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Letter from the Editor

It is an honour to be allowed to serve as the Editor of the Singapore Dental Journal (SDJ).

One of the hardest things the editorial team had to think about was what the role of our journal is or should be. Perhaps, Education and Information may be the most important roles of our journal since it is published once a year. Given that most dentists do not have access to the full papers of many academic dental journals, we thought that several reviews a year might be useful. We have successfully invited three reviews from scholars recommended by the Editorial team. We are ever grateful to them for taking the time and making the effort to write for us, to these authors my heartfelt gratitude and deepest appreciation.

From one of the reviews in this issue, you will see that, thus far, even for something that we have done for a very long time and may think that the procedure is backed up by solid research and science - pulp capping, the evidence from dental research falls short of the strict standards required for it to be recognized as evidently scientific. Note here that when considering if something we do is scientific, we are using the yardstick known as the hierarchy of evidence.

It was fortuitous that a member of our team had been keeping up with developments with worldwide attention on mercury hygiene issues. A review was invited from an author who conducted original research on mercury hygiene and its effects on dentists in Singapore in the early 1980s. As you are already aware, with signing of the Minamata Convention, a statement of the Ministry of Health announced that the undergraduate dental curriculum will phase out the teaching of dental amalgams (as reported by Today, online version dated 10 October 2013, sighted on 30 October 2013, available from <http://www.todayonline.com/singapore/mercury-poisoning-fears-prompt-new-guidelines-silver-dental-fillings>). Going into the future, it is likely that the use of amalgams will be reduced and will eventually die out in Singapore. Here again, though there may be evidence that

mercury is very toxic in specific experiments or case reports, note that on the scale of the hierarchy of evidence, there is insufficient evidence to say mercury in amalgams is poisonous and as a result of that should be discontinued in humans. Note that saying that there is no solid scientific evidence to say that it is not poisonous does not mean that mercury or amalgam use is entirely safe.

Another review was invited from the local experts on stem cell research in dentistry and what it might mean to the future of dental practice. We hope you will find the review useful and informative.

The editorial team met again recently and we have drawn up a list of things that we will try to do to increase publishing in the SDJ by local authors. The team led by the Convenor of the Editorial committee, Dr. Terence Jee, will consult with all stakeholders concerned and put these plans into action. Already my predecessors had worked hard to position the SDJ so that it is indexed in Index Medicus and hence when articles are published in our journals, the articles can be found through search via PUBMED. Being indexed is very important as what is published get exposure to other researchers in the field. We know we have to work very hard to maintain this However; plans are just that - plans till we have agreement by all stakeholders concerned. If we are successful in our plans we should have a steady stream of publications by local authors. When that happens, we hope to be able to put out two issues a year and eventually have the journal listed in more credible literature publication indices and would then attract more international authors to publish with us. If you have any suggestions, please do not hesitate to write to SDJ Administrator at sdj@sda.org.sg

We hope that you will all give us your full support and work towards ensuring that our journal remains relevant and useful to the membership.

Best wishes for a bright, cheerful and healthy year in 2014.

Editor
SUM Chee Peng

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Review

Treatment of pulps in teeth affected by deep caries – A systematic review of the literature

Gunnar Bergenholtz*, Susanna Axelsson, Thomas Davidson, Fredrik Frisk, Magnus Hakeberg, Thomas Kvist, Anders Norlund, Arne Petersson, Isabelle Portenier, Hans Sandberg, Sofia Tranæus, Ingegerd Mejare

Varsaparken, Gothenburg, Sweden

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ABSTRACT

Background: This systematic review assesses the effect of methods commonly used to manage the pulp in cases of deep caries lesions, and the extent the pulp chamber remains uninfected and does not cause pulpal or periapical inflammatory lesions and associated tooth-ache over time.

Study design: An electronic literature search included the databases PubMed, EMBASE, The Cochrane Central Register of Controlled Trials and Cochrane Reviews from January 1950 to March 2013. In addition, hand searches were carried out. Two reviewers independently evaluated abstracts and full-text articles. An article was read in full if at least one of the two reviewers considered the abstract potentially relevant. Altogether, 161 articles were read in full text. Of these, 24 studies fulfilled established inclusion criteria. Based on studies of at least moderate quality, the quality of evidence of each procedure was rated in four levels according to GRADE.

Results: No study reached the high quality level. Twelve were of moderate quality. The overall evidence was insufficient to assess which of indirect pulp capping, stepwise excavation, direct excavation and pulp capping/partial pulpotomy, pulpotomy or pulpectomy is the most effective treatment approach for teeth with deep caries.

Conclusions: Because of the lack of good studies it is not possible to determine whether an injured pulp by deep caries can be maintained or whether it should be removed and replaced with a root canal filling. Both randomized studies and prospective observational studies are needed to investigate whether a pulp exposed to deep caries is best treated by measures intended to preserve it or by pulpectomy and root filling.

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Introduction

Pulpal inflammation, caused by deep caries infection, can be clinically managed either by an attempt to preserve the tissue, or remove it and root-fill the tooth. Considerable controversy exists on the issue and it is frequently maintained that pulp capping/pulpotomy procedures should be considered for minimally affected teeth where the exposure occurred through healthy and non-carious dentine (for review see e.g. [1-3]). The advantage of preserving the pulp is nevertheless obvious in cases with large pulp chambers and underdeveloped roots because pulpectomy arrests root development. The dentinal walls in the root canal will then be thin and increase the risk of root fracture. Thus, from a biological, patient and cost perspective, especially in young teeth, the route of retaining all or some of the pulp can be seen preferable.

There are several modes to preserve pulpal vitality in teeth with deep caries. In recent years indirect pulp capping has been advocated in several reports (e.g. [4,5]). By leaving the deepest layer of carious dentine undisturbed, the aim of this method is to avoid exposure of the pulp and thus enhance what is believed to be a better long-term outcome. Favourable outcome studies have been reported [4-6]. Carious tissue may also be completely removed, either at the same appointment (complete caries excavation) or in one or more treatment steps (stepwise excavation). In the latter case the objective is to allow the pulp an opportunity to recover, at the same time as a potentially unnecessary pulpal exposure may be prevented. If the pulp happens to be exposed, the wound can then be treated with a conservative procedure (direct pulp capping or partial or complete pulpotomy). The most radical approach is to remove the entire pulp (pulpectomy) and replace it with a root filling. In this report we examine the scientific support for the effect of these procedures, i.e. that the pulp chamber remains uninfected and does not give rise to pulpal or periapical inflammatory lesion and associated toothache.

Systematic review reports have recently addressed the area [7-9]. One propose is that the pulp capping has a reasonably good outcome in a short-term perspective [7], while others see advantages of step excavation or one-step

incomplete excavation in comparison to two-step incomplete or complete caries removal [8,9].

The present review is part of a more comprehensive systematic review first published in Swedish by the Swedish Council on Health Technology Assessment (SBU) covering methods of diagnosis and treatment in endodontics [10]. In 2012 an English translation was made available. SBU is an independent national authority for the critical evaluation of methods for preventing, diagnosing and treating health care problems.

The specific questions addressed in this report were

- How effective are the different methods for preserving the pulp in a vital, asymptomatic condition in teeth with deep caries?
- How effective is pulpectomy in comparison?
- What factors may influence healing after a pulpectomy procedure?

Material and methods

Electronic literature search included the databases PubMed, Embase and CENTRAL. All languages were accepted provided there was an abstract in English. Articles published between 1950 and 2010 were sought in the first series of searches. Considered were articles in all languages having at least a summary in English or Swedish. For this review, articles published between 2010 and 2013 were pursued by March 2013 and added. Also searches by hand were carried out. Table 1 describes the inclusion criteria and Table 2 the exclusion criteria for the selected studies.

The review process

Two assessors (GB and IM) examined independently the abstracts of the acquired studies. The objective was to identify studies, which were relevant to the three questions. The results were compared and full-text versions were ordered of all articles judged as relevant or "possibly relevant". The same

Table 1 – Inclusion criteria.

| | |
|--------------|--|
| Population | Deciduous or permanent teeth of all ages. While the response of the pulp of a deciduous tooth might be different from that of the permanent tooth, studies on deciduous teeth were accepted with respect to stepwise excavation and direct or indirect pulp capping to avoid losing important information. Studies calculating the cost effectiveness and cost benefit. Study type: randomisation controlled studies (RCT) quasi-RCT, controlled clinical studies (CCT) or prospective cohort studies with reference groups. Observation time ≥ 1 year, Attrition $\leq 30\%$ of included individuals |
| Intervention | Indirect pulp capping, direct pulp capping, partial pulpotomy, pulpotomy and pulpectomy. Pulp capping using various wound dressings. Pulp exposure after stepwise and immediate complete caries excavation. |
| Control | RCT, quasi-RCT, (CCT) or prospective cohort with reference group. Acceptable reference groups are groups within the cohort, e.g. age, size of pulp exposure, degree of root closure. |
| Outcome | Survival of the pulp, verified by absence of symptoms, sensibility testing, radiographic examination or closure of the roots in young teeth. With reference to studies on pulpectomy: the minimum allowable unit for effect measure is the individual tooth. |

Table 2 – Exclusion criteria.

| | |
|--------------|--|
| Population | Animal studies. Human experimental studies employing teeth with healthy pulps. Retrospective studies, observational studies (cohorts without comparison groups). Studies with undefined populations or small samples. |
| Intervention | Studies with traumatic lesions, pulpotomy in deciduous teeth, pulp dressings which devitalize pulpal tissue, apexification (closure of the root). |
| Control | Retrospective studies, prospective observational studies without reference groups. |
| Outcome | Studies with undefined outcome measures. |

Table 3 – Criteria of high, moderate and low quality study.

| | |
|---------------------------------|---|
| High: small risk of bias | RCT with adequate (generalizable) patient spectrum, consecutive inclusion, number of eligible patients reported, adequate randomization method, power calculation, one tooth/patient; outcome measures defined and validated with clinical and radiological data with at least two blinded investigators to outcome, reliability test reported; follow-up time: ≥ 2 years for primary teeth; ≥ 5 years for permanent teeth; attrition analyzed if $< 30\%$; precision of results reported; data presented in four-fold tables, adequate data analysis. |
| Moderate: moderate risk of bias | RCT or CCT (cohort study with comparator group) not fulfilling all requirements for high quality but adequate patient spectrum; outcome measures defined and validated against clinical and radiographic data at least one blinded investigator to outcome; follow-up time: ≥ 1 year for primary and permanent teeth; attrition analyzed if $> 30\%$; adequate data analysis; if CCT: important (known) confounders at baseline controlled for. |
| Low | Studies not fulfilling criteria for moderate quality, e.g. high risk of bias, retrospective study. |

two assessors then examined independently the full-text versions. In order to determine whether a study warranted inclusion in the third phase of the review process, predetermined inclusion and exclusion criteria were applied. The reasons for exclusion of a study were noted. Studies judged by at least one of the assessors to fulfil, or possibly fulfil the inclusion criteria were selected for inclusion in the final review.

