Chapter 13

Combinatorial Approaches in Tissue Engineering: Progenitor Cells, Scaffolds, and Growth Factors

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Summary

Tissue engineering (TE) has the potential of improving the quality of life for many thousands of people throughout the world. One particular area which presents an exciting challenge in this field is the repair and regeneration of cartilage where traumatic injuries and arthritis result in pain and restriction of movement. In order to achieve this goal of tissue engineering cartilage, there are three necessary components: cells for the generation of tissue, a scaffold to support growth and that degrades as the extracellular matrix is generated and a bioactive factor to stimulate the correct biological signals in vivo for tissue growth and integration with native cartilage. Over the last number of years there has been significant interest in progenitor stem cells for TE applications. Mesenchymal stem cells (MSCs) have been shown to differentiate into a range of tissues including bone, cartilage, muscle, tendon, skin and fat. Furthermore, a number of biocompatible biodegradable materials have been identified as carriers for these cells for transplantation. Once transplanted, however, these cell seeded scaffolds require the necessary signals to survive and assimilate in their host environment. Therefore, a number of bioactive factors including growth factors and cytokines are also required for cartilage repair and regeneration. In this chapter, the combinatorial approach of progenitor cells, biodegradable scaffolds and bioactive factors is discussed in relation to cartilage TE. The challenges for the future including cell sources, osteochondral defects and gene therapy are also examined.

Keywords: Biodegradable Biocompatible Scaffolds, Cartilage, Growth Factors, Mesenchymal Stem Cells, Tissue Engineering

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Background

Degenerative diseases represent a critical issue, especially in the aged where it is predicted that the next thirty years could see the population over 60 grow by 50% in the European Union (1). This in turn requires the need for more organs, joints and other body parts which will have to be replaced in order to maintain a reasonable quality of life. However, while the development of synthetic materials and artificial devices has improved the quality of life for many thousands of people, the issue of biocompatibility and foreign body response still remains an issue that has not been fully resolved. TE presents an excellent opportunity to address these issues with the development of the next generation of biomaterials. One such area that seeks to benefit from TE is cartilage. Our hypothesis is that, where cartilage damage is beyond self-repair, progenitor stem cells supported by biodegradable scaffolds and chondrogenic growth factors can sense and respond to their host environment and thereby promote cartilage repair and regeneration.

Essentially, cartilage is composed of chondrocytes embedded in an extracellular matrix composed of collagen and proteoglycans (Fig. 1). During foetal development the human skeleton is mostly cartilaginous in nature. In adult life there are three types of cartilage found in the human body, namely hyaline, white fibrocartilage and yellow elastic fibrocartilage. Hyaline cartilage covers the articulating surface of bones and is also found in the nasal, costal, trachea and bronchial and all other regions with temporary cartilage. White fibrocartilage is composed mainly of collagen and is found in the intervertebral disks, articular disks and the lining of the bony grooves that lodge tendons, while yellow elastic fibrocartilage contains a rich elastin network and is found in the external ear, larynx, epiglottis and the apices of the arytenoids (2).

