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Gap Junction Intercellular Communication: A Review of a Potential Platform to Modulate Craniofacial Tissue Engineering

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Abstract

Defects in craniofacial tissues, resulting from trauma, congenital abnormalities, oncologic resection or progressive deforming diseases, may result in aesthetic deformity, pain and reduced function. Restoring the structure, function and aesthetics of craniofacial tissues represents a substantial clinical problem in need of new solutions. More biologically-interactive biomaterials could potentially improve the treatment of craniofacial defects, and an understanding of developmental processes may help identify strategies and materials that can be used in tissue engineering. One such strategy that can potentially advance tissue engineering is cell–cell communication. Gap junction intercellular communication is the most direct way of achieving such signaling. Gap junction communication through connexin-mediated junctions, in particular connexin 43 (Cx43), plays a major role bone development. Given the important role of Cx43 in controlling development and differentiation, especially in bone cells, controlling the expression of Cx43 may provide control over cell-to-cell communication and may help overcome some of the challenges in craniofacial tissue engineering. Following a review of gap junctions in bone cells, the ability to enhance cell–cell communication and osteogenic differentiation via control of gap junctions is discussed, as is the potential utility of this approach in craniofacial tissue engineering.

Keywords

bone tissue engineering; gap junctions; connexin; cell-cell communication; bone marrow stromal cells

INTRODUCTION

Defects in craniofacial tissues, resulting from trauma, congenital abnormalities, oncologic resection or progressive deforming diseases, present a formidable surgical challenge, and restoration of these tissues is a subject of active clinical concern.[1–3] In addition to leaving patients with aesthetic deformities, craniofacial defects may be uncomfortable to the patient and directly affect function. Thus, structure, function aesthetics and pain must all be managed effectively, resulting in a formidable challenge. In addition to problems associated with cranial and facial tissues, 15% of the US population has periodontal disease severe enough to warrant surgery.[1] It is further estimated that 9–15 million people in the US experience temporomandibular joint (TMJ) disorders,[4] and more than 10% of the US population experiences jaw or facial pain due to TMJ disorders or osteoarthritis, with an average of 1–2 work days/month lost.[4]

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In many cases, tissues of the TMJ also need to be repaired or regenerated because of structural deficiencies.[5] There is, however, inadequate guidance regarding patient-selection criteria for these procedures.[6] For cases involving TMJ restoration, there are several neural and vascular structures near this joint that may become compromised, causing joint pathology, and adding complexity and risk to the surgery. Surgical treatment of TMJ, periodontal, and other craniofacial defects is therefore not predictable and does not fully restore function to the tissues in all cases.[1] Collectively, therefore, defects associated with orofacial tissues may result in aesthetic deformity, pain and reduced function, and represent a substantial clinical problem in need of new solutions.

Techniques to repair orofacial skeletal defects parallel the accepted surgical therapies for bone loss elsewhere in the skeleton, and include autografts,[3,7,8] allografts,[7,8] and synthetic materials.[8–11] Each of these reconstructive and/or regenerative strategies, however, has limitations and lacks clinical predictability. Only a minimal amount of tissue can be harvested for autografts, the harvesting procedure may lead to donor site discomfort and morbidity, and it may be difficult to form this tissue into desired shapes, particularly in the craniofacial region.[12,13] Autografting, the current “gold standard” for bone regeneration, has failure rates as high as 30%.[12] Allografts have the potential of transferring pathogens.[3,14] Freeze-drying, demineralization, and irradiation, which reduce immunogenic potential, can also reduce structural integrity, leading to graft fracture.[3] Other complications with autografts and allografts include unreliable incorporation, resorption, and non-union of the graft/bone interface.[12–15] Induction of new bone by growth factors requires large amounts of recombinant material, which may not be realistic in cases of massive defects.[16] Additionally, successful use of growth factors relies on the presence of a sufficient population of undifferentiated progenitor cells capable of responding to the inductive cues provided by the growth factor.[17] Synthetic materials are primarily designed to be permanently implanted. Long-term complications include stress shielding, leading to loosening, and mechanical or chemical breakdown of the material itself.[8–11] Complications with synthetic materials are especially well documented for TMJ prostheses.[4] Demographics on total joint replacements, such as TMJ replacements, indicate that 25% of the more than 200,000 procedures performed each year are revisions.[18] Many TMJ patients have had multiple surgeries, and the greater the number of surgical procedures performed on the TMJ, the less the chance for improvement.

Of particular importance with the use of synthetic materials is that most problems manifest themselves at the biomaterial/tissue interface, in part because the tissue has the ability to functionally adapt, whereas the synthetic material does not. More biologically-interactive biomaterials could potentially improve the clinical treatment of craniofacial defects.

A more biological alternative to the permanent implantation of synthetic materials is a cell transplantation approach where a three-dimensional natural or synthetic construct provides a temporary substrate for cells to organize, grow, differentiate, and form a functional extracellular matrix and new tissue.[10,11,19,20] Bone regeneration is a complex process that requires autocrine, paracrine, and endocrine signals, positional cues, cell–matrix interactions, mechanical forces and cell–cell contacts to mediate the formation of a complex 3D architecture and function. Therefore, an understanding of developmental processes may help identify strategies that can be used in tissue engineering.