The review evaluated the relevance and the methodological quality such as study design, internal validity (reasonable guarantee against systematic errors), analysis of the results, statistical power and generalizability. In order to ensure uniform, transparent and reproducible assessment

with limited subjectivity, appraisal sheets were used, specifically structured for various study designs and research question. After appraisal, each study was rated for methodological quality (high, moderate, or low; Table 3). When there was lack of consensus about the quality of a study, the articles were appraised by the entire project group. In cases where the appraisal concerned a paper in which a member of the project group was an author, or had any other kind of association with the content of the study, the entire expert committee participated in the final evaluation. Finally, important facts from the included studies were summarized and tabulated.

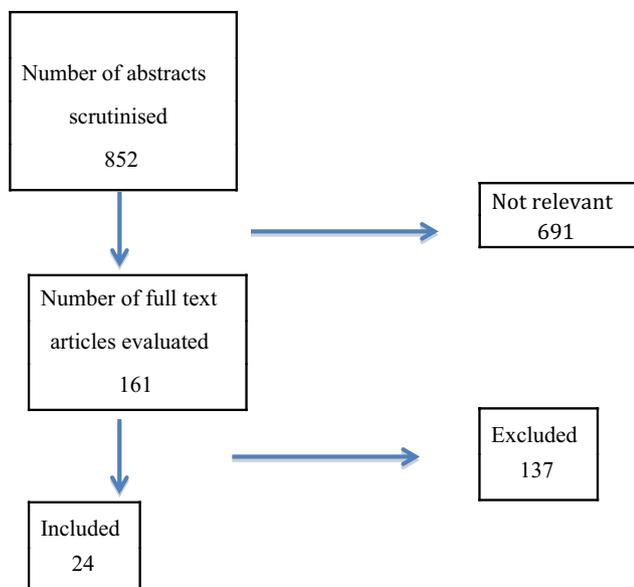


Fig. 1 – Flow diagram of literature search.

The result of treatment (the effect measure) was ascertained by determining that healing of either pulp or periapical tissue had occurred. For stepwise excavation and pulp capping or partial pulpotomy, these criteria were applied:

- asymptomatic tooth;
- positive response to sensitivity testing;
- radiographically normal periapical conditions; and
- continued root development in immature teeth.

Criteria for lack of healing included:

- pain and tenderness in the tooth and
- necrotic pulp as indicated by clinical and radiographic observations.

For teeth treated by pulpotomy or pulpectomy, the outcome was evaluated primarily on the basis of radiographic examination. Subjective symptoms were noted in addition, as well as other clinical findings, which indicated the development of a root canal infection.

Selection of studies

The literature search yielded 852 abstracts, of which 691 were considered irrelevant. In all the 161 full-text articles were assessed according to the predetermined criteria for inclusion/exclusion described above. Articles, which met the inclusion criteria, were scrutinized and assessed with the aid of the appraisal form and rated. Twenty-four studies were finally included (Fig. 1). Yet only those, which were rated, at least of moderate quality, were used for assessment of the overall quality of evidence (see below). With the main reason for exclusion the excluded studies were listed in an Appendix (can be requested from SBU, E-mail address: <http://www.sbu.se>). The same apply to included studies given a rate of low quality.

Rating quality of evidence

The quality of evidence of the accuracy of each methodological procedure was rated in four levels according to GRADE [11,12]:

- High (++++): based on high- or moderate-quality studies containing no factors that weaken the overall judgement.
- Moderate (+++o): based on high- or moderate-quality studies containing isolated factors that weaken the overall judgement.
- Limited (++oo): based on high- or moderate-quality studies containing factors that weaken the overall judgement.
- Insufficient (+ooo): the evidence base is insufficient when scientific evidence is lacking, the quality of available studies is low or studies of similar quality are contradictory.

GRADE amounts to asking how much confidence one can have in a particular estimate of effect. Is it built on solid ground, or is it likely that new research findings will change the evidence in the foreseeable future? The rating starts at high, but confidence in the evidence may be lowered for several reasons, including limitations in study design and/or quality, inconsistency or indirectness of results, imprecision of estimates and probability of publication bias. Any disagreements about inclusion/exclusion criteria, rating quality of individual studies or quality of evidence of test methods were solved by consensus.

Results

Exposure and healing of pulps on various caries excavation procedures

Four randomized controlled studies of moderate quality found that the risk of pulp exposure increased with immediate complete excavation of caries compared with stepwise excavation (relative risk=2.2 (95% CI=1.6;3.0)) or indirect pulp capping [5,13–15]. A very recent multi-centre RCT of moderate quality [4] observed better success rate for indirect pulp capping than stepwise excavation after an observation period of 3 years, 91% versus 69%. This report included 22 operators and a total of 299 teeth. Selection was based on caries to or deeper than half the distance to the pulp. A single centre RCT of 94 primary and 60 permanent mandibular molars of 4–15 year old individuals observed no difference in pulpal exposure between indirect pulp capping and stepwise excavation, while more pulpal exposures occurred after direct caries excavation [5]. Pulpal healing rate in the study varied from 95% (direct caries excavation) to 100% (indirect pulp capping). Table 4 gives details on the studies evaluated moderate quality for vital pulp treatment.

Direct pulp capping

Two randomized controlled studies [13,16] and a prospective cohort study [17], all of moderate quality, investigated healing of pulps in teeth with either asymptomatic or symptomatic

Table 4 – Study details of vital pulp treatment of deep caries lesions. Effect (pulp exposure and/or healing) of one-visit treatment (incomplete or complete excavation) or two visit treatment (stepwise excavation). IPC= 1-visit indirect pulp capping leaving caries behind permanently, SWE= 2-visit stepwise excavation, DCE = 1-visit direct complete excavation, NS = not statistically significant, CI = confidence interval.

| First author, Year, Country, Reference | Study design Setting Sample size Tooth types | Inclusion criteria | Intervention Outcomes Blinding Follow-up Drop-out rate | Main findings | Study quality Comments |
|--|---|--|---|---|--|
| Björndal, 2010, Denmark [13] | RCT, multi-centre. Operators: not reported. Sample size: 292. Incisors, premolars and molars. | Age: > 18 years, lesion depth: radiographically, $\geq 3/4$ of the dentin, mild to moderate pre-treatment pain accepted. | SWE or DCE. Outcome 1: pulp exposure/ no pulp exposure. Outcome 2: healing/ no healing. Blinding: observers of radiographs blinded to treatment. Follow-up: 1 year. Drop-out rate: 7%. | Pulp exposure: SWE: 25/ 143=17.5%; DCE: 43/ 149=28.9%; difference: 11.4% (CI: 1.2; 21.3). Healing: SWE: 106/143=74.1%; DCE: 93/149=62.4%; difference: 11.7%; ($p < .04$). Pre-treatment pain: less healing. | Moderate. Short follow-up time. |
| Leksell, 1996, Sweden [14] | RCT, single-centre. Operators: $n=6$. Sample size: 134 teeth/116 subjects. Posterior permanent teeth. | Age: 6–16 years (mean=10.2), lesion depth: pulp exposure expected with DCE. Provoked or transient pain before treatment accepted ($n=14$). | SWE or DCE. Outcome 1: pulp exposure/ no pulp exposure. Outcome 2: healing/ no healing. Blinding: observers of radiographs blinded to treatment. Follow-up: > 1 year (mean 3.6 years). Drop-out rate: 5–15%. | Pulp exposure: SWE: 10/ 57=17.5%; DCE: 28/70=40%. *Relative risk: DCE/SWE=2.3 (CI: 1.65; 2.91). Healing: SWE, unexposed pulps: 40/40, exposed pulps: not reported. DCE: not reported. | Moderate. Randomization procedure not reported. |
| Magnusson, 1977, Sweden [15] | RCT, single-centre. Operators: not reported. Sample size: 55 teeth/55 children. Primary molars. | Age: 5–10 years. Lesion depth: Supposedly thin layer of softened carious dentin remaining on the pulpal floor. Transient pain before treatment accepted. | SWE or DCE. Outcome: pulp exposure/ no pulp exposure. | Pulp exposure: SWE: 8/ 55=15%; DCE: 29/55=53%. *Relative risk: DCE/SWE=3.6 (CI: 2.9; 4.3). | Moderate. Quasi-randomized. No follow-up of the two interventions. |

Table 4 (continued)

| First author, Year, Country, Reference | Study design | Inclusion criteria | Intervention | Main findings | Study quality |
|--|--|--|---|---|--|
| | Setting | | Outcomes | *Calculations not reported, by the author(s). CI=95% | Comments |
| | Sample size Tooth types | | Blinding Follow-up Drop-out rate | | |
| Maltz, 2012, Brazil [4] | RCT, multi-centre. Operators: n=22. Sample size: 299 teeth/233 subjects. Permanent molars: 62% 1st, 33% 2nd, 5% 3rd molars. | Age: 6-53 years (median 14 years). Lesion depth: radiographically $\geq 1/2$ of dentin thickness. Absence of clinical/radiographic symptoms. | Drop-out rate: not reported. IPC, 1visit, SWE (2-visit). Outcome: healing/no healing. Follow-up: 3 years. Blinding: evaluator of results blinded to treatment not reported. Drop-out rate: 29%. | Healing: IPC: 102/112=91%; SWE: 70/101=69%; Difference stat sign ($p=.004$). SWE completing treatment: 74/84=88%. SWE not completing treatment: 2/17=13%. One surface restorations higher success rate (OR=5.2). | Moderate. Subjects in SWE group not completing treatment (2nd visit) included in the main analysis. |
| Orhan, 2010, Turkey [5] | RCT, single-centre. Operators: n=1. Consecutive enrolment not reported. Sample size: 154 teeth/123 subjects. 94 primary 2nd molars, 60 permanent mandibular 1st molars. | Age: 4-15 years. Lesion depth: radiographically $\geq 3/4$ of dentin thickness. Absence of clinical/radiographic symptoms. | IPC (1visit), SWE (2 visit);DCE (1visit). Outcome1: pulp exposure/ no pulp exposure. Outcome 2: healing/no healing. Follow-up: 1 year. Blinding: 2 observers of radiographs blinded to treatment. Drop-out rate: not reported. | Pulp exposure: IPC: 3/50=6%; SWE: 4/49=8%; DCE: 12/55=22%. *Relative risk: DCE/SWE=2.7 (CI: .9:7.7); IPC vs. SWE: NS; IPC vs. DCE: $p < .02$; IPC+SWE and DCE: $p=.008$. Healing: IPC: 100%; SWE: 98%; DCE: 95%. | Moderate. Short follow-up time. Randomization procedure suboptimal. |

pulps. In one of the studies, circa a third of the teeth were extracted for histological examination [17]. Two of the studies reported a lower frequency of successful pulp capping in permanent teeth with clinical and/or radiographic signs of pulpitis at the time of treatment compared with teeth without such signs [16,17]. Most of the patients with symptoms had persistent toothache. For permanent teeth without preoperative symptoms of pulpitis, the clinically assessed healing rate was around 80% and for symptomatic teeth, around 60% (relative risk=2.07). In one of the studies the failure rate for pulp capping in the group with preoperative symptoms of pulpitis was in fact greater than that reported in Table 2 because 17.5% (24/137) of the teeth were assessed as failures only 3 days posttreatment on the grounds of persistent toothache [16]. Because of subsequent loss to follow-up, these teeth were not included in the analysis. The difference in healing rate between teeth with and without preoperative symptoms was thus greater than reported in the study.

