AN INSIGHT INTO CLINICAL ADVANCEMENTS OF REGENERATIVE DENTISTRY

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CONTENTS

INTRODUCTION

Regenerative dentistry is a rapidly evolving field in dentistry. According to the Global oral health status report issued by WHO in 2022, oral diseases are the most widespread non-communicable diseases affecting almost half of the world's population (45% or 3.5 billion people worldwide). These includes dental caries and periodontal diseases. But still; the complete rehabilitation of disease-affected tissues is not yet achieved.

Current treatment options offer to control the disease process to obtain symptomatic relief or/and restoration of tissue by artificial materials. As adult human teeth are not replaced naturally, it is necessary to find ways to achieve a fully functional recovery of teeth affected by oral diseases and conditions. Many researchers worldwide are working on advancing regenerative medicine and tissue engineering field due to the recent advancements in bio-medical engineering research.

Regenerative dentistry is expanding rapidly because of it's promising nature of unravelling curative and restorative treatment approaches for disease conditions. It is well known that oral and craniofacial tissues have limited capacity to regenerate spontaneously and restore to their original state once they are severely damaged, such as in the case of dental caries, severe maxillofacial pathology, or trauma. Hence, dental and craniofacial tissue engineering has been researched and developed over the decades.

DEFINITION

Regenerative dentistry is a new speciality of dentistry that aims to regenerate injured or diseased tissue or the whole functional dental organ using biologically based approaches. Tissue engineering is the foundation of regenerative dentistry which mainly focuses on 3 key components:

1) Stem cells
2) Bioactive molecules and
3) Biomaterials, which act as scaffolds to promote cell growth and differentiation[1].

The optimal combination action of these 3 components is known to enhance the reparative potential of the resident cells of the tissue by promoting the migration of more stem cells towards the site of injury and propagating the overall regenerative or reparative process. Dental tissue−derived stem cells (DSC) are especially significant in tissue regeneration. Numerous interventional approaches have been developed to enhance the efficacy and applicability of these strategies, mainly: Cell-free and Cell-based approaches.

- Cell-free approaches aim to recruit resident cells, including stem cells, via bioactive molecules embedded in biomaterials or scaffolds to enhance the regenerative process. This phenomenon is also referred to as Cell Homing.

- Cell-based approaches, on the other hand, involve the administration of exogenously cultured autologous or allogeneic stem cells into the affected tissue to mediate regeneration.

CLINICAL ADVANCEMENTS OF REGENERATIVE DENTISTRY

1)Regenerative endodontics (dentin-pulp complex regeneration)

Pulpodentin complex regulates and maintains dentinogenesis and tooth vitality throughout life. The human pulpodentin complex has the capacity to mineralise and to increase the rate of dentinogenesis in response to caries and trauma. The success rate of direct pulp capping to restore the structure of tooth with dental materials, especially following a carious pulpal exposure is extremely low. The weak regenerative capacity of human pulpodental complex is main reason for the need for pulpotomy or tooth extraction following symptoms of chronic pulpitis.

The development of regenerative dental procedures originated in 1952 by B.W.Hermann[3]

The goal of regenerative endodontics is to:-

a) Recover normal pulp function in teeth with reversible pulpitis &
b) Regeneration of pulp-dentinal complex in irreversibly inflamed or necrotic teeth.

Pulp Revascularisation is currently considered the only regenerative endodontic treatment approach in clinical practice.

Pulp revascularisation refers to the revascularisation of an immature permanent tooth with an infected necrotic pulp and apical periodontitis/abscess that can promote root development.[2]

This procedure involves chemical disinfection of the root canal using intracanal medicaments and antibiotics followed by induction of bleeding to generate a favourable regenerative niche.

The resulting blood clot acts as a scaffold that facilitates homing of stem cells, macrophages, and fibroblasts, whilst hard tissue deposition reinforces the dentinal walls. However, this may not be considered pulp regeneration as it gives rise to bone, cementum, and periodontal ligament like fibrous tissue.

