Cell-Based Bone Reconstruction Therapies—Cell Sources

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Over the last few decades, reconstructive surgery has shifted from a resection-oriented approach toward strategies focusing on repair and regeneration of tissues. As the main aim of maxillofacial reconstruction has been the restoration of bone form and function, surgeons used artificial tissue substitutes in the early decades of bone reconstruction. These artificial materials significantly improved the ability of surgeons to restore the form and, to some extent, the function of defective bones. Despite the fact that every artificial material has specific disadvantages, the use of biomaterials is a common treatment option in clinical practice even today. Due to the more detailed understanding that exists concerning transplantation of cells and tissues, autogenous grafts are the second mainstay in clinical practice. However, the main disadvantage of using autogenous grafts is donor site morbidity and donor shortage. Research is currently in progress into the use of cell-based approaches in reconstructive surgery, since cells are the driving elements for all repair and regeneration processes. Various cell populations have been reported on in the relevant literature. These cells can be classified according to differentiation capacity and the tissue from which they originated. In this review, unrestricted cells, multipotential progenitor cells, determined cells, and genetically modified cells are described systematically, and their advantages as well as limitations are discussed. (More than 50 references.) INT J ORAL MAXILLOFAC IMPLANTS 2006;21:890–898

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Over the last few decades, reconstructive surgery has shifted from a resection-oriented approach toward strategies focusing on repair and regeneration of tissues. As the main aim of maxillofacial reconstruction has been the restoration of bone form and function, in the early decades of bone reconstruction surgeons used artificial tissue substitutes containing metals, ceramics, and polymers to maintain skeletal function.[1] These artificial materials significantly improved the ability of surgeons to restore the form and, to some extent, the function of defective bones. Despite the fact that every artificial material has specific disadvantages, the use of biomaterials remains a common treatment option in clinical practice. More detailed understanding exists concerning transplantation of cells and tissues; thus, autogenous grafts are the second mainstay in clinical practice. The advantages of transplanting the body's own tissues are such that autogenous tissue transplantation can be considered the gold standard in bone reconstruction. The reason for the primacy of tissue grafts over nonliving biomaterials is that they contain living cells. The main disadvantage of using autogenous grafts is donor site morbidity and shortage of graft material.[2] Research into the use of cell-based approaches in reconstructive surgery is currently in progress, since cells are the driving elements for all repair and regeneration processes. As they synthesize and assemble the extracellular matrix, cells can be considered the basic unit that is needed for a biological regeneration strategy. Living cells can be used in a variety of ways to restore, main-

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Table 1 Enhancing Bone Formation by Cells

Methods	Main cell source	Clinical example
Transfer of cells in tissue blocks	Autologous cells	Iliac crest
In situ cell activation	Autologous cells	BMP-7 in spine fusion surgery
Implantation of isolated cells	Autologous or heterologous cells	Preclinical
Implantation of extracorporeally generated tissue constructs	Autologous or heterologous cells	Preclinical

tain, or enhance tissue functions.[3,4] There are 4 principal ways in which cells are used to enhance bone formation (Table 1): transfer of cells as tissue blocks, in situ cell activation, implantation of isolated or cultured cells, and implantation of an extracorporeally generated tissue construct. In a broader sense, genetic engineering is another cell-based regeneration therapy, since the action of genes is directly related to the presence of living cells. Introduction of genetic information into cells, effected by different measures, is in its preclinical stage but may become a therapeutic option in the near future.

Transfer of cells located in an autologous bone tissue specimen is a common method of cell-driven repair strategy. It has some advantages, since bone cells have a high mechanical competence. Additionally, the transplantation process supplies the organic and anorganic components of bone tissue for the remodeling process. Because the relation between the "active" cellular elements and the remaining tissue components is shifted toward the latter, the main disadvantage is significant—and to some extent unnecessary—donor site morbidity. For in situ regeneration, new tissue formation is induced in cells located in the defect using specific measures. This approach is independent of a donation of bone from other skeletal sites, as stimuli are used to activate the body's own bone cells and promote local bone formation.[5] Cellular implantation can be considered as the classic method of cell-based reconstruction therapy in a narrow sense. Individual cells or small cellular aggregates from the patient are transferred to the bone defect without using an artificial "space holder." Cells are commonly placed in a suspension or hydrogel when they are placed in the defect. A gel (which would be considered a scaffold material) is often used more to hold cells in place than to support the 3-dimensional location of the cells.[6] For extracorporeal tissue engineering, a 3-dimensional tissue is grown in vitro using cells within a scaffold. After cell growth and differentiation, ideally in a mechanically stable scaffold, such hybrid materials are then implanted into the defect site.[7,8]

