8 The Illustrative Role of Cells in Cartilage Repair

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8.1 Introduction

Autologous chondrocyte implantation (ACI) introduced over 25 years ago has been a milestone treatment for the articular cartilage defects and has produced hyaline cartilage like repair and excellent clinical results [1, 2]. However, the need for two surgical procedures and cell engraftment issues has long been major shortcomings, leading to the development of alternative cell sources such as the mesenchymal stem cells.

Compared to chondrocytes, stem cells hold advantages in terms of securing a large number of cells as well as a differentiation potential for various tissue types. In order for stem cells to be reliably used in clinic, key issues must be addressed regarding the actual survival and continuing chondrogenic differentiation of the transplanted cells.

Implanting the cells from an outside or an inside source to the defect area will lead to the following sequences of events. The cells should attach to the subchondral bone and then shall proliferate as they are stimulated by surrounding stimuli such as the growth factors mechanical stimuli, etc. In addition, the cells should differentiate into chondrocytes, secrete extracellular matrix, and eventually repair cartilage tissue.

Ongoing research regarding stem cells aim to improve the survivorship and differentiation of the transplanted cells by providing a favorable environment as well as stimulation of endogenous stem cells, thereby improving the currently existing surgical methods. A variety of biomaterials are being used to enhance the engraftment of the endogenous or the implanted cells. Researchers and clinicians must understand the mode of action, pros and cons, and posttransplantation behavior of each biomaterial of interest in order to appropriately utilize them.

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8.2 The Illustrations

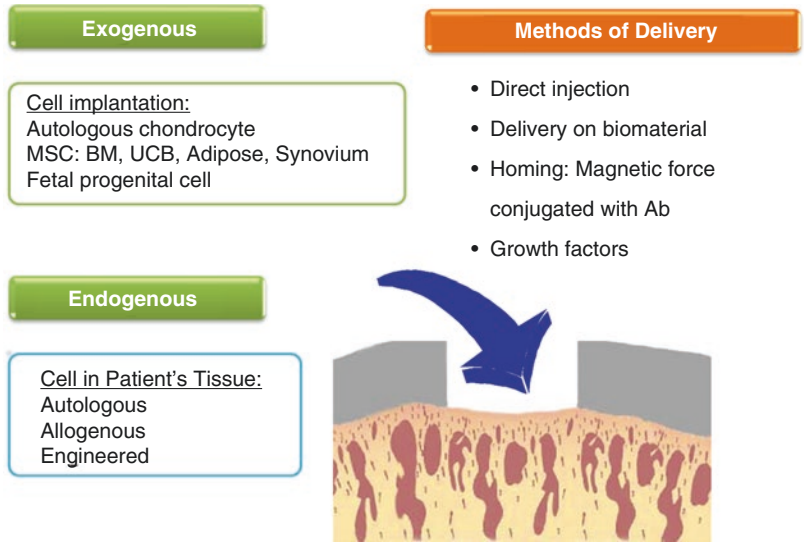


Figure 8.2.1: Cartilage Repair: Different Source of the Cells. The biologic sources for cartilage repair largely includes the use of endogenous cells or the exogenous cell by multiplication. To induce repair by endogenous stem cells, a bone marrow stimulation method is often used. For exogenous cells induced repair, cells that are proliferated by culture are used, such as chondrocytes, mesenchymal stem cells, fetal progenitor cells, etc. The exogenous cells may be implanted to the defect area by direct injection [1, 3] or by seeding into the biomaterials [4–10] or can be navigated by attaching with a magnetic bead [11, 12] or an antibody [13, 14]

