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Embryonic Versus Mesenchymal Stem Cells in Cartilage Repair

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Abstract

As our population changes osteoarthritis and cartilage defects are becoming more prevalent. The discovery of stems cells and their ability for indefinite regeneration has revolutionised the way cartilage problems are viewed. Tissue engineering has been shown to be the ideal way of repairing articular cartilage lesions, i.e. back to native tissue. The two main types of stem cells being investigated in chondrogenesis are embryological and mesenchymal stem cells. Research into embryological stem cells has been surrounded by controversy because of tumour formation and damaging embryos during the harvest of cells. We discuss the use of embryological and mesenchymal stem cells in cartilage repair and the various factors involved in the differentiation into chondrocytes.

Stem cells are cells with the ability to renew themselves over long periods of time. They are present in all multicellular organisms, and also have the ability to differentiate into many cell types. There are two main types of stem cells, embryonic and adult stem cells. Embryonic stem cells, (ESCs), are derived from four main sources, cloned embryos, unused in vitro fertilised stem cells, aborted / miscarried embryos or existing stem cell lines. Adult stem cells are present to maintain homeostasis of a certain group of cells, for example haematopoietic stem cells forming red blood cells.

In 1963 Becker et al, [1], published a paper in Nature demonstrating self-renewing cells in mouse bone marrow. Since then stem cell research has taken on many different fields and had problems with ethical controversy. Apart from a distinction between embryonic and adult stem cells, they can be divided into their differentiation potential or potency, (figure 1). Omni/Totipotent can differentiate into embryonic and extra-embryonic stem cells, produced by fusion of an ovum and sperm; Pluripotent cells can differentiate into any of the three germ layers,

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(endoderm, ectoderm and mesoderm), and are produced from totipotent cells; Multipotent cells can differentiate into a certain tree of cells, an example being haematopoietic stem cells or Mesenchymal stem cells; Oligopotent cells can only differentiate into a few cell types, such as lymphoid stem cells; Finally unipotent stem cells can only differentiate into one cell type, but unlike normal cells have the ability of self-regeneration.

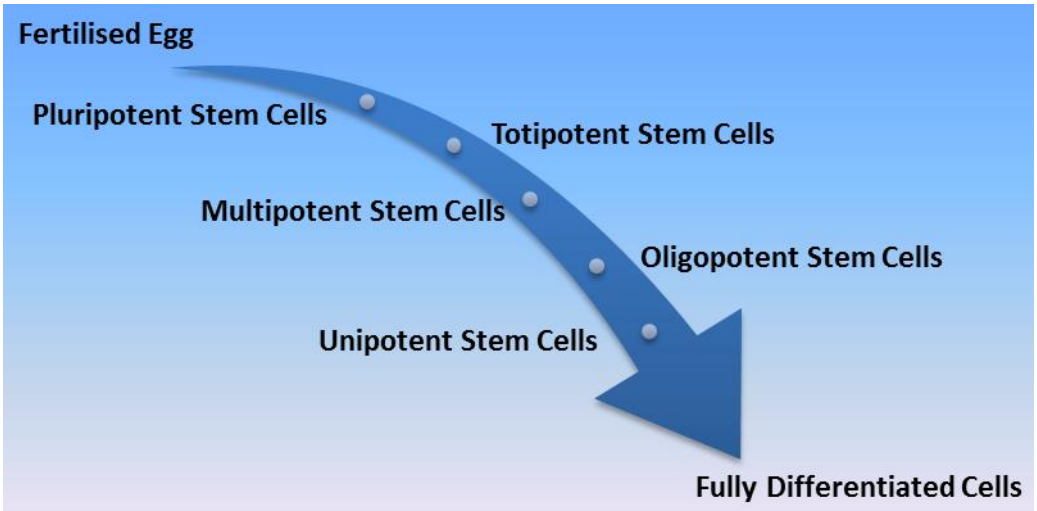

Figure 1. The Potency of Stem Cells.