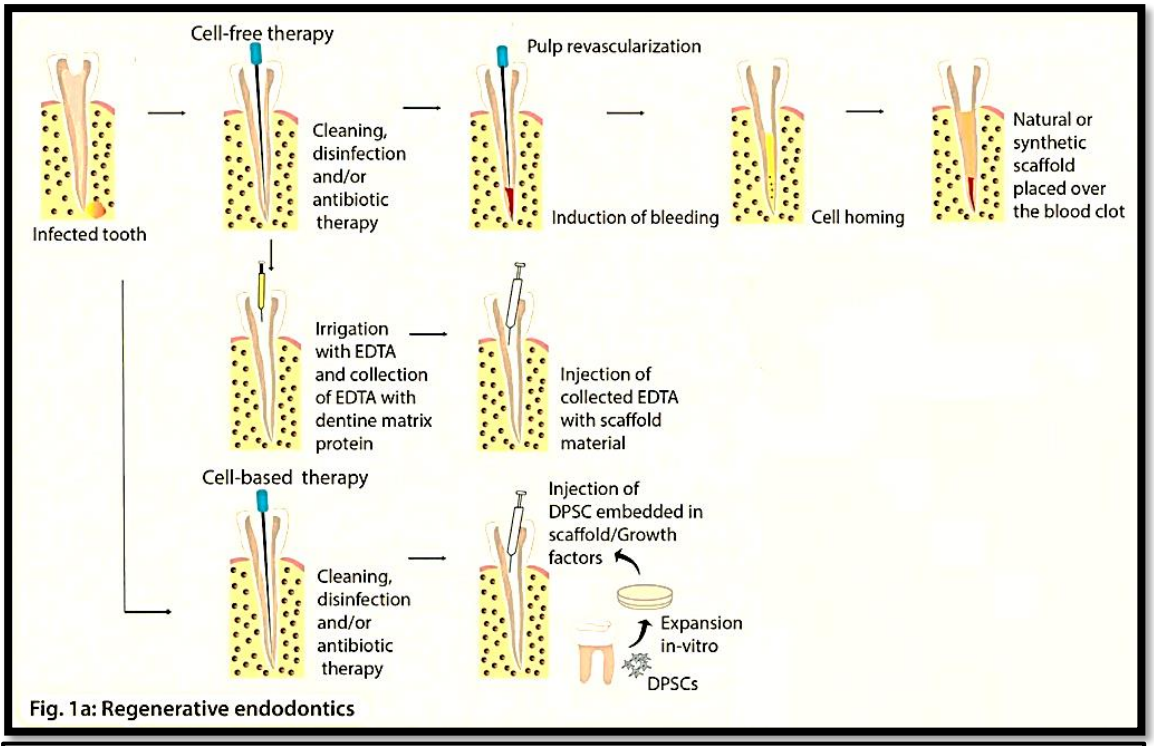


Fig. 1a: Regenerative endodontics

Source: Galler KM, Widbiller M (Cell-free approaches for dental pulp tissue engineering)

