Osteoconductive carriers for integrated bone repair

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Symposium

Symposium on osteoconductive carriers

Biologics are an increasingly important area of interest to spine surgeons throughout the world. As we search for improved osseous healing in our spinal fusions and explore the efficacy of the biological products available, we need to have a basic understanding of the information available on each class of product and how they may work so that we can optimize the desired effects in our patients. As scientists, we need to look beyond the advertising and marketing descriptions of each product and turn to the literature to properly evaluate the data and determine the best way to utilize them for our patients. It is also important to understand the mechanisms of action of each class of biologics so that we can implement the proper usage in the correct clinical scenarios and achieve the optimal benefits.

In this issue, we present a symposium looking at some osteoconductive biologics and their mechanisms of action. In the first discussion, we find an excellent basic review of the overall field which will hopefully give the readers a strong foundation for the class of products, and explain why they are important and how they act at a basic level in biologic environments where bone growth is desired. The second discussion presents the history and present state of one class of osteoconductive materials, which will serve as a general overall update in the area. Our third and final discussion is a specific study in the cervical spine of one biologic material. I hope the reader finds this to be a nice overall symposium on this focused area, and I would like to congratulate the authors for contributing to this valuable discussion.

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Osteoconductive carriers for integrated bone repair

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Abstract

Successful bone repair is judged in achieving restitution of space and mechanical integrity, and in regaining function. When the biology or anatomy are insufficient to attain a full repair, therapeutic use of graft material has been used to omit compliance features such as strain tolerance, reduced stiffness, and attenuated strength, and instead promote primary or membranous-type bone formation within the physical approximation of a graft material. The challenge of most conductive materials is that they emerge from a static platform and in placement force the living system to adapt to placement, dimension, different properties, and eventually are only successful in degradation and replacement, or in integration. The synergy and interdependency between adhesion, ECM, and proteolysis are important concepts that must be understood to engineer scaffolds capable of holding up to standards which are more than cell decoration. Moreover, the reactive specificity to loading, degradation, therapeutic delivery during absorption remains a key aim of both academic and industrial designs. Achieving conductivity comes with challenges of best fit integration, delivery, and in integrated modeling. The more liquid is the delivery, the more modular the components, and adaptive the matrix to meeting the intended application, the more likely that the conductivity will not be excluded by the morphology of the injury site. Considerations for osteoconductive materials for bone repair and replacement have developed conceptually and advanced parallel with a better understanding of not only bone biology but of materials science. First models

of material replacements utilized a reductionist-constructionist logic; define the constituents of the material in terms of its morphology and chemical composition, and then engineer material with similar content and properties as a means of accommodating a replacement. Unfortunately for biologic systems, empiric formulation is insufficient to promote adequate integration in a timely fashion. Future matrices will need to translate their biological surfaces as more than a scaffold to be decorated with cells. Conductivity will be improved by formulations that enhance function, further extended from understanding what composition best suits cell attachment, and be adopted by conveniences of delivery that meet those criteria.

Keywords: Bone repair; Grafting material; Geometric fidelity; Regenerative capacity; Bio-instructive

Surgeons have applied the principles of tissue engineering for years; they transplant and shift bone and other tissues within a patient to promote regenerative potential. The advent of technology for fabricating structures (eg, matrices, TCP bone fillers, etc.) with geometric fidelity, compositional consistency, and tissue-specific identity offers further promise that regenerative potential of tissue and whole organ systems can be achieved. While attaining scar tissue might be sufficient for soft tissue applications such as skin or in muscle or tendon, achieving an outcome in most orthopedic indications that is not mechanically solid and weight-supporting would be insufficient.

Implicit in the strategy of repairing bone is a need to gain restitution of space, achieve mechanical integrity, and regenerate functional continuity. When the biology or anatomy are insufficient to attain a full repair, therapeutic use of graft material has been used to omit compliance features such as strain tolerance, reduced stiffness, and attenuated strength, and instead promote primary or membranous-type bone formation within the physical approximation of a graft material. In the broadest sense, 3 basic components are required of a graft: osteoprogenitor cells, osteoinductive factors, and an osteoconductive matrix or scaffold.

A more critical assessment would discriminate cell lineage and capacity for differentiation, define metabolic support with regard to defined vascular and immunogenic transparency, and evaluate material properties such as surface area-to-volume ratio, porosity, and modeling and degradation capacity during early integration. Any of these subcategories is subject to further reduction, and in the context of a short overview of appropriate carriers, this discussion has been limited to conductive properties that offer advantages for bone repair and might facilitate the delivery of cells, or mineral, or even consider cytokines for adjunctive intervention. Within the domain of osteoconductivity, shape, composition and matrix turnover are inextricable components.

