and harvested for histological examination for 26 weeks. New bone formation was greatly promoted compared to composites built under normal atmospheric pressure (*16*,*17*). Torigoe *et al*. (*18*) in 2007 modified this system for bone regeneration and also obtained positive results compared to conventional seeding methods. Moreover, they examined the effect of various low-pressure conditions (50-760 mmHg) and various processing periods (1-10 min) on the proliferative and osteogenic capabilities of bone marrow-derived rat stromal cells. Interestingly, the optimal pressure of these two experiments was different, which might be due to the different pore diameters and the overall size of the scaffolds used. In 2008, Lin *et al*. (*19*) applied this method when co-culturing vascular endothelial cells with mesenchymal stem cells on porous β-TCP to promote vascularization of bone tissue engineering. They found many more new capillary vessels formed in the center areas and osteogenesis was enhanced at the same time, which indicated that low-pressure could be a fit for vascular endothelial cells and may improve angiogenesis of tissue engineering.

Low pressure cell seeding can also be integrated into other seeding systems such as perfusion, centrifugation or bioreactor systems, to better promote seeding efficiency. Wang *et al*. (*20*) reported in 2006 that low pressure seeding of bone marrow stromal cells on β-TCP, together with medium perfusion can produce more uniform and extensive new bone formation in bone tissue engineering. Combinations of different seeding methods and utilization of other techniques to facilitate cell seeding such as surface modification of scaffolds could be a principle strategy in tissue engineering in the future (*21*-*23*).

On the other hand, in spite of the recent advances, there are still some important issues left to be investigated further. First, the fate of the cells after treatment with low pressure should be followed, especially the long term effects on cell differentiation or de-differentiation, and cell function. Second, the safety issue is also critical. Cell viability after low pressure treatment, and possible genetic mutation and carcinogenesis should be addressed. Third, the problem of how to combine low pressure with other methods more effectively also involves further understanding of the seeding mechanisms and elaborate designs of these systems.

2. Conclusions

An ideal method for cell seeding should not only yield efficient and uniform cell distribution throughout the scaffold but also should not impair cells. If such a method does not need complicated equipment, is easy to carry out, is universal for all kinds of scaffolds and cell lines, and can be integrated with other methods, it will surely enhance tissue engineering and promote the efficiency of regenerative medicine.

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