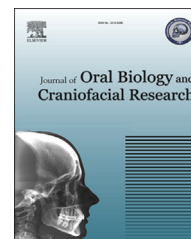


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Review Article

TMJ Bioengineering: A review



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ABSTRACT

Regeneration using scaffolds, growth factors, and stem cells is being investigated worldwide. Pubmed search for scaffolds for condyle resulted in 102 articles, of which 24 analyzed Temporomandibular joint (TMJ) scaffolds and only 6 evaluated hydroxyapatite scaffolds. 17 articles report studies on TMJ disc regeneration.

The ideal bone construct for repair should be able to replicate the lost structure, restore function, be harmless, reliable and biodegradable. Scaffolds act as carriers for mesenchymal stem cells and/or growth factors and are useful for cell adhesion, migration, proliferation, and differentiation. Gene therapy has also led to the accelerated effective bone regeneration. The major materials used as scaffolds are natural or synthetic polymers, ceramics, composite materials, and electrospun nanofibers.

Mesenchymal stem cells are responsible for the formation of virtually all dental, oral, and craniofacial structures. Tissue-engineered bone can possess the customized shape and dimensions. It has the potential for the biological replacement of craniofacial bones.

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1. Introduction

The craniofacial structure consists of bone, cartilage, soft tissue, nerves, and blood vessels. Acquired defects after cancer surgeries, trauma as well as congenital or developmental deformities require a reconstructive procedure as the bones of the craniofacial region support the rest of the elements. The procedures used today for temporomandibular reconstruction are mostly autologous, allogenic, or alloplastic, with variable clinical outcomes and morbidities.¹ Distraction histogenesis has emerged as a possible alternative to regenerate ramus condyle unit.²

Regeneration using osteo-conductive scaffolds, osteoinductive growth factors, committed progenitor cells and stem cells is being investigated by researchers and surgeons alike.³ Osteo-conduction and osteo-induction are very important features for bone tissue scaffolds. Osteo-induction implies the

process of the conversion of non-osseous cells into bone-forming cells, whereas osteo-conduction is the process by which implanted scaffold supports the bone growth.⁴

Pubmed search for scaffolds for condyle resulted in 102 articles, of which 24 analyzed Temporomandibular joint (TMJ) scaffolds and only 6 evaluated Hydroxyapatite (HA) scaffolds. 17 articles report studies on TMJ disc regeneration. The ideal bone construct for repair should be able to replicate the lost structure, restore function, be harmless, reliable and biodegradable i.e. should degrade during the process of tissue regeneration and replaced with fully functional tissue.

2. Scaffolds

Scaffolds are the mechanical constructs that act as carriers for cells and/or growth factors. The main role of scaffolds is to

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simulate the extracellular matrix for cell adhesion, migration, proliferation, and differentiation.⁵

2.1. Biomaterial for scaffolds

The major materials used in craniofacial tissue engineering are natural and synthetic polymers, ceramics, composite materials, and electrospun nanofibers.⁶ Biomaterial to be used as a scaffold must possess sufficient mechanical strength, large pore volumes and pore interconnectivity to allow continuous tissue in growth, and transport properties to allow the influx of nutrients and elimination of waste products.⁷ Randomly positioned pores contribute to better cell seeding and better cell aggregation in the designed scaffolds.⁸ Natural scaffolds like collagen type I, chitosan, calcium alginate, hyaluronic acid, and composites have been shown to be osteoconductive, but with problems like lack of mechanical strength when implanted, risk of infection, immunogenicity, and rapid degradation rate.^{9–14}

Bone contains 85% calcium phosphate, hence ceramics such as hydroxyapatite (HA), tricalcium phosphate (TCP), and composites such as biphasic calcium phosphate (BCP), have been widely investigated for bone scaffolds. The HA ceramics are well suited as biomaterials because of their biocompatibility, not eliciting an inflammatory response, lack of immune reaction, and easy radiographic assessment. TCP demonstrates a too fast degradation rate *in vivo*, whereas HA degrades too slowly, is not resorbed, and resides in the defect for several years after callus formation. BCP has more favorable degradation rates compared with TCP and HA. However, the problem with use of ceramics is their brittleness which makes them mechanically inadequate for load bearing.^{13,15}

Polymers include polyethylene glycol (PEG), polyglycolic acid (PGA), poly-L and poly-D, L-lactic acid (PLA), poly-DL-lactico-glycolic acid (PLGA), polycaprolactone (PCL), polyurethanes, and composites. Polymers are flexible and biodegradable through their hydrolysis or by means of cellular or enzymatic pathways when implanted. Polymers have low mechanical strength and hence are often combined with high-modulus micro or nanoscale ceramic constituents like HA.