Whereas transfer of the patient's own tissue and in situ stimulation rely on autologous cells, cell implantation, extracorporeal tissue engineering, and genetic engineering can be done with a wide variety of cells in different stages of cell differentiation and maturation. The intention of this review is to describe different cell-based strategies as related to maxillofacial bone reconstruction. The main focus is on the sources and features of the cells that can be used for such regenerative strategies. Information on the current state of preclinical and clinical approaches to improve the repair, regeneration, and reconstruction of defective or lost bone are given in a separate article in this issue of the journal.[9]

Autologous, allogenic, and xenogenic cells can all be used for tissue engineering. Each category can be subdivided according to whether the cells are in a more or less differentiated stage. Various mature cell lines as well as multipotential so-called mesenchymal progenitors have been successfully established[10] in bone tissue engineering approaches. Moreover, some researchers have used totipotent embryonic stem cells for tissue engineering of bone.[11,12] Additionally, other bone cell lines such as genetically altered cell lines (sarcoma cells, immortalized cells, nontransformed clonal cell lines) have been developed and used to evaluate basic aspects of in vitro cell behavior in nonhuman settings.

Three types of genetically unaltered natural cells have been used for bone engineering. In addition to completely undifferentiated cells, such as embryonic stem cells[11,12] or umbilical cord stem cells,[13] terminally differentiated cells, such as osteoblasts, have been used (Fig 1). It has long been known that the vast capacity of bone for regeneration is due to the presence of differentiated osteoblasts.[14] As the use of determined osteoblastlike cells—unlike the use of stem cells—does not raise legal issues, and there are no problems of immune rejection, determined bone cells currently can be considered the most important cell source in bone tissue engineering.

However, a third class of cells exists between undifferentiated and terminally differentiated cells. This

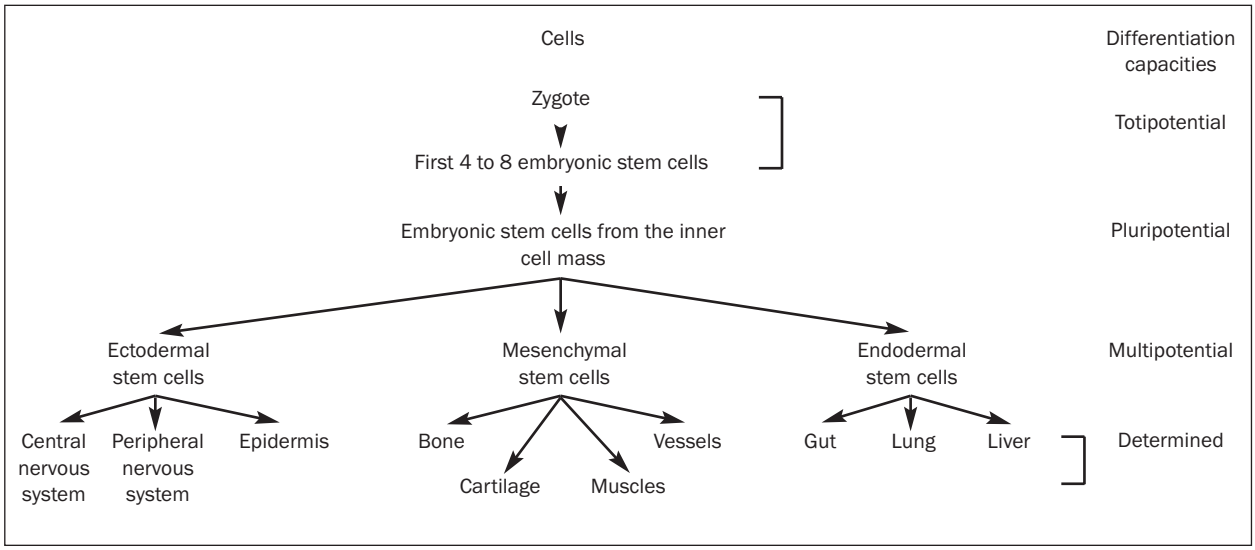

Fig 1 Differentiation cascade.

