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Review Article

Forging a New Path in Cleft Rehabilitation by Tissue Engineering – A Review

Rohit Raghavan, Sumitra S., Nadeem Abdul Rahman, Justin Baby

Department of Prosthodontics, Royal Dental College, Chalissery, Palakkad, Kerala

Email address:

drrovan @gmail.com (R. Raghavan), dineshsumi@gmail.com (Sumitra S.), nadhee4u4ever@gmail.com (N. A. Rahman), drjustinbaby@gmail.com (J. Baby)

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Abstract: Of all the birth defects, Cleft palate is among the most common and affects about one in 1,500 births resulting in medical, physical, developmental, social and emotional problems in affected children in addition to the high health care costs. Current treatment is based on surgical closure of the cleft followed by orthodontic dental care, speech therapy, bone grafting, and requires multiple surgeries spanning over 18 years. Thus, there is a pressing need to develop more effective methods of treatment to provide young patients with a safer option that will result in a complete closure of the palatal cleft shortly after birth. In this review, the application of the field of tissue engineering, involving the use of adult stem cells, such as mesenchymal stem cells from bone marrow and Adipose-derived Stem Cells (ASCs) seeded on currently available biomaterials is presented in the context of healing craniofacial defects like the cleft palate. This article presents the concise technique to generate new bone in cleft deformities, using stem cells. It also throws light on the work done by various researchers to regenerate bone in large defects.

Keywords: Bone Tissue Engineering, Cleft, Stem Cells, Growth Factors, Scaffolds

1. Introduction

Cleft lip and/or palate is considered the most prevalent of the common human congenital craniofacial birth defects. Cleft palate deformities occur when palatal shelves fail to fuse. These deformities are classified according to the extent of the palate involved. Failure of fusion of the primary and secondary palate leads to complete cleft palate, in which the palatal shelves also fail to fuse. Complete cleft palate is typically associated with uni- or bilateral cleft lip. The approximate incidence of Cleft lip and/or palate is 1: 700 live births. In addition, Cleft lip and/or palate is the second most common congenital malformation following clubfoot (Peter and Larsen, 2004) [1]

Cleft lip and/or palate are more often unilateral and more common in males than in females. Unilateral defects of the left side are more common than those of the right side. Cleft palate is more common in females and most often associated with other developmental anomalies. [1]

The ultimate goals of the treatment of clefts are to improve the function and quality of life. The management is very complex and involves multidisciplinary approaches such as orthodontics, maxillofacial, plastic surgery, prosthodontics, speech therapy and psychological departments.

The closure of the bony defects and stability of the maxillary arch are the crucial elements of the treatment plan. In 1972, Boyne and Sands [2] reported that smooth eruption of the canine to the bone transplant area was induced and normal arch form was obtained by autogenous iliac bone grafting before canine eruption. Bone grafting is done using autogenous and/or allogeneic grafts and is followed by dental implant placement. Bone grafting materials such as autogenous cortico-cancellous iliac crest, bone morphogenetic proteins and recombinant human protein have shown good results the in long term. [3]

Since then, autogenous iliac bone grafting has been frequently employed for the closure of bone defects at the cleft site. Bone grafting is performed preferentially during the

orthodontic treatment to enhance the success of dental implants. However, the related surgical procedures to collect the autogenous graft from iliac bone are quite invasive, causing large stress for the patients. Therefore, other artificial transplant materials for bone regeneration can be used. [4, 5]. This review article elucidates the process of bone tissue engineering using the key ingredients of tissue engineering - stem cells, scaffolds and growth factors.

2. Tissue Engineering

Due to the numerous drawbacks associated with bone grafting, the search is on for newer and less invasive techniques to regenerate tissues. Tissue engineering is a promising solution for a widespread range of defects and disorders. Development of biological and biomaterial sciences has put tissue engineering as a tool for regeneration of lost and damaged organs.

The term tissue engineering was coined at the National Science Foundation (N. S. F.) bioengineering meeting in Washington D. C., in 1987. [6] At a subsequent N. S. F. sponsored workshop, it was formally defined as

"the application of principles and methods of engineering and life sciences, to obtain a fundamental understanding of structural and functional relationships in novel and pathological mammalian tissues, and the development of biological substitutes to restore, maintain or improve tissue function" (Shalak& Fox, 1988).

