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Perspective

Bone voyage: An expedition into the molecular and cellular parameters affecting bone graft fate

Abstract

The demand for bone grafts in orthopaedic and craniofacial surgery is steadily increasing. Estimations suggest that about 500,000 are performed annually in the United States that include bone grafting as a component of the surgery, and the majority of these surgeries employ autografts. This perspective focuses on the biological events that occur during osseointegration of such bone grafts. Here, three key factors of graft osseointegration – the embryonic origin, the inclusion of skeletal progenitor cells, and the integrity of the recipient site – are discussed. Altogether, they form the foundation for survival of the bone graft and eventually for a positive clinical outcome of the procedure.

Keywords: Osteoprogenitor cell; Osteoblast; Transplant; Bone regeneration

Introduction

When the skeleton sustains damage, the basic challenge that faces the host is to perceive the injury and then repair the defect as quickly as possible. This is accomplished by recruiting skeletal progenitor cells to the injury site and by stimulating their proliferation. Once a sufficient population has been generated, the next task is to decelerate proliferation and induce differentiation of progenitor cells into osteoblasts. In most cases, this series of events proceeds unimpeded. There are, however, situations where bone repair is delayed or altogether arrested, cases where disease has left behind a cavity that weakens a skeletal element, or scenarios where degenerative processes compromise the stability of a joint. Orthopaedic and trauma surgeons can attest to the fact that these situations are not as infrequent as one would hope [1,2], and they must oftentimes resort to additional treatments to augment or accelerate bone regeneration. For these cases, the most frequently employed course of action is bone grafting. For example, 8% of all fractures, 7% of spinal disorders, and an astounding 70% of benign tumors require some sort of bone grafting procedure [3–5].

Most bone grafts performed today are autologous; that is, they are derived from a patient's own skeletal tissues. While there are certain advantages to this source of bone graft material (the most obvious being the lack of any immunogenic response), there are also obvious disadvantages. For example, if the patient has an underlying disease state that compromises their skeleton, clinicians are oftentimes reluctant to use autologous sources. There are other potential complications as well: donor site morbidity, risk of infection, and inadequate bone stock are frequently cited reasons for turning to other sources of bone graft material. Allogeneic grafts (a.k.a. allografts) are derived from cadaveric sources and can be obtained in any amount or shape [6,7] but their obvious shortcomings include the lack of skeletal progenitor cells in the graft material, and the potential for an immune reaction [8]. For these reasons, clinicians typically turn to autologous sources. But bone grafts are not always as successful as they should be, or that we hope they will be, and it is this pertinent issue which will be the focus of our perspective. We begin with a seemingly simple inquiry: just where does the graft go, when the bone grafting is done?

What is in a graft?

The fate of the graft depends in large part on which component of the transplant you are interested in. Mineralized matrix is always incorporated in a bone graft but to varying amounts depending on the origin of the graft. For example, when the graft is taken from the bone marrow cavity then small trabeculae are incorporated into the transplanted tissue. When mechanical stability is required then the matrix is comprised of whole cortical bone [5]. Experimental data seem to indicate that transplanted mineralized matrix is largely resorbed through osteoclastic activity and then replaced by new bone [9].

In addition to transplanted mineralized matrix, autologous bone grafts contain cells. Some of these cells are from the periosteum and endosteum that adheres to the transplanted bone, others are osteocytes embedded in the transplanted bony matrix, and still other cells are derived from the bone marrow. These

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tissues − the periosteum, endosteum, and bone marrow − are thought to be sources of skeletal progenitor cells [10–12], but which is the richest source is entirely a matter of speculation.

We have a few ideas − but almost no data − about what actually happens to skeletal progenitor cells after transplantation. Grafted cells may differentiate into osteoblasts at the site of injury, and therefore contribute to the regenerate [3]. Alternatively, grafted cells may be the source of osteoconductive or osteoinductive signals, which in turn recruit host progenitor cells from the surrounding tissues to differentiate into osteoblasts. Both remain viable hypotheses, but since clinical success of a graft is judged by radiographic appearance [13], esthetic criteria, or functional readouts such as the ability to masticate [14], the fate(s) of grafted tissues and cells are rarely investigated. Since we do not know the ultimate fate of these putative skeletal progenitor cells contained within the graft, the next question is harder to address: can the osteogenic potential of a graft be enhanced?

Exogenous factors: can they enhance graft osseointegration?

