An Insight into CLINICAL ADVANCEMENTS OF REGENERATIVE DENTISTRY

ARATHI. L. JOHNY

ANEEZA V SUKUMARAN

ANEESURAHIMAN .P

DR SIYA K

DR SUHAS R NAYAK

CONTENTS

INTRODUCTION

Regenerative dentistry (RD) is a swiftly progressive branch in the field of evolving dentistry. WHO in the year 2022 published a Global oral health status report which stated that oral diseases are the most prevalent non-communicable diseases affecting almost half of the world's population. Dental caries and periodontal diseases are considered to be the predominant ones.

A well known fact that, the permanent teeth cannot be replaced naturally. But the existing treatment strategies offers symptomatic relief by controlling the disease process with the help of restorative materials. In order to restore the function and form of teeth in its original mode, it is necessary to find out newer methods.
Many researchers worldwide are working on advancing regenerative medicine and tissue engineering field.

Regenerative dentistry is expanding rapidly because of it's assuring nature of unravelling healing potential for various oral diseases. Since; oral and craniofacial tissues have minimal capacity to revive spontaneously and to re-establish their original form & function from dental caries, traumatic injuries of maxillofacial region. Henceforth, dental and craniofacial tissue engineering has been developed over time.

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. The main 3 components of regenerative dentistry comprises:

1) Stem cells
2) Bioactive molecules and
3) Biomaterials[1].

The advantages of 3 components in RD is to enhance the potential of resident cells by promoting stem cell migration towards the site of damage, there by enhance the overall regenerative and reparative mechanism. To enhance the efficacy of the technique various researchers had developed cell free and cell based approaches.

- **Cell-free approaches**: The aim of this approach is to enhance migration of stem cells with the help of bioactive molecules thereby the regenerative process. This is otherwise called as Cell Homing.

- **Cell-based approaches**: These includes administration of exogenously cultured stem cell into the affected tissue 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 dentistry in endodontics (dentin-pulp complex regeneration)

The lifetime vitality of a tooth is regulated by pulpodentin complex as well as the dentinogenesis. The capacity to mineralise and revascularize the dentin and pulp in reponse to caries and trauma has enormous advantages.

In 1952, Baden Wurttenberg Hermann[3] develop regenerative dental procedures to re-establish the normal functioning of pulp.

Considering clinical dentistry; the only regenerative procedure to follow is Pulp Revascularisation. Here we can revascularize an immature permanent tooth with an infected necrotic pulp or periapical abscess or apical periodontitis.[2]

This method deals with the use of intracanal medicaments and antibiotics to disinfect the root canal followed by stimulation of bleeding that give rise to favourable niche.

Thus formed blood clot acts as a framework which helps in homing of stem cells, macrophages, and fibroblasts. At the same time deposition of hard tissue takes place in the dentinal wall. Since it give rise to bone, cementum and PDL, but it is not considered as pulp regeneration.