class is believed to contain multipotential stem cells which are often called *mesenchymal stem cells (MSCs)*[15,16] or *adult stem cells*.[17] Whereas the situation of determined cells is well known to researchers and clinicians, some confusion exists about the use of so-called MSCs. Not only the origin but also the destiny and clinical usefulness of such cells have been questioned.

CAPACITY FOR DIFFERENTIATION

Do MSCs have the capacity to differentiate into different mesenchymal cell lineages? MSCs or "adult stem cells" are obtained from various tissues. Bone marrow,[16,17] adipose tissue,[18] vein wall,[19] peripheral blood,[17] fetal and maternal placenta,[20,21] periodontal ligament,[22] periost,[23] and trabecular bone[23,24] have all been described as sources of MSCs. However, the characteristics of stem cells, ie, the capacity for extensive replication without differentiation and a multilineage developmental potential to generate not only bone and cartilage but also tendon, muscle, fat, and marrow stroma, are not reported for all these cells. Many adult tissues contain populations of cells that have the capacity for renewal after disease, trauma, or aging. Are these cells multipotential stem cells, or is there a mixture of cell populations in the tissue, each of which can differentiate into only 2 or 3 lineages? In the relevant literature there are many reports about the multilineage potential of human MSCs, but the majority of these studies use all cells derived from various tissues to perform the differentiation experiments.[16,25–27]

Pools of such adult stem cells can exhibit multiple differentiated phenotypes under appropriate in vitro conditions. It is not clear whether these multipotential cells are active at the clonal level. Halleux and coworkers[15] performed a differentiation experiment with various clones derived from iliac crest biopsy specimens. Although all expanded clones showed osteogenic differentiation, only 50% of these cells underwent differentiation into the osteogenic, chondrogenic, and adipogenic lineage. These findings are in line with the data presented by other authors[28] and suggest that osteogenic differentiation represents a default pathway for bone marrow-derived cells and that a high percentage of these cells lack MSC characteristics. Recently, Guilak and colleagues analyzed the differentiation potential of human adipose-derived cells on a clonal level.[18] Eighty-one percent of the cell clones derived from subcutaneous adipose tissue in the abdomen and hips differentiated into at least 1 mesenchymal lineage. In addition, only 52% of the cell clones differentiated into 2 or more of the lineages. More clones expressed phenotypes of osteoblasts (48%) and chondrocytes (43%) than expressed phenotypes of adipocytes (12%). These data indicate that cells derived from different sources also contain a multipotential subpopulation along with other cells. These cells have the capacity to differentiate into different mesenchymal cell lineages. Since studies have shown that both adipose-derived cells[29] and bone marrow–derived cells[30] can also differentiate into ectodermal neural cells, the term *mesenchymal stem cell* seems inaccurate.

There is now evidence for the existence of cells with the capacity to differentiate into various mes-

enchymal and ectodermal lineages. However, there are no reports describing the determination of these progenitor cells in the tissue or cell cultures except clonal outgrowth. From a clinical point of view, this determination is not necessary when it is known that among cells derived from a specific source there are some progenitor cells that can differentiate into osteoblasts, chondrocytes, or adipocytes. No nomenclature based on the developmental origins or differentiation capacities of these cells has been entirely accurate. Therefore, for pragmatic reasons, terms that consider the source and known differentiation capacity of cell populations derived from different tissues should be used. With regard to bone tissue engineering, terms such as *adipose tissue–derived progenitor cells (ADPCs), bone marrow–derived progenitor cells (BMDPCs),* or *periost-derived progenitor cells (PDPCs)* might be more accurate.