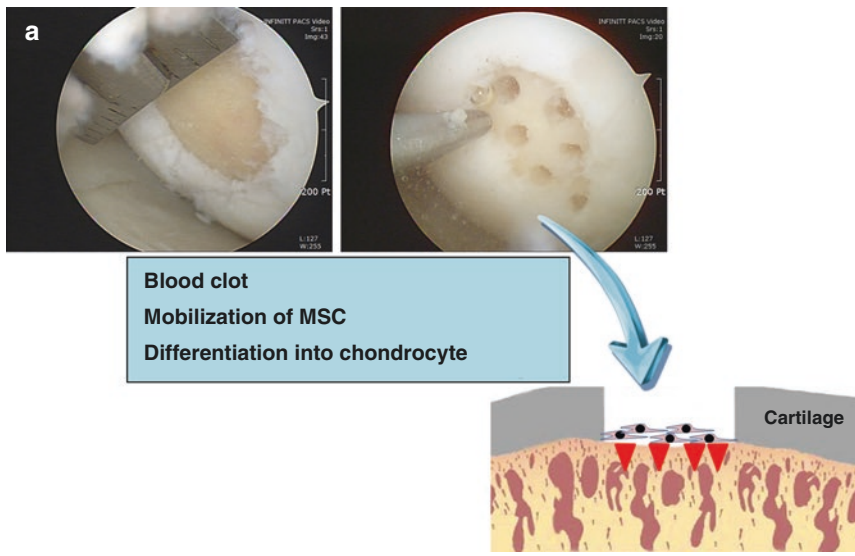
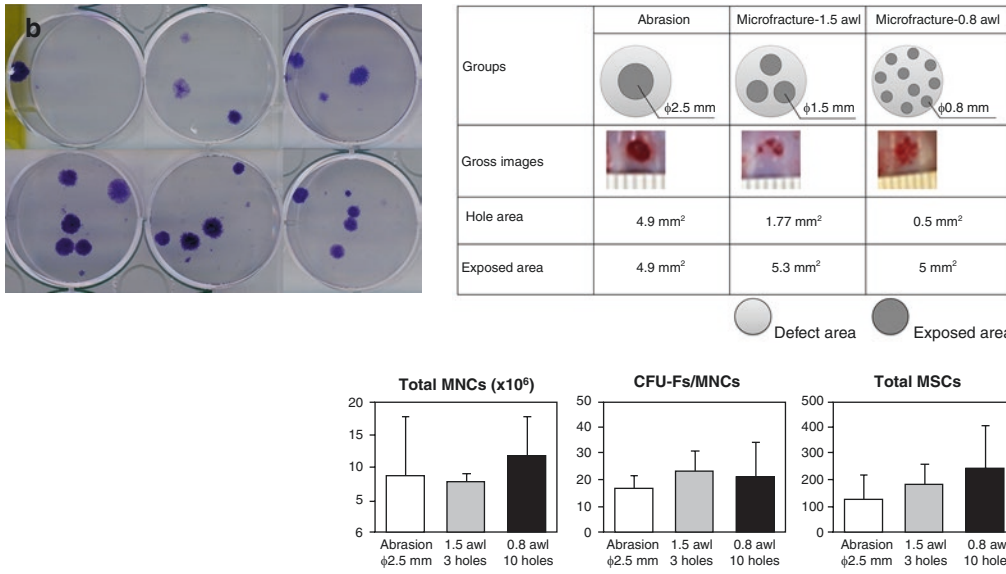


Figure 8.2.2: Cartilage Repair: The Endogenous Stem Cells from the Bone Marrow. The most commonly used method of accessing the endogenous stem cells is the bone marrow stimulation technique. (**a**) One such technique is the microfracture technique, that has been practiced relatively more commonly. This method requires making of multiple holes through the subchondral bone into the bone marrow using an awl, through which the stem cells in the marrow flow to the defect area. These stem cells undergo differentiation and proliferation within the blood clots (mesenchymal blood clot or super clot) to create a cartilage tissue [15, 16]. (**b**) The stem cells in the blood clot formed after a microfracture technique can be identified by colony-forming unit method. Stem cells attached to the culture plates are proliferated to form a colony, and the number of these colonies corresponds to the number of stem cells. The number of colonies varies depending on the diameter and the number of holes



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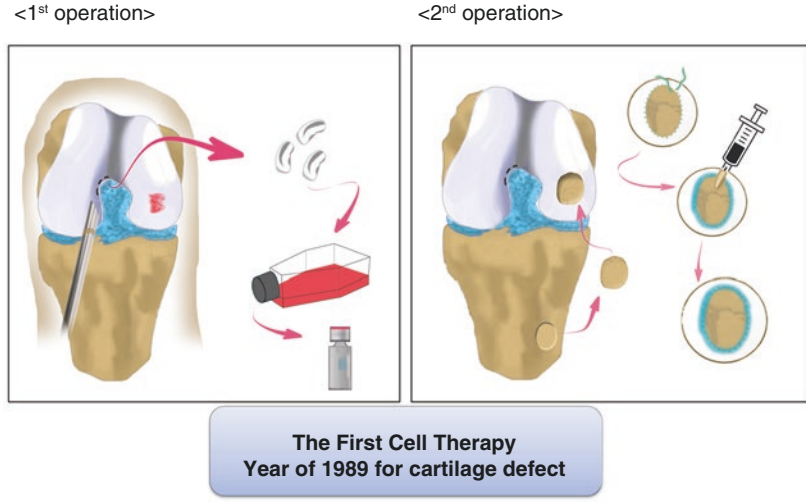