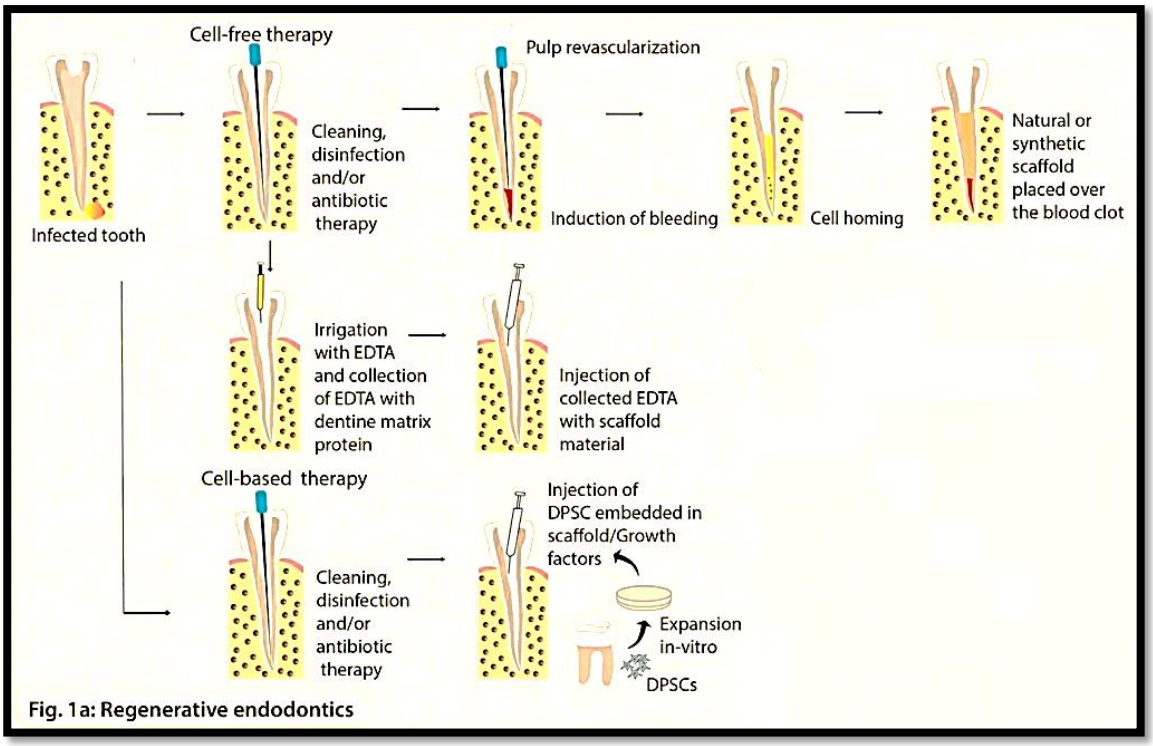
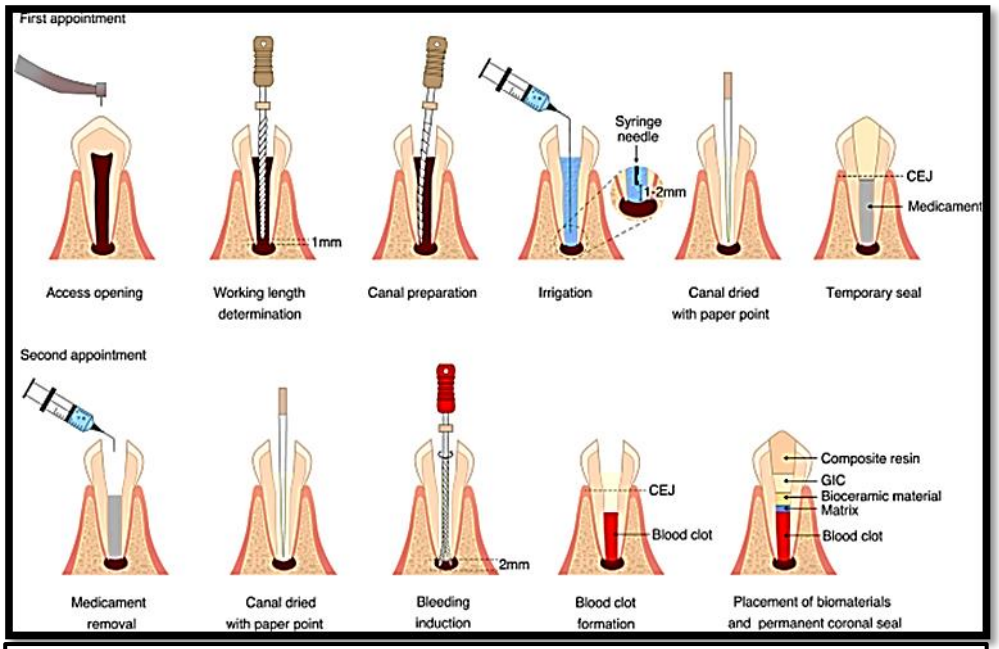