2.2. Fabrication of scaffolds

There are a lot of conventional techniques used for scaffold fabrication, such as solvent casting, particulate leaching, gas foaming, fiber meshes/fiber bonding, phase separation, melt molding, solution casting, and freeze drying. Conventional techniques allow to control the pore size, geometry, and distribution. However, the scaffolds made with conventional techniques have many imperfections that limit their role in tissue regeneration.

The need to introduce new techniques for scaffold fabrication led to the development of solid free-form fabrication techniques that include 3-dimensional printing, stereolithography fuse deposition modeling, 3D plotter, and phase-change jet printing. These techniques are based on using computer-aided design software which allows the fabrication of scaffolds with more precise external shape and internal morphology.

A recently developed technique, electrospinning, has shown promising results in obtaining micro and nanofibers from polymeric solutions or melts. Electrospinning systems can adjust mechanical properties as well as the size of the produced fibers. Nanofibers have a large surface area-to-volume ratio and can be processed so that they have high porosity; to allow delivery of protein coatings, drugs, or specific signaling molecules, cell infiltration, nutrient diffusion, and angiogenesis during the process of bone regeneration. Electrospun polymeric scaffolds for bone tissue engineering are most often made with PLA, PGA, PCL, silk fibroin, calcium phosphates, bioactive glass, and glass ceramics.¹⁴ Recently, rapid CAD–CAM prototyping of pure hydroxyapatite was used to replace temporomandibular joint condyles in sheep.¹⁵

3. Stem cells

A stem cell is self-renewable and capable of differentiating into at least two distinctive cell types, then only it can be defined as a stem cell. Self-renewal denotes that undifferentiated daughter cells are a precise replica and can further replicate many generations without losing their original characteristics.¹⁶ Cells of an immortalized cell line can replicate many generations, but are generally incapable of multi-lineage differentiation. Thus, cell lines are not stem cells.

Selective isolation and differentiation of Human embryonic stem cell (hESC), when cultured in feeder-free conditions, showed up-regulation of osteoblastic lineage markers and production of *in vitro* mineralized matrix when cultured in osteogenic differentiation medium. The implantation of these cells in critical-size calvarial defect in immune-deficient mice for 10 weeks resulted in new bone formation and partial repair of the calvarial defect.¹⁷

3.1. Mesenchymal stem cells (MSC)

MSCs are self-renewable and can differentiate into all cell lineages that form mesenchymal and connective tissues.¹⁶ The first successful isolation of bone marrow MSCs was described almost 4 decades ago.¹⁸ The isolation method was based on the adherence of MSCs to the plastic substrate of the cell culture plates. Homogenous populations of MSCs are isolated using flow cytometry, based on differential cellular features, are further purified and cloned. Size-dependent sieving from human bone marrow aspirates through a porous membrane also results in a homogenous cell population with the capacity of self-renewal and multi-lineage differentiation.¹⁹ Positive selection of MSCs with microbeads, combined with fluorescence-activated cell-sorting²⁰ or magnetic-activated cell-sorting,²¹ is another effective technique for isolation and characterization of MSCs. STRO-1 and CD146 (MUC18), are two early cell-surface markers for MSCs.²²

3.2. Dental pulp stem cells (DPSC)

DPSCs represent an adult stem cells population that is easily recruitable with low invasivity. These multipotent cells are able to differentiate in osteogenic, chondrogenic, myogenic, adipogenic and neurogenic lineage. Osteogenic differentiated

DPSCs express bone tissue specific proteins like Runx2, Osterix (Osx osteopontin (OPN), osteocalcin (OCN), bone sialoprotein (BSP) alkaline phosphatase (ALP), matrix extracellular phosphoglycoprotein (MEPE), DSPP and collagen type I. DPSCs have demonstrated production of calcified extracellular matrix and formation of nodular cell aggregates and nodular bone *in vitro*.²³