One aspect of developmental biology that could potentially be controlled to advance the engineering or repair of tissues is cell–cell communication. Cell-to-cell communication occurs via intercellular chemical and mechanical signals and is critical to maintain tissue homeostasis.[21,22] Gap junction intercellular communication (GJIC) is the most direct way of achieving such signaling, and is particularly important to maintain synchronized and cooperative behavior of cells in three-dimensional tissue.[23,24] Gap junction communication through connexin-

mediated junctions, in particular connexin 43 (Cx43), plays major and diverse roles bone development. Because of the ubiquitous nature of Cx43 throughout vertebrate cell types, with the exception of red blood cells, platelets, some neurons, and spermatozoids,[25] this protein provides a signaling platform that enables communication between similar cell types and also between different cell types.[26] Such an attribute makes connexins natural conduits for communication between osteogenic cells to enable proper bone modeling and re-modeling. Controlling the expression of Cx43 may provide control over cell-to-cell communication and may help overcome some of the challenges in craniofacial tissue engineering.

This review examines the roles of gap junctions, in particular Cx43-mediated gap junctions, in the function of osteoblasts, osteocytes, osteoclasts, and bone marrow stromal cells (BMSCs), and the role of gap junctions in cell–cell communication and bone formation. The ability to enhance cell–cell communication and osteogenic differentiation via control of gap junctions is discussed, as is the potential utility of this approach in craniofacial tissue engineering.

GAP JUNCTIONS AND BONE

Gap junctions are present in all types of vertebrate cells, with the few exceptions of red blood cells, platelets, and some neurons noted above.[25] This ubiquity makes it reasonable to consider gap junctions as a fundamental structure necessary for normal cell function.[27] As one example, gap junction communication plays a critical role in osteoblast differentiation. During osteoblastic differentiation, the expression of Cx43 increases and correlates with an increase in GJIC.[28] When GJIC is inhibited, delayed differentiation is observed, as well as a reduced ability to form a mineralized extracellular matrix.[29]

Composed of two juxtaposed hemichannels present on the surfaces of adjacent cells, gap junctions form a trans-cellular channel that permits the rapid and efficient propagation of ions, metabolites, and second messengers between adjoining cells. Each hemichannel, termed a connexon, is composed of six transmembrane proteins, termed connexins (Figure 1). Each connexin monomer is a polypeptide consisting of four transmembrane domains (two extracellular loops, one intracellular loop, and intracellular carboxylic and amino ends).[25] Each intercellular channel provides an aqueous pathway for the passage of intracellular ions and small molecules. Gap junction channels are permeable to ions, fluorescent dyes, and physiologically active molecules including amino acids, second messengers, and small peptides.[30,31] Twenty connexins encoded by a multigene family have been identified.[32] An interesting aspect of the various gap junctions is their apparent differences in perm-selectivity (molecular permeability and ion selectivity). Connexins play a major role in response to many mechanical, electrical, chemical and hormonal stimuli, and help regulate cell homeostasis as well as calcium signaling and differentiation.[33–35]

Of the 20 known connexins, only Cx43, Cx45, and, to a lesser extent, Cx46 exist in bone cells. The primary gap junction in bone is Cx43.[27,36,37] Cx45 gap junctions form smaller pores, only allowing diffusion of molecules < 0.3 kD in molecular mass (compared with < 1.2 kD for Cx43), and have poor transfer of solutes.[27,38] Cx45 gap junction channels possess relatively small and fixed conductances for gap junction channels.[38] It has therefore been suggested that Cx45 plays a smaller role in GJIC, relative to Cx43.[39] Cx46 is found in the cytoplasm of osteoblasts, but does not incorporate into the membrane, and therefore does not form gap junctions.[40] Cx43 produced gap junctions exist between osteoblasts as well as between osteocytes and osteoblasts. The extensive network formed by osteocytes is also dictated by their gap junctions.

Osteoblasts

Osteoblasts are the cells responsible for bone formation, originating from mesenchymal multipotent stem cells resident in the bone marrow.[41,42] Osteoblasts work in a coordinated fashion to produce the lamellar structure of bone by secreting collagen and non collagenous proteins such as osteopontin, osteonectin, biglycan, decorin, and osteocalcin, that serve as a template for controlled mineralization. Osteocalcin constitutes 10–20% of the noncollagenous matrix proteins. It changes its conformation in the presence of calcium, binds strongly to hydroxyapatite, and inhibits the formation of hydroxyapatite from amorphous calcium phosphate. Although the *in vivo* function of osteocalcin is unknown,[42] its affinity for bone mineral constituents and correlation to bone volume density implies a role in bone formation.[43]

