A regeneration-based, nonobturation root-canal treatment for fully-mature teeth: Six years' experience with "SealBio"

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Abstract

Objectives: To provide scientific evidence on the outcome of a large number of cases treated by SealBio over the longer follow-up period. **Materials and Methods:** One hundred and thirty-four teeth in 116 patients presenting with pulp and periapical disease were randomly recruited between 2009 and 2014. SealBio was performed, and cases were followed up at regular intervals up to 6-year. **Results:** Of the total 134 teeth treated, 16 teeth could not be followed up and 9 cases failed (7.62% of cases). In only 4 cases (approximately 3.38% of cases), the failure could be directly attributed to endodontic causes. In the remaining 5 cases, coronal leakage from under the crown margins or dislodged restoration was found after 3–5 years of treatment. **Conclusions:** SealBio was found to be a successful, nonobturation, regeneration-based endodontic treatment protocol. By cell homing of endogenous stem cells, a biological seal rather than an artificial seal with gutta-percha and sealer cement is possible to achieve. It is highly cost saving and easier to perform, in addition to other advantages, such as retreatment is much simpler, and postcore restoration is possible after SealBio treatment.

Keywords: Endogenous stem cells, homing of stem cells, nonobturation root canal treatment, periapical healing, regenerative endodontic treatment, SealBio

Introduction

Interest in "regenerative endodontics" has revived since 2001 when Iwaya *et al*.[1] introduced "revascularization" (later termed as "revitalization") as a treatment modality for immature, non-vital teeth. Enormous research is being undertaken all over the world on how best to improve the treatment outcome. In this direction, use of platelet-rich plasma and platelet-rich fibrin, preparation of effective scaffold utilizing nano technology, various growth factors, injectable scaffold, etc., are being tested. Now, the scientists are also debating if using regeneration-based procedures, "biological obturation" of root canals in fully mature teeth can be achieved, instead of using external artificial materials such as gutta-percha and sealer cement.[2] Another novel approach could be to achieve apical seal with biological tissues rather than plug the canal space with root canal obturating materials. This novel regeneration-based, non-obturation endodontic treatment protocol "SealBio" was reported in 2012.[3]

Root canal obturation requires multiple dental materials comprising solid core and sealer cement. Over the past 1½ century, all possible materials have been used to obturate root canal space; from wood sticks, silver cones, silver amalgam, gutta-percha, Resilon, etc. Grossman[4] as early as in 1958 had stated, "I doubt very much whether there is any hollow cavity in the body that has been plugged with as many different materials as the root canal of a tooth."

Toward achieving a biological obturation, research is under way to regenerate vital pulp in the root canal space after debridement and disinfection. Various attempts are being made to search for the best method to deliver stem cells, scaffold, and growth factors in the canal lumen so that it can regenerate the vital pulp as existed before pulp infection had occurred. However, the total pulp volume in permanent teeth is 0.38 cc with the mean being 0.02cc.[5] The functions of pulp listed are nutrition, defense, and repair. In a mature

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Access this article online	
Quick Response Code:	Website: www.contempclindent.org
	DOI: 10.4103/0976-237X.188541

How to cite this article: Shah N. A regeneration-based, nonobturation root-canal treatment for fully-mature teeth: Six years' experience with "SealBio". Contemp Clin Dent 2016;7:296-301.

tooth with fully developed root, both laterally and apically, the function of dentin deposition is not required. Therefore, regeneration of pulp in a fully mature tooth is not likely to be of much benefit. Mao stated that attempt to regenerate tissues and high cost may be justified for potentially life-threatening diseases such as Parkinson's disease, diabetes, and spinal cord injuries, but likely not for tooth regeneration.[6] Applying the same analogy, attempt to revitalize a fully mature tooth after endodontic treatment with extraneous stem cells, growth factors, scaffold, etc., and the cost involved is similarly neither required nor justified.

If a canal after cleaning, shaping, and disinfection, can be maintained in a disinfected state, without recurrence of infection, the apical pathology can be healed, and a biological barrier, composed of fibrous or cementum tissue over the root apex can be achieved (which is considered the most desirable outcome of conventional endodontic treatment) without obturation of root canals, will be highly advantageous and cost saving.

The novel, non-obturation, "regeneration-based" treatment protocol "SealBio" was conceived and developed in 2009. The patent application was filed in 2010 and Australian and US patents were granted in 2014 and 2015 respectively (Australian patent no. 2010355508 and US patent no. US 9,180,072B2). The technique has been tested over the past 6 years on 134 teeth in 116 patients. This paper reports on the philosophy of treatment, its application in varied clinical cases, outcome, failures, and retreatment considerations in the light of treated cases and explains the scientific basis of all the procedures employed with evidence from the existing endodontic literature.