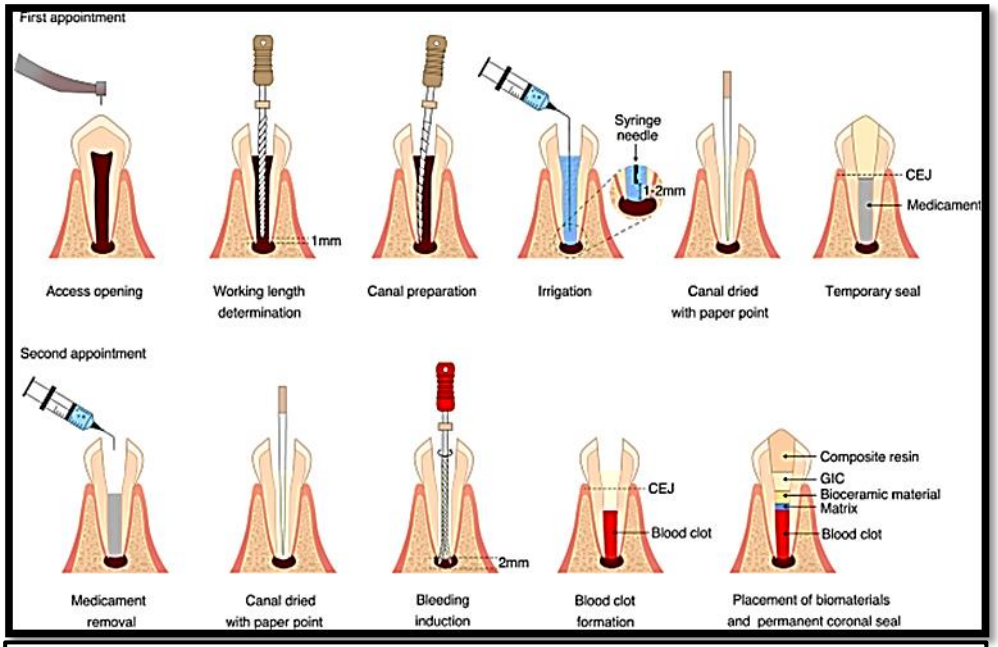
The above method of pulp revascularisation has been advanced into approaches where natural or synthetic scaffolds/biomaterials are used as adjuncts to aid in the revascularisation process. Some examples of this includes:-

a) Collagen scaffolds that are placed over the blood clot and platelet-rich fibrin (PRF) placed in the root canal to promote cell homing, differentiation of stem cells, entrapment, and slow release of angiogenic cytokines, including fibroblast growth

factor (FGF), vascular endothelial growth factor, and platelet derived growth factor (PDGF).

b) Platelet Rich Factor (PRF) is known to overcome many limitations of the basic method of bleeding induction, such as possible damages to Hertwig's epithelial root sheath resulting in impaired or no continued root development in immature teeth.
c) In the recent times, Dentine has been identified as a rich source of growth factors favourable for pulp regeneration, many of which are determined to be signalling molecules. The effect of these proteins on dental pulp cells has been observed in terms of chemotaxis, increased gene expression of odontoblast phenotype, improved mineralisation, and enhanced neurogenesis and angiogenesis.

d) Based on in vivo studies, Galler et al have suggested a regenerative endodontic approach that can be attempted in the clinical setting, which involves Root canal preparation and Disinfection, Irrigation with ethylene diamine tetraacetic acid (EDTA), and collection of EDTA with dentine matrix proteins followed by mixing of collected EDTA with a scaffold material and injection into the root canal. The purpose of EDTA conditioning; not only the removal of the smear layer but also the release of the bioactive molecules embedded in dentin and exposure of dentin's collagenous structures to facilitate cell adhesion.



Source: Wei et al, International journal of oral science(Published in 2022)

All the above treatment approaches are cell-free methods targeting endogenous cell homing, a few cell-based regenerative endodontic approaches are emerging into clinical practice.

(i) Transplantation of autologous DPSCs of human exfoliated deciduous teeth (SHED) in teeth with pulp necrosis due to traumatic dental injuries has shown regeneration of 3D pulp tissue along with blood vessels and sensory nerves at 12 months post-treatment.

(ii) Further, regenerative endodontic procedures using allogeneic human umbilical cord mesenchymal stem cells in a plasma derived biomaterial in mature permanent teeth with apical lesions have shown increased sensitivity and blood flow in 12 months follow-up, highlighting the clinical safety and efficacy of allogenic endodontic regenerative cell therapy.

2) Periodontal Tissue Regeneration

The advances in the biology of wound healing and periodontal regenerative technologies are applied to improve long term clinical outcomes of teeth that are periodontally compromised by intrabony or inter radicular defects. The treatment objective is to obtain shallow, maintainable pockets by reconstruction of the destroyed attachment apparatus and thereby also limit recession of the gingival margin.[4]

Periodontal therapy aims to restore the affected soft and hard tissue components of the periodontium to their original architecture and function. In general, periodontal regeneration is selected to obtain:

1. An increase in the periodontal attachment of a severely compromised tooth
2. A decrease in deep pockets to a more maintainable range
3. Reduction of the vertical and horizontal components of furcation defects.