Osteoblastic cells respond to many stimuli, including hormones, growth factors and mechanical loading.[42,44–46] Gap junction communication also plays a prominent role in the differentiation and function of these cells and their response to stimuli. The most studied skeletal cells with regard to gap junctions are osteoblasts. The role of gap junctions in osteoblasts has been demonstrated by electron microscopy,[47,48] histology[49,50] and dye transfer studies.[51] During osteoblastic differentiation, the expression of Cx43 increases and correlates to an increase in GJIC.[28] When GJIC is inhibited, delayed differentiation is observed as well as a reduced ability to form a mineralized extracellular matrix.[29]

Osteocytes

An ostoecyte is formed when an osteoblast becomes trapped in the matrix it secretes. Osteocytes form an extensive network throughout bone and are connected in the canaliculi via gap junctions.[27,41,48] The main role of gap junctions in osteocytes has long been hypothesized to be the diffusion of signals, such as calcium, after the osteocyte responds to mechanical perturbation. Both fluid flow and mechanical perturbation elicit communication responses through osteocyte gap junctions.[52,53]

Osteocytes are hypothesized to regulate bone anabolic functions via coordinated signaling among bone cells. Such signals originate from osteocytes, and are passed along to other osteocytes, as well as to osteoblasts by gap junctions. The mechano-sensing role of osteocytes plays a critical role in remodeling,[54] and fluid flow or mechanical perturbation can induce the opening of Cx43 channels in osteocytes.[52,53]

Osteoclasts

Osteoclasts are the cells responsible for bone resorption. They originate from precursors within the monocyte/macrophage system and arise from hematopoietic mononuclear cells in bone marrow. Osteoclasts are multinuclear cells and have a morphology highly specialized for bone resorption.[55] The actively resorbing osteoclast has a specialized area facing the bone surface. Bone resorption takes place upon osteoclast activation following signals from osteoblasts and their precursors. The receptor activation of RANKL is partly responsible for the differentiation of progenitors into osteoclasts.[55,56]

Osteoclasts are the least studied bone cell type with respect to gap junctions. Osteoclasts also express Cx43,[50] with both the formation of these cells and communication with other cell types dependent on this expression.[57] A role for Cx43 in the fusion of the monocyte like precursor cells into (multinucleated) osteoclasts has been suggested.[57] Treatment of osteoclasts with gap junction inhibitors reduces the number of osteoclast-like cells,[57–59] while increasing the number of non-fused precursor cells. Furthermore, the osteoclasts that are formed show reduced ability to resorb bone.

The typical effect PTH and Vitamin D have on stimulating osteoclast activity is inhibited by blocking gap junctions.[57–59] PTH can have anabolic or catabolic effects on bone. When administered intermittently, PTH leads to increased bone formation.[60] However, chronic administration of PTH stimulates the generation of new osteoclasts (osteoclastogenesis) and leads to an increase in osteoclast number and activity.[61] Because of coupling between osteoclasts and osteoblasts, anabolic effects of PTH emerge from a single action, the stimulation of osteoclastic bone resorption. As examples of how blocking gap junctions may potentially play a therapeutic role and affect bone regeneration, an upregulation of Cx43 mRNA has been detected in PTH-stimulated bone resorption, but when gap junctions are blocked, PTH and 1,25-$(OH)_2D_3$ stimulated osteoclast pit formation are inhibited.[62] On the other hand, a lack of Cx43 in osteoblasts leads to suboptimal acquisition of peak bone mass, and hinders the bone anabolic effect of PTH.[63]

Bone Marrow Stromal Cells

Bone marrow is where hematopoiesis occurs in close contact with the stromal microenvironment, which supports hematopoietic stem cell growth and differentiation. The bone marrow stroma provides structural and functional support for hematopoiesis and is composed of a variety of cell types with stem cell like characteristics that can differentiate into bone, cartilage, adipose tissue and hematopoietic support tissue.[64] These cells are self-renewing and highly proliferative. The marrow cavity serves as a critical domain for progenitor cells and interactions between bone and stem cell populations.[65] BMSCs, in particular, provide the essential support for hematopoiesis through both direct contact with cell surfaces and stromal cell derived soluble mediators. BMSCs are essential for the maintenance of bone turnover throughout life, as progenitors for endosteal osteoblasts. Bone has been generated *in vivo* following transplantation of these cells.[66–68]

The maintenance of BMSC stem cell properties and the possibility to reprogram their commitment is of primary interest, given their potential use in regenerative medicine. Gap junctions form between bone lining cells and BMSCs.[48] Gap junctions also exist between BMSCs,[69] although to a lesser extent than other bone cell types. Gap junctions also play a role in defining the hematopoietic environment.[70] Connexin-mediated coupling in the stroma modulates the ratio ratio of proliferation to differentiation in hematopoietic precursors. In addition, connexins have been identified as mediators in stromal cell-endothelial cell interactions.[70] This opens the possibility of translating this aspect of developmental biology into regenerative medicine therapies since bone marrow stroma can receive developmental cues via gap junctions. For example, co-culture of endothelial cells with BMSCs increases the expression of alkaline phosphatase and type I collagen, suggesting that the physical interaction between osteogenic and angiogenic cells via gap junctions supports osteogenesis.[70] As another example of the potential for the controlled use of gap junctions as a tissue engineering approach, overexpression of Cx43 in BMSCs leads to higher expression of alkaline phosphatase and osteocalcin.[71] The importance of GJIC in BMSC differentiation is further supported by studies involving the transfection of BMSCs with a Cx43 mutation gene (Cx43Δ7). This gene possesses all of the structural properties of a connexin hemichannel with the exception that once coupled it does not permit GJIC.[72] Transfected cells show reduced GJIC compared to control BMSCs, resulting in lower expression of the bone differentiation markers alkaline phosphatase and osteocalcin.[71]

