Sri Lanka Dental Journal

The Official Publication of Sri Lanka Dental Association

Volume : 52
Number : 03
December : 2022

ISSN 2950-6662

DEFEAT PAIN IN 30 SECONDS*

helps to stop it from coming back**

SENSITIVE EXPERT by Signal

1323 (TOLL FREE) Call us

Rs. 320/- onwards

* Based on in-vivo study on finger application

** With twice a day brushing.

Unilever

Sri Lanka Dental Journal

Volume 52 ***Number 03*** ***December 2022***

The **Sri Lanka Dental Journal** is a refereed journal published three times a year by the Sri Lanka Dental Association.

Editorial Office

Office of the SLDA,
Professional Centre,
275/75, Prof. Stanley Wijesundara Mawatha,
Colombo - 7.

Correspondence regarding editorial matters, articles, reviews and news items should be addressed to the editors, SLDJ, Prof. N.S. Soysa, Professor in Pharmacology, Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya, niroshanis@dental.pdn.ac.lk, Tel: 0094 76 8818205 and Prof. B.S.M.S. Siriwardena, Professor in Oral Pathology, Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, samadaranis@dental.pdn.ac.lk, Tel: 0094 812397536

Correspondence regarding advertisements and financial matters should be addressed to Dr. Malcolm Stanislaus, 50, Hekitta Cross Road, Hendala, Wattala. Tele: 011 - 2930368

ISSN 2950-6662

Typeset & Layouts:

EM Creations
227/7, Temple Road, Uduwana, Homagama.

C O N T E N T S

Sri Lanka Dental Journal

Volume 52 *Number 03* *December 2022*

GUEST EDITORIAL

Regenerative Potential of Dental Pulp Stem Cell 'Secretome'

Dental stem cells, a type of mesenchymal stem cells (MSCs), have been extensively studied over the last decade and reported to hold a promising therapeutic potential in regenerative medicine and tissue engineering approaches. These cells can be easily isolated from various tooth-related soft tissues such as dental pulp of permanent & deciduous teeth, apical papilla, periodontal ligament and gingival tissue compared to many other types of MSCs which require costly and invasive techniques.

Dental pulp being highly saturated with stem cells and readily available from third molars or premolars extracted for iatrogenic purposes bring a significant advantage. Human dental pulp stem cells (DPSCs) possess the MSC properties including the capacity of selective attachment to solid surfaces, in vitro colony formation and expression of mesenchymal markers and negative expression for hematopoietic antigens (1). Human DPSCs can be differentiated in to multiple cell lineages including odontoblast, chondrocyte, adipocyte, osteoblast, neuronal, hepatocyte among many others. In addition to differentiation potential, DPSCs also have demonstrated neuroprotective and immunomodulatory abilities (1).

Taken together, human DPSCs are highly valued MSCs with fewer limitations in moral, ethical or safety related concerns compared to embryonic stem cells. Accordingly, human DPSCs have exhibited success in dental pulp,

bone and periodontal tissue regeneration in vivo. However, there are multiple limitations in applying stem cell therapies clinically. Mostly, these limitations are due to the absence of clinical grade standard protocols for culture conditions and cell expansion. Furthermore, optimal dosage of MSC infusion for a given application is still under debate.

In order for successful regeneration of the target organs, stem cells should be able to migrate to the tissue of interest, differentiate in to required cell type and secrete broad-array of bioactive factors via secretome (1). It has been evident that transplanted MSCs do not commonly become part of the repaired tissue. Emerging evidence indicate that most of the therapeutic effects of MSCs are mediated exclusively by releasing of bioactive molecules known as 'secretome'.

Secretome can be divided into conditioned medium and (CM) and extracellular vesicles (EVs). The EVs are further categorized into exosomes (EXs), microvesicles (MVs), and apoptotic bodies by origin, size and composition (2). Bioactive factors included in a secretome could consist of various chemokines, cytokines, growth factors, proteins, free nucleic acids (miRNAs, mRNAs, IncRNAs), and lipids (sphingolipid, cholesterol, ceramide). Although secretome could be specific for a given cell type, it could change with altered physiological and pathological conditions. CM is usually produced by culturing stem cells in serum-free medium until 60-80% confluency and collecting supernatant and frozen to preserve biological properties. It was recently demonstrated that CM obtained from human DPSCs could induce cell survival by releasing anti-apoptotic factors. Furthermore, CM from dental MSCs contain angiogenic growth factors including vascular endothelial growth factor (VEGF)-A, fibroblast growth factor (FGF)-2 and platelet derived growth factor (PDGF) (3). These growth factors can promote angiogenesis and play a fundamental role in formation of new bone and repair/ regeneration of dental tissue. For instance, VEGF secreted by DPSCs is one of the critical factors necessary for repairing and remodeling the vascular network (3).

Additionally, dental MSC-CM not only contains multiple bioactive factors that initiate differentiation of neuronal cells and enhance neural growth but also is involved in reduction of neurotoxicity. Hence there have been various attempts to utilize dental MSC-CM in therapies for diseases involving central nervous system (4). For example, in an in vivo model of Alzheimer's disease, CM derived from dental MSCs has shown neuroprotective effects, promoted anti-apoptotic properties, and modulated neuro inflammatory effects. Parkinson's disease (PD) is also a common neurodegenerative disease that causes motor or non-motor disorders. As human DPSCs and stem cells from human exfoliated deciduous teeth (SHED) release neurotrophic factors that stimulate neuroregenerative effects, secretome obtained from these cells are currently under investigation as a therapy for PD (4). Similarly, secretome of hDPSCs and SHED is recognized as a potential therapy in spinal cord injury due to their ability to promote neurogenesis by inducing recruitment of neuronal cells and maturation. In addition, the secretome from human DPSCs, generates neuroprotective microenvironment that nurture the neurons by enhancing neurogenesis, axonal growth, and re-myelination. Also, by promoting cell metabolism, it prevents degeneration and apoptosis. Moreover, Schwann cells that play a key role in maintaining neuronal health have shown inhibited apoptosis and increased proliferation in the presence of dental MSC-CM (4).

In addition to therapy for neurodegenerative diseases, dental MSC-CM is commonly used in tissue engineering applications, mostly in bone regeneration. However, majority of these approaches are still at the experimental status. Dental MSC-CM enhances the migration and proliferation of bone forming stem cells by upregulation of osteogenic genes. It is

also reported that dental MSC-EXs improve osteogenesis/angiogenesis while EVs derived from dental MSCs promote healing of bone defects by enhancing osteoblast differentiation, and improving osteochondral regeneration (1). New bone formation is highly dependent on blood supply. Therefore, the potential of dental MSC derived secretome to stimulate angiogenesis via paracrine angiogenic factors is a key regulator of bone formation.

Regenerative therapies are recently directed to articular tissue repair, especially articular cartilage of temporomandibular joint. In an animal model, it has been demonstrated that SHED-CM could promote the regeneration and repair of articular cartilages when affected by osteoarthritis by proliferation of the multipotent polymorphic cell layer and production of cartilage matrix (5). It was also found that SHED-CM can inhibit the articular degradation cascade and reduce further inflammatory damage. During therapy for osteoarthritis using secretome, chondrocytes have downregulated the inflammatory pathways and produced inflammatory cytokines exhibiting enhanced anti-inflammatory ability. Moreover, in another study, when human DPSC-CM was injected to joint cavity of rheumatoid arthritis induced mice, joint symptoms were relieved with reduction of synovial inflammation, cartilage damage and bone erosion (5).

Combination of dental MSC-CM secretome with scaffolds is one of the most popular approaches in tissue engineering (6). These CM impregnated 3D scaffold-based cultures have been used to stimulate the integration of stem cells into host tissues. Scaffolds incorporated with secretome can even be used as a cell-free mean of tissue engineering. Number of in vivo experiments has demonstrated that scaffolds loaded with dental MSC secretome can be successfully used in regeneration of dental pulp, bone, cartilage, or spinal cord injury. However, selection of scaffolds for regenerative purposes must be carefully decided to match with the biological and physical parameters of the host tissue (6).

Taken together, it is clear that the therapeutic action of mesenchymal stem cells is largely mediated by the paracrine action through secreting soluble factors and EVs. This secretome nurtures the surrounding environment and provide a supportive microenvironment to heal the damaged tissue and initiate intrinsic regenerative process. Research conducted on dental MSC secretome has identified its unique ability to release biomolecules that mediate cell-to-cell signaling for a longer duration in the regenerative process following disease or injury. This significant clinical potential could be utilized as an alternative to cell-based therapy. Although, further mechanisms and actions of dental MSC secretome are yet to be fully explored, therapy based on the secretome is one of the viable next options in regenerative medicine.

1. Han Y, Zhang L, Zhang C, Dissanayaka WL. Guiding Lineage Specific Differentiation of SHED for Target Tissue/Organ Regeneration. Curr Stem Cell Res Ther. 2021;16(5):518-534.

2. Thalakiriyawa DS, Jayasooriya PR, Dissanayaka WL. Regenerative Potential of Mesenchymal Stem Cell-Derived Extracellular Vesicles. Curr Mol Med. 2022;22(2):98-119.

3. Han Y, Gong T, Zhang C, Dissanayaka WL. HIF-1α Stabilization Enhances Angio-/Vasculogenic Properties of SHED. J Dent Res. 2020 Jul;99(7):804-812.

4. Gugliandolo A, Mazzon E. Dental Mesenchymal Stem Cell Secretome: An Intriguing Approach for Neuroprotection and Neuroregeneration. Int J Mol Sci. 2021 Dec 31;23(1):456.

5. Matheus HR, Özdemir ŞD, Guastaldi FPS. Stem cell-based therapies for temporomandibular joint osteoarthritis and regeneration of cartilage/osteochondral

defects: a systematic review of preclinical experiments. Osteoarthritis Cartilage. 2022 Sep;30(9):1174-1185.

6. Dissanayaka WL, Zhang C. Scaffold-based and Scaffold-free Strategies in Dental Pulp Regeneration. J Endod. 2020 Sep;46 (9S):S81-S89.

Waruna L Dissanayaka
BDS, PhD, PDipMDPath, FHEA
Assistant Professor and Assistant Dean
(Electives & Experiential Learning)
Faculty of Dentistry, The University of Hong Kong
Secretary, IADR-SEA Division

Research Article

Sri Lanka Dental Journal 2022; 52(03) 103-109
Received: 20.11.2022 | Revised: 12.12.2022 | Accepted: 13.12.2022

pH of some commercially available mouthwashes in Sri Lanka

BDSN Neris, MAMU Nethmini, HBY Perera, KNK Ranasinghe, MP Paranagama, KSND Gunawardhana

Abstract

Objective: Out of the numerous properties of mouthwashes, acidity of them can be potentially erosive and harmful to oral tissues. Despite the increased awareness and usage of mouthwashes, studies on the pH of mouthwashes in Sri Lanka are scarce. Hence, this study was conducted to analyse the pH of several commercially available mouthwashes in Sri Lanka regardless of their efficacy as antimicrobial agents.

Materials and methods: Ten brands of commonly used commercially available mouthwashes in Sri Lanka were selected and the pH was analysed. The experiments were performed in triplicates using three different batches of each brand of mouthwash.

Results: The pH of mouthwashes ranged from 2.69 to 6.09 and 80% of mouthwashes had a pH less than 5.5, the critical pH for enamel dissolution. None of the mouthwashes had pH $\geq$ 7.00.

Conclusion: The present study revealed that the majority of tested mouthwashes have low pH values. It indicates that they are potentially erosive if not used properly. These findings may be useful in the selection of mouthwashes and further studies are required to explore the other properties essential for proper mouthwash selection.

Keywords: pH, mouthwashes, Sri Lanka

Introduction

Oral health is an important and integral part of general health, and good oral hygiene is essential for oral health. To maintain good oral hygiene, mechanical plaque removal by regular brushing and interdental cleaning is necessary. When only these methods alone are not sufficient to maintain oral hygiene at an optimum level, mouthwashes are used as adjuncts.

The use of mouthwashes was reported from as far back in history as 2700 BC, even before the invention of toothbrushes[1]. They are liquid products that are used to rinse the mouth to maintain oral health by preventing, relieving and curing oral conditions such as dental caries, dental erosion, halitosis, gingivitis, periodontitis and mucositis[2]. They can be broadly categorised into two main types as therapeutic and cosmetic mouthwashes[3]. Therapeutic mouthwashes are those with active ingredients such as fluoride, chlorhexidine, and cetylpyridinium chloride, which help to control conditions like plaque,

BDSN Neris Faculty of Dental Sciences, University of Peradeniya.
MAMU Nethmini Faculty of Dental Sciences, University of Peradeniya.
HBY Perera Faculty of Dental Sciences, University of Peradeniya.
KNK Ranasinghe Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya.
MP Paranagama Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya.
KSND Gunawardhana (Correspondence) Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya.
ORCID: 0000-0003-2618-5050
First 3 authors contributed equally

periodontal diseases, dental caries or halitosis while cosmetic mouthwashes have mainly a mouth freshening effect[4]. They have an added advantage over other methods used in the maintenance of daily oral hygiene measures since they can reach many areas of the oral cavity which cannot be reached during brushing and flossing.

A huge diversity of mouthwashes is commercially available in Sri Lanka as prescription or over-the-counter (OTC) products in pharmacies, supermarkets and even grocery shops. From these mouthwashes, patients and oral health professionals have to select the most appropriate one for a particular need. When selecting a mouthwash, it is important to consider the prevailing oral condition, the risk for occurrence of other oral diseases in the patient and especially its safety and efficacy[5].

When considering the safety and efficacy of mouthwash, one of the essential parameters to be considered is its pH. It is because the deviation of the oral pH from its optimum pH required for the maintenance of healthy oral tissues is detrimental to oral health. Generally, soft and hard tissues of the oral cavity are coated with saliva maintaining a neutral healthy environment. It has been found that salivary pH of 7 creates a healthy dental and periodontal environment with little or no incidence of dental decay and calculi formation[6].

It has been reported that a pH value equal to or less than 5.5 is considered critical for enamel dissolution[7] while several studies have reported that the critical pH for dissolution of dentin is to be in the range of 6-6.9[8-10]. Therefore, when the oral pH drops below 7 and continues for a longer period, it can predispose to conditions such as dental caries, dental erosion and halitosis[11]. In contrast, when the oral pH value increases above 7.6 it creates an alkaline environment in the oral cavity which facilitates the formation of dental plaque crystals[6].

Mouthwashes have their inherent pH and thus have the potential to affect the neutral environment of the oral cavity. It has been reported that the pH of mouthwashes is determined by their active ingredients such as essential oils, antibacterial peroxidases, sodium bicarbonate and flavours[10].

Even though several studies have been carried out to analyze the pH of commercially available mouthwashes in other countries[12-15], research done on this topic in Sri Lanka is scarce. Moreover, many manufacturers do not indicate the pH of their products which causes a gap in the knowledge about their pH values. Hence, it's essential to have sufficient knowledge of the pH level of mouthwashes in the Sri Lankan market, as this will help dental healthcare professionals to select and prescribe the most appropriate mouthwashes to patients as well as customers to select mouthwashes based on their chemical properties to get maximum benefits.

Therefore, this study aims to assess the pH of ten commercially available, commonly used mouthwashes in Sri Lanka and fill this knowledge gap which existed for a long time.

Materials and Methods

Ten commercial brands of mouthwashes comprising various active ingredients were selected for this study and analysed in the Biochemistry Laboratory of the Department of Basic Sciences, Faculty of Dental Science, University of Peradeniya. Three different batches of each mouthwash were purchased from different supermarkets and pharmacies in Sri Lanka as over-the-counter products. All the information about the composition, manufacturing date, expiry date and other relevant information were obtained from the packaging.

Determination of pH

A digital pH meter (Horiba, Japan) was used to measure the pH of mouthwashes. The instrument was calibrated using pH 4.01, pH 7.01, and pH 10.01 buffer solutions before analyzing the mouthwashes. The pH of each mouthwash was

analysed immediately upon opening the bottle and data were collected by a single examiner. To measure the pH of mouthwashes, the electrode of the pH meter was dipped in 10ml of each mouthwash sample until the reader gives a stable value. All the readings were triplicated. Since there were three bottles from each brand, nine readings were recorded for each brand and the mean of the nine readings was considered as the pH of the mouthwash.

Data analysis

The pH values of mouthwashes were entered into Microsoft Excel and data were expressed as Mean ± Standard Deviation (SD).

Ethical consideration

Ethical clearance for this study was obtained from the Ethics Review Committee of the Faculty of Dental Sciences, University of Peradeniya.

Results

The selected mouthwashes were coded from A to J (Figure 1). Out of the ten brands of mouthwashes, five mouthwashes (A, B, C, H and J) have been manufactured locally while D, E, F, G, and I mouthwashes have been imported. The active ingredients of selected brands of mouthwashes as listed on the labels are shown in Table 1.

Table 2 shows the distribution of mean pH values

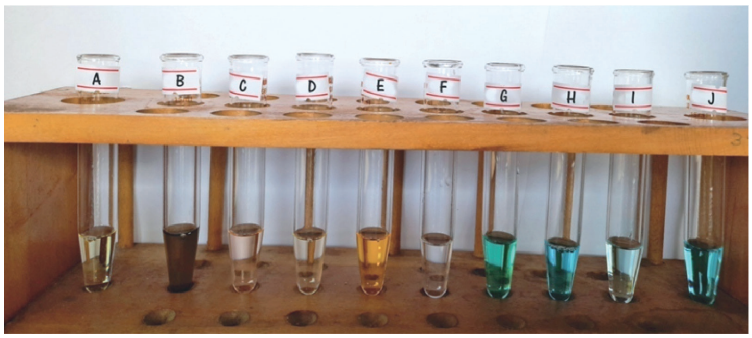


Figure 1. Selected ten brands of mouthwashes for the study with their codes

Table 1. Composition of the mouthwashes used in the study as mentioned on the label

Code	Locally manufactured or Imported	Active Ingredients
A	Locally manufactured	Sodium benzoate, Sodium fluoride, Thymol, *Syzygium aromaticum* (clove) extract
B	Locally manufactured	Natural plant extracts
C	Locally manufactured	Castor oil, Bisabolol, Spearmint, Clove oil, Cinnamon oil
D	Imported	Benzoic acid, Eucalyptol, Methyl salicylate, Thymol, Sodium benzoate, Menthol
E	Imported	Sodium fluoride (200ppm), Sodium phosphate, Phosphoric acid
F	Imported	0.2% W/V chlorhexidine digluconate, Castor oil, Peppermint oil
G	Imported	Chlorhexidine gluconate 0.2% W/V
H	Locally manufactured	Thymol, Benzoic acid, Menthol, Eucalyptol , Methyl salicylate
I	Imported	Chlorhexidine gluconate 0.2% W/V
J	Locally manufactured	PEG-40 hydrogenate castor oil, Sodium fluoride, Menthasachalinensis (Mint) extract

Table 2. Distribution of mean pH values of mouthwashes with their standard deviation (SD)

Mouthwash	Mean pH ± SD
A	5.83 ± 0.23
B	2.69 ± 0.12
C	4.82 ± 0.27
D	4.20 ± 0.05
E	4.09 ± 0.04
F	6.09 ± 0.02
G	4.40 ± 0.10
H	3.68 ± 0.08
I	5.12 ± 0.08
J	4.79 ± 0.02

and standard deviation of mouthwashes analysed in this study. The pH values ranged from 2.69 to 6.09 and a total of eight brands of mouthwashes had pH values less than the critical value, 5.5.

According to the study findings, mouthwash B, which consisted of natural plant extract showed the lowest pH value (2.69) and mouthwash F, which contained 0.2% chlorhexidine digluconate showed the highest pH value (6.09). None of the mouthwashes had a pH value around or above the neutral pH of 7.00.

Discussion

Even though mouthwashes have been used for many years, recently, there is a marked increment of research activities to evaluate the properties and active ingredients of them all over the world. These mouthwashes consist of various formulations where the acidity of the formulations can vary according to the constituents and play a major role in the outcome that is gained with the usage of mouthwashes. Hence, the present study evaluated the pH of ten brands of commonly used commercially available mouthwashes in Sri Lanka.