Due to many limitations of the conventional treatment approaches including nonsurgical and surgical periodontal debridement and autologous and connective tissue grafts, the current trend of periodontal therapy has shifted towards "TRUE" regenerative rather than reparative approaches.

(i) One such approach is Bone grafts introduced as alloplastic material (synthetic fillers), autografts, allografts, and xenografts that can induce bone formation.

(ii) Guided tissue regeneration is a widely practiced therapeutic approach currently considered as the gold standard of periodontal tissue regeneration. This method is superior to

conventional periodontal treatment. GTR has varying success rates depending on the technique and properties of the barrier membrane used. Microbial colonisation and rupture of the membranes have been identified as potential causes for poor treatment outcomes.[5]

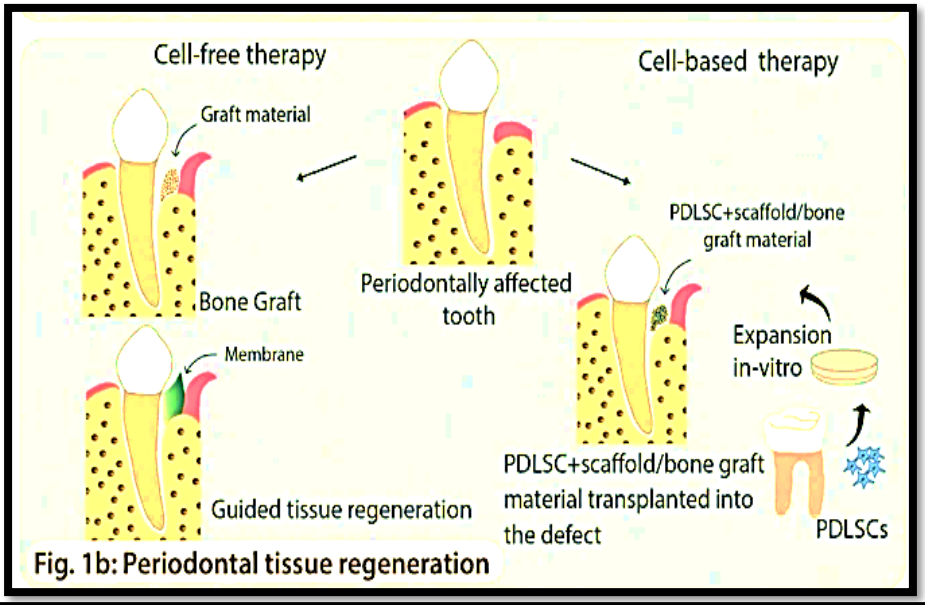


Fig. 1b: Periodontal tissue regeneration

Source: Shalini HS, Vandana KL. Direct application of autologous periodontal ligament stem cell niche in treatment of periodontal osseous defects: a randomized controlled trial.

To overcome these limitations, composite membranes of polycaprolactone and gelatin loaded with zinc oxide nanoparticles have been fabricated, which significantly reduces the growth of Staphylococcus aureus over time while leaving the ability of periodontal cells to repopulate unaffected.

(iii) Use of PRF with open flap debridement/bone graft was reported to lead to significant improvements in clinical attachment levels and radiographic bone fill.

(iv) In contrast, growth factors such as FGF-2, PDGF, and bone morphogenetic protein-2, in conjunction with various

biomaterials, have also demonstrated improved clinical outcomes.

(v) Several clinical trials have been conducted on treating periodontal defects with cell-based regenerative approaches. These include transplantation of autologous human periodontal ligament stem cells isolated from extracted third molars mixed with bone grafting material or gelatin scaffolds into the periodontal defects following open flap debridement. This has shown favourable outcomes in terms of tooth mobility, clinical attachment loss, and bone density on postsurgical follow-up over extended periods.