Another randomized controlled study compared the outcomes of pulp capping and partial pulpotomy in adults after an observation period of 1 year. The healing rates were the same for both treatment approaches and much lower than the one reported in the two studies described above (33%) [13]. Only teeth with very deep carious lesions were included in the study. The study also found a higher risk of failure in cases of preoperative toothache. The number of patients with and without toothache was not reported.

Partial pulpotomy

A randomized controlled study of moderate quality [18] and a cohort study of low quality [19] reported healing rates of 91–94% for young permanent teeth without preoperative signs or symptoms. The follow-up period in both studies was 2 years. No studies investigating long-term healing frequency were identified. The previously cited study [13] reported much lower (33%) pulpal survival after 1 year of follow-up. Table 5 presents details on the studies assessing moderate quality for pulp capping and partial pulpotomy.

Pulpotomy

Few studies were carried out on pulpotomy. Very recently, March 2013, a 12-month prospective clinical follow-up examination of moderate quality was published on molars showing a high outcome rate and similar to pulpectomy [33]. It is a multicentre study comprising subjects recruited in 23 health care centres of five Medical Universities in Iran. Treatments were conducted in 407 patients and only 16% were lost to follow-up. While being a study on general dentists, it failed to indicate procedures for pain assessment, patients age distribution in the two study arms, and the quality of the root fillings. Blinding of assessors to treatment outcome was not stated.

Wound dressings

The effect of different wound dressings for treatment of exposed pulps was compared in six randomized controlled studies [16,18,20–23]. One study of moderate quality

investigated the effect of Ledermix, an anti-inflammatory non-steroidal compound, calcium hydroxide and zinc oxide eugenol in direct pulp capping [16]. After an observation period of 2 years, there were no significant differences between the four dressings. Two studies of moderate quality compared calcium hydroxide paste with “mineral trioxide aggregate” (MTA) as dressings after direct pulp capping and partial pulpotomy, respectively [18,22]. After observation periods of 2–3 years, no difference was disclosed with respect to healing. Thus, there is limited scientific evidence that MTA has equal effect as calcium hydroxide paste. Two studies of low quality compared different calcium hydroxide containing compounds for indirect pulp capping and found no differences after a 1-year observation period [21,23]. A randomized controlled study of low quality compared adhesive resin with calcium hydroxide paste as a dressing for indirect pulp capping in deciduous teeth with deep carious lesions [20]. The observation period was 1.5 years. The results disclosed no differences between the materials.

Pulpectomy

A randomized controlled study of moderate quality compared the outcome of pulpectomy in one or two treatment sessions (calcium hydroxide was used as a root canal dressing between the appointments) [24]. A majority of the teeth in the study were affected by caries and had symptoms because of pulpitis. The healing rate was 93% and similar in both treatment groups with a follow-up time up to 3 years. A single dentist specialized in endodontics carried out the treatments and this limits the extrapolation of the results.

A controlled clinical study of low quality found that at 1 year follow-up, teeth with positive bacterial samples at the time of root filling had a poorer, statistically non-significant treatment outcome than teeth with negative bacterial samples [25]. After an observation period of 3.5–4 years, it was noted that the outcome for teeth with positive bacterial samples was significantly lower than that for teeth with negative samples. Dental students under supervision carried out the treatments in this study. Significantly more treatment failures were noted after 3.5–4 years than after 1 year of observation.

Comparison of methods

Except for the recent study on pulpotomy from Iran it was not possible to identify randomized or non-randomized controlled studies of at least moderate quality, comparing different methods aimed at preserving the vitality and functional capacity of the whole or part of the pulp. This means that there is room for well-planned, well-conducted studies comparing the outcomes of indirect pulp capping, direct pulp capping, partial pulpotomy and pulpotomy. There is also need for more studies comparing these methods with pulpectomy.

Comparison of immature and mature permanent teeth and between tooth types

One study of moderate quality observed no statistically significant difference in healing rate after direct pulp capping

Table 5 – Study details of vital pulp treatment of deep caries lesions. Healing after pulp capping and partial pulpotomy.

| First author, year, Country, Reference | Study design | Inclusion criteria | Intervention (I) | Main findings | Study quality/comments |
|--|--|--|--|---|--|
| | Setting | | Outcomes | *Calculations not reported by the author. CI=95% | |
| | Sample size | | Blinding | | |
| | Tooth types | | Follow-up | | |
| | | | Drop-out rate | | |
| Björndal, 2010, Denmark [13] | Multicentre (n=6) RCT. Consecutive enrolment. Operators: n not reported. | Age: ≥ 18 years (mean=30 years). Depth of primary lesion: radiographically ≥ 3/4 of the dentin; pulps exposed after stepwise (n=25) or direct complete excavation (n=43). Mild to moderate pre-treatment pain was accepted. | I1. Direct pulp capping; I2. Partial pulpotomy. Outcome: healing/no healing. | I1: 7/22=31.8%; I2: 10/29=34.5%; Difference NS. Presence of pre-treatment pain significantly associated with treatment failure. | Moderate Short follow-up time. Small sample. |
| | Sample size: 51 teeth/51 subjects. | | Blinding: 2 observers of radiographs blinded to treatment. | | |
| | Premolars and molars. | | Follow-up: 1 year. Drop-out rate: 12%. Pre-operative lesion depth: Repeated measurements by one observer. | | |
| Nyborg, 1958, Sweden [17] | Single-centre cohort study with two comparison groups. Consecutive enrolment. Operators: n=1. | Age: < 15 years (n=124), adults (n=101). Deep caries lesions. | I: Direct pulp capping; I1: no sign of pulpitis; I2: signs of pulpitis. Outcome: healing/no healing: (a) clinical and (b) histological. | Clinical vs. histologic findings: difference NS. Healing clinical/radiographic: I1: 106/124=86%; I2 (prolonged pain, pain at night): 9/20=45%. *Relative risk: I2/I1=3.79 (CI: 3.21; 4.37). | Moderate Small sample in one subgroup Unclear exclusion criteria for I2. |
| | Sample size: 225. | | Blinding: clinical/radiographical: yes. Histological: not reported. | | |
| | Permanent teeth: incisors (n=48), premolars (n=61), molars (n=116). Clinical study (n=144). | | Follow-up: (a): > 3 years: 82%, (b): > 2 years: 72%. | Healing histologic: I1: 55/69=80%; I2: 0/5=none (uncertain assessment, n=1). | Limited external validity (one operator). |
| | Clinical and histologic study (n=81). | | Drop-out rates: (a) 4% and (b) 6%. | | |
| Shovelton, 1971, England [16] | Multi-centre (n=8) RCT. | Age: 15–44 years. Premolars and molars with exposed vital pulps due to caries. | I: Direct pulp capping with different dressing materials. | Healing: no difference between dressing materials. | Moderate |
| | Operators: n not stated. | | I1. No preoperative pain. One step procedure. | 1 Year follow-up. I1: 164/200=82%; I2: 48/67=72%. | Possible confounding (age, type of pulp exposure: caries or accidental). |

| | | | | | |
|-----------------------------|---|--|---|--|---|
| | Sample size: 412 subjects/412 teeth. | | I2. Preoperative pain. Two step procedure. Teeth with unsuccessful temporary treatment (no pain relief) considered unsuccessful. Outcome: healing/no healing. | *Relative risk: I2/I1: 1.58 (CI: 1.09; 2.06). | High drop-out rate at 2 year- follow-up. |
| | Premolars and molars. | | Blinding: independent, blinded outcome examiner. Follow-up: 1 and 2 years. Drop-out rates:1 year: 31%, 2 years: 47%. | 2 Year follow-up. I1: 115/154=75%; I2:33/51=65%. | |
| Qudeimat, 2007, Jordan [18] | Single-centre RCT. | Age: 6.8–13.3 years (mean 10.3 years). | Partial pulpotomy. I1: capping with MTA; I2: capping with calcium hydroxide. Outcome: Healing/no healing. | Healing: No difference between dressing materials. | Moderate |
| | Operator: n=1. | Carious pulp exposures with no history of pain. | Blinding: independent outcome examiners. Follow-up: 25–46 months (mean 35 months). Drop-out rate: 19–21%. | I1: 26/28=93%. I2: 21/23=91%. | Limited external validity (one operator). |
| | Sample size: 43 subjects/63 permanent 1st molars. | | | | |
| Tuna, 2008, Turkey [22] | RCT (quasi-randomised; split mouth model), consecutive enrolment. | Age: 5–8 years. | Direct pulp capping. I1: capping with MTA; I2: capping with calcium hydroxide. Outcome: Healing/no healing. | Healing: no difference between dressing materials. | Moderate |
| | Operators: not reported. | Pulp exposure from deep dentin caries, exposure less than 1 mm, red colour, haemostasis evident in 2–3 min, no other clinical or radiographic pathology (no spontaneous pain). | Blinding: outcome examiners blinded to materials. Follow-up: 2 years. Drop-out rate: 8/50=16%. | I1: 22/22. I2: 20/20. | Randomization procedure not described. Power analysis unclear. |
| | Sample size: 50. | | | | External validity unclear. |
| | Primary molars. | | | | |

in permanent teeth of young (<15 years) and older individuals (≥ 15 years) [17]. Nor did the study find any statistically significant difference in healing rate with respect to tooth type: molars versus premolars versus incisors. There were however, numerical differences and incisors had the highest healing rate and premolars the lowest. The study of Björndal et al. [13] noted that teeth with unexposed pulps after stepwise excavation had a greater healing rate in individuals <50 years than in those >50 years of age [13]. Statistically the difference was of borderline significance. Because of the insufficient scientific support, it is not possible to conclude with respect to the influence of patient age or type of tooth on the outcome of direct pulp capping. One study of low quality showed no differences in healing rates between deciduous and permanent teeth following direct or indirect pulp capping [23].