All 10 brands of mouthwashes tested in this study showed pH values below 7, ranging from 2.69 (B) to 6.09 (F). According to the results, it could be assumed that there is a higher chance for the commercially available mouthwashes in Sri Lanka to have an acidic pH. As salivary pH ranges from 6.2 to 7.6 with 6.7 being the average in a healthy individual [6], mouthwashes in this range are conducive to the oral environment. However, non of the mouthwashes tested in the present study has a pH value within that favourable range.

A similar study conducted using eleven brands of mouthwashes in the Indian market has found that the pH values of their mouthwashes were in the range of 4.01 to 6.58 while 54.5% of the mouthwashes had pH values lesser than the critical value of 5.5[13].

Another in vitro study that evaluated the pH of mouthwashes in Sulaimani/ Iraq Markets has found that the pH range of their mouthwashes was 4.4 to 7.85. Among these mouthwashes, 26.7% had a pH value lesser than 5.5 while 26.7% had a pH value greater than 7.00[14].

A study conducted on the mouthwashes in the Brazilian market has recorded the lowest and the highest pH values as 3.56 and 7.43 respectively[12]. Seventy percent of those mouthwashes were reported to have a pH value above 5.5 while in the present study it was only about 20%.

In contrast to the aforementioned international studies, the present study had a higher percentage of mouthwashes (80%) with a pH value lesser than 5.5. Intriguingly, unlike in many other studies, mouthwashes with a pH value above 7 were not found in the present study. When compared with the results of other studies, the present study had the mouthwash with the lowest pH value of 2.69.

It has been shown that low pH values in mouthwashes containing essential oils with some concern for dental erosion despite their beneficial properties like antibacterial and anti-inflammatory effects[5,16]. Clove oil, peppermint

oil, thymol, eucalyptol, methyl salicylate and bisabolol are such essential oils or compounds of essential oils commonly found in mouthwashes[2,17]. Accordingly, it can be assumed that low pH values of the mouthwashes A, C, D, F, H and J are due to their essential oil component. Similarly, mouthwash B which shows the lowest pH also possesses natural plant extracts rich in essential oils while the low pH of mouthwash E could be due to its phosphoric acid content. It has been reported in the literature that the optimal antimicrobial effect of chlorhexidine gluconate was achieved within the pH ranges of 5.5 to 7[18]. Even though the mouthwash F, G, and I tested in the present study contained chlorhexidine, only the mouthwash F was within that pH range.

Use of mouthwashes with a pH below the critical pH for enamel dissolution for longer durations may cause harm to dental tissues and thereby increasing the risk of developing dental caries[12]. In addition, they may cause corrosion of orthodontic wires[14]. Moreover, acidic mouthwashes were found to affect dental materials such as resin composites and GIC, by altering their chemical properties and causing chemical degradation[19]. As evidence suggests, mouthwashes like B with an extremely low pH (2.69) may have a higher potential of developing dental erosion, dental caries and corrosion of dental materials with prolonged use. Hence, it is better to avoid prolonged usage of mouthwashes with such a low pH, as much as possible.

However, it has been reported that the taste of commercially available mouthwashes may increase salivary flow preventing the chance of enamel dissolution due to their low pH[2]. Such findings suggest that the pH of mouthwashes alone is not a good indicator to measure their erosive potential as biological fluids like saliva neutralise sudden pH changes[20].

Further, in an acidic medium, the incorporation of fluoride into calcium hydroxyapatite crystals becomes more efficient and the resulting calcium fluorapatite is more acid-resistant. It minimises demineralization and promotes the remineralization of dental hard tissues. Therefore, slightly acidic, fluoride-containing mouthwashes are reported to be more beneficial over alkaline fluoridated mouthwashes[21]. Hence, out of the fluoride-containing mouthwashes (A, E & J) tested in the present study, mouthwash A could be considered beneficial over the rest of the mouthwashes due to its pH being above critical pH and the presence of fluoride.

In contrast, mouthwashes containing sodium bicarbonate have been reported to be beneficial because of their alkaline pH and their ability to increase reduced salivary pH through their buffering action[5]. However, none of the mouthwashes used in the current study contained bicarbonates as an active ingredient indicating another possible factor for their low pH.

By considering most of the facts discussed and evidenced in the literature, it is not justifiable to declare that all the mouthwashes used in the current study are harmful to dental tissues relying only on their pH values. To select or recommend a suitable mouthwash, a complete analysis of their other chemical and physical properties, antimicrobial activities, fluoride content and toxicity studies is crucial. Hence, further studies are recommended on the above-mentioned aspects to provide a complete picture of commercially available mouthwashes in Sri Lanka.

Conclusion

All the mouthwashes tested in this study have pH values below 7. Among them, only two mouthwashes exhibited pH values above the critical pH (5.5) for tooth demineralization. Thus these two mouthwashes may not be potentially erosive. The rest of the mouthwashes are more suitable to be used as short to medium-term adjuncts and never to be used as pre-brushing rinses due to their low pH. Further in vitro and in vivo studies are crucial to have a better

understanding of the beneficial and harmful effects of commercially available mouthwashes.

Acknowledgement
The authors would like to thank Mr. MGB Gangadhara and Mr. RMA Rathnakumara for their technical assistance in this study.

References

1. Jardim JJ, Alves LS, Maltz M. The history and global market of oral home-care products. Braz Oral Res. 2009; 23(Suppl 1): 17–22. doi: 10.1590/s1806-83242009000500004.

2. Radzki D, Wilhelm-Węglarz M, Pruska K, Kusiak A, Ordyniec-Kwaśnica I. A fresh look at mouthwashes-What is inside and what is it for? Int J Environ Res Public Health. 2022; 19(7): 3926. doi: 10.3390/ijerph19073926. PMID: 35409608; PMCID: PMC8997378.

3. Zero DT. Dentifrices, mouthwashes, and remineralization/caries arrestment strategies. BMC Oral Health.2006; 6(Suppl 1): 1–13. doi: 10.1186/1472-6831-6-S1-S9.

4. Takenaka S, Ohsumi T, Noiri Y. Evidence-based strategy for dental biofilms: Current evidence of mouthwashes on dental biofilm and gingivitis. Jpn Dent Sci Rev. 2018; 55(1): 33–40. doi: 10.1016/j.jdsr.2018.07.001.

5. Farah CS, McIntosh L, McCullough MJ. Mouthwashes. Aust Prescr. 2009; 32: 162–4. doi: 10.18773/austprescr.2009.080.

6. Baliga S, Muglikar S, Kale R. Salivary pH: A diagnostic biomarker,J Indian Soc Periodontol. 2013; 17(4): 461–5. doi: 10.4103/0972-124X.118317.

7. Bowen WH. The Stephan Curve revisited, Odontology, 2013; 101(1): 2–8. doi: 10.1007/s10266-012-0092-z.

8. Sung YH, Son HH, Yi K, Chang J. Elemental analysis of caries-affected root dentin and artificially demineralized dentin. Restor dent Endod. 2016; 41: 255–61. doi: 10.5395/rde.2016.41.4.255.

9. Neel EAA, Aljabo A, Strange A, et al. Demineralization-remineralization dynamics in teeth and bone. Int J Nanomedicine. 2016; 11: 4743–63. doi: 10.2147/IJN.S107624. PMID: 27695330.

10. Delgado AJ, Olafsson VG, Donovan TE. pH and erosive potential of commonly used oral moisturizers. J Prosthodont. 2016; 25(1): 39–43. doi: 10.1111/jopr.12324. Epub 2015 Jul 27. PMID: 26216576.

11. Pontefract H, Hughes J, Kemp K, Yates R, Newcombe RG, Addy M. The erosive effects of some mouthrinses on enamel. A study in situ. J Clin Periodontol. 2001; 28(4): 319–24. doi:10.1034/j.1600-051x.2001.028004319.x.

12. Cavalcanti AL, Ramos IA, Leite RB, et al. Endogenous pH, titratable acidity and total soluble solid content of mouthwashes available in the Brazilian market. Eur J Dent. 2010; 4(2): 156–9. PMID: 20396446; PMCID: PMC2853830.

13. Vivek S, Shwetha R. Endogenous pH, titratable acidity of commercially available mouthwashes in the Indian market. Int J Clin Trials. 2015; 2: 20–4. doi: 10.5455/2349-3259.ijct20150204.

14. Garib BT, Tofiq SA, Fakhruldeen ZE. pH of commercially available brands of toothpaste and mouthwashes in Sulaimani/Iraq markets: An in vitro Study. Sulaimani Dent J. 2019; 6(2): 45–52.

15. Qureshi S, Mili´c L, Petrovi´c B, et al. The measurement of contact angle, pH, and conductivity of artificial saliva and

mouthwashes on enamel, Glass-Ionomer and Composite dental materials. Materials (Basel). 2022; 15 (13), 4533. doi.org/10.3390/ma15134533.

16. Claffey N. Essential oil mouthwashes: A key component in oral health management. J Clin Periodontol. 2003; 30(Suppl 5): 22–4. doi: 10.1034/j.1600-051X.30.s5.8. x.

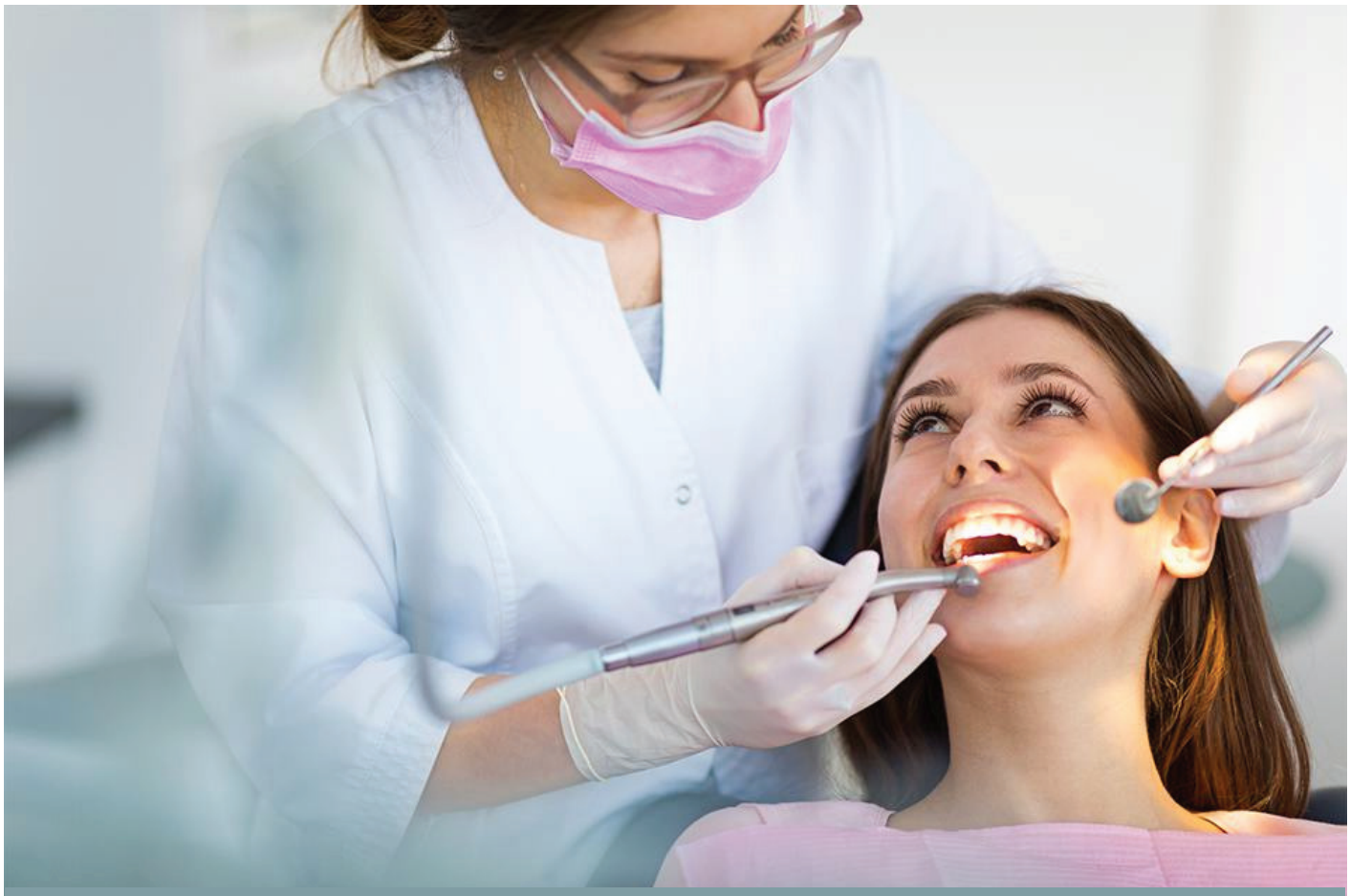
17. Tongnuanchan P, Benjakul S. Essential Oils: Extraction, bioactivities and their uses for food preservation. J Food Sci.2014; 79(7): 1–19. doi: 10.1111/1750-3841.12492.

18. Mohammadi Z. Chlorhexidine gluconate, its properties and applications in endodontics. Iran Endod J. 2008; 2(4), 113–25. PMID: 24265633; PMCID: PMC3834637.

19. Soares LES, Soares ALS, De Oliveira R, De Oliveira S. The effects of acid erosion and remineralization on enamel and three different dental materials: FT-Raman spectroscopy and scanning electron microscopy analysis. Microsc Res Tech.2016; 79(7): 646–56. doi: 10.1002/jemt.22679.

20. Belardinelli PA, Morelatto RA, Benavidez TE, Baruzzi AM, Blanc SALD. Effect of two mouthwashes on salivary pH, Acta Odontol Latinoam. 2014; 27(2): 66–71. doi: 10.1590/S1852-48342014000200004.

21. Hellwig E, Lussi A. Oral hygiene products and acidic medicines. Monogr Oral Sci. 2006; 20: 112–18. doi:10.1159/000093358.



The 1st line Drug of Choice for Dental Infections

Axcil®

Amoxycillin 125mg/5ml
for oral suspension USP & Amoxycillin Capsules
USP 250 mg / 500 mg

Best Choice of Amoxycillin Brand..!

EFFICACY GUARANTEED HIGH QUALITY

Astron Estd. 1956

QUALITY HEALTHCARE | TRUSTED EXCELLENCE | GLOBAL STANDARDS

Manufactured & Marketed by
Astron Limited, 688, Galle Road, Ratmalana. Tel : 011 2148799, 011 5566741 Fax : 011 2636173 E-mail : marketing@astron.lk Web : www.astron.lk

Review Article

Sri Lanka Dental Journal 2022; 52(03) 110-127
Received: 11.11.2022 | Revised: 28.11.2022 | Accepted: 01.12.2022

Tissue Repair and Immunomodulatory roles of Mesenchymal Stromal Cells: An Overview

NS Soysa, D Thalakiriyawa, WL Dissanayaka, CNRA Alles

Abstract

Mesenchymal stromal cells (MSCs) are multipotent, non-hematopoietic cells with self-renewing capabilities and clonogenicity. They are a heterogeneous cell population characterized by plastic adherence, plasticity, and cell surface markers which distinguish them from hematopoietic stem cells(HSCs). Many biological properties, including mobilization and homing into the effete tissue, differentiation into tissue-specific cells, secretion of trophic factors, and tissue repair, make these cells an attractive therapeutic strategy in ameliorating a broad range of disease conditions including bone diseases such as osteogenesis imperfecta, stroke, myocardial infarction, diabetes to name a few. Being immune privileged, MSCs exert their effect on both innate and adaptive immunity. Above all, the immunomodulatory effect of these cells may overcome the problems of rejection associated with conditions like Graft-Versus-Host Disease (GVHD). Therefore, the rational use of mesenchymal stromal cell-based therapy might be the centerpiece in regenerative medicine in the foreseeable future.

Key words: Mesenchymal stromal cells, tissue repair, immunomodulation, paracrine activity.

1. Introduction

Mesenchymal stem/stromal cells (MSCs),initially identified in the bone marrow (BM), are non-hematopoietic, multipotent cells that are capable of self-renewing and differentiating into various cell lineages. Evidence for the existence of cells with self-renewal ability and mesodermal lineage differentiation was first revealed by Friedenstein *et al*., in 1970[1]. The term mesenchymal stem cells was coined by Caplan[2] for these cells because of their potential to differentiate into cells of the mesenchymal lineages. MSCs are harvested from a multitude of adult tissues such as bone marrow, peripheral blood, adipose tissue, dental pulp, and periodontal tissues, as well as fetal tissues such as the umbilical cord and placenta[3]. Diverse procedures and different approaches used to isolate MSCs from varying sources cause ambiguity as to how to specifically identify these cells as MSCs. Therefore, three criteria were proposed by the International Society for Cell Therapy (ISCT) to identify human cells as MSCs[4]. The criteria include the ability of the cells to adhere to plastic in culture, *in vitro* tri-lineage differentiation into osteoblasts (bone), adipocytes (fat), and chondroblasts (cartilage), and the expression of certain surface markers by flow cytometry. The cells should ideally

NS Soysa (Correspondence) — Department of Oral Medicine and Periodontology, Faculty of Dental sciences, University of Peradeniya, Sri Lanka. ORCID: 0000-0002-6187-9341

D Thalakiriyawa — Applied Oral Sciences and Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong.

WL Dissanayaka — Applied Oral Sciences and Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong.

CNRA Alles — Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Sri Lanka.

express cell markers such as CD73, CD90, and CD105 in greater than 95% of the culture and should not express hematopoietic cell markers such as macrophage antigen CD11b, monocyte, and macrophage antigen CD14, B-lymphocyte antigens CD19 and CD79, leukocyte antigens CD34 and CD45, and MHC class II antigen HLA-DR in more than 2% of the culture.

The functional versatility of MSCs includes their ability to mesodermal lineage differentiation into osteoblasts, chondrocytes, and adipocytes[5]. In addition, there is a plethora of reports to show the ability of the MSCs to differentiate into endodermal and ectodermal lineage cell types, including vascular smooth muscle cells, nerves, hepatocytes, lung parenchymal cells, epithelial cells, etc. However, the *in vitro* differentiation of MSCs largely depends on the culture conditions and many factors including growth factors and chemical as well as physical factors. Last two decades have seen the employment of MSCs in forming cartilage or bone to cure the related conditions. Furthermore, the ability of the MSCs to differentiate into vascular smooth muscle cells and their secretion of 'trophic' factors has been used to revascularize ischemic myocardium. More interestingly, other functional and biological features such as immunomodulation and tissue repair have brought the MSCs to the forefront of managing immunological disorders such as graft-versus-host disease (GVHD). This review is an attempt to address the biological properties of MSCs including immunomodulation and tissue repair effects.

2. Biological properties of MSCs

Biological and functional properties of MSCs include their ability to proliferate, self-renew, mediate intercellular communication, maintain the hematopoietic niche, tissue repair, induce mobilization and homing, differentiate and trans-differentiate, secrete paracrine factors, immunomodulate and so on.

2.1 Clonogenicity and self-renewal capacity

The ability of a single cell to give rise to identifiable offspring is known as clonogenicity. It can be one type or several types of differentiated cells. MSCs self-renew by asymmetric or symmetric cell division giving rise to identical cells and daughter cells, which invariably progenerate into various types of mature cells with specific functions[6]. *In vitro,* MSCs can divide a finite number of times in culture. It is estimated that the maximum number of population doublings that MSCs can achieve is around 30 to 40,[7,8] depending on the donor. And then, usually, the cells enter senescence or 'Hayflick limit'.

