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Case Report

Treatment of intrabony defect using silk fibroin as GTR with Xenograft

Archana R Sankar[1], Sheela Kumar Gujjari[2]

[1]Postgraduate student, Department of Periodontology, JSS Dental College & Hospital, JSSAHER, Mysuru, India
[2]Professor and Head, Department of Periodontology, JSS Dental College & Hospital, JSSAHER, Mysuru, India
Correspondence:sheelagujjari@gmail.com

Abstract

Background: Although various treatment strategies have been tried, the use of bone grafts along with a barrier membrane has been the gold standard in treating the intrabony defects for periodontal regeneration. In vivo animal studies and a few in vitro studies have revealed the importance of silk fibroin in bone tissue engineering and has also shown its potential as a scaffold for periodontal ligament cells. The main objective of the case report was to assess the clinical and radiological outcomes of silk fibroin membrane with a xenograft in the treatment of intrabony defects. Results: A male patient reported with a complaint of food lodgment in the right lower back tooth region. On examination there is a pocket depth of 5mm with grade II furcation irt 46. Phase I therapy followed by open flap debridement was done. Intrabony defect was filled using xenograft and silk fibroin membrane was used as GTR membrane. Reduction in probing pocket depth with gain in CAL was noticed clinically. Radiographs were taken to further analyze the bone fill using AUTOCAD software at the end of 12 months. Conclusion: Silk fibroin when used as GTR with xenograft in treating grade II furcation defects has given good clinical improvements in periodontal regeneration. It can thus be considered as a better material for periodontal regeneration with further longitudinal clinical trials in tissue engineering in the field of Periodontology.

Keywords: Intrabony defects, Silk fibroin, Bone graft, Bone tissue engineering.

Introduction

Periodontal disease is an inflammatory and infectious disease affecting the tooth supporting structures leading to tooth loss when left untreated. The molars show greater amount of periodontal destruction and highest frequency of tooth loss [1].

Periodontal regeneration deals with the reconstruction of injured or lost tooth-supporting tissues i.e. cementum, periodontal ligament and alveolar bone in such a way that the architecture and function are restored to normal [1].It is a well-established fact that the cells from the periodontal ligament have the potential for regeneration of the tooth attachment apparatus during the disease/repair process [2].In addition, bone grafts have been used as gold standard to regenerate the lost bone around the tooth. Of the various bone graft materials, xenograft has been used predominantly due to its structural similarity to human bone thereby improving the osteoconductive properties when compared to that of synthetically derived materials. Added advantage of xenografts over autografts is there is no requirement of a donor site Apart from the bone grafts, guided tissue regeneration (GTR) using various barrier membranes either resorbable or non resorbable has brought a revolution in the periodontal regeneration [2]. There is ample evidence stating that the periodontal regeneration is better when it was attempted with combination of bone graft and barrier membrane when compared to flap surgery alone or flap surgery with bone graft [4,5,6].When both GTR and bone grafts alone have shown to be promising in furcation fill and intrabony defects, it also has been proved that a combination of bone graft and GTR shows a greater amount of defect fill [7,8,9,10].

With the emergence of tissue engineering, these barrier membranes have undergone various modifications without compromising its properties specially to promote sel-ective cell proliferation within the defect and should be absorbed at a rate that parallels regenerative tissue formation within 4 weeks to 6 months. There are very few membranes which fulfill these requirements, hence the search is on for an innovative material from a natural

New insights have shown that silk could be the futuristic regenerative material due to its remarkable mechanical properties, biocompatibility and biodegradability (after a period of 12 months) [11]. It is a novel biomaterial which has evolved from insects such as Bombyxmori silkworm, mites, spiders and beetles, has proved to be a promising material as a scaffold in tissue engineering [12].