Systematic reviews

A systematic review of low quality compared the effect of various wound dressings [26]. The authors' concluded that the results did not support proposals to change currently accepted practice. Another systematic review [27] had included four studies, two of which investigated the survival of restorations after complete or incomplete removal of dentinal caries [28,29]. These studies did not specifically investigate teeth with deep carious lesions and did not meet our inclusion criteria. The other two studies were included and are tabulated in the report [14,15].

Cost-analysis

A modelling study of moderate quality investigated the costs and benefits of direct pulp capping compared with pulpectomy [30]. With the support of the decision analysis, the authors concluded that if the healing rate for pulp capping is greater than 56%, then this and not pulpectomy should be chosen. The analysis considered only direct costs for the procedures. The long-term effects of the treatment (e.g. risk of toothache) and the patients' preferences were not assessed.

Discussion

The result of this systematic review shows that there are substantial gaps in our knowledge base with respect to treatment of the vital pulp exposed to deep caries. Hence, the report is unable to offer a clear answer to the question of whether indirect pulp capping, stepwise excavation, direct pulp capping/partial pulpotomy, pulpotomy or pulpectomy is the most effective treatment for this kind of cases. Indirect pulp capping and stepwise excavation certainly lead to fewer pulp exposures than direct complete caries excavation. Whether this results in a higher survival rate for the pulp over time has not been thoroughly investigated.

The studies reporting the outcomes of direct pulp capping upon deep caries in general have short follow-up times. While retrospective studies have indicated a poorer outcome over time [31], the long-term survival of the pulp is not well confirmed. It is furthermore not well known whether pulp

capping or pulpectomy offers a greater potential to attain non-infectious conditions and thus the health of the periapical tissues and asymptomatic teeth long-term. There are almost no studies at all of health economic aspects of different treatment options. Such studies should consider both patient satisfaction and direct and indirect costs.

The presence of preoperative pain (toothache), particularly over a longer period and if it has caused sleep disturbance, appears negative for the outcome of pulp capping. Yet, pain is difficult to properly assess. The experience is subjective and is modified by both physical and psychological factors. Thus, measurement of pain can easily be erroneous. The three studies, which evaluated the result of pulp capping in relation to preoperative toothache, have differing and in part imprecise definitions of toothache, which makes it difficult to compare the results. It has been proposed that dichotomizing toothache/no toothache is the most relevant and this was the basis for the current report [32]. Data shows that the healing rate after pulp capping is lower in cases of preoperative toothache. The evidence is limited and better-designed studies evaluating the importance of preoperative toothache are required. An important further question is how data such as the patient's age, tooth type, a combination of preoperative symptoms and clinical observations e.g. presence, persistence and character of toothache, the extent and depth of the carious lesion, the location of the pulp exposure, its size and the tendency of the pulp to bleed can be applied to make a well-informed choice between pulp preservation procedure and pulpectomy.

If the pulp tissue is directly exposed, some type of wound dressing is usually applied. Even restorative materials (e.g. resin composite) have been used to cover the wound. Over the years calcium hydroxide has been the most commonly employed wound dressing. Despite its high pH, it creates conditions conducive to healing of the pulp tissue. Other wound dressings contain steroids, with or without antibiotics, but were not accounted as these agents are not routinely used in Sweden. In recent years promising results have been reported for "mineral trioxide aggregate", MTA. Studies comparing different types of dressings for the exposed pulp (calcium hydroxide paste, cement containing calcium hydroxide, MTA, Ledermix and zinc oxide eugenol), disclose no clear difference in treatment outcome. MTA and calcium hydroxide paste were comparable in two studies [18,22]. Yet patients in these studies were of very young ages. Our review found no support for other materials.

The outcome of treatment of a deep caries lesion, with or without pulpal exposure, depends largely on how extensively the pulp is infected at the time of treatment. The outcome may also depend on the age of the patient, the treatment approach (indirect pulp capping, direct pulp capping, etc) and the choice of material applied to the exposed pulp tissue. The capacity of the restorative material to prevent leakage of bacteria is yet another important factor.

The primary aims of pulpectomy are to prevent infection of the pulp chamber, to maintain the health of the periapical tissues and to ensure asymptomatic conditions. In order to achieve these results, proper asepsis during treatment, effective removal of the pulp tissue and dense fill of the instrumented root canal are regarded critical measures in order to

prevent the development of root canal infection. Complicated root canal anatomy and the skill of the operator may also influence the outcome. The impact of these and other treatment variables on the outcome including the length of the follow-up period has not been satisfactorily explained and were not possible to investigate in this systematic review.

Calcium hydroxide has also been considered to provide a beneficial treatment effect after pulpectomy. The material is then used as an intermediate dressing in the instrumented canal between appointments. Whether this measure improves the treatment result is still the subject of debate.

On the basis of this analysis the following conclusions on the evidence-graded results can be drawn:

- Limited scientific support exists for the claim that pulpal exposure occurs twice as frequently during direct complete caries excavation as in stepwise excavation (⊕⊕□□).
- Insufficient scientific basis endures to allow an evaluation of whether there are differences in pulpal survival rates following immediate complete caries excavation and stepwise excavation (⊕□□□).
- The scientific basis is contradictory with respect to healing following direct pulp capping when the pulp is exposed during excavation of deep caries. In two studies, the short-term (1-3 year) healing rate was 80-85% in asymptomatic teeth. Another study on adults with very deep carious lesions, including patients with preoperative toothache, reported a much lower healing rate after 1 year (33%) (⊕□□□).
- Limited scientific support exists for preoperative toothache to be associated with increased risk of failure after direct pulp capping (⊕⊕□□).
- Insufficient scientific basis endures to allow an evaluation of the effect of indirect pulp capping, i.e. when the innermost layer of carious dentine is permanently left in situ (⊕□□□).
- There is no scientific basis for assessment of whether indirect pulp capping, stepwise excavation, direct pulp capping, partial pulpotomy, or pulpotomy offers the best potential for maintaining the pulp in a vital and asymptomatic condition.
- Limited scientific evidence exists that there is no difference in treatment effect between "mineral trioxide aggregate" (MTA) and calcium hydroxide as wound dressings on an exposed vital pulp (⊕⊕□□).
- There is insufficient scientific evidence to determine the influence of age and type of tooth on survival of the pulp following direct pulp capping (⊕□□□).
- There is insufficient scientific basis on which to assess whether it is more advantageous to preserve all or some of the pulp in teeth with deep caries than to undertake a pulpectomy and root filling (⊕□□□).
- There is a no scientific basis on which to assess the treatment outcome after pulpectomy and root filling.
- There is insufficient scientific evidence to determine whether the number of treatment sessions is of importance for the outcome of pulpectomy and root filling (⊕□□□).
- There is no scientific basis on which to assess which other factors might be of importance for the treatment outcome of pulpectomy and root filling.
- Insufficient evidence exists for the cost effectiveness and cost benefit of the various procedures (⊕□□□).

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Review

What and where are the stem cells for Dentistry?

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ABSTRACT

Disinfection of root canals followed by the replacement of the infected or inflamed pulp tissues by inert materials is the foundation for treating irreversible damaged dental pulps. The management of pathological conditions of the periodontium is mainly based solely upon infection control via the reestablishment of oral hygiene, scaling and root planing to control inflammation which stops progressive bone loss. As one may see, the clinical management of endodontic and periodontal diseases has not changed drastically despite the development of new materials, techniques and medicaments. Tissue engineering is a multi-disciplinary field focused on the development of materials, techniques and strategies to improve or replace damaged or lost biological functions and tissues. As the tissue engineering field progresses, “scaffolds”, “suggest pathways” and “stem cells” abandoned their role as technical words exclusively used by scientists and slowly assume a part in the language of students, educators, clinicians and patients. However the unfamiliarity with some of the concepts can lead to misinterpretations of the current status and overexcitement about future applications of stem cells for dental-related tissue regeneration. This paper will present a panorama and the future challenges on the path to use of stem cells for endodontic and periodontal tissue regeneration.

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Introduction

Stem cells are clonogenic cells capable of self-renewal and are classified according to their potency, that is, the range of cell types they can differentiate into. This is directly related

to the stages of cell division and differentiation of the human embryo during different stages of development which begin with a zygote, a totipotent cell that divides into identical totipotent cells in the first hours from the fertilization of an egg by a sperm. Totipotent stem cells can form embryonic

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and extra-embryonic tissues and have the greatest differentiation potential. In fact, they can differentiate into each of the more than 200 cell types of the adult body [1].

As development advances, the totipotent cells undergo differentiation and segregation into more limited cell lineages [1]. After reaching a 16-cell stage, they differentiate into cells that will finally convert to either the trophoblasts (first embryonic epithelium) or embryoblast. The latter is the blastocyst's inner cell mass that contains pluripotent embryonic stem cells (ESC) that can differentiate into any of the three germ layers (endoderm, mesoderm or ectoderm). As the ESCs have such outstanding differentiation potential, their clinical problems may be fraught with immunological rejection and ethical concerns. In 2006, a groundbreaking approach allowed researchers to induce pluripotency in somatic cells by introducing four transcription factors (OCT4 and SOX2 with the combination of either KLF4 and MYC or NANOG and LIN 28) into dermal fibroblast. This discovery has shed light on the possibility of obtaining autologous pluripotent embryonic-like stem cells without the need of dealing with nuclear transfer and embryos, the so-called induced pluripotent stem cells (iPSC) [2].

Additionally, multipotent or adult stem cells are undifferentiated cells that provide some or the entire major specialized cell types to allow the repair and maintenance of the tissue where they reside. Multipotent stem cells are able to cross lineage boundaries and differentiate into multiple, but limited number of cell types. For instance, a multipotent blood stem cell is a hematopoietic cell that can differentiate into several types of blood cell types (such as neutrophils and lymphocytes) but cannot differentiate into brain cells, bone cells or other non-blood cell types. In the bottom of the differentiation hierarchy are the oligopotent and unipotent stem cells, that can differentiate into a few cell types or only reproduce their own phenotype, respectively [1].

