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Commentary

Insights into *in vitro* environments for human cartilage tissue engineering

Novel methodologies addressing the damages to human cartilage tissues using *in vitro* tissue-engineered autologous grafts have progressed to a clinical application in select conditions. However, still several challenges remain requiring significant improvisation. The *in vitro* expansion of chondrocytes, though is a challenging process owing to their limited proliferation capacity, slow doubling time and a higher extracellular matrix to cell ratio[1,2], compared to other tissues such as neurons or cardiomyocytes, it is relatively simpler to grow and transplant them for a successful engraftment into the defective area, as these do not require activation by electrical signals or membrane potentials to make them functional. In this issue, Balakumar *et al*[3] have used human autologous bone marrow cell-free extract (BME) to culture human iliac apophyseal chondrocytes, in which maintenance of hyaline phenotype in the BME supplemented cultures for nearly 21 days was demonstrated in contrast to the cultures using foetal bovine serum (FBS) which could not maintain the hyaline phenotype[3]. Although the authors have utilized only human iliac apophyseal chondrocytes, which are physiologically capable of *in vivo* proliferation until puberty[4], but they have also included the references pertaining to adult articular-derived chondrocytes in their discussions and while deriving conclusions.

Expansion of chondrocytes in culture requires several inputs. One amongst them is the culture medium which provides nutrition to the cells and forms the main focus of the study by Balakumar *et al*[3]. The other two important inputs are scaffolds and physical forces and stimuli. Different kinds of extracellular matrices, both natural and synthetic scaffolds, have been used in the *in vitro* cartilage regeneration. Hydrogel polymers custom tailored to suit the cartilage providing optimal tissue delivery have been employed earlier[5,6], which combine the effects produced by chemical moieties with those produced by physical forces of an orbital shaker, to yield stable three-dimensional (3D) hyaline cartilage tissue *in vitro*. Physical forces such as dynamic compression and the associated fluid flow-induced shear, tissue shear and hydrostatic pressure[7] have also been utilized for cartilage tissue engineering. Several groups have employed various methodologies to introduce dynamic compression during *in vitro* cell culture, and the results have been significant in giving rise to tissues which mimic the native cartilage[8,9]. The effects of fluid flow-induced shear stress on cartilage tissue engineering have been investigated using bioreactors systems such as spinner flasks and perfusion culture systems[10]. In addition, electrical stimuli have also been used to stimulate protein synthesis in cartilage explant cultures[11].

After successful *in vitro* expansion of chondrocytes using appropriate culture media, stimuli and scaffolds, the laboratory grown tissue has to be transplanted clinically in place of a defective, dysfunctional or damaged cartilage tissue, which is the ultimate goal. A review of clinical results of such studies reveals that in spite of various methods employed to *in vitro* culture, post *in vivo* transplantation, the transplanted cartilage in articular cartilage injuries reverts to a fibrocartilage, and this is a major impediment in providing a long-term disease-free state in patients undergoing even established procedures such as the autologous chondrocyte implantation[12,13]. In studies which were able to maintain hyaline phenotype *in vitro* using several other cartilage regeneration techniques and employing cells other than chondrocytes such as mesenchymal stem cells, the end result was the development of a relatively higher fibrocartilaginous tissue in the defect rather than a hyaline tissue, after *in vivo* transplantation[13].

The present study by Balakumar *et al*[3] has shown that it is possible to maintain the hyaline phenotype of the cartilage tissue *in vitro* for nearly 21 days with BME supplementation. However, the ultimate goal of

tissue engineering can be achieved only when *in vivo* transplantation of the hyaline cartilages developed *in vitro*, using methodologies such as the one described by Balakumar *et al*[3], are attempted and the hyaline cartilage phenotype is found to be maintained *in vivo* for a long period of time even after being subjected to various physical forces, and finally be able to yield a disease-free status. Before a clinical translation is planned on lines of the work of Balakumar *et al*[3], the following major points need further consideration:

(*i*). The authors have used chondrocytes from children undergoing hip surgery for dysplasia of hips, and the implications of the results on studying chondrocytes obtained from dysplastic joints may not hold valid for healthy chondrocytes, and this point needs to be analyzed by future studies on healthy joint chondrocytes.

(*ii*). The number of samples studied by Balakumar *et al*[3] is only four, and the age group from which the samples were derived was between two and nine years. More samples from donors of different age groups need to be studied to add value to the clinical relevance of the study.

(*iii*). Cartilage damage is a component that occurs as a varied spectrum across the age group of patients, manifesting as sports injury in the young, degenerative in the elderly, *etc*. Therefore, when similar applications are considered in a varied age group of patients, how far their bone marrow extract will supplement the *in vitro* growth of cells is a major question because bone marrow stem cells have shown to deplete with ageing[14].

(*iv*). The bone marrow is a multifunctional tissue producing millions of cells on a day-to-day basis, with several of the bone marrow produced cells reaching the target by circulation, resulting in damage repair along with actively performing their function of replenishment of blood cells. Therefore, the principle behind breaking of the bone marrow barrier to utilize its extract to grow cartilage cells (which actually are not directly supplied by vascular supply) and this effect of vascular or avascular components of the BME in producing nutrition to chondrocytes must be thoroughly studied. The reason for taking the bone marrow for a different high-value component or protocol such as the lifesaving haematopoietic stem cell transplantation in comparison to *in vitro* culture of chondrocytes should be justified. The clinical implications of depriving the bone marrow and necessary clinical procedure-related complications pertaining to the procedure described by Balakumar *et al*[3] to obtain BME also needs consideration.

(*v*). A thought process on comparing how the effect produced by such a high-value BME can, in fact, be provided using scaffolds[5,6,15], physical forces,[5,7] *etc.*, for maintaining the hyaline cartilage phenotype has to be considered. In addition to evaluation of BME, effects of alternatives to FBS such as human platelet lysate, insulin transferrin selenium, human serum, serum-free defined medium and others need to be evaluated, or the comparative data from the literature have to be analyzed to justify the advantages of BME.

With 3D bioprinting revolutionizing the field of regenerative medicine, bioprinted cartilage has already become a reality[16] and engineering of complex organs such as lung and heart would be possible using decellularized extracellular matrix scaffolds[17]. Factory made cartilages will not be a distant dream in the path to create such ready-to-use cartilage tissues. Emphasis should be laid on the *in vitro* tissue culture conditions including the use of right culture methodologies, culture media and growth factors as has been attempted by Balakumar *et al*[3]. Furthermore, excluding FBS will provide animal protein-free culture methodologies which will be highly useful in clinical translation of the methodologies. Hence, efforts like this need to be encouraged for achieving stable, ready-made and custom-tailored cartilage tissues which would be a boon to address a spectrum of cartilage-related disorders in future.

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