Tissue bioengineering in orthopedics

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Summary

The use of cells for the purpose of orthopedic tissue engineering started more than 300 years ago. The first attempt of bone grafting was reported in 1668 by the Dutch surgeon Job-Van Meek'ren. In 1867, Ollier performed a series of experiments using transplanted periosteum and concluded that transplanted periosteum and bone remained alive and formed new bone. The osteogenic potential of transplanted bone marrow was later documented by Goujon in 1869, then by Macewen in 1881. Efforts of Albee and Phemister highlighted further the utility of bone transplantation for the healing of fractures and bone defects. The techniques for autografting pioneered by these individuals remained largely unchanged until today. Advances in understanding of the biology of osteogenic cells, the availability of many highly purified peptide growth factors, and the capacity to create highly specialized implantable materials have launched an explosion of new advances in bone grafting and bone regeneration, all under the banner of tissue engineering. This new field is rapidly expanding the armamentarium of orthopedic surgeons in every setting in which bone healing is required. Composites of cells and matrices are at the core of this revolution.

KEY WORDS: bone; stem cells; tissue engineering.

Core concepts in tissue engineering of bone

The ultimate goal of bone tissue engineering is to create a bone-healing response in a precise anatomic area in which bone is desired. Clinical success further requires that the bone formed becomes integrated structurally with the surrounding skeleton and that the bone formed remodels reliably to provide the mechanical properties of load bearing and fatigue resistance necessary for durable and effective function.
There are several basic tenants of tissue engineering, but among the first principles is that all new tissue regeneration requires the presence of cells that are capable of forming the tissue desired. Using the example of bone formation, one must be confident that the site contains a sufficient number of osteogenic progenitors to produce the bone desired. A sufficient number of osteogenic progenitors may be present locally in many tissue sites. If the number of osteogenic progenitors is deficient, however, one must either engineer the site to provide the necessary population or use some stimulus to recruit the necessary population of cells to the site. Assuming that progenitors are present in sufficient numbers, several other objectives must be met. First, the necessary cells must be distributed throughout the grafted volume to ensure that the bone-healing response is contiguous and integrates with the adjacent skeleton. To accomplish this, the site must be filled with a matrix or material that facilitates the attachment, migration, and differentiation of osteoblastic progenitors, to allow the bonehealing response to progress throughout the site. A matrix or material having these properties is considered osteoconductive.
Given a sufficient competent cell population and the means of distributing the cells necessary for transducing the bone-healing response throughout the grafted volume, it is also necessary that the cells in the graft site receive stimuli that allow them to progress toward a bone phenotype. As discussed subsequently, the population of osteogenic cells is, in fact, a pleuripotent population, capable of differentiation along pathways leading to many other tissue types. As a result, signals from soluble growth factors secreted as autocrine or paracrine factors or stimuli from bioactive molecules contained in or on the surface of an implanted matrix can influence strongly the end result of tissue formation in a graft site. Many signals are present in the normal cascade of events in osteoblastic differentiation, which modulate proliferation, determination, and differentiation. As a group, the stimuli from these growth factors and adhesion molecules are termed osteoinductive stimuli.
Most of the graft materials that are currently in use possess one or more of these properties: osteogenic cells, osteoconductive matrix, or osteoinductive stimuli. These same properties can be found in a variety of synthetic materials and purified growth factors. Using these tools, it is the goal of tissue engineers to design combinations of existing materials, or new materials, that can be used clinically to create an optimal bone healing environment and grafting methods that equal or exceed the efficacy of existing methods. Such methods may be able to reduce the cost of existing procedures or allow entirely new approaches to treatment in some settings.
Before concluding a discussion of core concepts in tissue engineering, two other concepts, which are critical to the success of any bone-healing response, need to be mentioned: vascularity and mechanical stability. These two factors are largely in the hands of the surgeon who prepares the patient and the graft site for a procedure requiring bone tissue regeneration. Even in the setting of optimal materials combining osteogenic, osteoconductive and osteoinductive stimuli, the fate of cells in a graft site depends on preparation of the tissue bed to ensure a good blood supply and optimal preservation of local osteogenic cells through meticulous tissue handling. The vascular bed acts as a vehicle to deliver inflammatory cells, which secrete stimulatory factors. Vascularity also transports oxygen and nutrients to the repair site and acts as a

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conductive pathway for endothelial cells and osteoblastic progenitors. Patient management and patient selection also remain critical: specifically the need to optimize nutritional and health status preoperatively and to avoid pharmacologic agents that may inhibit the bone-healing response (such as nonsteroidal anti-inflammatory drugs, steroids, and nicotine) during the perioperative period.