Figure 8.2.3: Cartilage Repair: The Cultured Chondrocytes. The autologous chondrocytes implantation (ACI) is a typical biological method of cell therapy, first reported in 1994. It is two-stage surgery, with the cartilage biopsy collected primarily and then chondrocytes multiplied to minimum of more than five million cells [1]. As a second-stage surgery, the patient's periosteum is harvested to cover the chondral defect, and then the multiplied chondrocytes are injected into the cavity created by it

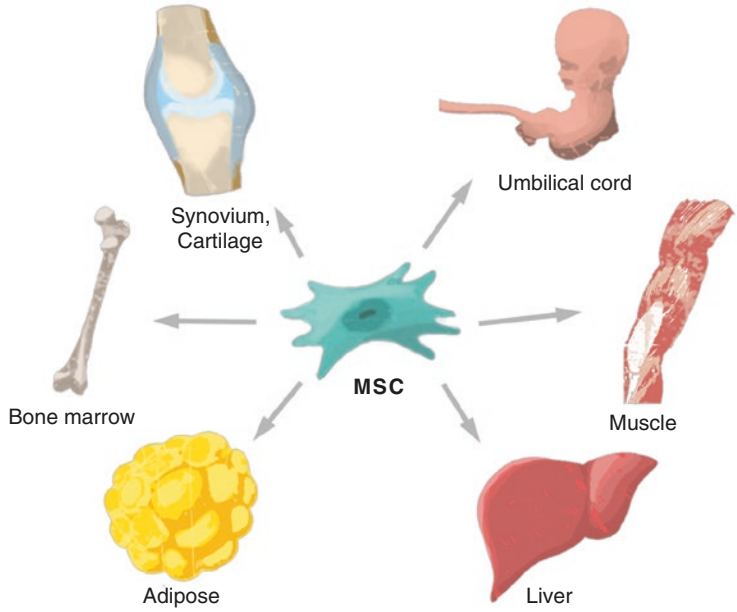

Figure 8.2.4: Cartilage Repair: The Sources of the Stem Cells. There are many different sources of the stem cells, from the embryonic stem cells to the induced pluripotent stem cells. Mesenchymal stem cells (MSC) are known to exist in all the tissues of the body. As far as stem cells for cartilage repair are concerned, the research on stem cells taken from the following tissues have been reported: umbilical cord, umbilical cord blood, fat tissue, bone marrow, synovium, fetal cartilage, iPS, and the embryonic stem cell [17, 18]. For cartilage regeneration, MSCs derived from the bone marrow are studied the most, and MSCs originated from the fat tissues or the umbilical cord blood have been started in clinical application. MSCs cells derived from patient's synovial tissue are reported to have the most potent cartilage differentiation, and their clinically effective application is being studied [17–21]