2.2 Intercellular communications

Cell-to-cell communication or cellular crosstalk is important for proper coordination among different cells within their microenvironment. Similarly, in response to various stimuli in the environment, MSCs modulate the activity of neighboring cells. One mechanism of MSCs employed in modulating other cells is by secretion of soluble factors such as cytokines, chemokines, and growth factors, as well as by cell fusion[9]. Using a co-culture system of human MSCs with heat-shocked human small airway epithelial cells (SAECs), the latter study showed that MSCs fused with epithelial cells while some cells showed nuclear fusion as well[9]. Besides the soluble factors and cell fusion, another important mode of intercellular communication is the exchange of microparticles which are plasma membrane-derived vesicles/ exosomes that are <1 μm in diameter and are secreted by cells. By using MSCs derived from human embryonic stem cells (hESCs) Chen *et al.,*[10] showed that MSCs secrete microparticles enriched in miRNA that were essentially a subset of those in MSCs. Quesenberry and Aliotta[11] have shown that once microvesicles (MV) are moved from lung to marrow cells, the MSCs assume the characteristics of pulmonary epithelial cells having their respective mRNA[11]. It seems that the alteration of cells by transfer of MVs is responsible for the conversion of bone marrow stem cell phenotype by the cells of the liver, brain,

and heart[11], suggesting that MV transfer among cells causes a continuous genetic modulation of MSCs (continuum model) and change their plasticity. Thus, the preceding observations show that MSCs exert multiple effects on their neighboring cells by affecting their proliferation, survival, matrix modeling, and so forth.

2.3 Maintenance of hematopoietic niche

The stem cell niche is referred to as the micro-environment where stem cells reside and receive stimuli that determine their fate. It is regarded as the place where extrinsic signals interact and integrate to influence stem cell behavior rather than a physical location[12]. The stem cell niche consists of stromal cells, extracellular matrix, stimuli in the form of soluble signaling which comes from the adjacent support cells, neural inputs, and blood vessels that provide nutritional support and carry systemic signals[13,14] (Figure 1) which are necessary for the self-renewal and differentiation. MSCs provide a supportive environment for hematopoiesis by cell-to-cell contact via direct contact with the hematopoietic stem cells (HSC) and by secreting growth factors as well as cytokines that are vital for HSC growth and differentiation. Moreover, MSCs synthesize several components of the extracellular matrix including collagens, fibronectins, and other glycosaminoglycans[15,16]. Furthermore, MSCs are important to maintain and conserve the network of sinusoids (Figure 1).

According to earlier studies, it is believed, that HSCs are in contact with MSCs and bone-lining osteoblasts suggesting that MSCs provide the support necessary for homeostasis of HSCs. Furthermore, the growth factors secreted by MSCs such as granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), and lymphocyte

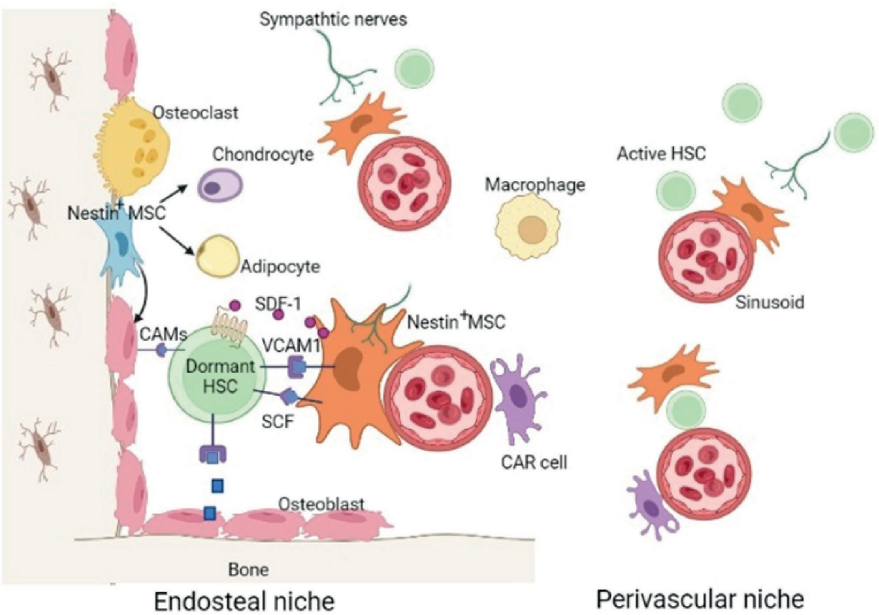


Figure 1. Hematopoietic support provides by MSCs. Hematopoietic Stem Cells (HSCs) reside in two niches of the bone marrow. HSCs in the endosteal niche are quiescent where Nestin^{+} MSCs along with osteoblasts maintain the quiescent state of HSCs through factors such as CXC chemokine ligand-12 (CXCL-12), vascular cell adhesion molecule 1 (VCAM-1), Angiopoietin-1(Ang-1) and stem cell factor (SCF). In addition, MSCs differentiate into tri-linage cells giving rise to osteoblasts, adipocytes, and chondrocytes. Nestin^{+} MSCs along with CAR cells and sinusoidal endothelial cells maintain HSC in perivascular niche.

inhibitory factor (LIF) are necessary to support hematopoiesis, indicating that MSCs can be used in *ex vivo* amplification of several hematopoietic cells for therapy[17]. Interestingly, studies have revealed that only a small subpopulation of HSCs resides in the endosteal niche (Figure 1) whereas HSCs are abundantly associated with the sinusoidal endothelium or perivascular niche. Moreover, recent findings indicate that Nestin-expressing mesenchymal cells which are located both near the endosteum and towards the center act as HSC niches[18,19].

Another mechanism of support provided by MSCs on HSCs is the secretion of soluble factors such as the stromal cell-derived factor-1(SDF-1 or CXCL12) and stem cell factor (SCF). The Nestin$^+$ MSCs have shown to express several HSC maintenance markers like VCAM1, CXCL12 and SCF prominently (Figure 1). The alliance between Nestin$^+$ MSCs and HSCs is discernible when depletion of MSCs causes enhanced mobilization of HSCs whereas treatment with G-CSF decreases the Nestin$^+$ MSC expression of aforementioned HSC maintenance factors[20].

2.4 Tissue repair

Because of the unique properties pertaining to MSCs such as their ability to move from their niche in the BM into the circulation and then incorporate into the injured site (engraftment), MSCs are extensively employed as therapeutic agents in regenerative medicine. Though earlier studies were based mainly on conditions which are related to bone, at present, the center of interest has moved to a multitude of other conditions including GVHD, myocardial infarction, sepsis, diabetes, stroke, etc. The dual functions, namely, cell replacement and cell 'empowerment' served by MSCs are important for the therapeutic effect exerted by these cells[21]. Further, a new therapeutic modality has recently emerged using MSC-derived exosomes and modified MSCs. Currently, there is promising research evidence to show that MSC-derived exosomes can render significant therapeutic effects on disease models such as myocardial infarction[22], hepatic fibrosis[23,24] and cancer[25]. The ability of MSCs to differentiate into organ-specific cells enables the repair of the effete tissue and restores the functions of the organ to an acceptable level. The potential of MSCs to secrete soluble paracrine factors, which are necessary for the proliferation and survival of resident cells, and for their immunomodulatory and repair factors, stimulate a host repair response that is necessary for cell empowerment.

2.5 Mobilization

The recruitment of MSCs to a site of injury involves several sequential processes, beginning with sensing of signals, movement from the site of administration (storage niche) into the circulation (mobilization), then homing from the circulation to the injured tissue and *in situ* proliferation and differentiation[26] (Figure 2). Even though the mechanisms behind the mobilization of MSCs are yet to be delineated, one mechanism that is implicated in the mobilization of MSCs from their niches is thought to occur because of the release of cytokines and chemokines to the circulation from the remote injured tissues. SDF-1 and its cognate receptor CXCR play a crucial role in the mobilization of MSCs from their niche. Using a rat myocardial infarction model, Zhang *et al*.,[27] demonstrated that overexpression of MSCs with CXCR4 and SDF-1 improved cardiac function, promoted neo-myoangiogenesis, and prevented left ventricular remodeling in the rodents. Similar results were obtained when MSCs were overexpressed with IGF-1, which increased SDF-1 and stem cell migration, angiomyogenesis, and improvement of cardiac functions of infarcted rat hearts[28]. A recent study revealed that pretreatment with SDF-1 increased the migration of Wharton's jelly-derived mesenchymal stem cells and embryonic stem cells, and augmented the skeletal muscle regeneration[29]. Osteopontin, a cytokine which is produced in increased amounts in response to injury and inflammation in various tissues, was also found to be involved in increasing migration of mesenchymal stem cells[30]. In addition, physical factors such as

mechanical strain, shear stress, matrix stiffness, and microgravity are also proven to affect migration of mesenchymal stem cells[31].

2.6 Homing

Homing is the movement of marrow stem cells from the administration site to a site of marrow space or an injury site, depending on the tissue condition. MSCs are attracted to the injury site after they migrate out of the bone marrow and then differentiate into organ-specific mature cells and exert their trophic effects on the injured tissue. Homing of cells to the injured site from the circulation involves multi-step processes, which include rolling of cells on the endothelium, adhesion, transendothelial migration, extravasation, and migration towards the injured tissue,which is similar to that employed by leukocytes migrating to sites of inflammation (Figure 2).

It has been substantiated by many studies that once administered, MSCs are distributed to many sites in animal bodies, including the lung and liver. The injected MSCs can be detected in the circulation within the first hour[32] and then engrafted into bone, marrow, and skin[33], suggesting that MSCs have the ability to home to various tissues without specificity[15]. However, chronic inflammation seems to change the homing of MSCs by directing them towards the immune cells[15]. A number of studies have revealed that basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), and transforming growth factor β1 (TGF-β1), are critical in the process of homing of mesenchymal stem cells for the purpose of tissue regeneration[31].

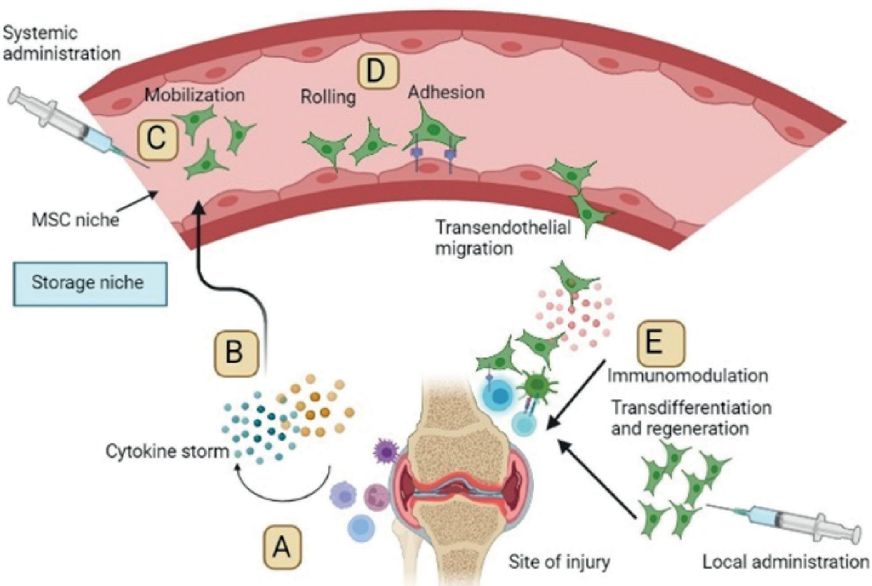


Figure 2. A&B. Tissue damage causes release of cytokines and chemokines into the circulation. C. MSCs mobilize from their bone marrow niche to the circulation to home to the injured tissue. In addition, MSCs can be either directly administered to systemic circulation or be injected at the site of injury. D. Homing is a multi-step process involving rolling of cells on the endothelium, adhesion, trans-endothelial migration, and extravasation. E. MSCs act by direct cell-to-cell contact and through secretion of trophic factors to regenerate the tissue back to its pervious condition before the injury. MSCs have the ability to differentiate and transdifferentiate into the organ-specific cells as well.

2.7 Paracrine activity

In the earlier days, the therapeutic action of mesenchymal stem cells was known to be exerted through migration to the target tissue, engraftment, and infusion. However, recent studies have elucidated that much of the therapeutic effects of MSCs are orchestrated through their paracrine activity; through the secretion of a wide array of growth factors, cytokines, chemokines, etc. Herein, the latest research focus has been on the paracrine activity of MSCs, which is mediated through various types of extracellular vesicles[34]. The use of extracellular vesicles presents many advantages over the use of their cellular counterparts, such as lower immunogenicity, the inability to form tumors directly, higher safety profile as well as more efficient migration to the target tissues following infusion[35].

MSCs secrete various paracrine factors, which are also known as trophic factors, that influence the microenvironment necessary for tissue regeneration (Figure 3). The tissue regenerative potential of MSCs could be attributed to the ability of MSCs to remodel the extracellular matrix making it more receptive to the proliferation of organ-specific stem cells and the synthesis of factors that will act on inhabiting cells[36]. It has been demonstrated that the synthesis of these paracrine factors occurs under hypoxia brought about by ischemia[37]. MSCs have shown to exert the following effects in tissue repair.

i. increase migration of other cells towards the site of repair by secreting SDF-1

ii. release of growth factors, such as IGF and EGF, to enhance the proliferation of resident stem cells

iii. reduction of inflammatory cytokines, such as IFN-γ and TNF-α thus, reduce the

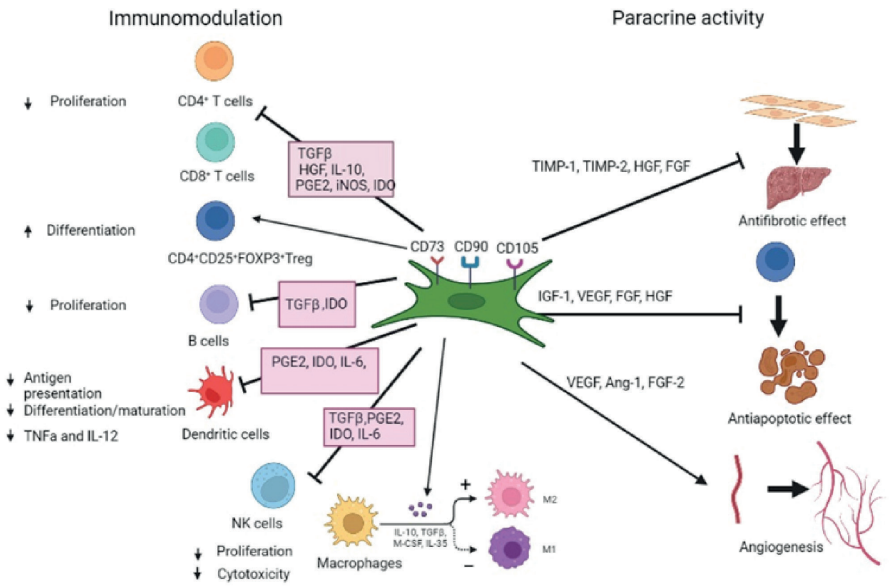


Figure 3. Paracrine and immunomodulatory effects of MSCs on different immune cells. MSCs exert immunomodulatory effects by way of suppressing the effects of T cells, B cells, dendritic cells (DCs), neutrophils, and NK cells through the secretion of various cytokines. Nevertheless, MSCs promote the proliferation and differentiation of Treg cells and promote macrophage polarization toward M2 type. Paracrine activity of MSCs are by secreting factors which are necessary for certain cellular processes at sites of injury such as chemoattraction, progenitor cell proliferation, angiogenesis, and differentiation into lineage specific cells.

inflammatory response

iv. secretion of pro-survival factors and induce anti-apoptotic effect
There is ample evidence to show that MSCs prevent the apoptosis of neighboring resident cells by secreting anti-apoptotic factors (Figure 3). Efforts to evaluate the mechanisms underlying this prevention of cardiomyocyte apoptosis in ischemic heart disease show that MSCs (ASCs) do so by secreting, IGF-I and VEGF[38]. This paracrine effect of MSCs was substantiated by the study done by Mirotsu *et al.,*[39], who showed that Akt-transfected MSCs enhance the survival of cardiomyocytes by secreting, frizzled protein-2. In addition, the studies done by Wang *et al.,*[40] demonstrated that MSCs exert neuroprotection by secreting anti-apoptotic SDF-1. It has been demonstrated that hypoxic preconditioning of MSCs increases the secretion of pro-angiogenic and pro-survival factors such as hypoxia-inducible factor-1, erythropoietin, VEGF, angiopoietin-1, Bcl-2, Bcl-xL, and Flk-1[41].

v. Secretion of angiogenic factors/pro-angiogenic effect
MSCs secrete many pro-angiogenic factors such as VEGF, angiopoietin-1 (Ang-1), FGF- 2, FGF-7, PDGF, and cytokines including IL-6 and TNF-α[42,43]. By using various heart disease models, it has been shown that MSCs improve cardiac function by secreting various soluble mediators. Non-invasive intramuscular administration of MSCs and MSC-conditioned media were able to restore cardiac function in the hamster heart failure model[44]. This is in accord with the study by Kinnaird *et al.,*[42], who showed that MSCs augment the collateral blood flow by secreting arteriogenic cytokines such as VEGF and bFGF. Though MSCs are capable of differentiating into smooth muscle and endothelial cells, collateral remodeling brought about by MSCs is mainly through paracrine mechanisms but not through incorporating into the developing collaterals[37]. The mechanism by which MSCs exert this pro-angiogenic effect is believed to occur by activating the JAK-STAT3 pathway[45]. Furthermore, proteomic analysis has shown that MSCs secrete cysteine-rich protein 61 (Cyr61)/CCN1, which causes neovascularization *in vivo*[46]. In addition to the enhancement of angiogenesis, MSCs express junction proteins, including occludin, thereby potentiating microvascular integrity[43].

Furthermore, a study conducted by Takeuchi *et al.,* in 2019[47] revealed that extracellular vesicles from a conditioned medium of bone marrow-derived MSCs promote bone regeneration through angiogenesis mediated mainly through the secretion of VEGF.

vi. anti-fibrotic effects
Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) play an important part in tissue remodeling and fibrosis formation. MSCs secrete high levels of both TIMP-1 and TIMP-2 and exert a net inhibitory effect on the proteolysis of the vascular environment by keeping the tissue MMP activity under control and protecting the vascular environment from outside MMPs[48] as well. The studies by Dixon *et al.,*[49] showed that lower concentrations of MSCs reduce collagen content by enhancing the collagen degradation in MI in the sheep model. Furthermore, they showed that MSCs change the dynamics of collagen, MMP, and TIMP levels in a concentration-dependent manner. This finding is substantiated by Mias *et al.,*[50], who showed that MSCs modulate cardiac fibroblasts and reduce the cardiac fibrosis following MI. Also, MSCs are shown to attenuate myocardial fibrosis by secreting hepatocyte growth factors in the rat model of heart failure[51]. Further, it has been demonstrated that MSCs secrete VEGF and

reduce renal fibrosis in rats[52,53]. Moreover, a study done by Maria *et al.,* in 2015[54] highlights that MSCs counteract the effects of oxidation-induced damage and fibrotic processes in HOCl-injected mice. These effects were mainly found to be mediated through the tissue modulation of ECM remodeling, inflammation, and antioxidant machinery.

vii. antioxidant effects

Growth factors and certain cytokines like IGF and IL-6 have shown to protect cells from free radicals. The fact that MSCs also secrete these factors is a clear indication that MSCs also exert antioxidant effects. By secreting superoxide dismutase 3 (SOD3), MSCs directly exert antioxidant effects and cause neuroprotection[55]. A study by Kim *et al.,*[56] has shown that adipocyte-derived MSCs activate fibroblasts by paracrine mechanism and protect them from oxidative stress.

2.8 Immunomodulation

MSCs interact with all types of immune cells and exert their immunomodulatory effects by several mechanisms (Figure 3). MSCs are immune-privileged andthey express several types of receptors, including MHC class I which is necessary to communicate with T-cells (57). Due to the absence of expression of T-cell co-stimulatory surface antigens such as CD40, CD80, and CD86 (MHC class II), T-cells do not identify the transplanted MSCs as foreign (58). Though HLA class II is not present on the cell surface as determined by flow cytometry, analysis of whole-cell lysates by western blot showed that HLA class II is present intra-cellularly (59). Furthermore, culturing MSCs in the presence of IFN-γ which mimics the inflammatory milieu, has been shown to induce cell surface MHC class II molecules. However, the expression levels were markedly reduced when cells were cultured in a differentiation medium (59). The absence of lympho proliferation, which was observed, seems to be not due to the lack of co-stimulation (60). However, MSCs inhibit proliferation of majority of immunologically competent cells such as B cells which respond to antigens by secreting immunoglobulins, and other cells such as dendritic cells (DCs) and natural killer cells (NKs) which cause cell-mediated immunity and exert inhibitory effects on them directly (61) by coming into contact with them and/or by releasing soluble factors which inhibit their immunological effects.