3.3. Stem cells from human exfoliated deciduous teeth (SHED)

Pulp consists can be extracted from the exfoliated deciduous tooth. Twelve to twenty cells from pulp of each exfoliated incisor formed adherent colony clusters with extensive proliferative capacity.²³ After implantation into immunocompromised mice, with HA/TCP as a carrier, SHED differentiated into odontoblast like cells that formed small dentin like structures. These results suggest that SHEDs are distinctive from DPSCs with respect to odontogenic differentiation and osteogenic induction.²⁴

3.4. Periodontal ligament stem cells (PDLSCs)

Stem cells have been identified in human periodontal ligament (PDLSCs) and found to generate structures that resemble the native tissue when implanted into nude mice.²⁴ After a 3 week culture with an adipogenic-inductive cocktail, PDLSCs differentiated into Oil-red-O-positive, lipid-laden adipocytes.²⁵ Upon 4 week osteo/odontogenic inductions, alizarin-red-positive nodules formed in the PDLSC cultures, similar to MSCs and DPSCs. Thus, the PDLSCs have the potential for forming periodontal structures.

4. Cell seeding in scaffold

Although MSCs clone in the culture media, in the scaffold their characteristics may change. Evidence shows that MSCs seeded on PLA scaffolds when used for reconstruction of bony defects in pig mandibles showed a uniform radio-density on the radiographs, and interface between native bone and constructs was indistinct.²⁶

Furthermore, cultivation of bone marrow derived stem cells (BMDSC) in an autogenous fibrin and platelet-rich clot and membrane with a mineral base of β TCP and HA were able to lead to callus formation and bone regeneration when implanted in a maxillary bone after massive deficiency.²⁷

It has been demonstrated that co-culture of human embryonic stem cell (hESC) derived cells with osteo-conductive material, such as HA/TCP, may increase their osteogenic potential. Eagle medium with fetal bovine serum, dexamethasone, and ascorbate has shown to promote more frequent bone formation, although α modified media was seen to promote teratoma formation in 12- to 20-week-old transplants.²⁸

5. Osteochondral constructs

Simultaneous regeneration of cartilage and bone is a great challenge. A hydrogel system was designed was constructed

as two layers to simultaneously induce the endogenous regeneration of hyaline cartilage and subchondral bone. Chondro-inductive transforming growth factor-b1 (TGF-b1) was placed in one layer and the osteoinductive bone morphogenetic protein-4 (BMP-4) in second layer, via affinity binding to the matrix. Human MSCs were seeded in the bilayer system, which differentiated into chondrocytes and osteoblasts in the respective layers, confirming the activity of TGF-b1 and BMP-4.²⁹

Differentiation of MSCs into chondrocytes and osteoblasts was also observed when a bilayered gene-activated HA/chitosan-gelatin osteochondral scaffold seeded with MSCs, was implanted in a rabbit knee osteochondral defect, where plasmid transforming growth factor (TGF) β 1-activated the scaffold for chondrogenic layer and plasmid bone morphogenetic protein (BMP) 2-activated the scaffold for osteogenic layer. Localized gene delivery can also influence the single-type stem cells to differentiate into different lineages, which is of great importance in regeneration of tissues that consist of various cell types.³⁰

Another study documented that after differentiation of MSCs from rat bone marrow into osteogenic and chondrogenic cells *in vitro*, the cells were seeded in PEG hydrogel in 2 stratified layers and implanted in the dorsum of immunodeficient mice. 8 weeks after transplantation the results showed that the condyles were formed *de novo*, with the presence of both osteogenic and chondrogenic cells.³¹ After 12 weeks, the obtained condyles showed further tissue maturation and phenotypic growth of both cartilage-like and bone-like tissues.³²

Yet another study was conducted to engineer an osteochondral implant by promoting endochondral ossification in one layer of a bilayered construct and stable cartilage in the other, the top half of bilayered agarose hydrogel was seeded with culture expanded chondrocytes and the bottom half with MSCs. Constructs were cultured in chondrogenic medium for 21 days and thereafter were either maintained in chondrogenic medium, transferred to hypertrophic medium, or implanted subcutaneously. The bilayered co-culture appeared to suppress hypertrophy and mineralization in the osseous layer, as the hypertrophic factors were found to induce mineralization of the osseous layer *in vitro* as well as *in vivo*. This approach represented a potential new strategy for the osteochondral regeneration.³³