Materials and Methods

The study was conducted at the Centre for Dental Education and Research at the All India Institute of Medical Sciences, New Delhi. Ethical clearance was taken from the Institute Ethics Committee (P-06/January 21, 2009 dated February 12, 2009), and the study was registered with Clinical Trial Registry of India (CTRI) and Universal Trail Registry Number (UTRN) of WHO (CTRI/2010/091/001030, June 28, 2010, and UTRN WHO TEMP UTRN 093019259-2306201023293507). The patients who presented with either irreversible pulpitis or acute and chronic apical periodontitis, in reasonably good health, were randomly recruited, irrespective of age and gender during 2009–2014. The procedure was explained in detail, and a written informed consent was obtained from all the patients. The treatment was initiated in the tooth/teeth requiring endodontic therapy. Access cavity was prepared, and chemo-mechanical preparation was performed under aseptic conditions using instrumentation with crown-down technique and copious irrigation with 2.5% sodium hypochlorite. Apical patency was maintained throughout the cleaning and shaping procedure. As recommended in conventional root canal treatment, the canal was enlarged 3 sizes larger than the initial apical file size. Intracanal dressing of either triple antibiotic paste of ciprofloxacin, metronidazole, and tetracycline (in cases treated during 2009–2011) or calcium hydroxide (later since 2011) was given, and the cavity was sealed with intermediate restorative material. In the next visit after 5–7 days, if the clinical signs and symptoms were resolved and on opening, the canals were found dry and clean, the following steps were done to perform SealBio:

- The apical third of the canal was further enlarged to 2–4 sizes larger than the master apical file, which is termed as "apical clearing."[7] It is the dry reaming of the apical third without transportation of apical foramen or the canal. It helps in removing the loose debris from the apical third and the recesses, deltas, and accessory canals, which are the areas where bacteria hide and escape disinfection process
- After copious irrigation, "apical foramen widening" was done with gradually increasing size of K-file till size #25. This helps to clean the cemental part of the canal[8] as well as ensures smooth passage of instrument for overinstrumentation to be performed as the next step
- After "apical clearing" and "apical foramen widening," a final wash with Betadine was done and the canals were dried with sterile paper points. This was done to ensure that the final over-instrumentation was done by passage of an instrument through a clean, disinfected canal and to eliminate the possibility of pushing any traces of contaminant from the root canal to the periapical tissues. Intentional over-instrumentation into the periapical region was done with #20 K-file by giving gentle 2–3 clockwise turns and then withdrawn by giving counter-clockwise rotation, to induce bleeding and subsequent clot formation near the apical foramen.
- A calcium sulfate-based cement (Cavit, 3M ESPE USA) was introduced into the access cavity and with a hand plugger, condensed into the cervical third of the root canals. Access cavity was then appropriately restored with either silver amalgam/composite/cermet cement and full coverage coronal restoration was given, where indicated, at subsequent appointment. The patient was recalled every 6-months for clinical and radiographic evaluation.

Results

A total of 134 teeth in 116 patients were recruited; 76 men and 40 women. The age range was 12–80 years, mean age being 46.84 years. The cases were followed up for up to 6-year, which is shown in Table 1.

Criteria for success and failures

The cases were evaluated by clinical and radiographic evaluation. Clinical criteria included - tooth asymptomatic and functional, intraoral swelling, and sinus, if present pre-operatively, had healed. On radiographic evaluation, complete healing or decrease in the size of radiolucency. If the periapical area was normal at the start of treatment (as in

Table 1: Follow-up of treated cases

6 years	5 years	4 years	3 years	2 years	1 year	6 months	Lost to follow-up	Total
12	15	21	16	20	18	16	16	134

the cases where only pulpitis was the presenting complaint), no lesion should develop subsequently after the treatment.

Of all the cases treated, 16 cases were lost to follow-up. Only 9 cases (7.62 % of cases) failed. However, in only 4 cases, the cause of failure could be directly correlated to endodontic cause (approximately 3.38% of cases). In the remaining 5 cases, coronal leakage from under the crown margins or dislodged restoration was found, leading to periapical lesion with or without clinical symptoms, after 3–5 years of treatment. In these cases, retreatment was performed, in some cases, of only the exposed canal. Hence, the novel technique was successful in approximately 97% of the cases.