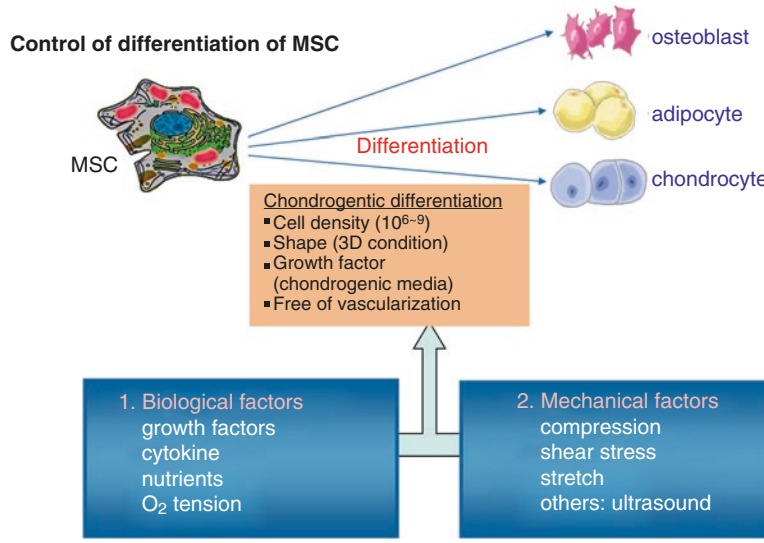

Figure 8.2.5: Factors Controlling Differentiation of Stem Cells into the Chondrocytes In Vitro/In Vivo. Stem cells can differentiate into numerous cells of lineage by biologic or mechanical factors in vitro and in vivo. To differentiate into chondrocytes, the cell density should be high, incubation must be done under the conditions of three-dimensional culture, and chondrogenic media that promotes chondrogenesis should be used [22]. Vascularization during chondrogenesis in vivo should be inhibited if possible, as it can lead to dedifferentiation and calcification [23]. Certain dynamic culture conditions involving compression, shear stress, stretch, and ultrasound improve chondrogenesis [24, 25]