Concerning differentiation capacity, 3 different groups of cells can be determined (Table 2):

- Unrestricted cells able to differentiate into all or nearly all lineages, eg, embryonic stem cells (ESCs) and unrestricted somatic stem cells (USSCs)
- Multipotential progenitor cells, eg, ADPCs, BMDPCs, and PDPCs
- Determined cells, eg, osteoblasts and osteocytes

Beside these cells, which are found in natural tissues, there are genetically modified cell lines (Table 2). The various cell lines are described here as they are used in cell-based reconstruction therapies on a basic, preclinical, and clinical level.

Table 2 Classification of Cells Used for Bone Tissue Engineering

Natural cells	Genetically modified cells
Unrestricted cells	• Osteosarcoma cell lines
• Embryonic stem cells (ESCs)	• Intentionally immortalized cell lines
• Unrestricted cord blood cells (USSCs)	• Nontransformed clonal cell lines
Multipotential cells	
• ADPCs	
• BMDPCs	
• PDPCs	
• Blood vessel-derived progenitor cells	
• Placenta-derived progenitor cells	
Determined cells	
• Preosteoblasts	
• Lining cells	
• Osteoblasts	
• Osteocytes	

UNRESTRICTED CELLS

ESCs are the major representative cell line in this group. These cells were first isolated and grown in culture more than 20 years ago.[31] ESCs are routinely derived from the inner cell mass of blastocysts and represent pluripotential embryonic precursor cells that give rise to any cell type in the embryo (Fig 1). ESCs have historically been maintained in coculture with mitotically inactive fibroblasts.[32–34] This coculture system is unnecessary if the medium is supplemented with leukemia inhibitory factor (LIF).[35,36] In the absence of LIF, ESCs differentiate into a morphologically mixed cell population manifesting genes characteristic of endoderm and mesoderm.[37] By definition ESCs have the potential to differentiate into every cell lineage. Significant progress has been achieved in inducing murine and human cells to differentiate into particular types of cells, such as cardiomyocytes,[38] neurons,[39,40] and smooth muscle cells.[41] Specifically, it has been shown by various investigators that ESCs can differentiate into osteogenic cells under selective culture conditions.[11,12,42]

The most common way to initiate osteogenic differentiation in ESCs is to supplement the medium with dexamethasone, ascorbic acid, and β-glycerol phosphate.[32,42] Moreover, cytokines such as vitamin D3 or BMP-2 have been found to promote osteogenic differentiation in ESCs.[12]

An advantage of using ESCs instead of tissue-derived progenitor cells is that ESCs are immortal and could potentially provide an unlimited supply of differentiated osteoblast and osteoprogenitor cells for transplantation. In contrast, the capacity of cells derived from adult tissues for proliferation, self-renewal, and differentiation decreases with age.[43,44]

One major challenge in the use of ESCs for osteoregenerative therapies is overcoming immunological rejection from the transplant recipient. Interestingly, Burt and colleagues performed ESC transplantation in major histocompatibility complex (MHC)–mismatched mice without clinical or histologic evidence of graft-versus-host disease (GVHD).[34] In addition, recent data indicate the potential of ESCs to offer a possible solution for low-risk induction of tolerance without immunosuppression.[45] Moreover, it might be possible to downregulate the antigenicity of ESCs through suppression of MHC gene expression.[11] Another concern is that the cultivation and transplantation of such stem cells are accompanied by tumorigenic differentiation. It has been shown that undifferentiated ESC cells give rise to teratomas and

teratocarcinomas after implantation in animals; this potential misdevelopment constitutes a major problem for the clinical use of such cells.[46]

Opposition to the use of embryonic stem cells because of moral and religious concerns presents an obstacle to progress in cell-derived replacement treatment.[47,48] Objections are mainly raised because experimentation with embryonic stem cells derived from human blastocyst requires the destruction of human embryos. The current debate on the ethical status of the human embryo has focused on its fragility, its defenselessness, and the degree of respect it should merit in light of recent medical progress. It is generally accepted that the blastocyst contains a complete set of genetic instructions and the capacity for the epigenetic determinations needed to develop into a human being. However, whether absolute respect for individual human life should begin at conception is controversial.[47,48]

Recently, another possibly unrestricted cell line was detected by Kögler and colleagues.[13] This cell population was obtained from human cord blood and showed homogeneous differentiation into osteoblasts, chondroblasts, adipocytes, hemapoietic cells, and neural cells. These cells were termed *unrestricted somatic stem cells (USSCs)*. No tumor formation was observed on implantation of these cells in animals, but it is unclear whether USSCs lead to GVHD in immunocompetent hosts.