Discussion

Rationale for non-obturation

The concept of obturating root canals was based on the hollow tube theory given by Rickert and Dixon.[9] Their theory was based on observation in experimental animals that when hollow plastic tubes were implanted, inflammatory response was seen at both the open ends of the tube. It was inferred that the open ends encourage fluid movement in and out of the tube and hence evoke inflammation. Therefore, to prevent inflammation, both the ends of the tube must be sealed. The tube was likened to an unobturated root canal and hence the root canal obturation was considered mandatory. However, Goldman and Pearson[10] refuted the hollow tube theory. Their results showed no inflammation at the open ends of the implanted tubes when they were sterile and clean. The majority of the implants showed the presence of fluids inside the lumens of the tubes. This fluid was compatible with normal cellular function in adjacent tissues even after a long period of placement. From the results, it was concluded that the presence of space, even if fluid-filled, is not sufficient to initiate and perpetuate an inflammatory reaction. Klevant and Eggink[11] showed that stagnant tissue fluid and sterile necrotic pulp tissue do not sustain inflammation at the periapex.

Although the hollow tube theory no longer holds, the concept of root canal obturation has stuck. It is believed that it is impossible to completely clean all the canal space, and the remaining bacteria will cause reinfection of the canal and periapical tissues at a later date. Therefore, root canal obturation in three-dimension is mandatory to entomb the remaining bacteria and to prevent future reinfection.[12]

However, it has now been recognized that root canal system can be adequately disinfected, and microbial density can be lowered below the threshold level, required to initiate or perpetuate periapical infection. Grossman[13] had stated that an optimum concentration was necessary to initiate or sustain periapical inflammation. Body's defense mechanism can take care of residual infection. Sequera in 2011[14] stated that for any microbial species to cause disease, they have to reach a population density (load), conducive to cause tissue damage. It was demonstrated that reducing the microbial count to $<10^3$–10^4 colony-forming units, which is not detected on culture, would suffice to resolve the infection.

"Apical clearing" and "apical foramen widening" are intended to maximize debridement and results in reduced number of apical ramifications and bacterial load in the apical third.[15] Apical foramen widening removes the bulk of contaminated cementum.[16]

Sabeti *et al*.[17] had documented that there was no difference in periapical healing in teeth with and without obturation following complete disinfection, if the coronal seal of the sterile canal was maintained.

Healing following "SealBio"

The healing of periapical lesion is seen as a result of body's response to disinfected root canal space and decreased bacterial burden. Ostby demonstrated that healing response with deposition of cellular cementum and fibrous connective tissue in the apical third of the root canal had better resistance to infection and is advantageous over inert root filling materials.[18]

In "SealBio" technique, bleeding is induced by intentional over-instrumentation. The novelty of the present treatment protocol is that over-instrumentation is performed in fully formed, mature teeth after minimal apical foramen widening. The traditional text of endodontic literature contraindicates violating the apical foramen for fear of pushing necrotic debris and microorganisms from root canals into the periapical tissues and inducing inflammation in the periapical region.[19]

In the periapical region, periodontal ligament (PDL) stem cells, PDL progenitor cells (PDLPs), stem cells from the apical papilla, and bone marrow mesenchymal stem cells are commonly present,[20] which can be recruited by over-instrumentation into the scaffold provided by the blood clot, close to the apex of the root. The reason regenerative procedure succeeded in adults and in the presence of large periapical lesions could be explained by the fact that: a) the MSCs recruited in the regenerative endodontic procedure are either less prone to aging ("persisting MSCs") or elude aging indefinitely ("perennial MSCs")[21], b) the quiescent, non-proliferative adult MSCs can be stimulated by the signals triggered either by tissue damage or by different growth factors released[22] and c) the presence of inflamed periapical progenitor cells (iPAPCs)[23] and infection survived stem cells from

apical papilla (SCAP)[24] in teeth with large periapical pathology, helped the regenerative procedures in adults.

Organization of blood clot results in the release of various growth factors, which regulate multiple events involved in wound healing such as chemotaxis, cell adhesion, proliferation, and differentiation.[25] Of the many growth factors, platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor α and β, fibroblast growth factor, and bone morphogenetic proteins 2, 3, and 7 are important for regeneration of apical tissues.[26,27] The stem cells then differentiate into various forming cells such as fibroblast, cementoblasts, and osteoblasts which lay down fibrous/mineralized tissue over the apex; hence, the term "SealBio" - sealing with biological tissues.

There are two methods of tissue engineering; stem cells can putatively be isolated and expanded *in vitro* or can be stimulated *in situ*. Since the former is highly technically demanding and cost-intensive and as mentioned earlier, not justified for pulp–dentin regeneration in fully mature tooth, bioactivation of adult stem cells in the organ and the neighboring tissues is more practical for clinical implementation.[9] "SealBio" confirms to this philosophy of regeneration-based treatment protocol.