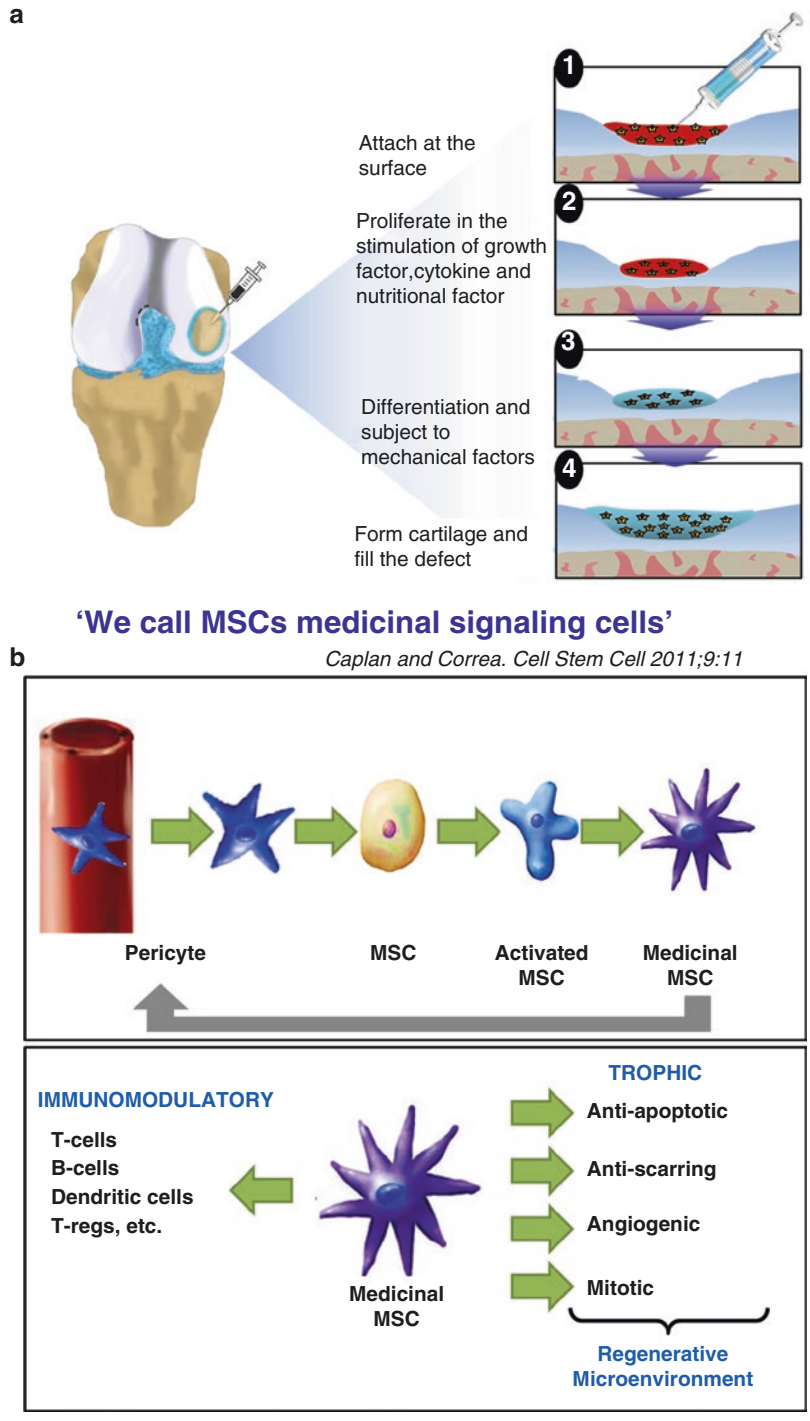

Figure 8.2.6: The Mode of Action of the Transplanted Cells. (**a**) In the classical concept, implanting the cells from an outside source or from an inside source to the defect area will inevitably lead to the following mechanisms. The cells attach on to the surface of the defect to the subchondral bone. The implanted cells proliferate as they are stimulated by surrounding stimuli such as growth factors, cytokine, mechanical stimuli, and other nutritional factors released by neighboring tissues. Cells differentiate, synthesize, and secrete extracellular matrix, eventually filling the cartilage defect with the repaired cartilage tissue. (**b**) The mechanism of cartilage repair by stem cell implantation is being interpreted differently than in the past. According to many current studies, stem cells work like drugs. Since the survival of transplanted cell is very limited, the cells release biologically active factors (paracrine factors) to promote tissue regeneration and inhibit fibrosis, apoptosis, and inflammation by modulating the survival, proliferation, migration, and gene expression of the cells around them

Title/Journal	Cell type	Animal	Duration
Behavior of transplanted bone marrow-derived GFP mesenchymal cells in osteochondral defect as a simulation of autologous transplantation/ Journal of Histochemistry & Cytochemistry 53(2):2-7-216,2005	Autologous MSC	Rat 12-week-old female GFP transgenic rats, genetically identical with wild-type rats.	▪GFP positive cells were observed in the regenerated tissues for 24 weeks although GFP positive cells decreased in number with time
Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion/ Frontiers in Immunology 3: 1-8, 2012	Allogeneic MSC	▪MSC donors: DsRed C57BL/6 mice ▪MSC recipients: would-type C57BL/6 mice	▪DsRed-MSC were present in lung after 1h, but strongly reduced after 24h. ▪No living DsRed-MSC were detected in cultures of any of the other tissues established at 1, 24, or 72h after MSC infusion
Survival of transplanted rat bone marrow-derived osteogenic stem cells in vivo/ Tissue Eng. Part A 7(7-8): 1147-1156, 2011	Autologous MSC	▪Isogenic adult male rats (Lewis)	▪On day 3, cell numbers had decreased by more than two-thirds of day 1. ▪On day 14, cells could no longer be identified
Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord/ Nature Medicine 5(12): 1410-1412, 1999	Xenogenic ESC	▪Adult long evans female rats	▪At 2-5 weeks after transplantation, ES cell-derived cells were found in aggregates or dispersed singly through-out the injury site
Bone marrow-derived mesenchymal stem cell transplant survival in the injured rodent spinal cord/ Journal of Bone Marrow Research 2(2): 1000146, 2014	MSC		▪0-52%: 1 week after transplantation ▪0-8%: 1 month after transplantation ▪In some cases, presence of MSCs up to 2 months and even 3 months ▪Usually no or very few cells survive at 2 or 3 months
Human adipose-derived stromal/stem cells demonstrate short-lived persistence after implantation in both an immunocompetent and an immunocompromised murine model/ Stem Cell Research & Therapy 5(6):142, 2014	Xenogenic ADSC	▪Immunocompetent wildtype mice: C57BL/6NCr ▪Immunocompromised mice: Athymic NCr-nu/ nu)	▪The persistence of human ASCs is correlated to the percent ERV-3 amplicational. ▪Human ASCs survive for less than 3 weeks after injection

Figure 8.2.7: The Survival of Transplanted Cells. Numerous researches have shown that regardless of the method in which the cells are transplanted, including injection, surgery, or transplantation with the use of biomaterials, the number of cells significantly decreases in a matter of weeks [26–30]. This time-dependent decrease in cell number is also observed in both autologous and allogeneic cells. The mode of action of the transplanted cells, limited by the time within the transplantation site, may not be due to the continued differentiation and functioning of the transplanted cells