GENETIC MODIFICATIONS

Genetic modifications have been made to stem cells, osteoprogenitor cells and bone cell lines to elucidate the role of connexin-mediated gap junctions *in vitro*, and genetically modified mice have been created to evaluate the functional role of gap junctions and connexins *in vivo*.[73–77]

Cx43 knockout mice exhibit osteoblast dysfunction, delayed ossification and defects in shape and mineralization of the appendicular and craniofacial skeletons.[73] These mice die prenatally due to defects in the heart leading to swelling and blocking of right ventricle outflow.[75] Cells obtained from the cranium of these mice show delayed differentiation and mineralization in culture.[76] To understand the role of communication through Cx43 in bone cells *in vitro*, the osteosarcoma cell line UMR has been used as a model.[53,77] UMR cells do not express Cx43, but do express Cx45. By transfecting UMR cells with the Cx43 protein, the cells then respond to calcium stimuli in a manner similar to osteoblasts, supporting the significance of Cx43 in osteoblast differentiation and the ability to increase GJIC via genetic modification.

Given the important role of Cx43 in controlling development and differentiation, this basic biological information has the potential to be translated into regenerative medicine applications. For example, significant increases in GJIC and osteogenic differentiation *in vitro* occur following the overexpression of Cx43 in BMSCs.[71] The opposite is true for cells expressing the deletion mutant Cx43Δ7, where GJIC is similar to that of a negative control group with the gap junction uncoupler 18α-glycyrrhetinic acid, suggesting a dominant negative effect with non-mutant Cx43 structures. Transplantation of cells transfected with a CX43 lentivirus shows that overexpression of Cx43 also significantly increases the volume fraction of regenerated bone relative to bone regenerated from BMSCs.[71] These results suggest a prominent role of GJIC in bone formation *in-vivo* that can be used as a strategy in bone tissue engineering.

MECHANISTIC ROLES OF GJIC IN BONE BIOLOGY

Gap junctions have been implicated in many important mechanisms in bone formation, including the regulation of signaling molecules, such as ERK, response to growth factors, such as BMP-2, and diffusion of paracrine agents, such as PTH.[33,78–80] A generalized mechanism for the role of connexin in mediating cell–cell communication in bone cells can be developed (Figure 2). The gap junctions serve mostly as a conduit to amplify the effect of a primary stimulus, which can be mechanical, electrical, or biological. Once this primary stimulation is sensed by cells, the response will be a secondary messenger (e.g., Ca^{2+}, cAMP, IP_3) that is typically elicited by release or by exciting channels that allow such secondary messengers to permeate into the cells.[81,82] These secondary messengers then evoke a response in the host cell, but can also permeate through the gap junctions to elicit the same response in a neighboring cell. Such a secondary messenger can also modulate the transfer of signals (communication), usually by phosphorylation of the connexin structure. An example of this mechanism is the ERK signaling cascade with connexin response elements (CxRE) of transcription.[83,84] The ERK cascade is activated by secondary messengers as part of a response to some extracellular cue. In the presence of gap junctions, these secondary messengers activate the cascade in adjacent coupled cells. In this example, these messengers converge to recruit a transactivator (SP1) to promote transcription of CxRE, which leads to production of differentiation markers.[84–87]

There are also GJIC-independent roles of connexin in bone formation, such as signaling through the ERK cascade in osteoblasts, in which alendronate (which stimulates ERK cascade) flows from the extracellular medium to the inside of the cell through the connexin hemichannel.[88] This review will not go into detail on such signaling mechanisms, as other reviews have been written about these topics,[27,36,37,88,89] but will examine some of the mechanisms by which gap junctions modulate calcium signaling, since calcium signaling may be controlled to enhance cell based tissue engineering, and may also be a mechanism of biological response to bioactive ceramics.