Coronal seal

If coronal seal is broken, bacteria or their endotoxin can reach the apex of the tooth within 20 days, and it is recommended to per form retreatment in such cases.[28,29] In SealBio technique, calcium-sulfate-based cement was placed on the floor of the access cavity and pushed into the cervical third of the canals with hand pluggers. It has the property to expand on setting in the presence of moisture, which provides an effective seal and in case retreatment was required, it is easy to gain access to the canals.

Figure 1: (a) Tooth #16 in a 58-year-old male periapical lesion at both mesial and distal root. (b) At 3-year follow-up, complete healing and normal bony architecture are evident (compare with Figure 2a). (c) At 6-year follow-up, the periapical area has remained normal

All the limitations/drawbacks associated with root canal obturation, as listed below, are eliminated in the non-obturation protocol of "SealBio:"

- The obturating materials act as foreign materials; if extruded beyond the confines of the root canal, have the potential to impair periapical healing or to cause a foreign body reaction[30]
- The effectiveness of root fillings to attain a fluid-tight seal has been questioned as experimental studies have shown microleakage at various interfaces of obturating materials[31]
- Obturation is demanding on operator's skill; if not performed well, it can result in under or over-filling, poor lateral condensation and can have voids, which would result in microleakage and reinfection at a later date[32]
- Iatrogenic mishaps during obturation such as excessive extrusion of sealer cement into the periapical space, invading the maxillary sinus or the inferior alveolar canal, causing severe pain or paresthesia have been reported[33,34]
- It requires obturation materials such as gutta-percha and sealer cement and various delivery systems, which adds to the total cost of treatment
- If retreatment is required, removal of the old root canal obturation materials from the root canals requires several instruments and retreatment kits, solvents, additional time, and can cause iatrogenic complications such as strip perforation.

Retreatment considerations

The failure cases could be categorized as reinfection (2), cystic lesion expanding (1), and periodontal cause (1).

In all the cases of retreatment, re-entry into the canal was found to be very easy. With a probe, the calcium sulfate-based cement could easily be felt and removed with files and irrigation to clean and retreat the canal.

Thus, the novel protocol of "SealBio" can be considered as a simple, easy to perform, cost saving, regeneration-based endodontic treatment protocol [Figures 1 and 2]. The other major advantages are: (i) posts can be used in

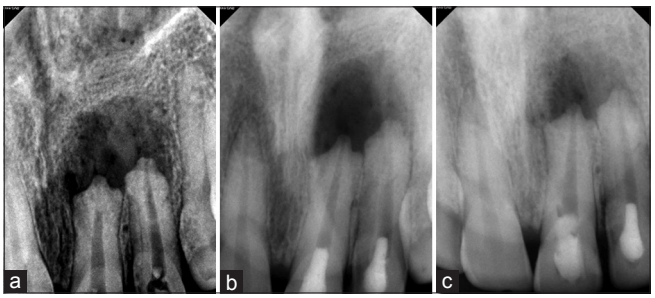

Figure 2: (a) Large periapical lesion associated with teeth #21 and 22 in a 45-year-old male. Significant apical root resorption of both the teeth is evident. (b) Follow-up radiograph at 6-month, showing significant healing (compare with Figure 3a). (c) At 2-year, bony trabeculae seen filling-in the apical radiolucency

cases treated by SealBio, as the canals are left empty and only an apical barrier is expected to form at the apical end [Figure 3], unlike in cases of revascularization/revitalization (another regeneration-based protocol for immature, nonvital teeth), where the canal is filled with vital tissues. (ii) As the cleaning and disinfection are augmented with "apical clearing" and "apical foramina widening," better healing response was observed, even in cases of large cyst-like lesions [Figures 4 and 5]. (iii) In case retreatment is required, it is easy to re-enter, as the canal is empty unlike removal of all the obturating materials, as required in conventional endodontic treatment. [Figure 6].