The concept of stem cell niches was introduced in 1978 by Schofield, and describes the environment immediately surrounding stem cells in their naïve state. [2] The niche involves not only the stem cells but the non-stem cells and extracellular matrix which are in direct contact. This surrounding microsystem of cells and soluble molecules keeps the stem cells undifferentiated until they are required for regeneration.

This review will discuss the use of stem cells in cartilage repair and how this repair process can be enhanced. Most of the current work is on animal models and therefore the review of clinical studies will be concise. The use of embryonic stem cells will be briefly discussed but since mesenchymal stem cells have mainly been studied in the literature, this will be the main focus of this paper.

Embryonic Stem Cells

ESCs are gathered from the inner cell mass of a blastocyst and are pluripotent. This means they are theoretically able to form any cell in the body. Their tissue engineering potential is endless, but usage and study has been halted by ethical and safety concerns. The use of ESCs for chondrogenesis is not as common as mesenchymal stem cells, (MSCs). [3]

There are several reasons why ESCs have not been fully investigated in cartilage tissue engineering. The major concern is of tumour formation, but there are also ethical controveries regarding the use of pluripotent stem cells. In 2003 ESCs were injected into a mouse knee joint, which subsequently formed a teratoma and destruction of the entire joint. [4] There has been no further advancement in embryonic stem cell research which has led to the lack of its use in clinical research.

The controversy surrounding the use and culture of ESCs is linked to their source, the blastocyst. This is formed in early embryogenesis after the morula and eventually will form the embryo. Subsequent harvesting of ESCs can be damaging to the blastocyst and therefore the embryo and effectively a human life, (if they are human ESCs). It has recently been discovered that several transcription factors, oct-4, sox-2, klf4, c-myc are important in stem cell differentiation and induction of pluripotency. [5] These factors may play an important role in eliminating the need for ESC sources as differentiated cells can be induced into pluripotent cells using these factors. [6] Another recent discovery is the ability to

form an ESC line from a single cell from a blastocyst without damaging embryo development. [7]

Oldershaw et al [8] have reported on a chemically defined, efficient, scalable and reproducible protocol for differentiation of human ESCs toward chondrocytes that should facilitate studies of chondrocyte differentiation and of cell replacement therapies for cartilage repair. These developments are steps in the right direction but, until the ethical and tumorgenic complications can be resolved, further study must take place before chondrogenic applications. MSCs do not suffer from the ethical problems as their source can be from adults and even the target treatment patient. Some studies have shown MSCs to be tumour inducing in certain situations and others have shown them to be tumour suppressive. [9]

Mesenchymal Stem cells

MSCs are multipotent cells with the ability to differentiate into: chondrocytes forming cartilage, osteoblasts forming bone, adipocytes forming fat cells, myocytes forming muscle cells, fibroblasts forming skin or tendons and ligaments, astrocytes forming neural cells and stromal cells forming bone marrow. [10-15] There are several sources from which MSCs can be isolated from; Bone marrow, adipose tissue, skeletal muscle, synovium, umbilical cord, amniotic fluid and peripheral blood in small amounts. [16-19] MSC renewal is the cells ability to preserve the undifferentiated stem state. This process is controlled by extracellular signalling mainly by cytokines and growth factors; leukaemia inhibitory factor, fibroblast growth factor, epidermal growth factor, platelet derived growth factor all play a role. [20,21].

Treatment of articular cartilage defects has an important role for MSCs. Focal full thickness articular defects in the knee are commonly treated by marrow stimulation treatment techniques, such as abrasion arthroplasty, microdrilling or microfracture. These procedures populate the articular defect with pluripotential mesenchymal stem cells that form fibrous cartilage and have demonstrated mixed early results and only fair to poor long-term results. [22] Currently autologous chondrocyte implantation, (ACI), is limited by the poor proliferation capacity of the chondrocytes and the possibility of dedifferentiation once implanted. [22] Although the cartilage produced is hyaline-like cartilage that more closely recreates the wear characteristics and durability of normal hyaline cartilage than the fibrous or fibrocartilage repair tissue formed by the earlier described marrow stimulation procedures, it does have disadvantages. ACI is a two stage procedure with an initial arthroscopy required for cell harvest and second procedure involving an arthrotomy and implantation.