MULTIPOTENTIAL PROGENITOR CELLS

As previously described, this category includes a heterogeneous group of cells. The multipotential progenitor cells (MPCs) acquire specific phenotypes depending on their maturation during differentiation. Stem cells and precursor cells arise in the embryo, and at least some of these cells appear to persist in the adult organism, where they contribute to the replacement of lost cells in the remodeling and repair of skeletal tissue. MPCs serve as the major reservoir for different classes of cells. Stromal cells have multilineage differentiation capacity. Progenitors with restricted developmental potential, such as fibroblasts, osteoblasts, chondrocytes, and adipocyte progenitors, are generated from these cells.[16,49] Maturation of cells takes place through stages of proliferation, commitment, progression, and differentiation in the organism in order to generate the various tissues. Numerous hormones and cytokines regulate the cell during the differentiation process from progenitor to mature cell. Among these, members of the transforming growth factor superfamily are the most potent inducers and stimulators of osteogenic differentiation. For bone development, bone morphogenetic proteins (BMPs) not only stimulate osteoprogenitors to differentiate into mature osteoblasts but also induce nonosteogenic cells to differentiate into osteoblast lineage cells (for review see Kale and Long[50]). Other factors (dexamethasone, prostaglandines) also play important roles in the regulation of osteoblast differentiation in an orchestrated way.

At some point in this process, a cell becomes an osteoblast. The mature osteoblast phenotype is characterized by the ability of the cells to synthesize a bone matrix that will finally mineralize. Additionally, osteoblasts express various phenotypic markers, such as high alkaline phosphatase (ALP), and synthesize collagenous (predominantly collagen type I) and noncollagenous bone matrix proteins, including osteocalcin.[51] However, in the vast majority of published studies regarding the differentiation capacity of MPCs, only ALP or osteocalcin expression as well as calcium deposition were measured as markers for osteoblasts. These markers do not completely prove the existence of a mature osteoblast.

Since it is known that MPCs have, in part, different characteristics depending on the tissue from which the cells are obtained,[20] MPCs are here described with regard to their source:

- **BMDPCs:** BMDPCs are commonly obtained from femoral or iliac bone. Frequently, it is not feasible to obtain sufficient amounts of bone marrow with the requisite number of progenitor cells using marrow aspiration. In addition, the age-related decrease in bone marrow components, accompanied by a partial loss of precursor cells,[44,52] is a frequent clinical limitation to obtaining sufficient numbers of BMDPCs. Through the use of appropriate culture protocols, BMDPCs can be induced to differentiate so as to yield osteoblasts, chondrocytes, and adipocytes.[15,16,25,53] Surprisingly, when implanted in the brain, BMDPCs may also differentiate to become astrocytes, which indicates an enormous degree of plasticity.[54] However, only 50% of isolated cells show a multilineage potential.[15] Regarding their osteogenic potential, the potential of BMDPCs to differentiate into osteoblasts appears comparable to that of ADPCs.[25] BMDPCs and ADPCs both show high expressions of the surface markers CD29, CD44, CD105, and CD90. Both cell populations are negative for the hematopoetic stem cell marker CD34.[27] Interestingly, Shimko and colleagues[55] compared BMDPCs with ESCs with respect to the mineralization process. Whereas the BMDPCs exhibited a more characteristic osteoblastlike phenotype and a mineral that was low in calcium, ESCs mineralized considerably.