Fig. 1a: Regenerative endodontics

The previously discussed re-vascularizing procedures has been progressed into various approaches where natural or synthetic biomaterials used as adjuncts. Examples are:-

a) In root canal cases, cell homing and differentiation of stem cells can be promoted with the help of placement of Collagen scaffolds over the blood clot.
b) To overcome the limitation of conventional method of bleeding induction Platelet Rich Factor can be used to overcome damages to Hertwig's epithelial root sheath as result of impaired or incomplete root development.
c) Dentine acts as a rich source of growth factors which helps in the regeneration of pulp. These growth factors acts as signalling molecules play a vital role in improved mineralisation, increased regeneration and enhance neurogenesis and angiogenesis.

d) Evidences from in vivo studies conducted by Galler et al concluded that regenerative approach can also be attempted in the clinical dentistry, which involves the Root canal procedures.



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

Emerging techniques in dentistry utilises both the cell- free and cell – based methods for a quick, effective, and successful clinical practice.

(i) Teeth with a necrotic pulp has been transplanted with an autologous DPSCs of human exfoliated deciduous teeth shows regeneration after 12 months with a healthy pulp tissue ,blood vessels and sensory nerves.

(ii) Regenerative cell therapy in endodontics found to be safer and efficient as the apical lesions shows increased sensitivity and blood flow at 12 months post treatment.

2) Regeneration Of Periodontal Tissue

Regenerative techniques in the field of Periodontics appears to have improved long term clinical outcomes especially intrabony and inter radicular defective cases. The objective of the treatment is to reconstruct the defective attachment apparatus along with restoration of periodontal pockets and gingival margins[4].

The aim of Periodontal therapy is restoration and reconstruction of hard and soft tissue components of the periodontium into its normal form and function.

Goals of periodontal regeneration:

1. To improve the attachment of periodontium in a severely compromised tooth
2. To decrease the pocket depth in a range which is manageable
3. To reduce the furcation defects in a vertical as well as horizontal manner.

Reparative approaches got outdated due to the limitations in conventional methods which includes both surgical and non-surgical treatment. Regenerative treatment got wide acceptance in periodontal therapy than reparative procedures as the demerits overlaps its benefits:

(i) Introduction of bone graft as an alloplastic material can induce bone formation.

(ii) One of the golden standard which was widely practiced in periodontal tissue regeneration was Guided Tissue Regeneration. The success rate of GTR depends on the techniques and properties of the barrier membrane used. Colonisation of microbes and membrane rupture has considerable impact over the treatment outcome.[5]