MSCs have multiple sources and are easily amplified making them a superior alternative. In recent studies it has been shown that bone marrow derived MSCs, (BMMSCs), have more chondrogenic potential than adipose derived MSCs, (AMSCs), and are as effective as chondrocytes in the treatment of articular cartilage defects. Huang et al, [23], in 2005 directly compared BMMSCs and AMSCs and found up to 5000 fold higher expression of type II collagen and 120 per cent more proteoglycan deposition from BMMSCs, $p<0.05$. Im et al, [24], performed a similar analysis but compared the histological scores. They found scores of 6.5 for BMMSCs and 4.3 for AMSCs ($p=0.023$), showing that BMMSCs have greater chondrogenic potential. More recently Vidal et al [25] have demonstrated the superior chondrogenic potential of BMMSCs over AMSCs using eleven horses. Not only was the type II collagen synthesized earlier but a hyaline matrix developed earlier in the BMMSC group. Nejadnik et al [26] in 2010 compared ACI using chondrocytes and BMMSCs in a cohort study. They found some improvement in the BMMSC group over the chondrocyte group especially in physical role functioning (using the SF-36 health questionnaire). Furthermore the chondrocyte group patients aged less than 45 scored significantly better than those older than 45, and this age related effect was not seen in the BMMSC group. There are some concerns over the use of BMMSCs as seen by Peltarri et al in 2006, [27]. They demonstrated endochondral calcification in the implanted chondrocytes from BMMSCs, resulting in bone formation and ultimately cartilage loss.

The differentiation of MSCs to final cell type is influenced by intrinsic and extrinsic factors. Autocrine regulation or intrinsic factors are signals from the cell itself, and paracrine regulation or

extrinsic factors are cuing from the surrounding niche cells. [28,29] The niche is thought to be located perivascularily allowing for easy migration. [30] This signalling process dictates the development and the position of the cells and implies migratory capacity of MSCs. MSCs have been shown to migrate to sites of injury, following systemic infusion in animal models. [31-33] It has also been shown that MSCs have retarded osteoarthritic progression and cartilage destruction in a caprine model. [34]

The regulation of the signalling processes allowing migration of MSCs to injured tissues is not fully understood. Chemokines are extrinsic factors which have an important homeostatic function in MSC migration. [35] Stromal cell derived factor-1, (SDF-1), mediates MSC movement and is regulated by its receptor, chemokine receptor type 4, (CXCR4). [36,37].

Though chemokines are involved in stem cell migration, growth factors are thought to control MSC differentiation into its multiple cells types. Bone morphogenic proteins, (BMPs), transforming growth factors, (TGFs), growth and differentiation factor, (GDF), are important in the formation of new cartilage and bone throughout the body and many different types have been discovered. *In vitro* studies have demonstrated that BMP-2/4/6, TGF-β1/β3, GDF-5 induce rapid chondrogenesis in MSCs and this has been confirmed by several *in vivo* studies. [38-42] Insulin-like growth factors, (IGFs), have also been studied to enhance cartilage repair with stem cells. Its structure is similar to insulin and it can also stimulate cell growth. In cartilage homeostasis, it regulates proteoglycan synthesis and the extracellular matrix degradation thereby maintaining the overall structure. [43] It has been shown to increase both MSC chondrogenesis and collagen type II content, when compared to TGF-β3 alone. [44,45] There are only limited studies on IGF due to the experimental stage of its discovery in this process. Finally fibroblast growth factor, (FGF), and platelet rich plasma, (PRP), have also been investigated in MSC chondrogenesis. FGF-2 has been investigated as an addition to other growth factors in the expansion phase and has shown to increase the chondrogenic potential of MSCs. PRP is a mixture of growth factors and cytokines which is spun down from samples of venous blood. Therefore the main constitutes of PRP are actually TGF and IGF. These can increase chondrogenesis separately, but PRP has been shown to increase MSC proliferation, chondrocyte differentiation and increase collagen production. [46,47] There are a few studies comparing growth factors directly with one another, and the ones which do, predominately compare factors from the same family. The importance of these factors in MSC differentiation and chondrogenesis is obvious but the early nature of the studies means that best synergistic combination is yet to be discovered.