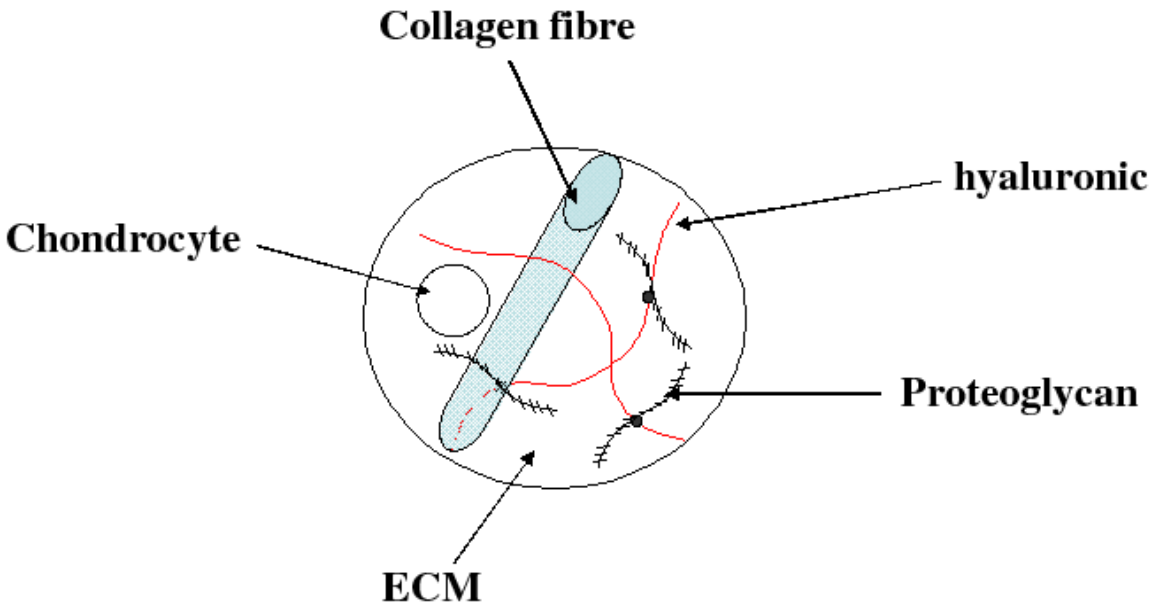

Fig. 1: Schematic diagram illustrating the key components of articular cartilage

As shown in Figure 2, articular cartilage is quite porous and is composed of water, cells, collagen fibres and an ECM. The cells and matrix form a layered structure comprised of the superficial layer, the transitional layer, the radial layer and the calcified layer. In the human body, articular cartilage is exposed to tensile, compressive and shear forces. Under stress, the fluid moves in and out of the tissue and alters the properties with fluid movement. The collagen fibres are strong and stiff in tension, while the proteoglycans are strong in compression. With respect to the mechanical properties of cartilage itself, the compressive stiffness and resistance of cartilage depends on the water and proteoglycan content of the tissue. The greater the percentage of proteoglycan present, the greater is the compressive resistance. The tensile strength of cartilage depends on the percentage of collagen present. As the percentage of collagen increases, the tensile strength of cartilage also increases (3). As people grow older the percentage of collagen increases, and this has an effect on the mechanical properties of cartilage.

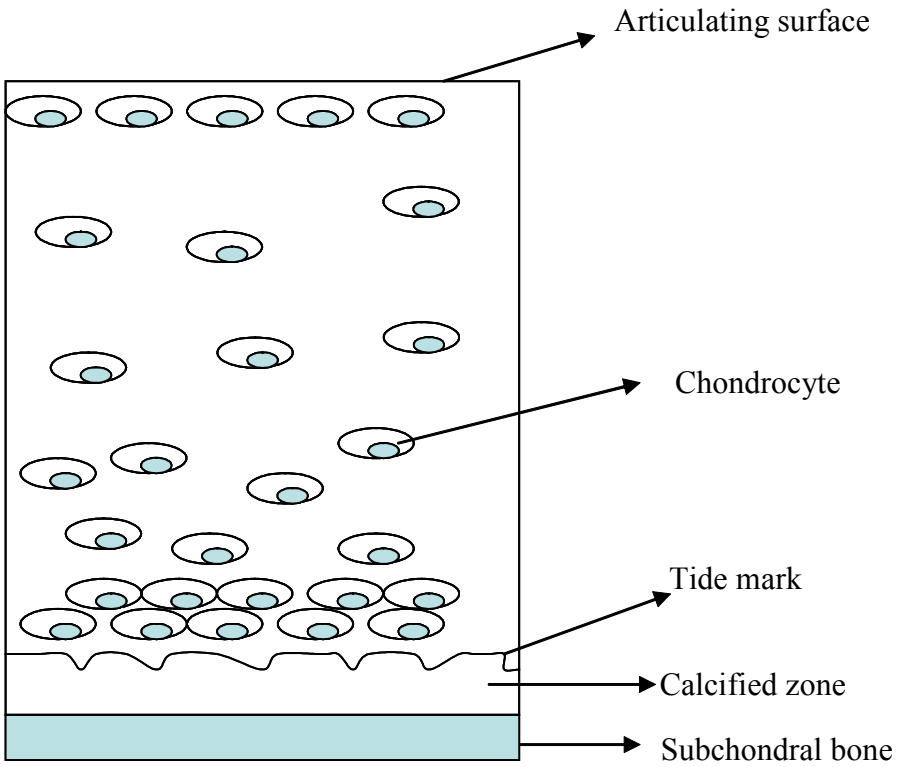

Fig. 2: Cross section of articular cartilage illustrating the location of chondrocytes