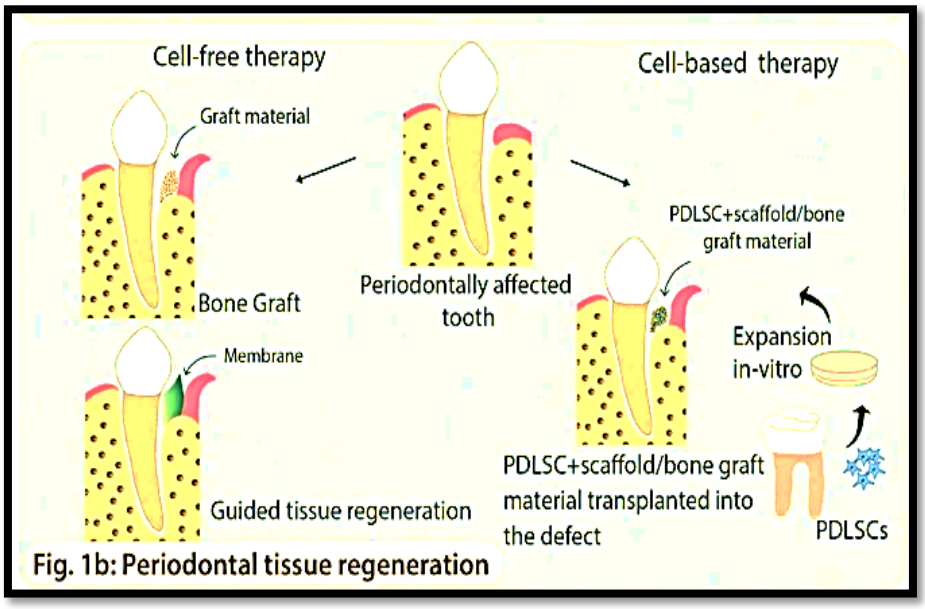


Fig. 1b: Periodontal tissue regeneration

Composite membranes of PCL and gelatin along with ZnO-NPs are produced enormously to overcome their drawbacks which significantly reduces the number of S.aureus, without altering the capability of periodontal cell proliferation

(iii) For improving CAL and radiographic bone fill, Platelet Rich Factor is used with open flap debridement/bone graft procedure.

(iv) Growth factors like Fibroblast Growth Factor -2 (FGF-2), Platelet Derived Growth Factor(PDGF), and BMP-2(Bone Morphogenic Protein-2) are used along with certain biomaterials gives better results.

(v) In cases of mobile tooth, clinical attachment loss and post treatment bone density during follow up period; transplantation of autologous human PDL stem cells taken from extracted 3rd molar is mixed with gelatin scaffold or bone graft material is inserted into the defects after open flap surgical procedure.

3) Regeneration Of Temperomandibular Joint (TMJ)

One of the most common site which is frequently affected by pathologies is Temperomandibular Joint. The pathologies are: autoimmune disorders, disc derangement disorders, and osteoarthritis associated with TMJ.

The treatment approaches varies in the sense sometimes it helps to relieve the symptoms successfully and cases are there, where the complete recovery is not possible. Treatment modalities include conservative treatment such as: intra- articular injections, occlusal splints, arthrocentesis or arthroscopy in mild or moderate cases to radical curative methods like open joint surgery for severe cases.

Therapeutic benefits of DSCs had broadly used in the in-vivo experiments. Studies conducted in animal models leads to the wide use of Bone Marrow Mesenchymal Stem Cells (BMMSCs) in TMJ-OA. The advantages are improved pain relief, increased mouth opening and chewing efficiency.

(i) One of the effective treatment of TMJ-OA is the intravenous administration of serum-free conditioned media of SHED, which can inhibit cartilage destruction and inflammation. It can also promote the regeneration of condylar cartilage and subchondral bone.

(ii) Experiments conducted in animal models shows that exosome therapy helps to control the level of inflammation in TMJ. Exosomes is derived from mesenchymal stem cells along with

Platelet Rich Plasma; which helps in the proliferation leads to healing of subcondylar cartilage and bone.