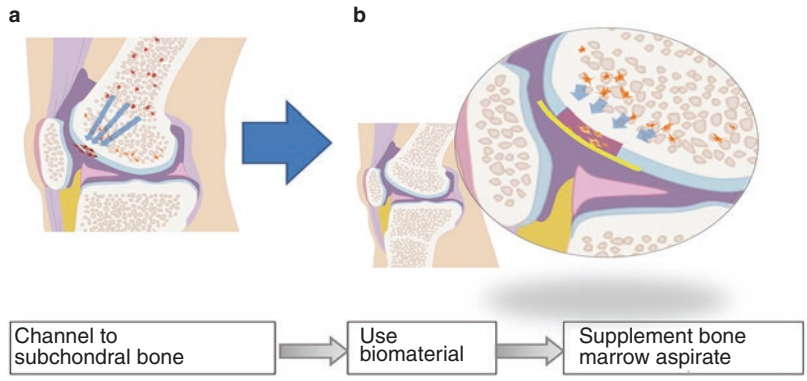

Figure 8.2.8: Supporting the MSC in the Defect Area with Biomaterials. (**a**) Blood clots that contain stem cells and fill the defect areas can be easily lost by gravity, by a shear force or washed by the synovial fluid. Also, cells present in the synovial fluid and cytokine can kill or disrupt the differentiation process. (**b**) Thus, to avoid this, a method of either covering the defect area or scaffolding the defect area; is being sought. Alternatively, increase in the stem cell numbers that participate in regeneration can be done via transplantation of MSCs extracted from the bone marrow or adipose tissue to the area of the defect [31, 32]

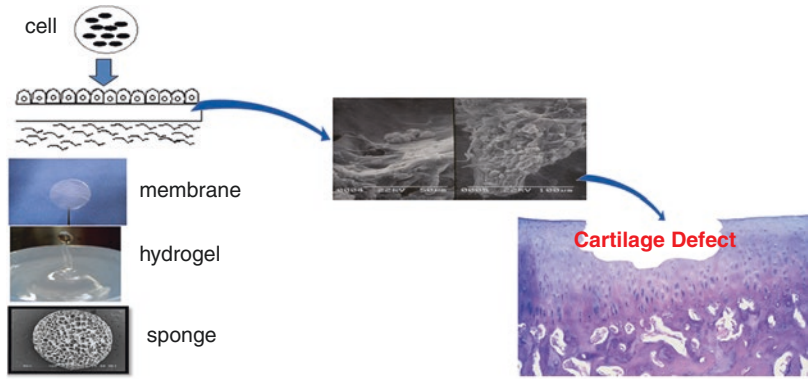

Figure 8.2.9: Concepts of Biomaterials in the Cartilage Repair. The downside of the cell therapy is that the efficiency of engraftment is reduced by the leakage of implanted cells from the damaged area. The survivorship and differentiation success rate are seriously threatened in the unfamiliar environment of the implant. Biomaterials, when used as a carrier of implanting cells, can increase the efficiency of engraftment and survivorship by providing a beneficial environment for growth and differentiation of the grafted cells. Biomaterials used with cells can be membrane-type, gel-type, and three-dimensional scaffold-type. Scaffold with growth factor is also used to promote a differentiation into the cartilage cells