The oral cavity harbors various types of multipotent stem cells such as PDLSC (periodontal ligament stem cell) [3], SCAP (stem cells from apical papilla) [4] and dental follicle stem cells [5,6] (Table 1). Considering oral mucosa and gingiva, recognized reservoirs for mesenchymal stem cells [7-9]. Although bone marrow stem cells are well-established and are considered the gold standard for research targeting stem cells therapies, the oral cavity can provide stem cells with simple and less invasive procedures (especially compared to

bone marrow aspiration), under local anesthesia and without esthetic damage. Additionally, dental pulp stem cells (DPSC) and stem cells from human exfoliated deciduous teeth (SHED) can be found in the dental pulp of permanent and deciduous teeth, respectively [10,11]. As the latter are retrievable from naturally exfoliated teeth, which are one of the only disposable post-natal human tissues, the interest towards SHED has increased [12]. In fact, primary teeth offer a second chance to those parents who have not opted to save the umbilical cord for possible future needs. Due to its ability to cross lineage boundaries, stem cells from dental pulp are also being investigated aiming at cardiac repair [13] and the regeneration of the central nervous system [14].

Shinya Yamanaka has been recently awarded The Nobel Prize in Physiology or Medicine 2012 for generating iPSC in 2006 [2]. The recent advances in iPSC technology for dental applications are in early stages of developments [15-17]. Although iPSC can be generated without using virus, the protocols are costly and technically challenging. In fact, the processes to generate iPSC using small molecules, PiggyBac transposons, minicircle systems or episomal systems are still under development and optimization and demand high-end technology. Thus, creating conditions to use iPSC for future dental applications is far from and the studies published so far are mostly exploratory [17,18].

Stem cells for periodontal and dental pulp tissue engineering

The routine endodontic treatment stands for the disinfection and filling the root canal with synthetic and, most of the time, inert materials. This procedure frequently removes additional sound dentin decreasing the fracture resistance of the remaining structure [12]. Other strategies in Endodontics, such as the use of mineral trioxide aggregate (MTA) or calcium hydroxide (Ca(OH)₂) to induce the formation mineralized tissue in incomplete root apices are not clearly predictable [19].

Stem cells offer a new perspective targeting dental pulp regeneration and further development of root structure. Both SHED and DPSC can differentiate into odontoblasts *in vivo* [10]. DPSCs were capable to regenerate a dental-pulp-like complex composed of soft-fibrous tissue, mineralized matrix and

Table 1 – Stem cells from oral sources.

| Year of isolation | Population | Characteristics | Ref. |
|-------------------|---|--|------------------|
| 2000 | Dental pulp stem cells (DPSC) | Ability to regenerate a dental pulp-like complex | [11,20] |
| 2003 | Stem cells from human exfoliated deciduous teeth (SHED) | Dental pulp and dentin regeneration in full length root canals | [10,22,23,25,49] |
| 2004 | Periodontal ligament stem cells (PDLSC) | Form functional both cementum-like mineral and periodontal ligament including Sharpey's fibers | [3,32] |
| 2005 | Dental follicle stem cells | Capability to differentiate into adipogenic, chondrogenic and osteogenic lineages | [5,6,47,53,54] |
| 2006 | Stem cells from apical papilla (SCAP) | Osteo/dentinogenic and neurogenic differentiation | [4,26] |
| 2009 | Stem cells from gingiva | Capacity to differentiate into neural cells and chondrocytes and to modulate immune cells | [55,56] |

odontoblast-like layer able to deposit reparative dentin-like structure on the surface of human dentin [11,20,21]. In 2008, a proof-of-principle work showed that SHED holds the potential to regenerate the dental pulp. Human tooth slices (1 mm-thick) were seeded with biodegradable scaffolds with SHED and transplanted into immunodeficient mice for 28 days. After this period, the space once occupied by the scaffold was fully replaced for a dental pulp-like tissue with morphologic characteristics similar to a natural human dental pulp [22]. Later, using tetracycline staining and confocal microscopy, it was possible to show that SHED could differentiate into functional odontoblasts capable of generating tubular dentin [23].

Although these are exciting results, a stem cell-based approach in regenerative Endodontics needs to fulfill the requirement of regenerating dental pulp in the whole three dimensional geometry of root canals. Recently, we were able to proliferate SHED within a full length root canal and to engineer a dental pulp with soft tissue containing blood vessels and connective tissue capable of depositing mineralized tissue on dentin walls *in vivo* (Fig. 1) [24,25].

Although SHED and DPSC seem to be the natural candidate for functional dental pulp regeneration, SCAP also rises as an alternative once they have the capacity to undergo osteo/dentinogenic and neurogenic differentiation and present expression pattern of osteo/dentinogenic markers and growth factor receptors similar to those observed in DPSC [4]. Notably, it was possible to regenerate typical dentine structure *in vivo* by seeding human SCAP into a hydroxyapatite/tricalcium phosphate (HA/TCP) root-shaped constructs [26]. Moreover, this cell type was able to further induce root formation in cases of apexogenesis in infected immature tooth with periradicular periodontitis or abscess [27,28].

Other areas that may benefit from the development of stem cell therapies is Periodontology. Up to date, infection control allied to either guided bone regeneration or/and bioabsorbable matrices are the main therapies used to regenerate the periodontium tissues and although favorable

clinical outcomes have been reported, regenerating the support tissues remains challenging [29-31].

The level of expression of scleraxis (a transcription factor highly expressed in mesenchymal progenitors involved in chondrogenic and/or osteogenic differentiation) by PDLSC suggests that these cells may exhibit unique properties compared with other mesenchymal stem cells such as bone marrow stem cells (BMSC) and DPSC [32]. Transplantation of PDLSC mixed with ceramic particles into subcutaneous pockets of mice demonstrated the aptitude of these cells to form both cementum-like mineral and periodontal ligament including Sharpey's fibers [3]. One study showed that it was possible to regenerate the root and periodontal ligament-like tissue using the co-transplantation of PDLSC with SCAP into the tooth sockets of miniature pigs. The mineralized root-like structure formed was encircled with periodontal-like tissue and was capable of supporting a porcelain crown and restoring normal tooth function [26].

As one could observe, the selection of the cell type to be used as the impellent power to initiate and propagate the regeneration is of paramount importance. One example of this can be illustrated by the attempts of using dental pulp stem cells for periodontal tissue regeneration. The implantation of DPSC into periodontal defects resulted in inconsistent patterns of regeneration reported by different studies. Some have demonstrated that implantation of DPSC enhanced regeneration through the generation of well-formed vascularized bone [33,34]. Furthermore, it has been shown that mixing DPSC and Ca(OH)₂ led to more regeneration due to the increase of the proliferation and mineralization of dental pulp stem cells [33]. Contrariwise, another study that compared the regenerative potential of PDLSC, periapical follicular stem cells and DPSC, showed that the latter failed to increase the extent of regeneration in defects when compared to the control groups that did not receive any stem cells [35].

Similarly to BMSC, PDLSC were shown to be able to modulate immune responses and inflammatory reactions [36]. Using a swine model it was possible to reverse periodontitis by transplanting allogenic PDLSCs into experimental periodontal bone defects due to the low immunogenicity and immunosuppressive function of the stem cells used [37]. This was also true for humans that have periodontal intra-bony defects regenerated using autologous periodontal ligament-derived cells, including PDLSC. These studies inspire that PDLSC transplantation may be an interesting for the treatment of human periodontitis [38].

Stem cell research does not intend to extinguish safe, established and reliable treatments (e. g. titanium implants) but aims for new alternatives that may benefit patients even more. The tried and tested concept of osseointegration has certainly been one of the most important advances over the past 30 years in Dentistry and the multi-million dollar market of dental implants is inclined to keep increasing. Despite of the unequivocal qualities (such as affordable cost and design flexibility), titanium implants has one major drawback: the lack of periodontal ligament. This can compromise prosthetic rehabilitations because natural teeth and implant have different mobilities and it is impossible for the patient to balance the contact and load sensations as the implant is integrated into bone via an ankylosis type contact and is

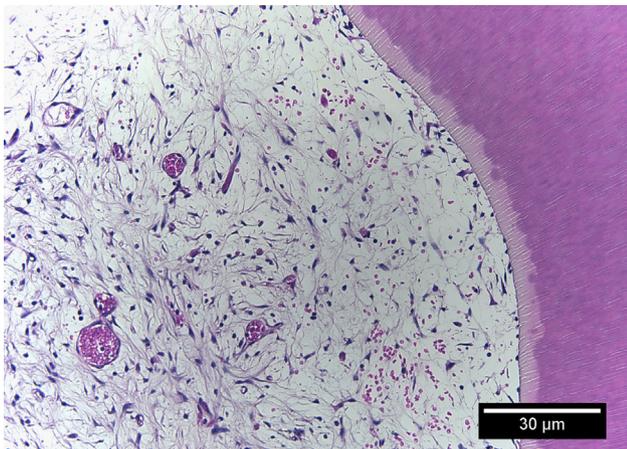


Fig. 1 – Engineering of a dental pulp-like tissue with SHED seeded in tooth slice and transplanted into the subcutaneous space of an immunodeficient mouse for 35 days. Further immunohistochemistry analysis for GFP confirmed that tissue engineered with the root canals is primarily populated with SHED [25].

without proprioceptors [39]. Furthermore, the most relevant long-term failure mode of dental implants is peri-implantitis that has an overall frequency of 8% [40]. The development of new strategies such as the bio-root may provide an alternative for titanium implants in cases where the existence of functional periodontal ligament can benefit the long-term success of prosthetic rehabilitation [3,26]. However, one of the major concerns is the costs involved in both development and implementation of these new approaches. History has shown that most of the revolutionary technologies became more affordable with popularization. The price of one gigabyte, for example, dropped from US\$ 200,000 in 1980 to US\$ 0,07 in 2013. Hopefully this can be also true for future tissue engineering approaches under development in Dentistry.

Future challenges for stem cells in Dentistry

Stem cell-based tissue engineering demands the fine orchestration of three fundamental elements: scaffold, cells, and signaling pathway [24].