3) Temporomandibular joint (TMJ) regeneration

TMJ is a frequent site of pathology in the oral and maxillofacial (OMF) region. The most frequent TMJ pathologies include: disc derangement disorders, autoimmune disorders, and osteoarthritis associated with TMJ (TMJ-OA).

The treatment of TMJ-OA at present depends on its severity. It ranges from conservative treatment such as occlusal splints, intra-articular injections, arthrocentesis, or arthroscopy in mild or moderate cases to radical curative methods such as open joint surgery for severe cases. These approaches often relieve symptom successfully but don't facilitate permanent recovery, hence the need for novel regenerative curative treatment strategies for TMJ-OA.[6]

(i) The use of Bone Marrow Mesenchymal Stem Cells (BMMSCs) in TMJ-OA has been widely studied in animal models and proceeded to clinical trials where BMMSCs have been

inoculated into the osteoarthritic TMJ resulting in improved pain relief, increased mouth opening and chewing efficiency. The therapeutic action of DSCs in TMJ-OA is still being explored via in vivo experimentation.

(ii) Intravenous administration of serum-free conditioned media of SHED can inhibit cartilage destruction and inflammation whilst promoting the regeneration of condylar cartilage and subchondral bone. This evidence suggests that intra-articular stem cells may also be an effective treatment option for TMJ-OA.

(iii) TMJ- OA has also been experimentally treated with mesenchymal stem cell (MSC)− derived exosomes and platelet-rich plasma in animal models, which have shown that exosome therapy controls the level of inflammation in TMJ followed by expression of the matrix and proliferation leading to healing of subcondylar cartilage and bone.

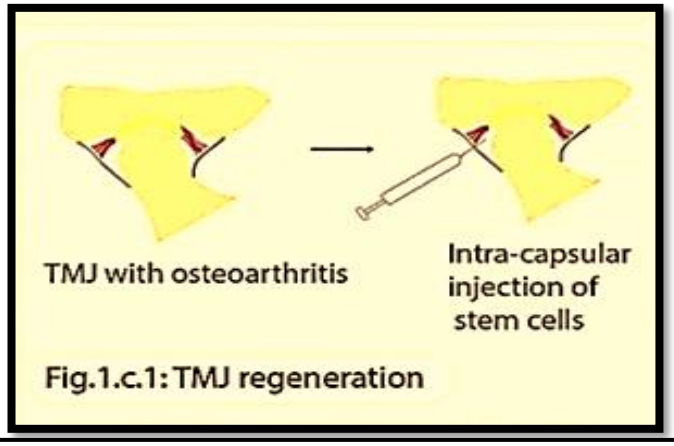


Fig.1.c.1: TMJ regeneration

Source: - De Riu G, Vaira LA, Carta E, et al. Bone marrow nucleated cell concentrate autograft in temporomandibular joint degenerative disorders,Zhang M, Yang H, Lu L, et al. Matrix replenishing by BMSCs is beneficial for osteoarthritic temporomandibular joint cartilage.

4) Craniofacial tissue engineering

(A) **Regeneration of Bone**: Reconstruction of OMF structures is challenging due to the various tissues involved, functional complexity that requires fine neuromuscular coordination and aesthetic demands. OMF defects could result from trauma or surgical resection owing to benign or malignant tumours, infections, or developmental anomalies.

Bony defects in the OMF region can be reconstructed using autologous bone grafts, allografts, demineralised bone matrices, hydroxyapatite calcium phosphate, bone morphogenetic proteins, collagen scaffolds, and bone marrow aspirate concentrate.