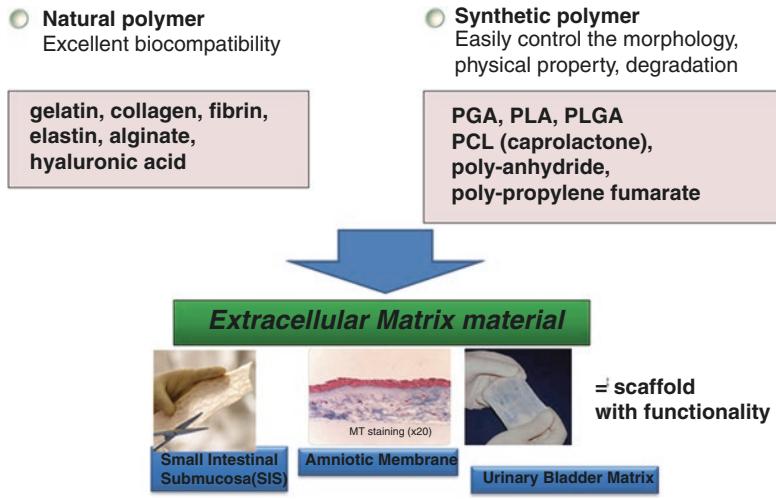

Figure 8.2.10: The Optimal Biodegradable Biomaterial. Biomaterials include natural polymers and synthetic polymers. Each has its advantages and disadvantages. While natural polymers exhibit superior biocompatibility, the physical and chemical processing and degradation control are difficult. While the synthetic polymers have advantages in controlling morphology, physical property, and degradation, biocompatibility may not be good, such as the occurrence of posttransplant inflammation. Biomaterials that are made of extracellular matrix of tissues are useful as a complement to their strengths and weaknesses. The biggest strength of these materials is their biocompatibility with the strong biologic functions. The small intestinal mucosa, amniotic membrane, the urinary bladder matrix, and cartilage are various examples of currently used clinical applications

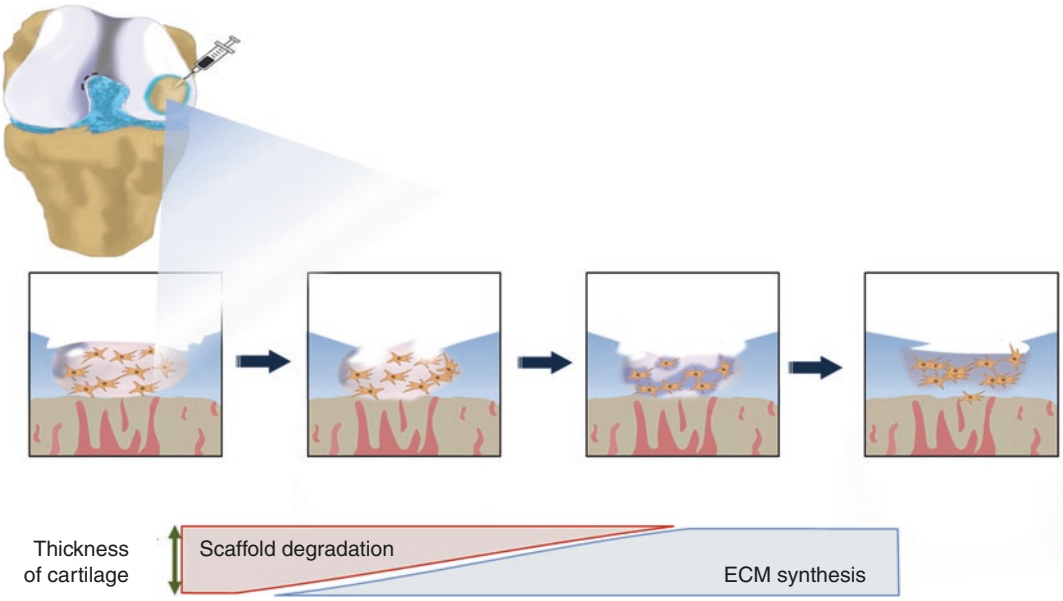

Figure 8.2.11: The Balanced Degradation of the Biomaterial. The stem cells that are transferred into the biomaterials proliferate and differentiate, and they secrete an extracellular matrix. The biomaterials must degrade to accommodate the extracellular matrix that is produced by the cells. When biodegradation of the biomaterial occurs rapidly, the mechanical support becomes mechanically weak, while a slow degradation can inhibit the production of the extracellular matrix. This harmonized degradation of biomaterials is called "balanced degradations"

8.3 Take-Home Message

Stem cells can either originate from cultured autologous, allogeneic cells, or endogenous cells from the patients' bone marrow or other niche tissue. Stem cells have many beneficial qualities compared to adult cells making them an attractive treatment modality. Continued survival and differentiation toward cartilage requires the stem cells to be exposed to certain biological cues and chondrogenic environment, which still requires a further research.

We need a clear understanding of the differentiation process after stem cell transplantation. Understanding of an in vivo stem cell behavior and differentiation mechanism will lead to more advanced cell therapies that will maximize the differentiation process toward cartilage. As for the biomaterials, each material (whether synthetic or natural origin) holds unique advantages and disadvantages. Change in the biomaterial itself after transplantation, such as degradation, should continually support the differentiation and survival of the transplanted cells.

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