Tissue engineering has been used to provide new alternatives for bone reconstruction. Tissue engineering uses three dimensional bone-like scaffolds that are loaded with bone cells that are planted in the bony defect for bone reconstruction. [1]

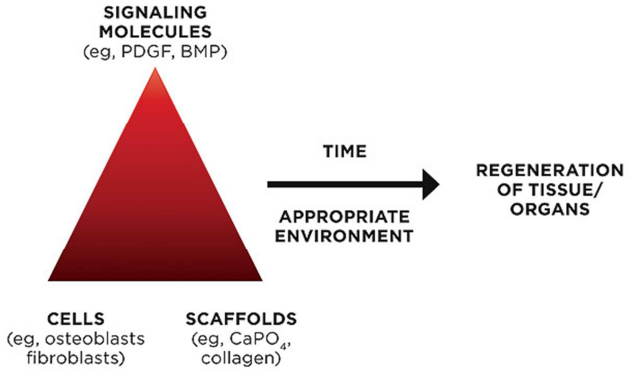

Figure 1. *Triad for tissue regeneration.*

Three elements (cell, scaffold, and growth factor) are believed to be crucial for successful tissue regeneration. The three key elements of tissue engineering are [7, 8]

1. Morphogenic signals such as growth factors and differentiation factors. These factors play an important role in the multiplication and differentiation of stem cells into the specifically needed type of cells.
2. Responding stem cells which are originally harvested from the patient and preserved under good conditions to maintain their special ability to differentiate into a wide range of cells.
3. Scaffold of extra cellular matrix, which provide these cells with the environment and mold to grow into what we want them to become and function.

3. Stem Cells

Stem cells are the foundation cells of every organ in the body. The term stem cell was proposed for scientific use by Russian histologist Alexander Maksimov in 1908. Stem cells are defined by three main characterstics[10]

(1) self-renewal, or the ability to generate at least one daughter cell with characteristics similar to the initiating cell,
(2) multilineage differentiation of a single cell, and
(3) in vivo functional reconstitution of a given tissue or cell type.

4. Stem Cells Types and Sources

Stem cells are immature, undifferentiated cells that can divide and multiply for an extended period of time, differentiating into specific types of cells and tissues. Autogenous stem cells are derived from the patient being treated, while allogenous stem cells are derived from other individuals

The process by which stem cells are derived from one type of tissue and differentiate into other types of tissue is referred to as plasticity or transdifferentiation. Multipotent stem cells consist of three major types—ectodermal, mesodermal or mesenchymal and endodermal. The two main categories of stem cells are embryonic stem cells and adult stem cells, defined by their source.

5. Adult Stem Cells

Adult stem cells [11, 12, 13] are defined as the undifferentiated cells that are found in a differentiated adult tissue, residing in a specific area of each tissue where they remain quiescent in the body until they are activated by epigenetic and/or environmental factors, such as mechanical forces, disease, or trauma

Though many sources of adult stem cells have been identified today, to the craniofacial surgeon interested in using tissue engineering, there are two exciting sources of stem cells: bone marrow and adipose tissue.

6. Mesenchymal Stem Cells (Mscs)

The identification of pluripotent MSCs[14-20] in the bone marrow stroma over 25 years ago has led researchers to a variety of exciting research avenues. Capable of differentiating to multiple mesodermal lineages, including bone and cartilage, MSCs have become a standard in the field of adult stem cell biology and in regenerative medicine**.** Multiple studies have reported the formation of bone tissue

both *in vitro* and *in vivo* upon the combination of MSCs and 3D scaffold supports.

Mesenchymal stem cells [4] account for 0.001~0.01% of the subcellular component in bone marrow, having the potential to differentiate into multiple mesenchyme lineages such as chondrocytes, adipocytes, and osteoblasts by appropriate biological stimuli. The pain score [4] was significantly lower in CLP patients who underwent bone marrow puncture from iliac bone than in those who underwent conventional surgical separation of iliac bone marrow, suggesting that the bone regeneration using MSCs can relieve stress of patients.