Silk contains two proteins namely fibroin and sericin and studies have demonstrated their potential in regeneration, but the allergic nature of sericin has reduced its application in the field of medicine. Another characteristic of silk, it can be processed different forms such as hydrogels, films, Nano fibers and nanoparticles with a controlled degradation rate by beta sheet content during processing(not assessed in the present case)[13].

Though, there are animal studies in regenerative medicine using silk fibroin there is sparse or no evidence on usage of silk fibroin on humans as a scaffold material in regeneration [14-23].In this case report an indigenous silk fibroin membrane was developed and used as scaffold to regenerate the lost periodontal structures.

Fabrication of Silk Fibroin Membrane

Materials:

1. Species of Bombyx.mori cocoons obtained from Satellite Silkworm Breeding Station, Gobichettipalayam, Tamil Nadu, India.
2. Sodium bicarbonate, Calcium chloride, Ethanol and Hydroxyl Propyl Methyl Cellulose were purchased from Padmashree chemicals, Mysuru.
3. Cellulose membrane was obtained from Sigma Aldrich.

All the materials used for the study were of pharma grade and were **Generally Regarded As Safe (GRAS).**

Step 1: Extraction of Silk fibroin from Bombyxmori [24]

Bombyx mori silkworm cocoons were cut into small pieces and were placed in a beaker of 1 liter ultrapure water and boiled for half an hour. To the heated silk fibers,2grams of sodium bicarbonate was added occasionally and stirred with a glass rod. After cooling, the silk fibers were washed with water to remove the excess sodium bicarbonate followed by squeezing to remove water. The degummed silk fiber was stored at room temperature (can be stored for a long term period) and wrapped in an aluminum foil.

Dialysis of degummed silk fiber

A dialysis bag (cellulose membrane) was presoaked in ultrapure water for 24 hours. The silk fibers cium chloride. Ethanol-Water which was used as a predissolving solvent mixture for the silk fibers. The soaked silk fibers were then placed in cellulose membrane (dialysis cassette was replaced) and dialysed against ultrapure water for 4 days.

Centrifugation

Silk fibers and the supernatant was removed from the dialysis bag after centrifugation at 4000rpm at 4°C for 40 minutes in ultra-low centrifuger. The resultant viscous solution was then stored in screw capped tube.

Step 2: Preparation of the membrane

The extracted viscous solution was mixed with 0.2% hydroxyl proplyl methyl cellulose (HPMC) and was poured into petridishes with gentle tapping for even dispersion of contents. The petridishes were placed in a vacuum driver (Memmert Company) for one day at 39°C to get a membrane. The obtained membrane was gamma sterilized for 2 hours using cobalt 60 at 15kGy. (fig-1)

Case Report:

A male patient of aged 46yrs reported to the Department of Periodontology, JSS Dental College & Hospital, Mysuru with the chief complaint of food lodgment and pain in the right lower back tooth region with no relevant medical history. On intra oral examination, there was bleeding on probing with a probing pocket depth (PPD) of 6mm in relation to distal of right mandibular first molar (tooth no.46) with grade II furcation involvement.

Pre-surgical phase:

Pre surgically, patient was emphasized on the importance of oral hygiene and underwent Phase I therapy comprising of scaling and root planing. Then, patient was sent for routine hematological investigations and surgery was scheduled after 4 weeks. All the measurements were recorded using Williams graduated periodontal probe to the nearest millimeter.

Surgical phase

After asepsis defect site was anesthetized using 2% lidocaine with 1:80,000 adrenaline. Using blade No.12 intra crevicular incisions were given and a full thickness flap (conventional flap) was raised. Thorough manual debridement including root planing was done at the defect site using Gracey curettes. After instrumentation, the surgical area was irrigated thoroughly with saline to remove any remaining detached fragments. Any adherent granulation tissue on the flap if present was trimmed. A 2 walled intrabony defect was noted distal to 46 and received xenograft (colocast) and silk fibroin as a GTR membrane was placed 1-2mm apical to the cementoenamel junction to prevent its exposure as a result of post-

operative tissue shrinkage. (fig 2).The flaps were repositioned with simple interrupted suturing using 3-0 silk sutures and periodontal dressing (Coe-Pak) was placed.