A number of strategies have been employed to improve the osteogenic capacity of bone grafts. For example, bone marrow aspirates or platelet-rich plasmas are oftentimes added to the graft material in hopes of increasing the number of skeletal stem or progenitor cells [15,16]. When bone marrow or platelet-rich plasma augmentation is contraindicated (usually because of an underlying disease state) surgeons may resort to the addition of growth factors. Two classes of growth factors that are considered for this function include bone morphogenetic proteins (BMPs [17–19]) and vascular endothelial growth factor (VEGF [20]). Precisely how these growth factor additions affect the behavior of the graft, however, is unclear. Does the exogenous growth factor act on cells within the graft, and either induce their proliferation or stimulate their differentiation into osteoblasts? As their name implies, BMPs were at one time thought to stimulate the differentiation of cells into osteoblasts [21] but that function has been called into question more recently. And although VEGF is widely recognized as an angiogenic factor [22–24], VEGF receptors including Flk, Flt, and the neuropilins are expressed by osteoblasts and osteoblast precursors [25].

Rather than acting directly on cells in the graft, another possibility is that exogenous growth factors may act in an osteoinductive manner by recruiting host cells to the recipient site. Growth factors added to a bone graft might also induce neoangiogenesis, and indirectly induce bone formation by host cells. Remarkable as it sounds, there are almost no data that directly address how exogenous growth factors affect the function of a bone graft. Once again, since the success of a bone graft is typically judged by clinical readouts such as radiopacity or joint stability, we can only guess as to what actually happens when growth factors are incorporated into a bone graft. In the last few years, a number of new techniques have been perfected that allow researchers to permanently label cells prior to transplantation [26]. What this means is that bone grafts could be performed with these labeled cells, and then the fates of the cells could be assessed at multiple time points following grafting. The ready availability of multiple non-invasive imaging methods (i.e., CCD cameras, MRI [27]) means that the survival, proliferation, and differentiation of engrafted cells could be assessed. Until now, however, these novel cell labeling techniques and innovative imaging modalities have not been exploited to answer these questions.

We have discussed the inclusion of growth factors that may enhance the osteogenic potential of a bone graft, but there may be intrinsic differences in the various tissues that are incorporated into bone grafts. For example, the embryonic origin of the skeletal progenitor cells contained within the bone graft, or the response of skeletal progenitor cells to mechanical stimuli may influence the success or failure of a graft. Some of these potential differences have been suggested by *in vitro* studies that explore the behavior of osteoprogenitor or skeletal progenitor cells, but only a few have addressed this question using *in vivo* models. In the following paragraphs, we summarize this limited literature and suggest areas where research may provide some much-needed clues into how bone grafts succeed, or equally important, why they can fail.

Graft sources: all bones are not created equal

Whether it is the coccyx or the crista galli, visual inspection of the skeleton will lead an astute observer to conclude that all osseous tissues look remarkably similar (Fig. 1). This summation will be bolstered by histological analyses, which show equivalent staining of mineralized tissues in the head, the limbs, and the spine (Fig. 1). Even molecular analyses indicate that, once cells commit to a chondrogenic or osteogenic lineage they differentiate using the same molecular machinery [28].

Given these indisputable observations, is there any reason to suspect that skeletal progenitor cells derived from the facial skeleton are different from those that are derived from the appendicular (limb) skeleton? A number of investigators have (indirectly) addressed this question and when considered together, their data support the hypothesis that neural crest- and mesoderm-derived cells represent unique populations of skeletal progenitors (reviewed in [11,12,29]). If the skeletal progenitor cells from these two sources are unique, then presumably grafts derived from the two sources will also be distinctive with regards to their regenerative potential.

The first unique feature among osteoblasts from various parts of the body is their response to mechanical stimuli [11,30], which might be anticipated based on the weight-bearing function of the appendicular and axial skeletons relative to the cranial skeleton (reviewed in [31]). Even within a single skeletal element, however, periosteal osteoblasts are dissimilar from endosteal osteoblasts, both in terms of their response to molecular signals and their response to mechanical forces (reviewed in [32–35]). For example, when skeletal injury sites are subjected to mechanical forces, the periosteum reacts by forming endochondral bone, while the endosteal osteoprogenitor cells directly differentiate into matrix depositing osteoblasts [36,37]. The different fate decisions of osteoprogenitor cells occurred at similar strain levels, indicating that these cell types respond to mechanical stimuli in a characteristic manner that is governed by their endosteal or periosteal origin.