The primary function of articular cartilage is to allow ease of movement between articulating bones. This is achieved by the presence of synovial fluid, which reduces the coefficient of friction to the order of 0.001-0.06. Other functions of cartilage include wear resistance and dissipation of mechanical energy to reduce damage upon impact. However, traumatic injuries and degenerative diseases such as rheumatoid arthritis and osteoarthritis can result in cartilage wear, damage, failure and hence reduction in joint movement and load – bearing ability. Due to the fact that cartilage is avascular, once damaged it is very difficult to repair. Current methods of articular cartilage repair include joint resurfacing techniques, such as microfracturing, drilling, scraping, shaving and autologous grafting (4, 5). In autologous grafting, adult chondrocytes are isolated, expanded *in vitro* and transplanted into the required area using a scaffold or as cell suspension. Once such method of grafting is called autologous cartilage transplantation (ACT), where chondrocytes are injected in a cell suspension to the cartilage defect and a progenitor rich periosteal flap is sutured over the affected area (4-6). However, to date there has been very little success in the area of articular cartilage repair. Only in cases where damage to cartilage is accompanied by damage to the

subchondral bone does the formation of small fibrous tissue repair occur (5). However, this thin tissue layer is fibrous and does not have the same mechanical properties as native hyaline cartilage.

One method of addressing this problem of cartilage repair and regeneration involves TE, where the prospect of developing patient specific cartilage without adverse foreign body reactions is possible. The critical parameters required for such a system are illustrated in Fig. 3 and a combinatorial approach employing cells, scaffolds and growth factors is proposed. To create cartilage tissue *ex vivo,* it is necessary to attract cells to a scaffold and for these cells to multiply, differentiate and organise to form normal healthy cartilage as scaffold degrades *in vivo*. Therefore, we believe that the identification of the ideal cells, scaffolds and growth factors are crucial in the development of tissue engineered cartilage.

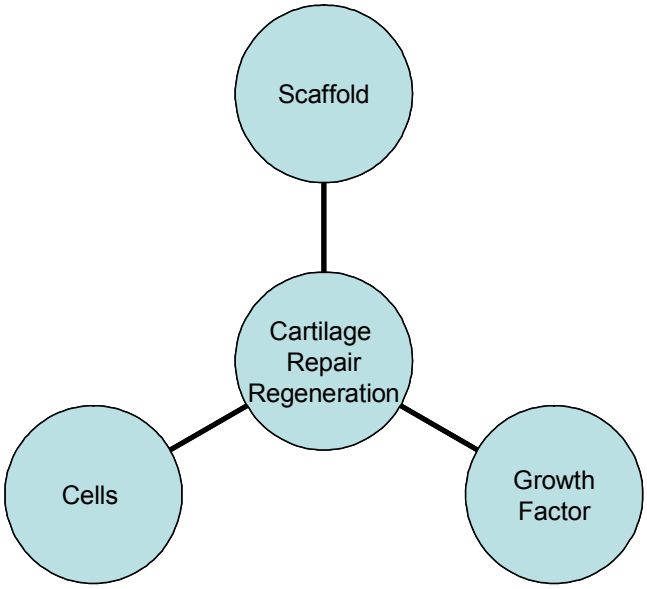

Fig. 3: Parameters required for cartilage repair regeneration using a combinatorial approach

Cells

In order to generate tissue engineered cartilage, the cells chosen will have to be able to respond to their environment, differentiate, form new tissue and integrate with native tissue. To develop a tissue suitable for transplantation, it is necessary to isolate healthy cells, expand these cells *ex vivo* using cell culture methods, incorporate them into three dimensional scaffolds and introduce them to growth factors (Fig. 4).