The mechanical environment is also important to the success of the repair. The mechanical environment in a graft site is an important biologic stimulus, which, in part, determines the pathway of differentiation selected by cells in the graft site. For example, micromotion may direct local and chemoattracted connective tissue progenitors toward a fibrocartilaginous pathway, resulting in nonunion. Bone is favored in a site in which tissue strain is limited.

Using the overall conceptual framework just described, this article reviews the current concepts related to osteoblastic progenitor cells and osteoconductive matrices, used alone or in combination. A more detailed discussion of the concept of osteoinduction and the possible applications of osteoinductive materials in tissue engineering is presented in another article in this issue.

Designing composites of cells and matrices

Why consider cell-matrix composites?

All successful bone healing requires the presence of a sufficient number of osteoblastic progenitor cells. This concept is important because in many clinical settings the number of local osteoblastic progenitors may be limited. As a result, implanting an osteoconductive material alone or even an osteoconductive material with one or more osteoinductive growth factors may not be adequate to accomplish a reliable and optimal bone-healing response. Settings that are likely to be deficient in osteoblastic cells include sites of large bone defects, sites containing extensive scar tissue from previous surgery or trauma, sites of previous infection or radiation, sites in which local bone may be diseased, or sites with compromised vascularity. Systemic conditions, such as diabetes or metabolic bone disease, or pharmaceutical agents, such as nicotine, systemic glucocorticoids, or chemotherapy, may also limit the number or function of progenitor cells. These conditions and agents are common in clinical practice.

In contrast, in many clinical settings, there may be no need to deliver osteogenic cells. A satisfactory clinical result may be predictable, for example, when grafting of contained metaphyseal defects, fresh metaphyseal fractures, or other settings in which small-volume grafts are placed in healthy tissue beds. Even in these settings, however, addition of osteogenic cells may still be additive by increasing the rate of bone formation or the amount of bone formed.

Which cells should be included?

Given the several sources of osteoblastic cells and many available options for osteoconductive matrix materials, one of the next opportunities in bone tissue engineering is to optimize the combination of matrices and cells. Because it is the osteoblastic cell that does the work of bone formation, an appropriate first step in this process is defining the questions related to cell delivery. Do cells need to be added to the graft? If so, when? If so, how many cells are needed? Is it necessary only to add osteoblastic progenitors, or might addition of other cells contribute to the success of a graft? If so, what other cells?

Osteoblastic cells or osteoblastic progenitors are the obvious candidate for inclusion in a cell matrix composite for bone tissue engineering. Osteogenic cells alone may not be optimal, however. Under normal physiologic conditions, osteoblastic progenitors interact with many cells. Some of these interactions may be critical for optimal progression of bone regeneration. Platelets and growth factors secreted by platelets play a large role in establishing the biologic environment in an early fracture callus. Similarly, other selected cell types, including monocytes, T cells, and vascular endothelial cells, may be desirable partners for osteoblastic progenitors in an engineered graft site.

What source of cells should be used?

Any of the potential sources of osteoblastic progenitors discussed earlier could likely be used for bone grafting with similar efficacy. Of all of the available sources of osteoblastic cells for bone grafting, however, the only one that does not require an open surgical procedure or the added time, cost, and risk of growing cells *in vitro* is bone marrow harvested by aspiration. Bone marrow aspiration from the pelvis has little risk or morbidity. The principal disadvantage is that the bone marrow-derived osteoblastic progenitors harvested by aspiration are diluted markedly by peripheral blood, often 20 to 40 fold lower than the concentration present in autogenous cancellous bone. This dilution can be minimized by reducing the aspiration volume to 2 mL or less but cannot be eliminated.