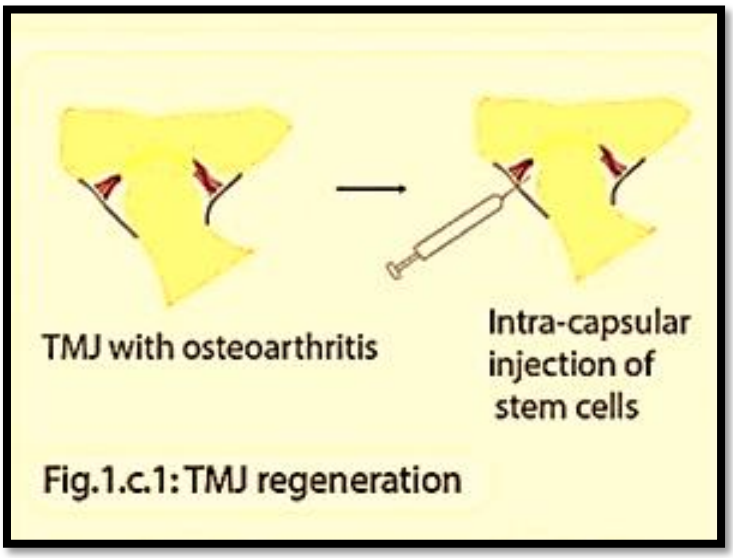


Fig.1.c.1: TMJ regeneration

4) Tissue engineering in Craniofacial Region

(A) **Bone Regeneration**: Oral and maxillofacial structures are the ones which are structurally and functionally complex due to their location (where esthetics and neuromuscular coordination is a requisity). Most of the OMF defects are due to trauma or surgical resection owing to developmental anomalies, infections, benign or malignant tumours.

Reconstruction can be achieved with the help of Bone Morphogenic Proteins, allografts, autogenous bone grafts,

hydroxyapatite calcium phosphate, demineralized bone matrices, collagen scaffolds, and bone marrow aspirate concentrate.

(i) Iliac bone graft and costochondral rib bone graft are the most commonly used graft for reconstructon. In cases of extensive bony defects such as in segmental mandibular resection- Microvascular free fibula flaps are used as the conventional autologous grafts.[7]

(ii) To optimise a bone graft; it is necessary to prolong the viability and stimulate the process of mineralization. New approaches for such improvements include graft vascularisation, via growth factors and bone marrow stimulation.

(iii) To improve the longevity of the implant in resorbed alveolar ridges; bone augmentation procedure should be done before the placement of implants.

(iv) To improve the treatment outcomes; customised 3D printed nanohydroxyapatite (3DHA) block grafts are incorporated with growth factors can be used in Autogenous bone grafts.

(B) **Nerve regeneration**: In surgical practice nerve injuries are very common in the orofacial region as a result of trauma, tumours, or iatrogenic causes. The most commonly involved nerves are: Inferior alveolar nerve (IAN), lingual nerve, infraorbital nerve, and facial nerve. Nerve conduits and autologous nerve grafts are used to reconstruct such nerve gaps.

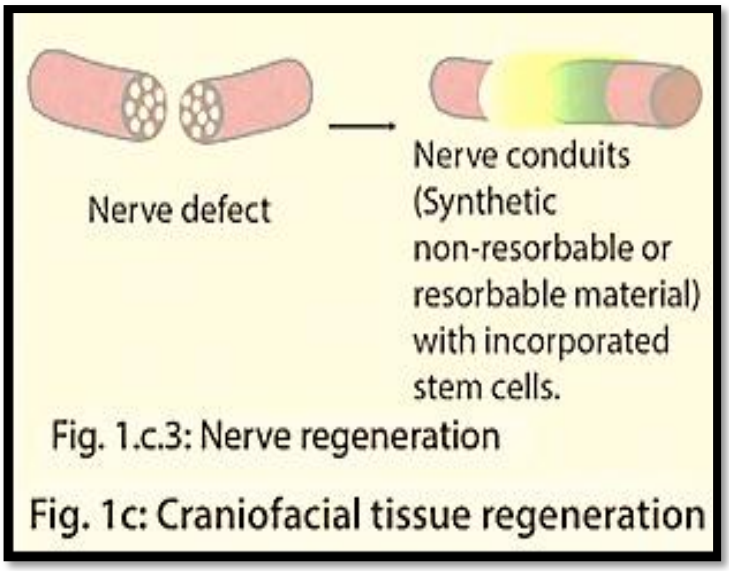


Fig. 1.c.3: Nerve regeneration

Fig. 1c: Craniofacial tissue regeneration

(i) Treatment outcomes with nerve conduits were poor due to the absence of cells in the autologous nerve graft. These nerve conduits were made of synthetic non- resorbable materials.

(ii) Clinical trials in animals shows a favourable outcome with the use of nerve conduits advanced with the incorporation of Schwann cells or stem cells. This found to enhance nerve regeneration via improved cell survival, differentiation, and extracellular matrix (ECM) formation.[8]

(iii) For the regeneration of peripheral nerves; gingival MSCs have been identified as a potential cell source.