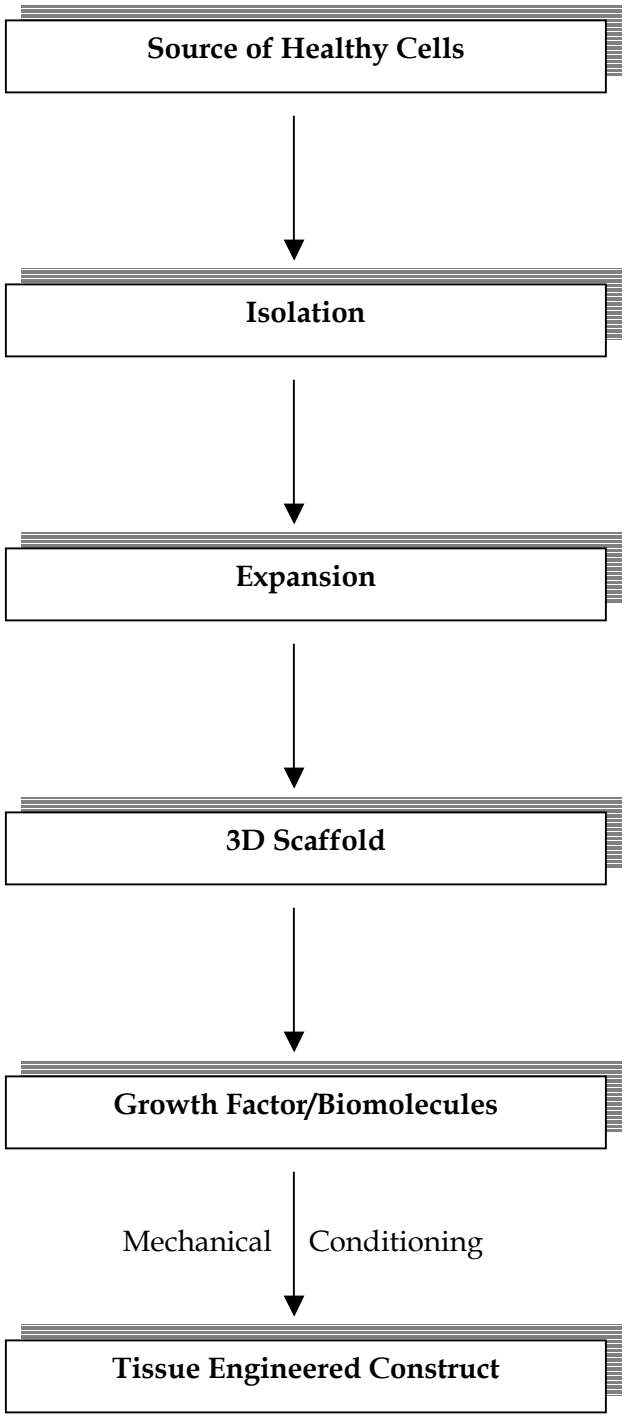

Fig. 4: Proposed route to obtaining a tissue engineered construct

Autologous chondrocytes have been isolated from healthy articular cartilage sites using tissue biopsy methods (5). While redifferentiation *in vitro* is possible, it is problematic and requires transforming growth factors such as TGFβ1 to induce chondrogenesis (4). However, success to date has been limited due to low numbers of cells available and the restricted ability of these cells to proliferate, differentiate and regenerate tissue. Cells from non- articulating surfaces such as nasal and rib cartilage have also been examined. Nevertheless, more research is required to determine if these cells are suitable for articular cartilage applications (4).

Over the last decade there has been significant interest in progenitor cells such as pluripotential embryonic stem cells and multipotent adult stem cells to regenerate or repair damaged tissues (2, 4, 5, 7-22). Embryonic cells from human fetal tissue have the capacity to differentiate into any tissue or organ (5, 14, 23-34). However, the practical use of embryonic stem cells is restricted due to ethical and political considerations. Adult stem cells such as mesenchymal stem cells (MSC) are less problematic in terms of ethical issues and have been shown to differentiate into a variety of mesenchymal tissue (Fig. 5) including, bone, cartilage, muscle, tendon, fat and connective tissue, thereby making them very attractive for tissue engineering applications (16). These progenitor cells can be found in bone marrow, adipose tissue and muscle and are known to decrease in frequency with age. At present it is difficult to quantify exact numbers or specify exact locations for stem cells and their descendants since all the surface markers for these cells have not been completely identified (8). Risbud *et al.* (16) reported that the frequency of MSCs in bone marrow varies between $1:10^4$ and $1:10^6$. MSCs can be isolated from bone marrow and expanded to large numbers in culture, which makes them very attractive for TE applications. Over the last number of years, surface markers for the isolation and characterization of MSC have been developed, thereby allowing the use of MSCs in TE.