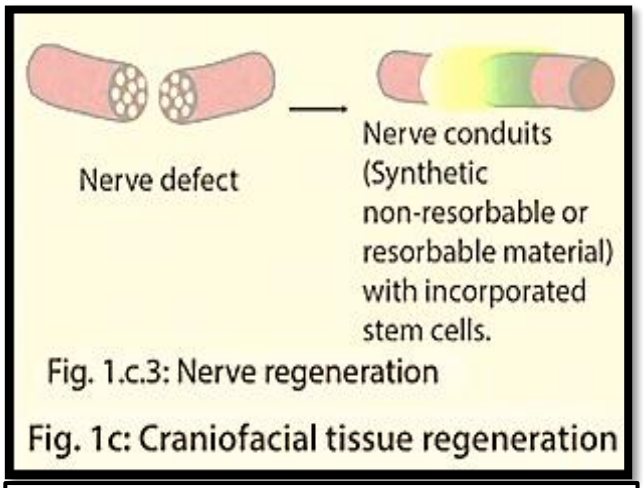
(i) Autologous grafts frequently delivered include iliac bone grafts and costochondral rib bone grafts. However, the treatment of choice for reconstructing extensive bony defects, such as in segmental mandibular resection, consists of conventional autologous grafts that can be delivered as microvascular free fibula flaps.[7]

(ii) Many approaches have been introduced to optimise the bone graft, prolong its viability, and promote mineralisation. Improvement of graft vascularisation via growth factors and stimulation of bone marrow and adipose tissue-derived MSCs are a few such approaches.

(iii) Bone augmentation before dental implant placement in resorbed alveolar ridges is a critical step that contributes to the longevity of the implant.

(iv) Whilst the gold standard of bone grafting materials is considered autogenous bone grafts, customised 3D printed nanohydroxyapatite (3DHA) block grafts with incorporated growth factors have been introduced into clinical practice with promising outcomes.

(B) **Nerve regeneration**: Nerve injuries in the orofacial region are often the result of trauma, tumours, or iatrogenic causes and frequently involve the inferior alveolar nerve (IAN), lingual nerve, infraorbital nerve, and facial nerve. In current surgical practice, autologous nerve grafts and nerve conduits are used to reconstruct nerve gaps.



Source: 2. Ducic I, Yoon J. Reconstructive options for inferior alveolar and lingual nerve injuries after dental and oral surgery

(i) Nerve conduits were initially made of synthetic non-resorbable material and then advanced to conduits composed of resorbable materials such as collagen. However, their

treatment outcomes are not reported to be as successful as autologous nerve grafts due to the absence of cells.

(ii) The newest advancement is focused on incorporating Schwann cells, or stem cells, into the conduit to promote better regeneration. Herein, nerve conduits constructed with cell spheroids and 3D bioprinted conduits have been used in animal studies with favourable outcomes as they enhance nerve regeneration via improved cell survival, differentiation, and extracellular matrix (ECM) formation.[8]

(iii) Gingival MSCs have been identified as a potential cell source for 3D bioprinting of scaffold-free nerve constructs for the regeneration of peripheral nerves.

(C) **Salivary gland regeneration:** Currently, individuals with impaired salivary gland function are symptomatically treated with artificial saliva and sialagogues. Salivary gland regenerative treatment would be ideal for these patients.

(i) The use of DSCs in salivary gland regeneration has reported in the clinical setting.

(ii) However, studies have shown that umbilical cord−derived MSC intravenous infusions result in increased salivary flow rate and alleviate other Sjogren syndrome−related symptoms.

(iii) Intraglandular injection of adipose-derived MSCs (AMSCs) has shown improved salivary flow rate, increased acinar and ductal areas, and a decrease in fibrosis in irradiated individuals

highlighting the potential of MSCs in salivary gland regeneration.[9]

Even though only a few regenerative therapeutic approaches in regenerative dentistry are currently in clinical practice, many studies are being conducted with promising results that pave the way for the discovery of more advanced treatment approaches.