Postoperative care:

Manifestations of any allergic reaction like irritation, itching etc were observed for 1 hour post surgery, subsequent to which the patient was discharged after postoperative instructions and asked to use Charters method of tooth brushing. Antibiotics [Amoxcicillin 500mg] and analgesics [Aceclofenac + Paracetamol] combinations were prescribed for 5 days and was advised to be taken only if it was necessary on a 'need to treat' basis.

Post-surgical evaluation:

At regular intervals, the patient was recalled to evaluate his oral hygiene maintenance and the periodontal pack and sutures were removed after a week.Radiographic parameters were assessed using conventional IOPAR with x-ray mesh gauge at baseline and 12 months (**fig 3a,b).** Grid with markings of $1mm^2$ was used to assess the bone fill at the defect site and also to reduce scattered radiation to improve image contrast. differences between preoperative and post-operative changes could be calculated. The radiographs were subjected to AUTOCAD ver. 2017 software for the analysis of furcal and intrabony defect bone fill.

Discussion

There is sufficient evidence from various systematic reviews, meta-analysis and Cochrane database, regarding the use of GTR membrane along with a bone graft in the treatment of intrabony defects and in grade II furcation defects.

Delivery of slow, sustained controlled release of bioactive molecules while maintaining the space at the intrabony defects plays a vital role during periodontal regeneration. Upon considering the available data silk can address these needs and can act as GTR. Silk fibroin protein in silk has the properties like self-assembly, mechanical toughness, processing flexibility, biodegradability and biocompatibility, when prepared and processed indigenously under ambient aqueous conditions to prevent loss of bioactivity of the drugs then the purpose would be served as equal to GTR [13]. In this case report there was a reduction in PPD from baseline to 12 months from 6mm to 3mm with clinical attachment level gain which could be due to the complete debridement with result of true periodontal regeneration via new attachment or of healing by repair through flap. This is in accordance with a study where intrabony defects on lower first molars showed improvement in clinical attachment level (CAL) when treated with bone graft and a barrier membrane[25].

Fig 1: silk fibroin membrane

Fig 2: bone graft and silk fibroin membrane

(a)

(b)

Fig 3: (a) bone at baseline, (b)- bone fill at 12 months

Although X ray grid can evaluate bone changes, AUTOCAD is more precise and can give values up to decimal points[26,27,28].Changes in bone fill were detected using Grid. AUTOCAD was used in the present case report and this is in accordance to a study where regeneration of intrabony sites treated with Perioglass and PRF were determined using AUTOCAD 2007 which was precise in measuring bone fill [29].

There was notable bone area fill at the site (6.4mm at 12 months) which may be due to the porous nature of the scaffold used which supported the attachment, proliferation, and differentiation of human mesenchymal stem cells thereby making it an ideal material for bone regeneration[30].

Another study showed the growth of mineralized bone matrix on silk scaffolds was a result of enhanced differentiation and faster recruitment of cells. New Zealand white rabbits when treated with silk membranes of 0.5mm thickness resulted in effective bone regeneration. The processed silk fibroin membrane used in this study had a thickness of 1.1mm which would have contributed to increase in bone area fill [31,32].

A lthough evidence states that tissue engineering is possible with silk, further studies are required to confirm its efficacy. More longitudinal studies are required to assess the relation of thickness of silk and regeneration.

Conclusion

Silk fibroin throws a light to expand the family of regenerative barrier membranes with an enhanced performance for bone regeneration in the field of dentistry. It can thus be considered as a better material for periodontal regeneration with further longitudinal clinical trials.

Future considerations:

In this case report degradability of the membrane, porosity and cytotoxic effects were not assessed. Using this membrane in more cases would enhance the use of silk as membrane in the periodontal regeneration.

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