The scaffold is a temporary structure used to provide a three-dimensional environment where cells can proliferate, differentiate and generate the desired tissue [41]. It needs to allow cell attachment and migration, enable the influx of oxygen to maintain the cell metabolism and preferably permit the sustained delivery of growth factors. The scaffold must present physical properties to support and protect cells from the environmental mechanical stresses. On the other hand, the material is expected to degrade once it has served its purpose of providing a template for regeneration in a rate compatible with the new tissue formation. Finally, the handling characteristics need to fulfill the clinical situation that they will be used. For Endodontics, injectable scaffolds are the logical choice once cells can be spread in the whole space available disregarding anatomical characteristics such as saliences, recesses and even curved canals [12,24]. Considering periodontal regeneration, fiber mesh layers or powder compaction may be preferred to provide a support matrix for the ingrowth of alveolar bone and periodontium, avoiding gingival epithelium growth into the defect to be regenerated [42]. In order to obtain these desired qualities, fine-tuning of physical characteristics and chemo/mechanical properties of the scaffolds to be used is critical.

Obtaining and banking stem cells is an expanding business. Laboratories worldwide offer the whole package of collecting and storing dental stem cells for a lower cost as compared to cord blood stem cells. To maintain cell viability, collection kits are prepared to keep cells alive and shelter freshly extracted teeth from temperature changes. Even with these precautions, the time passed from gathering to arrival at the processing laboratory must be shorter than 40 h [43]. Although the complete substitution of deciduous to permanent teeth lasts 7 years, providing a good window of opportunity to obtain SHED, only incisors and canines with at least one third of root left are known to contain a sufficient number of stem cells that allow separation and multiplication [43]. DPSC are obtained from permanent teeth and the possibilities to gather them are restricted to teeth assigned to

be extracted, such as third molars and pre-molars due to orthodontics reasons. Conversely, SCAP collected from just one tooth are capable of providing a large number of stem cells probably because they have high proliferative potential, reflected in high telomerase activity [44].

Cell signaling is part of a complex system of communication that rules cell activities and organizes their interactions [24]. Extracellular signal molecules can act over both short or long distances and different cell types may have various reactions to a given extracellular signal molecule. Even the level of response greatly depends on the concentration and exposure time of the cell to the stimuli [45]. One example of how complex these interactions can be illustrated when analyzing the role of bone morphogenetic proteins (BMP). BMP-2 and -7 were reported to play a role in the differentiation of PDLSC and dental follicle stem cells and in the regeneration of the periodontal attachment apparatus [46,47]. Furthermore, these BMPs are strongly involved in the odontoblastic differentiation processes [48,49]. Both BMP-2 and 7 are known to present inductive effects in reparative dentinogenesis [50,51] however it has been shown that SHED responded potently to BMP-2 and more modestly to BMP-7 while undergoing through odontoblastic differentiation. Furthermore, using neutralizing antibodies for BMP's, it was possible to show that BMP-2 signaling and not BMP-7 was actually required for odontoblastic differentiation [49]. By observing the different effects of only two members of the BMP family used for distinct purposes, it is possible to visualize that many interactions among the plethora of naturally occurring molecules in different stages of cell differentiation make the signalling system extremely complex to be fully simulated in the laboratory.

Conclusion

The development of stem cells technologies and biomaterials can change the archetype where lost or damaged tissues will not be treated using only materials tolerated by the body but congregating biomaterials and biological principles to deliver to the body its original construction. However, stem cell-based therapies are still far from leaving laboratory benches for the chairside [52]. Identifying specific growth factors and their mechanisms in the natural processes will definitely help improve our understanding of molecular triggering and regulating processes among stem cells, scaffold, bioactive molecules and host tissues is necessary to achieve proper tissue regeneration. The investments in basic research and education, allied to the rise of commercial services to isolate and to provide stem cells and development of new materials to support their growth and differentiation shed a light in the promise for using stem cells as a clinical practice in the future.

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Review

Health and safety in the dental clinic – Hygiene regulations for use of elemental mercury in the protection of rights, safety and well-being of the patients, workers and the environment

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ABSTRACT

The rules governing the use of metallic mercury, a toxic and hazardous chemical, is in most jurisdictions identical to widely accepted standards and practices for handling the same chemical in industry for the protection of humans and their work environment. There cannot be exceptions solely for the practitioner dentists and their patients. Any workplace must be safe for both workers and visitors. The latter being dental patients waiting in the dentist's work environment. We reviewed the literature for toxic health effects of elemental mercury upon humans and present information about the Minamata Convention convened by the United Nations Environment Programme. A study conducted among dentists in Singapore and their personal work environment almost 30 years ago contributed to the workplace standard for elemental mercury, which was reduced, and is still currently enforced as a global standard. We recommend that dentists, with a large alternative battery of restorative materials today, make selection of a restorative material a more seriously considered choice, and not to make use of amalgam without the proper use of personal protective equipment for themselves (members of the dental operating team) and their patients, (amalgam traps and judicious monitoring of their workplace air quality). Mercury is ubiquitous in our presence due to human activities; any reduction in the dentists' workplace contributes to a global reduction.

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1. Introduction

Mercury is ubiquitous. Mercury occurs naturally in the environment and exists in several forms [1]. Elemental mercury is used in many industrial processes and manufactured products, including but not limited to, manufacture of soaps, detergents, and fluorescent bulbs, in production of sulphuric acid, in gold mining, in batteries and so on. All forms of mercury, namely, metallic or elemental forms as used in dentistry for the manufacture of dental silver amalgam during restorative dentistry; organic forms as existing in fish, pesticides and other bonded-chemicals and inorganic mercury, at times mercuric oxide used as the red coating for traditional herbal remedies, are present in our human environment through usage. Consequently mercury is present in our human environment from manufacturing to waste disposal and finally as waste in our midst. This mercury could be in the air we breathe, in the food we consume, also in antiseptics or antifungals we come in contact with daily as hand wash, or in vaccinations as the preservative Thiomer-sal[®] [2] found in vaccines.

In the 1980s, the first author conducted a study of the neurobehavioral effects of mercury of 98 actively-practising Singapore dentist volunteers, dentists who were occupationally exposed to elemental mercury [3]. As the range of dental materials was then limited, some of these dentists used mercury and amalgam almost exclusively as the only restorative material for all posterior teeth in their practices, where aesthetics was not a prominent patient consideration. Note also that patients in those days were not as demanding with regards to anaesthetics as they might be today. Although the "controls" were also investigated, their mercury data remain unpublished as part of a doctoral thesis within the National University of Singapore Medical Library archive.

2. Human exposure to different forms of mercury

This hygiene fact from the US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry is well acknowledged

"A person can be exposed to mercury from breathing in contaminated air, from swallowing or eating contaminated water or food, or from having skin contact with mercury. Not all forms of mercury enter your body easily, even if they come in contact with it; so it is important to know which form of mercury you have been exposed to, and by which route (air, food, or skin). When you swallow small amounts of metallic mercury, virtually none (less than 0.01%) of the mercury will enter your body through the stomach or intestines, unless they are diseased. ...When you breathe in mercury vapours, (from elemental mercury), however, most (about 80%) of the mercury enters your bloodstream directly from your lungs, and then

rapidly goes to other parts of your body, including the brain and kidneys. Once in your body, metallic mercury can stay for weeks or months. When metallic mercury enters the brain, it is readily converted to an inorganic form and is "trapped" in the brain for a long time. Metallic mercury in the blood of a pregnant woman can enter her developing child [1]".

Mercury crosses the placental barrier easily to affect the developing foetus. What effects this may have is however only reflected in the case study reports. However research conducted by the author showed neurobehavioral changes in test subjects even when they were exposed to very low levels of mercury, levels below that established for applications in industry and dentistry. In that study we examined 96 24-49 year-old dentists and compared them to 56 control subjects. The results however, apply only to adults [4].

In a case report of an accident involving four adults in 1983, including a pregnant woman and her new-born infant, Lien et al. reported that although the baby was born without reportable abnormalities within 26 days of the accidental exposure to mercury vapour, the baby had blood levels of mercury that were comparable to the mother indicating direct and free transfer of the metal across the placental barrier [5]. This study adds to the evidence that breathing in mercury vapour crosses the placental barrier and mercury crosses into the foetus when mother is exposed to mercury vapour. Likewise, mercury crosses the blood brain barrier easily to affect the developing foetus. What effects this may have, have not been fully elucidated. However, since in the research conducted on adults, even very low levels of exposure, levels below that established as safe by authorities, led to neurobehavioral changes in adults, the question of how much mercury is acceptable in air in the operatory as we use amalgam is raised.

Hygiene considerations for mercury used in dentistry for the manufacturing of dental amalgam, comprising 50% metallic mercury, must have no exceptions from similar industrial applications in terms of health and safety regulations. Mercury used is identical for both dentistry and chlor-alkaline industry, or in the industry manufacturing fluorescent tubes or mercury-fumed street lighting bulbs, commonly seen along roads and highways. It is certain that mercury has extensive applications for products that result in human benefits. Along with this, humans are also exposed to the well documented toxic effects, similar as the mercury leaching from the dental amalgam fillings in our oral cavities and also a constant source of dentist's work environmental pollution wherever mercury is stored and used.

Dufault et al. [6] reported that many food products are now made using such mercury-cell chlor-alkaline industry applications. They concluded that with respect to total mercury exposure in children and sensitive population, consumption of high fructose corn syrup also had insidious mercury ranging from 0.005 to 0.670 µg mercury/g of sweetener.

They reported that average daily consumption was approximately 50 g/person in the US in 2009.

3. What are the toxic effects of elemental mercury that require attention?

The US's Centre for Disease Control, published this review in 1999 and updated that in 2006, stating

"The nervous system is very sensitive to mercury ... Permanent damage to the brain has also been shown to occur from exposure to sufficiently high levels of metallic mercury. ... Metallic mercury vapours or organic mercury may affect many different areas of the brain and their associated functions, resulting in a variety of symptoms. These include personality changes (irritability, shyness, nervousness), tremors, changes in vision (constriction (or narrowing) of the visual field), deafness, muscle incoordination, loss of sensation, and difficulties with memory [1]".

Similarly, in the same review [1] based upon occupational exposure of elemental mercury at higher concentrations, as in the chlor-alkali industry, where chlorine and alkali are products from the electrolysis of seawater using pans of elemental mercury as the electrode, the toxic effects were stated as

"Short-term exposure (hours) to high levels of metallic mercury vapour in the air can damage the lining of the mouth and irritate the lungs and airways, causing tightness of the breath, a burning sensation in the lungs, and coughing. Other effects from exposure to mercury vapour include nausea, vomiting, diarrhoea, increases in blood pressure or heart rate, skin rashes, and eye irritation. Damage to the lining of the mouth and lungs can also occur from exposure to lower levels of mercury vapour over longer periods (for example, in some occupations where workers were exposed to mercury for many years). Levels of metallic mercury in workplace air are generally much greater than the levels normally encountered by the general population. Current levels of mercury in workplace air are low, due to increased awareness of mercury's toxic effects. Because of the reduction in the allowable amount of mercury in workplace air, fewer workers are expected to have symptoms of mercury toxicity".