The shape of the biomaterial template is critical to the success of an osteoconductive carrier. The essence of shape permeates more than just the 3-dimensional (3-D) form of the material and is part of the molecular domain as well. A central tenet of biomineralization is that nucleation, growth, morphology, and aggregation of the inorganic crystals of bone are regulated by organized assemblies of organic macromolecules. The close spatial relationship of hydroxyapatite crystals with type-I collagen fibrils in the early stage of bone mineralization is a relevant example. Hydroxyapatite is a natural mineral component of hard tissue, composing 60–70% of bone. It is also evident that combining hydroxyapatite with protein does not render the macroscopic form of bone nor impart its characteristic properties. Unlike fabricated materials that can be developed from components with predictable properties, biological systems control desired properties with an intrinsic rationale that discriminates essential from nonessential factors.[1] Living organisms avoid geometric randomness by segregating structures that resonate with function. Anatomical variations that do not result in significant input to the whole organism remain "neutral" with regard to selection pressures. Within the context of "more demand – more function" equilibrium is the eventual arbiter of change—biologic systems are not static but in a constant shifting response to their stimulation. To a large extent the symmetry of the stimulus imposes an order of stability.

The challenge of most conductive materials is that they emerge from a static platform and, in placement, force the living system to adapt to placement, dimension, and different properties, and eventually are only successful in degradation and replacement or in integration. Materials that have been developed for orthopedic applications and made available as grafting substitutes include cancellous and cortical allograft bone, ceramics such as sintered coralline matrices, hydroxyapatite and tri-calcium phosphate, demineralized bone matrix, bone marrow, composite polymer grafts, and recently various combination carriers endowed with growth factors. Complications include availability, cost, variable biological absorption profiles, brittleness, immune stimulation, as well as the economic reality of regulatory hurdle. Polymers, in and of themselves, constitute a nearly uncontainable universe of bone application potentials. Many natural and synthetic polymers have wide use in bone engineering and material development. Among the best known and characterized of the synthetic polymers are polycaprolactone (PCL), polyethylene glycol (PEG), poly(L-lactide) (PLLA), and polyglycolide (PGA) and copolymers such as poly(lactic-*co*-glycolic acid) (PLGA). Natural polymers such as collagen and hyaluronic acid (HA) also have been

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widely defined for potential use in bone applications. Early use of these polymer matrices was widely considered little more than place holders for cells, in essence armatures for placing cells and maintaining tissue dimension during the healing process.

In the context of understanding matrix-cell interaction, a demand for new and more sophisticated matrices has been fostered. Rather than merely defining a place for cells to rest, materials now play an active role in guiding tissue development. Although bone can appear de novo, it more often develops from accretion on a scaffold of matrix that contains appropriate vascular and compositional arrangement. As such, both 2-dimensional (2-D) and 3-D patterns have been shown to enhance osteoconductivity.[2] Bone has significantly more matrix than cells, and cell regulation through anchorage dependent mechanisms is an established premise.[3–5] Compensatory mechanisms for changing sensitivity to mechanical stimulation have been shown to undergo adaptive or kinetic regulation, likely tied directly to osteoblast attachment to immobilized molecules in the extracellular matrix (ECM). Extracellular matrix molecules promote cell spreading by resisting cell tension, thereby promoting structural rearrangements. Ideally, the evolution of new materials will provide more than a substrate affording tissue compatibility and define a scaffold that will be not only be structurally enhancing but conductively optimum for bone formation.

An early strategy for enhancing primary binding sites in bone tissue engineering was to include integrin polypeptide sequences in the backbone.[6] Integrins are cell-surface glycoprotein receptors which mediate interactions between similar and different cells as well as between cells and extracellular matrix proteins. These interfaces are involved in physiological processes, such as embryogenesis, hemostasis, wound healing, immune response, and formation/maintenance of the tissue architecture.[7–9] As has so often been the case with efforts to structure natural systems, further observation has resulted in better definition of the material needs.[10] The ECM serves a dual role to the extent that the provisional matrix must not only serve the foundation for bone repair but also mediate a biophysical barrier to prevent fibroblast invasion and generation of scar tissue.[11,12] Given the need for cell migration and cell situation in proximity to the area, one of the important challenges to achieve in optimizing matrix conductivity is understanding the pericellular environment and the regulation of proteases, vascular invasion, receptor specificity, and cell attachment and differentiation. The synergy and interdependency between adhesion, ECM, and proteolysis are important concepts that must be understood to engineer scaffolds capable of holding up to standards that are more than cell decoration. Moreover, the reactive specificity to loading, degradation, therapeutic delivery during absorption remains a key aim of both academic and industrial designs. Achieving conductivity comes with challenges of best-fit integration, delivery, and in integrated modeling. The more liquid the delivery is, the more modular the components are; and the more adaptive the matrix is to meeting the intended application, the more likely that the conductivity will not be excluded by the morphology of the injury site.