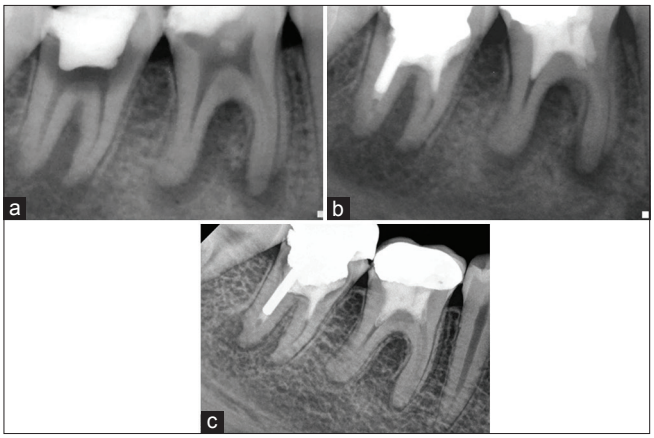

Figure 3: (a) X-ray showing large periapical lesions associated with teeth #36 and 37 in a 17-year-old girl. (b) SealBio was performed in both the teeth. The second molar required post and core restoration due to severe coronal destruction. Follow-up radiograph at 2-year showing significant healing (compare with Figure 4a). (c) Follow-up at 3-year shows normal bony architecture around both the root apices

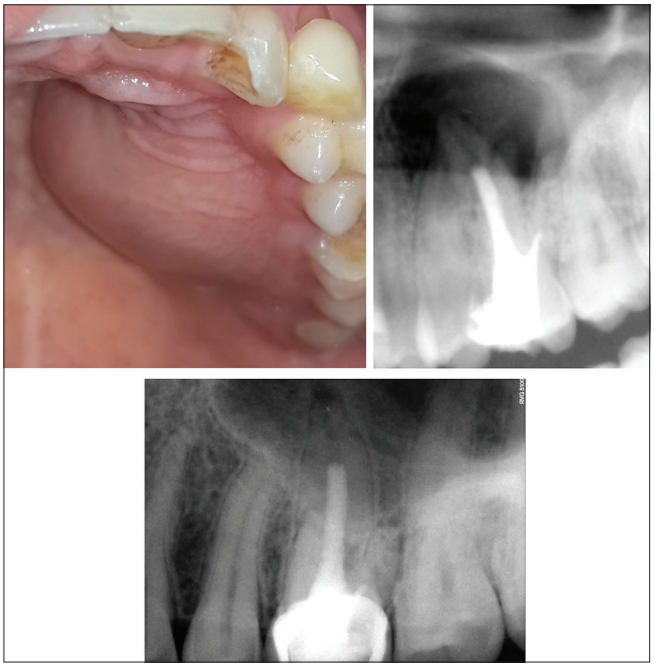

Figure 5: (a) Large, fluctuant swelling on left side of palate associated with carious tooth # 26. (b) Periapical X ray immediately after "SealBio. Note large cyst-like periapical radiolucency. (c) At 2-years follow-up, complete healing of the lesion is seen

Although the efficacy of this procedure is established in clinical endodontics by the large number of cases treated and long-term follow-up, further evidence may be established by histologic examination of the apical root end, to evaluate the type of tissue deposited to seal the apical end. Furthermore, the treatment outcome with SealBio needs to be compared

Figure 4: (a) Tooth #36 in an 18-year-old boy, showing a very large cyst-like radiolucency associated with distal root and also a smaller lesion on the mesial root. (b) At 6-month after SealBio, significant healing of both the lesions can be seen (compare with Figure 3a). (c) Radiograph at 3-year follow-up shows almost complete healing

Figure 6: (a) Tooth #36 in a 32-year-old male, immediately after SealBio. Note the wide root canal space, periapical lesions on both roots and root resorption, more marked on mesial root. (b) Patient reported 1½ years after SealBio, with diffuse bone expansion. Intraoral X-ray showed a large radiolucency around the mesial root. It was decided to perform retreatment only in the mesial root. (c) At 2½ years postretreatment, the apical lesion shows healing at the mesial root

with conventional endodontic treatment with obturation of root canals, for which the study is under way.

Conclusion

Long-term follow-up of treated cases has proved the efficacy of "SealBio" as non-obturation of root canals does not cause re-infection, if proper coronal seal is provided. The technique is highly effective, simple to perform and cost and time saving, as obturation is not required. Additional benefits are that retreatment is much easy and allows the use of post and core restorations, as no vital tissue is in the canal space.

Acknowledgment

The author wishes to acknowledge Dr. Vijay Kumar, Assistant Professor for the assistance during preparation of the first draft of the manuscript and Dr. Ajay Logani, Additional Professor, Department of Conservative Dentistry and Endodontics, Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi, for initial reviewing the manuscript. The assistance of all the postgraduates, senior residents, and research officers is acknowledged for their very valuable help in radiographic evaluation of pre- and post-treatment cases. I would also like to acknowledge Ms. Kiran for her secretarial assistance in record keeping and patients' recall during the entire study period.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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