2.8.1 T cells

It has been shown that MSCs prevent proliferation and differentiation of T cells in response to various stimuli such as mitogens and alloantigens (CD3/CD28 etc.)[62]. Additionally, they prevent T cell proliferation by halting cells in the cell cycle G(0)/G(1) phase but not by apoptosis[63,64]. MSCs affect the proliferation of the T helper (CD4) as well as cytotoxic T cells (CD8) and nullify the ability of memory T cells to acknowledge the antigen in a dose-dependent manner[65]. The inhibitory effect on cytotoxic lymphocytes by MSCs is believed to occur via paracrine mechanisms and is found to be exerted by the release of transforming growth factor beta (TGF-β) and hepatocyte growth factor (HGF). In addition, MSCs inhibit the cytotoxic lymphocyte-mediated lysis when added at early time points 66. The MSC-induced immunosuppression is believed to have been brought about by concerted secretion of cytokines and chemokines such as IFN-γ and TNF-α[67], TGF-β, IL-10, HGF (hepatic growth factors), PGE2 (prostaglandin E2), NO[67], to name a few. The secretion of heme oxygenase-1[68] and nitric oxide synthase (iNOS)[68,69] by MSCs are also supposed to bring about the MSC-mediated immunosuppression on T cells. Moreover, a study done by Meisel *et al.,*[70] demonstrated that when cultured with IFN-γ MSCs induce indolamine 2,3-dioxygenase (IDO) by giving rise to the degradation of tryptophan, which suggests that IDO-mediated tryptophan metabolism is responsible for the prevention of T cell proliferation mediated by MSCs.

In addition, it has been demonstrated that MSCs induce the production of anti-inflammatory cytokines by T cells while down-regulating the secretion of pro-inflammatory cytokines, giving rise to a tolerant phenotype[71]. MSCs induce the formation of $CD4^+CD25^+FoxP3^+$ Treg cells, subsequent to the suppression of T cell proliferation by secreting human leukocyte antigen-G5(HLA-G5) in IL-10 dependent manner[72]. MSCs also induce the proliferation of $CD4^+CD25^+FoxP3^+$ Treg cells[73] while inhibiting the differentiation of $CD4^+$ T cells into TH1 and TH17 subset of T cells[74]. By using antibodies against ligands of programmed death 1 (both PD-L1 and PD-L2) and its cognate receptor PD-1, it has been demonstrated that MSCs down-regulate T cell proliferation[75] through direct communication with T cells and by secreting TGF-β indirectly. GVHD is an example where the inhibitory effects of MSCs on T cells translate into their clinical use.

2.8.2 Dendritic cells (DCs)

MSCs prevent the differentiation and maturation of dendritic cells (DCs) from monocytes (MCs). This is believed to be brought about by arresting the cell cycle at G0/G1 phase[76]. Substantiating this observation, Zhang *et al.,*[77] has shown that MSCs prevent the upregulation of differentiation and maturation markers such as CD1a, CD40, CD86, and CD83. In addition, MSC-mediated secretion of IL-6, M-CSF, and PGE2 has shown to cause the downregulation of DC maturation markers[78,79]. This is important because the secretion of cytokines like IL-12 by mature DCs and surface expression of co-stimulatory molecules are necessary for T cell immune response, whereas immature DCs render T cells into an anergic phenotype[78]. In addition, MSCs can impair the migration of DCs with downregulation of molecules associated with DC migration, such as CCR7 and CD49dβ1.This would also decrease their ability of antigen presentation and inflammatory cytokine secretion and make them less efficient in T cell activation (80). Besides arresting DCs in immature phenotype, MSCs cause DCs to secrete more IL-10 and reduce the secretion of inflammatory mediators like TNF-α, IFN-γ and IL-12. This creates a suppressive milieu, thereby preventing T cell activity and allogeneic rejection (81). Using umbilical-cord-derived MSCs (UC-MSCs), Deng *et al.,*[82] reported that MSCs induce DCs to produce an immunologic tolerant phenotype by upregulating SOCS1 via the secretion of IL-6 and activating JAK-STAT pathway, which subsequently elicits the generation of Treg cells[82]. A newer study has revealed that by secreting TNFα-stimulating gene (TSG)-6, MSCs inhibit the DC maturation and function as well[83].

2.8.3 B cells

B cells are important effector cells in adaptive immunity that play major roles in autoimmunity, antibody secretion, and in activation of the complement system. Research has shown that MSCs can regulate B cell proliferation and differentiation and inhibit B cell apoptosis. MSCs are also capable of indirectly suppressing the adaptive immune response by downregulating dendritic cell-mediated antigens. Peripheral blood-derived B cells co-cultured with MSCs in the presence of various B cell stimuli have shown that MSCs inhibit B cell proliferation by forestalling the cell cycle at the G0/G1 phase. Furthermore, MSCs inhibit the B cell mediated synthesis of immunoglobulin via paracrine mechanisms (84). It has been further delineated that depending on the type and dose of stimuli and source of B cells, MSCs can either exert inhibitory or stimulatory effects on B cells (85). In addition to the above, MSCs exert their immunomodulatory effects through the induction of regulatory B cells (Bregs), the major type of immunosuppressive B cells, and promote the secretion of IL-10 and thereby exert immunosuppressive effects,especially in patients with autoimmune diseases or solid organ transplantation. In a study done in 2017, Gupte *et al.,*(86) reported that co-culture of adipose tissue-derived MSCs from potential kidney donors with peripheral blood PBMCs of donors induced IL-

10-secreting B cells, which shows the promise of cell therapies following transplantation.

2.8.4 Natural killer cells (NKs)

Natural killer cells play a vital role in innate immunity. Their function is mainly exerted through cytotoxic activity and the production of cytokines such as IFN-γ. Unlike pretreated NKs with cytokines such as IL-2 and IL-15, the freshly isolated NKs cannot kill MSCs. It is believed that the cytolytic activity of NKs on MSCs occurs via the cell surface expression of NK receptors such as NKp30, NKG2D, and DNAM-1 which are stimulated by their cognate ligands expressed on the cell surface of MSCs[87,88]. MSCs have been shown to reduce IFN-γ secretion by NKs cultured with IL-2 and the cytotoxicity effect of NKs when it is cultured with IL-15. The latter effect is believed to take place via paracrine activity of MSCs by secreting TGF-β and PGE2, as well as direct cell-to-cell communication[89].

A novel mode of interaction between NKs and MSC was reported in a recent study where extracellular vesicles isolated from fetal liver-derived MSCs diminished NK cell proliferation, activation, and differentiation mostly via their surface-associated TGF-β which downregulated the NK cell surface stimulatory receptors NKG2D and Nkp30[90]. Even though there is a great number of studies supportive of the immunosuppressive action of MSC on NKs, there are only a few reports on the stimulatory effect of MSCs on NKs. These findings can be attributed to variations in the source of MSCs, different culture conditions as well as the different ratios of MSCs to NKs used in these studies[91].

2.8.5 Macrophages

Macrophages are immune cells that play a vital role in inflammation, tissue repair, and regeneration. MSCs induce the transformation of macrophages into an anti-inflammatory type 2 (M2) cells from a proinflammatory type 1 (M0) cells. This effect of MSCs is exploited in the treatment of sepsis with MSCs[92]. Furthermore, MSC-induced T cell apoptosis via coupling of FASL and cognate receptor FAS leads to the secretion of TGF-β by macrophages, resulting in the induction of Treg cells[93]. A recent report shows that through direct contact and in the presence of M-CSF, MSCs enhance the survival of monocytes which are able to differentiate into a novel type of macrophages (M^{MSC})[94]. The differentiation into these functional macrophages has shown to occur in a PGE-2-dependent manner. In addition, these differentiated macrophages are able to secrete IL-10, IL-1β and copious amounts of TGF-β. Co-culture of these macrophages with activated NKs was able to downregulate the NK markers such as CD69, CD25, and NKp44 but not NKG2D, NKp30, and NKp46. Moreover, NK-mediated IFN-γ secretion was markedly reduced by these polarized macrophages through the combined effect of IL-10 and TGF-β secretion, showing the important mechanism how MSCs regulate the adaptive immunity (94). M^{MSC} were also able to increase the number of Treg cells while downregulating the $CD8^+$ T cells highlighting the MSC-immunoregulatory effect on adaptive immunity[94]. Further, it was reported in a recent study that bone marrow MSC-derived extracellular vesicles attenuated myocardial ischemia/reperfusion injury in mice via shuttling miR-182,which modifies the polarization status of macrophages[95].

2.8.6 Neutrophils (PMNs)

Neutrophils are a type of short-lived leukocytes that play a key role in innate immunity. They will undergo programmed cell death after engulfing and destroying pathogens, and thereby, maintaining the balance of cellular homeostasis[96]. MSCs have shown to prevent the apoptosis of resting and stimulated neutrophils directly as well as indirectly via the secretion of IL-6[97]. Toll-like receptor activation (TLR-3 and TLR-4) by their cognate ligands enhances the survival and activity of PMNs by BM-MSCs as well MSCs derived from other tissues, such as adipose tissues[98]. Using MSCs derived from parotid glands (tissue-specific MSCs) and peripheral

blood-derived neutrophils, Brandau *et al.,*[99] demonstrated that MSCs, when challenged with LPS, showed enhanced motility and expression of a panel of chemokines. Moreover, LPS stimulation increased the recruitment of PMNs in an IL-8 and macrophage migration inhibitory factor (MIF)-dependent manner and increased their survival. The enhanced activity of recruited PMNs is believed to be via the secretion of MIF by MSCs. They concluded that tissue-specific MSCs are responsible for the initiation of local immune response by recruiting PMNs before immune cells invade the infected tissue[99].

Conclusion

MSCs hold great promise in regenerative medicine with their autologous/allogeneic transplantation potential due to their immune-privilege properties and immune modulation effects they exert on other immune cells. The ability of MSCs to transdifferentiate out of their exclusive lineage and to assume a phenotype of resident cells in repair of effete tissue is one of the most attractive aspects of MSCs. In addition, they overcome the moral and ethical issues involved in using embryonic stem cells. However, depending on the source of MSCs, the differentiation versatility and functional equivalence of each type of cell might differ; hence, there is no global stem cell type that can be used in all disease conditions. Therefore, depending on the disease condition, the appropriate stem cell type should be carefully selected before applying it to regenerative therapy.

Acknowledgement

The authors gratefully acknowledge Mr. E.K.P.S Epa, Assistant Librarian, Faculty of Dental Sciences, the University of Peradeniya, for their assistance in checking the manuscript for plagiarism. The authors would like to acknowledge the assistance of Ms. Monica Jayasekara of ELTU, Faculty of Dental Sciences, for language editing of the manuscript.

References

1. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970 Oct;3(4):393–403.

2. Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991 Sep;9(5):641–50.

3. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. Cell Stem Cell. 2008 Apr 10;2(4):313–9.

4. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315–7.

5. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999 Apr 2;284(5411):143–7.

6. Majumdar S, Liu ST. Cell division symmetry control and cancer stem cells. AIMS Mol Sci. 2020;7(2):82–98.

7. Banfi A, Bianchi G, Notaro R, Luzzatto L, Cancedda R, Quarto R. Replicative aging and gene expression in long-term cultures of human bone marrow stromal cells. Tissue Eng. 2002 Dec;8(6):901–10.

8. Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I. Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion. Stem Cells. 2004;22(5):675–82.

9. Spees JL, Olson SD, Ylostalo J, Lynch PJ, Smith J, Perry A, et al. Differentiation, cell

fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma. Proc Natl Acad Sci USA. 2003 Mar 4;100(5):2397–402.

10. Chen TS, Lai RC, Lee MM, Choo ABH, Lee CN, Lim SK. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res. 2010 Jan;38(1):215–24.

11. Quesenberry PJ, Aliotta JM. The paradoxical dynamism of marrow stem cells: considerations of stem cells, niches, and microvesicles. Stem Cell Rev. 2008 Sep;4(3):137–47.

12. Ferraro F, Celso CL, Scadden D. Adult stem cels and their niches. Adv Exp Med Biol. 2010;695:155–68.

13. Spradling A, Drummond-Barbosa D, Kai T. Stem cells find their niche. Nature. 2001 Nov 1;414(6859):98–104.

14. Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell. 2006 Jan 27;124(2):407–21.

15. Lazennec G, Jorgensen C. Concise review: adult multipotent stromal cells and cancer: risk or benefit? Stem Cells. 2008 Jun;26(6):1387–94.

16. Mehrasa R, Vaziri H, Oodi A, Khorshidfar M, Nikogoftar M, Golpour M, et al. Mesenchymal stem cells as a feeder layer can prevent apoptosis of expanded hematopoietic stem cells derived from cord blood. Int J Mol Cell Med. 2014;3(1):1–10.

17. Majumdar MK, Thiede MA, Haynesworth SE, Bruder SP, Gerson SL. Human marrow-derived mesenchymal stem cells (MSCs) express hematopoietic cytokines and support long-term hematopoiesis when differentiated toward stromal and osteogenic lineages. J Hematother Stem Cell Res. 2000 Dec;9(6):841–8.

18. Nagasawa T, Omatsu Y, Sugiyama T. Control of hematopoietic stem cells by the bone marrow stromal niche: the role of reticular cells. Trends Immunol. 2011 Jul;32(7):315–20.

19. Méndez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010 Aug 12;466(7308):829–34.

20. Ehninger A, Trumpp A. The bone marrow stem cell niche grows up: mesenchymal stem cells and macrophages move in. J Exp Med. 2011 Mar 14;208(3):421–8.

21. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol. 2014 Nov;15(11):1009–16.

22. Ma T, Chen Y, Chen Y, Meng Q, Sun J, Shao L, et al. MicroRNA-132, Delivered by Mesenchymal Stem Cell-Derived Exosomes, Promote Angiogenesis in Myocardial Infarction. Stem Cells Int. 2018;2018:3290372.

23. Jiang W, Tan Y, Cai M, Zhao T, Mao F, Zhang X, et al. Human Umbilical Cord MSC-Derived Exosomes Suppress the Development of CCl4-Induced Liver Injury through Antioxidant Effect. Stem Cells Int. 2018;2018:6079642.

24. Qu Y, Zhang Q, Cai X, Li F, Ma Z, Xu M, et al. Exosomes derived from miR-181-5p-

modified adipose-derived mesenchymal stem cells prevent liver fibrosis via autophagy activation. J Cell Mol Med. 2017 Oct;21(10):2491–502.

25. Phinney DG, Pittenger MF. Concise Review: MSC-Derived Exosomes for Cell-Free Therapy. Stem Cells. 2017 Apr;35(4):851–8.

26. Salem HK, Thiemermann C. Mesenchymal stromal cells: current understanding and clinical status. Stem Cells. 2010 Mar 31;28(3):585–96.

27. Zhang D, Fan GC, Zhou X, Zhao T, Pasha Z, Xu M, et al. Over-expression of CXCR4 on mesenchymal stem cells augments myoangiogenesis in the infarcted myocardium. J Mol Cell Cardiol. 2008 Feb;44(2):281–92.

28. Haider HK, Jiang S, Idris NM, Ashraf M. IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1alpha/CXCR4 signaling to promote myocardial repair. Circ Res. 2008 Nov 21;103(11):1300–8.

29. Kowalski K, Kołodziejczyk A, Sikorska M, Płaczkiewicz J, Cichosz P, Kowalewska M, et al. Stem cells migration during skeletal muscle regeneration - the role of Sdf-1/Cxcr4 and Sdf-1/Cxcr7 axis. Cell Adh Migr. 2017 Jul 4;11(4):384–98.

30. Hirano Y, Aziz M, Yang WL, Wang Z, Zhou M, Ochani M, et al. Neutralization of osteopontin attenuates neutrophil migration in sepsis-induced acute lung injury. Crit Care. 2015 Feb 26;19:53.

31. Fu X, Liu G, Halim A, Ju Y, Luo Q, Song AG. Mesenchymal Stem Cell Migration and Tissue Repair. Cells. 2019 Jul 28;8(8):E784.

32. Koç ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol. 2000 Jan;18(2):307–16.

33. Horwitz EM, Gordon PL, Koo WKK, Marx JC, Neel MD, McNall RY, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc Natl Acad Sci USA. 2002 Jun 25;99(13):8932–7.

34. Mendt M, Rezvani K, Shpall E. Mesenchymal stem cell-derived exosomes for clinical use. Bone Marrow Transplant. 2019 Aug;54(Suppl 2):789–92.

35. Börger V, Bremer M, Ferrer-Tur R, Gockeln L, Stambouli O, Becic A, et al. Mesenchymal Stem/Stromal Cell-Derived Extracellular Vesicles and Their Potential as Novel Immunomodulatory Therapeutic Agents. Int J Mol Sci. 2017 Jul 6;18(7):E1450.

36. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem. 2006 Aug 1;98(5):1076–84.

37. Kinnaird T, Stabile E, Burnett MS, Epstein SE. Bone-marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. Circ Res. 2004 Aug 20;95(4):354–63.

38. Sadat S, Gehmert S, Song YH, Yen Y, Bai X, Gaiser S, et al. The cardioprotective effect of mesenchymal stem cells is mediated by IGF-I and VEGF. Biochem Biophys Res Commun. 2007 Nov 23;363(3):674–9.

39. Mirotsou M, Zhang Z, Deb A, Zhang L, Gnecchi M, Noiseux N, et al. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell-released paracrine factor mediating myocardial survival and repair. Proc Natl Acad Sci USA. 2007 Jan 30;104(5):1643–8.

40. Wang F, Yasuhara T, Shingo T, Kameda M, Tajiri N, Yuan WJ, et al. Intravenous administration of mesenchymal stem cells exerts therapeutic effects on parkinsonian model of rats: focusing on neuroprotective effects of stromal cell-derived factor-1alpha. BMC Neurosci. 2010;11:52.

41. Hu X, Yu SP, Fraser JL, Lu Z, Ogle ME, Wang JA, et al. Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. J Thorac Cardiovasc Surg. 2008 Apr;135(4):799–808.

42. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res. 2004 Mar 19;94(5):678–85.

43. Zacharek A, Chen J, Cui X, Li A, Li Y, Roberts C, et al. Angiopoietin1/Tie2 and VEGF/Flk1 induced by MSC treatment amplifies angiogenesis and vascular stabilization after stroke. J Cereb Blood Flow Metab. 2007 Oct;27(10):1684–91.

44. Shabbir A, Zisa D, Suzuki G, Lee T. Heart failure therapy mediated by the trophic activities of bone marrow mesenchymal stem cells: a noninvasive therapeutic regimen. Am J Physiol Heart Circ Physiol. 2009 Jun;296(6):H1888-1897.

45. Wang Y, Abarbanell AM, Herrmann JL, Weil BR, Manukyan MC, Poynter JA, et al. TLR4 inhibits mesenchymal stem cell (MSC) STAT3 activation and thereby exerts deleterious effects on MSC-mediated cardioprotection. PLoS ONE. 2010;5(12):e14206.

46. Estrada R, Li N, Sarojini H, An J, Lee MJ, Wang E. Secretome from mesenchymal stem cells induces angiogenesis via Cyr61. J Cell Physiol. 2009 Jun;219(3):563–71.

47. Takeuchi R, Katagiri W, Endo S, Kobayashi T. Exosomes from conditioned media of bone marrow-derived mesenchymal stem cells promote bone regeneration by enhancing angiogenesis. PLoS One. 2019;14(11):e0225472.

48. Lozito TP, Tuan RS. Mesenchymal stem cells inhibit both endogenous and exogenous MMPs via secreted TIMPs. J Cell Physiol. 2011 Feb;226(2):385–96.