The concept of molecular self-assembly for the development of new biomaterials comes from close observation and modeling of events well known in biology.[13] In the most primal of function form, DNA sets a foundation that resonates in microfilament and microtubule assembly, in its self-complementary double-helix annealing, and in lipid membrane development. Self-assembling properties are also found in proteins that are critical to forming the extracellular matrix of connective tissues such as collagens, laminins, and fibronectins.[14–16] Within the assembled matrix, key sequences have been shown to promote cell adhesion, cell migration, endothelial cell monolayer development, and the inhibition of angiogenesis. Enrichment of sequences in defined and specific synthetic approaches has been shown to facilitate desired integration of cells to scaffolding.[17] Among the more known extensions of applied self-assembly potential has been the development of Matrigel (BD Biosciences, San Jose, California) by Hynda Kleinman at the NIH; countless studies have used the material as a standard for 3-D matrix studies.

Semino offers a review of future platforms for designer matrices.[18] With a strong basis founded in work with self-assembling hydrogels,[19–21] the sentinel element of future application promises the ability to extend structural and biomechanical similarities of matrices that additionally provide instructive capacity for cells as a regulatory template that specifies cell signaling. Most likely, the matrices and gels will need to play complementary roles—first optimizing the conditions for conduction of the proper cells to the repair site and, second, amplifying and possibly enhancing specific intentions in compromised patients.

One such application might be in consideration of patients who use tobacco. Evidence of nicotine interfering with bone healing in the spine is well known.[21–23] With knowledge of an endothelial nicotinic acetylcholine receptor (nAChR) being instructive to endothelial proliferation, survival, migration and tube formation in vitro, it might be possible to fine-tune the hydrogel or self-assembling matrix to block exogenous nicotine receptors that retard this angiogenic pathway. Traditional drug delivery systems have been based on synthetic polymer materials, or animal-derived collagen, which may contain residual growth and/or viruses from animal tissues. Peptide hydrogels are ideally suited for drug delivery as they are pure, easy to design and use (eg, non-toxic, nonimmunogenic, bio-absorbable), and can be applied locally to a particular tissue.

Since Zhang first discovered peptide hydrogels in the early 1990s, numerous applications have been developed that show promise in regenerative medicine.[24] Composed of self-assembling amino acid chains (peptides), the gel is about 99% aqueous by volume and offers a deliverable, low-viscosity solution for reaching difficult anatomy. Cou-

pled to needed flow and deliverability qualities and to the formulation of the lattice hydrogels, it is possible to deliver molecules specific to accentuating conductivity, reducing potential inhibition, and exaggerating the biophysical properties of matrix. These gels can be chemically engineered to release proteins from the gel over hours, days, or even months, and the gel itself is eventually broken down into harmless amino acids, which are the building blocks of proteins. While not offering the initial strength needed for weight bearing, important aspects of conductivity needed to support the repair and integrated regenerative modeling are found.

A potential to exploit the instructive capacity of the hydrogels in conduction with polymerizing, or even physically-static, conductive scaffolds seems to offer opportunity for synergism. Critical to therapeutic adoption for bone repair products are attributes such as immediate mechanical properties, functional biological activity during integration that does not weaken the construct, and a resorption profile that is metabolically benign. Additionally, each therapeutic application must consider the trade-off gained in attaining a tight apposition to the walls of the defect thus achieving mechanical solidarity and avoiding structural gaps in the delivery that might predispose the repair to fibrous interposition with a safe, with off-the-shelf, cost-effective application.