MSCs are also known as a colony forming unit-fibroblasts and since their discovery by Friedenstein et al in 1971 there has been a greater understanding of these cells potential. [48,49] In order to isolate MSCs correctly a cell surface antigenic profile is required especially if Mesenchymal Progenitor cells, (MSPCs), are being isolated, which are only unipotent and occasionally omnipotent. To date the best known surface marker is the antigen Stro-1. Stro-1 Positive cells have been shown to differentiate into chondrocytes, osteoblasts, adipocytes, smooth muscle cells. Stro-1 Negative cells do not contain MSCs, thereby confirming Stro-1 as a marker for MSCs. [50,51] However Stro-1 expression is not exclusive to MSCs and when these cells are expanded, stro-1 expression is lost. [52,53]

There have been many other markers identified for MSCs: Vascular cell adhesion molecule-1 (CD106), CD44, CD45, CD73, Endoglin (CD105), CD90/Thy-1 and Sca-1/Ly6. [54,55] from this non-exhaustive list, CD73 and CD106 seem to show the most promise as combined markers with Stro-1 for MSCs. Considerable research is being performed to isolate markers that may identify MSCs/MSPCs that have a better ability for chondrogenesis. Aicher et al, [56], have recently shown that CD146 is a reliable marker for identifying MSCs with a greater osetogenic potential. Currently no such marker has been isolated to identify MSCs with greater chondrogenic potential but research is still ongoing.

Conclusion

Articular cartilage defects are notoriously difficult to treat, mainly due to their poor intrinsic ability for self-repair. Apart from the obvious avascular nature of this tissue the other contributing

factors are the loading forces, (direct and and particularly shear), the articular cartilage in the knee has to endure. There are many different types of treatment options for these defects, but the one which anecdotally seems to show the most promise is tissue engineering.

The most significant scientific discovery for tissue engineering was the discovery of stem cells, and the varied potency, (figure 1). This discovery makes any sort of tissue engineering possible, but the correct processes for harvesting, expanding and implanting cells needs to be delineated. There has already been forging work into ESC culture and new stem cell lines without embryo damage. As these discoveries are still in there early stages they still require more investigation. This leads onto the promise of MSCs in cartilage repair, due to the ready source from the patient themselves.

The early results of clinical studies in the use of MSCs in cartilage repair are encouraging. [57,58] This could also potentially be an arthroscopic procedure, given the right scaffold, and that is attractive prospect the morbidity associated with an arthrotomy is diminished. There has been a huge volume of experimentation into understanding the differentiation process of stem cell into chondrocytes and how this process can be enhanced. The failure to isolate a single factor has led to randomised controlled trials comparing standard cartilage repair techniques with multiple MSC repair techniques.

Research on the use of MSC in cartilage repair is very much in its infancy. The ideal source of MSC is yet to be discovered as well as the best combination of growth factors to enhance chondrogenesis and form stable hyaline-like repair tissue. Although we have concentrated on bone marrow and adipose tissue as potential sources of MSCs, these cells have been found in number of tissues including cartilage [59]; cartilage MSCs, although small in number and difficult to expand may represent the optimum source for cartilage repair. The current evidence points to MSCs as an excellent source for repair of cartilage defects but there is definitely a long way till this becomes the gold standard treatment option.

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