49. Dixon JA, Gorman RC, Stroud RE, Bouges S, Hirotsugu H, Gorman JH, et al. Mesenchymal cell transplantation and myocardial remodeling after myocardial infarction. Circulation. 2009 Sep 15;120(11 Suppl):S220-229.

50. Mias C, Lairez O, Trouche E, Roncalli J, Calise D, Seguelas MH, et al. Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction. Stem Cells. 2009 Nov;27(11):2734–43.

51. Li L, Zhang Y, Li Y, Yu B, Xu Y, Zhao S, et al. Mesenchymal stem cell transplantation attenuates cardiac fibrosis associated with isoproterenol-induced global heart failure. Transpl Int. 2008 Dec;21(12):1181–9.

52. Semedo P, Correa-Costa M, Antonio

Cenedeze M, Maria Avancini Costa Malheiros D, Antonia dos Reis M, Shimizu MH, et al. Mesenchymal stem cells attenuate renal fibrosis through immune modulation and remodeling properties in a rat remnant kidney model. Stem Cells. 2009 Dec;27(12):3063–73.

53. Tögel F, Cohen A, Zhang P, Yang Y, Hu Z, Westenfelder C. Autologous and allogeneic marrow stromal cells are safe and effective for the treatment of acute kidney injury. Stem Cells Dev. 2009 Apr;18(3):475–85.

54. Maria ATJ, Toupet K, Bony C, Pirot N, Vozenin MC, Petit B, et al. Antifibrotic, Antioxidant, and Immunomodulatory Effects of Mesenchymal Stem Cells in HOCl-Induced Systemic Sclerosis. Arthritis Rheumatol. 2016 Apr;68(4):1013–25.

55. Kemp K, Gray E, Mallam E, Scolding N, Wilkins A. Inflammatory cytokine induced regulation of superoxide dismutase 3 expression by human mesenchymal stem cells. Stem Cell Rev. 2010 Dec;6(4):548–59.

56. Kim WS, Park BS, Sung JH. The wound-healing and antioxidant effects of adipose-derived stem cells. Expert Opin Biol Ther. 2009 Jul;9(7):879–87.

57. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007 Nov;25(11):2739–49.

58. Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation. 2003 Feb 15;75(3):389–97.

59. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol. 2003 Oct;31(10):890–6.

60. Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, Simonetti DW, et al. T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. J Biomed Sci. 2005;12(1):47–57.

61. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol. 2008 Sep;8(9):726–36.

62. Rasmusson I, Ringdén O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. Exp Cell Res. 2005 Apr 15;305(1):33–41.

63. Jones S, Horwood N, Cope A, Dazzi F. The antiproliferative effect of mesenchymal stem cells is a fundamental property shared by all stromal cells. J Immunol. 2007 Sep 1;179(5):2824–31.

64. Glennie S, Soeiro I, Dyson PJ, Lam EWF, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood. 2005 Apr 1;105(7):2821–7.

65. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood. 2003 May 1;101(9):3722–9.

66. Rasmusson I, Ringdén O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. Transplantation. 2003

Oct 27;76(8):1208–13.

67. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008 Feb 7;2(2):141–50.

68. Chabannes D, Hill M, Merieau E, Rossignol J, Brion R, Soulillou JP, et al. A role for heme oxygenase-1 in the immunosuppressive effect of adult rat and human mesenchymal stem cells. Blood. 2007 Nov 15;110(10):3691–4.

69. Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T, et al. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood. 2007 Jan 1;109(1):228–34.

70. Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood. 2004 Jun 15;103(12):4619–21.

71. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005 Feb 15;105(4):1815–22.

72. Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, et al. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. Stem Cells. 2008 Jan;26(1):212–22.

73. Wang Y, Zhang A, Ye Z, Xie H, Zheng S. Bone marrow-derived mesenchymal stem cells inhibit acute rejection of rat liver allografts in association with regulatory T-cell expansion. Transplant Proc. 2009 Dec;41(10):4352–6.

74. Luz-Crawford P, Kurte M, Bravo-Alegría J, Contreras R, Nova-Lamperti E, Tejedor G, et al. Mesenchymal stem cells generate a CD4+CD25+Foxp3+ regulatory T cell population during the differentiation process of Th1 and Th17 cells. Stem Cell Res Ther. 2013;4(3):65.

75. Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, et al. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol. 2005 May;35(5):1482–90.

76. Ramasamy R, Fazekasova H, Lam EWF, Soeiro I, Lombardi G, Dazzi F. Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. Transplantation. 2007 Jan 15;83(1):71–6.

77. Zhang W, Ge W, Li C, You S, Liao L, Han Q, et al. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. Stem Cells Dev. 2004 Jun;13(3):263–71.

78. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE. Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. J Immunol. 2006 Aug 15;177(4):2080–7.

79. Djouad F, Charbonnier LM, Bouffi C, Louis-Plence P, Bony C, Apparailly F, et al. Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. Stem Cells. 2007 Aug;25(8):2025–32.

80. Consentius C, Akyüz L, Schmidt-Lucke JA, Tschöpe C, Pinzur L, Ofir R, et al.

Mesenchymal Stromal Cells Prevent Allostimulation In Vivo and Control Checkpoints of Th1 Priming: Migration of Human DC to Lymph Nodes and NK Cell Activation. Stem Cells. 2015 Oct;33(10):3087–99.

81. Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. J Inflamm (Lond). 2005 Jul 26;2:8.

82. Deng Y, Yi S, Wang G, Cheng J, Zhang Y, Chen W, et al. Umbilical cord-derived mesenchymal stem cells instruct dendritic cells to acquire tolerogenic phenotypes through the IL-6-mediated upregulation of SOCS1. Stem Cells Dev. 2014 Sep 1;23(17):2080–92.

83. Liu Y, Yin Z, Zhang R, Yan K, Chen L, Chen F, et al. MSCs inhibit bone marrow-derived DC maturation and function through the release of TSG-6. Biochem Biophys Res Commun. 2014 Aug 8;450(4):1409–15.

84. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, et al. Human mesenchymal stem cells modulate B-cell functions. Blood. 2006 Jan 1;107(1):367–72.

85. Rasmusson I, Le Blanc K, Sundberg B, Ringdén O. Mesenchymal stem cells stimulate antibody secretion in human B cells. Scand J Immunol. 2007 Apr;65(4):336–43.

86. Gupte KS, Vanikar AV, Trivedi HL, Patel CN, Patel JV. In-vitro generation of interleukin-10 secreting B-regulatory cells from donor adipose tissue derived mesenchymal stem cells and recipient peripheral blood mononuclear cells for potential cell therapy. Biomed J. 2017 Feb;40(1):49–54.

87. Poggi A, Prevosto C, Massaro AM, Negrini S, Urbani S, Pierri I, et al. Interaction between human NK cells and bone marrow stromal cells induces NK cell triggering: role of NKp30 and NKG2D receptors. J Immunol. 2005 Nov 15;175(10):6352–60.

88. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood. 2006 Feb 15;107(4):1484–90.

89. Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells. 2006 Jan;24(1):74–85.

90. Fan Y, Herr F, Vernochet A, Mennesson B, Oberlin E, Durrbach A. Human Fetal Liver Mesenchymal Stem Cell-Derived Exosomes Impair Natural Killer Cell Function. Stem Cells Dev. 2019 Jan 1;28(1):44–55.

91. Moloudizargari M, Govahi A, Fallah M, Rezvanfar MA, Asghari MH, Abdollahi M. The mechanisms of cellular crosstalk between mesenchymal stem cells and natural killer cells: Therapeutic implications. J Cell Physiol. 2021 Apr;236(4):2413–29.

92. Nemeth K, Mayer B, Mezey E. Modulation of bone marrow stromal cell functions in infectious diseases by toll-like receptor ligands. J Mol Med. 2010 Jan;88(1):5–10.

93. Akiyama K, Chen C, Wang D, Xu X, Qu C, Yamaza T, et al. Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. Cell Stem Cell. 2012 May 4;10(5):544–55.

94. Chiossone L, Conte R, Spaggiari GM, Serra M, Romei C, Bellora F, et al. Mesenchymal stromal cells induce peculiar alternatively activated macrophages capable of dampening

both innate and adaptive immune responses. Stem Cells. 2016 Mar 26;

95. Zhao J, Li X, Hu J, Chen F, Qiao S, Sun X, et al. Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization. Cardiovasc Res. 2019 Jun 1;115(7):1205–16.

96. Salami F, Tavassoli A, Mehrzad J, Parham A. Immunomodulatory effects of mesenchymal stem cells on leukocytes with emphasis on neutrophils. Immunobiology. 2018 Dec;223(12):786–91.

97. Raffaghello L, Bianchi G, Bertolotto M, Montecucco F, Busca A, Dallegri F, et al. Human mesenchymal stem cells inhibit neutrophil apoptosis: a model for neutrophil preservation in the bone marrow niche. Stem Cells. 2008 Jan;26(1):151–62.

98. Cassatella MA, Mosna F, Micheletti A, Lisi V, Tamassia N, Cont C, et al. Toll-like receptor-3-activated human mesenchymal stromal cells significantly prolong the survival and function of neutrophils. Stem Cells. 2011 Jun;29(6):1001–11.

99. Brandau S, Jakob M, Hemeda H, Bruderek K, Janeschik S, Bootz F, et al. Tissue-resident mesenchymal stem cells attract peripheral blood neutrophils and enhance their inflammatory activity in response to microbial challenge. J Leukoc Biol. 2010 Nov;88(5):1005–15.

Help your patients reclaim life's small pleasures

SENSODYNE

Works deep inside the tooth to soothe sensitivity [4-10*]

Works in 60 seconds [1-3#]

For 24/7 protection [4,6,9,10*]

Images are for representation purpose only.

When used as directed, individual results may vary. *With twice-daily brushing. When used as directed on the pack. Individual results may vary.

References: 1. Mason S, Burnett GR, Patel N, *et al.* Impact of toothpaste on oral health-related quality of life in people with dentine hypersensitivity. *BMC Oral Health.* 2019;19:226. **2.** Mason S, Hughes N, Sufi F, *et al.* A comparative clinical study investigating the efficacy of a dentifrice containing 8% strontium acetate and 1040 ppm fluoride in a silica base and a control dentifrice containing 1450 ppm fluoride in a silica base to provide immediate relief of dentin hypersensitivity. *J Clin Dent.* 2010;21(2):42-48. **3.** GSKCH Study Z4010663: A three-day pilot study investigating the efficacy of two toothpastes in immediately reducing dentinal hypersensitivity. **4.** Holland GR, Narhi MN, Addy M, *et al.* Guidelines for the design and conduct of clinical trials on dentin hypersensitivity. *J Clin Periodontol.* 1997;24(11):808-813. **5.** Silverman G. The sensitivity-reducing effect of brushing with a potassium nitrate - sodium monofluorophosphate dentifrice. *Compend Contin Educ Dent.* 1985;6(2):131-136. **6.** Troulloss ES, *et al.* Potassium Nitrate Containing Dentifrice Study. Ref Type: Block Drug Study Report, No. 525, 22 May 1992. Data on file. **7.** Nagata T, *et al.* Clinical evaluation of a potassium nitrate dentifrice for the treatment of dentinal hypersensitivity. *J Clin Periodontol.* 1994;21(3):217-221. **8.** Axelrod S, Minkoff S. Desensitising dentifrice study. Ref Type: Block Drug Study Report, 30 Nov 1981. Data on file. **9.** Silverman, G. Desensitising dentifrice study. Ref Type: Block Drug Study Report, BLK-86-01, Sept 1986. Data on file. **10.** Jeandot J, *et al.* Efficacy of toothpastes containing potassium chloride or potassium nitrate on dentin sensitivity. *Clinic.* 2007;28:379-384.

For the use of Registered Medical Practitioners or a Hospital or a Laboratory only.

SmithKline Beecham (Pvt.) Ltd. Level 34, West Tower, World Trade Center, Colombo 01, Sri Lanka.

PM-LK-CSYL-22-00004

Case Report

Sri Lanka Dental Journal 2022; 52(03) 128-135
Received: 07.10.2022 | Revised: 22.11.2022 | Accepted: 24.11.2022

Carcinoma of breast metastatic to the mandible: Report of a case and review of the literature

RP Ekanayaka, PS Atapattu, EDPM Hettiarachchi, DD Edirisinghe

Abstract
Tumours can metastasize to the oro facial region and affect the jaw bones, soft tissue and salivary glands. The oro facial region metastases are rare and represent approximately 1% of all oral malignancies. These metastatic lesions could be the first evidence of an undiscovered malignancy at a distant primary site, and early detection of such metastases is a diagnostic challenge both clinically and histopathologically. Here we report a case of metastatic breast carcinoma in the mandible in a 58 yearold female, in which the presence of primary tumour was not known at the time of diagnosis of the jaw lesion.

Introduction
Metastasis is the most formidable hallmark of cancer that is responsible for the majority of cancer related deaths[1]. Breast cancer is the most commonly occurring cancer worldwide, with 2.26 million new cases in 2020, contributing 12.5% of the total number of new cases diagnosed in 2020[2]. Breast cancer is the most common cancer among females in Sri Lanka and the age standardized incidence is 24.7 per 100,000 in 2010[3].

The overall 5- year relative survival rate for breast cancer is 90%. Despite this relatively high 5-year survival rate, distant metastasis serves as the principal cause of mortality among breast cancer patients[4]. The most common site of distant breast cancer metastases is bone, and around 70% of individuals with metastatic breast cancer harbor bone metastases[4].

However overall metastases to the oro-facial region are rare and comprise only 1% of all oral malignancies[5]. The jawbones are more frequently affected than the oral soft tissues, while metastases to the mandible are more frequent than maxilla[6].

And the breast is the most common primary site for tumors that metastasize to the mandible[5].

Jawbone metastases occasionally could be the first evidence of an undiscovered primary malignancy[5,6]. Early detection of such metastases is challenging for both the clinician and the pathologist, yet of great clinical significance as the diagnosis of the relevant primary tumor site will always follow the detection of the metastasis.

The present case illustrates the importance of considering a metastatic lesion in differential diagnosis of tumor arising in jaws, even though the metastases to the oro-facial region are rare.

Case report
A 58 year old female presented to the Department of Oral and Maxillofacial Surgery of District General Hospital, Embilipitiya, Sri Lanka with a progressive swelling in the right side

RP Ekanayaka (Correspondence) — Department of Oral Pathology, Faculty of Dental Sciences, University of Sri Jayewardenepura/ Oral Pathology unit, Teaching Hospital Karapitiya, Galle. ORCID: 0000-0002-1073-0846
PS Atapattu — Oral and Maxillofacial Surgery unit, District General Hospital, Embilipitiya.
EDPM Hettiarachchi — Histopathology unit, District General Hospital, Embilipitiya.
DD Edirisinghe — Oral and Maxillofacial Surgery unit, District General Hospital, Embilipitiya.

mandible of five weeks duration. Furthermore, the patient reported a three-day recent history of pain associated with the jaw swelling. Her past medical, dental and surgical histories were unremarkable.

Extra-oral examination revealed a diffuse, non tender, bony hard swelling involving mainly the right side mandible and measuring about 6x4cm in its greatest diameter. A significant facial asymmetry was noted (Figure 1a and 1b). There were no signs of neurological manifestations such as lip or tongue paresthesia. Also, there was no cervical lymphadenopathy and the mouth opening was normal.

Intra orally a well-defined firm swelling was noted in the right side posterior mandibular buccal sulcus in relation 46, 47, and 48 region. The swelling measured 5 x 3 cm in size with no apparent mucosal changes (Figure 2). It was hard in consistency and non tender on palpation. Teeth 45,46,47 and 48 were missing, while teeth 42,41,31 and 32 were mobile. Lateral oblique radiograph of right mandible revealed a multilocular radiolucent lesion with relatively well defined margins (Figure 3).

Based on the history, clinical and radiological findings a provisional diagnosis of ameloblastoma in the right mandible was considered, along

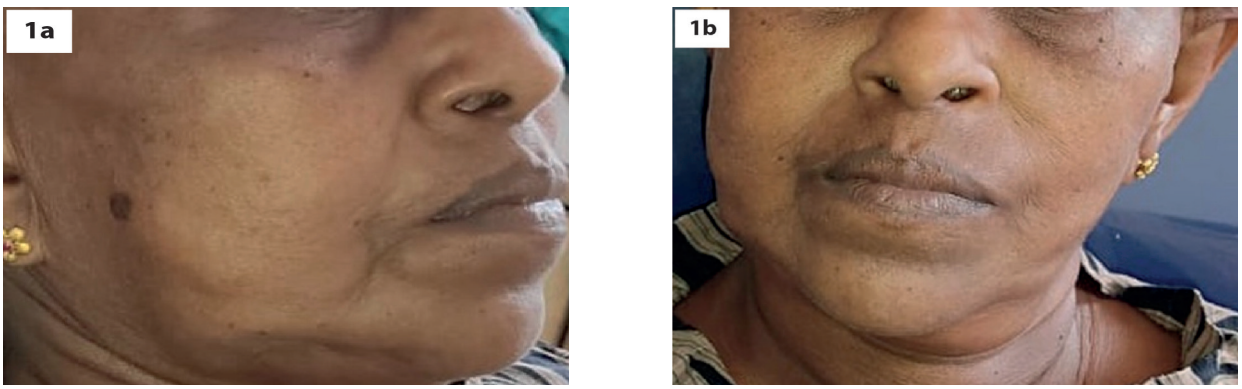


Figure 1. Extra oral view showing a diffuse swelling in right side mandible (a) and facial asymmetry (b)

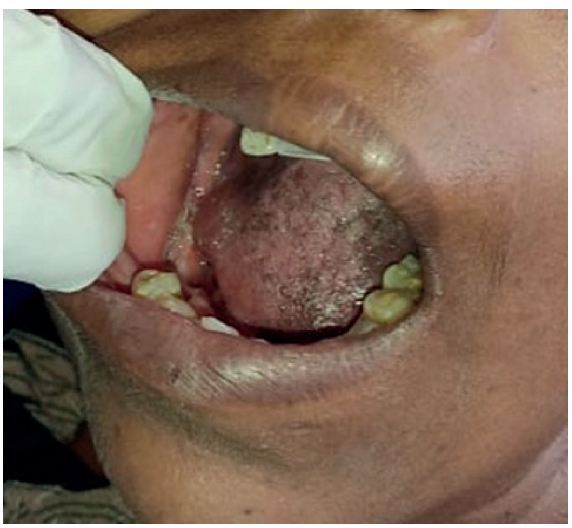


Figure 2. Intra oral view showing a swelling located over the buccal sulcus of the right side mandibular molar region

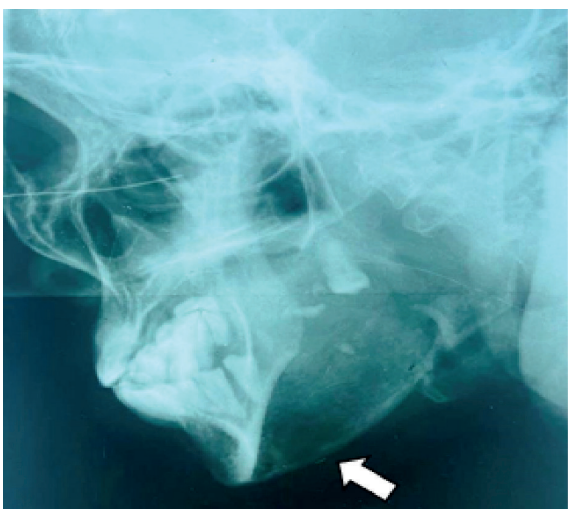


Figure 3. Lateral oblique radiograph showing a multilocular radiolucent lesion with relatively well defined margins in right mandible.

with the differential diagnosis of odontogenic keratocyst.

An incisional biopsy was performed under general anesthesia and the specimen was submitted for histopathological analysis.In intraoperative exploration of the lesion, it was noticed that the gross appearance of the lesion was not compatible with such of ameloblastoma or odontogenic keratocyst.