One interesting material in early stages of development is an osteconductive calcified triglyceride with remarkable bone-like mechanical properties.[25] Initially a liquid created by combining fatty acids and calcium carbonate, the material is touted as an isothermal, non-toxic alternative to methyl methacrylate. When used as a bone void filler, carbon dioxide generated during the curing process extends a porosity that supports bone integration, and material adhesive qualities stabilize the now-filled margins of the defect at the interface of the native tissue. Although adhesive, the material is readily molded to any shape. As formulated, the material is conductive, resorbable, has an isothermal curing temperature, and during consolidation achieves porosity similar to that of bone. More critical to the adoption may be the fact that block material achieves similar mechanical characteristics to bone within 24 hours.

Future matrices would seem to benefit from the synergy gained by incorporating amphiphilic hydrogel moieties that support appropriate cell differentiation within the structural sufficiency provided by the solid material. Secondary domains of improvement could include the addition of stem cells into the matrix. As a first consideration, sourcing autologous adult stem cells to support regeneration and integrate bone voids would provide appropriate cells in conjunction with the osteoconductive matrix. One of the key values in this pairing is to provide inflammatory insulation gained by the use of stem cells during the repair process and hasten the conversion of matrix. Gains made in immune modulation are particularly important in building skeletal material, where the need to sustain weight-bearing support is critical to stabilizing the repair.

Considerations for osteoconductive materials for bone repair and replacement have developed conceptually and advanced parallel with a better understanding of not only bone biology but of materials science. First models of material replacements utilized a reductionist-constructionist logic: define the constituents of the material in terms of its morphology and chemical composition, and then engineer material with similar content and properties as a means of accommodating a replacement. Unfortunately for biologic systems, empiric formulation is insufficient to promote adequate integration in a timely fashion. Early hydroxyapatite formulations required years to remodel, and rather than providing an embracing source of mechanical similarity, they challenged the body to overcome the insulation of difference instead of integrating the interface.

Bone conduction and a better understanding of materials enhancing skeletal repair has evolved from scalar understanding as a science in translational medicine. Knowledge of how cells attach to matrix remains a distinct part of the continuum recognizing how tissues respond to force and have optimized insight into what drives the biological assembly of tissues. That concept, in turn, reflects the cell mechanics of what is required to neutralize strain and necessary for the body to adapt a neutral biology. Commercially successful osteoconductive matrices will still need to provide sufficient mechanical stability for skeletal repair. Moreover, future matrices will need to translate their biological surfaces as more than a scaffold to be decorated with cells. Conductivity will be improved by formulations that enhance function, further extended from understanding what composition best suits cell attachment, and be adopted by conveniences of delivery that meet those criteria.

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A brief summary of 15 years of research on beta-tricalcium phosphates

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Porosity enhances cellular ingrowths and bone tissue formation but impairs the mechanical strength of calcium phosphate ceramics.[1] We have measured the compressive strength of hydroxyapatite (HA) ceramic with increasing porosity rates 20–60% and pore size 5–400 nm. After mathematical reconstruction of the data, the results showed that both the total porous volume and pore size of the ceramics influenced the mechanical strength, and that appropriate control of these characteristics allows for designing calcium phosphates implants with suitable mechanical strength and bone ingrowths capacities for bone grafting, even in load bearing applications.

In the early 90's, very little clinical data was available on β-tricalcium phosphates (β-TCP) ceramics as bone substitutes.[2] Considering 2 groups of patients, we have evaluated the quality of fusion with β-TCP (50% porosity) versus allograft (cortico-cancellous chips) in postero-lateral fusion from a consecutive series of 54 idiopathic scoliosis by means of clinical and radiological evaluation over a period of up to 4 years. Tricalcium phosphates resorption was total after 2 years, while allograft fragments were visible on x-rays at the same delay. Loss of correction was 8% in the allograft group and 2% in the TCP group. Loss of correction did not progress after 6 months in the TCP group and after 2 years in the allograft group. These findings supported the clinical use of TCP ceramics in posterior lateral (PL) grafting and confirmed absorption of the material over time.

β-TCP ceramics promote bone healing and are absorbed over time by surrounding cells and tissues.[3] What is the fate of the ions dissolved from the material in vivo? We have implanted nuclear radioactivated β-TCP ceramics (50% porosity) in the femoral condyle of rabbits for 1, 3, and 9 months and the implants were studied using histology, histomorphometry, and radio counting (autoradiography) techniques. Over time, the pores were invaded by connective then bone tissue (80%) and resorption of the implant (40%) was visible both at the outer surface and inside the pores. Bone formed inside the pores was radioactive and the radioactivity measured was consistent with the theorical mineral content of woven bone formed in the early stages of endochondral ossification, suggesting that the ions dis-

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