Tissue formation and vascularization of anatomically shaped human tibial condyles ectopically with a dimension of 20 × 15 × 15 mm has been reported using a composite of PCL & HA scaffold with an overlaying layer of 1 mm of PEG-based hydrogel. hMSCs were seeded in both layers, other group had hMSCs derived osteoblasts in lower part and hMSC-derived chondrocytes in upper layer. After 6 weeks of subcutaneous implantation, hMSC generated significantly more blood vessels, larger-diameter vessels, but hMSC-derived osteoblasts yielded mineralized tissue in microchannels. Significantly more cells were present in the cartilage layer seeded with hMSCs. However, chondrocytes were present in safranin-O-positive glycosaminoglycan matrix in the cartilage layer seeded with hMSC-derived chondrogenic cells.³⁴

6. Gene therapy

Gene therapy is based on the transfer of genetic material (non-viral or viral) into living cells for regeneration of tissues. Non-viral gene transfer, *transfection*, is done by chemical or physical delivery of gene material. The methods include injection of naked DNA, electroporation, particle bombardment, and cationic liposomes. Transfection is dependent on cellular transport systems and expression of the host cell. Non-viral vectors have the advantage of being safe and have the ability to introduce large segments of DNA.

Viral gene transfer, *viral transfection* or *infection*, despite their higher transfection efficacy in transmitting the genetic material to the host, is somewhat limited in gene therapy for tissue regeneration due to the toxicity of the viral vector, their control and gene expression. Viral vectors undergo genetic modifications before use in gene therapy. The most used viruses as vectors are adenoviruses, adeno-associated viruses, retroviruses, and herpes simplex virus.

The combined use of BMDSCs transfected with hBMP-2 and vascular endothelial growth factor (VEGF) 165 gene and natural coral scaffolds has led to the effective bone regeneration of orbital defects in rabbits.³⁵ Other studies have shown that gene delivery of the osteogenic BMP-2 via an adenoviral vector in BMDSCs seeded in mandibular defects, revealed high expression levels of BMP-2 protein, which induced osteogenic differentiation of these cells *in vitro* and induced bone regeneration after transplantation.³⁶

Basic fibroblast growth factor (bFGF) can increase the mRNA expression levels of osteoblast differentiation factor, activity of alkaline phosphatase (ALP) and induce differentiation of MSCs *in vitro* as well as *in vivo*. However, bFGF has a short half-life *in vivo*. BMDSCs with transfected bFGF gene showed efficacy in forming bone in craniofacial defects in New Zealand rabbits after distraction osteogenesis. The bone mineral density and bone mineral content in the group treated with transfected BMDSCs was higher than the control group.

Runx2 is a bone-specific transcription factor with the ability to stimulate osteoblast differentiation. Runx2-engineered MSCs displayed enhanced osteogenic potential and osteoblast-specific gene expression *in vitro* and *in vivo* in critical-size calvarial defects and increased both bone volume fraction and bone mineral density.

SATB2 gene, which is expressed in branchial arches, is responsible for preventing craniofacial abnormalities and defects in osteoblast function. When transfected into murine adult stem cells, SATB2 significantly increases expression levels of bone matrix proteins, osteogenic transcription factors, and VEGF. The transplantation of SATB2-overexpressing adult stem cells from calvarial bones in mandibular defects showed excellent rates of osteogenic differentiation and bone formation compared with adult stem cells that did not have overexpression of SATB2.

7. TMJ Bioengineering

Ideal engineered constructs for mandibular condyle regeneration must have integrated bone and cartilage layers in a

single osteochondral construct to meet the demands for anatomic, structural, and functional regeneration.