Calcium Signaling

Cells typically do not act in isolation, as they coordinate their activities with surrounding cells. In addition to extracellular synaptic, mechanical and hormonal signaling, intercellular communication through gap junctions, with secondary messengers such as calcium, plays an important role in cooperative cell activity.[90] Intercellular calcium signaling is the generalized mechanism by which cells can coordinate activities within a cell population or control the activity of other cells, and can be elicited in response to many diverse stimuli, including electrical, mechanical and hormonal.[91,92] Transient and oscillatory elevations in extracellular calcium concentration initiate or modulate many activities, such as cell growth, cell motility and secretion of matrix producing proteins.[92] The intercellular propagation of calcium signals is common among many cell types, including epithelial cells, chondrocytes, fibroblasts and cardiac cells. Therefore, intercellular calcium is a mechanism utilized by a variety of cells, including bone cells, to coordinate activities based on some initial stimuli.[92–94]

Calcium Signaling in Bone

Although many investigations have examined the role of calcium in bone formation,[53,92,95,96] the mechanisms of intercellular calcium signaling in bone cells are still unknown. Several studies have examined time dependent bursts of intercellular calcium by gap junctions and paracrine mechanisms in different cell types.[22,53,77,97,98] These studies have shown a two prong mechanism to calcium signaling in osteoblasts. As previously mentioned, UMR cells do not express Cx43. Another osteosarcoma cell, ROS, does express Cx43, but has no purinergic (PSY family) receptors. The P2Y containing UMR cells produce a high burst of calcium release at early time points following mechanical stimulation, whereas Cx43 containing cells had lower, but longer lasting intercellular calcium flux. When both cell types were transfected to express either Cx43 or P2Y, the cells behaved as normal osteoblasts, suggesting that Cx43 and P2Y signaling mechanisms are integral to proper osteoblastic signaling, and also suggesting that the time-dependence of calcium signaling could be employed in the temporal control of delivering cues to cells in tissue engineering.

Increased intracellular calcium concentration and faster onset of osteogenic differentiation occur in BMSCs that have higher GJIC due to overexpression of Cx43.[71] Enhanced retention of intracellular calcium also occurs in cells overexpressing Cx43, when excited with higher extracellular calcium levels.[71] BMSCs transfected with the connexin mutation (Cx43Δ7) exhibit the opposite effect; a delayed onset of intracellular calcium elevation and more rapid return to its basal level.[71] Collectively, these studies suggest that overexpressing Cx43 in BMSCs can be used to enhance calcium signaling through higher GJIC. Figure 3 shows a potential mechanism for the role of calcium communication through gap junctions in bone formation. The finding that overexpression of Cx43 also modulates the cellular response to extracellular calcium may have implications in tissue engineering since bioactive ceramics may affect cell function via release of soluble factors (e.g., Ca ions).[99]

CONTROLLING GAP JUNCTIONS: APPLICATIONS IN TISSUE ENGINEERING

Having discussed some of the principles of gap junction structure and function, we now discuss potential strategies and examples of how controlling GJIC could be applied in tissue engineering. Although the specific examples and focus of this review are on bone regeneration, many of the strategies could be extended to other cells, tissues and organs.

The three classical tissue engineering approaches, conduction, induction and cell transplantation can all potentially be exploited to improve cell–cell communication, resulting in enhanced cell differentiation and tissue regeneration. Osteoblasts and their precursors respond to many stimuli, including insoluble and soluble signals from bio-materials

(conductive tissue engineering strategy), growth factors (inductive strategy), and mechanical loading.[10,11,20,42,44–46,99] Because GJIC also plays a prominent role in the differentiation and function of osteoblasts and their response to stimuli, it is possible to design materials or present signals to cells that enhance GJIC.

One example of the potential for the controlled use of gap junctions in tissue engineering involves a cell transplantation approach, in which cells are transfected with a Cx43 lentivirus.[71] Overexpression of Cx43 in BMSCs leads to significant increases in GJIC, and elevated expression of alkaline phosphatase and osteocalcin *in vitro*, indicative of enhanced osteogenic differentiation.[71] Transplantation of cells transfected with a Cx43 lentivirus also shows that overexpression of Cx43 significantly increases the volume fraction of regenerated bone relative to the amount of bone regenerated from transplantation of control BMSCs.[71] These *in vitro* and *in-vivo* results suggest that increasing GJIC can be used as a strategy to enhance bone tissue engineering.

In addition to the direct control of GJIC via endogenous means, exogenous control may also be achieved. Exogenous approaches include altering cell seeding and density, and altering the chemistry and solubility of the biomaterial onto which cells are seeded.[71,100] Micromass and filtration seeding of BMSCs onto 3D scaffolds also lead to increases in GJIC and osteogenic differentiation *in vitro*, and an increased amount of bone formation *in vivo* compared to cells that are seeded statically.[71]

Gap junctions also modulate calcium signaling.[22,53,77,97,98] Control of calcium signaling may therefore be another means of enhancing cell based tissue engineering. Such control could be achieved via direct manipulation of cells, or indirectly via cell-biomaterial interactions. The latter approach could exploit the solubility of calcium-containing biomaterials, such as calcium-phosphate ceramics, glasses and alginates, and capitalize on the fact that soluble factors released from bioceramics can modulate osteoblast differentiation.[9,10,99] Intracellular calcium concentrations are increased in BMSCs that have higher GJIC due to over-expression of Cx43.[71] Intracellular calcium also increases in cells overexpressing Cx43 when excited with higher extracellular calcium levels.[71] These findings therefore suggest that GJIC and intracellular calcium can be manipulated by controlling the solubility of calcium-containing biomaterials which interact with cells.