The confirmation of toxic effects relies upon animal studies, and not solely upon observations in humans,

"To protect the public from the harmful effects of toxic chemicals and to find ways to treat people who have been harmed, scientists use many tests. One way to see if a chemical will hurt people is to learn how the chemical is absorbed, used, and released by the body; for some chemicals, animal testing may be necessary" [7].

There is now sufficient data in global occupational safety databases to support this fact: mercury is highly toxic to humans, yet a controversy exists.

The US FDA webpage at the time of writing this article (2013) offered consumers the following hygiene advice:

"FDA has reviewed the best available scientific evidence to determine whether the low levels of mercury vapour associated

with dental amalgam fillings are a cause for concern. Based on this evidence, FDA considers dental amalgam fillings safe for adults and children ages 6 and above. The amount of mercury measured in the bodies of people with dental amalgam fillings is well below levels associated with adverse health effects. Even in adults and children ages 6 and above who have fifteen or more amalgam surfaces, mercury exposure due to dental amalgam fillings has been found to be far below the lowest levels associated with harm. Clinical studies in adults and children ages 6 and above have also found no link between dental amalgam fillings and health problems [8]".

From the above, we see that amalgam restorations are deemed to be safe, by the FDA, in children 6 years old and above. However, there have been reports that eating and chewing releases mercury from fillings [9,10].

It seems contradictory therefore that there should be regulations enacted for chronic inhalation exposure of mercury as enforced by the US Environmental Protection Agency (EPA) [11], when another US government agency the FDA deems it safe for amalgam to be used in fillings. While this political debate rages on globally, the authors here are of the view that mercury, whether used in dentistry or industry, is still mercury. Safe hygienic principles are required whenever the dentist's personal choice is to use dental amalgam as his restorative material. There cannot be any exceptions from industrial practice for the practising dentist for safe handling of mercury. A comprehensive review of the effect of mercury on humans and animals has been published in the Journal of the Federation of American Societies for Experimental Biology (FASEB) 1995 and the article is available free online at <http://www.ncbi.nlm.nih.gov/pubmed/7737458>. The effects of mercury on the immune system, kidney glomerular physiology, intestinal bacteria of both humans and animals, amongst others, are discussed.

4. Global conventions, regulations and the dental operatory environment

A vast and substantial data had been presented to any interested reader for the safe handling of this toxic chemical: elemental mercury, because use of mercury is as old as antiquity. In the following section, we elaborate the rationale for the growing database of pharmacokinetics and pharmacodynamics attributes linked to mercury's safety and along with this the regulations to ensure the safety, health, rights and well-being of all workers, including the practitioner-dentist, and their patients. The patients may be told to be exposed to a lesser degree in the toxic environment of the dentist's operatory, but the rationale of public health administration based upon the scientific principles from hygiene, is that patient has a right to know of their environment, be this work environment or just for the visit to their dentist. In addition, the patients are duly required to be fully informed under clinical practice ethics as under clinical research situations, of what may happen to mercury that was implanted as dental amalgam restorations in their dentition – permanent or deciduous.

The waiting room of the dental practitioner is required to be environmentally assayed for presence of safe levels of elemental mercury vapour on a daily or periodic basis whenever mercury is present in the clinic. We, as humans, have a right to know and to be duly informed of our environment – work or recreational or homes and places we visit from time to time, including a shopping centre. In a Singapore law, this is within the scope and ambit of our Workplace Safety and Health Act 2006, revised Chapter 354A in 2009. However, the safety of placement of mercury within the dental amalgam (device) for restorative purposes is not in this legislation, but is regulated separately as detailed above by the US FDA, as a Class II medical device.

The environment of the dental clinic in the US is governed by the Occupational Safety and Health Administration (OSHA), which published a pamphlet about standards for dealing with mercury in the work environment [12]. The Singapore legislations, as revised Chapter 354A, were enacted after much consultation with the OSHA regulations. In this aspect, one will note the emphasis on air quality and methods of mopping up mercury spills. The current OSHA permissible exposure limit (PEL) for mercury vapour is 0.1 mg/m³ of air as a ceiling limit. A worker's exposure to mercury vapour shall at no time exceed this ceiling level. From the perspective of hygiene, another index for assessing human exposure based upon personal dosimetry is preferred and more realistic, namely that of inhaled mercury toxicity. This is because the ceiling PEL may not be at all reflective of the practising dentist's real-time exposure. The preferred standard for safety is the EPA's "Reference concentration for Chronic inhalation exposure" [10] index computed by personal dosimetry breathing zone studies.

Similar in design to the first author's dosimetry study of 1992, a more recent dosimetry study of 180 dentists, Ritchie et al. [13], reported that dentists were found to have on average urinary mercury levels four times that of control subjects, dentists were significantly more likely to have suffered from kidney disorders and that dentists were advised to put greater emphasis on the safe handling of dental amalgam within their practice environment by periodic hygiene surveillance using personal dosimetry monitoring. He further commented, 122 (67.8%) of the 180 surgeries visited had environmental mercury measurements in one or more areas above the Occupational Exposure Standard (OES) set by the Health and Safety Executive of UK. In the majority of these surgeries the high levels of mercury were found at the skirting and around the base of the dental chair. In 45 surgeries (25%) the personal dosimeter measurement (i.e. in the breathing zone of dental staff) was above the OES. Note the UK's OES is the same concentration as the EPA's Reference Concentration for Chronic Inhalation Exposure (RfC), at 25 µg/m³ air for 8 h a day, 40 h per week; this occupational hygiene standard for "lowest-observed-adverse-effect level" (LOAEL: 0.025 mg/m³ air) was derived from several studies, one of which was by the first author [3]. Note that the EPA's IRIS for the RfC stated "no-observed-adverse-effect level" (NOAEL) as "None" [8]. This means that mercury is very toxic at any concentration, even at the minimum lowest as yet undetermined because of limitation of our diagnostic tools.

Mercury hygiene practice in dentistry should be similar to that in industry and should have the same regulations as

they are about prevention of the same thing – mercury poisoning and long term health effects of mercury exposure amongst workers. To this end we should all realise that the United Nations Environment programme has a Global Mercury Partnership, the aims of which include promoting the development of national inventories of mercury uses and releases; developing strategies for enhanced outreach and risk communication activities to reach at-risk populations, including sensitive populations; increasing public awareness and promotion of mercury-free products, technologies and processes, using and/or with environmentally friendly alternatives; promoting application and sharing of information on best available techniques and measures to reduce mercury emissions from point sources, among others [14]. In another United Nations convention, namely the Minamata Convention of 2013, it was specifically agreed that

"Certain kinds of non-electronic medical devices such as thermometers and blood pressure devices are also included for phase-out by 2020. Governments approved exceptions for some large measuring devices where currently there are no mercury-free alternatives. Vaccines where mercury is used as a preservative have been excluded from the treaty as have products used in religious or traditional activities. Delegates agreed to a phase-down of the use of dental fillings using mercury amalgam".

Independently, the European Union, the US and Japan have all declared bans on export of mercury since about 2008 [15].

What do all these activities mean to practising dentists in Singapore? It is quite obvious that over next decades it would be more difficult to obtain mercury and hence as a profession we need to work at being good at using alternative restorative products and remain as successful as before in restoring teeth. The United Nations Environment Programme (UNEP) has published a pamphlet on "Mercury Use in Healthcare Settings and Dentistry" and is available online as a downloadable portable document file [16].

In that document, dentists, including their clinic operatory support staff, will learn how to store mercury and how to mop up spills of mercury. It also advises removal of amalgam fillings in chunks rather than grinding it down completely, use of finer mesh to trap amalgam waste (100 rather than 40 units for sieve traps) which were endorsed for ISO 14011 Environmental Management audit procedures, namely, use amalgam traps that are certified ISO 11143. For a detailed description and management of dental amalgam wastes, some countries have legislations that waste water emitted from any dental clinic be subjected to audits for compliance, and cannot exceed 100–2000 ppm of mercury in waste water [17].

The UNEP also stated – *not to place or remove amalgam fillings in pregnant ladies*. It further stressed,

"Treat extracted teeth with amalgam fillings as amalgam waste. Waste amalgam should be kept sealed in plastic containers. Waste amalgam may be disposed by licenced waste disposal companies who will recycle mercury and other metals".

Similarly, the American Dental Association has published a 2007 pamphlet on the "Best Management Practices for Amalgam

Waste” and this is available online as a downloadable portable document file [18].

5. Safe removal of dental amalgam restorations

The measurable level of mercury in blood and plasma is correlated with the number of surfaces of fillings that are of amalgam in the oral cavity. Higher number of fillings of amalgam is correlated with a higher blood and plasma levels of mercury. Upon removal, within 3–48 h, there was a rise in the level of mercury in blood and plasma. Thereafter, there was a decline [19]. It is therefore important to follow a strict protocol to reduce patient exposure to mercury during removal. It had been recommended that hair covers, body drape, eye protection and rubber dam together with a high vacuum suction be used during amalgam removal [20]. Water coolant is important during removal as more mercury vapour is released from amalgams when the temperature increases.

It may be important for signage to be displayed so that everyone, workers and visitors are cautioned about the presence, from storing, using and disposing, of elemental mercury in the workplace, namely the dental clinic.

6. Dentist's choice in use of dental amalgam and informed consent documentation from patients as a good practice procedure

Despite the advantages that bonding seems to provide, various studies comparing longevity of fillings of amalgam and composites have shown that amalgam is the more tolerant material and despite poor technique, such as poor moisture control during placement, is as lasting as composite restorations. Roulet [21] reported that amalgam shows excellent longevity data with studies up to 20 years; the average annual failure rate was 0.3–6.9%. Posterior composites were in the same range (0.5–6.6%); however, the study times were much shorter (max. 10 years). However, it was pointed out that composite restorations took longer to place. A more recent study comparing amalgams versus composites in posterior teeth showed that amalgam restorations lasted statistically significantly longer [22]. When composites failed, they deteriorated rapidly. It is no wonder therefore that dental schools continue to teach and practitioners continue to choose the use of amalgam restorations.