The challenge in TMJ Bioengineering is to promote matrix synthesis and tissue maturation of stem-cell-derived chondrogenic and osteogenic cells in biocompatible and bioactive scaffolds, which may be possible by incorporating an array of growth factors and/or transcription factors separately for chondrogenesis and osteogenesis. The mechanical properties of the tissue-engineered mandibular condyle must match with that of an anatomic condyle for *in situ* implantation into the human TMJ. Also, the tissue-engineered mandibular condyle must have a remodeling potential.³⁷

Osteoblasts, obtained after differentiation of porcine bone marrow MSCs in adequate medium, can lead to the formation of the bone construct resembling mandibular condyle after seeding on biodegradable PLGA scaffold.³⁸ Calf osteoblasts and chondrocytes seeded on PGA and PLA scaffolds and implanted into subcutaneous pockets on the dorsum of athymic mice, showed positive results in mandibular condyle tissue regeneration. After 12 weeks of implantation, the analyzed bone structure had a condylar shape, and microscopic examination showed the formation of trabecular bone and hyaline cartilage on the articulating surface.³⁹

Stem cells from human umbilical cord when seeded onto PGA, after 4 weeks of culture in growth medium containing chondrogenic factors, showed their ability to produce components of the extracellular matrix, such as collagen type I, II, glycosaminoglycans, and to double their number.⁴⁰ Recently, NEL-like molecule 1-modified autogenous BMDSCs when seeded on PLGA composite, showed the potential to rapidly regenerate bone and cartilage tissue after transplantation in large osteochondral defects of goat condyles. 6 weeks after transplantation, the fibrocartilage was regenerated, and the regeneration of subchondral bone native articular cartilage occurred at 24 weeks.⁴¹ PLGA microspheres with a gradient transition between cartilage-promoting and bone-promoting growth factors showed newly formed bone in mandibular condylar defects, 6 weeks after transplantation in New Zealand white rabbits.

Insulin-like growth factor (IGF) I and TGF- β 1 have demonstrated promotion of increased secretion of collagen I, glycosaminoglycans, and cellular proliferation during mandibular condyle regeneration, when applied on the self-assembled constructs of TMJ disc *in vitro*.⁴² TGF- β 1 also showed some positive effects on the production of extracellular matrix and cellular proliferation in studies with constructs of TMJ disc.⁴³

Hydroxyapatite/collagen block has been successfully used with platelet-rich plasma in temporomandibular joint ankylosis in children and adolescents to regenerate a new functioning condyle.⁴⁴ However, a long-term evaluation is required to prove its efficacy.

The positive effects of low-intensity pulsing ultrasound on mandibular condyle regeneration demonstrated enhanced formation of bone and cartilage tissue and their integration.⁴⁵

8. TMJ disc Bioengineering

The earliest such study was performed in rabbit disc where cultured cells were used in collagen I meshes.⁴⁶ Later hyaline

cartilage was engineered in the shape of a human TMJ disc.⁴⁷ Four years later, Girdler harvested hyaline cartilage cells along with chondroprogenitor cells and cultured them to form disc.⁴⁸ Recently, human and porcine disc cells have been cultured in 2 dimensions on expanded polytetrafluorethylene monofilaments, PLA monofilaments, polyamide monofilaments, and natural bone mineral blocks.⁴⁹

Recent studies have identified that a scaffold of non-woven PGA mesh in combination with cell seeding technologies, could provide an engineered disc.⁵⁰ Three growth factors: insulin-like growth factor-I, basic fibroblast growth factor and transforming growth factor- β 1 have been assessed in maintaining disc-like tissue in culture.^{51,52}

In another study, a scaffold material composed of porcine-derived extracellular matrix, configured to mimic the shape and size of the TMJ, was implanted in a canine model of bilateral TMJ discectomy. The results showed the formation of site-appropriate, functional host tissue resembling native TMJ disk.⁵³

Polyglycerol sebacate (PGS), a biocompatible, biodegradable elastomer, was used as a porous scaffold material for the TMJ disc, where goat fibrochondrocytes were seeded at three seeding densities (25, 50, 100 million cells/mL scaffold), respectively, and cultured. The results showed that cell seeding density and culture time, both effect the biochemical and biomechanical properties of PGS scaffolds. The findings demonstrated PGS as a favorable scaffold material for TMJ disc engineering.⁵⁴

9. Conclusion

Craniofacial tissue engineering is an emerging field where researchers and clinicians together are in search of a possible solution to regenerate the lost craniofacial structure. MSCs are responsible for the formation of virtually all dental, oral, and craniofacial structures. Tissue-engineered bone can possess the customized shape and dimensions. It has the potential for the biological replacement of craniofacial bones. The possibility of regenerating a neocondyle is currently being investigated. Several meritorious studies have been successful in *in vitro* fabrication of a TMJ disc.

Conflicts of interest

The author has none to declare.

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