On gross examination, the specimen consisted of multiple fragments of cheesy looking friable tissues, white in color, together measuring 20x15x10 mm. On histology, H&E-stainedsections revealed prominent hyalinized tissue with foci of calcifications and bony trabeculae. Thin strands and small nests of epithelial cells were present, scattered within the hyalinized tissue (Figure 4, 5a & 5b). Central comedo - type necrosis was observed within the epithelial cell nests (Figure 6). Vague ductal differentiation was noted in places (Figure 7). There was no evidence of mitoses or significant cytological atypia in the epithelial cells.

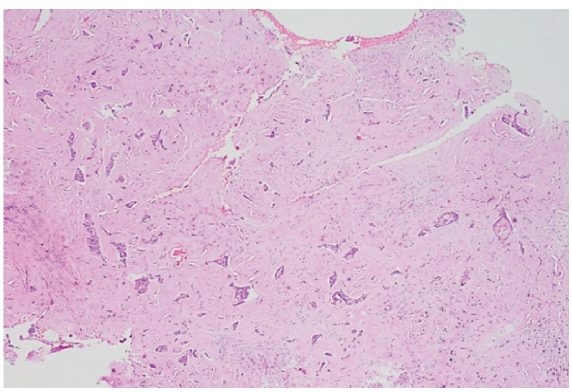


Figure 4. Characteristic tumour view consisting of thin strands and small nests of epithelial cells scattered within prominent hyalinized tissue. (H & E x 40)

Immunohistochemically,the initial diagnostic panel included cytokeratin 7 (CK7), cytokeratin 20 (CK20) and cytokeratin 19 (CK19). Tumor cells showed a strong and diffuse cytoplasmic positivity for CK7 (Figure 8a), whilst CK20and CK 19 were negative. Once the CK7/CK20 expression profile was established, additional organ specific markers were performed. However, considering the limited availability of immunohistochemical markers currently, a minimal set of markers for carcinoma identification were evaluated. Ascattered positive nuclear staining for Estrogen Receptor (ER) and Progesterone Receptor (PR) was observed (Figure 8b and 8c). Thyroid transcription factor 1 (TTF-1) was negative. Furthermore,the tumor

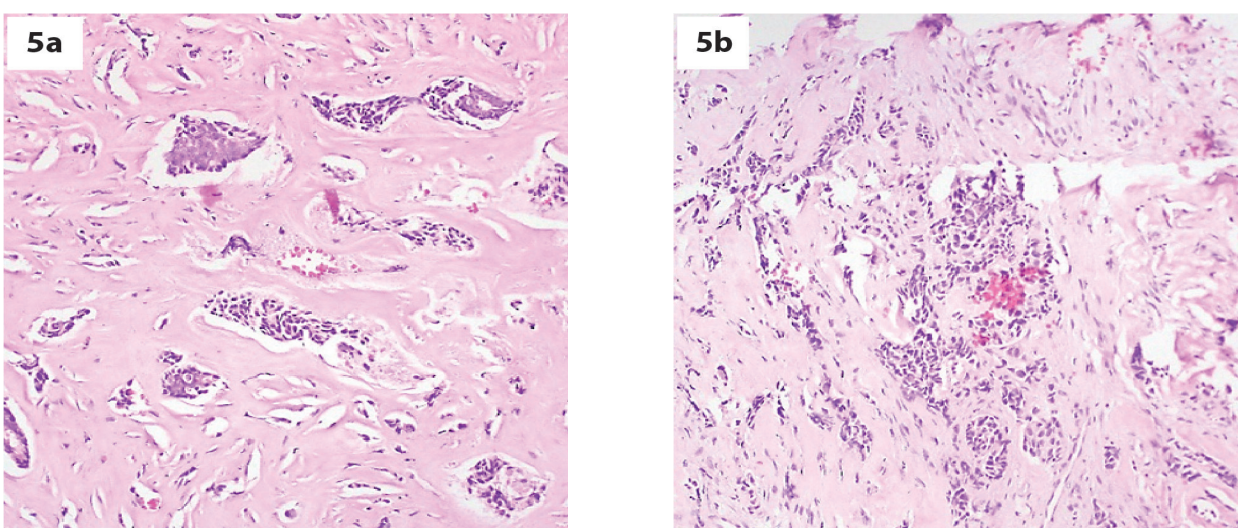


Figure 5. (a) & (b) High power view highlighting the epithelial cells arranging into thin strands and small nests (H & E x 100)

was immunonegative for subsequently performed HER2. The histopathological features and immunoprofile of this case were most suggestive of a metastatic adenocarcinoma of breast origin. Further clinicopathological correlation, along with systemic investigation and work up were therefore recommended.

Subsequent to the diagnosis the patient was referred to the General Surgery Unit of PGH Embilipitiya, and an ultrasound scan of both breasts and axillae was performed, followed by CECT of Chest/Abdomen and Pelvis. The ultrasound scan revealed a Birads category 5 lesion in R/S breast with suspicious right axillary lymphadenopathy, and the CECT further reported a lesion in the right breast extending to the nipple and the skin measuring 3.5x2.7x1.9 cm with enlarged lymph nodes in right axilla measuring 2x1.5 cm, both of which served to confirm the initial histological assertion of presence of a primary breast adenocarcinoma. No other distal metastases were identified in the CECT of chest/abdomen and pelvis. The subsequent ultrasound guided FNAC reported a C4 lesion in the right breast and the histopathological evaluation of true cut biopsy from right breast tissue revealed an invasive breast carcinoma of no special type (NST). A prompt referral was arranged to the oncology unit of PGH Badulla, and first cycle of chemotherapy was completed without serious complications. The patient is currently continuing her chemotherapy treatment with simultaneous close monitoring.

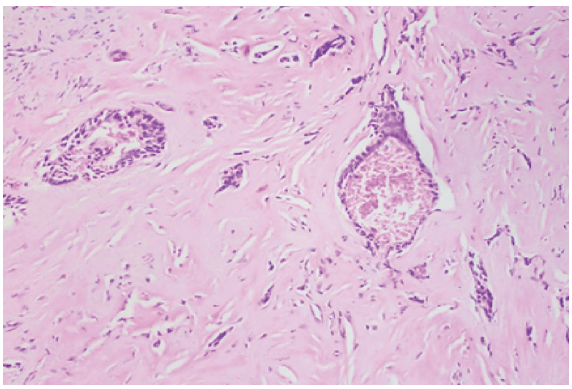


Figure 6. Central comedo- type necrosis within the epithelial cell nests (H & E x 400)

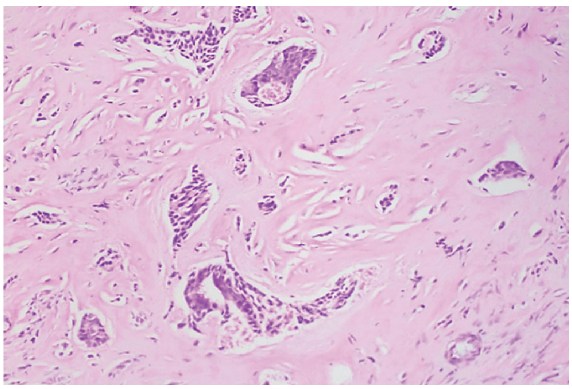


Figure 7. High power view highlighting the presence of vague ductal differentiation (H & E x400)

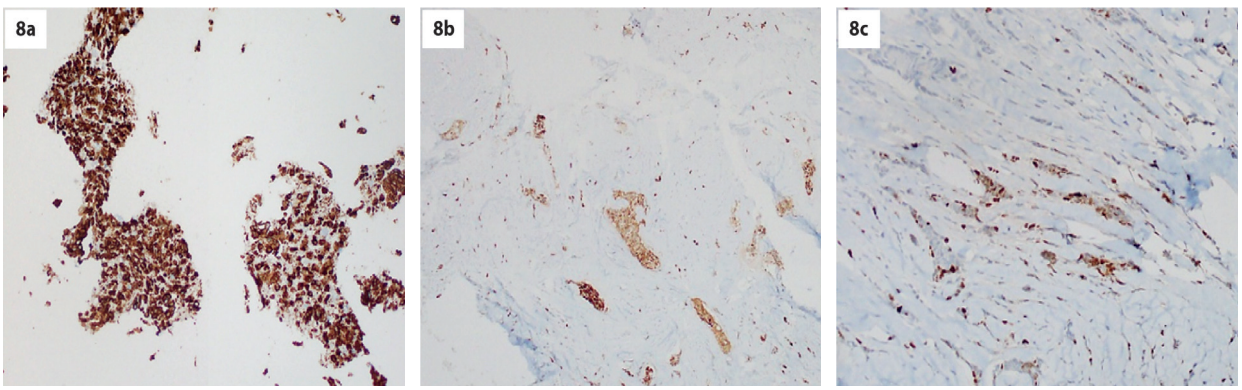


Figure 8. Immunostains showing strong diffuse cytoplasmic positivity with CK 7 (a) (x400) and scattered nuclear positivity with ER (b) and PR (c)

Discussion

Metastases to the oro-facial region are uncommon and comprise approximately 1% of all oral malignancies[5]. The jaw bones, particularly the mandible, are more frequently affected than the oral soft tissues, at a ratio of 2:1. The attached gingiva is the most commonly affected soft tissue site[6]. In accordance with the patient demographics available in the literature, most metastatic tumors to the oro-facial region are seen in patients between the fifth and seventh decades of life[6].

There is no sex predilection in jaw bone metastases, while an average male to female ratio of 2:1 is reported in oral soft tissues[5,6]. The case presented herein is of a progressive swelling of five weeks duration in the right side posterior mandibular buccal sulcus of a 58-year-old female. The demographic findings of the present case are compatible with those in the literature.

The most common primary tumor sites for oro-facial metastases are lung, kidney, liver and prostate for males; and breast, genital organs, kidney and colorectum for females. Lung is the most common primary site for tumors that metastasize to the oral soft tissues; and to the jaw bone metastases breast is the commonest primary site[6,7].

In majority of the patients, primary tumor is known at the time of diagnosis of the metastatic lesion[8]. However, in about 23% of cases the metastatic lesion in the oro-facial region is the first indication of an undiscovered tumor at a distant primary site[5,6]. In the present case, the primary tumour site was not known at the time of diagnosis of the metastatic lesion in the mandible. With subsequent systemic investigations the presence of a primary breast carcinoma was identified, which is on par with the commonest primary tumour site reported for jaw bone metastases.

According to the literature, in total <1% of mandibular malignancies are metastases of primary tumors elsewhere in the body. However, the actual incidence of metastatic lesions in the mandible may be higher, since radiographic surveys of the jaws are not performed routinely and also because the jaws are seldom examined during autopsies[8,9]. Within the skeleton, bones with haematopoietically active marrow are the preferred sites for metastatic deposits, and jaw bones have relatively little active marrow especially in the elderly. This paucity of active marrow in the mandible together with its increased bone density and poor vascularity, account for the relative rarity of mandibular metastases[10].

Reviewing 114 cases retrospectively, D' Silva et al reported that the common primary sites of metastases to the mandible are breasts (25%), lungs (13%), prostate (10%), colon (7%), thyroid gland (3%), and kidneys (3%)[9]. Jaw bones lack lymphatic vessels;therefore, tumors metastasize to the jaws via blood stream by embolization, and these metastases prefer haematopoietically active bone marrow[10]. According to the literature metastases to the mandible are more frequent than maxilla, and comprise 80% of all jaw bone metastatic lesions[11]. The most favored metastatic sites within the mandible are the molar (55%) and premolar (38%) regions, followed by the mandibular angle, retromolar trigone area and condyle in descending order[11]. This mandibular predilection may be related to the presence of relatively larger amount of hematopoietic bone marrow in the mandible rich with sinusoidal vascular spaces, accompanied by branching of the local blood vessels and slowing of the blood flow[12]. The patient discussed herein presented with a metastatic lesion in the molar region of right side mandible (in relation 46, 47, and 48). Hence with reference to the location of the metastatic lesion within the mandible, the findings of the present case were compatible with those in the literature.

The clinical presentation of the metastatic lesions in the mandible includes rapidly progressive bony swelling with associated tenderness, pain, ulcer

and bleeding. Sometimes tooth mobility, trismus and even pathological fractures are present[13]. Paresthesia of the lower lip and chin is considered an important sign of mandibular metastasis. It is described in the literature as Numb Chin Syndrome (NCS) and the nerves associated are the inferior alveolar nerve and its terminal branch, the mental nerve. Numbness of the teeth and mucosa may also occur[13,14]. However, in the present case swelling and pain were the main complaints and the patient did not describe of any paresthesia.

It is reported that metastatic lesions in the mandible may appear with a variety of symptoms, and the clinical differential diagnosis of such lesions include ameloblastoma, primary intraosseous squamous cell carcinoma, osteosarcoma and temporomandibular disorder. Nevertheless, in patients with a history of malignancy metastatic deposits must be considered high on the list of differential diagnoses.

Due to its rarity and its highly varied clinical presentation the definitive diagnosis of a mandibular metastatic lesion, particularly in the absence of a known primary tumor elsewhere, is challenging for both the clinician and pathologist[15].

Radiographically, metastases to the mandible may present as osteolytic radiolucent lesions with irregular and ill-defined margins. While metastases from kidney, lung, and breast cancers are more often osteolytic, metastases from prostate cancer mostly form osteoblastic lesions with a radio-opaque or a mixed radio-opaque radiolucent appearance[5,9,16]. And according to Hirshberg et al 5.4% of all jaw bone metastases do not show any significant radiographic change[5]. As per literature the radiological differential diagnosis of metastatic lesions to the mandible includes odontogenic cysts, osteomyelitis, advanced periodontal bone loss and primary bone or odontogenic malignancy[13].

The present case also revealed a multilocular radiolucent lesion in the right side mandible. At the same time, on contrary to the common presentation it showed relatively well defined margins. However, the use of radiographic findings in the diagnosis of metastatic lesions in the mandible is limited, as they do not exhibit a pathognomonic radiographic appearance.

As both clinical and radiological features of metastatic lesions in the mandible are not pathognomonic, the definitive diagnosis of such lesions and their origin is based on histological and immunophenotyphic examination of a biopsy specimen from the lesion.

Histopathologically, the jaw metastases often appear poorly differentiated and their site of origin cannot be determined with certainty by means of H & E staining alone, hence the use of immunohistochemistry is imperative for definitive diagnosis[17].

Immunoprofiling with CK7 and CK 20 facilitates initial categorization of the tumor in terms of site of origin. Breast carcinoma consistently shows negativity for CK 20 whilst showing strong positive immunoreactivity for CK 7[18].

CK 7 and CK 20 immunostains are best used together in narrowing down the possible sites of primary tumors. Additional site specific immunohistochemical markers and molecular markers are useful adjuncts for the determination of the site of origin of the metastases[18]. The strong and diffuse nuclear positivity of the present tumor for ER and PR prompted the definitive diagnosis of metastatic adenocarcinoma of breast origin.

Breast cancer is the most commonly occurring cancer in women both in the developed and less developed world, and the world's most prevalent cancer overall. In 2020, there were more than 2.26 million women diagnosed with breast cancer and 685,000 deaths worldwide. And as of the end of 2020, there were 7.8 million women alive, who

were diagnosed with breast cancer in the past 5 years[2].

Breast cancer occurs in women of any age after puberty. However, the risk of breast cancer increases with certain factors including increasing age, use of alcohol and tobacco, obesity, family history of breast cancer, and postmenopausal hormone therapy. Nevertheless, approximately half of the breast cancers develop in women who have no such identifiable risk factors[2, 19].

Breast cancer is the most common cancer among females in Sri Lanka. The age standardized incidence of female breast cancer in Sri Lanka is 24.7 per 100,000 in 2010 and highest incidence is seen among women of 60 to 64year age group. And the female breast cancer incidence in Sri Lanka is gradually increasing by approximately 4% per year[3].

Metastatic breast carcinoma is most often reported in bone, lung, and liver. When affecting the oral cavity, the metastatic deposits from breast cancer preferentially spread to jaw bones. For instance, in women, 40% of jaw bone metastases originate from the breast, compared with 25% of soft tissue metastases[4]. Metastasis to the jaw bones is a late complication and frequently a part of generalized spread with associated multiple co-existent metastatic deposits[8].

According to the literature, metastasis to the mandible is usually associated with a poor overall prognosis. The majority of patients diagnosed with metastatic deposits in mandible die within a year of detection of the metastases[17]. And distant metastasis is the principal cause of mortality among breast cancer patients, despite their relatively high 5-year survival rate[4].

Since jaw bone metastases usually are evidence of a widespread disease, the management of metastatic breast carcinoma of the mandible is primarily palliative, and may include chemotherapy, radiotherapy, endocrinotherapy, targeted therapy and rarely surgical intervention at both primary and metastatic tumor sites. Palliative therapy is mainly aimed at reliving pain while extending the survival time of the patient[20,21].

In conclusion, though rare it is important to consider distant metastases in the differential diagnosis of a jaw lesion, even if the presence of primary tumor is not known at the time of detection of the jaw lesion.

References

1. Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y. Molecular principles of metastasis: a hallmark of cancer revisited. Signal Transduct Target Ther. 2020; 5(1): 28.

2. World Cancer Research Fund International. Cancer trends-Breast cancer statistics. London, UK: 2022.

3. Fernando A, Jayarajah U, Prabashani S, Fernando EA, Seneviratne SA. Incidence trends and patterns of breast cancer in Sri Lanka: an analysis of the national cancer database. BMC Cancer. 2018; 18(1): 482.

4. Chen MT, Sun HF, Zhao Y, Fu WY, Yang LP, Gao SP, Li LD, Jiang HL, Jin W. Comparison of patterns and prognosis among distant metastatic breast cancer patients by age groups: a SEER population-based analysis. Sci Rep. 2017; 7(1): 9254.

5. Hirshberg A, Leibovich P, Buchner A. Metastatic tumors to the jawbones: analysis of 390 cases. J Oral Pathol Med. 1994; 23(8): 337-41.

6. Hirshberg A, Shnaiderman-Shapiro A, Kaplan I, Berger R. Metastatic tumours to the oral cavity - pathogenesis and analysis of 673 cases. Oral Oncol. 2008; 44(8): 743-52.

7. Hirshberg A, Buchner A. Metastatic tumours

to the oral region. An overview. Eur J Cancer B Oral Oncol. 1995; 31B(6): 355-60.

8. Siriwardena, Jayasooriya PR, Ratnayake P, Tilakaratne WM. Metastatic tumors of the oral and maxillofacial region: A retrospective analysis of 18 cases. Merit Res J BiochemBioinform. 2013; 1(1): 1-4.

9. D'Silva NJ, Summerlin DJ, Cordell KG, Abdelsayed RA, Tomich CE, Hanks CT, Fear D, Meyrowitz S. Metastatic tumors in the jaws: a retrospective study of 114 cases. J Am Dent Assoc. 2006; 137(12): 1667-72.

10. Hashimoto N, Kurihara K, Yamasaki H, Ohba S, Sakai H, Yoshida S. Pathological characteristics of metastatic carcinoma in the human mandible. J Oral Pathol Med. 1987; 16: 362-7.

11. Thiele OC, Freier K, Bacon C, Flechtenmacher C, Scherfler S, Seeberger R. Craniofacial metastases: a 20-year survey. J Craniomaxillofac Surg. 2011; 39(2): 135-7.

12. Barnes L. Metastases to the head and neck: an overview. Head Neck Pathol. 2009; 3(3): 217-24.

13. Kumar G, Manjunatha B. Metastatic tumors to the jaws and oral cavity. J Oral MaxillofacPathol. 2013; 17(1): 71-5.

14. Ryba F, Rice S, Hutchison IL. Numb chin syndrome: an ominous clinical sign. Br Dent J. 2010; 208(7): 283-5.

15. Jham BC, Salama AR, McClure SA, Ord RA. Metastatic tumors to the oral cavity: a clinical study of 18 cases. Head Neck Pathol. 2011; 5(4): 355-8.

16. Akinbami BO. Metastatic carcinoma of the jaws: a review of literature. Niger J Med. 2009; 18(2): 139-42.

17. Neville BW, Damm DD, Allen CM, Chi AC. Oral & Maxillofacial Pathology. 4th Edition, WB Saunders, Elsevier, Missouri. 2016; 622-7.