(C) **Salivary gland regeneration:** Artificial saliva and sialogogues are the symptomatic treatment measures employed in individuals with impaired salivary gland function. Salivary gland regeneration will be of great benefits in such patients.

Dental stem cells and MSCs shows great advantages in patients with impaired salivary gland. In diseases such as Sjogren syndrome and other related syndromes; Intravenous infusions of Umbilical cord derived MSC which result in increased salivary flow rate.

Salivary flow rate can also be improved in acinar and ductal areas with the intra glandular injection of adipose-derived MSCs (AMSCs) in case of fibrosis of the glands and ducts.[9]

More advanced treatment approaches are essential in the field of regenerative dentistry to enhance better treatment outcomes as well as for the promising results in future.

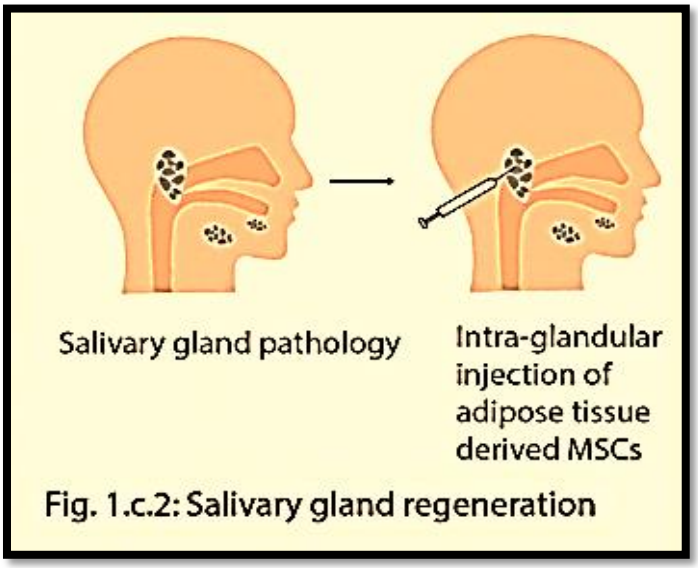


Fig. 1.c.2: Salivary gland regeneration

Recent and Future Advancement in regenerative dentistry

1) Cell sheath, Spheroids and Organoids

In clinical dentistry, DSC therapy has limited outcomes because the cells are administered in mono-dispersed form; which results in poor retention or rapid cell lysis at the site.[10]

(i) A cell-free approach in regenerative dentistry is Cell sheets/sheath. It acts as a scaffold by providing strength and support for the stem cells to enhance cell proliferation and differentiation. They forms high density sheet structures and extracellular matrix. ECM maintains inter-cellular as well as cell-matrix connections.

(ii) A dense, 3D cell aggregates in an extracellular matrix is Spheroid. They are capable of producing powerful Secretome which enhances angiogenesis, alleviates inflammation, and promote the process of cell repair and regeneration.

(iii) A cell-based regenerative approach called Organoid; which is fabricated by in- vitro generation of 3D tissue constructs. They have same microanatomy and functions of the corresponding tissue from in- vivo method via embryonic stem cells or adult stem cells, and induced pluripotent stem cells (iPSCs).

2) 3D Bioprinting

Even though organoid fabrication is considered accurate, they have numerous drawbacks also. The limitations of organoid fabrication can be overcome by a more sophisticated method known as 3D bioprinting.

3D Bioprinting is a more advanced technology which has the ability to produce customised 3D replicas using Computer- aided design (CAD) digital models.[11]

In the upcoming future; we can expect 3D printed organs which is readily available for transplantation.

3)Layered scaffold

Layered scaffold cells are most frequently used in periodontal tissue regeneration. This technique facilitates the development of multiphasic scaffolds. They are generated layer-by-layer using CAD technology into which stem cells, growth factors and biomaterials are incorporated.