Despite its advantages, informed consent is required in some jurisdictions (by regulations) during placement of dental amalgams, much similar to the clinical research environment. In Europe, the federal governments of Norway, Finland, Denmark, and Sweden have enacted legislation requiring that dental patients receive due process of documentation with regards to adequate informed consent information provided prior to their decision for receiving the type of dental restorative material that will be used and implanted. This is prior to the actual amalgam placement by the dentist. In the US, a few state governments have enacted similar informed consent legislation for dental patients receiving dental restorations. These state legislations were enacted by Maine, California,

Connecticut, and Vermont. There is a similar need for informed consent procedure for dentists who use mercury amalgam restorative material as well as technical considerations in such information during removal of dental amalgam restorations [23].

While such regulations does not apply in Singapore and we have not yet enacted these regulations, it is in the prudent opinion of the author that we take and document full informed consent for using any restorative material. Informed consent here means giving a patient adequate information concerning the materials, providing adequate opportunity for the subject to consider all options, responding to the subject's questions, ensuring that the subject has comprehended this information, obtaining the subject's voluntary agreement to choose a material and continuing to provide information as the subject or situation requires. Patients should also be informed that bleaching teeth with amalgam restorations risks increasing the release of mercury vapour from amalgams [24]. Note that composites are not free of hazards. Composites leach oestrogenic monomers into the environment in concentrations at which biologic effects have been demonstrated in in vivo experimental models [25].

The US's Centres for Disease Control and Prevention published a free booklet “NIOSH Pocket Guide to Chemical hazards” from the US' National Institute for Occupational Safety and Health (NIOSH) which is applicable to the constant surveillance of the workplace for any air-borne mercury levels for alerting the inhabitants of the clinic to dangerous levels and what preventive steps to be taken to control and avoid such hazardous situations. Note that the dental clinic could be located within a shopping centre or in the hospital with lots of humans potentially being exposed. The CDC has evidence to label elemental mercury inhalation as “Lung Damaging Agent”.

In this booklet and the Emergency Response Card [26,27] data, the dental practitioner using dental amalgam is advised to read about measurement and monitoring methods, as in personal dosimeter for the operator and the dental chairside assistants. There are now rapid reading assays to gather such data. Next, the type of personal protective equipment, ranging from respirators and facemasks with filters, some reusable and others single use types, are also listed. This Emergency Response Card contains the different types of PPE to be used for the various alert levels. It is advisable for all practising dentists using mercury as restorative material, to read and train their staff for workplace hygiene maintenance on a weekly basis.

The FDA has provided guidance that amalgams should be used in adults and children 6 years of age and above. Mercury is highly toxic. Its immediate effect from inhalation is as appropriately named “Lung Damaging Agent”. When mercury is allowed to enter the dental clinic, the chances of such exposure is ever present. The only way to prevent such occurrence is to eliminate the use of such materials for restorative dentistry applications. Amalgam has been used as fillings for about 150 years and has served dentistry well. As mercury is ubiquitous in the environment it will always be measurable in blood and urine. Though a number of patients have reported hypersensitivity to amalgams, the large majority of patients with amalgam fillings have not, albeit neurobehavioral deficits may affect those chronically exposed to mercury,

such as dentists. When choosing to use amalgams, dentists should have the safety of their team members and patients in mind and should conduct audits with respect to amalgam hygiene and make the choice to be safe.

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Case report

Double papilla repositioned flap for the treatment of isolated recession – A case report

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ABSTRACT

Root coverage is achieved by many procedures like free gingival autografts, connective tissue grafts and pedicle grafts. Several studies state that root coverage using connective tissue grafts have high success rates but have disadvantages like creation of second surgical site and post-operative color harmony is less. Although Cohen and Ross reported more than 85% success in covering denuded roots, the degree of success varies among other clinicians. The double papilla pedicle graft has limited usefulness. The double papillae pedicle graft is most appropriate in those cases where esthetics demand a close tissue color match and where the papillae are large and have shallow gingival grooves.

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Introduction

Gingival recession is defined as the apical displacement of the gingival margin in relation to the cemento-enamel junction (CEJ) [1], resulting in patient complaints such as poor esthetics, dentin hypersensitivity, and inability to perform proper oral hygiene procedures [2]. The understanding and knowledge of different conditions of denuded root surfaces is paramount for predictable root coverage [3]. Several classifications of gingival recessions were proposed, but two classifications are helpful for predictability of root coverage procedures. In 1960, Sullivan and Atkins gave a classification which was not useful to predict outcome of root coverage procedures [4]. In 1985, Miller gave a classification, taking into consideration the anticipated root coverage [5]. Various surgical procedures are indicated for treatment of gingival recessions [6].

Cohen and Ross [7] introduced the method in which bilateral interdental papillae are used as donor tissue for localized root coverage. In this technique, there is less chance of flap necrosis and suturing is easy because interdental papillae are thicker and wider than labial gingiva on single root surface.

Single surgical site, excellent post-operative color harmony, requirement of small amount of donor tissue, less damage to interdental bone is the advantages of the double papillae technique.

Although Cohen and Ross reported more than 85% success in covering denuded roots, the degree of successes varies among other clinicians [8]. The double papillae pedicle graft is most appropriate in those cases where esthetics demand a close tissue color match and where the papillae are large and have shallow gingival grooves.

Case report

A female patient aged 42 years reported to the Department of Periodontics, Manipal College of Dental Sciences, Mangalore with a chief complaint of recession of gum and mild hypersensitivity in relation to the right maxillary canine.

On extra oral examination there were no palpable lymph-nodes, face was bilaterally symmetrical and lips were competent. On intraoral examination, the tooth showed gingival marginal recession with a 4 mm loss of attachment (Fig.1)

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on the facial aspect without loss of interdental papilla. Patient had developed mild sensitivity 3 months ago because of the gingival recession. According to Miller's classification, the defect was classified as Class-I gingival recession.

Surgical procedure

A curette was used to root plane the exposed root surface. A no. 15 blade was used to make a V-shaped incision on the recipient site.

A horizontal incision was made on the mesial and distal interdental papilla coronally (Fig. 2). Two vertical incisions reaching the alveolar mucosa were made on the line angle area of the adjacent teeth. A partial-thickness pedicle flap that included sufficient interdental papilla bilaterally was prepared.

A partial-thickness flap (about 3 mm) was reflected from the crest of the osseous dehiscence area till the alveolar mucosa in the apical for easy flap migration. After removal of the marginal tissue, tetracycline was applied to the exposed root surface for about 5 min for root biomodification (Fig. 3). Interrupted and sling sutures were used to stabilize the mesial and distal papillae using a 5-0 silk suture (Fig. 4). Hemostasis was achieved by applying pressure for 5 min. A periodontal dressing was placed. Post-surgical instructions were given to the patient. The patient told to refrain from brushing the operated area and was advised to use chlorhexidine gluconate mouth wash of 0.2% twice daily for two weeks.



Fig. 1 – Pre-op photo showing 3mm recession depth.



Fig. 2 – Picture showing incisions.

Results

10 days post-surgery, the sutures were removed and the area was examined. The surgical site showed complete root coverage (Fig. 5). Oral hygiene instructions were reinforced and the patient was recalled after three months and nine months (Fig. 6). The surgical site showed complete coverage and also the donor site healed completely. The pedicle graft healed uneventfully with excellent color matching with the adjacent tissue. It showed no-signs of inflammation and was firm and attached to the root surface. The patient was admitted in the hospital for knee surgery, hence could not come for oral



Fig. 3 – Root biomodification.



Fig. 4 – Sutures placed.



Fig. 5 – 3 months post-op.



Fig. 6 – 9 months post-op.

prophylaxis for the removal of stain associated with long term use of chlorhexidine mouthrinse.

Discussion

This case report presents double papillae pedicle graft surgical technique for the treatment of isolated or single tooth marginal tissue recession. Double papillae pedicle graft has shown excellent root coverage if the indications of this technique are followed.

This technique has been used as a replacement to free gingival autografts where second surgical site is not necessary. Sometimes with free gingival autografts, blood supply and graft stability may be jeopardized unlike pedicle grafts [9].

The double papillae graft involved a V-shaped incision outlining the area of recession. This incision allows the removal of a wedge of marginal tissue. It also provides a fresh wound surface for tissue approximation. Vertical incisions were given from line angles to extend beyond the mucogingival junction. Sharp dissection was then done to reflect partial thickness flaps and was then sutured together.

Rubelman modified this technique in 1977. He advocated making the initial V incision so that one side had an external bevel and the other an internal one. The flap edges then overlap when sutured together. Rubelman began suturing at the apical aspect of the graft as did Cohen, but used a continuous locking suture rather than the interrupted ties recommended by Cohen. As a part of the continuous locking

suture, he included a sling suture, a connective tissue suture, and an oblique suture.

Although Cohen and Ross reported more than 85% success in covering denuded roots, the degree of successes varies among other clinicians.

Partial thickness double papillae pedicle graft along with connective tissue surgical technique is proposed as an alternative for better functional and aesthetic outcome [10]. However, disadvantage of this technique is again two surgical sites.

In this case, the root coverage using double papillae pedicle graft is predictable and the second surgical intervention is avoided.

Conclusion

In cases of single tooth or isolated Millers class I and II gingival recession, predictable root coverage and color matching with adjacent tissues can be obtained with the double papillae pedicle graft technique.

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Instructions to Authors

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Journal articles:

1. Chew MT, Sandham A. An assessment of orthodontic treatment using occlusal indices. *Singapore Dent J* 2001;24: 9–16.
2. Smith RN, Rawlinson A, Lath D, Elcock C, Walsh TF, Brook AH. Quantification of dental plaque on lingual tooth surfaces using image analysis: reliability and validation. *J Clin Periodontol* 2004;31:569–73.
3. Olszewski R, Reychler H. Limitations of orthognathic models surgery: theoretical and practical implications. *Rev Stomatol Chir Maxillofac* 2004;105:165–9. [In French]

Books:

1. Stevens J. *Applied Multivariate Statistics for the Social Sciences*, 3rd edition. New Jersey: Lawrence Erlbaum Associates, 1996.
2. Sapp JP, Eversole LR, Wysocki GP. Infections of Teeth and Bone. In: Sapp JP, Eversole LR, Wysocki GP. *Contemporary Oral and Maxillofacial Pathology*, 2nd edition. St Louis: Mosby, 2004:70–93.

Report:

1. Committee on Mercury Hazards in Dentistry. *Code of Practice for Dental Mercury Hygiene*. London: Department of Health and Social Security, 1979, publication no. DHSS 79-F-372.

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