18. Krishna M. Diagnosis of metastatic neoplasms: an immunohistochemical approach. Arch Pathol Lab Med. 2010; 134(2): 207-15.

19. Wild CP, Weiderpass E, Stewart BW, editors (2020). World Cancer Report: Cancer Research for Cancer Prevention. Lyon, France: International Agency for Research on Cancer. Available from: http://publications.iarc.fr/586

20. Stavropoulos MF, Ord RA. Lobular adenocarcinoma of breast metastatic to the mandibular condyle. Report of a case and review of the literature. Oral Surg Oral Med Oral Pathol. 1993; 75(5): 575-8.

21. Poulias E, Melakopoulos I, Tosios K. Metastatic breast carcinoma in the mandible presenting as a periodontal abscess: a case report. J Med Case Rep. 2011; 5: 265.

Clogard

Natural Salt

STRONG TEETH | HEALTHY GUMS

Case Report

Sri Lanka Dental Journal 2022; 52(03) 136-139
Received: 20.11.2022 | Revised: 10.12.2022 | Accepted: 12.12.2022

Plexiform Neurofibroma in Submandibular Salivary Gland; a rare case report

KWIS Priyankara, Padma Silva, RLPR Liyanage

Abstract
A 21-year-old girl presented with a slow growing painful swelling in the right-sidesubmandibular region for three-year duration. Magnetic Resonance Imaging (MRI) of the neck showed a well-defined ovoid mass which was non-metastasizing and locally aggressive in submandibular region. Provisional diagnosis was benign neural tumour. The histopathology revealed a spindle cell tumour with wavy nuclei with nerve fascicles, confirming a plexiform neurofibroma within the submandibular salivary gland. Plexiform neurofibromaswithin major salivary glandsare considered as rare. However, reported cases mostly were in the parotid gland. In this report, a case of plexiform neurofibroma in the submandibular salivary gland is presented which is extremely rare.

Key words: Plexiform Neurofibroma, Submandibular Salivary gland

Introduction

Plexiform neurofibroma is a non-metastasizing, locally aggressive, invasive, benign peripheral nerve sheath tumor which surrounds multiple nervous fascicles[1]. It arises from multiple nerves as bulging and deforming masses.Different variants of neurofibromas such as plexiform type, solitary type, may occur as a part of the syndrome of Neurofibromatosis type I or Von Recklinghausen's disease (NF-1)[2,3]. However there are single or multiple neurofibromas with no evidence of the syndrome. Neurofibroma causes cosmetic and functional deformities in the head and neck region. Plexiform neurofibromas of the major salivary glands are extremely rare, often presenting in the parotid gland[4,5]. This report presents even a rare case where the tumor affected the submandibular gland. According to the English literature only seven cases have been reported[6] (Table 1).

Case report

A 21-year-old female presented to the oral and maxillofacial clinic with a main complaint of a painful swelling in the right-side submandibular region. It was a slowly progressive for three-year duration showing gradual increase of the size.During the mealtime, there was no changes in the size. There was no evidence of anesthesia, paresthesia, loss of general or taste sensation. It was found that she had no history of fever episodes, no recent significant weight loss, no headaches in the morning, no bone pain.

On examination, cardinal signs of the right-side submandibular region were negative. It was non-tender and non-pulsatile, firm in consistency, oval shaped, 4 x 2 cm size lump, with a well-defined border. This lump was not attached to the mandible or to the overlying skin or to the underlying tissues and structures. (Figures 1) It was bimanually palpable. There were no

KWIS Priyankara (Correspondence) National Dental Teaching Hospital. ORCID: 0000-0001-9798-850X
Padma Silva National Dental Teaching Hospital
RLPR Liyanage National Dental Teaching Hospital

discharging sinuses intra orally or extra orally.

The base line investigation (Full Blood Count, C- Reactive protein, Renal Function Test) values were within normal range. The radio-opaque shadow was suggestive of salivary calculi in the region of the submandibular gland of the plane radiographs (Lateral oblique right-side mandible and Dento panoramic tomography (DPT)).The provisional diagnosis was made as chronic sialadenitis with sialolithiasis. Ultrasound Scan (USS) guided Fine Needle Aspiration Cytology (FNAC) of the lump reported it as an inflammatory reactive lymph node. Therefore, further radiological investigations were carried out with a Magnetic Resonance Imaging (MRI) and it revealed that there is no sialadenitis, but suggestive of neurofibroma (Figures 2).

Further examination was carried out to exclude the syndromes associated with neurofibromas and found no café-au-lait spots on the skin and no multiple freckles in the axillary or groin region. She had no other lesions anywhere else in the

Table 1. Plexiform neurofibroma cases reported in the literature

Author Year Reference	Age of patient	Gender	Symptoms	Histological Diagnosis
Weitzner S.(1980)[5]	3 years	F	Submandibular mass with multiple neurofibromatosis	Plexiform neurofibroma
Tsutsumi T-A(1996)[8]	29 years	F	Submandibular mass	Plexiform neurofibroma
Derekey S, (2000)[7]	21 years	M	Submandibular mass with multiple neurofibromatosis	Plexiform neurofibroma
Bourgeois J-M, (2001)[4]	3 years	M	Submandibular mass with multiple neurofibromatosis	Plexiform neurofibroma
Aribandi M, (2006)[6]	6 years	F	Submandibular mass with multiple neurofibromatosis	Plexiform neurofibroma
Shekar TY, (2010)[10]	15 years	M	Submandibular mass	Plexiform neurofibroma
Anjana A. (2014)[11]	20 years	F	Submandibular mass	Plexiform neurofibroma

Figure 1. Clinical presentation showing a right submandibular gland swelling.

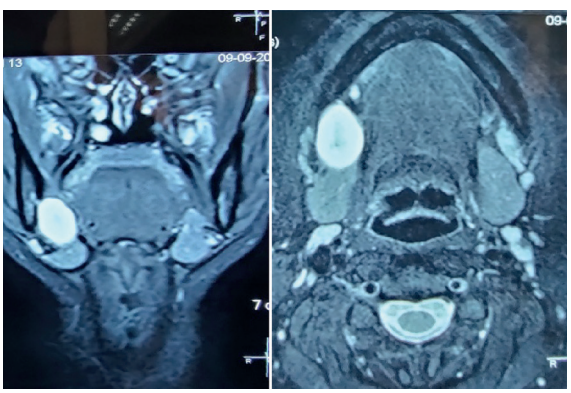


Figure 2. Well-defined ovoid mass 35 x 15 x 15mm at the anterior aspect of submandibular gland in T2 Head and Neck MRI Coronal and Axial sections

body. Her parents and siblings were denied of this kind of presentation. She had no learning disabilities.

Surgical excision was done, and it was a gelatinous nodule, attached to submandibular salivary gland capsule with greyish white to greyish yellow areas. Nocalculus was found within the gland. (Figures 3)

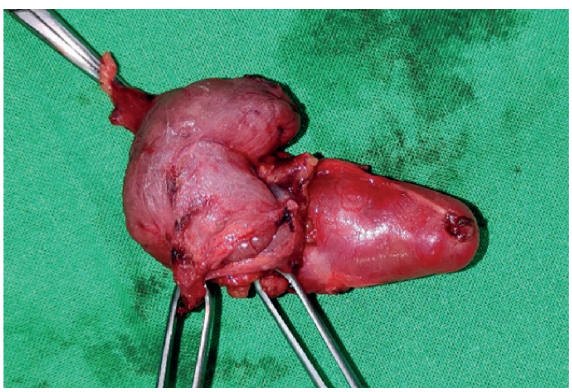


Figure 3. Macroscopic specimen

Histopathology revealed a circumscribed well-delineated, non-capsulated lesion comprising spindle cells in a loose myxoid stroma. It was composed of nerve cells, fibroblasts and perineural cells arranged in fascicles. Cells showing eosinophilic cytoplasm and centrally placed, elongated serpentine nuclei with tapering ends. There was no atypia. Immunohistochemical studies with S-100 protein showed positive expression. It was confirmed as Plexiform neurofibroma. The submandibular gland was normal in histology.

Discussion

Neurofibromas are benign peripheral nerve sheath tumours which are developed from non-myelinating Schwann cells[7]. It is also an autosomal dominant and genetically inherited disease. It can be presented in local discrete, generalized neurofibromatosis, and plexiform forms. The fifth cranial nerve and extremities are the common sites of origin of plexiform neurofibromas.

Neurofibromas represent in only 0.4% of all salivary gland neoplasms[6]. Plexiform neurofibromas of the salivary glands are very rare but usually presenting in the parotid gland. Tumours of neurogenic origin are extremely rare in submandibular gland.

The pathogenesis of neurofibroma is diffuse enlargements of multiple fascicles of the nerves and its branches which leads to thickening of nerves. These fascicles grow along nerves and extends into the surrounding tissue. Although they are slow growing, they are also locally infiltrating benign tumors. But they have a greater chance of developing malignancy when they are located deeply. When these lesions become large, they may compress trachea and pharynx. Compression of the spinal cord also can happen when it is extending into the spinal canal. In pregnancy and during puberty, these lesions grow rapidly[8].

Plexiform neurofibromas associated with 5-15% patients with neurofibromatosis-I[9]. They undergo malignant changes in 2% of the cases. Local somatic mutation can cause the tumour in Type- I neurofibromatosis patients which may not genetically transmit the disease to next generation[4].

Conclusion

Plexiform neurofibromas are painful, hypertrophic benign lesions with a malignant transformation rate of 2%. Diagnosis of Plexiform neurofibroma can be narrowed down with relevant history and examination with special investigations like MRI. The definitive diagnosis is based on histopathological examination. Neurofibromas are neurogenic in origin and 5 - 15% of cases are associated with Type - I neurofibromatosis. Its occurrence in submandibular salivary gland is very rare and can mimic other pathologies in submandibular gland and submandibular lymph nodes.

References

1. Geller M, Bonalumi AF, França FC, et al. Neurofibromatose. Síndrome de Von Recklinghausen: histórico, diagnósticos, examesclínicos e laboratoriais. JBM 1998;75:15-21.

2. Neely JG. Neoplastic involvement of the facial nerve. Otolaryngol Clin North Am 1974;7:385-96.

3. Batsakis JG. Tumors of the head and neck. Clinical and pathological considerations. 2nd ed. Williams and Wilkins: Baltimore 1979;313-33.

4. Bourgeois JM, Radhi J, DenEi L. Plexiform neurofibromas of the submandibular gland in a child. Can J Gastroenterol 2001;15:835-37.

5. Weitzner S. Plexiform neurofibromas of the submandibular gland in children. Oral Surg Oral Med Oral Pathol 1980;50:53-7.

6. Aribandi M, Wood WE, Elston DM, Weiss DL. CT Features of plexiform neurofibroma of the submandibular gland. AJNR Am J Neuroradiol2006;27:126-8.

7. Derekay S, Safali M. Plexiform neurofibroma of submandibular gland. J LaryngolOtol2000;114:643-5.

8. Tsutsumi T, Oku T, Komatsuzaki A. Solitary plexiform neurofibroma of the submandibular salivary gland. J LaryngolOtol1996;110:1173-75.

9. Neurofibroma plexiform tumors. Available from: http://www.madisonsfoundation. org/content/3/4/display.asp.[Last accessed on 2005 Dec 11].

10. Shekar TY, Gautam G, Prrabhala S and Gole S. Plexiform neurofibroma: A rare tumor of submandibular salivary gland. J Surg Tech Cse Rep 2010; 2(2): 81-3.

11. Anjana A; Ramesh N, Som DS, Kusum N. Plexiform Neurofibroma of Submandibular gland: A Case Report in Young Adult. SAJCRR 2014; 3(1): 695-699.

Case Report

Sri Lanka Dental Journal 2022; 52(03) 140-145
Received: 20.09.2022 | Revised: 12.10.2022 | Accepted: 01.12.2022

Prosthetic rehabilitation of a patient with partial glossectomy due to squamous cell carcinoma - A case report

EMKS Ekanayake, RM Jayasinghe, IP Thilakumara, D Leuke Banadara

Abstract

Treatment for oral carcinomas may cause loss of anatomical structures and affects individual's mastication, speech, swallowing, esthetics, and psychological wellbeing. Further, tongue has a very important role in sensation of various stimuli including taste and self-cleansing. Prosthetic rehabilitation helps to minimize such concerns and is important to improve the quality of life of the patients.

Glossal defects can be classified as partial or total. Factors influencing the rehabilitation of a glossal defect are depend on volume of tongue excised (lesser or more than 50%), presence or absence of teeth, type of surgical procedure (glossectomy with mandibulectomy or maxillectomy), effects of chemo radiation therapy and residual functions of the tongue. Patients with partial glossectomy (i.e., < 50% of tongue removed) suffer minimal functional impairment and require no prosthesis.

Resection of the tongue usually needs an immediate reconstruction with various types of flaps and grafts. Prosthetic rehabilitation for restoration of excised tissues and re-establishment of oral functions of such patients are key challenges. This task is even more challenging when glossectomy together with segmental mandibulectomy since retention and stability of a mandibular prosthesis is greatly affected by the position of the tongue. This report elaborate a case of a 46-years-old male patient following partial glossectomy due to squamous cell carcinoma on lateral border of the tongue, radical neck dissection and reconstruction with a pectoralis major mucocutaneous flap, who was rehabilitated with a lower metal partial denture with a modified lingual flange.

Introduction

Oral squamous cell carcinoma (OSCC) is a major oncological problem in many regions of the world due to the risk factors such as tobacco in forms of chewing or smoking, alcohol consumption and Human Papiloma Virus. It accounts for 16.5% of all cancers in Sri Lanka with a male: female ratio of 4:1. Nearly a 5% of OSCC are diagnosed in young pateients.[1] Tongue is one of the five commonest sites of OSCC.[2] Treatment for tongue SCC may vary from surgical excision, chemotherapy, radiotherapy, to palliative care. These treatments may cause loss of anatomical structures, and affects individual's mastication, speech, swallowing, esthetics, sensation of various stimuli including taste and psychological wellbeing. With the limitation of tongue movements self-cleansing ability gets compromised leading to dental caries and periodontal diseases. It also affects the denture retention when the patients are rehabilitated with a removable prosthesis.

Glossal defects can be classified as partial and

EMKS Ekanayake Faculty of Dental Sciences, University of Peradeniya. ORCID: 0000-0002-9322-5633
RM Jayasinghe Faculty of Dental Sciences, University of Peradeniya.
IP Thilakumara Faculty of Dental Sciences, University of Peradeniya.
D Leuke Banadara Faculty of Dental Sciences, University of Peradeniya.

total defects. Resection of the tongue is usually reconstructed with various types of flaps such as myocutaneous flaps which replace a bulk of tissues lost and free flaps which preserve the flexibility of the natural tongue. Prosthetic rehabilitation is required to restore the excised tissues and to re-establish oral functions. There are few factors affecting the prognosis of rehabilitation. They are volume of the tongue excised (less than 50% or more than 50%), presence or absence of teeth, other surgical procedures combined, such as mandibulectomy, maxillectomy, effects of chemo radiation therapy, and residual movements of the tongue.

However, Removal of more than 50% of tongue requires rehabilitation with either palatal or lingual augmentation prosthesis. Total glossectomy requires total glossal prosthesis[3]. Glossal prosthesis can be attached to the mandibular partial denture or the complete denture[4].

There are several types of prostheses, and they can be classified depending on the number of pieces it possesses. One such example is one-piece tongue prosthesis. There is one-piece prosthesis either for swallowing or speech depending on the function which is compromised. Prosthesis for swallowing has a middle trough to facilitate food bolus passage. Anterior and posterior elevation molded with lingulo-alveolar and lingulo-velar articulation is used for speech prosthesis.The two-piece prosthesis consists of two parts where the base part is contact with the floor of the mouth and a top part attached to the denture replacing the tongue. The commonest prosthesis, that is used is palatal augmentation prosthesis. It allows modification of the space of Donder and reduces the space in the oral cavity and allows contact between palate and the remaining tongue. It facilitates mainly speech followed by swallowing to some extent. Palatal ramps are incorporated into the prosthesis tominimize deviation. Mandibular augmentation prosthesis with guidance flange is also fabricated in certain instances to improve occlusal contacts minimizing deviation. The materials used for this prosthesis are mainly acrylic resins and silicon.

Case reports

A 46- years- old male referred from oral maxillofacial team to the department of Periodontology, Faculty of Dental Sciences, University of Peradeniya, to improve oral hygiene. Patient had undergone right side hemiglossectomy with modified radical neck dissection of the same side for OSCC in 2018. Reconstruction have been done with pectoralis major with mucocutenous flap. He had diabetes mellitus and COPD. He had habits ofbetel chewing and cigarett smoking.

On general examination, he was ill looking with asymmetric face and competent lips. There was a scar on right side of the lip and show a depression. Intra orally the gingiva was moderately inflamed with moderate gingival swelling on maxillary arch and a generalized gingival recession (Figure 1). The mucocutaneous flap was intact with the presence of hair follicles. About 50% of the remained tongue was with satisfactory residual tongue movements.

His hard tissue examination revealed full complement of the maxillary teeth were intact with lower partial dentate status.Teeth present were 18, 17, 16, 15, 14, 13, 12, 11, 21, 22, 23, 24, 25, 26, 27, 28, 32, 33, 34, 35, 36, 37, and 38. There were betel stains all over the dentition and plaque and calculi were in abundance. A mild attrition was visible all over the teeth. Assessment of the occlusion showed that he had stable static contacts in maximum inter-cuspation. No deviation or rotation of the mandible was observed. An adequate vertical space was present to replace teeth in the mandible up to tooth 46.

Despite the claims made by the patient regarding quitting the habits, it was revealed that he was continuing betel chewing occasionally. He was advised to stop betel chewing completely. Patient

Figure 1. Extra oral view and Intra oral view before the prosthesis.

was also motivated and educated regarding the oral hygiene practices following disclosing plaque. His brushing technique was monitored and corrected in each visit. Inter dental cleaning aids were introduced for the lower teeth such as end tufted brush. Diet counselling was done following diet analysis and fluoride mouth rinse was recommended on daily basis and varnish application in 3 months' intervals time.

Re-mineralizing agents such as tooth moose was recommended, and fissure sealants of the remaining molars was carried out. Prophylactic scaling was done to remove plaque and calculi. Following the detailed periodontal assessment, root surface debridement was done for the areas having deep pockets until they all were healed completely. Even though root canal treatment of 38, has been planned,it was extracted due to a severe pain.

The restorative phase was started with the mandibular metal partial denture construction. As the patient had almost 50% of tongue substances and the tongue movements were preserved to some extent, there was no need to replace the tongue substances. However, as the patient had difficulty in mastication, it was necessary to replace the missing teeth with a prosthesis. Over several treatment options, a removable metal denture was decided to be the most appropriate option to replace missing teeth. However, the retention would be compromised with the lack of lingual sulcus. Therefore, a modified lingual flange was planned to obtain maximum retention and stability.

Radiologically, there were generalized mild bone loss and extensive dentinal caries in 38 (Figure 2)

Primary impression was taken of the mandibular arch with a maxillary stock tray with irreversible hydrocolloid. The definitive impression tray was made with light cured acrylic. Border molding

was done with green stick compound in the edentulous saddle area. Definitive impression was taken with regular body silicone following tooth preparation for the metal denture (Figure 3).

The metal framework was consisted of distal rests on 37, 36 and 35, Mesial rests on 37 and 34, occlusally approaching ring clasp on 37, occlusally approaching C clasp on 35, gingivally approaching I bar on 33, lingual plate was planned to have more bracing action over lingual bar major connector and the metal saddle extended up to 46. A wax occlusal rim was attached to the metal framework and occlusal records were obtained (figure 4) and then thedenture was finished with acrylic teeth and flanges (figure 5).