7. Bone Marrow Derived Stem Cells (Bmscs)

BMSCs [21] consist of both hematopoietic stem cells that generate all types of blood cells and stromal cells (MSC) that generate bone, cartilage and other connective tissues and fat. BMSCs are currently the most common commercially available stem cell. They can be isolated from bone marrow aspiration or from the collection of peripheral blood-derived stem cells following chemical stimulation of the bone marrow, by means of subcutaneous injection, to release stem cells.

8. Adipose Tissue Derived Stem Cells (Ascs)

ASCs [22-27] are typically isolated from lipectomy or liposuction aspirates. They have been differentiated into adipocytes, chondrocytes, myocytes, and neuronal and osteoblast lineages, and may provide hematopoietic support. ASCs have an advantage in that adipose tissue is plentiful in many individuals and is easily accessible and replenishable. The advances in bone-tissue engineering [28] using marrow-derived mesenchymal stem cells (MSCs) offers the clinical opportunity to directly place appropriate numbers of osteogenic cells in desired extra-skeletal spaces to direct bone formation. MSCs are rare cells resident among the bone marrow that can be selectively isolated from an aspirate and expanded several million-fold to generate tissue engineering devices containing relatively high numbers of cells.

9. Scaffolds

Scaffolds are constructs, which are used as a support structure allowing the tissues / cells to adhere, proliferate and differentiate to form a healthy bone / tissue for restoring functionality. [29] An ideal scaffold is expected to provide chemical stability and physical properties, matching the surrounding tissues with respect to cell compatibility, adhesion performance, cell proliferation, controlled degradation, and mechanical strength.

At a basic level, tissue engineering scaffolds can be broken down into three groups: autografts, allografts, and xenografts.

Autografts which are taken from a different site in the same patient come with associated donor site morbidity. Many laws and the various histocompatibility issues preclude the use of xenografts. This leaves the allografts.

Allografts can be organized into two groups:

Natural and Synthetic.

NATURAL -The natural category is a broad-range category that includes bone powders, chips and fragments. Processed [30] to remove the cellular components, natural materials are osteoconductive but poorlyosteoinductive, thus decreasing the response.

An alternate natural allograft is demineralized bone matrix (DMB). [31] It is the decellularized, organic component of bone. DMB is a concentrated source of bonemorphogenic proteins (BMPs) and has been used in numerous animals systems since its initial description in 1965. Though it is easily available commercially from tissue banks, the widespread use of DMB in humans is restricted the immunologic properties of donor DMB is unknown.

10. Synthetic

As an alternative to the natural scaffolds, a wide range of synthetic materials are now being used, mainly due to their easy availability. These include ceramics, calcium phosphates and polymers. [11]

Table 1. *Synthetic bone engineering composites.*

Scaffold type	Commercial name
Chitosan (POLY 1, 4 d-Glucosamine)	
Ceramics	
Hydroxy Appatite/HA	
Sintered HA	
Biomimetic HA	
Bioglass	
Calcium phosphates	
β-Tricalcium phosphates	Eg Cellplex
biphasic calcium phosphates(eg HA/TCP)	
Synthetic polymers	
Poly(lactic-co-glycolic) acid	
Poly-1-lactic acid	
CNI-HA	Eg Healos
Treated metals- titanium, tantalite	
Composites	
CNI/β-TCP, CNI-HA	
PLA/HA/CNI Sponges	Eg COLLAGRAFT Eg Ceraform
PLGA/HA	
Gelatin/Chitosan	
PLA/Chitosan	

CNI – Collagen Type I, HA – Hydroxy apatite, PLA – Poly-1-Lactic Acid, PLGA - Poly(lactic-co-glycolic) acid, TCP – Tricalcium Phosphate

11. Growth Factors

Growth factors were first identified [32] by Murray PE, Gracia-Godoy F and Hargreaves KM in 2007. They are proteins that bind to receptors on the cell and induce cellular proliferation and/or differentiation. They are used to control SCs activity and to induce regeneration of damaged tissues.