Controlled GJIC can also be used in tandem with another agent, such as bone morphogenetic proteins (BMPs) to create a synergistic effect and enhance tissue formation.[71] In the case of BMP-7, the distribution of messengers generated by its binding to the type I receptor may be transferred through the gap junctions to neighboring cells that may have not been exposed to the stimuli, even though Cx43 expression is not being modulated. This dynamic allows transcription mechanisms to develop in cells that prompt differentiation, which can lead to tissue formation. Therefore, BMP-7 and gap junctions generated by Cx43 work synergistically when BMP 7 prompts secondary messengers, such as calcium and other transcription factors, that can be distributed through gap junctions.[71]

The mechano-sensing role of bone cells plays a critical role in bone remodeling.[35,54] Therefore, controlling fluid flow or mechanical strain can also potentially induce the opening of Cx43 hemichannels in osteocytes and other bone cells.[52,53] allowing for enhanced cell–cell communication and bone formation. A premise of functional tissue engineering is to provide mechanical cues to cells as a means of enhancing differentiation and tissue formation. Mechanical stimulation of cells in monolayer enhances gap junction function.[35,54,101–103] Therefore, the mechanisms underlying the enhanced tissue regeneration that occurs when cell-seeded 3D scaffolds are subjected to mechanical stimulation[104] may also be gap junction mediated.

At the core of translating the developmental biology insights about gap junctions into tissue engineering applications is balancing the ubiquitous nature of gap junctions with the goal of orchestrating a bone-specific effect. The desired effect of manipulating gap junctions in tissue engineering is to control cell-to-cell communication. The local microenvironment dictates what specific molecules propagate, as well as which specific cells are involved in cell–cell communication. To date, bone specific effects have been orchestrated by the use of closed systems *in vitro*, in which a well defined population of bone cells or their precursors have been directed in culture and cross-talk between bone cells and adjacent populations of other cell types has been avoided.[22,53,71,77,97,98] Subsequent implantation into a more open system *in vivo* then capitalizes on the ability of the already directed bone cells to enhance regeneration. By the time of implantation, the transient effects of enhancing GJIC may diminish, so potential undesirable communication with the host can be avoided.[71] A second example of controlling gap junction manipulation is in stromal-endothelial and bone-endothelial co-cultures, in which communication between two cell types has been achieved.[70] Thus, in a controlled osteogenic microenvironment, at least, enabling cell-to-cell communication through control of gap junctions enables a greater degree of osteogenic differentiation.

SUMMARY

Gap junctions serve as conduits between cells that transfer information to neighboring cells in the form of a secondary messenger (such as calcium) following a primary stimulus. Cx43 is almost completely ubiquitous in all cells, and is the gap junction protein that is predominant in bone cells. This protein is responsive to fluid shear, mechanical perturbation, hormones, growth factors, and other secondary messengers. Furthermore, Cx43 serves both gap junction-dependent and independent functions. Cx43 proteins play a significant role in controlling bone formation, and therefore have the potential to serve as a platform for regeneration of craniofacial structures. Controlling the expression of Cx43 may provide control over cell-to-cell communication which, in turn, can lead to larger and more uniform volumes of regenerated tissue.

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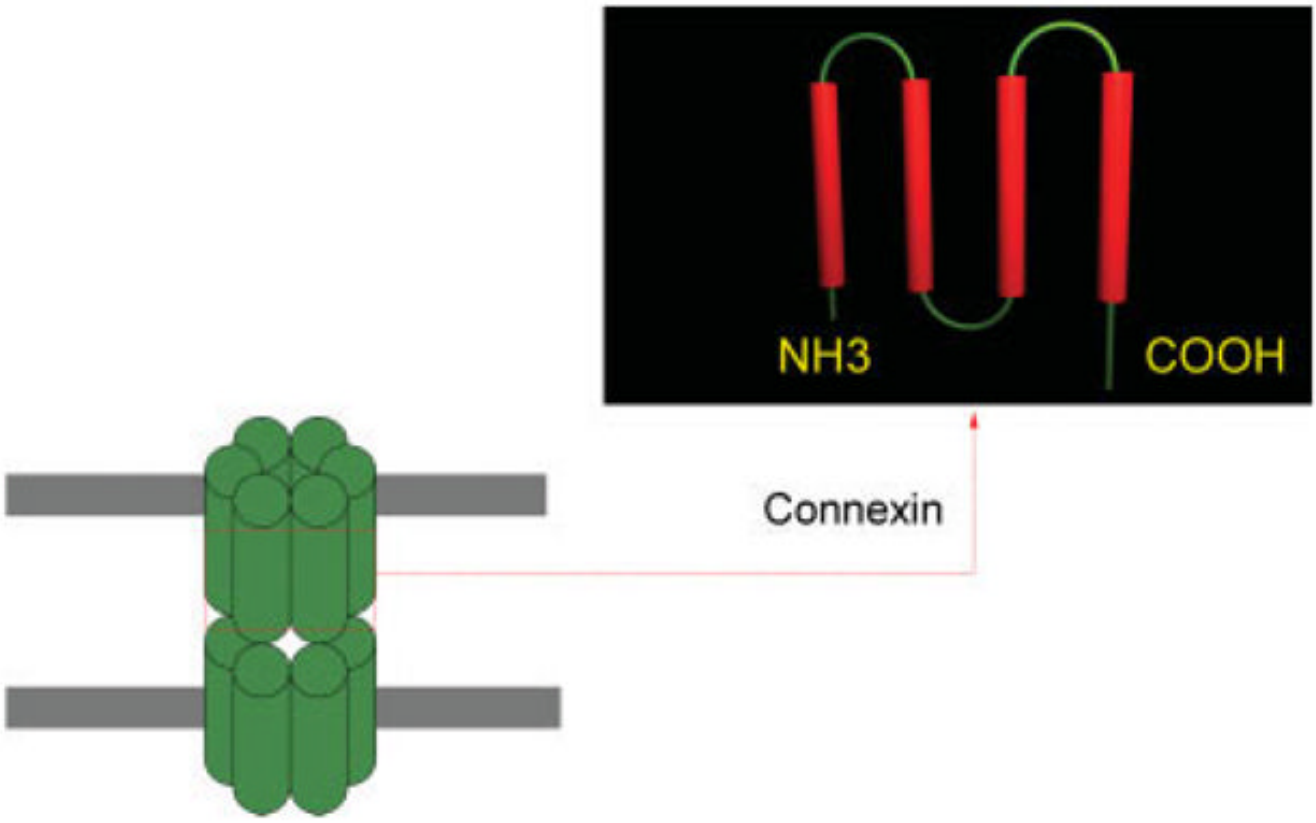