Tissue regeneration is guided by layered scaffolds, which are designed according to their hierarchial structure. Thus for sophisticated tissues; it is the best method to be considered and it also helps in establishing connections between soft and hard tissues.[12]

4) Exosomes

Exosomes secreted by Mesenchymal Stem Cells are current therapeutic alternative for the use of cell regeneration. They are considered to be viable and cell- free

The physiologic or pathologic status of cells at the time of secretion is directly proportional to the biological function of exosomes. It includes: immune response modulation, signal transduction, and epigenetic modification.[13]

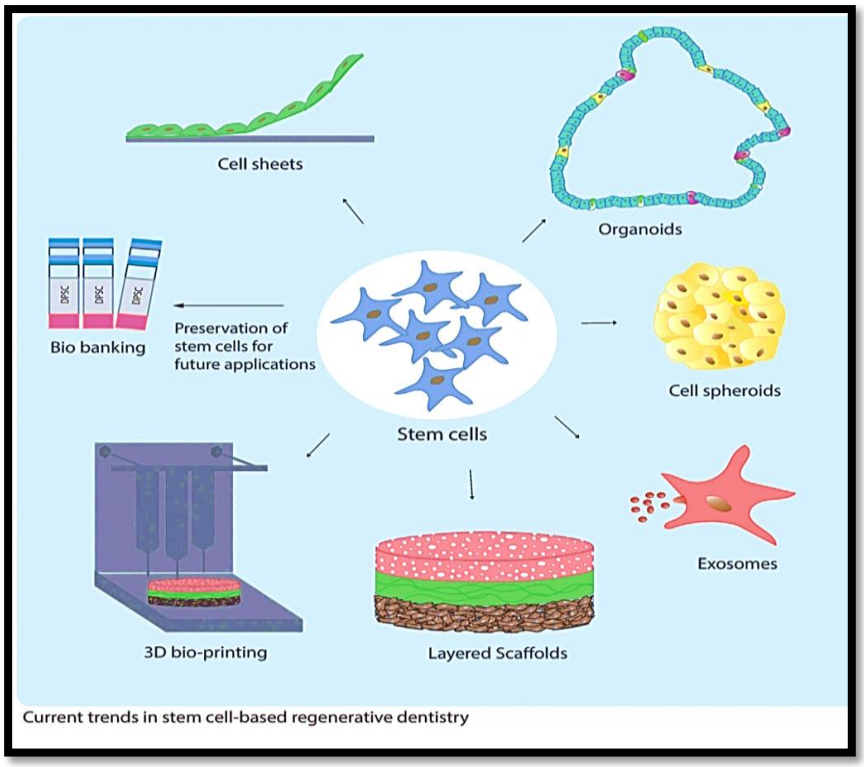
Low immunogenicity, high drug loading capacity, biocompatibility, specificity and stability, and lack of cytotoxicity made them superior over Cell therapy.

In in- vitro and in- vivo models; DSC-derived exosomes have greater potential for regeneration of dentine-pulp and soft tissues.

5) Biobanking / Cell banking

Dental stem cells need to be expanded in- vitro or should be cryopreserved because they hold greater potential in therapeutic field. They may cause many potential hazards such as chromosomal abnormalities, senescence, and microbial contamination. So, long term in-vitro culture is not recommended.

These risk factors has been eliminated using Cell banking. It has the ability to preserve the cells at their most potent stage for future applications. Its safety and effectiveness are ensured by International guidelines.[14]



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

Challenging Aspects in Regenerative dentistry

Regenerative dentistry has undoubtedly advanced a lot and had made many innovative discoveries over these years.

However,due to various safety and ethical issues, studies based on stem cells have been frozen at the level of animal studies and have not move further to clinical trials (Especially in the stem cell administration).