However, their differentiation was incomplete, and the extracellular matrix did not exhibit extensive type I collagen and osteocalcin. Finally, there seem to be significant interindividual differences between various donors regarding osteogenic differentiation capacity.[56]

- **ADPCs:** ADPCs are derived from adipose tissue, which is routinely available in large quantities through liposuction surgery. The yield of ADPCs after expansion is relatively high and averages about 400,000 cells per milliliter of lipoaspirate tissue.[57] However, at a clonal level only 50% of these cells differentiate into 2 or more mesenchymal lineages.[18] In addition, ADPCs can also differentiate along nonmesodermal pathways.[18] In both of these respects, BMDPCs are comparable with ADPCs. Particularly, the ability of ADPCs to differentiate into osteoblasts is similar to that of BMDPCs. It has also been demonstrated that ADPCs can form osteoid matrices in vivo.[58] Taken together, ADPCs are an alternate source of MPCs that are obtainable with minimal discomfort.
- **PDPCs:** Although progenitor cells obtained from bone marrow or adipose tissue are used in most studies, periosteum is also described as a source for bone tissue engineering.[23] PDPCs are reported to be more proliferative than BMDPCs. PDPCs generate progenitor cells committed to 1 or more cell lines with an apparent degree of plasticity and interconversion.[59,60] Despite the ease of obtaining periosteum pieces surgically, this technique also has the disadvantage of requiring operative harvest.

MPCs Derived From Other Sources

MPCs can also be obtained from sources other than the aforementioned cell populations. Recently, Covas and colleagues reported that cells detached from the internal surface of the saphenous vein can differentiate into osteoblasts, chondrocytes, and adipocytes. The surface markers are comparable to those of BMDPCs and ADPCs: CD29+, CD34–, CD44+, and CD90+.[19] Additionally, MPCs with multilineage differentiation have been isolated from peripheral blood. These CD34– cell lines can differentiate into osteoblasts, but angiogenesis and neovessel formation prevailed.[17] Moreover, different parts of the human placenta were studied for the presence of fetal and maternal MPCs. It was possible to culture the cells into various mesenchymal cell lineages, including osteoblastlike cells. Compared with BMDPCs, the expansion potency was higher from both fetal and maternal placenta-derived MPCs.[20]

DETERMINED CELLS

It has long been known that the vast capacity for regeneration of bone is due to the presence of differentiated osteoblasts.[14] Other cell populations found in bone (ie, osteoclasts, endothelial cells) certainly contribute to osteogenesis. However, the following focus on determined cells is predominantly on cells known to be involved in the mineralization process.

As the use of determined osteoblastlike cells, unlike the use of stem cells, does not raise legal issues, and there are no problems of immune rejection (as long as the source is autologous), determined bone cells can be considered the most important cell source in cell-based bone reconstruction therapies at present. Therefore, in current clinical practice, differentiated autologous osteoblastlike cells are the most desirable cell source. However, even these cells may be insufficient to rebuild damaged bone tissue in a reasonable time. A considerable number of cell divisions is needed to build a significant amount of tissue. Previous studies have regarded the propagation of adult mature cells in culture as a serious problem, because it was thought that most adult tissues contained only a minority of cells capable of effective expansion. However, in numerous recent investigations it has been shown that bone cells proliferate in culture without losing their viability (for review see Wiesmann and Meyer[61]). Various sources of determined bone cells can be used for cultivation. Cultures containing determined "osteoblastic" or "osteoblastlike" cells have been established from different cell populations in the lineage of osteogenic cells (preosteoblasts, lining cells, osteoblasts, and osteocytes; for review see Hutmacher and Sittinger[62]). Such cells can be derived from several anatomic sites using different explant procedures. Bone cell populations may be derived from cortical or cancellous bone, bone marrow, periosteum, or, in some instances, from other tissues. Isolation of cells can be performed with a variety of techniques, including mechanical disruption, explant outgrowth, and enzyme digestion.[63]

The *preosteoblast* is considered a precursor of osteoblasts and lining cells. Preosteoblasts share common phenotypical features of osteoblasts, such as ALP activity, but these cells do not express all markers of mature osteoblasts.[64]

Bone lining cells are synthetically more inactive compared with osteoblasts. The flat, thin, elongated cells cover bone surfaces where no significant remodeling takes place. A proportion of cells become embedded in bone; these cells, considered to represent the finally differentiated cell stage, are osteocytes.