Figure 1.
Schematic of gap junctions and connexins. Gap junctions are aqueous conduits formed by the docking of two hemichannels (connexons) in juxtaposed cells. Each hemichannel is composed of six transmembrane proteins (connexins). Each connexin monomer (inset) is composed of four transmembrane domains (two extracellular loops, one intracellular loop and intracellular carboxyl and amino ends). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

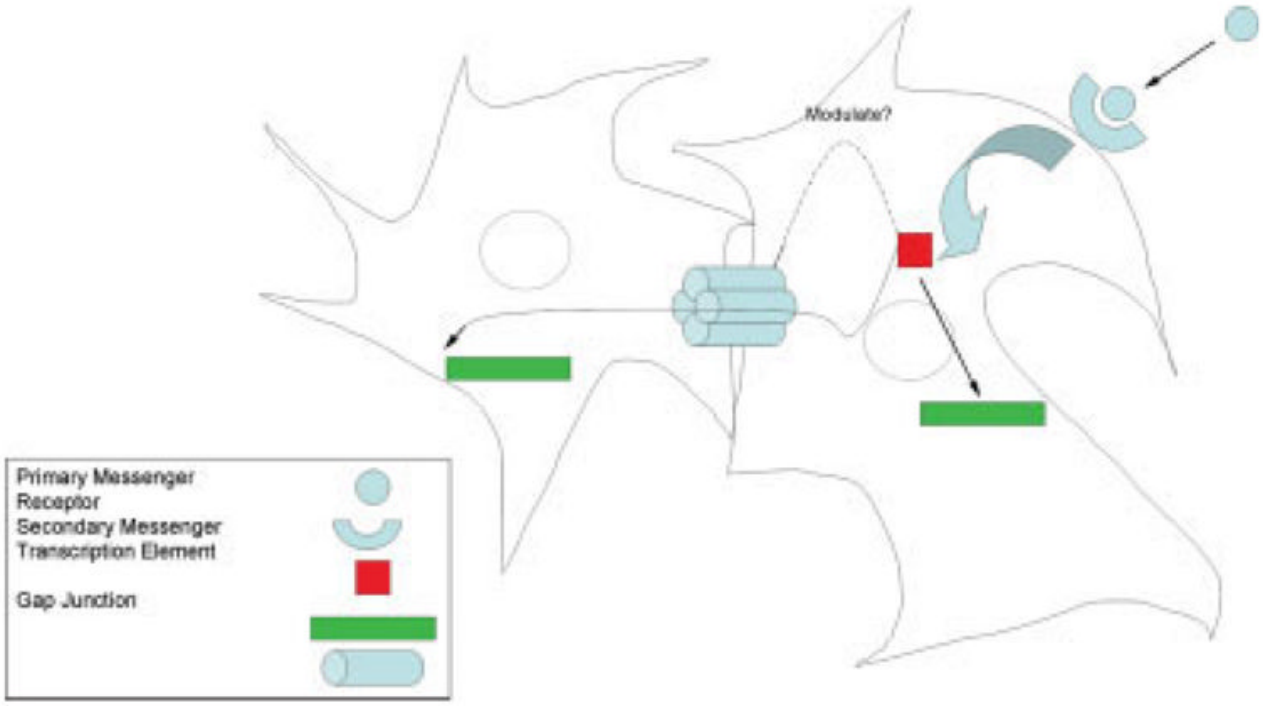

Figure 2.
Generalized mechanism for the role of GJIC. A primary messenger in the form of a hormone, growth factor, or mechanical stimulation elicits a response from the host cell. This response is the production or influx of secondary messengers (e.g., Ca^{+2}, cAMP, IP_3,) that enable the activation of a signaling cascade (e.g., ERK). This cascade produces a transcriptional response in the host cell. The secondary messengers can modulate the gap junctions to either open or close. If open, these secondary messengers will go through the gap junction channel and elicit the same response (as in the host cell) in adjacent cells, without the need for a primary messenger to stimulate that cell. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