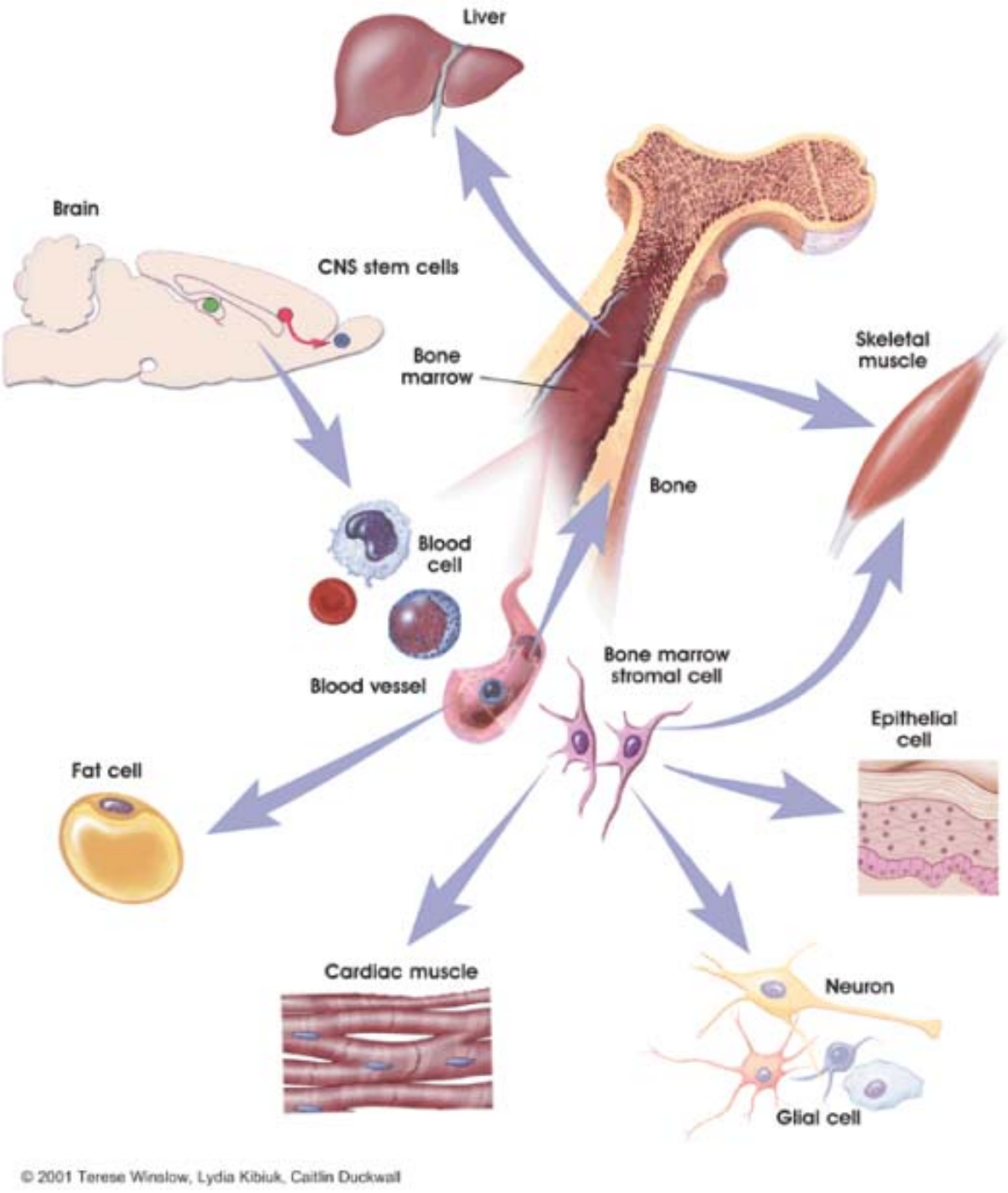

Fig. 5: Diagram illustrating MSC differentiation for a range of tissues including cartilage, bone, brain, fat, liver and muscle. (Reproduced with permission from NIH http://stemcells.nih.gov/infoCenter/stemCellBasics.asp).