The *mature osteoblast* phenotype is characterized by the capacity of the cells to actively synthesize a

bone matrix that will finally mineralize. Osteoblasts express various phenotypic markers such as high ALP and synthesize collagenous and noncollagenous bone matrix proteins, including osteocalcin.[51] Osteoblasts express receptors for various hormones, including parathyroid hormone,[65] 1α,25-dihydroxyvitamin D3 [1α,25(OH)2D3],[66] estrogen,[67,68] and glucocorticoids,[69,70] that are involved in the regulation of osteoblast differentiation.

Osteocytes are the most abundant type of bone cells. It is assumed that there are approximately 10 times as many osteocytes as osteoblasts in adult human bone.[71] Mature osteocytes are stellate-shaped or dendritic cells enclosed within the lacunocanalicular network of bone.

GENETICALLY MODIFIED CELLS

Beside the aforementioned naturally occurring cell populations, genetically modified cell lines are in use for evaluating basic scientific aspects of bone tissue engineering strategies. Genetically altered cells include osteosarcoma cell lines, intentionally immortalized cell lines, and nontransformed clonal cell lines.[72]

Osteosarcoma cell lines are known to display patterns of gene expression, modes of adhesion, and signal transduction pathways that in certain aspects resemble those of normal, nontransformed bone cells. Most of the osteosarcoma cell lines used do not, however, display a complete pattern of in vitro differentiation. The development of established clonal osteoblastlike cells from rat osteosarcomas (MG-63, UMR, and ROS series) provided cell lines that were homogeneous, phenotypically stable, and easy to propagate and maintain in culture.[73] They share many of the properties of nontransformed osteoblasts. But, as with cancer cells, these cells are transformed and display an aberrant genotype, have an uncoupled proliferation/differentiation relationship, and exhibit phenotypic instability in long-term culture. Therefore, these osteoblastlike cells do not reflect the normal phenotype of primary osteoblastlike cells. Thus, as substrate-dependent cell reactions are difficult to assess, these cells do not seem to be suitable for evaluating various aspects of tissue engineering and cannot be introduced into clinical engineering techniques.

Other approaches have used clonally derived immortalized or spontaneously immortalized cell lines (neonatal mouse MC3T3E1 and fetal rat RCJ cell lines[74]). Although none of these cell lines behave exactly alike, and their behavior in cell culture differs considerably,[75] they do have some common features (ALP activity, collagen type I production, bonelike nodule formation). Despite these common features, cells can be in different stages of growth and development under cell culture conditions. They therefore have differing phenotypic features depending on the cell culture situation. Conditionally transformed immortalized human osteoblast cell lines have been developed by various researchers with the aim of investigating the behavior of osteoblasts toward external stimuli. Xiaoxue and coworkers,[76] for example, assessed the generation of an immortalized human stromal cell line, which contains cells able to differentiate into osteoblastic cells. Concerning the use of immortalized cells in ex vivo approaches for evaluating basic cell reactions, it is important to recognize that all cell lines have the disadvantage of having unique phenotypes, so that their morphological sensitivity toward a changing environment (material surfaces, external stimuli) is impaired. In addition to these cell lines, some researchers have used viral or nonviral vectors to modify the expression genes (eg, BMP-2, BMP-7) in various multipotential or determined cells (for review see Franceschi and associates[77,78]).

When the features of genetically altered cells are considered, it becomes obvious that nontransformed osteoblasts and osteoblasts from primary cultures are advantageous in extracorporeal tissue engineering, since these cells display a well-defined inverse relationship of proliferation and differentiation.[79] Measures of osteoblast-specific matrix protein expression define valuable reference points for the study of regulated osteoblast physiology, especially when a substratum-dependent reaction is under investigation. In addition, the use of primary and nontransformed cells is advisable for assessing cellular reactions in preclinical testing.

All in all, numerous cell populations from various sources exist, each with inherent advantages and limitations. Up to now determined cells have been commonly used in clinical cell-based engineering strategies; however, further investigations may demonstrate the clinical feasibility and applicability of other cell lines in bone tissue engineering. It is predicted that the use of stem cells, whether unrestricted or multipotential, will play a major role in the future of bone tissue engineering.

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