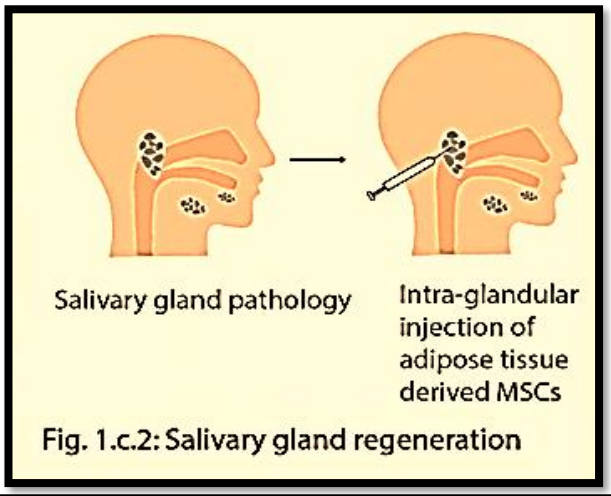


Fig. 1.c.2: Salivary gland regeneration

Source: Xu J, Wang D, Liu D, et al. Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjogren syndrome

Current trends and future perspectives in regenerative dentistry

1) Cell sheath, Spheroids and Organoids

Stem cell therapy has shown limited successful outcomes in clinical dentistry, as cells administered as mono-dispersed preparations are often poorly retained at the site or are subjected to rapid cell death.[10]

(i) Cell sheets are a scaffold-free cell therapy that forms high density sheet structures out of cells and their extracellular

matrix. The ECM here preserves intercellular and cell-matrix connections and caters to the function of a scaffold by providing strength and support for cells and a 3D structure for cell proliferation and differentiation.

(ii) Spheroids are dense, 3D cell aggregates that, in addition to having a well-formed network of cells in an ECM mimicking the microenvironment found in native tissues, are capable of creating a potent secretome that promotes angiogenesis, mitigates inflammation, and recruits host cells to enhance repair and regeneration.

(iii) Fabrication of Organoids is another cell-based regenerative approach that involves in vitro generation of 3D tissue constructs that mimic the complex microanatomy and function of the corresponding tissue in vivo using induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), or adult stem cells.

2) 3D Bioprinting

The limitations and challenges of organoid fabrication can be overcome in large part by 3D bioprinting.

3D Bioprinting is an advanced manufacturing technology capable of producing personalised 3D objects using standardised material based on Computer- aided design (CAD) digital models.[11] This cutting-edge technique involves a complex process where the exact positioning of biomaterials/scaffolds is done with cells embedded in a desired pattern with spatial control of functional component placement.

One of the most important aspects of 3D bioprinting is its ability to manipulate the delivery of cells and materials in complex fabricated

tissue-like constructs enabling it to maintain cell-to-cell growth interconnectivity for improved tissue regeneration.

It is expected that 3D printed organs readily available for transplant may be fabricated in the near future.

3) Layered scaffolds

The applications of layered scaffolds in dentistry are especially beneficial in periodontal tissue regeneration.

In this approach, 3D structures are generated layer-by-layer based on CAD, incorporating stem cells, biomaterials, and growth factors, facilitating the development of multiphasic scaffolds, with each layer designed to regenerate a specific section of the periodontium.

These scaffolds are designed according to a hierarchical architecture capable of guiding the tissue regeneration that takes place simultaneously. This is an ideal approach for tissues with sophisticated anatomy and is useful for establishing connections between soft and hard tissues.[12]

4) Exosomes

MSC-secreted exosomes are currently considered as viable, cell-free, therapeutic alternative for the use of cells.

The biological functions of exosomes depend on the cell's physiologic or pathologic status at the time of secretion and include immune response modulation, signal transduction, and epigenetic modification.[13]

Their advantages over cell therapy include low immunogenicity, high drug loading capacity, biocompatibility, specificity and stability, and lack of cytotoxicity.