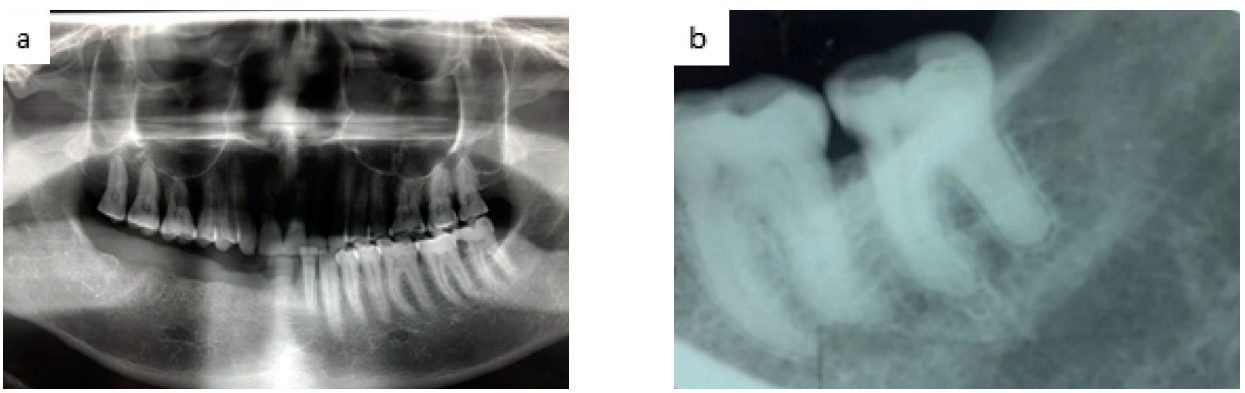


Figure 2. Generalized mild bone loss (a) and peri apical view of 38 (b)

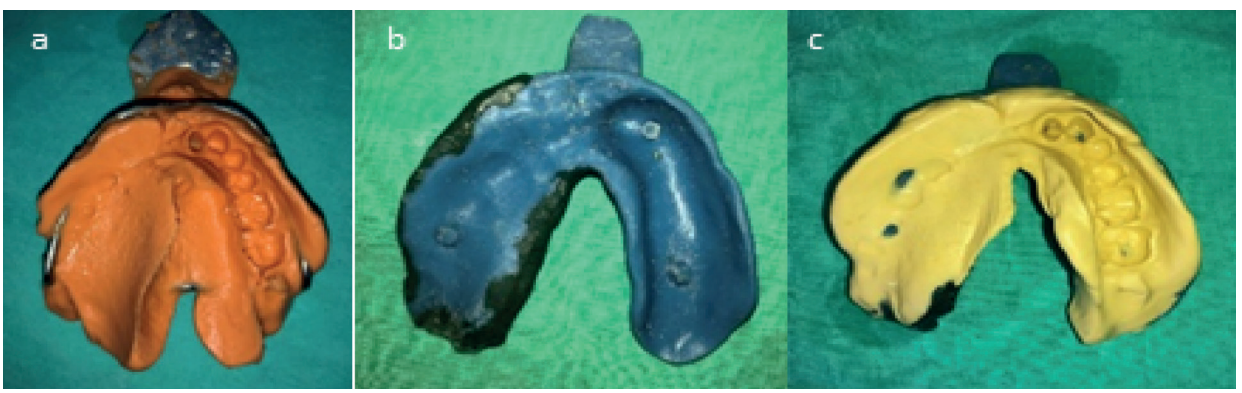


Figure 3. Primary impression (a,b) and definitive impression (c)

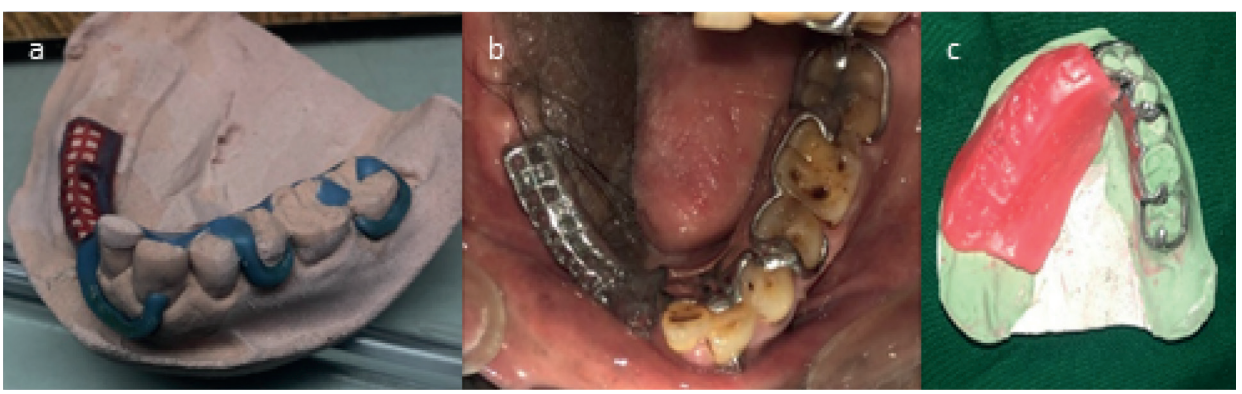


Figure 4. Metal framework (a,b) and wax record block (c)

Finally, a functional impression was taken with the tissue conditioner over the lingual flange area while the denture was inside the mouth. A pickup impression was taken with alginate and a stock tray.

The denture with modified lingual flange was processed with vulcanized silicone (Molloplast B). The patient was highly satisfied with the new prosthesis and the retention and stability were acceptable. It restored the patient's function and the esthetics (figure 6).

Discussion

Mandibular denture with tongue prosthesisreduces the size of oral cavity, there by improves resonance of the speech and directs food into esophagus. It also protects the underlying soft tissues. Also,it furtherdevelops acontact between residual tongue and palate during speech and swallowing. It improves the appearance and psychosocial wellbeing[5].

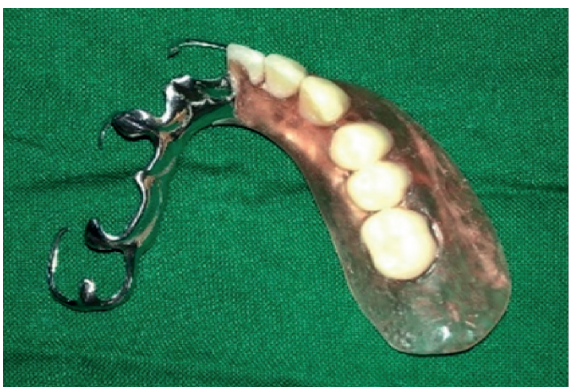


Figure 5. Finished denture with acrylic teeth

For the success of a conventional denture, properties such as retention, stability and support will be essential and to be fulfilled. Retention of the mandibular prosthesis was obtained with gingivally and occlusallyapproaching clasps. The support was optimized with widely distributed rests. Stability was achieved with guide plates, lingual plate, minor and major connectors.

Silicone was used over acrylic to modify the lingual flange in the denture. It can be polymerized simultaneously with acrylic. It also withstands the influences of oral environment without deterioration being non-irritant, odorless and tasteless[6].

The other treatment options are implant supported fixed or removable prosthesis. As there was no associated mandibulectomy, there was enough bone substances to place implants. Retention, support, and stability are achieved through the implants. Therefore, implant supported prosthesis will be a better choice of treatment. However, cost, time and surgical morbidity limit the treatment of choice.

Conclusion

Tongue prosthesis may not replace the internal structure of the tongue, but it merges with the residual anatomical structures and provides certain degree of comfort and function which

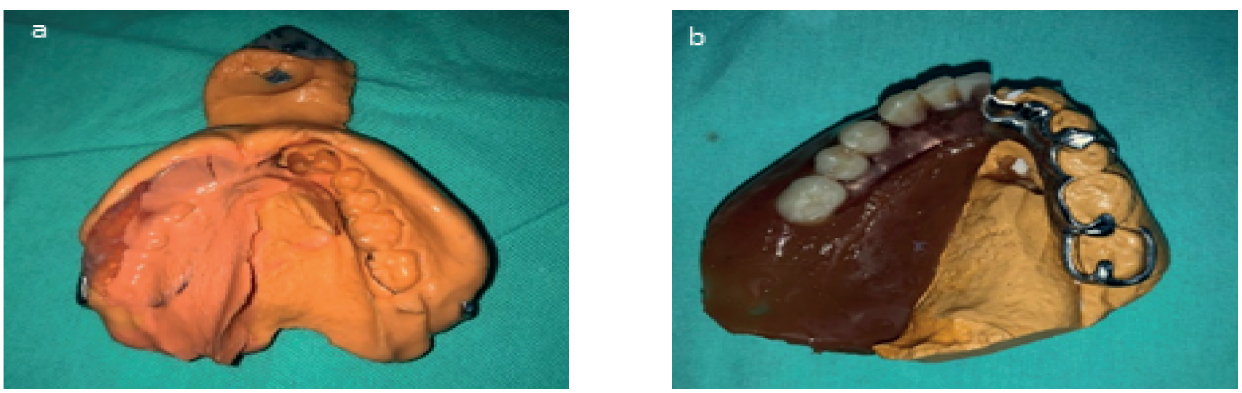


Figure 6. Pickup impression (a) and the finalized denture with modified lingual flange (b)

Figure 7. Intra oral view with the prosthesis and extra oral appearance

is called a 'functional prosthesis". In this case report a removable partial denture with a modified lingual flange has been provided to improve the quality of life of the patient.

References

1. B.S.M.S. Siriwardena, A. Tilakaratne, E.A.P.D. Amaratunga, W.M. Tilakaratne, Demographic, aetiological and survival differences of oral squamous cell carcinoma in the young and the old in Sri Lanka, Oral Oncology, Volume 42, Issue 8, 2006, Pages 831-836.

2. Dorsey J. Moore, Glossectomy rehabilitation by mandibular tongue prosthesis, The Journal of Prosthetic Dentistry, Volume 28, Issue 4, 1972, Pages 429-433.

3. Jiyoung Yoon, Siho Lee, Jiyoen Lee, Namsik, Prosthetic rehabilitation for a glossectomy patient - a clinical reportJ Korean AcadProsthodont 2013;51(4):347-352.

4. Balasubramaniam MK, Chidambaranathan AS, Shanmugam G, Tah R. Rehabilitation of Glossectomy Cases with TongueProsthesis: A Literature Review. J ClinDiagn Res. 2016;10(2):ZE01-ZE4. doi:10.7860/JCDR/2016/15868.7184.

5. Thosaporn W, Kintarak S, Subarnbhesaj A, Darling M, Kryshtalskyj E, Chiang CP, Shin HI, Choi SY, Lee SS, Aminishakib P. Oral cancer: A multicenter study. Med Oral Patol Oral Cir Bucal. 2018 Jan 1;23(1):e23-e29. doi: 10.4317/medoral.21999. PMID: 29274153; PMCID: PMC5822535.

6. Aramany MA, Downs JA, Beery QC, Aslan Y. Prosthodontic rehabilitation for glossectomy patients. J Prosthet Dent. 1982 Jul;48(1):78-81. doi: 10.1016/0022-3913(82)90050-6. PMID: 7050354.

Instructions to Authors

The Sri Lanka Dental Journal (SLDJ) publishes the following categories of articles which have relevance to dentistry and allied sciences.

Leading articles
One article per issue may be published on a current topic of interest, one's expertise or commentaries of general practice etc. These articles are commissioned by the Editors and may be considered at the Editors' discretion. They should not exceed 2500 words (excluding the abstract and references) and 20 references.

Reviews / Clinical updates
These are summaries of recent insights in specific research/clinical areas within the scope of SLDJ. Key aims of Reviews/clinical updates are to provide systematic and substantial coverage of a research or clinical area of wide interest or to present a critical assessment of a specified area. These articles must focus on recent research and on a topic that is timely and relevant to the field. No word/ reference limits.

Research Articles
Articles resulting from research work belong to this group. Results from routine clinical examinations or laboratory investigations will not be considered under this category. Subjects may vary from clinical trials to basic science research, historical analysis to dental economics. No word/ reference limits.

Short Reports
These include reports on current topics, modified techniques. New materials, practice management etc. Interesting results from routine, clinical work or laboratory investigations also may be accepted. They should not exceed 1500 words (excluding the abstract and references) and 10 references.

Case Reports
Reports such as of rare diseases or conditions, modifications to accepted treatment procedures, new management methods etc. may be included in this category. Case reports should make a contribution to existing knowledge and must have educational value or highlight the need for a change in clinical practice or diagnostic/ prognostic approaches. They should not exceed 2000 words (excluding the abstract and references) and 15 references.

Letters to Editor
Subjects unlimited, but may include short critique of published papers in the SLDA. Maximum 1000 words; no references.

Proceedings on Annual Sessions
Abstracts from annual sessions of the SLDA, other colleges and professional associations related to dentistry can be published under this category, upon request by such associations.

Submission of Manuscripts
Authors submitting a paper do so on the understanding that no part has been published before. That it is not being considered for publication elsewhere and that it has been read and approved by all the authors.

The journal does not hold it responsible for statements made by the contributors. The author alone is responsible for the statements made in his/her paper.

Submission of a manuscript means that authors automatically agree to assign exclusive copyright to the Sri Lanka Dental Association if and when the manuscript is accepted for publication.

The Authors can submit manuscripts and associated files as E-mail attachments to one of

the co-editors or the SLDA office. The editors will acknowledge receipt of submissions.

Manuscript Style
All manuscripts should be written in English and prepared in Microsoft Word format (.doc or .docx format). They should be typed in size 12 - Times New Roman fonts, double-spaced, single column, on A4 (2I2x297 mm) paper. Both British and American spellings are acceptable, provided this is consistent throughout the manuscript. All measurements must be given in SI units. Correct unit abbreviations should be used. All abbreviations should be defined in the text at first use.

Manuscripts not submitted in proper format may be returned without review. The manuscript should be prepared in the form of a single file containing the following items arranged in the order shown: Title page, Abstract, Main text, Acknowledgements, References, Figures & legends, Tables & legends.

Title Page
The title page should contain the following information in the order given: (1) a concise but informative title; (2) authors' full names (without degrees and titles): (3) authors' institutional affiliations: (4) name, address, telephone, telefax and electronic mail address of the author responsible for correspondence.

Abstract
All articles must contain an abstract of approximately 250 words. Do not use abbreviations and/or references in the abstract. The abstracts for research articles should be prepared under the following specified subtitles:

1. **Objective:** An introductory sentence indicating the objective and purpose of the study.

2. **Materials and Methods:** A description of experimental procedure including applicable statistical evaluation.

3. **Results:** A summary of the new. Previous unpublished data and results.

4. **Conclusion:** A statement of the study's conclusion

5. **Key words:** 3-5 key-words according to the Index Medicus should be provided.

Main text / body of manuscript
Manuscripts of research articles should include the following separate sections; Introduction, Materials & methods, Results, Discussion, Conclusion, Acknowledgements (if any), References.

Introduction
The introduction should carry sufficient background information on the subject of study.

Materials and Methods:
Procedures should be described in such detail as to make it possible to repeat the work. Sub-headings may be used to improve clearness. The authors should consider the ethical aspects of their research and ensure that the work has been approved by an appropriate Ethical Committee. Where applicable, a copy of the ethical clearance certificate should be attached. All human studies should have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and all subsequent revisions.

Results
The Results section should clearly and concisely present the findings of the research, as a rule in the past tense without subjective comments and reference to previous literature. The results should be supported by statistical or illustrative validation. For the sake of clarity this section may have subheadings.

Statistical Analysis
Since many scientific investigations rely on statistical treatment, authors are strongly urged

to consult a person with in-depth statistical knowledge. Manuscripts with a clear element of statistics are regularly refereed by the Journal's statistics consultant.

Discussion
This section should present the interpretations and /or implications of the findings in context of existing research and is the only proper section for subjective comments. Authors are strongly urged to avoid undue repetition of what has been reported in Results.

Conclusion
This should state clearly the main conclusions and provide an explanation of the importance and relevance of the study reported.

Acknowledgements
The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. The contribution of colleagues or institutions should also be acknowledged.

Tables
Tables should be self-contained and complement, but not duplicate, information contained in the text. Tables should be numbered consecutively in Arabic numerals. Each table should be presented on a separate sheet with a comprehensive but concise title above the table. Tables should be double-spaced and vertical lines should not be used to separate columns. There should be no left & right borders and no/ minimum internal horizontal lines to separate rows. Column headings should be brief, with units of measurement in parentheses; all abbreviations should be defined in footnotes. Footnotes to tables should be indicated by superscript lowercase letters (or asterisks for significance values and other statistical data) and included beneath the table body. Statistical measures such as SD or SEM should be identified in the headings. The table and its title/footnotes should be understandable without reference to the text.

Figures
All graphs, drawings, and photographs are considered as figures and should be numbered in Arabic numerals and cited in consecutive order in the text. All drawings and photographs should be supplied separately as JPEG files of adequate resolution (300 dpi or higher). Figures should be sized to fit within the column (75 mm), or the full text width (164 mm).

Each figure should have a legend which is concise but comprehensive: the figure and the legend must be understandable without references to the text. Include definitions of any symbols used and define/explain all abbreviations used and units of measurement. All legends should be typed together on a separate page.

References
The Vancouver system of referencing is used. References should be listed in a separate section immediately following the text. In the list, references should be numbered consecutively in the order in which they appear in the text; do not, alphabetise. Identify references in texts, tables and legends by Arabic numerals (superscripts). Cite the names of all authors when there are six or fewer; when more than seven list the first three followed by et al. Names of journals should be abbreviated in the style used in the Index Medicus.

Congress abstracts should not be used as references nor may "unpublished observations" and "personal communications" be placed in the reference list. References cited as "in press" must have been accepted for publication and not merely in preparation or submitted for publication.

Few examples of correct forms of references are given below.

Standard journal article
Bartlett IG, O'Keefe P. The bacteriology of the perimandibular space infections. J Oral Surg

1979; 37(6): 407-9.

Electronic journal article
Lemanek K. Adherence issues in the medical management of asthma. J Pediatr Psychol [Internet]. 1990 [cited 2010 Apr 22];15(4):437-58. Available from: http://jpepsy.oxfordjournals. org /cgi / reprint / 15 / 4 / 437 (If available, mention the DOI number at the end of the URL)

Unpublished article
Barker DS. Lucas RB. Localised fibrous growth of the oral mucosa. J Dent Res 1965: in press.

Books and other monographs
Becker A. Orthodontic treatment of impacted teeth. 3rd ed. Oxford, United Kingdom: Wiley-Blackwell;2012

Chapter in an edited book
Boyde A. Amelogenesis and the structure of enamel. In: Cohen B. Kramer KH (eds). Scientific Foundations of Dentistry. London: William Heinemann Medical Books Ltd.; 1976.p 335-352.

Chapter in a non-edited book
Speroff L, Fritz MA. Clinical gynaecologic endocrinology and infertility. 7th ed. Philadelphia: Lippincott Williams and Wilkins; 2005. Chapter 29, Endometriosis; p.1103-33.

No author given
International statistical classification of diseases and related health problems, 10th revision, vol J. Geneva: World Health Organisation, 1992; 550--564.

(Include the edition number after the book title for all editions except the first edition.)

Review policy

1. The Editorial Board reserves the right to publish or not submitted manuscripts, and informs the authors of its decision within a reasonable time frame. This decision is supported by an evaluation of the manuscripts based on a peer-review system, carried out in two consecutive stages:
   a) initial assessment by the Editorial Board, which decides on the manuscript's submission to the following stage of the process, involving its evaluation by anonymous reviewers; this decision is made taking into consideration criteria of relevance, interest and quality, defined according to the journal's editorial policy, as well as the manuscript's conformity to the submission guidelines contained in this document;
   b) detailed evaluation of the selected manuscripts by anonymous reviewers through a double-blind peer review system. Reviews resulting of this process will be used as a basis for the final decision on the manuscript's publication.

2. The Editorial Board may suggest revisions to authors, according to the recommendations made by the reviewers, and ask for a new evaluation of the revised paper in order to make the final decision on its publication.

3. In the case of manuscripts submitted to the second stage of evaluation, if they are not accepted for publication, editors will inform the authors of the reasons for this decision, generally attaching the reviewers' opinions and sending them to the authors.

4. Authors are fully responsible for the contents in published manuscripts.