Bone marrow-derived osteoblastic progenitors can be partly reconcentrated by selection of low-density cells using diffusion gradients or simple centrifugation to isolate a buffy coat. Experimental data indicate that methods of rapidly concentrating bone marrow-derived cells may improve the efficacy of cell matrix composites.

Alternatively, cancellous bone can be harvested by taking percutaneous core biopsy specimens with little morbidity. This approach has the advantage of maintaining the native concentration of osteoblastic progenitors and providing a small volume of matrix and other undiluted bone marrow cells. There are several disadvantages to this method of harvest, however. The volume of bone tissue available in this way is limited to a few cubic centimeters. There is greater risk of surgical complications and blood loss. Finally, one must still design a means of extracting the available osteoblastic progenitors from the bone obtained, then distributing them appropriately in the matrix to be implanted. The single cell suspension obtained from bone marrow by aspiration simplifies this last problem.

If large numbers of osteoblastic progenitors are needed, the use of *in vitro* culture and expansion of osteoblastic progenitors harvested from bone marrow is an available option. *In vitro* expansion of osteoblastic cells also opens up the opportunity to manipulate the cells obtained, using exogenous growth factors or by infection or transfection using naked DNA or using viral vectors1 to alter gene expression and presumably *in vivo* behavior after transplantation. These methods add significant expense to the preparation of a graft material, although such methods may be desirable or even critical for success in some settings.

Matrix, matrix structure, and surface chemistry

The concept of designing cell-matrix composites opens the opportunity to design or refine matrix materials to function specifically as delivery systems for transplanted osteoblastic progenitors. Potential matrix substrates include processed allograft bone, matrices formed from purified collagen or hyaluronic acid, synthetic polymers, calcium phosphate ceramics, and bioglasses. These materials and associated surfaces are likely not yet optimal as osteoconductive or osteoinductive materials. Improvements or design of new materials require specific chemical surface modifications, for example, addition of specific surface coatings to optimize biologic performance, such as adhesion molecules, or addition of defined growth factors linked to the matrix surface.

Cell density, diffusion, and cell survival

Optimal transplantation of cells into a graft bed requires knowledge and control of the relationship between the density of cells transplanted, the capacity of nutrients to diffuse through the matrix,

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and how these variables affect the outcome at the graft site. Because the initial survival of any transplanted cell depends on diffusion of nutrients and oxygen through the matrix, the porosity, pore size, and pattern of connectivity within the matrix become more important. This situation is particularly true as the volume of the graft increases, producing a longer diffusion path from the perimeter to cells at the center of a graft site.

The density of cells transplanted into a graft site is closely related to the problem of diffusion. Any viable cells transplanted into a graft site create a metabolic demand and compete with surrounding cells in the site. This situation inevitably lowers local oxygen tension, decreases pH, and reduces the availability of glucose and other nutrients. As a result, tissue engineering of larger-volume grafts likely requires not only transplantation of a sufficient number of selected cells but also may require means of limiting the transplantation of cells that are not critical to the end result. How many cells can or should be transplanted per unit volume and still contribute to an improved end result? This number depends on the graft setting, the matrix, and the metabolic demand of the cells being transplanted and needs to be examined experimentally in carefully designed and controlled *in vivo* experiments.

Conclusions

The ultimate goal of the orthopedic tissue engineer is to augment the body's repair mechanisms to stimulate the repair or regeneration of viable remodeling bone tissue. Improving knowledge of the biology of osteogenic cells and ability to harvest and manipulate these cells presents clinicians with the opportunity to harness capacity of these cells for targeted regeneration and repair of skeletal tissues. The attachment, migration, proliferation, and differentiation of these osteogenic cells can be influenced and modulated by selected bioactive molecules. The capacity to design specialized matrices that act as conductive scaffolds with defined structures, customized surface chemistries, and controlled degradation properties creates further opportunities to optimize the delivery and transplantation of highly selected osteogenic regenerative cells. Realization of the full potential of engineered matrix materials and cell-matrix composites can be expected to provide new and currently unavailable solutions to many clinical problems in skeletal reconstruction. These solutions should improve the quality of clinical outcomes and reduce the effective cost of care in many settings.

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