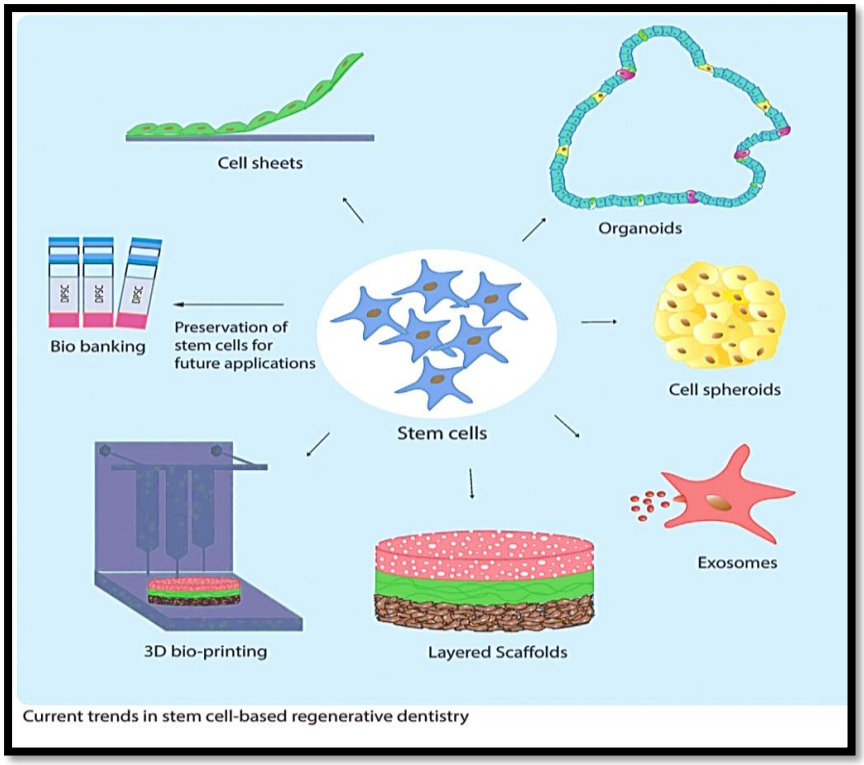
DSC-derived exosomes have shown great potential for dentine-pulp and oral soft tissue regeneration in in vitro and in vivo models.

5) Biobanking

DSCs hold great potential and promise for many clinical applications, but a large number of cells or their derivatives are required for therapeutic efficacy. Therefore, they need to be expanded in vitro and cryopreserved.

However, long-term in vitro culture may pose potential hazards such as chromosomal abnormality, senescence, and microbial contamination.

Cell banking is used to avoid these risks and to preserve the cells at their most potent stage for future applications. The quality control and maintenance of this multistep process is carried out and kept following international guidelines to ensure its safety and effectiveness.[14]



Source: -Zhao Z, Chen X, Dowbaj AM, et al. Organoids. Nat Rev Method

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Challenges in Regenerative dentistry

There is no doubt that regenerative dentistry has progressed a long way, and many groundbreaking discoveries have been made throughout the years.

However, most of the studies involving stem cells have halted at the stage of animal studies and have not proceeded to clinical trials due to numerous debatable safety and ethical concerns, particularly when it comes to the administration of stem cells.

Even though the risk of infection and immune rejection is minimal with the use of heterogeneous cells, the potential risks of undesired tissue formation, tumourigenesis, and metastasis represent a controversial issue that has not yet been resolved.[15]

Further, the experimental reproducibility of certain techniques, such as spheroid formation, is questionable as the exact mechanisms underlying these processes are not yet clearly understood.

In addition to the above, the limited availability of certain MSCs and the required clinical grade and modern, sophisticated technologies that may be limited to advanced clinics and laboratories may also curb the opportunities for the progression of these approaches from bench to clinic.

Summary and conclusion

Regenerative dentistry is a burgeoning field where progress has been expedited using MSCs, including DSCs, BMMSCs, and AMSCs. DSCs have gained popularity over recent years as they surpass widely used BMMSCs in non-invasive accessibility.

Hence, the current research trends are focused on using DSCs or their derivatives as adjuncts, along with other factors such as biomaterials and bioactive molecules that can optimise the performance of cells and mediate tissue regeneration.

CAD and 3D bioprinting have further revolutionised tissue engineering and regenerative approaches where patient-specific customised constructs can be created with high accuracy and precision.

Many of the recent achievements in regenerative dentistry are focused on restoring the structure of lost tissues. However, with the advancement of spheroid and organoid generation, significant levels of functional restoration could also be achieved in the near future.

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