The importance of MSCs for TE applications lies in their potential to be cultured *in vitro*, to proliferate and to possess specific cell surface marker proteins, adhesion molecules, cytokines and growth factors for differentiation (5). Nevertheless, there are still many questions that remain unanswered with regards to cell signalling mechanisms before a true understanding of the differentiation and repair mechanisms is achieved. To repair and regenerate cartilage, however, differentiation of MSCs into chondrocytes is necessary. To achieve this goal, MSCs have to respond to appropriate cell signals and bioactive molecules such as phenotype specific growth factors. Additionally, these cells have to be delivered to the specific area of interest without adversely affecting the cell signalling mechanism and differentiation. Once implanted these cells have to be able to respond to their environment and generate the required extracellular matrix. Herein lies one of the foremost challenges of producing tissue engineered cartilage with the same mechanical and physical properties of native cartilage from progenitor MSC cells.

Scaffolds

The primary function of the scaffold in TE is to provide a template to introduce the progenitor MSCs to the specific site of interest and to provide interim mechanical stability for tissue growth and integration. Additional requirements of the scaffold include the following:

- Provide a space to host cells – a three-dimensional (3D) structure is required
- Has the ability to transfer nutrients to the cells and remove their waste without adverse effects on the cells
- Should be biodegradable – at a rate comparable to extracellular matrix production
- Should be biocompatible
- Should be non-toxic
- Should be easy to manufacture

Over the last decade there has been significant interest in biocompatible biodegradable scaffold materials including synthetic biodegradable polymers such as polyglycolic acid (PGA), polylactic acid (PLA), copolymers of PLA and PGA (PLGA), polycaprolactone (4, 35-39) and natural polymer gels such as hyaluronic acid (HA), fibrin, alginate, collagen and agarose hydrogels (6, 9, 40-45).

In addition to the various polymers used as scaffold materials, there are also many different shapes, sizes and structural forms of 3D scaffolds including meshes, foams, fibres and sponges. However, the greatest challenge in the area of scaffold design and development lies in the areas of optimal pore size design and phenotype control. One approach which avoids the issue of immune response and examines the possibility of phenotype control involves the use of bio-based bio-mimetic scaffold materials. By incorporating bioactive molecules such as cytokines and growth factors into the scaffold material, it may be possible to assist chondrocyte differentiation and hence tissue growth and integration with native cartilage tissue. At present the authors are investigating the following method of scaffold design which involves the three-fold approach:
i) Biological Domain: to use biological substrates and factors that native cartilage tissue can sense and respond without an adverse immune response
ii) Biostructural Domain: to recreate mechanical properties that are intrinsic to the body, thereby removing any concerns of property mismatch in the damaged region.
iii) Bioarchitectural Domain: to create an architectural pattern where cells can recognise and respond to the appropriate signals for successful tissue growth and integration.

Growth Factors

Phenotype

As mentioned previously, MSC cells can differentiate into a range of mesenchymal tissue. For cartilage repair and regeneration, MSC progenitor cells have to differentiate into chondrocytes for the correct ECM generation. To ensure chondrogenic differentiation, the MSC cells will have to be able to attract and respond to the correct biological signals for tissue regeneration and repair (46).

Bioactive factors that regulate chondrocytes and cartilage development include transforming growth factor-beta 1 (TGF-1), insulin like growth factor-1 (IGF-1), growth and differentiation factor-5 (GDF-5), bone morphogenetic protein-2 (BMP-2), integrins and the receptors for these molecules (6). These bioactive factors can be delivered to the required specific site by a number of methods including the incorporation of these molecules into a scaffold for controlled release, by injection to the exact site or by the use of gene therapy.

In the area of bone tissue engineering bioactive factors such as bone morphogenetic proteins (BMP) have been shown to enhance osteogenesis (47-50). Bioactive factors also have an important role to play in the repair and regeneration of cartilage. The ultimate aim of this combinatorial approach is to promote articular cartilage repair or regeneration by the incorporation of MSC's and specific cell signalling molecules in a support matrix. By incorporating specific proteins into the scaffold matrix it is hoped to recruit *in situ* MSCs or stimulate implanted MSC's to promote chondrogenesis and encourage tissue growth and integration without an adverse immuno response. A more detailed discussion on the role of differentiation and growth factors is outlined by French *et al* in a previous chapter.