A critical component of osteoblastic progenitor cell differentiation and subsequent bone formation are osteoinductive growth factors. Many growth factors [33] are known to enhance bone regeneration. These include:

A) Bone morphogenic protein (BMP), which induces osteoblastic differentiation and bone mineralization,
B) Platelet-derived growth factor (PDGF), which promotes proliferation of connective tissue and muscle,
C) Fibroblastic growth factor (FGF), which promotes cellular proliferation,
D) Transforming growth factor beta (TGFB), used for tissue reparation, and
E) Epidermal growth factor (EGF), which promotes mesenchymal and epithelial cell proliferation.

12. Tissue Engineering In Craniofacial Defects

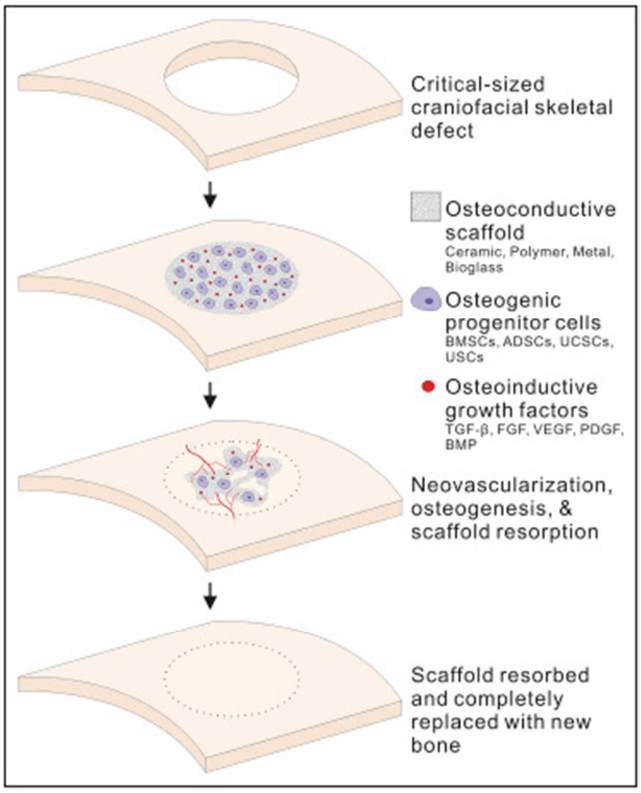

Figure 2. *Ideal modality [34] for craniofacial defect repair. The strategy involves growth factor-induced osteoblastic differentiation and bone formation within an osteoconductive and biodegradable scaffold.*

Several animal models have induced bone formation within long bone and cranial defects by using mesenchymal stem cells (MSCs) treated with BMP2. Early work by Peterson and Dragoo showed that ASCs, treated with BMP2, would be capable of forming bone within a cleft defect. However, this was contradicted byLeboy, who suggested that BMP2 may not promote osteogenic differentiation of human MSCs. The regenerative response with BMP 2 is several times lower than that in animal studies. This shows wide discrepancies between studies on animal models and in human tissues. Moreover, the effect of such a powerful growth factor as BMP2 in the craniofacial region of very young children remains undocumented. The use of growth factors to augment bone regeneration remains questionable.

So, various other approaches to regenerate the vast quantities of bone required to repair large defects, as in clefts, need to be studied. Research on three distinct approaches to bone regeneration used alone or in combination, shows promising results.

13. The "Scaffold-Driven" Approach—Biomimetic Apatites

The bioactivity of synthetic scaffolds can be limited. Studies have suggested that the bioactivity of scaffolds like PLGA or PLA can be strengthened through the formation of a layer of HA created through the immersion of 3D scaffolds in ionic solutions with compositions similar to blood plasma—called Simulated Body Fluid. These biomimetic apatites are composed of plate-like crystals of calcium phosphate capable of coating the entire 3D scaffold architecture. This improves their biocompatibility and biodegradability. This method was originally developed by Kokubo in 1990 and has undergone improvement and refinement by various other researchers. It is simple to perform, cost effective and can be applied to large surfaces with complex geometries.

14. The "Cell-Driven" Approach—The Pediatric Stem Cell

The use of pediatric stem cells in the repair of craniofacial defects involves the repair of the defect with the child's own stem cell. A small amount of adipose tissue from the child can be extracted using a simple syringe. These pediatric ASCs (pedASCs, *i.e.*, under 5 y) could be expanded in the lab and combined with the best possible scaffold for implantation into the defect. Yet, there is no current information available that studies pedASCs at an in-depth level.