An unresolved controversial issue is the use of heterogeneous cells in regenerative dentistry. It can cause potential hazards such as: undesired tissue formation, tumourigenesis, and metastasis.[15]

The exact mechanisms of spheroid formation is still a mystery. Also; the limited availability of MSCs and modern, sophisticated technologies may restrict the opportunities for the advancement of these approaches.

Conclusion

Regenerative dentistry is the recent emerging branch in dentistry accelerated using Mesenchymal Stem Cells (Dental Stem cells , Bone Marrow Mesenchymal Stem Cells, and Allogeneic Mesenchymal Stem Cells). DSCs have become more popular gained over the past few years since they excel BMMSCs in non-invasive approachability.

So, newer researches are mainly focused on using DSCs or their derivatives, accompanied by bioactive molecules and biomaterials that can enhance the function of cells and promote tissue regeneration.

Tissue engineering and regenerative approaches are further transformed dramatically by CAD and 3D bioprinting where customized patient specific constructs can be made accurately.

Nowadays, RD is targeted on restoring lost tissue structures. In the upcoming days there will be a significant increase in achieving a fully functioning teeth in every aspects in spite of any oral diseases.

REFERENCES

1) ©2023 The Authors. Published by Elsevier Inc. on behalf of FDI World Dental Federation. This is an open access article under the CC BY-NC-ND license., Blog published on “What is regenerative dentistry” by Sandeep Singh in Royal implants online portal

2) Peter E.Murray,Franklin Garcia-Godoy,Stem cells and Regeneration of pulpodentin complex

3) Hermann BW. On the reaction of the dentalpulp to vital amputation and calxyl capping(In German)Dtsch Zahnarztl Z 1952:7:1446-1447

4) Pierpaolo Cortellini And Maurizio S. Tonetti,Regenerative Periodontal Therapy In Clinical Periodontology And Implant Dentistry, 6th Edition

5) Dineshi Sewvandi Thalakiriyawa, Waruna Lakmal Dissanayaka Applied Oral Sciences & Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong

6) Kohnke R, Ahlers MO, Birkelbach MA, et al. Temporomandibular joint osteoarthritis: regenerative treatment by a stem cell containing advanced therapy medicinal product (atmp)—an invivo animal trial Int J Mol Sci 2021;22:1–16

7) Russell J, Pateman K, Batstone M. Donor site morbidity of composite free flaps in head and neck surgery: a systematic review of the prospective literature. Int J Oral Maxillofac Surg 021;50:1147–55

8) Ikeguchi R, Aoyama T, Tanaka M, et al. Nerve regeneration using the Bio 3D nerve conduit fabricated with spheroids. J Artifi Organs 2022;25:289–97.

9) Grønhøj C, Jensen DH, Vester-Glowinski P, et al. Safety and efficacy of mesenchymal stem cells for radiation-induced xerostomia: a randomized, placebo-controlled phase 1/2 trial (MESRIX). Int J Radiat Oncol Biol Phys 2018;101:581–92.

10) Griffin KH, Fok SW, Kent Leach J. Strategies to capitalize on cell spheroid therapeutic potential for tissue repair and disease modeling. NPJ Regen Med 2022;7(1):70

11) Nyirjesy SC, Heller M, von Windheim N, et al. The role of computer aided design/computer assisted manufacturing (CAD/CAM) and 3-dimensional printing in head and neck oncologic surgery: A review and future directions. Oral Oncol 2022;132:105976

12) Abedi N, Rajabi N, Kharaziha M, et al. Layered scaffolds in periodontal regeneration. J Oral Biol Craniofac Res 2022;12:782–97.

13) Kalluri R, Lebleu VS, Biology C. The biology, function, and biomedical applications of exosomes. Science 2020

14) Turinetto V, Vitale E, Giachino C. Senescence in human mesenchymal stem cells: functional changes and implications in stem cell-based therapy. Int J Mol Sci 2016;17:1164.

15) Volarevic V, Markovic BS, Gazdic M, et al. Ethical and safety issues of stem cell-based therapy. Int J Med Sci 2018;15:36–45.