Vascularization

In addition, chondrocytes also require oxygen and nutrients to survive. Although cartilage is avascular, blood vessels are required to keep cells alive and to advance tissue formation. To promote angiogenesis, bioactive factors such as fibroblast growth factor will have to be available for correct and fast-acting vascularization of the transplant (4), either through incorporation into the scaffold for controlled release, injection or through the use of novel gene vectors (51).

Mechanical Conditioning

One of the most important requirements of tissue engineered cartilage is the formation and integration of neo-tissue with mechanical properties similar to native cartilage. In recent years it has become increasingly apparent that biomechanical and biochemical stimulation of tissue engineered constructs is very important for tissue growth (44, 52-69). At present much attention is focussed on

the use of bioreactors to improve the mechanical and chemical properties of cartilage by mechanically conditioning cartilage constructs in a simulated physiological cartilage environment.

Challenges for the Future

TE of cartilage shows significant promise for many patients suffering from osteoarthritis, rheumatoid arthritis and damaged cartilage. However, at the present time, there are many challenges that need to be overcome before it becomes routine in clinical practice. One of the challenges in the area is cell source. MSCs can be isolated from bone marrow, and more recently it has been shown that MSCs can be isolated from adipose tissue and expanded in large numbers in culture. However it is also known that the number of MSCs decrease with age. This introduces the added problem of autologous cell availability in older people when osteoarthritis is major problem. The alternatives include the use of allogenic or xenogenic cells. Allogenic provide transplants the greater opportunity since human and animal MSCs appear to be immuno-privileged (5, 8). Indeed, the use of allogenic MSCs has its own problems, including storage, sterlization and ethical issues.

Another more interesting approach, which negates the need for cell isolation and transplantation, involves attracting the MSCs to the area of interest, and promoting chondrogenic differentiation *in situ*. This approach although challenging is not impossible due to recent significant progress that has been made in the area of gene therapy. In one of the first reports of a combined TE and gene therapy approach, Mason (70) developed a retroviral vector to introduce human bone morphogenic protein-7 complementary deoxyribonucleic acid into periosteal-derived rabbit mesenchymal stem cells. The results of this study indicated that the grafts containing bone morphogenic protein-7 gene modified cells consistently showed complete or near complete bone and articular cartilage regeneration at 8 and 12 weeks whereas the grafts from the control groups had poor repair as judged by macroscopic, histologic, and immunohistologic criteria. In the future it may be possible to generate gene vectors with the ability to attract chondrocytes to the specific region of interest and stimulate cartilage growth and integration with native cartilage. However, there are many fundamental questions

that need to be addressed before this method is routinely used. As mentioned previously, not all the marker proteins for MSCs have been identified, which presents a significant challenge in itself.

In many cases cartilage damage or degradation is accompanied by damage to the underlying subchondral bone. At present, transplantation of autologous chondrocytes results in the repair of osteochondral defects and the formation of a layer of fibrocartilage with inadequate mechanical properties. This represents an exciting challenge for TE. By incorporating the required bioactive molecules into scaffold materials, it may be possible to generate the correct biological signals to address this issue of bone and cartilage repair.

Although there has been remarkable progress in the area of scaffold design and development for cartilage TE applications, there is still much research to be completed before an ideal scaffold is constructed. Phenotype control is a major concern. As mentioned previously, cytokines and growth factors are being incorporated into scaffolds, but the quantity and release kinetics still raise many questions. For example, BMP-2 in low doses results in osteogenesis, but very high doses are required to cause the same effect in humans (5). With the advent of gene therapy, however, the scaffold may be employed in the future to provide structural support for tissue formation, while gene vectors may be recruited for phenotype control.

Osteoarthritis is a degenerative disease that results in the degradation of cartilage. If tissue engineered cartilage is to be successful, it has to last for a reasonably long time. This is an area where gene therapy can play a role in down-regulating the cytokines that cause cartilage degradation (5) and ensuring that the cartilage continues to repair and regenerate in the long term.

Conclusion

In summary, we believe that the key to cartilage repair and regeneration depends on a combinatorial approach involving the following components (1) the delivery of progenitor MSC cells to the specific site using a carrier or scaffold device (2) the response of these cells to specific bioactive factors, nutrients and environmental signals and most importantly (3) the growth and integration of the neo-tissue with the native tissue.

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