15. The "Gene-Driven" Approach—Molecular Signaling Within the Stem Cell

The regulation of differentiation in stem cells involves the expression of several genes. A precise investigation for revealing the gene expression profile and molecular signaling of MSCs for their osteogenic differentiation is required. Gene

expression studies of MSCs using genome eide association analysis revealed that the EphrinA-EphR pathway for femoral neck bone geometry is coordinated with osteogenesis. Epigenetic regulation is involved in MSc differentiation, and transcription regulation by RUN X2 is important for the osteogenic differentiation capacity of MSCs.

The process of palatogenesis[36] depends on highly coordinated, anatomically specific and precisely timed molecular signals for normal development. Among them, cell migration, proliferation, fusion, apoptotic, and differentiation events contribute to the complexity of craniofacial organization. In addition, multiple signaling pathways including sonic hedgehog, FGF, and transforming growth factor signaling complement each other. Aberration from any of this programming is likely to lead to pathogenesis of the palate, namely cleft palate.

GSK [38, 39] has been implicated as a key regulator of a wide variety of developmentally important molecular pathways including Wnt, nuclear factor of activated T-cells (NFAT), Hedgehog, and insulin signaling. These signaling pathways are essential components of many biologic responses and associated diseases, including embryonic development and cell fate determination, diabetes, neurodevelopment and neurodegeneration, psychiatric disorders, cell cycle regulation and cancer, hematopoiesis, and immunity.

Within the last several years, there have also been numerous reports of TGF-3's [40-42] role in palatogenesis. TGF signaling has long been recognized as a critical mediator of successful palatogenesis, and it will be interesting to follow further research in this field toward clinical translation into alternative strategies for the management of cleft palate.

Finally, Wnt signalling [43, 44] has recently received considerable attention for its role in craniofacial morphogenesis, including orofacial clefting.

Significant bone regeneration in a rabbit calvarial model [11] has been measured upon implantation of MSCs transduced with Sonic Hedgehog (Shh)—a key protein involved in craniofacial morphogenesis. Although these results are promising, the stem cell population must be carefully considered as Shh expressing ASCs were capable of regenerating bone within a calvarial defect but also appeared to induce the formation of large cyst-like structures. Canonical and noncanonicalWntsignalingpathways have also come under focus because of their well known role as regulators of embryologic patterning, stem cell fate and mesenchymal differentiation. Studies linking the LRP5 gene mutation and osteoporosis-pseudoglioma syndrome have suggested a connection between Wntsignaling and bone formation. Consistent with this, work in MSCs has linked Wnt3a induced signalling to a suppression of bone formation *in vitro* and *in vivo*. In contrast, increased bone regeneration in both mandibular and calvarial defects has been observed in MSCs isolated from craniofacial tissues overexpressing Wnt4. Because numerous signaling pathways, including the MAPK cascade, can be induced through integrin—matrix interactions in a variety of cells, it is not unreasonable to hypothesize the design of scaffolds that mimic the effect of growth factors through adhesion-based mechanisms, mediating signaling through specific "pro-osteogenic" signal transduction pathways.

16. Summary and Conclusion

Advances in craniofacial bone tissue engineering needs a thorough understanding of the physiology and molecular pathways involved in bone formation and remodeling. Innovations in material science and molecular biology have allowed tissue engineers to augment physiologic bone healing and make bone regeneration via scaffold/stem cell therapy a clinical possibility. Combining biomaterials, often with competing properties, to fabricate optimized scaffolds for use in craniofacial skeletal regeneration is representative of current research trends and the most promising strategy for tissue engineers and craniofacial surgeons. New advances unlocking the osteogenic potential of several stem cell types, as well as the discovery of more readily available stem cell sources (e.g., urine-derived stem cells), are also providing exciting prospects for craniofacial bone regeneration. Despite such advances in tissue engineering, craniofacial bone reconstruction is often complicated by scarring, osteomyelitis, osteonecrosis, or previous radiation damage. The combination of stem cells, growth factors, small molecules, and scaffold materials used in reparative bone tissue engineering will largely be guided by these and other complicating factors.

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