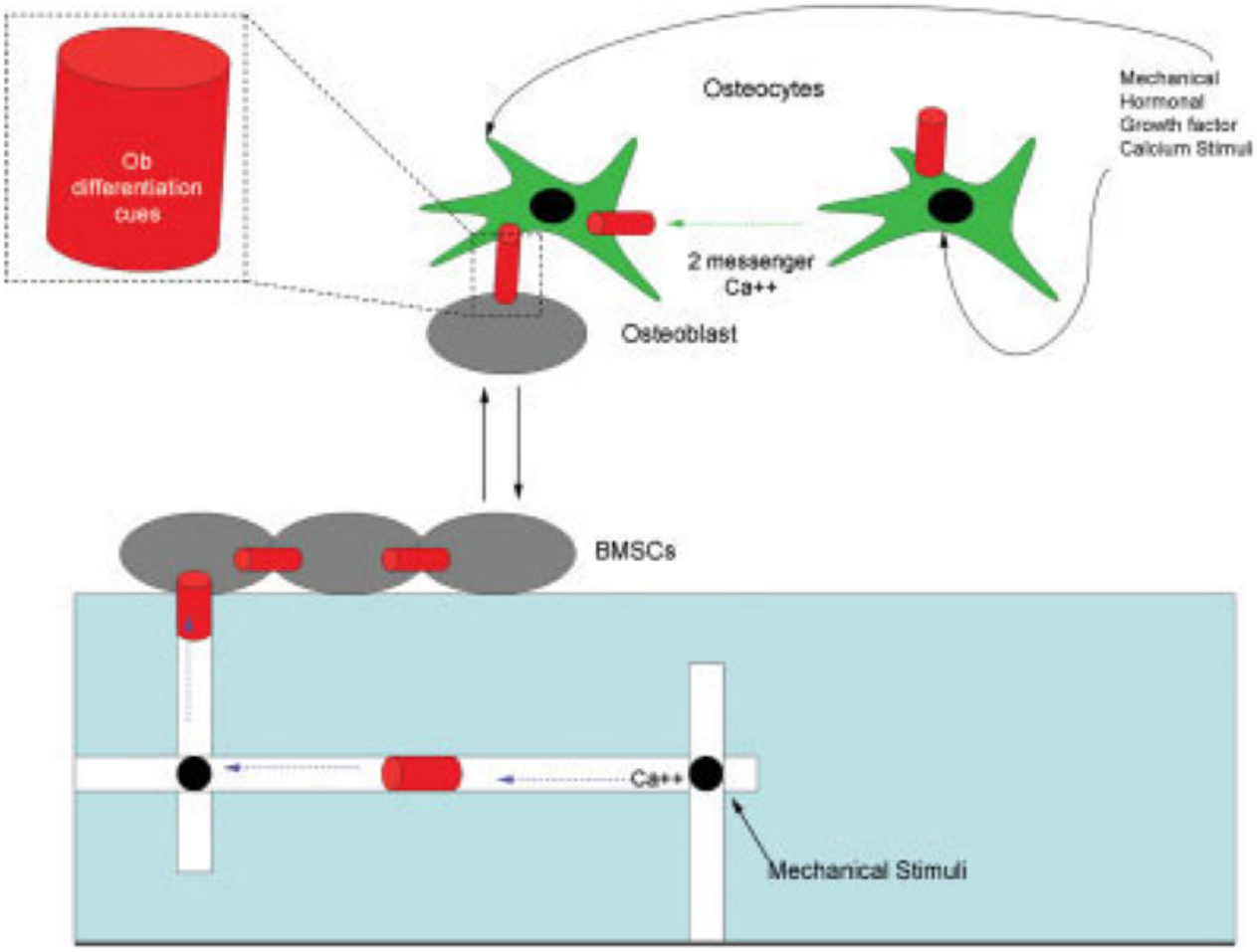

Figure 3.

Schematic of gap junction interactions between three types of bone cells: bone marrow stromal cells (BMSCs), osteoblasts, and osteocytes. First, BMSCs use gap junctions to communicate with one another. The expression of these junctions is less than in differentiated osteoblasts. Osteoblasts and BMSCs can communicate with each other, in which case BMSCs are likely to receive differentiation cues from osteoblasts. Osteoblasts are also able to communicate with osteocytes that are embedded in the bone matrix through these junctions, enabling communication between terminally differentiated cells in the mineralized matrix and cells that are in the lining and outside of the matrix. Osteocytes also communicate with one another via gap junctions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]